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Research Article

Lipid and fatty acid profile variations in *Perna indica* and *Perna viridis* of Kanyakumari district, South east and west coast of India

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Abstract

Variations of lipid and fatty acid profile of *Perna viridis* and *Perna indica* were assessed from Kanyakumari district. Lipid compositions varied and were measured as 8.92±0.03% in *P. viridis* and 6.73±0.01% in *P. indica*. The percentage composition of individual fatty acids as a ratio of total muscle lipids of *P. viridis* ranged from 0.11±0.01% to 25.09±0.03% whereas in *P. indica* it ranged from 0.10±0.01% to 33.2±0.05%. Highest proportions of fatty acids were of palmitic acid which was present in both species. The ratio of Omega-3 and Omega-6 fatty acids was 0.35±0.05 - 3.09±0.04 in *P. viridis* while in *P. indica* it was less than 1. Both the mussel species contained essential fatty acids particularly eicosapentaenoic acid and docosahexaenoic acid for promoting good health, as well as prevention and healing of diseases in humans. *P. viridis* and *P. indica* were recommended for human beings due to the presence of high content of unsaturated fatty acids.

Keywords: *Perna viridis*, *Perna indica*, Lipid, Fatty acid profile.

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Introduction

Seafood is an important diet for many people due to its unique nutritional composition. As the world population is growing, the per capita consumption of seafood is also increasing rapidly. Because of the health consciousness of modern people, they are interested in taking more seafood in view of its nutritional superiority to all other accessible foods. Next to fishes, molluscs form a good source of animal protein and have been highly esteemed as a delicious sea food. Generally, fish and shellfish meat is considered highly nutritious owing to its content of Omega- 3 fatty acids. The polyunsaturated fatty acids have been recognized as effective factors in human health and nutrition especially for cardiovascular diseases (Bruckner, 1992).

Lipid is the major source of metabolic energy and essential materials for the formation of cell and tissue membranes (Sargent, 1995). Aquatic animal fats are good sources of essential fatty acids that are not synthesized in the human body (Bhaskar, 2002; Babu *et al*., 2010). Seafood fatty acids have a very distinctive character compared to fatty acids from other sources. They consist of not only essential fatty acids, but also a significant source of unsaturated fatty acids such as omega-3 and omega-6 fatty acids especially EPA and DHA. These fatty acids play a vital role in human nutrition, disease prevention and health promotion (Frenoux *et al*., 2001). Long-chain polyunsaturated fatty acids (LC- PUFAs) are essential dietary compounds that have important physiological functions (Cook, 1996). Among their health benefits, a reduction in the risk of death from coronary heart disease with a daily consumption of 400 - 500 mg of EPA+DHA in healthy patients has been reported (Harris *et al*., 2008). In patients with schizophrenia and attention-deficit/ hyperactivity disorder, low plasma DHA levels have been observed (Riediger *et al*., 2009). On the other hand, DHA consumption during pregnancy and later in infant formulas results in improved brain and eye development in babies (Dobs and Edelstein, 2008).

Mussels of the genus *Perna* belong to the family Mytilidae or true mussels. This genus contains green and brown mussels and it is distributed in tropical, subtropical, warm temperate and cold temperate regions, mostly from the southern hemisphere, but also from northern Africa and the northern coasts of South America. They exist in the intertidal and shallow sub tidal habitats, including estuaries, mangroves and open rocky shores (Gosling, 2003). These mussels are both ecologically and economically important throughout their ranges and have long constituted an important source of human food (Tomalin and Kyle, 1998). In India, two species of mussels are present and are the Asian green mussel (*P. viridis)* and brown mussel (*P. indica)*.

The green mussel, *P. viridis* is widely distributed along the west coast (Rao, *et al.*, 1975) and southeast and west coasts of India (Alagarswami and Narasimham, 1973). The distribution of

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brown mussel *P. indica* is limited within a coastal stretch of about 150 km, roughly between Kanyakumari and Quilon but they are abundantly available in the rocky beaches of Southeast and Southwest coasts of India (Kuriakose and Nair, 1976). It is usually found at depths of 32 feet and fishermen hand pick these animals and collect them in special nets known as "Katcha valai". Coastal people exploit these highly nutritious resources for their shells for making lime in small scale industries.

Studies in relation to lipid contents and fatty acid profile of marine animal have increased not only because of the specific roles of the omega-3 fatty acids, in biochemical pathways and the development of gonads of animals (Mclean and Bulling, 2005), but also for the health benefits in human consumption (Simopoulos, 2000). Also it is essential to the reproductive biochemistry of both marine animals and humans (Mclean and Bulling, 2005). So far there are no considerable studies on marine mussel species with regard to their lipid and fatty acid profile. Though the mussel meat is being consumed by human beings, the present work was planned to study the lipid content and fatty acid profile of the meat.

