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Note

Deep-sea mud shrimp and shovel-nosed lobster from the Arabian Sea as prospective sources of long-chain polyunsaturated fatty acids

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ABSTRACT

Fatty acid profile of deep-sea mud shrimp (*Solenocera hextii*) and shovel-nosed lobster (*Thenus unimaculatus*) harvested from south-west coast (Arabian Sea) of India were evaluated and compared. Palmitic and oleic acids were the principle saturated fatty acids and monounsaturated fatty acids, respectively in lobster and shrimp species. *T. unimaculatus* contained greater concentrations of $C_{20\text{-}22}$ *n*-3 polyunsaturated fatty acids, such as docosahexaenoic acid and eicosapentaenoic acid. Higher *n*-3/*n*-6 polyunsaturated fatty acid ratio (greater than 4) in addition to considerably greater polyunsaturated/saturated fatty acid (>1.2) and hypocholesterolaemic/hypercholesterolaemic ratio (>2) were recorded in shovel-nosed lobster compared to the mud shrimp. Lesser thrombogenicity (≤0.5) and atherogenicity (<1.0) indices recognised *T. unimaculatus* as a desirable marine species for human nutrition.

Keywords: Atherogenicity index, $C_{20\text{-}22}$ *n*-3 polyunsaturated fatty acid, Deep-sea mud shrimp, Shovel-nosed lobster, Thrombogenicity index

Decapod crustaceans are one among the highly valued species in marine fisheries sector owing to their delicacy and prospective nutritional properties (Huys, 2003). Lobsters are deliberated as high-value seafood delicacy and have been in prodigious demand for several years in international markets, thereby commanding higher culinary values. The greatest number of economically important and taxonomically different lobster species are distributed in tropical marine waters, although predominant lobster catches were reported from the temperate waters of north-west and north-east Atlantic (Jeena, 2013). Commercially important lobster species (families, Scyllaridae and Palinuridae) are landed particularly along south-west, north-west and south-east coast lines. Scyllarid lobsters comprise about 8% of the global lobster production. *Thenus* (Leach, 1815) belonging to Scyllaridae is benthic and inhabit sea-sand from 10 to 50 m depth (Iamsuwansuk *et al*., 2012). The scalloped spiny lobster (*Panulirus homarus* Linnaeus, 1758) and shovel-nosed lobster (*Thenus unimaculatus*; Burton and Davie, 2007) are the major species that predominantly contributed to the lobster fishery of India.

Deep-sea shrimps inhabit the tropic to polar regions at depths of up to 5000 m (16,000 ft), and are represented by the families Pandalidae, Aristeidae, Solenoceridae, Penaeidae and Oplophoridae. *Aristeus alcocki*, *Metapenaeopsis andamanensis* (Wood-Mason, 1891) and *Solenocera hextii* (Wood-Mason and Alcock, 1891) are the important penaeid species contributing to about 20-40% of deepsea shrimp landing through the south-west coast and the peak landing was observed from October to March. Commercially important deepsea crustacean, *S. hextii* (family: Solenoceridae) is distributed in the Arabian Sea from the Gulf of Aden to the coasts of India including Bay of Bengal. It is landed by deepsea trawlers from a depth of 100-150 m during January-March from south-east and south-west coasts of India (Pillai and Thirumilu, 2007).

From the nutritional point of view, these deep-sea and inshore crustacean species are rich resources of polyunsaturated fatty acids (PUFAs), good quality proteins, minerals (selenium), essential amino acids and trace minerals, along with a potent natural antioxidant astaxanthin (Dayal *et al*., 2013). They have been focused owing to higher contents of $C_{20\text{-}22}$ *n*-3 PUFAs, particularly, eicosapentaenoic acid EPA (C20:5*n*-3) as well as docosahexaenoic acid DHA (C22:6*n*-3). Long chain PUFAs like linoleic acid (C18:2*n*-6), α-linolenic acid (ALA) (C18:3*n*-3), arachidonic acid (C20:4*n*-6), EPA and

DHA are called as biologically essential fatty acids (EFA) (Bharadhirajan *et al*., 2014).

