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Nutritional qualities of common edible cephalopods at the Arabian Sea

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Abstract

Nutritional composition of the edible portions of five commercially important species of cephalopods in the Arabian Sea was evaluated. The selected species included *Amphioctopus neglectus, Cistopus indicus, Uroteuthis duvauceli, Sepia pharaonis* and *Sepiella inermis*. The cephalopods were demonstrated to contain protein with balanced proportions of essential to non-essential amino acids (~ 1.2). *A. neglectus* was found to contain greater quantities of sulfur containing amino acids (23 mg/100 g) and lysine (36 mg/100 g) than other cephalopods, which indicated that the protein can effectively complement the limiting amino acids in our daily diets. The C_{20}–C_{22} long chain n-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, vital for human health, were found to be predominant in the edible part of the cephalopod species (119-360 mg/100g and 595-1211 mg/100g respectively). The n-3/n-6 polyunsaturated fatty acid ratio of *U. duvauceli* was significantly greater (~10, $P < 0.05$) than other cephalopods, and may consequently serve as a substitute to balance the greater admission of n-6 fatty acids in our standard utilization of vegetable oil. Fatty acid based atherogenicity and thrombogenicity indices (<0.8 and <0.3, respectively) were found to be ideal in cephalopods ensuring that they can be consumed without any risk for health.

Introduction

Class Cephalopoda represents the most advanced class of the phylum Mollusca, and include some interesting members, for example, squids, octopods, cuttlefishes and so forth (Boyle and Rodhouse, 2005). These species are exclusively marine, and are distributed throughout the seas of the world. The vast majority of them are free swimming shallow water predators characterized by rapid growth. More than 700 species are described today, and their number tends to increase every year as new species are discovered, particularly in tropical and polar seas (Sweeney and Roper, 1998). These species occupy a leading place among the exploited marine fishery resources in the world, and are receiving greater importance due to their increasing demand in export (Kreuzer, 1984).

The increasing demand for cephalopods in the international market is mainly due to the increased awareness of their nutritional qualities (Lee, 1994; Okuzumi and Fujii, 2000). Cephalopods were reported to contain greater protein content and more than 80 percent of their total body weight comprises the edible part for consumption by human beings (Lee, 1994). This speaks to a significant effect on the marketability of cephalopods, especially when contrasted with about 40 percent for shellfish and a maximum of 75 percent for teleosts (Kreuzer, 1984). The cephalopods were accounted for to be a rich source of long chain n-3 polyunsaturated fatty acids, essential amino acids, antioxidants and minerals, such as, selenium, which could not be acquired from other sources (Zlatanos *et al.*, 2006). In addition to this, their good sensory properties make them a preferred human delicacy.

The advancement of cephalopods as an important commercial fishery commodity in the international market occurred only during the recent decades (Boyle and Rodhouse, 2005). Declining catches in numerous conventional groundfish stocks have prompted expanded effort to develop the potential of non-traditional species, especially invertebrates, for example, the cephalopods. Information from the fifteen distinct FAO zones revealed that, except for the north-east Atlantic, the landings of cephalopod have significantly expanded in the course of the last twenty five years (Caddy and Rodhouse, 1998). These species were reported to be one-time spawners with a comparable shorter lifespan, and therefore, are favorably adapted for rapid growth (Boyle and Rodhouse, 2005). The production of cephalopods was less in the traditional fisheries, but after the introduction of trawling cephalopod exploitation experienced new strides. Cephalopod catches have expanded consistently during the last forty years,

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from 1 million metric tonnes in 1970 to about 4 million metric tonnes in 2010 (FAO, 2012), and the share of cephalopods (squid, cuttlefish and octopus) in world fish trade was 4 percent in 2010.

Cephalopods offer a good market price, and are the subject of global trade due to their good nutritive value. The major consumers of cephalopods are Japan, Spain, Republic of Korea, Italy, Portugal, Taiwan Province of China, and Hawaii (Shenoy, 1988; FAO, 2012). Asia reported the highest octopus production in 2010, at 2, 17, 506 tonnes (FAO, 2012). Due to their nutritional and market value, cephalopod mariculture practices of cephalopods have likewise demonstrated an increasing interest during the past few years (Lee, 1994). Considering the promising perspective for the utilization of cephalopod in the waters of the Arabian Sea, as a potential health food, studies on its biochemical composition began to receive considerable attention. Though, a couple of studies have been coordinated to the biological description, distribution, and proximate composition of the entire body or on distinctive body parts of cephalopods (Okuzumi and Fujii, 2000; Forsythe *et al.*, 2002; Thanonkaew *et al.*, 2006; Pierce *et al*., 2008), no published studies are available on their detailed nutritional composition. Also scanty reports are available on the fatty acid compositions and the seasonal variations in the fatty acid profiles of cephalopods (Ozyurt *et al.*, 2006). The cephalopods considered in the present study were *Amphioctopus neglectus* (Nateewathana and Norman, 1999), *Cistopus indicus* (Rapp, 1835), *Uroteuthis duvauceli* (D'Orbigny, 1835), *Sepia pharaonis* (Ehenberg, 1831), and *Sepiella inermis* (Van Hasselt, 1835), available in abundance at the waters of the Arabian Sea. The present study is designed to investigate in detail the biochemical profile of the edible parts of the cephalopods (mantles and tentacles) considered in the present study, and to compare the nutritional parameters among these species. The nutritional parameters taken into account were protein, lipid, fatty acids, amino acids, cholesterol, vitamins and minerals, keeping in mind the implication of such a variation for pharmaceutical products, food additives, and dietary health supplements. The health indices, for example, atherogenicity index, thrombogenicity index, and hypocholesterolaemic/ hypercholesterolaemic ratio of the edible parts of the cephalopods were taken into account to understand their nutritional qualities as healthy food for human consumption.

Materials and Methods

Study area and sample preparation

The study was directed during the monsoon months of June and July of the year 2014. A total of about 5 kg (mean weight) of each species, comprising of mature animals (the size of which were comparable to those available in the local market) were selected for the study. The cephalopod samples of practically identical body size of the individual species were collected from the collection site, before being sliced to about 500 g sub-samples. Since the nutritional evaluation of the edible parts, namely the mantle and tentacles were intended; the internal organs were removed before being stored in the container. The tissues were thereafter ground properly, and were stored at -80^{O}C for further analysis.

Fatty acids, cholesterol and fatty acid based nutritional indices

The extraction of the lipids in the tissues of the cephalopods was carried out by the Folch extraction method (Folch *et al*., 1957) using chloroform:methanol (2:1 v/v; 200 mL). The extracted lipids were determined gravimetrically. The fatty acid composition of the total lipids from the edible parts of cephalopods was determined as described elsewhere (Metcalf *et al.*, 1966; Chakraborty and Paulraj, 2009). GLC data were recorded on a Perkin-Elmer AutoSystem XL gas chromatograph (HP 5890 Series II, Perkin Elmer, Bridgeport Ave, Shelton, CT, USA) connected with a SP 2560 (crossbond 5% diphenyl-95% dimethyl polsiloxane) capillary column (100 m X 0.25 mm i.d., 0.50 μm film thickness, Supelco, Bellfonte, PA) using a flame ionization detector (FID) equipped with a split/splitless injector, which was used in the split (1:15) mode. The results were expressed as mg fatty acids/100 g of edible portion (FA/100g EP). The different ratios of fatty acid, indicating nutritional values of the edible portion of the cephalopods, namely, n–3/n–6, EPA/AA, DHA+EPA and PUFA/SFA were calculated.

The total cholesterol content in the edible tissues of cephalopods was determined spectrophotometrically (Varian Cary 50, Palo Alto, CA, USA) as described elsewhere (Wanasundara and Shahidi, 1999) with suitable alteration utilizing o-phthalaldehyde (50 mg/ dL in glacial acetic acid). The aggregate cholesterol content was ascertained from the standard curve of cholesterol, and expressed as mg/100 g EP.

