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Research Article

Seasonal variation of nutritional profiles of green mussel (*Perna viridis* L.) collected from the coastal areas of Bangladesh

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ABSTRACT

Marine mollusks are a valuable source of healthy food because they are low in calories and fats but high in proteins. Green Mussel, *Perna viridis* are highly esteemed sea food and considered a delicacy throughout the world. However, seasonal variation of nutritional profiles of green mussels along the coastal regions of Bangladesh is not well reported to date. Therefore, the present study was undertaken to analyze the seasonal (monthly) variations of nutritional profiles of green mussel collected from the Maheshkhali Channel, Cox's Bazar, Bangladesh. The proportion of protein was high and found to be varied seasonally from 16.3 -19.9% on wet basis (54.5-86.1% on dry basis). Among the ten essential amino acids, eight were found in the muscle of *P. viridis*. The fat content of green mussel was found to vary from 2.0-2.9 % on wet basis (6.7-12.6% on dry basis), with a high amount in the winter season and low amount in autumn. The fat of the green mussel contains higher amount of long-chain omega-3 (n-3) PUFAs such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, 22:6 n-3). The DHA and EPA contents of the green mussels were also found to be varied seasonally. The result showed that marine bivalve *P.viridis* is a valuable food source for human consumption, due to its high quality protein and well-balanced lipid profile.

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1. INTRODUCTION

Perna viridis, the Asian green mussel is a large (> 80 mm) bivalve, with a smooth, elongated shell typical of several mytilids. This is an economically important mussel, a bivalve which mainly harvested for food. The Asian green mussel is found in the coastal waters of the Indo-Pacific region. The mussels live in waters that are 11-32°C with a wide-ranging salinity of about 18-33 ppt. *P. viridis* grows fastest at 2 meters below the surface, in high salinity and high concentration of phytoplankton although it can tolerate a range of salinity and turbid water. The demand for protein rich food is increasing mainly in the developing countries, stimulating the exploration of unexploited or non-traditional resources. All types of marine molluscs are commercially valuable species and easy to cultivate in coastal areas. (Saritha *et al.*, 2015). The consumption of bivalve molluscs in Bangladesh, particularly the southern region, has increased in recent years in response to the higher availability from wild and cultured conditions (Kajal *et al.*, 2016). Bivalves in

coastal areas like green mussels, are excellent sources of n-3 polyunsaturated fatty acids (PUFAs) including the long-chained eicosa-pentaenoic acid (20:5n-3; EPA) and docosa-hexaenoic acid (22:6n-3; DHA) and essential minerals, balanced amino acids, and vitamins (Chakraborty *et al.*, 2014; Astorga Espana *et al.*, 2007). Recently, after the importance of *P. viridis* as a potential health food had been realized, studies on its biochemical composition began to receive considerable attention. No reports have yet been published about the essential nutritional composition and anti-oxidative properties of the green mussel under the wild and cultured condition in Bangladesh. Therefore, the data will provide useful information for food industries and mussel aquaculture.

2. MATERIALS AND METHODS

Sample collection

Green mussels (*Perna viridis*) were collected from Maheshkhali channel, Chawfaldandi, Cox's Bazar. Sample collection was started from January to December for analyzing the seasonal variation of nutritional composition and it was done at the end of every month. Samples were collected by diving into the water of the channel by local people and after collection those shells were brought to the laboratory by a box with ice. After bringing shells, analysis of nutritional composition gets started. 21 shells were collected to analyze nutrients composition where protein, lipid, ash and moisture were determined for every month and essential fatty acid profile and essential amino acid profile were determined for a seasonal basis.

Figure 1. Collected *P. viridis*

Sample Preparation

Once green mussels were collected from Maheshkhali channel, these were brought to the laboratory. In the laboratory, shell length, shell weight and muscle weight were measured. After these initial task, muscle of mussel was blended and dried for analyzing moisture content in wet basis. After drying, samples were used for further analysis such as protein, lipid, and ash on dry weight basis. Samples were sent to the Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram for analyzing the essential fatty acid profile and essential amino acid profile.