Present day humen diet comprises excessive amounts of *n*-6 PUFAs, which are the principal factor causing several lifestyle disorders, whereas elevated level of *n*-3 PUFA (a high *n*-3/*n*-6 ratio) exert suppressive reactions (Nisa and Asadullah, 2011). Therefore, evaluation of fatty acid composition becomes imperative when consumption of shrimps and lobsters are considered. Although there were reports on the fatty acid composition of varied species of shrimps and lobsters, the fatty acid composition of deep-sea mud shrimp *S. hextii* and shovel-nosed lobster *T. unimaculatus* have not been reported yet. In view of the above mentioned facts, it seemed essential to determine the fatty acid composition of *S. hextii* and *T. unimaculatus* to derive new information about their nutritive value.

The deep-sea shrimps and lobster samples were obtained from the fishing harbours of south-west coast of the Arabian Sea (coastal peninsular India) during the month of February. They were carried in ice boxes (-20°C) to the laboratory and thoroughly washed to remove debris, mucus and other particles. Further, they were identified as *T. unimaculatus* and *S. hextii* (Chakraborty, 2017a; Chakraborty, 2017b). The shrimps were defrosted, peeled and segregated into the edible portion or flesh (endoskeleton) and exoskeleton (head and outer body shell). The edible muscle tissues were used for fatty acid profiling. Analyses were carried out on male and female specimens, separately.

Lipids were extracted from 20 g (wet weight) of edible portion of shrimp and lobster samples of male and female specimens separately through Folch extraction procedure (Folch *et al*., 1957) utilising methanol: chloroform (1:2 v/v; 250 mL). The fatty acid methyl esters (FAME) were prepared from the aliquots of the extracted lipids from the edible portion of shrimps and evaluated *via* gas liquid chromatography (GLC). The data of GLC were obtained through a gas chromatograph (HP 5890 Series II; AutoSystem$_{XL}$ Perkin-Elmer, USA) attached with a SP®2560 (cross-bond 95% dimethyl-polysiloxane with 5% diphenyl substitution) capillary column (100 m × 0.25 mm *i.e.,* 0.50 µm, Supelco, Bellfonte, US) by a flame ionization detector (FID) containing a split/ splitless injector that was utilised in the split (1:15) mode (Chakraborty *et al*., 2010). GLC evaluations were performed by an oven temperature ramp method: 140°C for 1 min, going up at 30°C min^{-1} to 250°C, where it was retained for 1.0 min, followed by an elevation of 25°C min^{-1} to 285°C, where it was retained for 2.0 min, up until entire peaks was observed. The detector and injector were retained at 290 and 280°C, respectively. Nitrogen (ultra-high purity >99.99%) and hydrogen were utilised as the carrier gas at 25 cm s^{-1} flow rate and at a head pressure of 20 psi, respectively. FAMEs were analysed by comparing the retention times of known standards (Supelco™ 37 FAME Mix). Results were designated as percent cumulative fatty acids (% CFA).

Varied fatty acid ratios signifying nutritional standards of shrimp and lobster *viz.,* DHA/EPA, *n*-3/*n*-6, and PUFA/SFA were assessed and compared with the United Kingdom Department of Health recommendations (HMSO, 2001). The mean value of individual fatty acid was utilised to determine the summation of SFA, MUFA and PUFA.

To illustrate the thrombogenic and atherogenic effects of the edible parts of shrimp and lobster, the indices of thrombogenicity (TI) and atherogenicity (AI) were analysed by the equations as described earlier (Ulbricht and Southgate, 1991) as:

$$TI = (14:0 + 16:0 + 18:0)/ [(0.5\times \sum MUFA) + (0.5\times \sum n\text{-}6\ PUFA + (3\times\sum n\text{-}3PUFA) + (\sum n\text{-}3\ PUFA / \sum n\text{-}6\ PUFA)];$$

$$AI = (4 \times 14:0 + 18:0 + 16:0)/ (\sum MUFA +\sum n\text{-}3\ PUFA + \sum n\text{-}6\ PUFA).$$

The hypocholesterolaemic/hypercholesterolaemic (h/H) ratio was evaluated (Santos-Silva *et al*., 2002) as:

$$HH = (C18:1n\text{-}9 + C18:2n\text{-}6 + C20:4n\text{-}6 + C18:3n\text{-}3 + C20:5n\text{-}3 + C22:5n\text{-}3 + C22:6n\text{-}3)/ (C14:0 + C\ 16:0).$$

One-way analysis of variance (ANOVA) was performed with Statistical Program for Social Sciences (SPSS ver. 13.0, USA) to calculate significant differences among the means. The significant differences were designated as $p<0.05$ and values were allocated as mean of triplicates±standard deviation.