The indices of atherogenicity (AI) and thrombogenicity (TI) (Ulbricht and Southgate, 1991) have been calculated as:

AI = (4 × 14:0 + 18:0 + 16:0)/ (MUFA+Σn–3 PUFA +Σn–6 PUFA);

TI = (14:0 + 18:0 + 16:0)/ [(0.5 × MUFA) + (0.5 ×n–3 PUFA) + (3 × n–3 PUFA)+(n–3 PUFA /n–6 PUFA)]

The hypocholesterolaemic/hypercholesterolaemic (HH) ratio (Santos–Silva *et al.*, 2002) was calculated as:

HH = (18:1n–9 + 18:2n–6 + 20:4n–6 + 18:3n–3 + 20:5n–3 + 22:5n–3 + 22:6n–3)/ (14:0 + 16:0).

Protein and amino acids

The true protein contents of the edible portion of the cephalopods were estimated by the established method (Lowry *et al.*, 1951). The absorbance of the protein aliquot was measured at 660 nm in a UV–visible spectrophotometer (Varian Cary, USA) within 15 min against the reagent blank. The protein content of the sample was calculated from the standard curve of bovine serum albumin, and expressed as mg/100g of wet weight. Estimation of amino acid was carried out as described earlier (Chakraborty and Joseph, 2015). The samples (0.1 g) were hydrolyzed with HCl (6N, 10 mL) at 110°C in sealed glass tubes for 24 h on a multi–place heating mantle. The aliquot containing hydrolyzed amino acids was treated with redrying reagent (methanol 95%: water: triethylamine, 2:2:1 v/v/v), and thereafter pre–column dramatization of hydrolysable amino acids was performed with phenyl isothiocyanate (PITC or Edman's reagent) to form phenylthiocarbamyl (PTC) amino acids. The reagent was freshly prepared, and the composition of derivatising reagent comprised of methanol 95%:triethylamine: phenylisothiocyanate (20 µL, 7:1: l v/v/v, 70 µL methanol + 10 µL distilled water + 10 µL triethylamine +10 µL phenylisothiocyanate). The derivatized sample (PTC derivative, 20 µL) was diluted with sample diluent (20 µL, 5 mM sodium phosphate $NaHPO_4$ buffer, pH 7.4: acetonitrile 95:5 v/v) before being injected into reversed–phase binary gradient HPLC (Waters Corporation, Milford, MA 01757, USA) fitted with a column maintained at 38°C in a column oven to be detected by their UV absorbance (λ_{max} 254 nm). The mobile phase comprised of (A) sodium acetate trihydrate (0.14 M, 940 mL, pH 6.4) containing triethylamine (0.05%), mixed with acetonitrile (60 mL), and (B) acetonitrile: water (60:40 v/v). A gradient elution program, with increasing eluent B was employed for this purpose. An additional step of 100% eluent B is used to wash the column prior to returning to initial conditions. The standard (PIERS amino acid standard H; Thermo Scientific) was run before each sample injection. Samples (PTC amino acid derivatives) were injected in triplicate, and the output was analyzed using the BREEZE software (Waters).

The quantification of amino acids was carried out by comparing the sample with the standard (PIERS amino acid standard H; Thermo Scientific), and the results were expressed in mg/100 g edible portion. The total essential amino acids (TEAA), total non-essential amino acids (TNEAA), total amino acids (TAA), total aromatic amino acids (TArAA), total sulfur containing amino acids (TSAA) and the ratios of TEAA to TNEAA, TEAA to TAA, TNEAA to TAA and leucine/isoleucine (leu/ile) were calculated. The amino acid score (AS) for the essential amino acids was calculated using the formula: amount of amino acid per sample protein (mg/g)/amount of amino acid per protein in reference protein (mg/g), with respect to the reference amino acid requirements for adults (FAO/WHO/UNU, 2007).

Estimation of vitamins

Estimation of the fat soluble vitamins (A, D_3, E and K1) was carried out by a modified method of Salo–Vaananen *et al.* (2000). Briefly, the stock solutions (1, 10, 25, 50 and 100 ppm) of vitamin standards (Sigma–Aldrich Chemical Co. Inc, St. Louis, MO) were stored at –20°C except vitamin D_3, where the stock solutions were stored at 4°C. The lipids (0.1 g) were extracted utilizing the established method (Chakraborty *et al.*, 2014), before being hydrolyzed (KOH/MeOH 0.5 N, 2 mL). The hydrolyzed mixture (2 mL) was extracted with petroleum ether (fraction of 40–60°C, 15 mL) and washed with deionized water (2 × 10 mL) to make it alkali free. The non–saponifiable portion was concentrated under vacuum using a rotary evaporator (Heidolph Instruments GmbH and Co., Schwabach, Germany) at 50°C before being reconstituted in MeOH. The latter was filtered through a syringe filter (0.2 µm) before being injected (20 µL) in the HPLC (Shimadzu LC 20AD, Shimadzu Corporation, Nakagyo–ku, Japan). The HPLC system was equipped with a reverse phase column (phenomenex, C18 250 mm length, 4.6 mm i.d., 5 µm) that was housed in a column oven (32°C) and connected to a photodiode array detector. The gradient programme was as follows: 20% MeOH (HPLC grade) up to 3 min, which was increased to 100% in the next 5 min and held for 37 min with a complete run time of 45 min. The flow rate was 1 mL/min. Vitamin C was determined based upon the quantitative discoloration of 2, 6–dichlorophenol indophenol titrimetric method (AOAC, 2005). In brief, ascorbic acid was extracted from the fish fillet

(M, 15–20 g) using an acetic acid and metaphosphoric acid solution (HPO_3–CH_3COOH, 10 mL × 2). The extracts were transferred with distilled water into a known volume (B, mL) and filtered rapidly. The known volume (C, mL) of the above solution was pipetted out and titrated with the redox dye, 2, 6–dichlorophenol indophenol solution until the faint pink color persisted for 15 s.

Ascorbic acid was calculated as: $\{(A - A_0) \times D \times B \times 10\} / (M \times C)$,

where A = average volume for test solution titration (mL), A_0 = average volume for test blank titration (mL) and D = mg ascorbic acid equivalent to one mL indophenol standard solution. The vitamins A, D_3, K1 and C were expressed as µg/100g wet tissue, whereas vitamin E was expressed as mg/100 g wet tissue.

Estimation of minerals

Estimation of minerals was carried out by atomic absorption spectrophotometer (AAnalyst™ 200 spectrometer, Perkin Elmer, Bridgeport Ave, Shelton, CT, USA) following the di-acid (HNO_3/$HClO_4$) digestion method with suitable modifications (Chakraborty and Joseph, 2013). Phosphorus content was analyzed by an alkalimetric ammonium molybdophosphate method as described by AOAC official method 964.06 (AOAC, 2005). The minerals were expressed in mg/100 g wet tissue.

Statistical analyses

Statistical evaluation was carried out with the Statistical Program for Social Sciences 13.0 (SPSS Inc, Chicago, USA, ver. 13.0). Analyses were carried out in triplicate and the results were expressed as mean ± standard deviation. Descriptive statistics were calculated for all the studied variables. The means of all parameters were examined for significance by analysis of variance (ANOVA) with Scheffe´'s post-hoc analysis, and the level of significance for all analyses was $P \leq 0.05$. The mean variance in the data set was detected using principal component analysis (PCA).

