



Proximate, Amino Acid, Fatty Acid and Mineral Analysis of Clam, *Meretrix casta* (Chemnitz) from Cuddalore and Parangipettai Coast, South East Coast of India

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Abstract

The eulamellibranch bivalves, *M. casta* Chemnitz is an edible mollusc belonging to phylum Mollusca, class Bivalvia and family Veneridae. It is abundantly found in marine and estuarine water bodies at high rates in the local markets of Tamil Nadu along Indian coast. The clam meat is white and sweet and tasty. Whereas most food sources for the humans are provided from marine flora and fauna, now a days clams has been functionally used as another good source of food nutrients to man compared to marine animals. The bivalves are also known to be good sources for the provision of protein, carbohydrate, lipid, amino acids, fatty acids, vitamins and minerals. This study was conducted to assess the nutritional qualities of *M. casta* of the Cuddalore and Parangipettai in two different coasts. The total protein content was found to be varied between 45.67% and 30.021%. The carbohydrate concentrations were ranged from 4.21 to 15.67% and lipid were ranged from 5.63 to 1.11% respectively at the Station I and II. The total essential amino acids were ranged from 13.28% to 0.39% and non-essential amino acids were 8.36 to 1.08% at Station I and II. Total saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acids were determined. The vitamin A, vitamin D, vitamin E, and K of vitamins were 14.40IU, 200IU, 1.18 mg/g and 0.62 mg/g from the Station I and 8.200IU, 150IU, 1.06 mg/g and 0.18 mg/g found from Station II. Totally, 5 macro minerals and 2 trace minerals were detected from both the stations.

Keywords

Biochemical; Fatty acids; Vitamins; Minerals; Matti; Inflated umbones

Introduction

The molluscs are delicious and protein rich in importance next to fishes and prawns. Their nutritive value can be readily assessed by estimating the levels of protein, carbohydrate and fat in their body tissues. As the bivalves mostly constitute for the shell fishery, several studies were dealt with bivalves rather than gastropods [1]. The

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Meretrix spp. also forms a good source of food and cheap substitute for variety of man requirements such as food for the coast native people throughout the species range [2]. Molluscan proteins are rich in essential amino acids and they are required for the maintenance of growth, reproduction and synthesis of vitamins. The amino acid content varies not only from species to species but also from specimen to specimen and between different tissues. This variation depends upon the environmental conditions and the size of the individual species [3]. Aquatic animal fats are good sources of essential fatty acids that are not synthesized in the human body [4,5]. Molluscs are a good dietary source of vitamin B complex, Niacinamide and Folic acid. Minerals serve as components of bones, soft tissues, co-factors and co-activators of various enzymes important in human nutrition. As the world population is growing, the per capita consumption of seafood is also increasing rapidly. Because of health consciousness, the modern day man is interested in taking seafood more in view of its nutritional superiority than all other sources of food accessible to him. There remains no considerable study on marine bivalve with regard to their nutritive value [6,7]. Though marine bivalve are being consumed in islands other country and India. Hence, the present work was planned to study the proximate composition of *M. casta* through estimating their major biochemical components such as total protein, carbohydrate and lipid content in the whole body tissue apart from the amino acids, fatty acid profile, vitamins and mineral content.

Materials and Methods

The samples of the marine bivalve *M. casta* was collected from the sandy shore of (Station I) Parangipettai coast (Lat. 11°29' N and Long. 79°46' E) and (Station II) Cuddalore coast (Lat 11° 42' N Long 79° 46' E) and brought immediately to the laboratory for further studies. The study was conducted between January 2011- December 2011. The bivalves were kept in the glass trough in tap water for 24 hours, for emptying and cleaning the gutters. The shell was removed and the entire body tissue was dried at 55°C (constant temperature) for 24 hours in the hot air oven. Then, the dried meat was powdered and the required quantity of powder was taken for the estimation of total protein, carbohydrate, lipid, amino acids, fatty acids, vitamins and minerals.

Estimation of total protein

The Folin-Ciocalteu Phenol method of Lowry et al. was adopted for the estimation of total protein in the tissue [8].

Estimation of total carbohydrate

The estimation of total carbohydrate content, the procedure of Dubois et al. using phenol-sulfuric acid was followed [9].

