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Nutritional and Antioxidative Attributes of Green Mussel (*Perna viridis* L.) from the Southwestern Coast of India

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ABSTRACT
The present study reports the biochemical composition of *Perna viridis* from the southwestern coast of India. A balanced essential to nonessential amino acid ratio (0.7:1.1) along with the optimum and balanced quantities of vitamins, mineral nutrients, and low cholesterol contents characterized *P. viridis*. The *n*-3/*n*-6 polyunsaturated fatty acid ratio of *P. viridis* was found to be 3.7:5.3 and therefore may serve as an alternative to balance the higher intake of *n*-6 fatty acids. High oxyradical scavenging capacity (24–32%) and total phenolics (2–5 mg GAE^{-1}) suggest the nutraceutical potential of *P. viridis*.

KEYWORDS
Green mussel; *Perna viridis*; fatty acids; amino acids; vitamins; minerals; antioxidant activity

Introduction

The green mussel, *P. viridis*, is a bivalve mollusc (family *Mytilidae*) found in the coastal sea beds of the Arabian Gulf in south Malabar waters along the west coast of Peninsular India. In India, two species of mussels (green mussel *P. viridis* and brown mussel *P. indica*) support a traditional sustenance fishery in the Malabar and Konkan areas of Peninsular India. India has risen to one among the top 10 cultured mussel producing nations in Asia, with an annual production of 10,060 tons (Kripa and Mohamed, 2008). Mytilidae is a family of small to large saltwater mussels, marine bivalve mollusks in the order Mytiloida. It is the only family in the order.The Malabar Coast of Kerala is popularly called the "Mussel fishery zone of India" and accounts for the bulk of the mussel production in the country. The consumption of bivalve molluscs in India, particularly the south Malabar area, has increased in the recent years in response to the higher availability from wild and cultured conditions.

Bivalves in coastal areas, especially green mussels, are excellent sources of *n*-3 polyunsaturated fatty acids (PUFAs)—including the long-chained eicosapentaenoic acid (20:5*n*-3; EPA) and docosahexaenoic acid (22:6*n*-3; DHA) and essential minerals, balanced amino acids, and vitamins (Chakraborty et al., 2014; Astorga Espana et al., 2007). The antioxidant defense systems are of particular interest in the case of marine invertebrates particularly bivalve molluscs. Among the bivalves, mussels are considered to be a valuable food item as they constitute a rich source of antioxidants, essential for providing a balanced diet. They are also a very good source of biomedically important products. Aerobic organisms including *P. viridis* maintain constitutive antioxidant defenses, detoxifying, and scavenging the reactive oxygen species (ROS)—including superoxide radical (O_2^-), H_2O_2, peroxyl radical (RO_2), and hydroxyl radical (OH) that are continuously produced as a by-product of aerobic metabolism (Santovito et al., 2005). These compounds frequently produce structural and functional changes in lipids, proteins, and other biomolecules and are derived from the partial reduction of O_2. Bivalves were reported to repair of oxidative damage by ROS by increasing the expression of antioxidant molecules (Santovito et al., 2005).

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Among the different parameters of quality, biometric measurements and chiefly meat yield are important aspects of mussel marketability. Biochemical changes in the mussel from different sites and growth conditions may result from fluctuations of environmental parameters such as temperature, salinity, and oxygen levels and to the physiological status of the animals, depending on food availability, gametogenic cycle, and spawning (Livingstone, 2001). Earlier studies indicated the influence of environmental and nutritional conditions on the composition of bivalves (Murphy et al., 2002).

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. It is anticipated that the determination of the essential nutritional composition of wild *P. viridis* vis-à-vis cultured mussel will provide the necessary information concerning the nutrient value of this seafood for both the consumers and the researchers working on nutrient tables. No reports have yet been published about the essential nutritional composition and antioxidative properties of the green mussel under wild and cultured condition. The data will provide useful information for food industries and mussel aquaculture. Therefore, considering the promising perspective for the utilization of *P. viridis* and the need for knowledge of its biochemical composition, this work explores the influence of the growth conditions on the essential nutritional compositions of *P. viridis* harvested from different sites on the southwestern coast of India. The present study also directed to elucidate the antioxidant activities of wild and cultured *P. viridis* collected from two different study areas to understand the effect of growth conditions on antioxidant profile. The integration with the nutritional composition and antioxidant capacity was attempted for a more holistic assessment of the overall biological significance of wild and cultured mussels collected from different sites.

Materials and methods

Samples

P. viridis (green mussel) samples (wild and cultured) were collected from two different sites (Cochin and Kozhikode) along the southwestern coast of India. The first one is a relatively clean site on the intertidal rocky shore of the Sathar Island at Cochin (Lat: 9° 58′ N; Long: 76° 16′ E) on the southwestern coast of India, which is predominantly influenced by oceanic water from the Arabian Sea. During the same period, *P. viridis* samples were collected from Elathur (Lat: 11° 54′ 11.6″ N; 75° 12′ 21.8″ E), located about 10 km north of Kozhikode City, which is bounded by the Arabian Sea on the west and the Korapuzha River (Elathur River) on the north on the southwestern coast of India. Figure 1 illustrates the sampling locations of *P. viridis*. *P. viridis* from both sites were collected at comparable depths (2 m below the surface) to avoid confounding effects of depth on the fatty acid composition. Cultured mussels were stripped from the 1/4” rope at each site and transported for analyses following the protocol. Gender differences of the representative samples (n = 3 at each determination) were not treated. All live samples thus collected were immediately placed on ice in isothermic boxes for transportation. Upon return, these samples were cleaned of fouling organisms and placed in filtered (1.0 mm) seawater at ambient temperature. The tissues of mussels from the two sites were combined, minced, and the samples were stored in low temperature (–20° C) until further use for biochemical characterization.

Biometric parameters, condition indices, and meat yield of P. viridis

The *P. viridis* collected were measured for their biometrical parameters—viz., length, width, and thickness. Length (maximum measure along the anterior-posterior axis), width (maximum lateral axis), and thickness (depth of the maximum longitudinal axis) of randomly selected mussels were measured using a vernier caliper. The mussels were then weighed, opened by cutting the adductor muscle with a scalpel, and the wet meat and shell weight were noted. The tissues were oven-dried for 48 h at 60°C, and the dry weight determined. Condition indices are used to characterize the apparent health and quality of a biological

Figure 1. Sample collection site of green mussel (*P. viridis*; wild and cultured) from two different sites (Cochin and Kozhikode) along the southwestern coast of India. Sathar island at Cochin (Lat: 9° 58′ N; Long: 76° 16′ E) and Elathur (Lat: 11° 54′ 11.6″ N; 75° 12′ 21.8″ E) were selected as respective collection sites of *P. viridis*.

entity. Three condition indices (CI) were taken into account in this study as follows: (a) Economic CI (Imai and Sakai, 1961) as thickness × $[0.5\ (\text{length} + \text{width})]^{-1}$; (b) Booth's CI (Booth, 1983) flesh weight × total weight^{-1}; (c) Ecophysiological CI (Walne, 1976) as dry flesh weight/dry shell weight. The dry weight of the shells was determined by oven-drying at 80°C for 12 h. In brief, the whole weight of mussels was obtained on an electronic balance, before the meat was sucked. The dimensions of shells were measured using a vernier caliper. The meat from each mussel was weighed to obtain wet weight and dry weight, the latter after desiccation in a freeze-dryer for 24 h at −100°C.

Proximate composition

Moisture, crude lipid, crude fat, crude fiber, and ash content were determined by methods of the AOAC (1990).

True protein and amino acid composition of P. viridis

The true protein contents of *P. viridis* were estimated by the established method (Lowry et al., 1951). The protein content of the samples was calculated from the standard curve of BSA and 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 (λ_{max} 254 nm). The mobile phase eluents used were Eluents A and B, whereas Eluent A comprises sodium acetate trihydrate (0.14 M, 940 ml, pH 6.4) containing triethylamine (0.05%), mixed with acetonitrile (60 mL), and Eluent B was acetonitrile:water (60:40, v/v). A gradient elution program, with increasing Eluent B, was employed for this purpose. An additional step of 100% Eluent B is used to wash the column prior to returning to initial conditions. The detector was set at 0.1 absorbance unit full scale (AUFS) at 254 nm, and the column temperature was set at 38°C (± 1°C) in the column heater. Samples

(phenylthiocarbamoyl amino acid derivatives) were injected in triplicate, and the output was analyzed using BREEZE software (Trinity Consultants, Inc., Dallas, TX, USA). The quantification of amino acids was carried out by comparing the sample with the standard, and the results were expressed as mg/100 g wet tissue with mean ± *SD*.

