Nutritional Evaluation of Green Mussel *Perna viridis* (Linnaeus, 1758) and Brown Mussel *Modiolus modulaides* (Röding, 1798) From Panay Island, Philippines

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Abstract

Mussels are considered a low-cost protein source in the Philippines. The green mussel *Perna viridis* (Linnaeus, 1758) is widely consumed, while the brown mussel *Modiolus modulaides* (Röding, 1798) is not yet fully utilised as human food but used as animal food or fermented for human consumption. This study aims to determine the nutritional value of these mussels in terms of their proximate composition, amino acid, fatty acid, and mineral components. Results revealed that the moisture content of *M. modulaides* was significantly higher ($P < 0.05$) than *P. viridis*. In comparison, the crude protein content of *P. viridis* (11.39 %) was significantly higher ($P < 0.05$) than *M. modulaides* (9.19 %), but they have similar amounts of lipids. There were 18 amino acids detected in the two species with significantly higher ($P < 0.05$) total essential amino acids (EAA) and non-essential amino acids found in *P. viridis*. The most abundant EAA were leucine and lysine in *P. viridis* and *M. modulaides*. Results also revealed that the two mussel species are qualified for good quality protein claims with an essential amino acid index of >0.9 and digestible indispensable amino acid scores of >100 %. Palmitic and stearic acids were detected in both species, but eicosapentaenoic and docosahexaenoic acids were only detected in *P. viridis*. Sodium, potassium, iron, and calcium were also found in the two species. These results indicate that the two Philippine mussel species can be good sources of important amino acids, lipids and minerals for human and animal diets.

Keywords: proximate composition, amino acid, fatty acid, mineral content, mussels

Introduction

Mussels are among the large groups of invertebrates under Phylum Mollusca, with wide distribution in the coastal areas of the Indo-Pacific region. They are among the world's widely gathered seafood organisms, contributing largely to the overall marine bivalve production (Wijsman et al., 2019; Chakraborty and Joy, 2020). The demand for mussels as food is increasing especially in developing countries, because of their significance as a relatively cheaper source of protein for the human diet compared to other marine and terrestrial sources (Suplicy, 2020). In the Philippines, mussels are an essential source of animal protein and are consumed more than other bivalves because of a much lower market price and established consumer acceptability (Napata and Andalecio, 2011; Uba et al., 2020).

Given their importance as a health food, numerous studies have been conducted on the chemical composition of different mussel species worldwide. Green mussels, *Perna viridis* (Linnaeus, 1758), collected from the southwestern coast of India showed a protein content varying from 7.14–13.1 %, total lipid of 1.06–1.97 %, soluble carbohydrates of 0.84–3.78 %, and crude ash of 0.99–1.42 % (Chakraborty et al., 2016). The proximate composition of the edible tissue from the Mediterranean mussel, *Mytilus galloprovincialis* Lamarck, 1819 collected in Bulgaria contained 17.40–19.92 % protein, 1.40–2.89 % lipid, and 2.00–2.73 % carbohydrates (Merdzhanova et al., 2017). A *Mytilus* sp. from the northwest coast of Portugal and Spain were found to have higher carbohydrate content of 28 and 32 %, respectively, average total protein of 40 %, average total lipids of 10.45 %, and mean ash of 18.21 % (Oliveira et al., 2015).

All these studies indicate that mussels have high protein content, and their nutritional quality has also been elucidated and found to contain 18 types of amino acids with considerable proportions of essential amino acids and non-essential amino acids (Sengor et al., 2008; Saritha et al., 2015; Chakraborty et al., 2016). These findings emphasise the importance of mussels in human or animal nutrition because only about half of the more than 20 amino acids making up the protein are considered essential or those that cannot be synthesised by the human body and thus must be supplemented in the diet. The other critical nutritional components of mussels include fatty acids, vitamins, and essential minerals such as potassium, calcium, phosphorus, iron, copper, and zinc for the human body (Saritha et al., 2015).

Studies have also shown that mussels contain appreciable amounts of fatty acids omega-3 long-chain polyunsaturated fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have known good benefits to human health (Venugopal and Gopakumar, 2017; Carboni et al., 2019). In a study comparing the lipid, fatty acid, and sterol composition of New Zealand green-lipped mussel *Perna canaliculus* (Gmelin, 1791), and blue mussel, *Mytilus edulis* Linnaeus, 1758, both species were characterised by high proportions of phospholipids, triacylglycerides, free fatty acids, and sterols (Murphy et al., 2002). The study speculated that the differences in the lipid composition of the two species can be attributed to mussel's dietary and seasonal variabilities and developmental growth. A related study on the Mediterranean mussel, *M. galloprovincialis* found in Bulgaria showed that the proximate composition and bioactive lipid components are affected by seasonal variation, but the fatty acid composition characterised by a high amount of polyunsaturated fatty acids (PUFA) dominated by 22:6 omega-3 (n-3) remained high regardless of the season (Merdzhanova et al., 2017).