Materials and methods

A comparative study on lipid and fatty acid profile of marine mussel *P. indica* and *P. viridis* has not been carried out, while in this study both will be assessed to determine amount of individual fatty acids to recommend tp the people of different areas.

Collection of samples

Green mussel *P. viridis* and brown mussel *P. indica* were procured live from the Kanyakumari coasts fisher men. The collected mussels were transported to the laboratory in an ice box. Shells were broken and the meat was removed from the shell. It was thoroughly rinsed with deionised water to remove extraneous material and dried in a hot air oven at 50 to 70 ^{o}C for 24 hours. The well dried samples were powdered and used for the estimation of lipid and fatty acids.

Analytical method

Analysis of lipid

Lipid was estimated by the method of Folch *et al*. (1956). The dried samples were finely ground and the fat was extracted with chloroform and methanol mixture. After extraction, the solvent was evaporated and the extracted materials were weighed and the percentage of the lipid content was calculated.

Analysis of fatty acids

For the fatty acid analysis, the samples were ground finely with a pestle and mortar. Some 100 - 200 mg of the finely ground tissue sample was added to 1:1 ratio of chloroform: methanol and kept for 30 seconds. The residual matter was removed by filtering through Whatman No.1 filter paper. This was washed with 1ml of chloroform: methanol (2:1) to remove the inorganic substances from the

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combined extract by partition and treated with chloroform: methanol: water (8: 4: 3) where the lower phase was evaporated to dryness. The dried matter was subjected in a sealed test tube with 3% metabolic HCL at 80^{o}C for 18 hours. To this 2 ml of hexane and fatty acids methyl ester (FAME) obtained from methanol phase in hexane were added. The upper 1 ml of the hexane phase was collected in a micro vial. The residual fraction was dissolved in 10:1 of ethyl acetate and 1:1 aliquot was injected into a gas chromatograph (GC) (Agilant, 6890) equipped with flame identification detector and column HP ULTRA-2 (25m, 0.2mm ID) Kashiwa *et al*. (1997).

Statistical analysis

Data collected from this study were analyzed using the Excel XP 2007 computer software. In doing this, descriptive statistics such as means and standard deviations were computed. Two-way analysis of variance (ANOVA) was used to test the significance difference in amount of fatty acids from mussel species.

Results

Lipid

In the present study, lipid content was high in *P. viridis* (8.92±0.219%) and low (6.73±0.121%) in *P. indica*. Lipid composition varied between the two species but differences were not statistically significant (p>0.05) (Fig. 1).

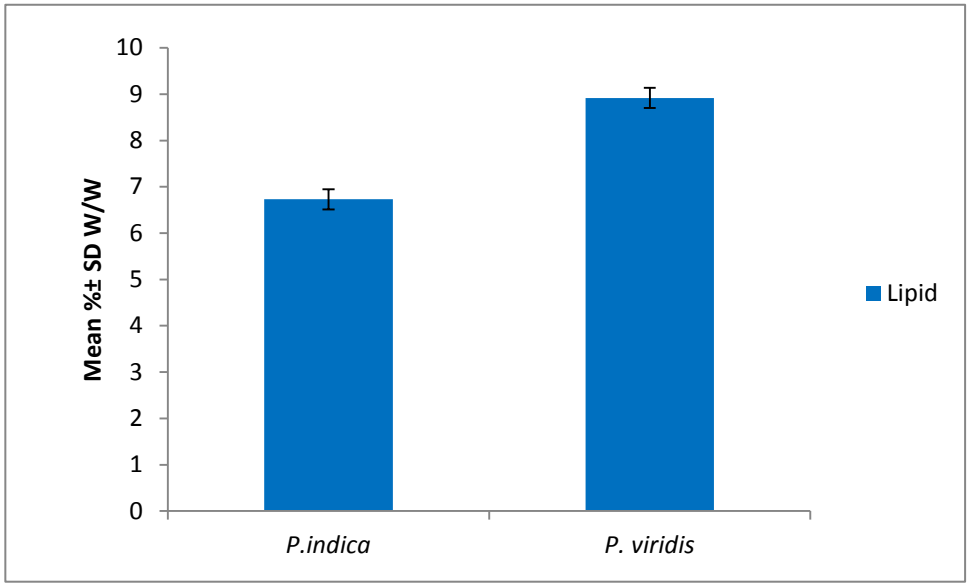

Figure 1: Presence of lipid content in *Perna indica* and *Perna viridis*.