Proximate composition analysis

Protein was estimated following the Micro-kjeldahl method as given in AOAC (1990). Fat was extracted from samples of dried meat in soxhlet extractant with petroleum ether and percentage determined (AOAC, 1990). Ash content was estimated by ignited in a silica crucible at 600˚C temperature in a muffle furnace until free from carbon and weighed accurately till the constant weight were obtained (AOAC, 1990). Moisture was estimated by drying the mussel meat for 24 hours in an oven maintained at 100-105˚C and then weighed till constant weight was obtained (AOAC, 2005). The protein, lipid and ash content of the samples were analyzed in triplicates on wet basis.

Amino acid composition of *P. viridis*

The true protein content of green mussel was estimated by the established method (Lowry *et al.*, 1951). The protein content of the samples was expressed as mg/100 g wet tissue. The estimation of amino acid was carried out using the Pico-Tag method as described earlier (Chakraborty *et al.*, 2013). The derivatized sample (phenylthiocarbamoyl 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 high- performance liquid chromatography (HPLC) and detected by their UV absorbance (λmax 254 nm). The quantification of amino acids was carried out by comparing the sample with the standard-, and the results were expressed as g/100 g muscle with mean ± SD.

Fatty acid (FA) profiling

The chloroform fraction was methylated by base -catalyzed transmethylation using 2M KOH in methanol and n-hexane. The hexane layer was separated and analyzed by GCMS. Gas chromatography was performed by a model FOCUS Gas Chromatograph, equipped with Polaris Q MS detector (Thermo Scientific, USA). The EI/GC–MS analyses were performed on a single-quadrupole mass spectrometer (Varian 1200L, Agilent Technologies, Santa Clara, CA, USA) under electron impact (EI, ionization energy 70 eV) conditions, with an on-column injector set at 110°C for confirmation of the fatty acid identification as described elsewhere (Chakraborty and Paulraj, 2007).

Statistical analyses

Statistical evaluation was carried out with the Statistical Program for Social Sciences Version 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were calculated for all the studied variables. Analyses were carried out in triplicate, and the means of all parameters were examined for significance ($p = 0.05$) by analysis of variance (ANOVA). The level of significance for all analyses was $p \leq 0.05$.

3. RESULTS AND DISCUSSION

Proximate composition

The seasonal variability of proximate compositions of green mussels is characterized by phases of accumulation and depletion of food reserves, reflecting the stage of gonadal development and availability of food. Moisture content in the present studies varied from 70.1 to 76.9% showed in figure 2. The minimum and maximum moisture contents were observed in winter and autumn, respectively. The possible reason for this variation is the presence of salts in the mussel tissues getting replaced by the moisture content. The salinity of the sea water is the decisive factor in controlling moisture content in mussel (Galtsoff, 1964). Parulekar *et al.* (1982) also reported the relationship between the salinity and water content in raft grown green mussel and stated that these mussels develop an iso-somatic internal medium to compensate for the considerable lowering of salinity during monsoon season. It is also reported that the rise in the moisture content synchronizes with the spawning activity of the mussel. The mussel remains in spent condition during monsoon month and this month moisture content was higher.

The crude protein content varied from 16.3–19.9% on wet basis (Figure 2). The minimum protein content was observed in the rainy and autumn season, and then start to increase with maximum in the season of winter. This variation in crude protein level coincides with the maturation of gonads. Nagabhushanam and Mane (1978) and Wafer et al. (1976) observed that increased protein content during pre-monsoon could be a mechanism of reserved storage to meet the spawning requirements.

In the present study, the fat values were varied from 2.0 to 2.9% on wet basis (Figure 3). The highest and lowest content found during the autumn and winter, respectively. Venkataramanand and Chabi (1951) stated that high lipid levels have a close relationship with intensive feeding and storage of fat. Low lipid values can also be found due to the initiation of gametogenesis and utilization of energy reserve for the development of gamets. During the investigation of Quasim *et al.* (1977), lipid content was reported to be comparatively high during the pre-pawning period. Low lipid content during the post-spawning could be attributed to the depletion of energy resources for spawning activities.

The ash content during the study period varied from 2.01 to 2.85% in wet basis (Figure 3). Ash values were high in the season of spring, following lower were in late autumn. It was found that low values of ash content coincided with the higher value of lipid and protein. The overall proximate compositions of green mussel are shown in Table 1.