Estimation of total lipid

The chloroform-methanol extraction procedure of Folch et al. was used for extracting lipid from the various body parts [10].

Estimation of amino acids

The experimental samples were finely ground for estimating the amino acids in the HPLC (Merck Hitachi L-7400) following the method of Baker et al. [11].

Fatty acid analysis

For fatty acid analysis, the samples were homogenized with chloroform: methanol (2:1 v/v) mixture and they were extracted using the method of Bligh et al. [12]. After the fat was extracted, they were esterified with 1% H₂SO₄ and fatty acid methyl esters were prepared by following the procedure of AOAC [13]. The identification and quantification of fatty acids were done using Gas Chromatography (Hewlett Packard 5890 model).

Estimation of vitamins

The fat soluble vitamins A, D, E and K and the water soluble vitamins B₁, B₂, B₆, B₁₂ and C were analysed in the HPLC (Merk Hitachi L-74000) following the method described by Sadasivam and Manickam [14]. The folic acid was estimated by following the calorimetric procedure of Sethi [15]. The pyridoxine, panthothenic acid and vitamin B₁₂ were estimated by following methods are suggested in USP NF 2000 Asian edition.

Estimation of minerals

The minerals were estimated by following the method of Guzman and Jimenez [16].

Results

Seasonal variations in proximate composition

The percentage concentrations of protein, carbohydrate and lipid contents of *M. casta* for the Station I and II are presented in Tables 1 and 2. The protein concentrations were varied between 45.67% and 30.021%. The carbohydrate concentrations were ranged from 4.21 to 15.67% and lipid were ranged from 5.63 to 1.11% respectively at the Station I and II during the study period. The highest protein concentration 45.67% was recorded in summer and lowest percentage 34.87 was recorded in monsoon at Station I. The maximum carbohydrate was recorded 7.1% in summer and minimum was recorded 4.21% in monsoon and maximum lipid was recorded 5.63 in summer and minimum was recorded 2.52% in a monsoon at the Station I (Figure 1). The observed maximum concentration of protein, carbohydrate and lipid for Station II were 39.58%, 15.53% and 3.52% during the summer and minimum was observed values were 30.021, 14.021 and 1.11% during the monsoon respectively (Figure 2).

Table 1: Seasonal variations in the proximate composition of clam *M. casta* at station I (Parangipettai coast) during the period of January 2011-December 2011.

Component	Jan	Feb	March	April	May	June	July	August	Sep	Oct	Nov	Dec
Protein	41.01	41.68	42.18	45.67	43.56	37.89	37.43	42.23	41.21	38.45	35.67	34.87
CHO	5.61	6.1	6.21	6.23	7.1	5.041	5.12	5.03	5.21	4.69	4.28	4.21
Lipid	4.23	4.65	5.21	5.63	5.1	3.31	3.76	4.25	4.75	3.23	3.1	2.52
Ash	2.78	3.12	3.34	3.56	3.26	2.65	2.67	3.03	3.1	2.68	2.52	2.41

Table 2: Seasonal variations in the proximate composition of clam *M. casta* at station II (Cuddalore coast) during the period of January 2011-December 2011.

Component	Jan	Feb	March	April	May	June	July	August	Sep	Oct	Nov	Dec
Protein	36.21	36.34	39.58	38.34	34.12	32.25	34.21	36.11	35.76	31.02	30.021	30.59
CHO	15.67	14.98	14.651	15.53	15.02	15.12	13.89	14.57	14.73	14.12	14.021	14.11
Lipid	2.85	3.21	4.34	3.52	4.32	2.24	2.97	3.51	3.65	2.02	1.11	1.21
Ash	3.3	3.23	3.34	3.87	3.52	2.57	2.45	3.36	2.33	2.31	2.26	2.3

Percentage composition of amino acids

The percentage composition of essential and non-essential amino acids were found in the tissues of the adult clam was shown in Table 3. The total essential amino acids were ranged from 13.28% to 0.39% and non-essential amino acids were 8.36 to 1.08% on dry matter basis. The maximum concentrations of EAA 9.14% and 13.28% were set by Lysine at the Station I and II, the minimum concentrations of 0.39 and 0.64% was set by tryptophan at Station I and II. Similarly the higher and concentration of NEAA 8.36% was accounted by glycine at Station I, 8.03% by glutamic acid at Station II and lower percentage of 0.39 and 0.64% were given by tryptophane from both the stations. Amino acid estimations were estimated using standard graph (Figure 3) and similar peaks were observed for both the stations (Figures 4 and 5).