These studies have established the nutritional importance of the different mussel species worldwide and have therefore emphasised the relevance of elucidating those of the relatively unknown species in the hope that they may also be utilised as good sources of essential nutrients for humans. The green mussel, *P. viridis* is the most popularly consumed among the mussel species in the Philippines. Due to its low production cost and ability to thrive in various settings, it is typically grown in estuarine and marine areas. In contrast, the brown mussel *Modiolus modulaides* (Röding, 1798) achieves sexual maturity within 3 months and spawns throughout the year. It can form dense aggregations called 'biogenic reefs' or 'beds,' which host several associated organisms. It is known to filter large amounts of water and offer spatial habitat for various other invertebrates, making it ecologically significant (Kent et al., 2017). *Modiolus modulaides* are emerging as invasive organisms in different parts of the country and have no known ecological or economic importance yet. Some local communities consume them directly as food or processed through fermentation.

This study aims to evaluate and compare the proximate composition, amino acid composition, and fatty acid profile of the two mussel species in the Philippines, the green mussel *P. viridis* and the brown mussel *M. modulaides*. The obtained information may help promote consumer acceptance as food and predict the feasibility of using this organism for other interests.

Materials and Methods

Sample collection

The two mussel species were identified according to morphological characteristics by experts from the University of the Philippines Visayas-Museum of Natural Sciences as *P. viridis* and *M. modulaides* (del-Norte et al., 2020; MolluscaBase, 2022). *Perna viridis* were collected in March 2019 from Buntod, Panay in Capiz, while *M. modulaides* were taken from Dumangas, Iloilo in February 2020 (Fig. 1). Sampling sites were chosen by considering the proximity and abundance of supply during sampling (Napata and Andalecio, 2011; Cebu, 2018). Mussels with sizes ranging from 6.0–8.5 cm shell length were selected as samples. A total of 250 kg per species were collected for all the analyses.

Fig. 1. Map showing the sampling sites for (a) *Perna viridis* and (b) *Modiolus modulaides*. The red arrowheads indicate the selected sites in Buntod, Panay and Dumangas, Iloilo.

Collected samples were sorted out, and dead mussels or those with shells open were discarded. The samples were cleaned with the byssus intact and then transported to the Seafood Processing Laboratory of Institute of Fish Processing Technology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas (CFOS, UPV) in Miagao, Iloilo. No icing was done during transport. Upon arrival at the laboratory, the samples were immediately shucked, and the meat was stored at -25 °C until analysis. All analyses used pooled samples of n = 10, and measurements were conducted in triplicates.

Determination of moisture, crude protein, lipid and ash content

The proximate composition of the mussel samples

was determined following the standard procedures found in the Association of Official Analytical Chemists (AOAC, 1990). The moisture content of mussel samples was determined using the oven-drying method, wherein about 5 g were weighed into a pre-weighed silica crucible. Samples were oven-dried at 100 °C and weighed until constant weights were obtained. Moisture content was calculated as follows:

$$\%Moisture = \frac{(initial\ wt.\ of\ sample + crucible) - (final\ wt.of\ sample\ from\ oven + crucible)}{initial\ wt.\ of\ sample} \times 100$$

The total nitrogen content of the mussel samples was analysed using the Kjeldahl method, which consists of digestion, distillation, and titration. Digestion was carried out in 250-mL Kjeltubes, where 0.5 g of freeze-dried mussel meat was added with 7 g Cu-Kjeltab and 12 mL concentrated H_2SO_4. The digestion was done at 420 °C for 75 min, and about 60-75 mL of distilled water was added after. Kjeltubes were placed in the distillation apparatus, pre-washed for 10 min and dispensed with 40 % NaOH. The receiver flask was prepared containing 25 mL of 4 % boric acid until 100 mL of distillate was collected. Finally, the receiver flasks were titrated with standard acid (0.1 N HCl) until the endpoint. Nitrogen content (%N) was then calculated using the formula:

$$\%\ N = \frac{\left(\frac{a}{b}\right) x\ Nacid}{W_s} \times\ 14 \times 100$$

where N is the normality of standard acid, a is the volume standard acid (mL), b is the volume of the blank (mL) and W_s is the weight of the sample (g). While the crude protein content was estimated by multiplying the nitrogen content with the factor 6.25.

A modified version of the Bligh and Dyer method (1959) was employed to determine the lipid content. The modifications involved a reduced sample size, only comprising 10 % of the original sample and using the solvents indicated in the original protocol. Briefly, 10 g mussel meat was macerated to a homogenous paste, then added with chloroform: methanol solution (2:1, v/v), transferred to an iodine flask and shaken to mix thoroughly. The homogenous samples were centrifuged for 25 min at 3,000 rpm. The resulting clear chloroform layer was collected into a pre-weighed clean and dry beaker, placed in the hood, and allowed to evaporate until apparently dry. Samples were then cooled in a desiccator, weighed, and the lipid content (%lipid) calculated using the formula:

$$\%Lipid\ = \left(\frac{W^3 - W_2}{W_1}\right) \times\ \frac{V_2}{V_1} \times\ 100$$

where W_1 = weight of the sample, W_2 = weight of the beaker, W_3 = weight of the beaker with lipid, V_1 = volume of the methanol added, and V_2 = volume of the chloroform added.