Table 1. Proximate Composition of *P. viridis*

Proximate Composition	Mean%±SD
Moisture	73.5±1
Protein	18.0±1
Lipid	2.5±2
Ash	2.4±1

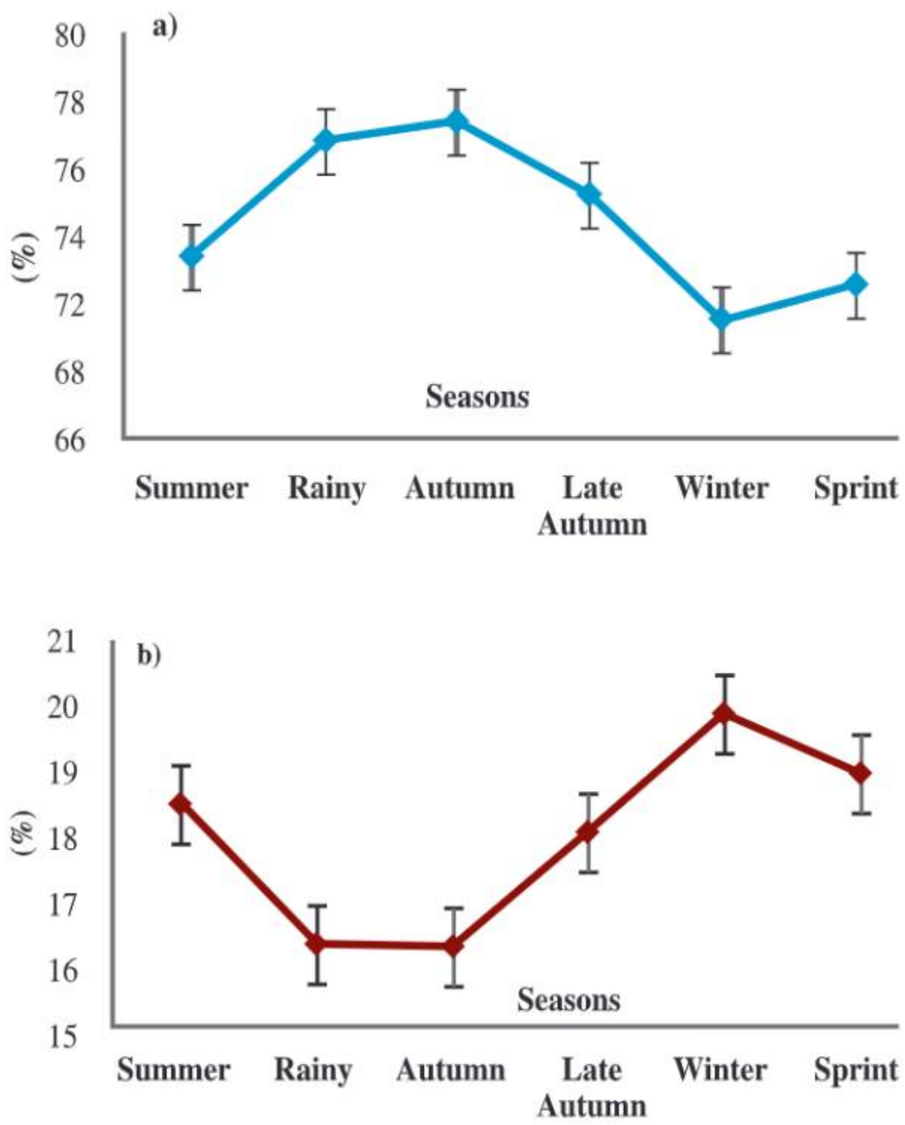

Figure 2. (a) Seasonal Variation of Moisture (%) and **(b)** Protein (%) in Wet Basis