Fatty acid profile

The profile of fatty acids obtained from the dried tissue samples were shown in Table 4. Totally, about 7 different fatty acids were found in *M. casta*, they are three saturated fatty acids (SFA), one monounsaturated fatty acid (MUFA) and three polyunsaturated fatty acids (PUFA). Among the SFAs C18:0 where the major acids. In MUFA, C18:1 and in PUFA three were the major acids found. The percentage availability of SFA content was 63.97% in the total lipid content of *M. casta* at the Station I and II respectively. The percentage of MUFA for the Station I and II were 14.63 and 13.85% respectively, the contribution of PUFA was 18.66 and 12.21% from the Station I and II.

Vitamin contents of *M. casta*

The concentrations of vitamins found in the dried tissue sample of *M. casta* was presented in Table 5. The observed results for fat soluble vitamins A, D, E and K were 14.40IU, 200IU, 1.18 mg/g and 0.62 mg/g from the Station I and 8.200IU, 150IU, 1.06 mg/g and 0.18 mg/g found from Station II. Similarly, the observed values for water soluble vitamins such as pyridoxine (vitamin B₆), Cobalamine (vitamin B₁₂) and vitamin C were 5.93 mg/g, 0.42 µg/g and 19.55 mg/g from the Station I and 3.64 mg/g, 0.13 µg/g and 26.38 mg/g from Station II respectively.

Mineral contents

The quantity of minerals present in the tissue varied significantly.

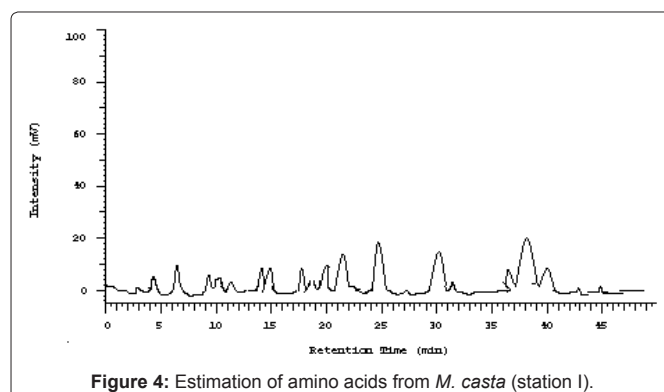
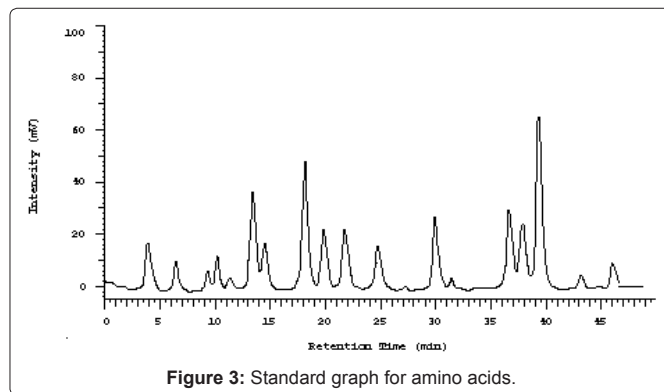
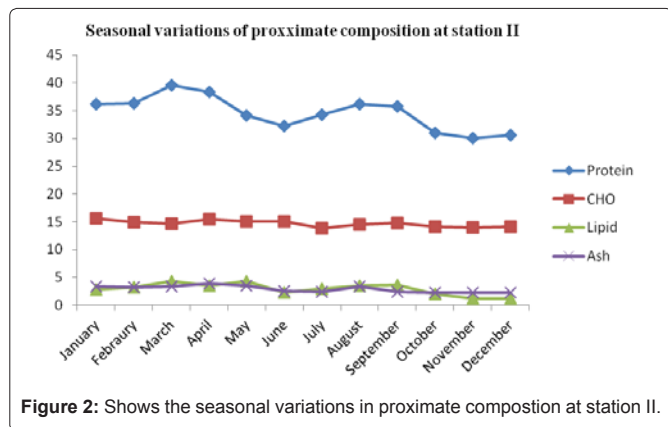
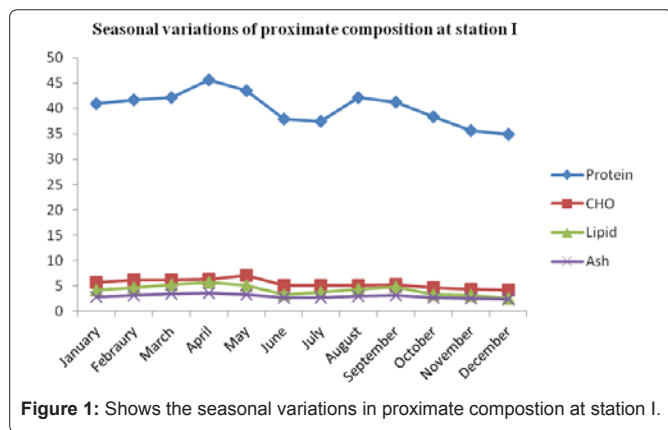
Totally, 5 macro minerals and 2 trace minerals were detected from both the stations. Among the macro minerals, calcium (345.5 mg/g), sodium (155.6 mg/g), magnesium (145.6 mg/g) and copper (2.33 mg/g) were observed at higher and lower levels at the Station I and calcium (286.12 mg/g), sodium (196.35 mg/g), magnesium (94.13 mg/g) and copper (2.33 mg/g), whereas potassium were in negligible level. The trace minerals such as iron (15.55 mg/g) and zinc (12.33 mg/g) from the Station I, values of those two metals 18.48 mg/g and 8.47 mg/g from Station II were estimated in this species (Table 6).