Results

Lipid, cholesterol, fatty acid profile, and nutritional indices of the cephalopods

Significant differences in lipid content were noted in the edible parts of cephalopods species ($P < 0.05$), wherein *S. pharaonis* and *A. neglectus* recorded greater content of lipid (2.5 mg/100 g EP)

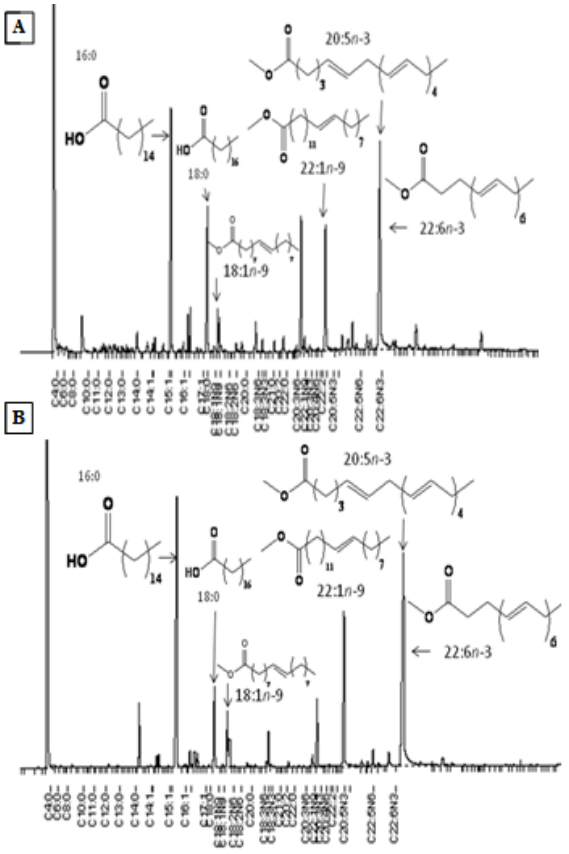

Figure 1. Indicative gas liquid chromatograms of fatty acid composition in (A) *A. neglectus* and (B) *U. duvauceli*

than different cephalopods. The cholesterol content of *A. neglectus* was found to be lesser (~100 mg/100 g EP) than those in *C. indicus* (128.6 mg/100 g EP) and *U. duvauceli* (191.6 mg/100 g EP) (Table 1). The fatty acid compositions of cephalopod species under study were presented in Table 1. The cephalopods demonstrated greater quantities of polyunsaturated fatty acids (PUFA) (595-1211 mg FA/100 g EP) followed by saturated (SFA) (398-879 mg FA/100g EP) and monounsaturated fatty acids (MUFA) (166-485 mg FA/100 g EP). *A. neglectus* demonstrated significantly greater contents of ($P < 0.05$) total SFAs (879 mg FA/100 g EP) than other cephalopod species (Figure 1A). Palmitic acid (16:0) was the predominant SFA found in cephalopods (161-455 mg FA/100 g EP) followed by stearic acid (18:0) (91-278 mg FA/100 g EP). The content of 18:0 was greater in *A. neglectus*, and contributed about 32 percent of the aggregate SFAs. The aggregate MUFA content in the edible parts of cephalopods was found to differ from 166-515 mg FA/100 g EP. Erucic acid (22:1n–9), oleic acid (18:1n–9), and palmitoleic (16:1n–7) were found to be the predominant MUFAs. The content of 22:1n-9 was significantly greater (72-322 mg FA/100 g EP) ($P < 0.05$) than 16:1n-7 and 18:1n-9 (Table 1). The fatty acid 22:1n-9 in *C. indicus* contributed to about 66 percent of the aggregate content of

Table 1. Lipid, cholesterol, and fatty acid composition (mg/100g EP) of the cephalopod species collected from the south west coast of India