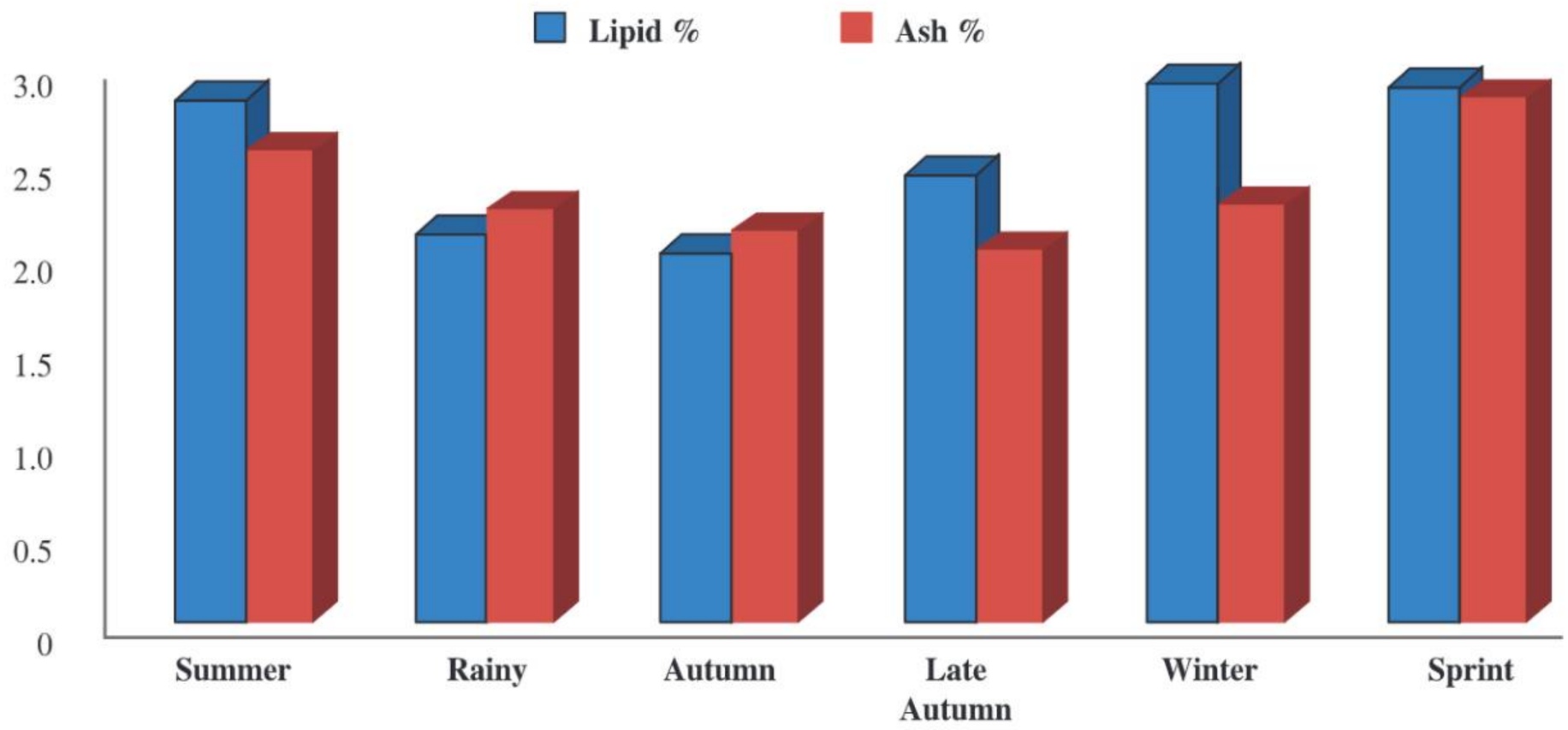

Figure 3. Seasonal Variation of Lipid and Ash (%) in Wet Basis

Amino Acid composition

The amounts of investigated essential and non-essential amino acids are presented in Table 2. Among the ten essential amino acids, eight were found in the muscle of *P. viridis*. Lysine was found to be peak as 3.96 (g/100g muscle) during winter whereas methionine was found to be low as 0.87(g/100g muscle) during the summer. The remaining amino acids like phenyl-alanine, histidine, arginine, leucine, threonine, isoleucine, valine and tryptophan were at intermediate level.

Among the non-essential amino acids glutamic acid level was high as 5.95(g/100g muscle) during the summer season and alanine level was low as 2.48(g/100g muscle) during the rainy season and rest of the non essential amino acids such as glycine, serine, glutamine, aspartic acid, tyrosine and glutamic acid were at transitional level.