Apart from their delicacy, deep-sea crustaceans provide high quality proteins, lipids, carbohydrates and minerals. Lipids are imperative in sustaining physiological and structural integrity of cellular and organelle membranes. They are the impending resource of vital nutrients and act as carriers of the fat soluble vitamins (Bhavan *et al*., 2010). An earlier report described that oil extracted from shrimp processing byproduct could be a rich source of *n*-3 PUFA and astaxanthin-esters and showed potential anti-adipogenic effects (Phadtare *et al*., 2021). In addition, lobster processing byproducts are rich in high value compounds, such as chitin, proteins, minerals, lipids and pigments. Extracts recovered from lobster processing byproducts were demonstrated to possess several functionalities and bioactivities, which were reported to be useful for numerous applications in functional food, pharmaceutical products and biomedicine (Nguyen *et al.,* 2017). Lipids and PUFAs contribute to the dietary quality, nutritional and sensory values of deep-sea crustaceans.

Table 1. Fatty acid composition (% cumulative fatty acids) of *S. hextii* and *T. unimaculatus*

Fatty acids	Abbreviated form	*S. hextii*		*T. unimaculatus*	
		Male	Female	Male	Female
Saturated fatty acids (SFA)					
Myristic acid	C14:0	1.22^{d}±0.01	2.74^{c}±0.01	4.33^{b}±0.03	6.70^{a}±0.02
Pentadecanoic acid	C15:0	7.38^{a}±0.01	5.23^{b}±0.01	3.40^{c}±0.01	0.55^{d}±0.01
Palmitic acid	C16:0	15.47^{b}±0.22	15.53^{b}±0.93	15.31^{b}±0.09	16.19^{a}±0.14
Margaric acid	C17:0	0.20^{c}±0.04	2.51^{a}±0.16	1.06^{b}±0.01	0.54^{c}±0.02
Stearic acid	C18:0	10.9^{a}±0.14	9.82^{a}±0.93	5.99^{b}±0.03	3.08^{c}±0.02
Total saturated fatty acids	ΣSFA	37.1^{a}±0.13	37.4^{a}±0.93	32.25^{a}±0.13	27.97^{b}±0.93
Monounsaturated fatty acids (MUFA)					
Palmitoleic acid	16:1*n*-7	5.23^{c}±0.03	5.39^{c}±0.04	7.26^{b}±0.13	10.99^{a}±0.06
Cis-vaccenic acid	18-1*n*-7	1.33^{a}±0.01	0.79^{b}±0.03	0.67^{bc}±0.01	0.08^{d}±0.04
Oleic acid	18:1*n*-9	14.02^{a}±0.18	15.32^{a}±0.29	11.02^{b}±0.01	9.94^{c}±0.16
Erucic acid	22:1*n*-9	5.75^{a}±0.88	6.05^{a}±0.16	5.08^{ab}±0.11	3.85^{c}±0.16
Total monounsaturated fatty acids	ΣMUFA	29.06^{a}±0.08	29.82^{a}±0.23	25.37^{b}±0.23	25.79^{b}±0.29
Polyunsaturated fatty acids (PUFA)					
Linoleic acid	18:2*n*-6	3.61^{a}±0.03	3.33^{b}±0.02	2.34^{bc}±0.10	1.79^{c}±0.16
Alpha linolenic acid	18:3*n*-3	0.27^{a}±0.03	0.18^{c}±0.01	0.26^{a}±0.02	0.12^{c}±0.01
Gamma linolenic acid	18:3*n*-6	2.49^{b}±0.16	2.57^{b}±0.08	3.90^{a}±0.03	0.46^{d}±0.23
Eicosadienoic acid	20:2*n*-6	3.33^{a}±0.01	1.91^{c}±0.01	2.26^{b}±0.01	1.52^{c}±0.01
Eicosatrienoic acid	20:3*n*-6	0.55^{a}±0.04	0.29^{b}±0.01	0.64^{a}±0.02	0.11^{d}±0.04
Arachidonic acid	20:4*n*-6	0.78^{cd}±0.03	1.18^{b}±0.04	2.47^{a}±0.13	0.65^{d}±0.01
Eicosapentaenoic acid (EPA)	20:5*n*-3	7.16^{c}±0.09	8.57^{c}±0.23	10.61^{b}±0.29	14.4^{a}±0.09
Docosapentaenoic acid (DPA)	22:5*n*-3	0.94^{c}±0.01	1.73^{a}±0.04	1.71^{a}±0.02	1.72^{a}±0.13
Docosahexaenoic acid (DHA)	22:6*n*-3	6.09^{c}±0.14	7.40^{c}±0.04	13.58^{b}±0.08	19.91^{a}±0.14
Total polyunsaturated fatty acids	ΣPUFA	26.68^{b}±0.14	27.91^{b}±0.09	38.0^{a}±0.09	40.76^{a}±0.23
n-3 PUFA	Σ*n*-3 PUFA	14.4^{c}±0.14	17.89^{c}±0.09	26.14^{b}±0.09	36.12^{a}±0.23
n-6 PUFA	Σ*n*-6 PUFA	10.76^{a}±0.01	9.21^{b}±0.01	11.54^{a}±0.01	4.47^{c}±0.01
Fatty acid indices					
n-3 PUFA to *n*-6 PUFA	Σ*n*-3/Σ*n*-6PUFA	2.19^{c}±0.53	3.03^{b}±0.84	3.18^{b}±0.94	4.52^{a}±3.13
Total DHA and EPA	DHA + EPA	13.19^{c}±0.14	15.97^{c}±0.23	24.19^{b}±0.09	34.27^{a}±0.02
PUFA to SFA ratio	ΣPUFA/ΣSFA	0.69^{c}±0.03	0.73^{c}±0.03	1.22^{b}±0.03	1.49^{a}±0.01
DHA to EPA ratio	DHA/EPA	0.85^{b}±0.01	0.86^{b}±0.03	1.28^{a}±0.03	1.38^{a}±0.01
Thrombogenic index	TI	0.42^{a}±0.01	0.49^{a}±0.02	0.26^{b}±0.01	0.21^{b}±0.01
Atherogenic index	AI	0.58^{ab}±0.01	0.64^{a}±0.01	0.61^{a}±0.01	0.67^{a}±0.02
Hypocholesterolaemic/ hypercholesterolaemic ratio	h/H	1.96^{a}±0.22	2.06^{a}±0.04	2.14^{a}±0.08	2.12^{a}±0.05