Total cholesterol content

The total cholesterol content in the tissue was determined by established method (Rude and Morris, 1973) using *o*-phthalaldehyde (50 mg/dL in glacial HOAc). The cholesterol content of the sample was calculated from the standard curve of cholesterol and expressed as mg/100 g wet tissue.

Estimation of minerals

Estimation of minerals was carried out by atomic absorption spectrophotometer following the di-acid (HNO_3/$HClO_4$) digestion method (Astorga Espana et al., 2007). The analyses of Ca, Mg, Na, K, Mn, Cu, Fe, and Zn were performed by flame atomic absorption spectrophotometry equipped with a hollow cathode lamp containing D_2 lamp background correction system. For Se, continuous flow hydride generator coupled with an atomic absorption spectrometer was used. The macro (Na, K, Ca, and Mg) and micro (Cr, Zn, Mn, Cu, Fe, and Se) minerals were expressed as mg/100 g wet tissue.

Estimation of vitamins

Estimation of fat soluble vitamins was carried out by the established method (Chakraborty et al., 2013). Vitamin C was determined based upon the quantitative discoloration of 2, 6-dichlorophenol indophenol titrimetric method as described (AOAC, 2005). The vitamins A, D_3, E, and C were expressed as IU/100 g sample and K_1 as μg/100 g wet tissue.

Fatty acid profiling

Lipid content and fatty acid composition were estimated following methods as reported elsewhere (Chakraborty and Paulraj, 2007). Fatty acid methyl esters (FAMEs) were identified by comparison of retention times with known standards (37 component FAME Mix, Supelco, Bellefonte, PA, USA). Results were expressed as percent of total fatty acids (% TFA). 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).

Total phenolic content

The amount of total phenolics, 2,2-diphenyl-1-picryl hydrazyl (DPPH)$^{\cdot}$ radical scavenging activity and degree of lipid peroxidation were determined as described earlier (Tai et al., 2011). The total phenolic content (TPC) was expressed as gallic acid equivalent (GAE) in mg/g wet tissue.

Antioxidant activities

The DPPH radical scavenging activity was expressed as total antioxidant radical scavenging activity (% TARSA) = {(absorbance of control − absorbance of sample) × 100}/absorbance of control. The degree of lipid peroxidation was measured colorimetrically in terms of thiobarbituric acid reactive substances, and the results were expressed in mM MDA equivalents/kg (mM MDA Eq/kg) of the

sample. The early stages of lipid peroxidation were determined by conjugated diene (CD) formation of unsaturated fatty acids, which were reported to absorb at 234 nm (Kim and Labella, 1987) and is proportional to the uptake of O_2 and formation of peroxides. The results of CD value were expressed by the following formula: CD (%) = (B × V)/w, where B = absorbance at 233 nm, V = volume of sample, and W = weight of sample in mg.

Statistical analyses

Statistical evaluation was carried out with the Statistical Program for Social Sciences Version 13.0 (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 Pearson correlation test was used to assess correlations between means. The level of significance for all analyses was $p \leq 0.05$.

Results and discussion

General

The present study provided a detailed biochemical profile of the green mussel *Perna viridis* collected from the wild and cultured conditions, for the first time, from two prominent mussel beds from different geographical regions of the southwestern coast of India (Cochin and Kozhikode). Considering the production trend of edible bivalves, where the contribution of cultured *P. viridis* is showing an increasing trend, information on the food qualities of cultured versus wild is of importance. The present study provided this valuable information. Differential growth conditions (wild and cultured) of *P. viridis* were therefore studied to understand their effect on nutritional indicators. This is essentially to develop the *P. viridis* nutritional biomarkers with respect to the collection area and growth condition for use in human food and nutrition. The data provide useful information for food industries and green mussel fishery.

Biometric measurements, percent meat yield, and condition index

The commercial quality and physiological state of bivalve molluscs are adequately described by the condition index (CI), a parameter of economic relevance reflecting the ecophysiological conditions and the health of *P. viridis*. The wild samples collected from Kozhikode showed significantly higher ($p < 0.05$) Imai and Sakai's CI than cultured samples (Table 1). The Booth's CI registered maximum value for cultured samples, collected from Kozhikode. A low value of CI for both wild and cultured samples collected from Cochin indicates that a major biological effort has been expended, either as maintenance energy under poor environmental conditions or disease, or in the production and release of gametes during spawning. The values obtained in this study indicated that cultured samples from Kozhikode were in better condition than Cochin samples. In the Goa region of the southwestern coast of India, *P. viridis* were reported to grow faster on ropes compared to those in natural beds (Qasim et al., 1977). Wild samples collected from Cochin exhibited significantly higher ($p < 0.05$) meat yield than recorded in cultured samples. The variations of percent meat and CI of *P. viridis* were statistically significant ($p < 0.05$). CI and meat content of green mussels were registered to be affected by a variety of environmental and endogenous factors—viz., water temperature, salinity, food availability, and gametogenic cycle of animals (Okumus and Stirling, 1998). However, the parameters of meat yield and condition index from wild and cultured *P. viridis* are within the range as reported earlier (Okumus and Stirling, 1998) and, therefore, appeared to be in good condition for consumption. Principle Component Analyses (PCA) were carried out to gain an overview of the similarities and differences among the various parameters of wild and cultured *P. viridis* collected from different geographical locations (Cochin and Kozhikode). The major nutritional and biochemical parameters of *P. viridis* vis-à-vis

Table 1. Biometric measurement (length, width, thickness); meat yield (%) condition indices; proximate and mineral composition; lipid, protein, and total cholesterol contents of *Perna viridis* collected from Cochin and Kozhikode.