Discussion

Biochemical studies are much more important from the nutritional point of view. The biochemical constituents in animals are known to vary with season, size of the animal, stage of maturity, temperature and availability of food etc. Biochemical components such as protein, carbohydrates and lipids are essential for body growth and maintenance. A protein is essential for the sustenance of life and exists in largest quantity of all nutrients as a component of the human body [17]. The present study revealed that the maximum level of protein content in *M. casta* is 45.67% and 39.58%. Similarly, the molluscs are reported to contain a high amount of protein ranging 40-78% [18]. Rajakumar observed the *Rapana rapiformis* protein value to be 44.8% in males and 46.1% in females [19]. Arularasan et al. reported that the protein ranged from 45.45% to 67.88% in males and 46.02% to 72.36% in females of *Strombus canarium* [20]. Babu et al. studied that the percentage of protein ranged from 19.25 to 27.9% in the Mesogastropod, *Bursa spinosa* [21]. The accumulation of proteins

Table 3: Essential and non essential amino acids of *M. casta* at station I & II during the period of January 2011-December 2011.

S. No	Amino acids in percentage		
	EAA	Station I	Station II
1	Phenylalanine	8.54	2.26
2	Lysine	9.14	13.28
3	Histidine	5.51	8.92
4	Methionine	6.13	6.04
5	Arginine	3.62	2.14
6	Leucine	7.18	6.62
7	Threonine	4.11	3.36
8	Isolucine	2.54	2.63
9	Valine	1.51	1.32
10	Tryptophan	0.39	0.64
NEAA			
11	Glycine	8.36	6.12
12	Serine	7.15	4.12
13	Glutamic acid	6.24	5.35
14	Cysteine	5.37	1.63
15	Glutamate	4.51	8.03
16	Alanine	4.16	3.04
17	Proline	3.43	2.32
18	Aspartate	2.26	4.36
19	Tyrosin	1.31	3.28
20	Asparagine	1.08	1.15



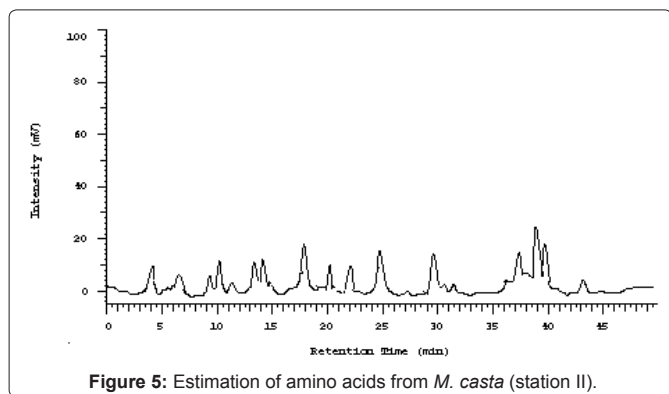


Table 4: Fatty acid profile of *M. casta* during the period of January 2011–December 2011.