	A. neglectus	*C. indicus*	*U. duvauceli*	*S. pharaonis*	*S. inermis*
Lipid	$2.50^{a} \pm 0.14$	$1.75^{b} \pm 0.05$	$1.75^{b} \pm 0.03$	$2.56^{c} \pm 0.12$	$1.30^{d} \pm 0.08$
Cholesterol	$103.65^{a} \pm 2.15$	$128.64^{b} \pm 5.02$	$191.65^{c} \pm 5.02$	$320.15^{d} \pm 12.0$	$307.51^{c} \pm 15.3$
Saturated fatty acids					
12:0	$8.47^{a} \pm 0.20$	$0.33^{b} \pm 0.01$	$2.88^{c} \pm 0.11$	$0.50^{b} \pm 0.04$	$2.89^{c} \pm 0.17$
14:0	$40.26^{a} \pm 1.12$	$15.77^{b} \pm 0.95$	$55.28^{c} \pm 1.12$	$24.02^{d} \pm 1.21$	$32.67^{c} \pm 2.21$
15:0	$21.99^{a} \pm 0.93$	$12.65^{b} \pm 0.75$	$9.32^{c} \pm 0.54$	$25.61^{d} \pm 0.92$	$22.25^{a} \pm 1.04$
16:0	$455.01^{a} \pm 13.5$	$160.73^{b} \pm 3.65$	$408.83^{c} \pm 3.03$	$368.94^{d} \pm 7.19$	$199.66^{e} \pm 7.45$
17:0	$70.66^{a} \pm 1.50$	$7.20^{b} \pm 0.42$	$15.58^{c} \pm 0.91$	$6.06^{d} \pm 0.43$	$12.99^{e} \pm 0.75$
18:0	$278.65^{a} \pm 10.3$	$253.92^{b} \pm 12.3$	$91.35^{c} \pm 1.56$	$264.77^{d} \pm 5.14$	$117.59^{e} \pm 5.48$
20:0	$1.23^{a} \pm 0.07$	$0.84^{b} \pm 0.02$	$2.86^{c} \pm 0.17$	$23.13^{d} \pm 1.32$	$3.03^{e} \pm 0.20$
22:0	$2.98^{a} \pm 0.15$	$2.20^{b} \pm 0.12$	$2.87^{a} \pm 0.15$	$2.30^{b} \pm 0.17$	$3.89^{c} \pm 0.14$
24:0	ND	$7.98^{a} \pm 0.34$	$2.03^{b} \pm 0.12$	$4.86^{c} \pm 0.25$	$3.29^{d} \pm 0.25$
[x]∑SFA	$879.25^{a} \pm 20.9$	$461.62^{b} \pm 12.5$	$591^{c} \pm 16.10$	$720.19^{d} \pm 12.7$	$398.26^{e} \pm 10.5$
Monounsaturated fatty acids					
14:1*n*-7	$15.85^{a} \pm 0.82$	$7.47^{b} \pm 0.31$	$2.15^{c} \pm 0.10$	$13.01^{d} \pm 0.81$	$3.34^{e} \pm 0.22$
15:1*n*-7	$13.94^{a} \pm 0.51$	$9.32^{b} \pm 0.44$	$1.99^{c} \pm 0.12$	$6.53^{d} \pm 0.22$	$4.42^{e} \pm 0.13$
16:1*n*-7	$53.26^{a} \pm 3.04$	$68.97^{b} \pm 1.18$	$13.69^{c} \pm 1.04$	$113.78^{d} \pm 1.30$	$40.54^{e} \pm 1.25$
18:1*n*-7	$2.96^{a} \pm 0.15$	$3.69^{b} \pm 0.24$	$0.33^{c} \pm 0.11$	$3.29^{b} \pm 0.15$	$2.77^{a} \pm 0.14$
18:1*n*-9	$131.26^{a} \pm 2.36$	$68.5^{b} \pm 1.92$	$83.43^{c} \pm 1.40$	$107.04^{d} \pm 1.50$	$27.37^{e} \pm 1.14$
20:1*n*-9	$25.52^{a} \pm 1.14$	$2.87^{b} \pm 0.11$	$0.16^{c} \pm 0.01$	$1.02^{d} \pm 0.07$	$3.39^{e} \pm 0.21$
22:1*n*-9	$228.04^{a} \pm 4.90$	$322.34^{b} \pm 12.3$	$71.74^{c} \pm 1.50$	$268.31^{d} \pm 14.6$	$83.48^{e} \pm 1.32$
24:1*n*-9	$6.23^{a} \pm 0.34$	$2.38^{b} \pm 0.15$	$7.46^{c} \pm 0.34$	$2.30b \pm 0.13$	$0.98^{d} \pm 0.02$
[y]∑MUFA	$477.06^{a} \pm 15.8$	$485.54^{a} \pm 12.1$	$180.95^{b} \pm 9.50$	$515.28^{c} \pm 11.5$	$166.29^{d} \pm 9.70$
Polyunsaturated fatty acids					
16:2*n*-4	$0.73^{a} \pm 0.02$	$1.17^{b} \pm 0.07$	$2.00^{c} \pm 0.14$	$1.51^{d} \pm 0.12$	$3.24^{e} \pm 0.15$
16:3*n*-4	$0.49^{a} \pm 0.01$	$1.00^{b} \pm 0.02$	$0.66^{a} \pm 0.02$	$1.51^{c} \pm 0.07$	$3.36^{d} \pm 0.20$
18:2*n*-6	$35.02^{a} \pm 1.46$	$14.1^{b} \pm 0.72$	$6.20^{c} \pm 0.4$	$65.26^{d} \pm 1.14$	$7.83^{e} \pm 0.30$
18:3*n*-6	$55.72^{a} \pm 2.12$	$58.05^{a} \pm 1.30$	$37.24^{b} \pm 1.20$	$67.52^{c} \pm 2.21$	$150.1^{d} \pm 5.23$
18:3*n*-3	$1.47^{a} \pm 0.07$	$1.34^{a} \pm 0.01$	$0.83^{c} \pm 0.05$	$3.03^{d} \pm 1.10$	$1.81^{a} \pm 0.07$
20:2*n*-6	$34.18^{a} \pm 1.10$	$9.44^{b} \pm 0.55$	$7.24^{c} \pm 0.32$	$79.52^{d} \pm 1.95$	$11.5^{e} \pm 0.71$
20:3*n*-6	$10.64^{a} \pm 0.62$	$1.35^{b} \pm 0.07$	$16.0^{c} \pm 0.64$	$5.59^{d} \pm 0.36$	$10.65^{a} \pm 0.51$
20:4*n*-6	$10.64^{a} \pm 0.51$	$22.24^{b} \pm 0.24$	$5.72^{c} \pm 0.25$	$9.14^{a} \pm 0.75$	$3.99^{d} \pm 0.15$
20:5*n*-3	$238.84^{a} \pm 15.5$	$135.57^{b} \pm 2.65$	$213.54^{c} \pm 11.8$	$360.96^{d} \pm 14.8$	$119.25^{e} \pm 2.65$
22:5*n*-3	$43.47^{a} \pm 2.21$	$41.92^{a} \pm 1.12$	$21.46^{b} \pm 1.32$	$17.33^{c} \pm 0.48$	$15.54^{d} \pm 0.73$
22:6*n*-3	$645.05^{a} \pm 12.7$	$374.68^{b} \pm 13.4$	$534.89^{c} \pm 15.2$	$599.90^{d} \pm 23.4$	$267.63^{e} \pm 7.56$
[z]∑PUFA	$1076.25^{a} \pm 66.0$	$660.86^{b} \pm 12.5$	$845.78^{c} \pm 12.4$	$1211.27^{d} \pm 55.9$	$594.9^{e} \pm 18.1$
∑*n*-3	$928.83^{a} \pm 26.3$	$553.51^{b} \pm 19.8$	$770.72^{c} \pm 25.1$	$981.22^{d} \pm 28.7$	$404.23^{e} \pm 10.1$
∑*n*-6	$146.2^{a} \pm 5.12$	$105.18^{b} \pm 2.45$	$72.4^{c} \pm 2.47$	$227.03^{d} \pm 6.89$	$183.97^{e} \pm 5.45$
∑*n*-3/∑*n*-6	$6.35^{a} \pm 0.31$	$5.26^{b} \pm 0.10$	$10.64^{c} \pm 0.65$	$4.32^{d} \pm 0.20$	$2.19^{e} \pm 0.10$
18:1*n*-7/*n*-9	$0.02^{a} \pm 0.00$	$0.05^{a} \pm 0.00$	$0.003^{b} \pm 0.00$	$0.03^{a} \pm 0.00$	$0.10^{c} \pm 0.02$
DHA + EPA	$883.89^{a} \pm 15.2$	$510.25^{b} \pm 14.6$	$748.43^{c} \pm 21.1$	$960.86^{d} \pm 29.8$	$386.88^{e} \pm 19.14$
EPA/AA	$22.44^{a} \pm 1.45$	$6.09^{b} \pm 0.44$	$37.33^{c} \pm 1.18$	$39.49^{c} \pm 1.24$	$29.88^{d} \pm 1.15$
∑PUFA/∑SFA	$1.22^{a} \pm 0.08$	$1.43^{b} \pm 0.07$	$1.43^{b} \pm 0.08$	$1.68^{c} \pm 0.11$	$1.49^{b} \pm 0.07$
Fatty acid-based nutritional indices					
Atherogenicity index	$0.57^{a} \pm 0.01$	$0.41^{b} \pm 0.01$	$0.70^{c} \pm 0.03$	$0.42^{b} \pm 0.01$	$0.59^{a} \pm 0.03$
Thrombogenicity index	$0.22^{a} \pm 0.03$	$0.19^{a} \pm 0.00$	$0.19^{a} \pm 0.01$	$0.17^{a} \pm 0.00$	$0.23^{a} \pm 0.15$
HC/HP ratio	$2.20^{a} \pm 0.10$	$3.70^{b} \pm 0.14$	$1.86^{c} \pm 0.10$	$3.40^{b} \pm 0.14$	$1.90^{c} \pm 0.07$

All samples were analyzed in triplicate (n=3) from pooled sub-samples, and expressed as mean ± standard deviation

Means followed by the different superscripts within the same row (a-d) indicate significant difference ($P < 0.05$). ND: non detectable

[x] ∑SFA, total saturated fatty acids

[y] ∑MUFA, total monounsaturated fatty acids

[z] ∑PUFA, total polyunsaturated fatty acids

fatty acids, which was found to be essentially more noteworthy than those recorded in other cephalopods.

The total content of polyunsaturated fatty acids (PUFAs) was found to be significantly greater ($P < 0.05$) in *S. pharaonis* (1211 mg FA/100 g EP) when contrasted with that in different cephalopods considered in the present study. The aggregate EPA and DHA content of the edible part of *S. pharaonis* were found to be significantly greater than those in other species of cephalopods. The n-3/n-6 PUFA proportion of *U. duvauceli* was found to be significantly greater (~10, $P < 0.05$) than those in the edible part of other cephalopods (Figure 1B). The mean total of n-3 PUFA content (404-981 mg FA/100

Table 2. Protein and amino acid composition (mg/100g wet weight) including essential amino acid score of the cephalopods