Table 2. Amino Acid Content of *P. viridis*

Parameters	Summer	Rainy	Autumn	Late Autumn	Winter	Spring
Essential Amino Acids (g/100g muscle)						
Arginine	2.98	2.55	2.96	2.64	2.78	2.68
Threonine	2.65	2.24	2.34	1.98	2.78	2.56
Valine	2.35	2.12	1.98	2.34	2.78	2.84
Methionine	0.87	1.12	1.24	1.88	2.34	2.14
Isoleucine	1.88	1.76	1.86	2.12	2.46	2.24
Lysine	3.66	3.88	3.46	3.52	3.96	3.44
Leucine	3.04	2.86	2.78	3.24	3.28	2.88
Histidine	1.69	1.34	1.68	1.88	1.94	1.66
Non-essential Amino Acids (g/100g muscle)						
Glycine	3.02	2.68	2.54	2.88	2.54	2.96
Tyrosine	2.17	2.25	2.43	2.02	1.86	2.04
Alanine	2.61	2.48	2.66	2.74	2.88	3.12
Aspartic acid	3.91	3.24	3.68	3.98	2.88	3.25
Serine	3.09	2.56	2.78	3.24	3.12	2.85
Glutamic acid	5.96	4.32	4.94	4.78	4.12	4.26

Fatty acid composition

There are wide variations in the FA profiles of the green mussels population in terms of total saturated (SFA), monounsaturated (MUFA) and polyunsaturated FAs (PUFA). In the present investigation, the major long-chain omega – 3 (n-3) PUFAs are eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, 22:6 n-3). Eicosapentaenoic acid (EPA, C20:5n-3) was found maximum during the summer and rainy seasonwhile remained minimum during winter. Besides, docosahexaenoic acid (DHA, 22:6 n-3) showed the highest and lowest amount as same as eicosapentaenoic acid (EPA, C20:5n-3). Monounsaturated fatty acids were in higher amount during autumn and late autumn whereas found in lower amount during winter. Saturated fatty acids were found maximum during winter, following minimum level during summer and rainy season. Figure 4 showed the fatty acids found in the muscle of *P. viridis* in the standard from the present study and Table 3 showed all types of FAs found in the muscle of green mussel. The measurement units of fatty acids were mg/100 g muscle. The variation of fatty acid contents of green mussels mostly related to the foods and breeding season.