S. No	Fatty acids		Carbon atom (n)	% of FA Station I	% of FA Station II
	SFA				
1	Palmitic acid		C16:0	15.35	16.82
2	Margaric acid		C17:0	12.41	11.32
3	Stearic acid		C18:0	36.21	31.35
Total				63.97	59.49
MUFA					
4	Oleic acid		C18:1	14.63	13.85
Total				14.63	13.85
PUFA					
5	Linolenic acid		C18:3	3.38	2.57
6	A Linolenic acid		C18:3	12.72	8.35
7	Stearidonic or Morocitic acid		C18:4	2.56	1.35
Total				18.66	12.27

in the body was higher during the summer in both the stations which reflect its correlation with gonad developmental stages.

The two peaks are observed in the protein concentration from both the stations during the study period, by one it was during April at the Station I and May at Station II. Following the spawning from April to May, the peaks started declining again the peak raised during post monsoon as second spawning season but the protein values 42.23% of the Station I and 36.11% at Station II was little lower than the previous spawning season. Suryanarayanan and Nair studied that the protein concentration is correlated with gametogenesis process [22]. The northeast monsoon period (Oct-Dec) appears to be most more unfavorable for *M. casta* when all its metabolic activities seem to be at their lowest. During post-monsoon (Jan-March), there is a buildup and during the breeding peak (April to June) the values reach the highest. During pre-monsoon (July-Sep), it seemed to be the second breeding peak at Vellar estuary [2].

Carbohydrates are major sources of energy in all human diets. The ratio of carbohydrate was less when compared to the other nutrients such as proteins and lipids in animal tissues, especially in aquatic animals [21]. Carbohydrate in the tissues exists as glycogen, free sugars and protein bound sugars, which serve as energy reserves for various metabolic processes. Nagabhushanam and Mane reported that in the molluscs generally the carbohydrate reserve may be utilized

under unfavorable conditions and the great variation found in the tissue indicate that the level of mobilized carbohydrate reserves may fluctuate widely and rapidly in response to fluctuation in condition [23].

In the present study, the percentage of carbohydrates in the body tissue of *M. casta* is 4.21% and 15.67% at Station I and II. Generally the *M. casta* carbohydrate content was found to be low as it is high when compared to other mollusks and there is no much fluctuation with the seasons. Similar findings were reported earlier by [7] estimated maximum levels (5.31%) of carbohydrate in *L. quadricentus* and (4.69%) in *N. pyramidalis*. Ramesh and Ayyakkannu studied to evaluate an increase trend in the carbohydrate level in the foot muscle (16%) from *Chicoreus ramosus* [24]. Shanmugam reported that the carbohydrate in *Pythia plicata* values from 0.84 % to 3.04% [25]. Arularasan have been reported that the carbohydrate values ranged from 10.49 % to 14.56 % in males and 9.15 % to 16.26 % in females *S. canarium* [20]. Periyasamy et al. studied that the carbohydrate value was maximum (16.85 %) in *Babylonia spirata* [26].

Lipids are highly efficient as sources of energy and they contain more than twice the energy of carbohydrates and proteins [17]. In the present study, lipid content of *M. casta* is 5.63 and 4.34%. Ananda Kumar et al. observed the value of lipid to range from 15.0% to 23.6% in *Hemifusus pugilinus* [18]. In *Chicoreus ramosus*, the lipid values assessed at 2% in foot muscle [24]. Rajakumar reported the lipid content in *Rapana rapiformis* ranged from 0.85-2.12% in male and 0.95-2.96% in female [19]. Nirmal has observed the highest level of lipid 10.38% in *Babylonia zeylanica* and 1.97% in *Pleuroploca trapezium* respectively [27].

The biological value of protein is obviously reflected upon its

Table 5: Vitamin content of *M. casta* (mg/g of the sample) during the period of January 2011–December 2011.

