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FULL LENGTH ARTICLE

Biochemical profile of oyster *Crassostrea madrasensis* and its nutritional attributes

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Abstract Oysters are highly esteemed sea food and considered a delicacy throughout the world. Yet this resource is not optimally utilised in several parts of the world. The aim of this study is to highlight its nutritional importance. Biochemical composition and nutritional attributes of oyster meat are discussed. Proximate composition, fatty acid and amino acid profiles and mineral content were determined in oysters (*Crassostrea madrasensis*). Moisture, protein, fat, carbohydrate and ash contents in the oyster were 82.64%, 9.41%, 3.25% 3.2% and 1.01%, respectively and it was rich in macro-minerals and trace elements especially selenium. Polyunsaturated fatty acids (PUFA) were highest of the total lipids among which eicosapentaenoic acid, docosahexaenoic acid and linoleic acid were the prominent fatty acids. The *n*-3/*n*-6 index was high indicating a predominance of *n*-3 fatty acids in the species. Total amino acid content was 99.33 g/100 g crude protein, of which, essential amino acid lysine was the most abundant. Valine had the lowest essential amino acid score (EAAS) (0.17) while threonine had the highest EAAS of 3.62. Chemical score was 17% and the lowest limiting amino acid was valine. Protein efficiency ratio, essential amino acid index and biological value of oyster were 3.92, 120.2 and 174.0, respectively which indicates that the protein is of superior quality. Data on biochemical composition, nutritional attributes and quality indices of *C. madrasensis* protein may prove important for future policies regarding exploitation of this species and for inducing favourable changes in consumer preferences.

Introduction

Oysters are marine animals belonging to the family *Ostreidae*. They are one of the best known and most widely cultivated marine animals. Oysters are highly esteemed sea food and considered a delicacy in USA, Europe, Japan etc. In India till recently oysters were consumed in the coastal areas only, mainly by fisherfolk and a few others to a limited extent. However, with the growing awareness for more nutritious food, demand for oyster meat has risen in the country among all classes of people. Yet there are places in Maharashtra, Goa

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and Karnataka on the west coast and Tamil Nadu, Pondicherry and Andhra Pradesh along the east coast with rich bivalve resources but their utilisation for human consumption is very negligible (Kripa and Appukuttan, 2003). The resources in these places are fished mainly for use in the construction industry for the manufacture of cement, calcium carbide, sand–lime bricks and lime. Ironically nutrition-rich oyster meat is discarded and the shell is used in these industries. Six species of oysters namely the Indian backwater oyster *Crassostrea madrasensis*, Chinese oyster, *Crassostrea rivularis*, West coast oyster, *Crassostrea gryphoides*, Indian rock oyster, *Saccostrea cucullata*, Bombay Oyster, *Saxostrea cucullata*, and giant oyster *Hyostissa hyotis* are found in India (James, 1992). The first four species mentioned above are of commercial value. Of the six species of oysters, the Indian backwater oyster *C. madrasensis* is the dominant species, more widely distributed, is euryhaline and inhabits backwaters, creeks, bays and lagoons and occurs in the coastal areas of the States of Orissa, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka and the Andamans. *C. madrasensis* is the common backwater oyster found in all estuaries and backwaters on the east-coast, but is consumed mostly in the southern region on the west coast. It occurs in abundance particularly in Ennore and Pulikkat areas in Chennai, Sonapur in Orissa and the Vembanad Lake in Kerala (James et al., 1993). Vast stretches of backwaters, estuaries and bays present along the Indian coast harbour a natural population of the oyster suggesting suitability of the habitat for oyster culture. Being filter feeders, the oyster converts primary production in water into nutritious sea food. Culture of these species is being carried out in several places in India. This paper describes profiling of its nutritional attributes with emphasis on its protein content and quality, with an aim to increase its popularity among consumers. Data on its biochemical composition may also prove important for future policy formulation for sustainable exploitation of this species.

Materials and methods

Sample collection and preparation

Cultured oysters were harvested in live condition from Moothakunnam in Ernakulam District of Kerala and depurated for a few hours. They were transported to the laboratory under iced condition in insulated styrofoam boxes and were thoroughly washed to remove slime and dirt. The surface water was blotted with filter paper, edible meat was separated from the shells and kept on ice for immediate use. The meat was homogenised by mincing and proximate composition, amino acid content, fatty acid content and mineral content were determined. Also tissue cholesterol and taurine content were estimated.