	Cochin		Kozhikode	
	Wild	Cultured	Wild	Cultured
Length (cm)	6.00 ± 0.01^a	8.10 ± 0.03^b	9.40 ± 0.07^b	6.33 ± 0.01^a
Width (cm)	2.59 ± 0.03^a	3.14 ± 0.03^b	4.10 ± 0.01^c	2.40 ± 0.14^a
Thickness (cm)	2.88 ± 0.09^a	3.96 ± 0.04^b	5.10 ± 0.01^c	1.90 ± 0.04^d
Meat yield (%)	28.4 ± 0.03^a	24.5 ± 0.04^b	27.9 ± 0.03^a	34.0 ± 0.01^c
Condition indices				
Imai and Sakai	0.63 ± 0.02^a	0.65 ± 0.05^a	0.86 ± 0.01^b	0.60 ± 0.02^a
Booth	0.28 ± 0.01^a	0.24 ± 0.04^b	0.28 ± 0.04^a	0.34 ± 0.02^c
Walne	0.06 ± 0.02^a	0.07 ± 0.01^a	0.06 ± 0.01^a	0.08 ± 0.01^a
Proximate composition (g/100 g wet tissue)				
Dry matter	10.68 ± 0.01^a	17.86 ± 0.01^b	20.6 ± 0.02^c	16.8 ± 0.01^b
Moisture	89.32 ± 0.52^a	82.14 ± 0.61^b	79.3 ± 0.60^b	83.1 ± 0.84^b
Crude protein	7.14 ± 0.07^a	12.02 ± 0.05^b	13.1 ± 0.18^b	11.9 ± 0.04^b
Crude fat	1.27 ± 0.04^a	1.90 ± 0.02^b	1.96 ± 0.02^b	1.72 ± 0.03^b
Crude ash	1.42 ± 0.03^a	1.71 ± 0.002^b	1.65 ± 0.02^b	0.99 ± 0.008^d
Crude fiber	0.012 ± 0.002^a	0.014 ± 0.001^b	0.08 ± 0.01^c	0.07 ± 0.007^d
Carbohydrates (soluble)	0.84 ± 0.12^a	2.22 ± 0.072^b	3.78 ± 0.31^c	2.08 ± 0.05^b
Acid insoluble ash	0.61 ± 0.01^a	0.09 ± 0.002^b	0.13 ± 0.01^c	0.07 ± 0.01^b
Lipid (%)	1.47 ± 0.10^a	$2.63 \pm 0\ .19^b$	2.00 ± 0.13^c	1.70 ± 0.16^d
Total cholesterol (mg/100 g)	96.6 ± 0.78^a	65.1 ± 0.32^b	58.61 ± 0.42^c	36.28 ± 0.42^d
True protein (mg/100 g)	183.06 ± 3.9^a	236.6 ± 5.38^b	103.9 ± 0.67^c	220.3 ± 1.83^d
Macrominerals (mg/100 g wet tissue)				
Na	1967.25 ± 14.32^a	1942.00 ± 26.05^a	2136.5 ± 1.59^b	1195 ± 2.12^c
K	1450.50 ± 84.73^a	1563.25 ± 87.24^b	1532.2 ± 3.80^b	697.5 ± 1.96^c
Ca	287.00 ± 13.41^a	427.65 ± 36.28^b	280.92 ± 0.53^a	1095 ± 9.2^d
Mg	64.33 ± 2.05^a	353.58 ± 8.49^b	69.2 ± 0.72^a	515 ± 1.45^c
Microminerals (mg/100 g wet tissue)				
Cr	ND	0.17 ± 0.02^a	0.365 ± 0.07^b	ND
Zn	1.55 ± 0.11^a	2.57 ± 0.19^b	3.03 ± 0.01^c	3.19 ± 0.01^c
Mn	ND	0.84 ± 0.05^b	0.27 ± 0.09^b	ND
Cu	0.05 ± 0.01^a	0.12 ± 0.01^b	0.26 ± 0.06^c	0.22 ± 0.01^c
Fe	6.10 ± 0.08^a	4.48 ± 0.25^b	10.19 ± 0.81^c	4.61 ± 0.07^b
Se	0.04 ± 0.00^a	0.03 ± 0.001^b	0.04 ± 0.01^a	ND
Vitamins				
Retinol A (IU/100 g)	11.89 ± 1.16^a	12.71 ± 0.85^a	8.20 ± 0.15^b	5.31 ± 0.08^c
Cholecalciferol D_3 (IU/100 g)	410.0 ± 17.1^a	442.0 ± 6.3^b	352.5 ± 4.3^c	412.8 ± 2.5^a
α-tocopherol E (IU/100 g)	0.15 ± 0.01^a	0.14 ± 0.02^a	0.13 ± 0.01^a	0.12 ± 0.01^a
Phylloquinone K_1 (μg/100 g)	2.72 ± 0.18^a	2.65 ± 0.21^a	1.26 ± 0.02^b	2.2 ± 0.14^c
Ascorbic acid C (IU/100 g)	12.14 ± 0.95^a	9.28 ± 0.56^b	12.74 ± 0.15^a	9.89 ± 0.08^b

All samples were analyzed in triplicate ($n = 3$) from a pooled sample (20 numbers) and expressed as mean ± standard deviation. Means followed by the different superscripts within the same row indicate significant difference ($p < 0.05$). ND = nondetectable.

different sampling locations/growth conditions were included as variables in a PCA model and illustrated in Figure 2. The loading plot obtained by performing PCA on conditional indices—viz., Imai and Sakai (IS), Walne, Booth—and meat yield, which were included as variables, are shown in Figure 2A. The formation of Group A which comprises conditional indices of Walne, meat yield (wild from Cochin and cultured from Kozhikode), and Booth (cultured from Cochin and wild from Kozhikode) showed a high positive correlation with PC1 (64.56%). PC2 describes the remaining variability (35.44%).

Biochemical composition of P. viridis in two different sites and growth conditions

Proximate composition

The proximate compositions of mussels are characterized by phases of accumulation and depletion of food reserves, reflecting the stage of gonadal development and availability of food. There was no marked variation in the contents with respect to fat, crude fiber, and ash from different regions of the experimental area, as well as growth conditions (cultured and wild). The crude protein content

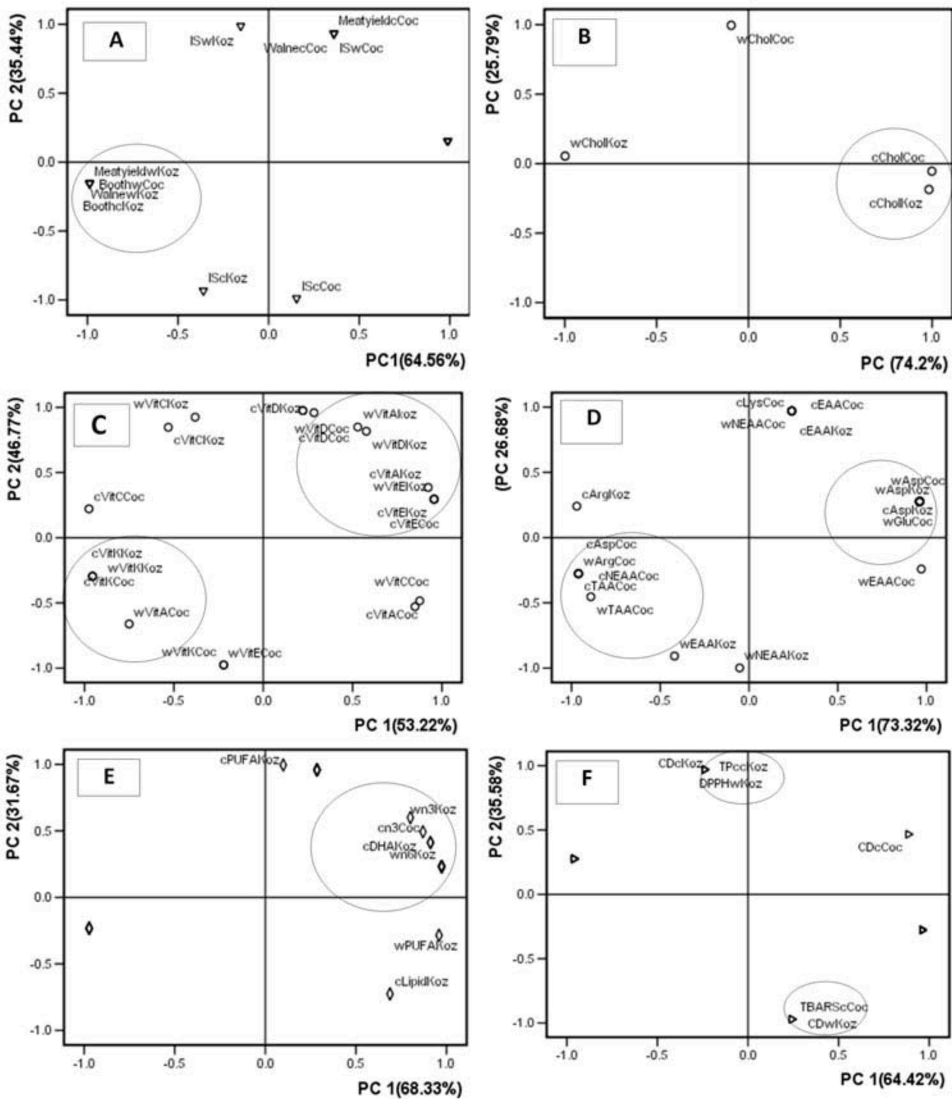

Figure 2. Correlation between various nutritional and antioxidative parameters of wild and cultured *P. viridis* from two geographical locations by loading plot diagrams (A–F) of various components (PC-1 and PC-2) in rotated space. Loading plot diagram (in PCA plot) showing the correlation of various condition indices (Imai and Sakai IS, Walne, and Booth) vis-à-vis meat yields. Here, cCoc and cKoz signify cultured *P. viridis* from Cochin and Kozhikode, respectively, whereas wCoc and wKoz imply wild *P. viridis* from Cochin and Kozhikode, respectively. The parameters with significant correlation ($p < 0.01$) are indicated within a circle (A). Loading plot diagrams showing the correlation of fatty acids (ΣPUFA, Σ*n*-3, Σ*n*-6, EPA, DHA) and Σcarotenoids of *P. viridis* (B). Loading plot diagrams showing the correlation between antioxidant indices (DPPH, CD, TBARS) of *P. viridis* collected from both Cochin and Kozhikode vis-à-vis total phenolic content, TPC (C). The loading plot of vitamins A, D2, E, K1, C (D). The loading plot of amino acids (Asp, Glu, Lys, Arg) vis-à-vis ΣEAA, ΣNEAA, ΣTAA and E/NE ratio (E). The loading plot diagram of cholesterol of wild and cultured *P. viridis* from Cochin and Kozhikode, respectively (F). Other notations are as indicated under Figure 2A.