For the ash content determination, 10 g of the sample was placed in a pre-weighed crucible and then incinerated in a muffle furnace at 600 °C for 4 h. The burned samples were cooled inside the desiccator and weighed. After the incineration process, the sample strongly adheres to the crucible. Hence, the weight of the crucible in the initial and final weighing was included to compute the final weight. The following formula was used to determine the per cent ash (%Ash):

$$\%\text{Ash} = \frac{(\text{initial wt.of sample+crucible}) - (\text{final wt.of sample from muffle furnace+crucible})}{\text{initial wt.of sample}} \times 100$$

Nitrogen free extract or soluble carbohydrates content determination

Nitrogen free extract (NFE) represents the soluble carbohydrates of the sample. NFE values from the samples were obtained by subtracting the total percentages of all the components already measured from 100 %.

$$\%NFE\ = 100\ \% - (\%M + \%CP + \%L + \%A)$$

where $\%M$ is the moisture content, $\%CP$ is the crude protein content, $\%L$ is the crude lipid content, and $\%A$ is the ash content.

Amino acid analysis

Mussel samples were sent to the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas for amino acid analysis, including essential, conditional, and non-essential amino acids. Hydrolysis was done by adding the mussel samples with 1.5 mL of 1:1 hydrochloric acid: propionic acid for 16 min at 160 °C. Mixtures were allowed to cool down at room temperature. Samples were diluted to 50 mL, and the mixture was adjusted to pH of 2.2 using 4N NaOH added drop by drop. The in-house protocol used in the analysis was based on AOAC (1996), and samples were analysed using Shimadzu LC-10A high-performance liquid chromatography-amino acid analyser (Shimadzu Prominence, Modular HPLC, Japan).

Nutritional evaluation of amino acids

The essential amino acid index (EAAI) is the geometric mean of egg ratios or the ratio of the essential amino acids in a food formulation relative to their respective amounts in whole egg protein (Oser, 1959), while the digestible (dietary) indispensable amino acid score (DIAAS) is obtained from the digestible indispensable amino acid (DIAA) content in 1 g protein of food and

the IAA reference ratio (FAO, 2013).

The EAAI based on the method of Oser (1951) and the DIAAS of the two mussel samples was calculated using the following formula:

$$EAAI = \sqrt[n]{a_1 \times a_2 \times \ldots a_n}$$

where a_n is the ratio of each essential amino acid in the investigated protein to its amount in the whole egg protein, and n is the amount of essential amino acid (EAA).

The DIAAS score was calculated using the formula:

$$DIAAS\ (\%) = \frac{mg\ of\ digestible\ dietary\ indispensable\ amino\ acid\ in\ 1\ g\ of\ test\ protein}{mg\ of\ the\ same\ amino\ acid\ in\ 1\ g\ of\ reference\ protein} \times 100$$

Lipid and fatty acid profiling and mineral determination

A total of 3 kg of each mussel meat sample was sent to the Department of Science and Technology-Food and Nutrition Research Institute (DOST-FNRI) in Taguig, Metro Manila, Philippines, for fatty acid profiling and mineral content determination. Gas chromatography protocol optimised by DOST-FNRI with Dani Master GC (Dani Instruments S.P.A., Italy) was used to determine the samples' cholesterol and fatty acids profile, based on the methods of Fletouris et al. (1998) and O'Fallon et al. (2007), respectively. The sodium and potassium content of mussel species were determined using the Thermo Fisher Scientific (USA) iCE3500 atomic absorption spectroscopy (AAS) using the AOAC 999.10 method, while the other mineral content of mussel species was determined using the Shimadzu (Japan) AA6300 AAS based on AOAC 985.35 method for iron, calcium, and zinc with slight modification. Mussel samples' mineral content and cholesterol were expressed as mg.100 g^{-1} and fatty acids (saturated and polyunsaturated) as g.100 g^{-1}.

Statistical analysis

All analyses were done in triplicate and presented as mean values ± standard deviation (SD). T-test was used for amino acid analysis, nutritional evaluation, vitamins, minerals, and fatty acid content analysis to determine differences between means. Differences were considered significant at $P < 0.05$. The IBM SPSS Statistics 20 was used for the analysis of the data.

Results

Proximate composition of mussels

Visual examination of the samples prior to analysis suggested typical physical characteristics of fresh mussels with light brown to deep orange colour, moist, plump, and intact tissues. Mussel shells were tightly closed and emitted a distinct seaweedy smell. Clumps of dark threads or byssus attached to the shell were apparent in all healthy mussels. Results of the proximate analysis of *P. viridis* and *M. modulaides* are presented in Table 1. The moisture content of *P. viridis* is higher, while its protein content is lower than that of *M. modulaides*. Significant differences ($P < 0.05$) in these compositions were observed in the two species. This indicates that *P. viridis* is a better option as far as protein content is concerned. However, the two species were found to have comparable lipid, ash, and carbohydrate contents and values that do not differ significantly ($P > 0.05$).