Individual fatty acids were expressed as percentage of total identifiable fatty acids. Data presented as mean values of three samples (mean±standard deviation). Row values with different alphabet subscripts (a-d) are significantly different ($p<0.05$).
ND: fatty acid identified as trace, but not integrated.

In the current study, fatty acid composition of *S. hextii* and *T. unimaculatus* in both the sexes are illustrated in Table 1, which comprised of unsaturated as well as saturated fatty acids. Saturated fatty acids are the principal sources or the storage form of energy in the species by reason of their elevated caloric content. No significant differences in SFA contents between the female and male population of *S. hextii* were apparent (37.1 to 37.4%, $p<0.05$) (Fig. 1), whereas the content of SFA was greater in the males of *T. unimaculatus* (~32%) than that exhibited by the females (~28%, $p<0.05$) (Fig. 2). Among the SFAs, palmitic acid (C16:0) were proportionately greater, and distributed evenly in both the species and sexes. Notably, palmitic acid is known to be a potent source of metabolic energy in shrimps and lobsters.

Oleic acid is recognised as the most important MUFAs in marine lipid (Nisa and Asadullah, 2011) and its content was found to be significantly greater in *S. hextii* (14-15%) than that recorded in the edible parts of *T. unimaculatus* (10-11%) ($p<0.05$) (Table 1). MUFAs could lower serum glucose and triglyceride level in hyperglycemia, and might decrease the susceptibility of low density lipid (LDL) oxidation (Reddy and Katan, 2004).