Chemicals

All reagents and solvents used in this investigation were of analytical grade. Standards like cholesterol, fatty acid methyl esters, amino acids, taurine, etc. were purchased from Sigma–Aldrich GmbH (Steinheim, Germany).

Biochemical analysis

Moisture

All analyses ($n = 6$) were carried out in triplicate. Moisture of the fish samples (10 g) was determined according to the AOAC (2000) method by drying in an oven at 105 °C ($n = 6$). Results were expressed as percentage of wet weight.

Ash

Ash content was determined by heating the sample (5 g) for 12 h in a silica crucible in a furnace at 525 °C ($n = 6$) according to the AOAC (2000) method. Results were expressed as percentage of wet weight. Minerals were assayed using the AOAC method. Macro elements were determined by flame photometry using working standards in the range of 10–40 ppm for each element (Na, K and Ca). Trace metals were determined by Varian Spectra-220 AA atomic absorption spectrophotometer. Samples were aspirated into the flame and the corresponding absorption of the characteristic radiation by each element was recorded. Values are expressed in ppm.

Protein

Total protein content in the homogenised samples (5 g) was determined using the Kjeldahl method (6). Results were expressed as percentage of wet weight ($n = 6$) basis.

Amino acids analysis

Total amino acid composition was determined following the method of Ishida et al. (1981) using a Shimadzu chromatograph LC-10AT vp high performance liquid chromatography (HPLC) equipped with an ion exchange column, quaternary pump, a 20 µl injection valve and a fluorescence detector. Mobile phase A contained sodium citrate and ethanol (pH 3.5) and B had sodium citrate and NaOH (pH 9.8). The flow rate was constant at 0.4 ml/min, and the column temperature was set at 60 °C. The fluorescence excitation and emission wavelengths were 340 and 450 nm, respectively. Samples were hydrolysed in 6 N HCl in evacuated sealed tubes at 110 °C for 24 h. After derivatisation by O-phthalaldehyde, amino acids were identified and quantified by comparison of their retention times with those of standards (Sigma). The results were expressed in terms of g amino acid per 100 g of crude protein.

Taurine estimation

Taurine content was estimated by the method described above. For the purpose of quantification, taurine standard was run separately and results were expressed in terms of mg taurine per g of tissue.

Nutritional parameters

Determination of nutritional parameters of oyster protein: Nutritional parameters were determined on the basis of the amino acid profile. Essential amino acid score was calculated: mg of essential amino acid/100 mg of test protein/mg of that amino acid/100 mg of FAO/WHO reference protein (Millward and Rivers, 1986). Chemical score i.e., the ratio of a gram of the limiting amino acid in oyster protein to the same amount of the corresponding amino acid in a reference diet (e.g.,

whole-egg protein) multiplied by 100; of oyster protein was calculated using the FAO/WHO (1991) reference pattern. Protein efficiency ratio (PER) was estimated according to the regression equations developed by Alsmeyer et al. (1974) as given below:

$$0.08084\ (X7) - 0.1094 \quad (1)$$

where X7 = Thr + Val + Met + Ile + Leu + Phe + Lys

$$0.06320\ (X10) - 0.1539 \quad (2)$$

where X10 = X7 + His + Arg + Tyr

Essential amino acid index (EAAI) was calculated according to Oser (1959) using as standard the amino acid composition of the whole egg protein published by Cheftel et al. (1985). EAAI is calculated using the ratio of the quantity of each essential amino acid in oyster protein to the quantity of the same amino acid in a reference protein and this ratio was multiplied by 100. Next, the log 10 of each ratio was calculated and the mean of the values is obtained. Finally the antilog of this mean was obtained. Biological value was calculated according to Oser (1959) cited by Mune et al. (2011) using the following equation:

$$BV = 1.09 \times EAAI - 11.7 \quad (3)$$

By convention, the essential amino acid pattern (g AA/100 g protein) for whole egg was used, which is: lysine (6.98), methionine + cystine (5.79), threonine (5.12), isoleucine (6.29), leucine (8.82), valine (6.85), phenylalanine + tyrosine (9.89) and tryptophan (1.49).

Lipid extraction

The estimation of crude fat content was done by continuous extraction of fat with petroleum ether according to AOAC method (2000). Total lipids were extracted according to the method of Folch et al. (1957), using chloroform/methanol (2:1). Aliquots of the chloroform layer extract were evaporated to dryness under nitrogen and the lipids were quantified gravimetrically.