Fatty acid profile

The fatty acid analyses of the lipid extracted from *P. viridis* and *P. indica* showed 30 fatty acids that were considered abundant. Among them ten were saturated fatty acids (SFA), six were monounsaturated fatty acid (MUFA) and fourteen were polyunsaturated fatty acids (PUFA) (Table 1).

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Table 1: Fatty acid profile of *Perna indica* and *Perna viridis.*

Fatty acid	*P.viridis* (Mean %± SD(W/W)		*P.indica* Mean %± SD W/W	
Saturated Fatty Acids (SFAs)				
(C08:0) Caprylic acid	ND		0.10± 0.01	
(C10:0) Capric acid	0.11±0.01		ND	
(C12:0) Lauric acid	3.2± 0.1		0.2± 0.02	
(C14:0) Myristic acid	5.1± 0.1		4.23± 0.03	
(C15:0) Pentadecanoic acid	1.62 ± 0.02		0.51± 0.08	
(C16:0) Palmitic acid	25.03 ±0.03	*	33.2± 0.05	NS
(C17:0) Heptadecanoic acid	3.82± 0.02		0.79 ± 0.09	
(C18:0) Stearic acid	7.21± 0.06		9.68 ± 0.08	
(C20:0) Arachidic acid	1.09± 0.04		0.62± 0.02	
(C22:0) Behenic acid	0.71± 0.06		0.55±0.05	
Mono Unsaturated Fatty Acids (MUFAs)				
(C14:1) Myristoleic acid	0.39± 0.01		0.55±0.05	
(C16:1) Palmitoleic acid	5.71± 0.01	NS	12.66 ± 0.06	NS
(C17:1) Heptadecenoic acid	0.7± 0.1		0.53 ± 0.03	
(C18:1) Oleic acid	25.09± 0.04		18.37 ± 0.07	
(C20:1) Gadoleic acid	2.0± 0.22		0.4±0.2	
(C22:1) Cetoleic acid	ND		ND	
Poly Unsaturated Fatty Acids (PUFAs)				
(C18:2) Linoleic acid	12.4± 0.2		10.9 ± 0.4	
(C18:3) Linolenic acid (omega3)	3.09± 0.04		0.7 ± 0.2	
(C18:3) Linolenic acid (omega6)	2.6± 0.1		0.52 ± 0.02	
(C18:4) Octadecatetraenoic acid	2.89± 0.09		0.31± 0.06	
(C20:2) Eicosadienoic acid	0.11± 0.02	NS	0.58±0.08	NS
(C20:3) Eicosatrienoic acid omega3)	0.37± 0.07		0.14±0.04	
(C20:3) Eicosatrienoic acid (omega6)	0.35 ± 0.05		ND	
(C20:4) Arachidonic acid (omega3)	0.92± 0.02		0.41±0.01	
(C20:4) Arachidonic acid (omega6)	0.86 ± 0.06		0.7±0.1	
(C20:5) Eicosapentaenoic acid	1.31± 0.03		0.93±0.03	
(C22:4) Docosatetraenoic acid	1.37± 0.12		0.61±0.06	
(C22:5) Clupanodonic acid	4.79 ± 0.09		1.71±0.01	
(C22:6) Docosahexaenoic acid	2.94± 0.04		2.57±0.07	
(C24:0) Lingnoceric acid	0.21± 0.02		0.13±0.03	

Means±standard deviation of triplicates,
* $p<0.05$ (Significant at 5% level)
NS - Non significant

Saturated fatty acids

Among the SFAs, Palmitic acid was the major fatty acid in *P. viridis* (25.03±0.03%) and *P. indica* (33.2±0.05%). The remaining SFAs Caprylic acid, Capric acid, Lauric acid, Myristic acid, Pentadecanoic acid, Heptadecanoic acid, Stearic acid, Arachidic acid and Behenic acid were present in the range of 0%, 0.11±0.01, 3.2±0.1%, 5.1±0.1%, 1.62±0.02%, 3.82±0.02%, 7.21±0.06%, 1.09±0.04% and 0.71±0.06% in *P. viridis*, respectively. In the case of *P. indica* they were 0.10±0.01, 0%, 0.2±0.025, 4.23±0.03%, 0.51±0.08%, 0.79±0.09%, 9.68±0.08%, 0.62±0.02% and 0.55±0.05%, respectively. The total availability of SFA contents were 47.89% and 49.88% in *P. viridis* and *P. indica*, respectively. There were significant differences (ANOVA, $p<0.05$) in the occurrence between the species but not significant variation (ANOVA $p>0.05$) between the SFAs.