S. No	Vitamins	Station I	Station II
1	Retinol (A)	14.40 IU	8.200 IU
2	Calciferol (D)	200 IU	150 IU
3	Tocopherol (E)	1.18 mg/g	1.06 mg/g
4	Vitamin (K)	0.62 mg/g	0.18 mg/g
5	Pyridoxin (B6)	5.93 mg/g	3.64 mg/g
6	Cobalamin (B12)	0.42µg/g	0.13µg/g
7	Vitamin (C)	19.55 mg/g	26.38 mg/g

Table 6: Minerals content of *M. casta* (mg/g of the sample) during the period of January 2011–December 2011.

S. No	Minerals	mg/g (St I)	mg/g (St II)
	Macro		
1	Calcium	345.5	286.12
2	Sodium	155.6	196.35
3	Potassium	13.4	10.64
4	Copper	2.33	1.15
5	Magnesium	145.6	94.13
Trace			
6	Iron	15.55	18.48
7	Zinc	12.33	8.47

essential amino acid concentration. In general, the shellfish has a balanced distribution of all essential amino acids required for an adult per day. In the present study *M. casta* showed the total essential amino acids were recorded 48.67 % and 47.21% and non-essential amino acids were recorded 43.87 % and 39.4% of the Station I and II respectively. Among the essential amino acids, Lysine is found high level in dry matter basis in bivalve tissue. The present study showed that a total of 20 amino acids which exhibit high levels of lysine followed by phenylalanine and histidine, based on the quantum of availability of EAA in the tissue of *M. casta*. The result revealed in this study showed that, the yellow clam *M. casta* meat is a potential source for food value due to high quality protein, as well as balanced essential amino acids. Similar observations were made by Ajaya Bhaskar, who reported that the total amino acids in *Perna viridis* is 95.76%, 98.4% in *C. madrasensis* and 65.17% in *M. casta* [4]. Arularasan studied the percentage of essential amino acids was more (80.97%) than those non essential amino acids (15.07%) from the Gastropod, *Strombus canarium* [20]. Babu et al. have been estimated the total amino acid was found to be 96.8%, among them the essential amino acids (EAA) 50.01% and non essential amino acids (NEAA) 46.79% and unidentified amino acids 3.2% in *Bursa spinosa* (Mesogastropod) [21]. Palpandi analyzed the amino acid content of *Nertita crepidularia*, out of a total of six amino acids recorded, four were essential amino acids (68.5%) and two non-essential amino acids (31.01%) respectively [28]. Periyasamy reported that the total of 10 essential and nonessential amino acids were from *B. spirata* (9.91 mg/g) [29].

In the present study, 7 different fatty acids were identified, 3 saturated fatty acids (SFA), 1 monounsaturated fatty acids (MUFA) and 3 polyunsaturated fatty acids (PUFA). Among the SFAs C18:0 where the major acids. In MUFA C18:1 and in PUFA C18:3 were major acids found. The high percentage availability of SFA contents was 63.97% and 59.49% respectively in *M. casta*. In the present study, *M. casta* showed the dominance of saturated fatty acids, Palmitic acid (18:0) which were found to be 36.21%. The *M. casta* could be a better alternative source (against tubercle bacilli and bactericidal effect), since it contains palmitic acids. Similar observations were made by [30] has been observed the Palmitic acid (16:0) contributed more than 10% in *Chlamys tehuilcha*. De Moreno et al. reported that the predominant fatty acids were 16:0 and 18:0 in *Mytilus plantensis* [31]. Zhukova and Svetashev observed the sum of saturated fatty acids ranged from 16.8 to 22.5% in five species of bivalves [32]. The oyster, *C. madrasensis* exhibited 48.2% of total SFA content and showed a similar trend of fatty acid profile as reported in *C. gigas* [33].

The second type of fat is MUFA. These fats are often referred to as "good" fats because studies have shown that they help in reducing blood cholesterol levels and protect against heart disease. The suggested fats are all monounsaturated or polyunsaturated fats because of their benefits to health. In the present observations *M. casta* showed 18:1 acid high levels of 14.63% Station I. Oleic acid (18:1) contributed more than 10% in *Chlamys tehuilcha* [30]. Gastropods have been found to contain 18:1 major fatty acid [34,35]. Abundance of 16:1 and 18:1 triacylglycerols of *Tapes decussatus* and *T. philippinarum* has been reported by Beninger and Stephan [36]. Zhukova and Svetashev observed a sum of monounsaturated acids ranged from 19.7% to 25.4% in five species of bivalves [32].