	A. neglectus	*C. indicus*	*U. duvauceli*	*S. pharaonis*	*S. inermis*
Protein	1202.74^{a} ± 4.56	1850.21^{b} ± 5.40	1951.39^{c} ± 8.20	1449.03^{d} ±7.80	1874.2^{b} ± 0.02
Essential amino acids					
His	12.30^{a} ± 0.50	16.01^{a} ± 0.80	6.57^{b} ± 0.32	4.31^{c} ± 0.10	3.19^{c} ± 0.12
Arg	73.13^{a} ±2.52	33.23^{b} ±1.20	15.11^{c} ± 0.20	9.41^{d} ± 0.60	6.07^{c} ± 0.20
Thr	30.15^{a} ± 1.68	12.18^{b} ± 0.50	6.48^{c} ± 0.30	4.27^{c} ± 0.20	2.11^{c} ± 0.10
Val	28.37^{a} ± 1.31	12.09^{b} ± 0.90	6.18^{c} ± 0.28	3.43^{c} ± 0.21	2.62^{c} ± 0.10
Met	22.10^{a} ± 1.47	7.35^{b} ± 0.58	4.41^{c} ± 0.19	2.15^{d} ± 0.17	1.32^{d} ± 0.07
Ile	33.12^{a} ± 2.56	13.05^{b} ± 0.94	7.21^{c} ± 0.61	3.47^{d} ± 0.28	2.44^{d} ± 0.12
Leu	46.57^{a} ±3.01	19.28^{b} ± 0.93	11.39^{c} ± 0.72	5.07^{d} ± 0.25	3.11^{d} ± 0.18
Phe	34.19^{a} ± 1.85	9.14^{b} ± 0.68	5.25^{c} ± 0.32	3.10^{cd} ± 0.20	2.25^{d} ± 0.17
Lys	36.26^{a} ± 2.15	19.11^{b} ± 0.82	12.51^{c} ± 0.43	4.07^{d} ± 0.29	3.37^{d} ± 0.21
TEAA	316.19^{a} ± 9.25	141.44^{b} ± 5.53	75.11^{c} ± 1.86	39.28^{d} ± 2.78	26.48^{c} ± 1.34
Non-essential amino acids					
Asp	45.12^{a} ± 1.80	25.08^{b} ± 1.25	15.21^{c} ± 0.53	6.30^{d} ± 0.38	3.15^{d} ± 0.22
Glu	74.28^{a} ± 4.67	33.16^{b} ± 0.99	17.04^{c} ± 0.89	9.39^{d} ± 0.64	5.18^{c} ± 0.35
Ser	28.38^{a} ± 1.65	15.46^{b} ± 0.63	6.14^{c} ± 0.17	3.08^{d} ± 0.21	2.16^{d} ± 0.12
Gly	34.73^{a} ± 1.28	19.61^{b} ± 0.90	8.45^{c} ± 0.50	4.18^{d} ± 0.33	2.28^{d} ± 0.15
Ala	25.45^{a} ±1.17	14.23^{b} ± 0.58	7.75^{c} ± 0.32	4.54^{d} ± 0.28	3.21^{d} ± 0.26
Pro	24.62^{a} ± 0.73	12.72^{b} ± 0.48	6.17^{c} ± 0.26	3.83^{d} ± 0.22	2.51^{d} ± 0.12
Tyr	11.46^{a} ± 0.68	6.82^{b} ± 0.43	4.35^{c} ± 0.21	2.27^{d} ± 0. 18	1.03^{d} ± 0.07
Cys	1.27^{a} ± 0.05	2.05^{b} ± 0.11	1.96^{b} ± 0.06	1.76^{b} ± 0.10	1.35^{a} ± 0.16
TNEAA	245.31^{a} ± 9.56	128.59^{b} ± 7.25	67.07^{c} ± 3.25	35.35^{d} ± 1.17	20.87^{c} ± 1.14
Ratios of amino acids					
TAA	561.5^{a} ± 8.32	270.03^{b} ± 1.20	142.18^{c} ± 6.20	74.63^{d} ± 1.02	47.35^{c} ± 0.96
TEAA/TAA	0.56^{a} ± 0.04	0.52^{a} ± 0.03	0.52^{a} ± 0.05	0.53^{a} ± 0.03	0.55^{a} ± 0.04
TNEAA/TAA	0.43^{a} ± 0.03	0.47^{a} ± 0.04	0.47^{a} ± 0.01	0.47^{a} ± 0.05	0.44^{a} ± 0.03
TEAA/TNEAA	1.29^{a} ± 0.04	1.09^{a} ± 0.08	1.12^{a} ± 0.06	1.11^{a} ± 0.07	1.26^{a} ± 0.05
TArAA	57.95^{a} ±1.52	31.97^{b} ± 1.54	16.17^{c} ± 0.98	9.68^{d} ± 0.54	6.47^{d} ± 0.20
TSCAA	23.37^{a} ± 0.85	9.4^{b} ± 0.67	6.37^{c} ± 0.13	3.91^{d} ± 0.26	2.67^{d} ± 0.18
Arg/Lys	2.02^{a} ± 0.15	1.74^{b} ± 0.09	1.20^{b} ± 0.06	2.31^{b} ± 0.14	1.8^{b} ± 0.14
Leu/Ileu	1.40^{b} ± 0.05	1.47^{b} ± 0.08	1.57^{b} ± 0.06	1.46^{b} ± 0.04	1.27^{c} ± 0.03
Essential amino acid score					
His	50.65^{a} ± 0.03	32.37^{b} ± 0.03	15.54^{c} ± 0.03	10.7^{d} ± 0.26	4.16^{e} ± 0.04
Thr	66.65^{a} ± 0.03	18.15^{b} ± 0.03	8.7^{c} ± 0.05	5.88^{d} ± 0.05	2.28^{e} ± 0.04
Val	68.56^{a} ± 0.18	16.58^{b} ± 0.25	7.85^{c} ± 0.05	5.37^{d} ± 0.05	2.07^{e} ± 0.01
Met + Cys	70.1^{a} ± 0.34	14.8^{b} ± 0.14	7.28^{c} ± 0.24	4.92^{d} ± 0.07	1.98^{e} ± 0.11
Ile	95.77^{a} ± 0.27	22.47^{b} ± 0.24	10.59^{c} ± 0.23	7.46^{d} ± 0.34	2.68^{e} ± 0.14
Leu	61.89^{a} ± 0.13	14.87^{b} ± 0.11	7.10^{c} ± 0.05	4.87^{d} ± 0.08	1.89^{e} ± 0.03
Phe + Tyr	63.8^{a} ± 0.16	13.57^{b} ± 0.25	6.44^{c} ± 0.18	4.51^{d} ± 0.29	1.70^{e} ± 0.05
Lys	51.79^{a} ± 0.21	17.44^{b} ± 0.18	8.44^{c} ± 0.18	5.57^{d} ± 0.13	2.51^{e} ± 0.27

TEAA, Total amino acids; TNEAA, total non-essential amino acids; TAA, total amino acids; TArAA, total aromatic amino acids; TSAA, total sulfur containing amino acids
Tryptophan was not determined.
Data are expressed as mean ± standard deviation (n = 3); Means followed by the different superscripts (a–d) within the same row (a-d) indicate significant difference ($P < 0.05$).

g EP) was found to be greater as compared with n-6 PUFA (72-227 mg FA/100 g EP) in the cephalopods. DHA (22:6n-3) was found to be the predominant n-3 PUFA in these species, which represented greater than half of the total PUFA content followed by EPA (20:5n-3, 119-361 mg FA/100 g EP). The major n-6 PUFAs were found to be 18:2n-6, 18:3n-6 and 20:2n-6. The AI and TI indices in the edible parts of cephalopods varied from 0.50–0.70 and 0.17-0.23, respectively. The HC/HP ratio was found to be significantly greater in *C. indicus* (3.7) as compared with that of the edible parts of different cephalopods considered in the present study ($P < 0.05$).

Protein content and amino acid composition of cephalopods

The protein content of the cephalopods was presented in Table 2. The protein content was found to vary between 1200 to 1950 mg/100 g. The more noteworthy protein content (1951.4 mg/100 g) was recorded in the edible parts of *U. duvauceli.* No significant differences in the protein contents in *C. indicus* and *S. inermis* were apparent (~1800 mg/100 g wet tissue) ($P < 0.05$) (Table 2). The edible parts of *A. neglectus* recorded lesser protein content (1202 mg/100 g) as compared to those in other species of cephalopods.

The amino acid contents of the cephalopods were presented in Table 2. The cephalopods were found to contain protein with balanced proportions of essential to non-essential amino acids (~ 1.2).