Cholesterol estimation

Cholesterol is estimated by the method of Rudel and Morris (1973). Standard cholesterol in the range of 10–50 μg/ml is taken to which ferric chloride and sulphuric acid are added to develop colour. Absorbance is read at 560 nm and a standard curve is made. Cholesterol content in oyster meat is extrapolated from the standard curve.

Fatty acid analysis

Fatty acids methyl esters (FAMEs) were obtained by the method described by Metcalfe et al. (1966). A fraction of the lipid extract was saponified with 0.5 N NaOH in methanol followed by methylation in 14% boron trifluoride in methanol (BF_3/MeOH). The methylated sample was then extracted with 8 ml *n*-hexane. All of these reactions were performed in quadruplet for each sample. The resulting methyl esters were analysed using an Agilent Gas chromatograph system 6890 N equipped with a flame ionisation detector (FID), a splitless injector and a polar fused silica capillary column (30 m * 0.25 mm i.d. * 0.25 μm film thickness). The temperature of the injector and the detector were 250 and 275 °C, respectively. Helium was used as a carrier gas with a flow rate of 1.5 ml/min. Peaks were identified by comparison of their retention times with FAMEs standards (Supelco).

Statistical analysis

Statistical analysis was performed using SPSS software, version 10.0. Values for each parameter analysed are means of triplicate determinations.

Results and discussion

Biochemical composition

Biochemical composition of a species helps to assess its nutritional and edible value in terms of energy units. Moisture, protein, crude fat and ash content of *C. madrasensis* are given in Table 1. *C. madrasensis* has revealed the following chemical composition: water (~82%), protein (~10%), fat (3.25%), carbohydrate (3.2%) and ash (1.01%). The main constituent of oyster flesh is water, which is tightly bound to the proteins in the structure in such a way that it cannot readily be expelled even under high pressure and is an index of freshness (Murray and Burt, 2001). The high protein content and the less than average lipid levels are similar to that found in species of fish. Oyster meat contains good amount of carbohydrate unlike in fin fish in which it is negligible. Several studies (Berthelin et al., 2000; Bacca et al., 2005) have stated the importance of the presence of glycogen in oysters which play an important role at the time of spawning. The ash content in oyster is slightly lower than in fish species. Oyster meat is rich in both macro elements and trace metals (Table 2). Potassium rich foods are considered to be healthy by convention (USDA, 2010) which is present in oyster in significantly high proportions. Trace metals like Se, Cu, Zn, Mn and Fe act as cofactors for enzymatic reactions in intermediary metabolism (Flemming, 1989) and Fe is an integral part of haemoglobin (Hb) which is essential for oxygenation–deoxygenation cycle of Hb. Selenium has been associated with protection of body tissues against oxidative stress, maintenance of defences against infection, and modulation of growth and development (Rayman, 2000). These minerals are present at significantly high levels in *C. madrasensis*.

Amino acids analysis

Table 3 shows the amino acid profile of *C. madrasensis*. Among essential amino acids, lysine (14.3 g%) content was the highest followed by threonine (12.3 g%); aspartic acid (11.8 g%) and histidine (7.7 g%) were present in high concentration among the non-essential amino acids. Such results are similar to that found by others authors (Murray and Burt,

Table 1 Proximate composition (% wet weight) of *C. madrasensis*.

Parameters	Wet tissue weight (%)
Moisture	82.64 ± 1.31
Protein	9.41 ± 0.85
Lipid	3.25 ± 0.32
Carbohydrate	3.2 ± 0.13
Ash	1.01 ± 0.06

Values expressed as means ± S.D.

Table 2 Macro minerals and trace elements (ppm) in oyster, *C. madrasensis*.

Macro minerals	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)		
	1170 ± 21	975 ± 13	309 ± 9	270 ± 7		
Trace minerals	Mn (ppb)	Cu (ppb)	Zn (ppb)	Fe (ppb)	Cr (ppb)	Se (ppb)
	0.81 ± 0.0	14.7 ± 0.6	95.5 ± 2	33.3 ± 1.1	ND	2.4 ± 0.6

Values expressed as means ± S.D.

Table 3 Amino acids profile (g/100 g crude protein) of oyster, *C. madrasensis*.

Amino acid	g/100 g
Asp	11.8
Thr	12.3
Ser	10.6
Glu	0.73
Pro	1.03
Gly	2.3
Ala	5.3
Cys	0.9
Val	2.6
Met	4.7
Ile	4.5
Leu	2.0
Tyr	5.9
Phe	4.1
His	7.7
Lys	14.3
Arg	6.4
Try	2.17
Total	99.33

Values expressed as means of three determinations.