varied from 7.14–13.1%; the minimum of 7.14% was recorded in Cochin (wild) and maximum of 13.1% in samples collected from Kozhikode (wild; Table 1). Proximate composition analyses in *Mytilus galloprovincialis* from different Spanish origins revealed moisture content as 79–83 g/100 g, lipid 1.4–2.1 g/100 g, and protein 6.5–10 g/100 g (Fuentes et al., 2009). An inverse relationship between moisture and protein content was apparent between Kozhikode and Cochin samples. Crude fat and ash recorded significant difference ($p < 0.05$) between wild and cultured samples, and their

fluctuations were also coincident with those of the crude protein. There appeared to be no significant differences in acid insoluble ash content of cultured samples collected from Cochin and Kozhikode ($p > 0.05$). It is of note that a positive correlation between CI and biochemical constituents of bivalves has been reported in different mollusc species (Orban et al., 2004).

Protein, lipid, and total cholesterol content

Bivalve molluscs were reported to provide an inexpensive source of lipid and protein with high biological value (Astorga Espana et al., 2007). The cultured samples collected from both the sites were found to possess significantly higher protein content ($p < 0.05$) than their wild counterparts (Table 1). The variations in protein contents appeared to be due to the differential availability of food (microalgae), which were reported to be major food sources of filter feeders—viz., bivalve mollusc *P. viridis* (Fernández-Reiriz et al., 1989). *P. viridis* from both locations and growth conditions were found to be rich in proteins with a low calorie count; they may therefore form an essential part of a healthy diet.

The cultured *P. viridis* harvested from Cochin registered higher lipid content (> 2.5%) than wild samples (< 1.5%; Table 1). It is generally accepted that water temperature and differences in salinities are principal environmental factors affecting growth and gonadal development of marine bivalves (Pazos et al., 1996), which is a major reason for the differences in lipid content. The marginal differences in the lipid content of tissues of *P. viridis* apparently depend on the variation in the availability of food, temperature, and microclimatic conditions as stated earlier. The higher relative percentage of diatoms (*Thalassiosira subtilis, Nitzschia seriata, Baciallria paradoxa*, and *Biddulphia mobilensis*) and dinoflagellates in their diet (*Ceratium furca, C. tripos, Gonyaulax spinifera*, and *Peridinium biconicum*) with high lipid content appeared to contribute to the lipid pool of *P. viridis*. The total lipid content of *P. viridis* in this study is in good agreement with earlier studies with *Mytilus edulis* and *Perna canaliculus* (Murphy et al., 2002), in which the total lipid content varied from 1.06–1.97% (wet weight).

Wild samples recorded significantly higher cholesterol content than cultured samples ($p < 0.05$) both in Kozhikode as well as Cochin, respectively. Though significant differences in cholesterol contents were observed between wild and cultured samples collected from Cochin and Kozhikode, the values are well within the limits and considered to be safe for consumption. The predominant diet of mussels is plankton, which contains various sterols that can be incorporated into mussel tissues; some sterols—viz., cholesterol, can be synthesized from plankton cholesterol precursors (Li et al., 2007). Earlier studies reported cholesterol as the predominant sterol present in mussels (Li et al., 2007). Cholesterol was the major sterol in *Mytilus edulis* (30%) and *Perna canaliculus* (29%; Murphy et al., 2002). The loading plot of cholesterol was analyzed by PCA (Figure 2B). Cholesterol of cultured origin from both sampling locations (Cochin and Kozhikode) is the dominant feature in PC1 (74.2%).

Mineral composition

Minerals are nutrients that are conserved by the body and play a significant role in metabolism in the human body. The present study revealed that Ca and Mg were higher than those reported in the literature for bivalve molluscs (Astorga Espana et al., 2007). The cultured green mussels registered significantly higher content of the macronutrients (Ca, Mg) than wild samples from both experimental locations ($p < 0.05$). Cultured samples collected from Kozhikode recorded significantly higher Mg content than cultured Cochin samples ($p < 0.05$). Ca and Mg are extremely important minerals that are often out of balance in persons with arteriosclerosis and thyroid diseases. Imbalances of these minerals can result in irregular heart rate. A normal person needs a Ca/Mg ratio of about 2:1. Alterations in the metabolism of Ca and Mg have been implicated in the pathogenesis of primary hypertension (Kisters et al., 2004). The cultured samples from Kozhikode were found to be superior in terms of the Ca/Mg ratio (2.13) and therefore may be referred to be an ideal diet for good health. The cultured samples from Kozhikode registered significantly lower ($p < 0.05$) Na than other samples and, therefore, rated high as health food. Reports linking excessive

Na intake to the genesis of hypertension and other related diseases like changes in vascular reactivity and changes in the renin–angiotensin system are well-known (Santos et al., 2006). The micronutrient element Zn was found to be significantly higher in samples collected from Kozhikode than Cochin. The cultured *P. viridis* was found to possess higher content of Zn than either the wild samples from both locations. Zn was found to be the second most abundant trace element in *P. viridis* (after Fe), and its role in the pathophysiology of disease is stimulating a great deal of interest (Coudray et al., 2006). Copper (Cu), which is also receiving growing attention for its role in alleviating oxidative stress, was found to be higher in samples collected from Kozhikode (~0.2 mg/100 g). Se, the antioxidant mineral element, was found to vary between 30–40 μg 100 g; this species is a valuable natural resource to combat free-radical induced disorders in the body. The variation of mineral content appeared to be due to the influence of several factors including food availability and differences in metal regulation. In addition, the differences in the mineral concentrations of the surrounding seawater could also influence their levels in *P. viridis* (Astorga Espana et al., 2007).

Vitamin composition

The fat soluble vitamins (A, D_3, E, and K1) and water soluble vitamin (vitamin C) are recorded in Table 1. All *trans* retinol underwent highly insignificant fluctuations, reaching maxima in cultured samples and minima in wild samples from Cochin. The levels of α-tocopherol, a vitamin with anti-oxidant properties, in *P. viridis* were low and showed insignificant fluctuations between wild and cultured samples collected from the experimental locations ($p > 0.05$). Significant differences were apparent in Phylloquinone (K1) content between wild and cultured samples collected from Kozhikode ($p < 0.05$). Similarly, significant differences in Cholecalciferol (D_3) content were realized between the wild and cultured samples collected from two different locations ($p < 0.05$). The loading plot of vitamins (Figure 2C) indicates that the vitamin E variables of both wild and cultured origin from Kozhikode are the dominant features in PC1 accounting for 53.2% of the total variability. The highest loadings on the second component (PC2) were defined by vitamin D_3 of both growth conditions (wild and cultured) of *P. viridis* from Cochin, representing 46.8% of the total variance. The values of transretinol content are within the limits to impart their beneficial effects. Vitamin D precursors constitute a large proportion of the unsaponifiable fraction of mollusc lipids. Among minor unsaponifiable components, HPLC analyses showed that oxygenated carotenoids, characterized by typical absorption spectra, were prevalent over the less polar, late-eluting, α-carotene and β-carotene. Due to the lack of suitable standard compounds, the early-eluting xanthophylls were not identified.