Table 3. Mineral and vitamin compositions of the cephalopods

	A. neglectus	*C. indicus*	*U. duvauceli*	*S. pharaonis*	*S. inermis*
Macrominerals					
Ca	73.72^{a} ± 2.02	54.04^{b} ± 2.01	72.82^{a} ± 2.01	108.41^{c} ± 2.10	79.73 ^{d} ± 4.02
P	86.07^{a} ± 1.60	66.33^{b} ± 0.49	85.63^{c} ± 1.31	80.4^{d} ± 1.46	72.37^{e} ± 2.40
Na	172.45^{a} ± 8.10	170.14^{b} ± 6.60	171.98^{c} ± 2.10	170.18^{b} ± 3.20	172.77^{a} ± 7.10
Mg	92.83^{a} ± 1.06	104.78^{b} ± 2.30	127.36^{c} ± 3.40	127.28^{c} ± 2.30	109.76^{d} ± 2.20
K	144.56^{a} ± 3.18	184.83^{b} ± 7.60	176.13^{c} ± 5.20	201.2 ^{d} ± 5.05	134.67^{e} ± 5.80
Microminerals					
Zn	13.08^{a} ± 0.64	14.17^{b} ± 0.87	7.21^{c} ± 0.30	15.18^{d} ± 0.23	8.34^{e} ± 0.46
Cu	3.55^{a} ± 0.15	7.54^{b} ± 0.54	1.33^{c} ± 0.09	5.57^{d} ± 0.32	5.33^{d} ± 0.20
Mn	6.39^{a} ± 0.27	6.51^{a} ± 0.39	6.57^{a} ± 0.37	6.92^{a} ± 0.44	6.43^{a} ± 0.37
Fe	7.44^{a} ± 0.31	6.60^{b} ± 0.32	10.42^{c} ± 0.77	7.79^{d} ± 0.36	8.45^{d} ± 0.31
Se	9.31^{a} ± 0.42	9.45^{a} ± 0.32	9.54^{a} ± 0.62	8.95^{b} ± 0.52	9.34^{a} ± 0.52
Mineral indices					
Na/K	1.17^{a} ± 0.06	0.93^{b} ± 0.04	0.94^{b} ± 0.03	0.87^{b} ± 0.05	1.37^{c} ± 0.07
Ca/P	0.86^{a} ± 0.01	0.81^{a} ± 0.03	0.85^{a} ± 0.02	1.35^{b} ± 0.60	1.10^{b} ± 0.05
Ca+P	145.46^{a} ± 3.5	120.87^{b} ± 4.01	158.87^{c} ± 3.90	166.59^{d} ± 2.90	159.49^{c} ± 2.60
Vitamins					
Retinol A	15.35^{a} ± 0.96	8.39^{b} ± 0.32	26.51^{c} ± 0.85	21.60^{d} ± 0.77	54.81^{e} ± 1.40
Cholecalciferol D_3	4.39^{a} ± 0.22	3.30^{b} ± 0.26	1.61^{c} ± 0.11	3.51^{b} ± 0.24	1.52^{c} ± 0.68
α–tocopherol E	11.43^{a} ±0.80	3.54^{b} ± 0.20	1.45^{c} ± 0.86	5.29^{d} ± 0.45	11.26^{a} ± 0.17
Phylloquinone K_1	0.01^{a} ± 0.00	1.58^{b} ± 0.12	0.01^{a} ± 0.01	0.26^{c} ± 0.01	ND
Ascorbic acid C	2.20^{a} ± 0.13	1.80^{b} ± 0.10	2.90^{c} ± 0.21	0.70 ^{d} ± 0.08	2.80^{c} ± 0.22

All samples were analyzed in triplicate (n=3) and expressed as mean ± SD
Mineral content was expressed as mg/100g EP except Se (µg/100g).
The vitamins were expressed in µg/100g except vitamin E that was expressed as mg/100 g.
The means followed by the same letter within the same row are not significantly different ($P < 0.05$), and differently shown letters in the same row are statistically different ($P < 0.05$).
ND: non detectable.

Among the different cephalopod species, *A. neglectus* exhibited significantly greater ($P < 0.05$) content of total essential amino acid (316 mg/100 g wet weight) followed by *C. indicus* (141 mg/100 g). The most abundant essential amino acid was found to be arginine in *A. neglectus* (73 mg/100 g wet edible tissue), followed by leucine (47 mg/100 g) and lysine (36 mg/100 g). Among the non–essential amino acids, glutamine constituted the major share in all cephalopods considered in the present study (Table 2). The total sulfated and aromatic amino acid contents were found to be significantly greater in *A. neglectus* (23 and 58 mg/100 g, respectively) when compared with different cephalopod species ($P < 0.05$). The arginine-lysine proportion was found to vary from 1.7–2.3. *S. pharaonis* showed significantly greater leucine/isoleucine proportion (1.57) when compared with those in other cephalopod species ($P < 0.05$, Table 2). The amino acid scores of the cephalopod species with respect to histidine, threonine, valine, methionine, aromatic amino acids, and lysine were shown in Table 2, and these scores were found to be significantly greater in *A. neglectus* as compared to those in different species ($P < 0.05$). The isoleucine score was found to be most noteworthy in the edible parts of *A. neglectus* (~ 96%).

Vitamin and mineral composition

The vitamin contents of the edible parts of cephalopods were shown in Table 3. The vitamin D_3 content was significantly greater ($P < 0.05$) in *A. neglectus* (4.4 µg/100 g EP) as compared to those in other species. The vitamin A content was significantly greater in *S. inermis* (~ 55 µg/100 g EP) than other species (lesser than 30 µg/100 g EP). The antioxidant vitamin E in *A. neglectus* and *S. inermis* (~ 11 mg/100 g EP) was found to be significantly greater than other cephalopods ($P < 0.05$). Vitamin C content was found to be significantly greater in *U. duvauceli* and *S. inermis* (2.8-2.9 µg/100g EP, $P < 0.05$) than other cephalopods considered in the present study.

The edible part of cephalopods used in this study was found to be rich in macro (K, Ca, Mg and P) and microminerals (Mn, Fe, Cu, Zn and Se) (Table 3). K and Ca were found in appreciable quantities in *S. pharaonis* (201 and 108 mg/100 g, respectively). A greater level of Mg was recorded in *U. duvauceli* (~127 mg/100 g). The Na/K ratio in the cephalopods ranged from 0.87–1.37. The Ca+P content was essentially more noteworthy in the edible portion of *S. pharaonis* (167 mg/100g) than other cephalopod

species ($P < 0.05$). *U. duvauceli* showed significantly greater content of Fe (10.4 mg/100 g) than recorded in other cephalopods ($P < 0.05$) (Table 3). The Se concentration was found to be significantly greater ($P < 0.05$) in *U. duvauceli, A. neglectus*, and *S. inermis* (greater than 9 µg/100 g wet weight) when compared with those in *S. pharaonis*.

Discussion

Cephalopods have been used as food by human beings from time immemorial because of their nutritive qualities and sensory characteristics (Zlatanos *et al*., 2006). They were a subject of artisanal fishery for a few a great many years. Greeks and Chinese have esteemed them as a vital food item since ancient time. The present study provided detailed biochemical compositions of five commercially important cephalopod species available in the Arabian Sea, and also demonstrated a detailed comparison on the nutritional parameters between them.

Lipid content in cephalopods was found to be lesser than those in marine finfish species (Njinkoue *et al*., 2002), and n-3 PUFAs contributed to the majority of lipids as likewise exhibited in prior reports (Ozyurt *et al*., 2006; Thanonkaew *et al*., 2006). Lipids are key in the reproductive phase of animals for gonad maturation, and in females it is certain to give vitality to consequent embryogenesis (Pollero *et al*., 1983). The lipid composition of cephalopods may be impacted by the lipid composition of the food and predominantly comprises of small fishes and other invertebrates. The present study demonstrated that *S. pharaonis* and *A. neglectus* had a more prominent lipid content (> 2 mg/100 g) than other cephalopods species (1.8-1.3 mg/100 g), but lesser when compared with other marine fish species (Njinkoue *et al.*, 2002; Peng *et al.*, 2013; Chakraborty *et al.*, 2014). The distinction in the lipid composition of cephalopods could be ascribed to different factors, for example, species and food availability (Rasoarahona *et al.*, 2005). Similar results were observed in several other species of cuttlefish, octopus and squid (Zlatanos *et al.*, 2006).