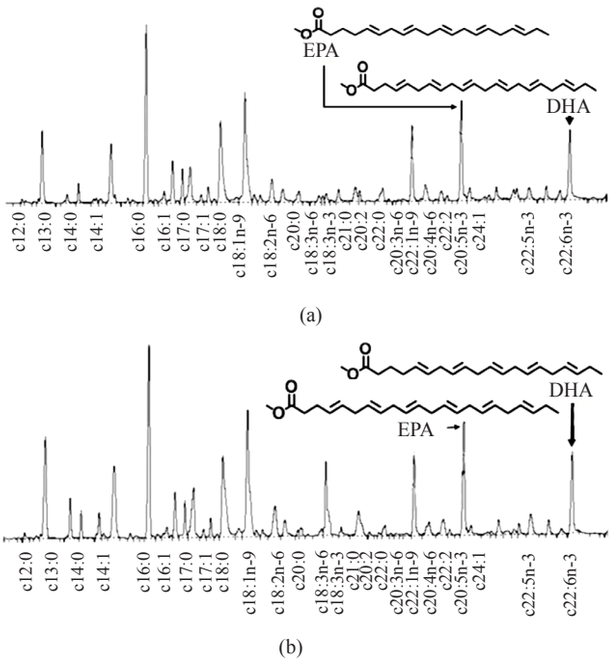

Fig. 1. (a) Gas chromatographic profile of fatty acid methyl esters (FAMEs) derived from the lipidic extract of the edible portion of *S. hextii* male. FAMEs were identified by comparison of retention time (R_t, min) with known standards. Results expressed as percent of cumulative fatty acids (% CFA). (b) Gas chromatogram of FAMEs resulting from the lipid-extract of the edible portion of *S. hextii* female

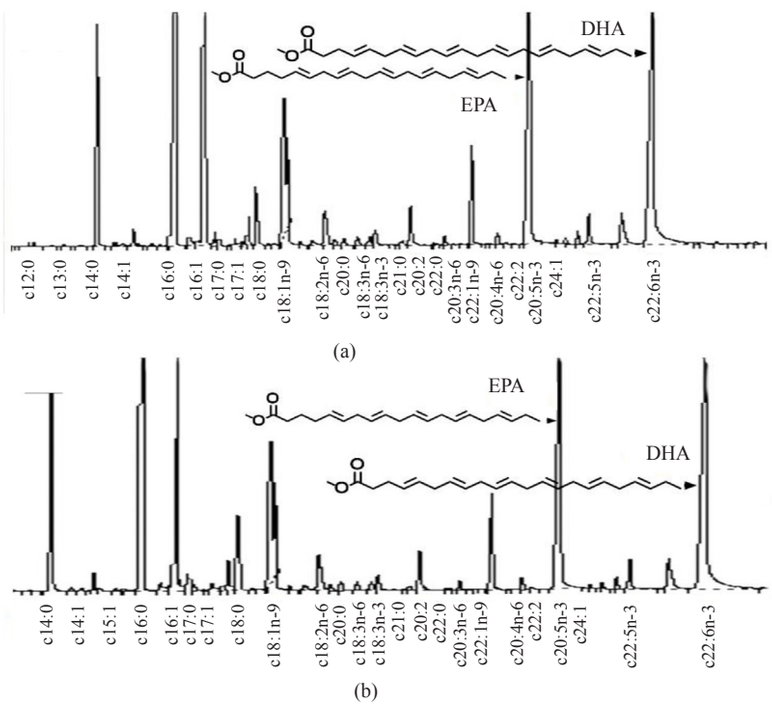

Fig. 2. (a) Gas chromatographic profile of FAMEs obtained from the lipidic extract of the edible portion of *T. unimaculatus* male. FAMEs were identified by comparison of retention time (R_t, min) with known standards. Results expressed as percent of cumulative fatty acids (% CFA). (b) Gas chromatogram of FAMEs ensuing from the lipid-extract of the edible portion of *T. unimaculatus* female