Table 4 Essential amino acid scores of oyster, *C. madrasensis* protein.

Essential amino acid	Essential amino acid score
Lys	2.47
Met + Cys	1.88
Thr	3.62
Ile	1.61
Leu	0.30
Val	0.17
Phe + Tyr	1.59
Try	2.00

Values are average of three determinations.

1969; Chukwuemeka, 2008) in fin fish. The concentration of lysine in oyster protein is 14.3 g per 100 g crude protein which is significantly higher than the FAO/WHO recommended reference lysine standard value of 5.8 g per 100 g of dietary protein for a 2–5 year child. An abnormally high content of leucine in a protein interferes with the balance of two amino acids namely, isoleucine and threonine and additionally hinders the absorption of isoleucine and tryptophan. Grain proteins like those of sorghum and maize contain a high proportion of leucine that becomes a precipitating factor for the manifestation of pellagra in nutritionally challenged subjects. Interestingly, in oyster meat, leucine is present at a low concentration of 2 g per 100 g crude protein. Table 4 shows the essential amino acid score (EAAS) of oyster protein. Valine and leucine had the lowest score of 0.17 and 0.30, respectively, while, threonine had the highest EAAS of 3.62 which implies that oyster protein has less content of valine and leucine than there is in FAO/WHO recommended protein pattern.

Chemical score is a value obtained by comparing the content of the most limiting amino acid in test protein with its content in reference egg protein. The chemical score obtained for oyster protein (Table 5) was 17.1% and the most limiting amino acid was valine (17.1%) and the 2nd limiting amino acid was leucine (30.3%). The protein efficiency ratio (PER) in *C. madrasensis* (3.49 and 3.92, Table 5) was significantly more than that of fin fish (2.7) and better than that of egg protein (3.0). EAAI of oyster protein was 120.2 which indicates that it is of high quality with respect to the presence of essential amino acids and is higher than that of fin fish (89.1) (Lim and Sessa, 1995). Biological value is a parameter that describes the excellence of a protein in terms of its essential amino acid content which was 174 for oyster protein; significantly higher than that of fin fish (83) (Ganoviak and Lipka, 1983; Ababouch, 2005).

Taurine

The freely occurring β-sulphonic amino acid, taurine was estimated using high performance liquid chromatography.

Table 5 Nutritional parameters of oyster, *C. madrasensis* protein.

	Chemical score	Limiting amino acids		PER		[c]EAAI	Biological value
		Lowest	2nd lowest	[a]PER_1	[b]PER_2		
C. madrasensis protein	17.1	Val (17.1%)	Leu (30.3%)	3.49	3.92	120.2	174

Values are average of three determinations.
[a] PER_1 (Protein efficiency ratio): 0.08084 (X7) -0.1094, X7 = Thr + Val + Met + Ile + Leu + Phe + Lys.
[b] PER_2: 0.06320 (X10) -0.1539, X10 = X7 + His + Arg + Tyr.
[c] EAAI: Essential amino acid index.