Fatty acids are vital components needed by the body for different metabolic and structural functioning. The edible portion of cephalopods considered in the present study was found to be rich in PUFAs, which was in accordance with the earlier reports showing that the adult cephalopods are rich in long chain PUFAs (Ozyurt *et al.*, 2006; Thanonkaew *et al.*, 2006). The SFAs were found to be present in appreciable quantities in the cephalopod species. It was reported that the SFAs with their high caloric content is fundamentally utilized as a source in the storage form of energy, and in this way, their concentration increases during the periods of enhanced feeding activity (Shirai *et al*., 2002). The C18 fatty acids, for example, 18:1n-9 was predominantly present in cephalopods, which is in concurrence with the prior findings that the marine lipids usually contain eighteen carbon atoms (Zlatanos and Laskaridis, 2007).

The investigation of the fatty acid composition of the cephalopods revealed the immense health benefits that this group of molluscs can provide. The total n–3 PUFA content in *S. pharaonis* (981 mg/100 g) was found to be significantly greater ($P < 0.05$) than other cephalopods used in this study. The PUFAs are vital biochemical markers of cephalopods adding to their significant nutritional qualities. The C_{20}–C_{22} long chain n-3 fatty acids, for example, EPA and DHA, which are vital for human well being, were found to be predominant in the edible part of the cephalopod species. The long chain n-3 PUFAs were found to be essential in the management of several health issues like cardiovascular sicknesses, hypertension, diabetes, joint inflammation, and other inflammatory diseases (Simopoulos, 2009). The relative nutritional value of the cephalopods can likewise be evaluated from the n-3/n-6 proportion. The UK Department of Health recommends an ideal proportion of n–6/n–3 at 4.0 (HMSO, 2001), and the ratio greater than 4.0 were considered as harmful to human wellbeing (Andrade *et al*., 2012). The n–3/n–6 proportion of the cephalopods in the present study was greater than 2 and, in this way, these species can be considered as a healthy diet. The n-3/n-6 polyunsaturated fatty acid ratio of *U. duvauceli* was found to be significantly greater (~10, $P<0.05$) than different cephalopods, and might subsequently serve as a substitute to balance the greater admission of n-6 fatty acids in our general utilization of vegetable oil. Since n-3 fatty acids can decrease the plasma lipids (Kinsella *et al.*, 1990), a more prominent dietary admission of food rich in n-3 fatty acids can lessen the danger of coronary heart diseases.

There is an increased use of n-6 fatty acids with a marked reduction in the use of the long chain C_{20-22} n-3 fatty acids because of the change in dietary patterns during the last few decades. This has created an imbalance in the n-6/n-3 ratio (Simopoulos, 2009) leading to the development of various inflammatory diseases. In this connection, the cephalopods, which are rich in n-3 fatty acid with the relatively lesser content of n-6 fatty acids, can be considered as an essential natural source of long chain n-3 PUFAs, and

also an alternative to the fishes. The present study additionally demonstrated that the cephalopods had a greater PUFA/SFA ratio than the recommended minimum of 0.45 (HMSO, 2001). However, the PUFA/SFA ratio cannot alone be an indicator of the atherogenic or the thrombogenic potential of the foods (Ulbricht and Southgate, 1991). Fatty acid based nutritional indices, for instance, the atherogenic and the thrombogenic potential of a particular diet relies on upon the relative contents of a particular set of fatty acids, which point out the global dietetic value of lipids. As a result, their potential effect on the development of coronary diseases can be anticipated from the atherogenicity and thrombogenicity indices (Jankowska *et al.*, 2010). Atherogenicity index is a marker of risk for cardiovascular maladies, whilst thrombogenicity index is an indicator of potential for blood platelets conglomeration. Lesser estimations of atherogenicity and thrombogenicity indices (<0.8 and <0.3, respectively) in the edible parts of the test cephalopod species were indistinguishable with the earlier studies related to the bivalves *Ruditapes decussates* and *Mytilus galloprovincialis* (Saba, 2011) subsequently ensuring that they can be consumed without any risk for health. The more prominent n-3 fatty acid content and hence the greater n-3/n-6 fatty acid proportion in *U. duvauceli* obviously added to lesser AI and TI indices in the edible muscles. It has been accounted for that because of the anti-atherogenic and anti-thrombogenic properties, the n-3 PUFAs assume a noteworthy role in protecting human beings from atherosclerosis and platelet aggregation (Barrento *et al*., 2010).

The principal component analysis was performed with respect to the lipid content and fatty acid indices of the candidate cephalopods. The loading plot was represented in Figure 2. PC1 displayed 63.51% variance, whereas PC2 demonstrated a variance of 36.48%. The lipid content and n-3 fatty acids of *C. indicus* showed positive correlation with atherogenicity index, while the aggregate n-3 fatty acid content indicated more noteworthy comparability with the thrombogenicity index of *A. neglectus*. The thrombogenicity index of *U. duvauceli* and its lipid content were found to be significantly correlated.

In aquatic molluscs, cholesterol is seen as a prominent sterol for membrane synthesis and in the production of other steroids involved in regenerative advancement (Murphy *et al.*, 2002). Earlier studies showed that the cholesterol content of the cephalopods ranged between 123 to 132 mg/100 g (Castro and Guerra, 1990). Vairamani (2010) reported a cholesterol content of 441 mg/100 g in the mantle tissue of the cuttlefish *S. inermis*.

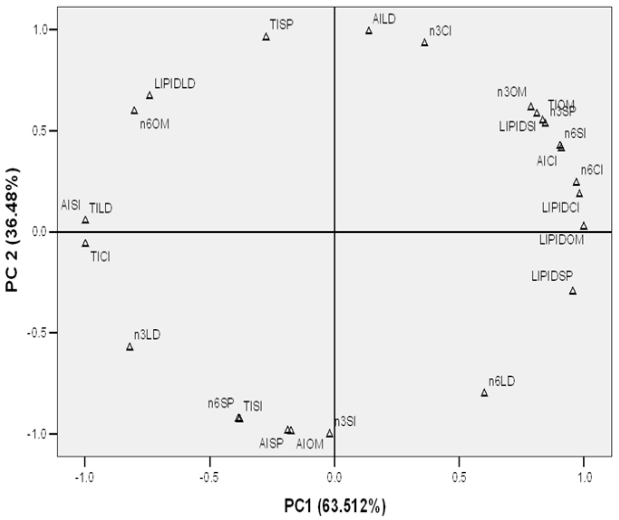

Figure 2. Loading plot diagram representing the correlation of lipid content and fatty acid indices of the cephalopods. LIPIDLD, LIPIDSI, LIPIDCI, LIPIDSP, LIPIDOM - lipid contents in *U. duvauceli, S. inermis, C. indicus, S. pharaoni*s, and *A. neglectus*, respectively; TILD, TISI, TICI, TISP and TIOM - thromrbogenicity indices of *U. duvauceli, S. inermis, C. indicus, S. pharaonis, A. neglectus*, AILD, AISI, AICI, AISP and AIOM - atherogenicity indices of *U. duvauceli, S. inermis, C. indicus, S. pharaonis* and *A. neglectus*; n3LD, n3SI, n3CI, n3SP and n3OM - total n-3 fatty acids in *U. duvauceli, S. inermis, C. indicus, S. pharaonis* and *A. neglectus*; n6LD, n6SI, n6CI, n6SP and n6OM - total n-6 fatty acids in *U. duvauceli, S. inermis, C. indicus, S. pharaonis* and *A. neglectus*

The cholesterol contents of octopus species (*C. indicus* and *A. neglectus*) considered in the present study were found to be within the threshold levels of lesser than 200 mg/100 g wet edible portion, when compared with those recorded in *S. pharaonis* and *S. inermis*, which recorded cholesterol content of about 300 mg/100 g.