Eicosapentaenoic (EPA), docosahexaenoic (DHA), eicosadienoic, gamma-linolenic and linoleic acid were found to constitute predominant share among the PUFAs studied. The DHA contents were significantly greater in *T. unimaculatus* (13.58% in males and 19.91% in females) ($p<0.05$) than those displayed by *S. hextii* (6.09% in males and 7.14% in females). Likewise, EPA was present in significantly greater amount in *T. unimaculatus* (10.61% in males and 14.40% in females) (Fig. 2), whereas 7.16% EPA was present in males and 8.57% in females of *S. hextii* (Fig. 1). Notably, C_{20-22} *n*-3 PUFAs, such as DHA and EPA were more concentrated in the females of *T. unimaculatus* (34.27% TFA) and consequently, consumption of shovel-nosed lobster could play an imperative role to prevent coronary heart diseases along with increased stress tolerance and membrane permeability (Breslow, 2006). Aggregate contents of PUFA constitute greater than 40% of the total lipids in the females of *T. unimaculatus*.

The *n*-3/*n*-6 ratio is recognised as a functional biomedical marker for evaluating the relative nutritional values of lipids derived from the marine sources (Ersoy and Şereflişan, 2010). The *n*-3 fatty acids regulate hypertension, impair platelet aggregation and inhibit pro-inflammatory and other physiological pathways associated with arterial sclerosis and vulnerable plaques (Anacleto *et al*., 2014). An increased *n*-3/*n*-6 fatty acid ratio is crucial to avert coronary heart disorder by plummeting plasma lipids and diminish cancer risk (Chakraborty *et al*., 2014). Notably, the females of *T. unimaculatus* displayed a larger *n*-3/*n*-6 fatty acid ratio (4.52) (Table 1). An increased *n*-3 PUFA could be attributed to the extended spawning phase of *T. unimaculatus* from September to April (Radhakrishnan *et al*., 2013).

A healthy diet should preferably possess a PUFA/SFA ratio beyond 0.45 (HMSO, 2001). The crustacean species considered in this study displayed greater values than the recommended limit, and thus, could be prospective sources of PUFAs. The PUFA/SFA ratio was highest (1.49) in the females of *T. unimaculatus*, even though *S. hextii* also displayed a higher ratio than the advocated threshold value (Table 1). Food with lesser PUFA/SFA ratio has been considered unsuitable for consumption because it could induce elevated blood cholesterol. However, this ratio alone may have restrictions since it relies solely on the chemical structure of fatty acid and considers that all SFA can induce the elevated blood cholesterol while disregarding the hypocholesterolemic effects of the PUFAs (Santos-Silva *et al*., 2002). In such circumstance, h/H could impart an apposite rationalisation of lipids, by way of lipid metabolism. The h/H ratio in *S. hextii* and *T. unimaculatus* studied were nearer or greater than 2 (Table 1), which was considerably higher than the anticipated threshold.

TI and AI are important parameters, which designate the metabolic effect of different fatty acids, and consecutively protect from arterial sclerosis and platelet aggregation by reason of the anti-atherogenicity and anti-thrombogenicity effects of *n*-3 PUFAs (Ulbricht and Southgate, 1991). The AI values were within the range between 0.51 and 0.61 in males of *S. hextii* and *T. unimaculatus*, whereas the values ranged from 0.64-0.67 in females of these crustaceans. It is notable that the TI values were significantly greater in *S. hextii* (0.42 in males and 0.49 in females) than those exhibited by *T. unimaculatus* (0.21-0.26) ($p<0.05$) (Table 1). Notably, the lower levels of TI and AI are referred for the highly nutritious diet, which can potentially reduce the risk of developing cardiovascular diseases (Tonial *et al*., 2014).

Fatty acid composition constitutes one of the vital indicators to ascertain the quality of food for human consumption and in this direction, decapod crustaceans could be used as high-health food item owing to appreciable content of C_{20-22} *n*-3 PUFAs. Among the deep-sea mud shrimp (*S. hextii*) and shovel-nosed lobster (*T. unimaculatus*) studied, the female lobsters were found to possess greater level of *n*-3 PUFAs (~36%) especially, DHA and EPA alongside lower level of SFA (~28%) compared to those displayed by *S. hextii*. Lower nutritional indices (TI, AI and h/H) in the edible part of *T. unimaculatus* imparts significance in cardioprotection and as high-health food. The results of the present study convey the importance of *T. unimaculatus* as a food component in human diet.

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