Amino acid composition and nutritional evaluation

The amino acid analysis results revealed that 18 amino acids are present in the two mussel species (Table 2). *Perna viridis* has higher EAA content of 28.32 ± 0.01 % and non-essential amino acid content (NEAA) of 48.25 ± 0.020 % compared to *M. modulaides* at 26.68 ± 0.005 % and 38.50 ± 0.005 % EAA and NEAA, respectively. *Modiolus modulaides*, however, has higher conditionally essential amino acid (CEAA) at 34.82 ± 0.41 % compared to the 23.43 ± 0.20 % NEAA found in *P. viridis*. Leucine and lysine were the highest EAA

Table 1. Mean proximate composition including moisture, crude protein, lipid, soluble carbohydrate, and ash content (wet weight basis) of *Perna viridis* and *Modiolus modulaides*.

Parameters (%)	Species	
	Perna viridis (Linnaeus, 1758)	*Modiolus modulaides* (Röding, 1798)
Moisture	81.81 ± 0.93[a]	83.56 ± 0.33[b]
Crude protein	11.39 ± 0.05[a]	9.19 ± 0.07[b]
Lipid	2.12 ± 0.67[a]	2.11 ± 0.33[a]
Soluble carbohydrate	1.95 ± 0.99[a]	2.33 ± 0.66[a]
Ash	2.73 ± 0.41[a]	2.82 ± 0.04[a]

Values are expressed as mean ± standard deviation (n = 10). Different superscripts (a,b) in the same row indicate significant differences ($P < 0.05$).

Table 2. Essential, conditionally essential, and non-essential amino acid content of *Perna viridis* and *Modiolus modulaides*.

Amino acids	Species	
	Perna viridis (Linnaeus, 1758)	*Modiolus modulaides* (Röding, 1798)
Essential amino acids (EAA), %		
Histidine	2.72 ± 0.00[a]	2.03 ± 0.00[b]
Isoleucine	3.42 ± 0.00[a]	2.32 ± 0.00[b]
Leucine	7.87 ± 0.01[a]	5.49 ± 0.00[b]
Lysine	0.24 ± 0.00[a]	5.65 ± 0.15[b]
Methionine	2.93 ± 0.00[a]	2.30 ± 0.00[b]
Phenylalanine	2.72 ± 0.01[a]	2.20 ± 0.00[b]
Threonine	4.22 ± 0.01[a]	3.06 ± 0.01[b]
Tryptophan	0.35 ± 0.00[a]	0.39 ± 0.09[a]
Valine	3.86 ± 0.01[a]	3.25 ± 0.00[b]
Total EAA	28.32 ± 0.01[a]	26.68 ± 0.01[b]
Conditionally essential amino acids (CEAA), %		
Arginine	0.59 ± 0.00[a]	14.40 ± 0.31[b]
Cystine	1.00 ± 0.16[a]	not detected
Glycine	21.66 ± 0.05[a]	18.41 ± 0.02[b]
Proline	0.18 ± 0.00[a]	2.01 ± 0.18[b]
Total CEAA	23.43 ± 0.20[a]	34.82 ± 0.41[b]
Non-essential amino acids (NEAA), %		
Aspartic acid	14.16 ± 0.01[a]	9.35 ± 0.00[b]
Alanine	8.75 ± 0.06[a]	8.69 ± 0.01[a]
Glutamic acid	16.06 ± 0.03[a]	13.00 ± 0.02[b]
Serine	6.18 ± 0.00[a]	5.15 ± 0.01[b]
Tyrosine	3.11 ± 0.01[a]	2.30 ± 0.00[b]
Total NEAA	48.25 ±0.02[a]	38.50 ± 0.01[b]

Values are expressed as mean ± standard deviation (n = 10). Different superscripts (a,b) in the same row indicate significant differences ($P < 0.05$).

components in *P. viridis* and *M. modulaides*, respectively, and both species have low tryptophan levels. Interestingly, lysine was detected highest among the EAA in *M. modulaides* but lowest in *P. viridis*. The two species have similar CEAA and NEAA profiles wherein they both contain high levels of glycine, glutamic acid and low levels of tyrosine. Cystine was not detected in *M. modulaides*, but it had a higher proline content than *P. viridis*. Overall, significant differences were observed ($P < 0.05$) between the two mussel species regarding total EAA and NEAA. However, no significant differences ($P > 0.05$) were found in tryptophan, alanine, and tyrosine.

Modiolus modulaides scored higher EAAI than *P. viridis*, but both obtained scores higher than 0.9 (Table 3). The highest EAAI among the amino acids was found in methionine (0.21 ± 01) in *P. viridis* and lysine (0.23 ± 0.01) in *M. modulaides*, while the lowest EAAI was found in histidine in both species. Except for tryptophan, the results revealed significant differences ($P < 0.05$) in total EAAI between the two species.