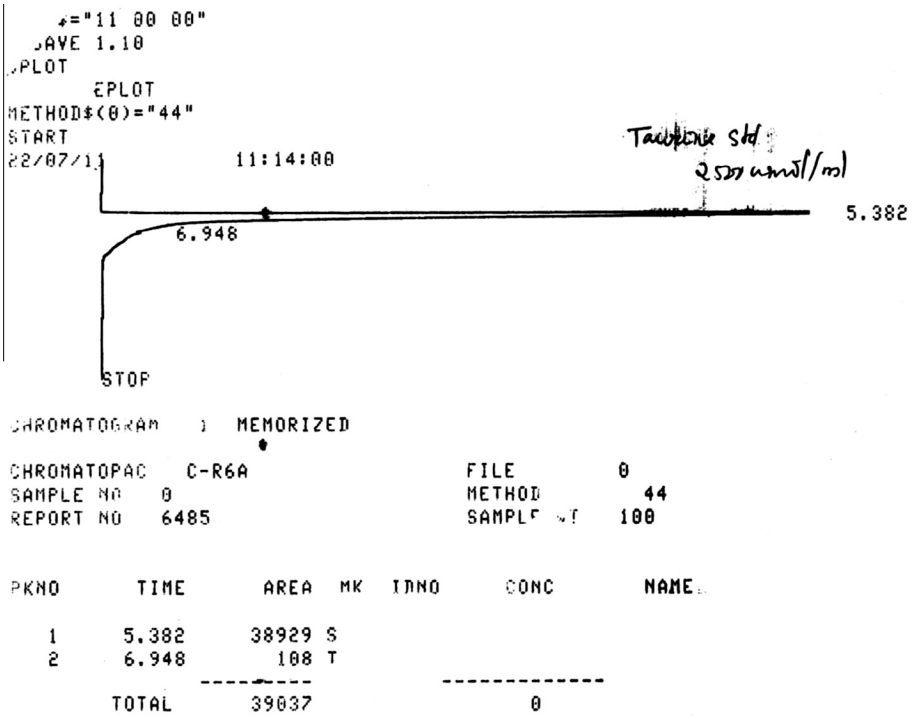

Figure 1 Chromatogram showing retention time and peak for standard taurine.

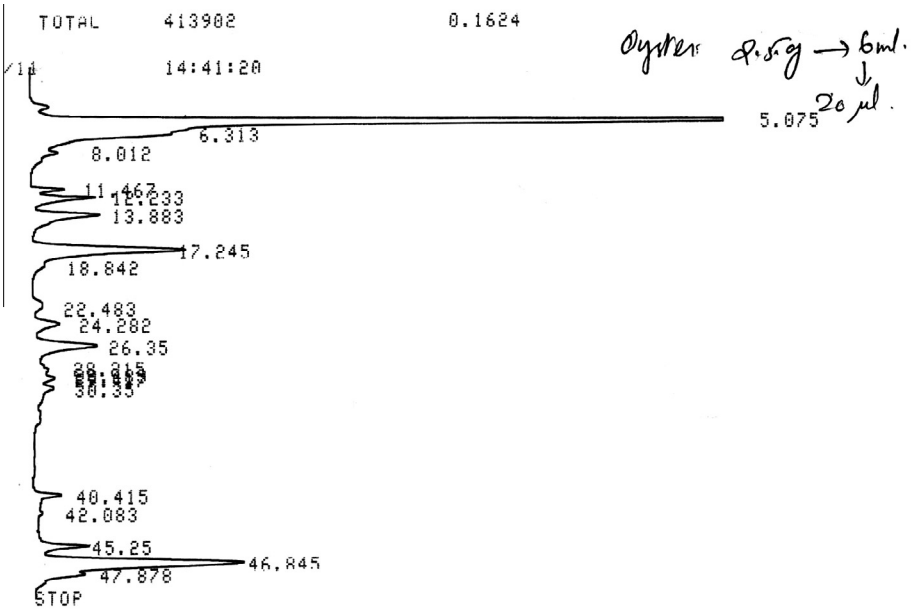

Figure 2 Chromatogram showing retention time and peak for taurine content in oyster meat.

Figs. 1 and 2 show the chromatogram indicating the peak and retention time of taurine standard and taurine content in the oyster sample, respectively. The taurine content is high in oyster meat 243 mg/100 g (Table 6) which is significantly higher than that of fish 40–85 mg/100 g (Divakaran, 2006). The ratio of taurine to cholesterol is an important index in foods and higher ratio is beneficial to the consumer (Choi et al., 2006). Taurine:cholesterol ratio is 2.3 in oyster (Table 6). Among taurine's many natural functions in living systems, is its hypocholesterolemic effect (Rijssenbeek et al., 2006). Taurine acts by conjugating bile acids that are formed from cholesterol synthesised in the liver and excreting them through bile. To replenish the excreted bile acids more endogenous cholesterol is converted to bile acids which results in lowering of cholesterol in the body. Thus taurine exhibits a hypolipidemic effect by stimulating hepatic bile acid synthesis from endogenous stores of cholesterol (Ogawa, 1996).

Table 6 Levels of taurine and cholesterol in oyster meat and taurine/cholesterol ratio.

	Taurine mg/100 g	Cholesterol mg/100 g
Oyster	243 ± 3.5	106 ± 2.2
T:C[a]	2.3	

Values expressed as means ± S.D.
[a] Taurine:cholesterol ratio.

Table 7 Fatty acids composition (mg/100 g tissue) in oyster, *C. madrasensis*.