Amino acid composition

A total of 17 amino acids was identified and quantified in the samples of cultured and wild *P. viridis* collected from two different locations is shown in Table 2. The cultured samples were found to possess higher essential amino acids than wild samples and present in quantities required for balanced nutrition. It is to be noted that the cultured samples collected from Kozhikode exhibited significantly higher essential amino acid content than wild samples ($p < 0.05$). Among the wild samples from Kozhikode, the most abundant essential amino acid was found to be phenylalanine followed by arginine and leucine. The study indicated that Kozhikode samples have significantly higher methionine content (115–93 mg/100 g) than Cochin (26–43 mg/100 g) samples. In general, the amino acid lysine is absent in cereals, and this amino acid constitutes a major share to the total essential amino acid pool in cultured *P. viridis* from both experimental locations. Threonine, another essential amino acid deficient in cereals, was found to be higher in *P. viridis* from Kozhikode as compared to the samples collected from Cochin. This was supported by earlier works of Wesselinova (2000), who reported that the amounts and types of amino acids in fish muscle were affected by location. It is, therefore, anticipated that cultured *P. viridis* is a good source to supplement the deficient amino acids in cereals. Iwasaki and Harada (1985) similarly reported lysine as one of the major amino acids in the muscles from marine origin. Among the nonessential amino acids in Kozhikode samples, negatively charged amino acid glutamate was observed to be the predominant. Glycine was found to constitute a major share, thereby signifying the potential capability of this species to withstand salinity and adverse stress.The results obtained from this study

Table 2. Amino acid composition (mg/100 g wet tissue) of wild and cultured *P. viridis* collected from Cochin and Kozhikode.

	Cochin		Kozhikode	
Amino acids	Wild	Cultured	Wild	Cultured
Essential amino acids (E)				
His	38.1 ± 1.58^{a}	29.36 ± 0.57^{b}	76.2 ± 1.69^{c}	12.12 ± 0.57^{d}
Arg	170.9 ± 1.89^{a}	245.9 ± 0.67^{b}	188.3 ± 1.15^{c}	444.7 ± 0.96^{d}
Thr*	61.40 ± 0.60^{a}	83.96 ± 2.35^{b}	153.1 ± 2.04^{c}	166.2 ± 1.62^{d}
Val*	59.95 ± 0.40^{a}	62.32 ± 4.27^{a}	96.92 ± 0.33^{b}	155.2 ± 1.60^{c}
Met*	25.81 ± 0.47^{a}	43.24 ± 1.06^{b}	114.7 ± 0.72^{c}	92.73 ± 0.83^{d}
Ile*	52.21 ± 0.54^{a}	44.65 ± 1.50^{b}	84.36 ± 1.27^{c}	145.6 ± 0.58^{d}
Leu*	116.3 ± 0.45^{a}	128.6 ± 7.63^{b}	182.5 ± 1.18^{c}	284.5 ± 0.91^{d}
Phe*	63.63 ± 0.63^{a}	54.89 ± 1.97^{b}	202.6 ± 1.24^{c}	139.0 ± 1.36^{d}
Lys*	91.30 ± 1.18^{a}	174.6 ± 0.66^{b}	164.1 ± 0.93^{c}	203.6 ± 1.98^{d}
ΣE	679.53^{a}	867.52^{b}	1,262.78^{c}	1,643.65^{d}
Nonessential amino acids (NE)				
Asp	179.7 ± 0.36^{a}	243.7 ± 0.12^{b}	223.16 ± 2.47^{c}	386.75 ± 1.82^{d}
Glu	242.1 ± 0.67^{a}	305.6 ± 2.51^{b}	308.91 ± 1.33^{b}	632.71 ± 1.23^{c}
Ser	92.16 ± 0.55^{a}	136.9 ± 0.85^{b}	206.62 ± 0.77^{c}	223.84 ± 1.46^{d}
Gly	185.9 ± 2.33$_{a}$	200.36 ± 2.72^{b}	166.22 ± 0.75^{c}	424.14 ± 1.54^{d}
Ala	87.77 ± 1.61^{a}	131.55 ± 0.78^{b}	110.94 ± 0.18^{c}	245.47 ± 1.47^{d}
Pro	77.44 ± 1.35^{a}	47.01 ± 1.38^{b}	136.44 ± 1.5^{c}	164.83 ± 1.00^{d}
Tyr	30.41 ± 0.37^{a}	41.10 ± 0.18^{b}	142.92 ± 0.72^{c}	87.21 ± 1.11^{d}
Cys	3.76 ± 1.60^{a}	3.58 ± 0.24^{a}	25.35 ± 1.11^{b}	39.24 ± 0.44^{c}
ΣNE	899.24^{a}	1,109.69^{b}	1,320.35^{c}	2,203.95^{d}
ΣE/ΣNE	0.76^{a}	0.78^{a}	1.11^{b}	0.74^{a}

Reversed-phase binary gradient high performance liquid chromatograph (HPLC, Waters RP PICO.TAG amino acid analysis system), fitted with a packed column (dimethylocatadecylsilyl bonded amorphous silica; Nova-Pak C_{18}, 3.9 X 150 mm maintained at 38 ± 1°C in a column oven) was used to analyze hydrolysable amino acids by their UV absorbance (λ_{max} 254 nm). The mobile phase eluents used were A and B; Eluent A comprises sodium acetate trihydrate (MeCOONa, 0.14 M, 940 mL, pH 6.4) containing TEA (Me_3N, 0.05%), mixed with CH_3CN (60 mL); and Eluent B used was acetonitrile:water (60:40, v/v). Data are means of duplicate analysis of pooled homogenates. Tryptophan was not determined, *Essential amino acid for humans.

showed that cultured *P. viridis* have well-balanced and high-quality protein source in the respect of E/NE ratio. Any ratio of E/NE amino acids higher than 1.0 is considered to be excellent, and therefore it can be concluded that *P. viridis* collected from Kozhikode is a good source of well-balanced proteins and a high-quality protein source in respect to E/NE ratio. The percentages of the major amino acids of *P. viridis* from different growth conditions (wild and cultured) and geographical locations were included as variables in a PCA and given as a loading plot in Figure 2D. The parameters included in the loading plot are aspartic acid, glutamic acid, lysine, arginine, ΣEAA, ΣNEAA, ΣTAA and E/NE ratio (Figure 2D). The highest loadings on the first component (PC1) were defined by variables—viz., glutamic acid, E/NE ratio of both growth conditions (wild and cultured) of *P. viridis* from the different geographical locations (Kozhikode and Cochin), lysine, aspartic acid, and TAA from *P. viridis* of wild and cultured origin from Kozhikode, representing 73.3% of the total variability. Lysine and the total EAA of cultured origin from Cochin dominates in PC2 and explains 26.7% of the total variance of the data set.

Fatty acid composition

The percentage compositions of fatty acids in *P. viridis* collected from different sites are given in Table 3. Cultured samples collected from Kozhikode recorded significantly lower ($p < 0.05$) total SFAs than wild samples. Mussel fatty acid profiles usually contain about ~30–40% SFA (Alkanani et al., 2007), a level which was found in the present study. The predominant SFA was recorded to be 16:0 (21–28% of total fatty acids, TFA), with a maximum recorded in samples collected from Cochin (≥ 28% TFA) and minimum in those from Kozhikode (~21% TFA). Palmitic acid (16:0) is the major SFA in mussels (Alkanani et al., 2007; Orban et al., 2002) and appeared to be insignificant, between the samples collected from cultured or wild condition (Table 3; $p > 0.05$). SFAs are used for energy storage, and therefore, their concentration increases during periods of enhanced feeding activity

(Gockse et al., 2004). Growth of mussels in the presence of readily available nonphytoplanktonic organic material can too result in the accumulation of a higher proportion of SFAs (Freites et al., 2002).