Polyunsaturated fatty acids are energy source and also function in

the body as components of membranes, modulators of gene expression and precursors for eicosanoids (self-healing agent). Gastropods have been found to contain 20:4, 20:5 and 22:5 as major fatty acids [30]. Zhukova and Svetashev observed a sum of polyunsaturated acids from ranged from 55.5 to 63.6% in five species of bivalves [32]. The total PUFA may account for about 15:25 of the total fatty acids, where 20:5 and 22:6 acids together accounted for about 90% of the total PUFA [37]. In the present study, *M. casta* showed the dominance of alpha linolenic acid (α C18:3) of polyunsaturated fatty acids which constituted 12.72%.

The clam *M. casta* showed the dominance of vitamin A and vitamin C which constituted 51.35 Stations I and 26.38 mg/g Station II. Nair and Mathew reported that fish contained approximately as much riboflavin beef. i.e. 0.2-0.3 mg/100, vitamin C to the extent of 1-5 mg/100 g and whole vitamin D ranged from 500-3000 IU/100g [37]. These findings are supported by the present study with respect to vitamin B level. The absorption of riboflavin by the larvae and adult pacific oysters of *C. gigas* in various feed experiments observed by Langdon et al. [38]. Ozden and Erkan have been studied the vitamin B mainly in muscle of gonad and eggs, while B2 was detected in the digestive gland, gonad and eggs [39]. They varied simultaneously in the three organs during the annual cycle. Ajaya Bhaskar estimated the vitamin level from three species of bivalve namely *P. viridis*, *C. madrasensis* and *M. casta* is found vitamin B1, B2 and B6 as 0.11, 0.31 and 0.31 respectively [4]. However, the present study vitamins B6 were reported as 5.93 mg/g in Station I and 3.64 mg/g Station II on DWB respectively.

Minerals also constitute important components of hormones, enzymes and enzyme activators in human nutrition [40]. Mineral deficiencies can cause biochemical, structural and functional pathologies which depend on several factors, including the duration and degree of mineral deprivation. The main aim of the present study was to ascertain the levels of nutritionally significant minerals and their variations in *M. casta*. Totally 5 macro minerals and 2 trace minerals were detected. The macro mineral calcium and magnesium (345.5 mg/g m and 286.12 mg/g m) at highest in Station I and II and other minerals such as sodium (196.35 mg/g m St. II), potassium and copper were also detected in *M. casta*. In the present study clearly indicate that the calcium was more in *M. casta*. Similar observations were earlier reported by Coombs who reported more than 40% of soluble copper and zinc in the oyster *Ostrea edulis* [41]. Lowe and Moore studied the localized Zn and Fe in lysosomes of a number of cell types in *Mytilus edulis* [42]. Rajagopal et al. reported the importance of Ca, Mg, and K in the human nutrition [43]. Gopakumar reported that the sea foods in general are excellent sources of I, Ca, P, Na, Fe, Zn and oysters are good sources of Fe and Cu [44]. Minerals in different species of pearl shells from the South China Sea were rich, particularly in Ca, P and Zn contents [45].

In general, seafood is one of the most nutritionally balanced foods. The seafood diet helps to control weight and goes a long way towards preventing heart diseases. Studies on fatty acid composition of commercial seafood in India are limited. This might be due to lack of awareness on the benefits of these nutrients particularly from molluscan meat. The nutritional values of bivalve do not bring the limelight so far, so consumption of these nutrient rich molluscs has not attracted attention. The results of the present study provide

information about the amino acids and fatty acid composition and also suggest the consumption of this bivalve, *M. casta*. It is rich in amino acids (lysine), fatty acids (Stearic acid and alpha linolenic acid). Further, the presence of amino acids (Lysine) and fatty acids (Stearic acid and alpha linolenic acid) in *M. casta* adds more value through the possibility. Therefore the balanced and healthy diet is a prerequisite for good health. In the present study clearly indicate that the *M. casta* are an important part of a balanced diet and contribute to a good nutritional status.

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