Mono unsaturated fatty acid

In the present study six monounsaturated fatty acids were recorded and the total percentages were 33.89%, 32.51% in *P. viridis* and *P. indica* respectively, among the MUFA, Oleic acid was found to be high (25.09±0.04 and 18.37±0.07%) followed by Palmitoleic acid (5.71±0.01 and 12.66±0.06%), Gadoleic acid (2.0±0.22 and 0.4±0.2), Heptadecenoic acid (0.7±0.1

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and0.53±0.03) and Myristoleic acid (0.39±0.01 and 0.55±0.05) were found in *P.viridis* and *P.indica* respectively. The total MUFA was higher in *P. viridis* than in *P. indica* but the differences were not statistically significant (p>0.05).

Polyunsaturated fatty acids

Present results showed totally 34.21% and 20.21% of PUFA recorded in 14 individual polyunsaturated fatty acids in *P. viridis* and *P. indica* respectively. Of them Linoleic acid was found to be high (12.4±0.2% and 10.9±0.4%) and Eicosadienoic acids was low (0.37±0.07) in *P. viridis.* Lingnoceric acid was found to be low in *P. indica* (0.13±0.03) followed by the Linolenic acid (3.09±0.04 and 0.7±0.2%), Linolenic acid (2.6±0.1 and 0.52±0.02%), Octadecatetraenoic acid (2.89±0.09 and 0.31±0.06), Eicosatrienoic acid (0.37±0.07 and 0.14±0.04%), Eicosatrienoic acid (0.35±0.05 and 0%), arachidonic acid (0.92±0.02 and 0.7±0.1%), eicosapentaenoic acid (1.31±0.03 and 0.93±0.03%), docosatetraenoic acid (1.37±0.12 and 0.61±0.06%), clupanodonic acid (4.79±0.09 and 1.71±0.01) and docosahexaenoic acid (2.94±0.04 and 2.57±0.07%) in *P. viridis* and *P. indica,* respectively.

Discussion

Lipids are highly efficient sources of energy and they contain more than twice the energy of carbohydrates and proteins (Okuzumi and Fujii, 2000). Lipids are also good source of essential fatty acids necessary for normal growth (Ponnusami, 1997). The fluctuation in the lipid content is highly variable between the species and which may be due to the variation in habitat and other factors (Kunusaki, 2000). Koftayan *et al*. (2011) reported 6.08 – 7.92% of total lipid content in *P. viridis* in different areas of the Eastern Venezuela. Chan *et al*. (2004) observed the total and neutral lipid contents of *P. viridis* were 6.17±0.71 and 2.71±0.42 mg $100mg^{-1}$, respectively. *P. viridis* exhibited good nutritional composition when compared with *Donax cuneatus* and *Meretrix meretrix* (Gopalakrishnan and Vijayavel, 2009). The lipid content showed a range of 8.09 to 12.62% in mussel and the average lipid content at 6.99% in *P. viridis* available in Kali estuary shows very high calorific values throughout the year (Salaskar and Nayak, 2011). Rajakumar (1995) reported lipid values (4.5 to 9.3%) were higher in female (0.95 - 2.96%) *R. rapiformis* than that of male (0.85 - 2.12%). In *L. quadricentus* the variation for lipid value was 0.79% (Thivakaran, 1988). In *C. rota* the lipid values ranged from 0.8-10.75% (Suryanarayanan and Nair, 1976). Anandhakumar (1986) recorded 15.0 to 23.6% of fat in *H. pugilinus.* Xavier Ramesh and Ayyakkannu (1992) reported 2% of lipid in foot muscle of *C. ramosus*. The value of lipid observed in the *R. rapiformis* ranged from 0.85 to 2.96% (Rajakumar, 1995). Nirmal (1995) has observed the highest level of lipid at 10.38% in *Babylonia zeylanica* and 1.97% in *Pleuroploca trapezium*. Periyasamy *et al.,* (2011) reported 9.3% and Thirumavalavan (1987) has reported that the range of lipid was 3 to 10% in *B. spirata*. Giese (1969)

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suggesting that a lipid value of 5% dry weight is a good estimate of structural lipid and it plays a role as reserves. Fatty acids are the fundamental structural components of lipids and they contain different kinds of fatty acids and they have three major roles in the body. They function as efficient energy stores, important building blocks of the cell membrane and are critically important as precursors of the hormones like compound such as prostaglandins, thromboxanes and leucotrienes involved in a variety of critical biochemical functions.