The wild samples collected from Cochin recorded significantly higher total MUFAs than cultured samples ($p < 0.05$; Table 3). However, no significant differences were apparent between wild and cultured samples harvested from Kozhikode ($p > 0.05$). MUFA was found to be highly dependent on 18:1*n*-9, the primary constituent that contributed a major share to total MUFA pool, registering maxima in wild samples (16–18% TFA) and minima (14–15% TFA) in cultured samples. The total content of MUFAs was found to be highly dependent on 18:1*n*-9, the primary constituent registered maximum in wild samples. The earlier studies reported that the fatty acid 16:1*n*-7 is believed to be a diatom marker (Alkanani et al, 2007), whereas 18:1*n*-9 is not restricted to a single phytoplankton group. This further supports the fact that a food ingested by *P. viridis* by filter feeding is directly reflected in their fatty acid composition. In an earlier study, Orban et al. (2002) did not observe consistent fluctuations of the MUFAs, particularly 16:1*n*-7. The other MUFA detected as 16:1*n*-7,

Table 3. Fatty acid composition of *P. viridis* collected from two different growth conditions (wild and cultured) and locations (Cochin and Kozhikode) of southwest coast of India.

	Fatty acids (% total fatty acids, TFA)			
	Cochin		Kozhikode	
Fatty acids	Wild	Cultured	Wild	Cultured
Saturated fatty acids				
16:0	28 ± 0.02^a	28.3 ± 0.09^a	21.25 ± 1.4^b	20.68 ± 0.04^b
17:0	1.32 ± 0.02^a	1.37 ± 0.06^a	0.12 ± 0.08^b	0.06 ± 0.12^c
18:0	8.84 ± 0.76^a	8.54 ± 0.15^a	4.83 ± 0.42^b	1.56 ± 0.58^c
20:0	0.22 ± 0.41^a	0.32 ± 0.36^b	1.09 ± 0.01^c	0.72 ± 0.04^d
22:0	ND	0.04 ± 0.02^a	2.11 ± 0.01^b	2.26 ± 0.02^b
24.0	1.18 ± 0.01^a	1.16 ± 0.01^a	2.68 ± 0.02^b	1.83 ± 0.07^c
ΣSFA	39.56^a	39.73^a	34.15^c	29.61^d
Monounsaturated fatty acids				
14:1*n*-7	0.22 ± 0.01^a	0.84 ± 0.01^b	1.57 ± 0.01^c	1.4 ± 0.02^d
16:1*n*-7	3.26 ± 0.05^a	2.95 ± 0.07^b	1.21 ± 0.18^c	1.39 ± 0.14^d
18:1*n*-9	17.6 ± 0.01^a	13.7 ± 0.04^b	15.49 ± 0.3^c	14.91 ± 0.49^d
22:1*n*-9	ND	ND	2.47 ± 0.04^a	3.74 ± 0.15^b
24:1*n*-9	0.55 ± 0.15^a	0.42 ± 0.26^b	3.38 ± 0.05^c	2.14 ± 0.03^d
ΣMUFA	21.63^a	17.91^b	24.03^c	23.57^c
Polyunsaturated fatty acids				
18:2*n*-6	1.54 ± 0.02^a	1.03 ± 0.01^b	1.09 ± 0.83^b	1.17 ± 0.04^c
18:3*n*-6	0.55 ± 0.01^a	0.46 ± 0.01^b	0.85 ± 0.001^c	1.03 ± 0.15^d
18:3*n*-3	1.68 ± 0.15^a	1.48 ± 0.16^b	1.03 ± 0.82^c	0.86 ± 0.01^d
18:4*n*-3	0.11 ± 0.01^a	0.98 ± 0.02^b	0.3 ± 0.05^c	0.42 ± 0.05^d
18:4*n*-6	1.01 ± 0.08^a	1.12 ± 0.17^b	1.15 ± 0.03^b	1.98 ± 0.01^c
20:2*n*-6	0.09 ± 0.03^a	1.05 ± 0.04^b	0.12 ± 0.08^c	0.39 ± 0.08^d
20:3*n*-6	0.77 ± 0.1^a	0.7 ± 0.02^a	0.54 ± 0.06^b	1.92 ± 0.04^c
20:4*n*-6	1.32 ± 0.03^a	0.26 ± 0.05^b	1.19 ± 0.02^c	0.86 ± 0.18^d
20:3*n*-3	0.21 ± 0.01^a	0.2 ± 0.02^a	0.25 ± 0.09^a	0.27 ± 0.03^a
20:5*n*-3	7.95 ± 0.46^a	7.38 ± 0.65^a	12.68 ± 0.05^b	12.84 ± 0.05^b
22:5*n*-3	0.55 ± 0.11^a	0.42 ± 0.06^b	2.41 ± 0.06^c	3.01 ± 0.03^d
22:6*n*-3	9.6 ± 0.05^a	8.96 ± 0.06^a	9.6 ± 0.16^a	9.87 ± 0.03^a
ΣPUFA	25.38^a	24.04^a	31.13^c	34.58^d
ΣC_{18}PUFA	4.89^a	5.07^a	4.42^b	5.46^c
ΣC_{20}PUFA	10.34^a	9.59^b	14.70^c	16.24^d
Σ*n*-3	20.1^a	19.42^a	26.27^b	27.27^b
Σ*n*-6	5.28^a	4.62^b	4.94^a	7.35^c
n-3/*n*-6	3.81^a	4.20^b	5.30^c	3.70^d
ΣPUFA/ΣSFA	0.64^a	0.61^a	0.91^b	1.17^c
22:6*n*-3/20:5*n*-3	1.21^a	1.21^a	0.76^b	0.77^b

ΣSFA = total saturated fatty acids; ΣMUFA = total monounsaturated fatty acids; ΣPUFA: = total polyunsaturated fatty acids. Data presented as mean values of three samples (mean ± *SD*). These values do not total 100% because minor fatty acids are not reported. ND implies nondetectable (or fatty acids present below 0.05%). Values followed by different superscripts within same row are significantly different ($p < 0.05$).

which is abundant in diatoms, and significant differences were apparent between those of wild and cultured samples ($p < 0.05$). The share of this fatty acid was found to be lower than those reported in literature (16:1*n*-7, 11–14%; Alkanani et al., 2007).

PUFAs are considered as the single most important nutritional indicator dictating the quality of *P. viridis*. The prevalence of PUFAs, amounting to about 24–34% of total fatty acids, over the MUFAs (18–24%) is evident in *P. viridis* harvested from both locations and growth conditions. These results were in accordance with mussels from different studies (Orban et al., 2004). Although other authors (Orban et al., 2002) have reported that the PUFA in mussels predominated over the SFAs, a wide range of variation in the percentages of the compounds in these molluscs has been detected, ranging from 29–48% for PUFA, 16–32% for MUFAs, and 23–45% for SFAs. The wild samples collected from Cochin showed insignificant differences in total PUFA content compared to cultured samples ($p > 0.05$; Table 3). Interestingly, the samples collected from Kozhikode registered significantly higher total PUFA content (31–35%) than those from Cochin (24–25%, $p > 0.05$). However, it is of note that a level of > 20% PUFA with LC-fatty acids are a source of balanced nutrition, and therefore, the samples from Cochin also satisfy the requirements of balanced nutrition. The marginal variability in fatty acid composition is due to the fact that the lipid levels and composition of marine bivalves depend on the biochemical and environmental conditions of seed development and environmental conditions, including the phytoplankton resources available (Fuentes et al., 2009).