Results of the calculated DIAAS in the mussel species are shown in Table 4. The EAA content in mussel samples was lower compared to the FAO/WHO scoring pattern. Results showed that the first limiting amino acid in the body wall of *P. viridis* was lysine (2.04 %), and the second limiting amino acid was tryptophan (30.53 ± 0.04 %). While the first limiting amino acid in *M. modulaides* was phenylalanine (30.72 ± 0.05 %), whereas the second limiting amino acid was valine (33.08 ± 0.12 %). DIAAS of the majority of the EAA is statistically different ($P < 0.05$) between two mussel species with higher values found in *P. viridis*.

Lipid and fatty acid profiling

Lipid and fatty acid analysis showed that levels of cholesterol present in *P. viridis* and *M. modulaides* were 54 mg.100 g^{-1} and 63 mg.100 g^{-1}, respectively (Table 5). Saturated fatty acids such as palmitic and stearic acids were detected at similar levels in the two mussel species. Polyunsaturated fatty acids such as eicosapentaenoic and docosahexaenoic acids were only detected in *P. viridis*. Trans fat and other saturated and unsaturated fatty acids were not detected in both mussel species. CSI and CI were also calculated to assess the dietary effect of the

Table 3. Essential amino acid indices (EAAI) of amino acids found in *Perna viridis* and *Modiolus modulaides* relative to the whole raw hen's egg as reference protein.

Amino acid (AA)	Reference protein	AA content (mg.100 g^{-1} protein)		EAAI	
		Perna viridis (Linnaeus, 1758)	*Modiolus modulaides* (Röding, 1798)	*Perna viridis*	*Modiolus modulaides*
Histidine	195	12.02 ± 0.15^a	10.92 ± 0.02^b	0.061 ± 0.06^a	0.056 ± 0.05^b
Isoleucine	129	12.77 ± 0.02^a	10.57 ± 0.02^b	0.10 ± 0.08^a	0.08 ± 0.07^b
Leucine	172	29.44 ± 0.10^a	24.99 ± 0.01^b	0.17 ± 0.01^a	0.15 ± 0.09^b
Lysine	125	0.98 ± 0.00^a	28.68 ± 0.03^b	0.01 ± 0.02^a	0.23 ± 0.01^b
Methionine	61	12.48 ± 0.02^a	11.90 ± 0.02^b	0.21 ± 01^a	0.20 ± 0.01^b
Phenylalanine	114	12.83 ± 0.11^a	12.60 ± 0.01^b	0.11 ± 0.09^a	0.11 ± 0.08^b
Threonine	99	14.34 ± 0.09^a	12.66 ± 0.07^b	0.15 ± 0.09^a	0.13 ± 0.09^b
Tryptophan	31	2.02 ± 0.02^a	2.75 ± 0.02^b	0.07 ± 0.02^a	0.08 ± 0.01^a
Valine	141	12.91 ± 0.07^a	13.23 ± 0.01^b	0.09 ± 0.07^a	0.09 ± 0.08^b
Average				0.11 ± 0.06^a	0.13 ± 0.07^b
Total EAAI				0.96 ± 0.06^a	1.13 ± 0.07^b

Values are expressed as mean ± standard deviation (n=10). Different superscripts (a,b) in the same row indicate significant differences ($P < 0.05$).

Table 4. Digestible indispensable amino acid scores (DIAAS) of the essential amino acids (EAA) found in *Perna viridis* and *Modiolus modulaides*.

EAA	WHO/FAO/UNU Pattern (mg. g^{-1} protein)*	mg AA g^{-1} sample		DIAAS (%)	
		Perna viridis (Linnaeus, 1758)	*Modiolus modulaides* (Röding, 1798)	*Perna viridis*	*Modiolus modulaides*
Histidine	16	12.02 ± 0.15^a	10.92 ± 0.02^b	75.09 ± 0.7^a	68.24 ± 0.06^b
Isoleucine	30	12.77 ± 0.02^a	10.57 ± 0.02^b	42.58 ± 0.08^a	35.22 ± 0.07^b
Leucine	61	29.44 ± 0.10^a	24.99 ± 0.007^b	48.27 ± 0.09^a	40.96 ± 0.2^b
Lysine	48	0.98 ± 0.00^a	28.68 ± 0.03^b	2.04 ± 0.00^a	59.75 ± 0.02^b
Methionine	23	12.48 ± 0.02^a	11.90 ± 0.02^b	54.25 ± 0.04^a	51.72 ± 0.08^b
Phenylalanine	41	12.83 ± 0.11^a	12.60 ± 0.01^b	31.28 ± 0.02^a	30.72 ± 0.05^b
Threonine	25	14.34 ± 0.09^a	12.66 ± 0.07^b	57.37 ± 0.14^a	50.60 ± 0.03^b
Tryptophan	6.6	2.02 ± 0.02^a	2.75 ± 0.02^b	30.53 ± 0.04^a	41.65 ± 0.02^b
Valine	40	12.91 ± 0.07^a	13.23 ± 0.01^b	32.28 ± 0.02^a	33.08 ± 0.12^b
Total	291	109.78 ± 0.09^a	128.27 ± 0.06^b	373.69 ± 0.01^a	411.94 ± 0.05^b

Values are expressed as mean ± standard deviation (n = 10). Different superscripts (a,b) in the same row indicate significant differences ($P < 0.05$).