The cephalopods were found to contain protein (1200-1950 mg/100g EP) with balanced proportions of essential to non-essential amino acids (~ 1.5) that demonstrated these species as good sources of protein with greater biological value. Any ratio of essential to non-essential amino acids greater than 1.0 is considered to be good, and in this manner, it can be inferred that the cephalopods can be considered as a well-balanced source of high-quality protein. The proteins derived from the marine molluscs were accounted for to be easily digestible, and are central to human growth and survival (Orban *et al.*, 2006).

U. duvauceli demonstrated significantly greater level of protein content (1950 mg / 100 g EP) when compared with the other cephalopod species. The more noteworthy protein content in cephalopods could be credited to their relatively greater rate of

protein synthesis (Moltschaniwskyj and Carter, 2010). In the present study, the amino acid profile showed that cephalopods are rich sources of crucial amino acids, which demonstrate the greater biological value of cephalopod proteins. The species belonging to the class Octopoda showed greater content of lysine and aromatic amino acids. Lysine is considered to be an important amino acid since it is the precursor for the de novo synthesis of glutamate, which is an important neurotransmitter in the mammalian nervous system. This amino acid accounted for to be the limiting amino acid in the cereal–based diets used as the staple food items in developing countries (Kim and Lall, 2000). Aromatic amino acids are the precursors for the synthesis of many neurotransmitters, for example, dopamine, serotonin, nor epinephrine etc., accordingly implying their role in the thyroxine metabolism (Logan *et al.*, 1987). The sulfur-containing amino acids are deficient in our staple diets such as cereals and pulses. Likewise, cysteine can be made from homocysteine but cannot be synthesised de novo in humans. *A. neglectus* was found to contain greater quantities of sulfur-containing amino acids (23.3 mg/100 g EP) and lysine (36.3 mg/100 g EP) than other cephalopods, which indicated that the protein derived from this octopus species can effectively complement the limiting amino acids in our daily diets. The arginine to lysine ratio is the most objective index to identify the cholesterolemic properties of a protein. Cephalopods exhibited an arginine to lysine ratio near to 2, and was comparable with those in vegetables and meat (Unusan, 2007), thereby indicating their importance to maintain the good cholestrolemic index. The cephalopods considered in this study displayed the leucine-isoleucine proportion as prescribed by FAO/WHO (FAO/WHO/UNU, 2007). The cephalopods demonstrated to possess good amino acid scores, an index of good quality of protein.

Cephalopods are also considered as a rich source of vitamins, which must be consumed on a regular basis in view of their key role in human health and metabolism. The antioxidant vitamin E in *A. neglectus* and *Sepiella inermis* (~ 11 µg/100 g EP) was found to be significantly greater than other cephalopods. This vitamin is an essential antioxidative supplement, and is considered to be vital to ensure PUFAs against their oxidative degradation. Vitamin C, the potent free radical scavenger, is a fundamental nutritional supplement for humans (Chakraborty and Joseph, 2015). But an additional external dietary source is required because it is not synthesized by human metabolism. It moreover helps the body to absorb iron and calcium, and assists in wound healing (Iqbal *et al.*, 2004). The vitamin C content reported in *A. neglectus, S. inermis,* and *U. duvauceli* were found to be greater than 2 µg/100 g wet weight, and was found to be more noteworthy than that in other shellfish species (Gopalakrishnan and Vijayavel, 2008). Vitamin D_3 is essential for the maintenance of normal blood levels of Ca and phosphate (Trivedi *et al*., 2003). A greater content of vitamin D_3 (4.4 µg/100g) along with significant quantities of Ca (74 mg/100 g) and P (86 mg/100 g) in *A. neglectus* demonstrated its significance in preventing osteoporosis in adults.

Minerals do not have any calorific value, but they are an essential part of the diet, and are required by the body to carry out various important metabolic functions. A number of them likewise form the active components of hormones, enzymes and proteins. Cephalopods are active predators and most part of the minerals is assumed to be incorporated through the diet (Lall, 2002). Some amount of minerals is also absorbed directly from sea water, general body surface and gills form the essential regions for this absorption. The amount of minerals accumulated can vary with the mineral composition of seawater and with changes in diet, as reported in our earlier literature (Chakraborty and Joseph, 2015).

Potassium and magnesium were the significant fundamental components found in greater quantities followed by calcium and phosphorous in the cephalopods. This is in connection with the findings that K, P, Mg and Ca are the significant components of cephalopods (Lourenco *et al*., 2009). Even though these elements are required by the body for maintaining the homeostasis (Lall, 2002), a greater concentration of them can also lead to various health problems. The lesser sodium-potassium ratio (≤1.0) of the cephalopods belonging to *C. indicus, U. duvauceli* and *S. pharaonis* showed the importance of these species as potential health food items, and are safe for utilization by individuals having cardiovascular ailments. It is of note that changes in Na-K ratio can prompt changes in osmotic offset of the body, prompting build or reduction in blood pressure (Murray *et al*., 2000). The K contents of the cephalopods were found to be greater than 100 mg/100 g EP, which is essential to maintain the osmotic balance and pH of the body liquid (Ensminger *et al*., 1995). It was noticed that the cephalopods in the present study were found to posses greater than 50 mg/100 g of Ca and Mg. Ca is responsible for bone development and maintenances, whereas, Mg is a vital cofactor to complete different biochemical responses in the body. Calcium and phosphorous are essential elements when the health of bones and teeth are concerned. Any food item having a Ca/P ratio of

≥ 0.5 is viewed as good (Chakraborty and Joseph, 2015). The cephalopod species were demonstrated a Ca/P ratio of ≥ 0.8, and thus might be considered as good sources of these essential minerals. The trace elements, for instance, Zn, Mn, and Fe function as cofactors of enzymes, and accordingly, ought to be supplied in sufficient amounts for maintaining the proper functioning of the body. These three elements were found to be well above the recommended dietary allowance in cephalopods (FAO/WHO, 2002).

Selenium has been associated with security of body tissues against oxidative stress, and is thought to give protection against disease conditions like cancer (Jackson *et al*., 2008). It is likewise a part of a component of several enzymes like glutathione peroxidase and other enzymes included in the regulation of thyroid hormone capacities. The suggested dietary allowance of selenium is 55 µg/day, for both men and women. The cephalopods were demonstrated to provide selenium of about 9 µg/100g EP, and were found to be present in greater quantities than in grains, fruits, vegetables (Levander and Burk, 1994), and various marine finfishes (10–20 µg/100g) (Chakraborty and Joseph, 2015). Likewise, the cephalopod species considered in the present study might be the rich storehouse of selenium, which is essentially required for combating oxidative stress induced diseases in the body.

Conclusions

The present study demonstrated that the commonly available cephalopods are a rich storehouse of key nutritional elements required by the human nutrition and metabolic pool. These deep sea species were found to be significantly valuable sources of protein with the more prominent content of essential/non–essential amino acid ratio. More noteworthy levels of C_{20-22} long chain n–3 polyunsaturated fatty acids, for example, eicosapentaenoic acid, docosahexaenoic acid, and greater n–3/n–6 fatty acid proportion demonstrated that these cephalopods are good sources of well–balanced diets. The ideal atherogenicity / thrombogenicity indices, hypocholesterolemic/ hypercholesterolemic ratio, and fatty acid /amino acid based health markers qualified these cephalopod species as potential health food. Notwithstanding this, these species were found to be the valuable source of numerous critical micro and macro minerals and vitamins, which are fundamental for the metabolic functioning of the body. The results obtained from this study will provide useful information for seafood industries and the utilization of the cephalopod species as potential health food.

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