Pollero *et al*. (1979) reported that the Palmitic acid contributed to more than 10% in *Chlamys tehuelcha.* Srilatha *et al*. (2013) reported *M. casta* showed the dominance of Palmitic acid (36.21%). De Moreno *et al*. (1980) reported that the predominant fatty acids in *Mytilus plantensis* were Palmitic acid and Stearic acid. Zhukova and Svetashev (1986) observed the sum of saturated fatty acids ranged from 16.8 to 22.5% in five species of bivalves. The oyster, *Crassostrea madrasensis* exhibited 48.2% of total SFA content. Langdon and Waldock (1981) showed a similar trend of fatty acid profile in *C. gigas*. Shanmugam *et al*. (2007) identified 36 individual fatty acids in clam *Donax cuneatus* and among them saturated fatty acids were the dominant fatty acids (35.28%).

The second type of fatty acid is mono unsaturated fatty acid and is often referred to as "good" fats because they help in reduce blood cholesterol levels and protect against heart disease. All the mono and polyunsaturated fatty acids are benefits to human health. Gastropods have been found to contain Oleic acid was the major fatty acid (Ackman *et al*., 1971; Johns *et al*., 1980). Among the total fatty acids oleic acid contributed to more than 10% in *Chlamys tehuecha* (Pollero *et al*., 1979). The earlier studied MUFA content was reported as 23% in the frozen Green mussel of *P. canaliculus* (Murphy *et al*., 2003).

Polyunsaturated fatty acids are energy sources and also function in the body as components of membranes, modulators of gene expression and precursors for ecosanoids. The marine animals are richest sources of PUFA and may account for about 15.25% of those total fatty acids, where EPA and DHA acids together accounted for about 90% (Nair and Mathew, 2000). Linoleic acid is a polyunsaturated fatty acid; it is called 18:2 (n-6). Previous studies have found evidence that α-linolenic acid has been assessed for its role in cardiovascular health to lower risk of cardiovascular disease (Fleming and Etherton, 2014). The weight of the evidence favors recommendations for modest dietary consumption of α-linolenic acid (2 to 3 g per day) for the primary and secondary prevention of coronary heart disease. Lack of linoleic acid and other omega-6 fatty acids in the diet causes dry hair, hair loss and poor wound healing (Duerksen and McCurdy, 2005). Linoleic acid is used in making soaps, emulsifiers and quick-drying oils and it has become increasingly popular in the beauty products industry because of its

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beneficial properties on the skin (Darmstadt *et al*., 2002).

The n-3: n-6 ratio has been suggested to be a useful indicator for comparing relative nutritional values of food oils. A higher ratio of n-3: n-6 PUFAs has often been cited as an index of better nutritional value (Zhao *et al*., 2010). In the present study the n-3: n-6 was higher in *P. viridis* compared to *P. indica*. These results indicate that mussel species contain a balanced lipid composition for nutritional purposes. Also the n-3 and n-6 polyunsaturated fatty acids have antagonistic effects in the human body. While n-3 fatty acids are precursors of vasodilators, platelet anti-aggregation and anti-inflammatory compounds, the n-6 are precursors of compounds with opposite effects. Pollero *et al*., (1979) reported Gastropods have been found to contain 20:4, 20:5 and 22:5 as major fatty acids, which is in conformity with the results of the present study

The LC-PUFA, particularly EPA and DHA have a significant therapeutic and nutritional value, and among other applications, they have been used for cardiovascular disease treatment (Kryzhanovskii and Vititnova, 2009) for babies in brain development (Schuchardt *et al*., 2010) and as hypotensors (Chen *et al*., 2009). In general, the mussel species in this study showed important concentrations of EPA and DHA. DHA values were higher than EPA values for the studied species; similarly, it has been reported for some aquatics species (Ackman *et al*., 1980). The total PUFA were high in *P. viridis* than the *P. indica* but the differences were not statistically significant (p>0.05).

Limitations

Studies on fatty acid composition of commercial seafood's in India are limited because the mussel species which are abundantly available in October to April and not throughout the year. This might be due to the lack of awareness on benefits of these nutrients. This study shows that total lipid content in the tissue of two species were relatively sufficient. Both species showed important concentrations of n-3 LC-PUFA, particularly EPA and DHA but *P. viridis* showed the highest content of EPA+DHA. It is an important food source to supply daily nutritional needs. The present study clearly indicates that the *P. viridis* and *P. indica* are an important part of a balanced diet and contribute to a good nutritional status. Further study to popularize the mussels in interior region and produced through aquaculture must execute.

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