*Amino acid scoring patterns for the older child, adolescent, and adult (amended values from 2002 WHO/FAO/UNU report).

Table 5. Fatty acid composition and cholesterol content of *Perna viridis* and *Modiolus modulaides* and their nutritional indices.

Lipid class and index	Content (g.100 g^{-1})	
	Perna viridis (Linnaeus, 1758)	*Modiolus modulaides* (Röding, 1798)
Palmitic acid (16:0)	0.20 ± 0.00^a	0.20 ± 0.00^a
Stearic acid (18:0)	0.10 ± 0.00^a	0.10 ± 0.00^a
Eicosapentaenoic acid	0.10 ± 0.00	ND
Docosahexaenoic acid	0.10 ± 0.00	ND
ΣSaturated fatty acid (SFA)	0.30 ± 0.14^a	0.30 ± 0.14^a
ΣPolyunsaturated fatty acid (PUFA)	0.20 ± 0.00	NA
Total fatty acid	0.50 ±0.00^a	0.30 ± 0.00^b
ΣPUFA/ΣSFA	0.67 ± 0.03	NA
Cholesterol (mg)	54.00 ± 0.00^a	63.00 ± 0.00^b
Cholesterol/SFA index (CSI)*	3.00 ± 0.00^a	3.45 ± 0.00^b
Cholesterol index (CI)**	5.26 ± 0.03^a	4.08 ± 0.00^b

Values are expressed as mean ± standard deviation (n = 10). Different superscripts (a,b) in the same row indicate significant differences ($P < 0.05$).

*CSI = (1.01 × SFA g.100^{-1} g ww) + (0.05 × cholesterol mg.100 g^{-1} ww).

**CI = 1.01(SFA g.100^{-1} g ww – 0.5PUFA g.100 g^{-1} ww) + (0.06 cholesterol mg.100 g^{-1} ww).

ND - Not detected, values are below the detectable level which is 0.01g.$100g^{-1}$; NA - Not applicable.

mussel lipid on cholesterol level. CSI and CI values were comparable but were statistically different between the two mussel species. The CSI of the fatty acids in *M. modulaides* was significantly higher ($P < 0.05$), while the CI was significantly higher in *P. viridis*.

Mineral composition

Table 6 shows that important dietary minerals such as sodium, potassium, iron, and calcium are found in both mussel species. Generally, the mineral content of *M. modulaides*, except calcium, was higher but not significantly different from that of *P. viridis* ($P < 0.05$).

Discussion

A straightforward approach to evaluating the nutritional value of a food is through determining its proximate composition in terms of its moisture, crude protein, lipid, and ash content. Proximate composition analysis is the simplest and most useful standard criteria adopted in international trade, particularly concerning regulations and commercial specifications (Biandolino et al., 2019). Results of the present study revealed that *P. viridis* and *M. modulaides* contain relatively high amounts of protein (9.19–11.39 %) and considerable levels of lipids (2.12 % and 2.11 %) in their tissues. In a study on the proximate composition of green mussels collected from the Southwestern coast of India reported by Chakraborty et al. (2016), the protein content of *P. viridis* ranged from 7.14–13.10 % and lipid content from 1.06–1.97 %. These protein content results agree with the present study's findings, while the lipids are lower than the values of the present study. Similarly, the Black Sea mussels *M. galloprovincialis* from Bulgaria contain crude protein content ranging between 17.80–19.92 % and lipid content of 1.40–2.89 % (Merdzhanova et al., 2017).

In other studies, the variability of protein content in mussels, which is also observed in most marine-derived food sources, may be influenced by certain factors such as developmental phases, spawning, regression, and resting cycles (Bongiorno et al., 2015; Merdzhanova et al., 2016). In the same way, lipid content is also influenced by the mussel's seasonality and life cycle stage (Murphy et al., 2002). Generally, changes in metabolic activity, location, sex, and spawning may result in biochemical changes in mussels. In the present study, the timing of the sampling for the season and life cycle was not considered, which may contribute to the variability of the results.

The amino acid analysis allows an investigation of the protein quality in terms of the nutritionally significant amino acids present in the food. This could supplement the relative quantitation derived from the proximate composition analysis. In the present study, 18 amino acids were detected in both *P. viridis* and *M. modulaides*. Similar findings were reported by Asha et al. (2014) in *Crassostrea madrasensis* (Preston, 1916) and by Karnjanapratum et al. (2013) in the Asian hard clam *Meretrix lusoria* (Röding, 1798), with results indicating that 18 amino acids were also found in these species. The results of the present study are also in consonance with the findings of several studies showing the abundance of leucine and lysine in different mussel species, such as freshwater mussels, *Anodonta pseudodopsis* (Locard, 1893), and *Unio tigridis* (Bourguignat, 1852), (Sereflisan and Altun, 2018), *Mytilus* sp. (Oliveira et al., 2015), *M. galloprovincialis* (Sengor et al., 2008) and Meti mussels *Batissa violacea* (Lamarck, 1818), (Jamaluddin et al., 2016).