The PUFA composition of *P. viridis* was characterized by the predominance of *n*-3 PUFAs, particularly 20:5*n*-3 (EPA) and 22:6*n*-3 (DHA), which constitute > 80% of total PUFAs. The total content of *n*-3 PUFAs was recorded to be significantly higher (26–27%) in *P. viridis* collected from Kozhikode than those collected from Cochin (19–20%; $p < 0.05$; Table 3). The presence of higher amount of 20:5*n*-3 and 22:6*n*-3 in Kozhikode samples than Cochin samples largely reflects the composition of the planktonic diet (De Moreno et al., 1980). EPA content was found to be significantly higher in samples collected from Kozhikode than those recorded in Cochin samples, and no significant differences ($p > 0.05$) were apparent between wild and cultured samples. No significant differences ($p > 0.05$) were realized in the share of DHA to the total fatty acid pool as registered in the samples collected from Cochin and Kozhikode under two different growth conditions (Table 3). It is to be noted that 20:5*n*-3 content of > 5% is good to provide a balanced nutrition with respect to fatty acids, and thereby indicating the *P. viridis* collected from Cochin is equally good as Kozhikode with respect to fatty acid composition. Kharlamenko et al. (1995) reported that a high 20:5*n*-3 content is a fatty acid marker characteristic of diatoms. The PUFA composition also concurs with numerous studies of mussels and other bivalve species (Alkanani et al., 2007; Orban et al., 2002). Bivalves are filter feeders with diets consisting largely of phytoplanktons that contain a high proportion of long-chain *n*-3 PUFAs, such as 22:6*n*-3 and 20:5*n*-3 (Li et al., 2007; Murphy et al., 2002). Phytoplankton is the most important food resource for molluscs and can be considered to be the most important source of unsaturated fatty acids—viz., $C_{20:5}$ and $C_{22:6}$ (De Moreno et al., 1976). Therefore, the quantitative and qualitative availability of food influences the unsaturation level and composition of fatty acids (Freites et al., 2002), even if green mussels are able to convert $C_{20:5}$ to $C_{22:6}$, as observed by Orban et al. (2002). The variations of 20:5*n*-3 and 22:6*n*-3 ($p < 0.05$), the fatty acids known to be synthesized by diatoms and dinoflagellates, may be related to the type of food ingested by *P. viridis* during their growth. The fact that lipids in mussel tissue are rich in $C_{20\text{-}22}$ PUFAs seems to suggest that food consists of phytoplankton elements. The New Zealand mollusc *M. canaliculus* is reported to contain a similar PUFA profile, dominated by 20:5*n*-3 and 22:6*n*-3.

A significantly higher proportion of C_{18} PUFAs were recorded in cultured *P. viridis* collected from Kozhikode than in wild samples ($p < 0.05$). A higher proportion of C_{18} PUFAs in cultured samples from both locations (> 5%) than in wild (< 5%) apparently indicated that they feed on the water column. This conclusion is supported by the fact that *Dunaliella* sp., a chlorophytan microlaga, which is characterized by C_{18} PUFAs (Volkman et al., 1998), is present in the water column. Interestingly, the cultured *P. viridis* recorded lower 20:4*n*-6 than those in wild counterparts.

The *n*-3/*n*-6 fatty acid ratio of *P. viridis* was found to be about 3.7–5.3, which is considerably higher than the health foods available in the market, and therefore, *P. viridis* may serve as an effective alternative to balance the higher intake of *n*-6 fatty acids. This ratio was also found to be directly proportional to the anti-inflammatory activities due to the fact that 20:5*n*-3 is a precursor to anti-inflammatory lipid mediators (E-series of resolvins, RvE1), whereas docosanoids (DHA, 22:6*n*-3) to the D-series of resolvins (RvD1) and protectins (neuroprotectin D1, NPD1). Proper balance of dietary *n*-3/*n*-6 PUFAs is integral to prevent chronic diseases, including cardiovascular diseases, by reducing ventricular arrhythmias, serum triacylglycerol concentrations, and atherosclerotic plaque growth. Reversing the *n*-3/*n*-6 ratio in a favorable manner is the necessary to retain a disease-free healthy populace. An earlier study conducted in this line concluded that a 20% reduction in overall mortality and a 45% reduction in sudden death were reported in subjects with preexisting cardiovascular diseases when given 850 mg *n*-3 fatty acids (Cordain et al., 2005). It is, therefore, apparent that higher dietary intakes of *P. viridis*, with a *n*-3/*n*-6 ratio of 4-5 as realized in the present study, may favorably alter the ratio resulting in preventing inflammatory and autoimmune diseases. This is potentially important because the ratios of these fatty acids in the tissues are determined largely by their ratios in the diet (Cleland et al., 2006).

The total content of *n*-6 PUFAs were recorded to be comparatively higher in cultured samples collected from Kozhikode than those collected wild (Table 3). This is apparently due to the fatty acids 18:4*n*-6 and 20:3*n*-6, which contributed a major share to the total *n*-6 PUFA pool. Among the *n*-6 PUFAs, 20:4*n*-6, 18:2*n*-6 and 20:3*n*-6 were the dominant fatty acids. Interestingly, the wild samples collected from the experimental locations recorded significantly higher 20:4*n*-6 than those collected under cultured condition ($p < 0.05$). However, it is of note that of the *n*-6 fatty acids, particularly 20:4*n*-6, was reported to have an important role in stress tolerance. Higher content of 20:4*n*-6 of wild *P. viridis* (> 1%) than that in cultured ones (< 1%) revealed the ability of the former to withstand adverse stress conditions prevailing in a wild environment. The fatty acid AA (20:4*n*-6), the 20 carbon *n*-6 PUFA was reported to be the key link between PUFAs and eicosanoid family of inflammatory mediators (series-4 leucotrienes LTB_4 and series-2 prostaglandins PGE_2) and pro-inflammatory cytokines (TNF-α) in a cascade of metabolic processes involving 5-lipoxygenase (LOX_V) and cyclooxygenase-II (COX_{II}; Calder, 2006) and PUFAs. A family of anti-inflammatory mediators such as resolvins derived from *n*-3 LC-PUFAs—viz., EPA and DHA produced via the COX_{II} reaction, and are considered to be anti-inflammatory (Calder, 2006).

Low levels of *n*-6 (5–7%) and high levels of *n*-3 PUFAs (19–27%) in *P. viridis* were apparent in the present study (Table 3). The average *n*-3/*n*-6 ratio was recorded as ~4 in samples collected from Cochin and 4–5 in Kozhikode samples (Table 3). No significant differences ($p > 0.05$) in the ratio between ∑PUFA/∑SFA were realized in the *P. viridis* collected from Cochin under wild or cultured conditions. The higher ∑PUFA/∑SFA ratio in cultured samples collected from Kozhikode was mainly contributed by *n*-3 fatty acids particularly 20:5*n*-3, 22:5*n*-3, and 22:6*n*-3 (Table 3). The loading plot which includes ∑PUFA, Σn3, Σn6, EPA, and DHA when analyzed by PCA are shown in Figure 2E. The first principal component accounted for 68.3% of the variability in the data set. The second principal component (PC2) accounted for 31.7% of the variance in the data set. This revealed a high correlation between ∑PUFA and an important *n*-3 fatty acid, DHA of green mussel collected from Cochin, despite different growth conditions. The higher negative loadings of the PC1 are for variables *n*-6, lipid of wild origin from Cochin, DHA of wild origin from Kozhikode, and EPA of cultured origin from Kozhikode, which are grouped as C, highlighting its inverse relationship with other components.

Antioxidant activities and phenolic contents of P. viridis

Antioxidant activities of the green mussel samples were conducted to evaluate its free radical scavenging properties. This is particularly important to assess *P. viridis* collected under different growth conditions on in-built antioxidant capacity to inhibit free radical formation in the body, and to underline their importance as nutraceutical supplements, and their use as a potential source to isolate potential molecules. This study was conducted to evaluate free radical scavenging properties of bivalve *P. viridis* and to compare their footprints under two different locations and growth conditions. The total phenolic contents vis-à-vis antioxidant and lipid peroxidation inhibitory potential of wild and cultured *P. viridis* collected from different sites are shown in Table 4.

Total phenolic content

P. viridis collected under wild conditions from Cochin and Kozhikode exhibited significantly higher titer of phenolics than cultured ones ($p < 0.05$; Table 4). The maximum peak in polyphenol extracts appeared near 280 nm, and the UV spectra were typical of polyphenols. The polyphenolic antioxidants were measured in this study in order to understand the antioxidant defenses in bivalve mollusc tissue. The marginally higher total phenolic contents in Kozhikode may possibly be related to the presence of amino acids—viz., aspartate, proline, lysine, and glycine—that can synthesize numerous substances including phenolics by involving themselves in protein or energy metabolism and/or transmethylation reactions thereby phenolic contents in bivalves.