Fatty acid	mg/100 g oyster meat
c12:0	11.5 ± 0.21
c14:0	4.71 ± 0.04
c15:0	2.07 ± 0.29
c16:0	27.8 ± 0.03
c17:0	11.9 ± 0.22
c18:0	30.6 ± 1.4
c20:0	91.6 ± 2.6
c21:0	2.13 ± 0.01
c23:0	5.80 ± 0.05
c14:1 *n*-7	1.42 ± 0.02
c16:1 *n*-7	28.0 ± 0.45
c17:1 *n*-7	1.87 ± 0.06
c18:1 *n*-9	9.53 ± 0.09
c20:1 *n*-9	16.7 ± 0.32
c22:1 *n*-9	22.6 ± 0.56
c18:2 *n*-6	11.5 ± 0.27
c18:3 *n*-6	4.71 ± 0.07
c18:3 *n*-3	11.9 ± 0.18
c20:2 *n*-6	2.07 ± 0.06
c20:4 *n*-6	27.8 ± 0.43
c20:5 *n*-3	112.0 ± 3.7
c22:6 *n*-3	91.6 ± 2.9
∑SFA[a]	188.1 ± 4.85
∑MUFA[b]	80.12 ± 1.5
∑PUFA[c]	261.58 ± 7.61
∑*n*-6 FA[d]	46.08 ± 0.83
∑*n*-3 FA[e]	214.9 ± 6.78
*n*3/*n*6	4.66
Total FA	584.2 ± 13.96

Values expressed as mean ($n = 6$) ± S.D.
[a] ΣSAT = sum percentage of saturated fatty acids (C12:0, C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C23:0).
[b] ΣMUFA = sum percentage of monounsaturated fatty acids (C14:1, C16:1, C17:1, C18:1 *n*-9, C20:1, C22:1 *n*-9).
[c] ΣPUFA = sum percentage of polyunsaturated fatty acids (C18:2 *n*-6, C18:3 *n*-6, C18:3 *n*-3, C20:2 *n*-6, C20:4 *n*-6, C20:5 *n*-3, C22:6 *n*-3).
[d] Σ*n*-6 = sum percentage of *n*-6 polyunsaturated fatty acids (C18:2 *n*-6, C18:3 *n*-6, C20:4 *n*-6, C20:2 *n*-6).
[e] Σ*n*-3 = sum percentage of *n*-3 polyunsaturated fatty acids (C18:3 *n*-3, C20:5 *n*-3, C22:6 *n*-3).

Cholesterol

Cholesterol content in oyster is 106 mg/100 g (Table 6) of oyster meat which is twice that of fish. The high cholesterol content is compensated by the presence of high taurine content in oyster as taurine is reported to have significant hypocholesterolemic effect.

Fatty acid analysis

Fatty acids composition of *C. madrasensis* is presented in Table 7. Polyunsaturated fatty acids (PUFA) constitute the majority of the fatty acids pool, followed by saturated fatty acid (SFA) and monounsaturated fatty acids (MUFA). The saturated fraction was 188.1 ± 4.85 mg/100 g with C20:0 being the most abundant fatty acid within this fraction, followed by stearic acid C18:0 and palmitic acid C16:0. Among mono-unsaturated fatty acids, C16:1 *n*-7 and C22:1 *n*-9 were in abundance than any other fatty acids (28.0 ± 0.45 and 22.6 ± 0.56 mg/100 g, respectively). PUFA content was the highest (261.58 ± 7.61 mg/100 g) of which *n*-3 PUFA was 214.9 ± 6.78 mg/100 g and *n*-6 PUFA was 46.08 ± 0.83 mg/100 g. Eicosapentaenoic, docosahexaenoic and linoleic acids were the prominent PUFA. The *n*-3/*n*-6 index was 4.66 which shows the occurrence of a high proportion of *n*-3 PUFA over *n*-6 PUFA in *C. madrasensis*. The ratio between *n*-3 and *n*-6 is a very useful index for comparing the nutritional value of fish lipid due to their human health effects on coronary heart disease, cancer and autoimmune diseases (Wang et al., 1990; Simopoulos, 2002).

Conclusion

C. madrasensis is comparable to fin fish with respect to its nutritional attributes with its protein being of high quality and its lipids being a good source of *n*-3 and *n*-6 fatty acids. The high levels of essential amino acids will make it a good food source in complementing cereals for weaning foods. Their high utilisable energy due to protein will prevent protein-energy malnutrition in their consumers. Thus it might be considered as a kind of aquatic food with high protein and low healthy fat. Biochemical composition and nutritional attributes of *C. madrasensis* may prove important for formulations of nutraceuticals and future policy regarding exploitation of this species.

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