Leucine and lysine were also abundant in other mollusc species, such as oysters *C. madrasensis* (Asha et al., 2014) and *Crassostrea gigas* (Thunberg, 1793) (Zhu et al., 2018), the clam *Meretrix meretrix* (Linnaeus, 1758) (Gopalakrishnan and Vijayavel, 2009), and the gastropod *Bursa spinosa* (Schumacher, 1817) (Babu et al., 2010). The high leucine content in the two mussel species has potential significance in studies exploring the biological activities of marine peptides.

The present study reports that the NEAA comprised most of the amino acids in both mussel species. The same result was reported by Babu et al. (2012) in the bivalve *Gafrarium tumidum* (Röding, 1798), where the NEAA was higher at 22.20 % compared to the EAA at 20.77 %. However, the study of Saritha et al. (2015)

Table 6. Mean mineral composition of *Perna viridis* and *Modiolus modulaides*.

Mineral	Species		US FDA standard*
	Perna viridis (Linnaeus, 1758)	*Modiolus modulaides* (Röding, 1798)	
Sodium (mg)	412 ± 2.83[a]	438 ± 2.83[a]	2,300
Potassium (mg)	144 ± 2.83[a]	191 ± 1.41[b]	4,700
Iron (mg)	17.20 ± 0.28[a]	34.60 ± 0.14[b]	18
Calcium (mg)	1.70 ± 0.00[a]	1.30 ± 0.00[a]	1,300

Values are expressed as mean ± standard deviation (n = 10). Different letters in the same row indicate significant differences ($P < 0.05$).

*United States Food and Drug Administration (USFDA) recommended daily value of vitamins and minerals to consume or not to exceed each day (March 2020). This USFDA reference guide applies to all conventional food and dietary supplements regardless of form.

revealed higher total EAA than NEAA in *P. viridis* harvested from the southwest coast of India. The difference with the results of the present study may be due to the differences in the environmental conditions of the sampling sites (food conditions and water and sediment parameters) that impact the amount of protein in mussel samples (Mititelu et al., 2022).

Among the NEAA, glutamic acid was found to have the highest concentration in the two mussel species. Its importance in human diets is associated with its involvement in flavour formation and is often used and produced in the form of sodium salt as monosodium glutamate (Lipnizki, 2010; Cui et al., 2014). The same property is found in aspartic acid, which in its naturally occurring form is supplied in food (Zgola-Grzeskowiak and Grzeskowiak, 2012). The presence of these two amino acids in *P. viridis* and *M. modulaides* may contribute to their savoury taste. This finding coincides with the study of Normah and Diyana (2018) in green mussel hydrolysate, with glutamic acid and aspartic acid acting as taste enhancers in peptides.

To determine the nutritional value of the detected amino acids, their EAAI was calculated. The EAAI measures the nutritional quality of the amino acid present in food and is calculated as a ratio of the EAA relative to their content in a protein reference such as an egg (Oser, 1959). Defined as the geometric mean of egg ratios or the ratio of the essential amino acids in a food formulation relative to their respective amounts in whole egg protein (Oser, 1959), this index indicates protein quality. Results of the present study show that the total EAAI of *P. viridis* (0.958 ± 0.06) and *M. modulaides* (1.129 ± 0.07) exceeded 0.90, indicating that they are both good-quality protein sources. According to the standards established by Oser (1959) and used in other studies, particularly in feedstuffs (Bunda et al., 2015, Kirimi et al., 2020), an EAAI of 0.90 is an indicator of good quality protein sources, while 0.80 and 0.70 indicate useful and incomplete protein, respectively.

When making claims for the protein content of food, the amino acid content should be assessed using the digestible indispensable amino acid score (DIAAS). The recommended nutritional reference value (NRV) for protein based on global standards is 50 g. The food must cover 10 % of NRV per 100 g (solids) to be considered as a protein "source" for nutritional claim (FAO, 2013). DIAAS cut-off values are necessary to distinguish between excellent or high protein (>100), good or source protein (75–99), and no claim (FAO, 2013). Food with DIAAS >75 is eligible for protein content claims (Marinangeli and House, 2017). Given that the DIAAS of the mussel species in the present study is >10 % of NRV per 100 g, they are qualified for protein nutritional claims. With DIAAS exceeding 100 %, it can be claimed that these mussels have excellent or high protein content. To date, there are limited studies on the nutritional evaluation of marine bivalves. This study is the first to present findings on the nutritional evaluation of amino acids in *P. viridis* and *M. modulaides* regarding the EAAI and DIAAS.