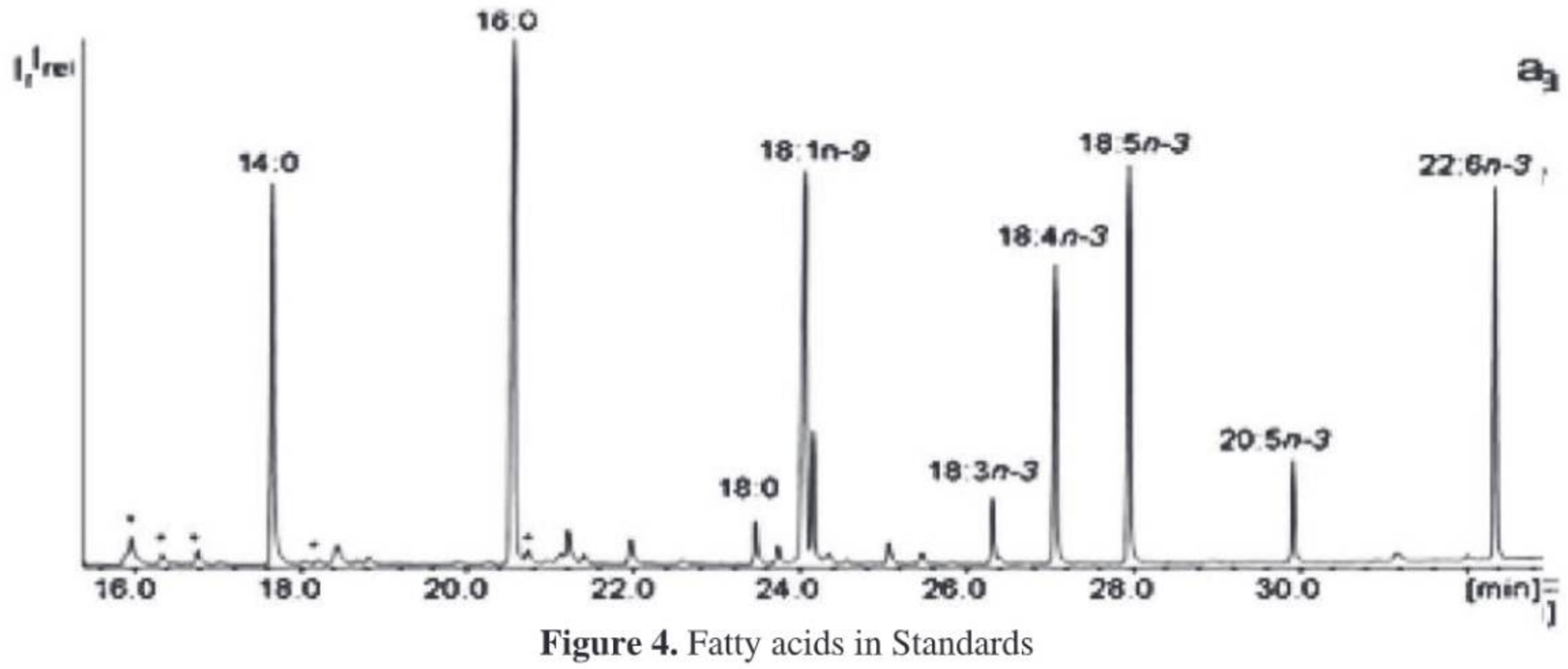

Figure 4. Fatty acids in Standards

Table 3. Fatty acid composition of *P. viridis* (mg/100 g muscle)

Parameters	Summer	Rainy	Autumn	Late Autumn	Winter	Spring
Saturated fatty acids	39.25±3.42	38.24±3.24	43.24±3.44	45.35±2.86	46.21±3.21	44.45±2.54
Myristic acids (C14:0)	5.88±1.12	6.12±0.78	8.21±1.24	8.88±1.15	--	6.23±0.87
Palmitic acids (C16:0)	30.63±3.12	28.12±2.65	29.94±3.14	30.35±2.38	39.17±2.84	31.64±2.52
Stearic acid (C18:0)	2.74±0.24	4.01±0.46	5.09±0.86	6.12±0.54	7.04±.0.89	6.58±0.46
Unsaturated fatty acids	60.75±4.22	61.76±4.22	56.76±3.84	54.65±3.64	53.79±4.21	55.55±4.86
Monounsaturated fatty acids	16.68±0.96	15.24±0.84	20.26±1.34	20.48±1.26	14.75±1.12	18.24±0.86
Myristoleic acid (C14:1)	0.88±0.12	1.24±0.14	1.36±0.22	0.96±0.12	0.72±0.06	1.14±0.16
Palmitoleic acid (C16:1)	10.26±1.46	8.9±0.84	12.98±1.34	14.62±1.06	14.02±1.12	12.24±1.24
Oleic acid (C18:1)	5.54±0.24	5.02±0.42	5.92±0.24	4.89±0.22	-	4.76±0.46
Polyunsaturated fatty acids	44.07±4.12	46.52±4.20	36.5±3.12	34.17±2.88	39.03±3.48	37.31±3.24
Linoleic acid (C18:2)	1.88±0.08	2.54±0.12	2.12±0.42	1.22±0.12	2.20±0.14	2.24±0.22
Linolenic acid (C18:3)	3.24±0.48	2.34±0.12	4.44±0.48	1.04±0.08	4.00±0.48	2.58±0.44
Arachidonic acid (C20:4)	3.12±0.38	2.36±0.24	1.96±0.24	2.84±0.84	13.32±1.24	2.12±0.26
Eicosapentaenoic acid (C20:5)	16.24±3.82	19.04±4.08	12.54±2.44	15.80±2.14	10.18±2.12	14.68±2.24
Docosahexaenoic acid (C22:6)	19.59±4.06	20.24±3.94	15.44±3.14	13.27±1.68	9.33±1.14	15.69±3.12

4. CONCLUSIONS

P. viridis 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. Their high utilizable 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 *P.viridismay* prove important for formulations of nutraceutics and future policy regarding the exploitation of this species.

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