2,2-Diphenyl-1-picryl hydrazyl (DPPH) activity

In general, *P. viridis* harvested from Cochin were less effective in quenching free radicals in this system (23.7–29%) with respect to DPPH scavenging activities in comparison to those collected from Kozhikode. Wild samples collected from both Cochin and Kozhikode recorded significantly higher DPPH scavenging capacity than cultured samples ($p < 0.05$; Table 4). This is supported by the higher free radical quenching ability of *P. viridis* harvested from Kozhikode than Cochin with respect to DPPH scavenging activities. Oxidative stress is considered to be a chemical threat to biological systems. Previous studies demonstrated the presence of anti-oxidative molecules, especially in mussels (Lemaire and Livingstone, 1993). Significantly *P. viridis* registered greater DPPH radical quenching capacity as compared to vitamin E and BHT, well-known antioxidants. The results are comparable with that published earlier (Moncheva et al., 2004) or with *Mytilus edulis* L. and some other species (Gaspic et al., 2002).

Lipid peroxidation inhibitory capacity

Lipid peroxidation inhibitory capacities of *P. viridis* tissues from two different growth conditions and locations was carried out by thiobarbituric acid reactive species (TBARS) and conjugated diene (CD) assays. The conjugated diene formation was found to be significantly ($p < 0.05$) lower in *P. viridis*

Table 4. Total phenolic content, lipid peroxidation inhibitory activity, and antioxidant activities of wild and cultured *P. viridis* collected from Cochin and Kozhikode.

	Cochin		Kozhikode	
	Wild	Cultured	Wild	Cultured
Total phenolic content (mg/g GAE)	5.22 ± 0.31[a]	2.38 ± 0.14[b]	5.26 ±0.31[a]	4.94 ± 0.48[c]
DPPH (% TARSA)	29.1 ± 0.89[a]	23.7 ± 1.82[b]	32.05 ± 2.3[c]	29.2 ± 1.20[a]
Conjugated diene (CD, %)	0.31 ± 0.15[a]	0.59 ± 0.06[b]	0.05 ± 0.01[c]	0.09 ± 0.01[d]
TBARS (mM MDA Eq/kg) activity	0.35 ± 0.02[a]	0.38 ± 0.06[a]	0.15 ± 0.02[b]	0.33 ± 0.06[a]

All samples were analyzed in triplicate ($n = 3$) from a pooled sample (20 numbers). Means followed by different superscripts within the same row are significantly different ($p < 0.05$). Data presented as mean values of three samples (mean ± *SD*).

collected from Kozhikode than those collected from Cochin. No significant differences in TBARS contents between wild and cultured samples of *P. viridis* from Cochin were observed ($p > 0.05$; Table 4). Lipid peroxidation was reported to be a major factor due to oxidative stress or disruption in the balance between prooxidant and antioxidant factors. It is of note that the wild samples collected from Kozhikode were found to have comparatively less TBARS than those collected under cultured conditions that appeared to be due to the differences in antioxidant activity. This reflects the ability of *P. viridis* to biosynthesize antioxidant molecules possibly from phytoplanktons available in their niches by filter feeding. The inbuilt mechanism of *P. viridis* helps to shield their lipids/fatty acids and thereby hinder their autooxidation leading to the formation of free radicals and oxidation of olefinic bonds in their metabolic system. Earlier reports indicated the MDA levels in soft mussel tissue extracts were in the range of 4.0–15.4 mol/mg protein (c), which was higher than the present results. However, there is scanty published literature about antioxidant activities of *P. viridis*, though some other types were investigated—viz., *M. edulis* L. (Sukhotin et al. 2002). Therefore, these results revealed valuable information regarding the potential of *P. viridis* tissues to elicit antioxidant principles and their reaction of different scavenging radicals.

The levels of CD values in samples of Kozhikode origin were found to be lower than those recorded in Cochin samples, which corroborates with the earlier results obtained from TBARS studies. The reasons behind lower TBARS and CD values of Kozhikode samples may be attributed to the antioxidant molecules and/or enzymes, which possibly harvest them from the phytoplanktons and an inbuilt mechanism to shield LC-PUFAs with multiple unsaturation, appeared to be developed by *P. viridis* to hinder their auto-oxidation leading to the formation of free radicals and oxidation of olefinic bonds. It is of note that total PUFA contents of Kozhikode samples were higher than of Cochin, with predominance of LC-PUFAs in the former than in later, which corroborates with the earlier observations. This functional interdependence is important for *P. viridis*, especially regarding their oxidative metabolism. The difference in the antioxidant activities in two different locations probably largely reflects the variations of temperature and composition of the planktonic diet (Filho et al., 2001).

Correlation analyses between antioxidative indices of P. viridis *collected from different locations and growth conditions*

The loadings of TPC, DPPH activity, CD, and TBARS collected from both Cochin and Kozhikode under both growing conditions are shown in Figure 2F. The loadings on PC1 (64.42%) are large for TPC, DPPH, and TBARS of wild origin from Cochin and TPC and DPPH of cultured origin from Cochin and are grouped as Cluster A. Evidently, TPC and DPPH activity of the Cochin samples were highly correlated with each other regardless of their growing conditions. The variables—viz., CD of wild origin from Cochin, TPC, and TBARS of wild origin from Kozhikode and DPPH and TBARS of cultured origin from Kozhikode—are grouped as Cluster B, and they have registered high negative loadings with PC1 highlighting its inverse relationship with other components. The loadings on PC2 (35.58%) are large for DPPH of wild origin from Kozhikode and TPC and CD of cultured origin from Kozhikode.

It is, in general, understandable that nutritional compositions are influenced by spatial variation and geographical locations apparently due to differential microalgal diversity and primary flora in the coastal food web, and this might be the reason for getting the minor variations. However, as apparent from the results, no marked variations in nutritional and antioxidant composition are realized with respect to both growth conditions and geographical variations (Cochin and Kozhikode). The ranges of nutrients are well within the range to get benefit out of them. Also of note is that the cultured *P. viridis* exhibited equal and sometimes better profile of nutritional composition when compared with their wild counterparts, and therefore, cultured samples can be used for consumption. This also assures the return of invested amount for the self-help groups involved in mussel farming. Moreover, there is no need to depend exclusively on the uncertain wild

samples to produce value added products from this species. This is the first report of nutritional profiles of tropical green mussel *P. viridis* from different growth conditions and geographical variations from the southwest coast of India, where this species is found to be abundantly available and is a very important food source. This study indicates this species as a source of balanced nutrition. The balanced composition of PUFA with higher *n*-3 PUFAs indicates its usage to combat inflammatory response and, therefore, as a potential source to produce a nutraceutical for good health. This particular study revealed the nutritional qualities and completeness of tropical green mussel *P. viridis* as a health food.

In conclusion, the present study provides insights in different biochemical and fatty acid variation of *P. viridis* collected from two different geographical locations on the southwestern coast of India (Cochin and Kozhikode) and under different growth conditions (cultured and wild). No significant differences in different nutritional and biochemical parameters under observation were apparent between *P. viridis* collected from different locations of the southwestern coast of India, and the cultured samples were found to be equal or sometimes better than their wild counterparts in terms of this food quality indicator. The minor differences in biochemical indicators could be attributed to the differential feeding patterns of *P. viridis* grown under wild and cultured conditions. It is apparent that spacial and growth conditions play a vital role in physiological mechanisms of *P. viridis* guiding fatty acid metabolism. High levels of PUFA including *n*-3 PUFAs, important in the human diet for their platelet anti-aggregating and blood pressure-reducing properties, low levels of *n*-6 PUFA, and relatively higher *n*-3/*n*-6 PUFA ratio values characterized *P. viridis*. The higher PUFA content of the samples collected from this mussel harvesting area also contribute to their potential to exhibit anti-inflammatory activities. *P. viridis*, by virtue of realizing a balanced E/NE ratio with considerable amount of lysine and threonine, is a well-balanced and high-quality protein source and a candidate species to supplement these amino acids in cereals. The optimum and balanced quantities of vitamins, mineral nutrients, and low cholesterol contents added to the good qualities of this particular species, and therefore, proved to be a desirable item in the human diet in the southwestern region of India. This study also highlights the potential antioxidant activity of *P. viridis* with respect to total free radical scavenging activity and lipid peroxidation to evaluate the suitability of *P. viridis* populations as health food and sources of valuable antioxidant principles.

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