In various molluscs, cholesterol generally makes up 2–90 % of total sterols. Plankton, which contains various sterols, is the predominant diet of mussels, and they can be incorporated into mussel tissues (Li et al., 2007). Earlier studies reported cholesterol as the predominant sterol present in mussels. For instance, cholesterol was the major sterol in *Mytilus edulis* Linnaeus, 1758 (30 %) and *Perna canaliculus* (Gmelin, 1791) (29 %) (Murphy et al., 2002). Notably, sterol components such as β-sitosterol and phytosterols in mussels, particularly in *P. canaliculus*, have generated anti-inflammatory activity in acute animal inflammation models (Li and Sinclair, 2002; Saltzman et al., 2017). Although not elucidated in the present study, this may indicate the bioactivity potential of sterols from mussels. In terms of the cholesterol content, the results of the present study agree with previous studies reporting that the total cholesterol of different mussel species ranged from 36.38 mg.100 g^{-1} to 96.6 mg.100 g^{-1} (Prato et al., 2010; Chakraborty et al., 2016; Merdzhanova et al., 2016). The cholesterol levels of the two mussel species can be considered low because they do not reach the limit for the recommended daily allowance for cholesterol intake of 300 mg per day or less (Sadler et al., 2013).

Reported saturated fatty acid (SFA) levels in mussels are within the range of 0.4–0.5 g.100 g^{-1} (Chakraborty and Joy, 2020), higher than those detected in *P. viridis* and *M. modulaides*. The difference in content profiles could be attributed to the developmental stages and the complex relationships between growth, reproduction, and food availability (Wang et al., 2011, Dernekbasi et al., 2015). Mussels consume more plankton during development, contributing to increased n-3 polyunsaturated fatty acid concentration in their gonads. Moreover, gonads acquire more lipids and saturated fatty acids during the mature and spawning stages, whereas the spent and resting stages had the lowest consumption. *Perna viridis* was collected in Capiz, with peak spawning and settling in April to May and September to October, while *M. modulaides* spawning occurs all year in Dacutan, Dumangas since spat can be found in large amounts in the environment. This reveals the influence of the environment, ecology, and physiology on the changes in the biochemical content of the two mussel species. Levels of SFA may also vary according to the amount of energy expenditure, such that high SFA levels could indicate lower energy expenditure (Stratev et al., 2017). In the context of this study, the two mussels examined may be in a state of high energy expenditure due to stress or spawning during collection, potentially contributing to their low SFA values. Specimens analysed in this study were harvested during their reproductive season when energy expenditure is high, thus, may explain the relatively lower SFA levels (Asaduzzaman et al., 2019).

Another significant finding of the present study is the

detection of EPA and DHA exclusively in *P. viridis*. These polyunsaturated fatty acids (PUFAs) play vital roles in the growth and development of the brain in infants and in lowering serum triacylglycerol and cholesterol levels, increasing membrane fluidity, and reducing thrombosis (Sun et al., 2002). PUFA from *P. canaliculus* reported by McPhee et al. (2007), also possesses anti-inflammatory activity. Comparatively, the levels of EPA and DHA detected in the two mussel species in the present study were lower than those observed in *Mytilus galloprovincialis* (Lamarck, 1819), which ranged from 0.495–0.662 g for every 100 g of mussel meat (Merdzhanova et al., 2016). Conversely, *P. viridis* has been reported as a prolific source of PUFAs at 61 % compared to the blue mussel *M. edulis* at 20–23 % (Chan et al., 2004). These PUFA were, however, not detected in *M. modulaides* in the present study but were found in other brown mussel species, such as *P. indica* (Dalin et al., 2017) and *M. galloprovincialis* (Prato et al., 2010).

The PUFA/SFA ratio is a standard used as an index when evaluating the relative nutritional values of seafood and in assessing diet's impact on cardiovascular health. A human diet with PUFA/SFA ratio of 0.45 is recommended to prevent the occurrence of cardiovascular diseases and some chronic diseases, such as cancers (Wołoszyn et al., 2020), while ratios below 0.45 may elevate blood cholesterol (Mapiye et al., 2011). Chen and Liu (2020) reported shellfish PUFA/SFA ratios between 0.20 and 2.10. In this study, the PUFA/SFA ratio exceeded 0.45 and was within the recommended range for shellfish. Thus, *P. viridis* can be considered to have a well-balanced and favourable fatty acid profile. Another mussel species, *Perna perna* (Linnaeus, 1758), had an almost similar PUFA/SFA ratio of 0.63 (Gualda et al., 2018). In *M. galloprovincialis*, the PUFA/SFA ratios varied from 0.69–3.08 and were also within the recommended range (Peycheva et al., 2021). Notably, when the same species, in the winter season, showed higher n-3 and n-6 PUFAs. Leading to increased PUFA/SFA ratios (Dernekbasi et al., 2015).

The dietary effect of mussel lipid consumption on serum cholesterol levels can be assessed using the CSI and CI. CSI defines the lipid quality of the food and is also a good indicator of atherogenic risk expressed on a scale of 1 to 100 (Di Renzo et al., 2017). The CSIs of *P. viridis* and *M. modulaides* in the present study (3.003 and 3.453, respectively), as well as their CIs (5.26 and 4.08, respectively), are comparable with reports for the Black Sea mussel lipids (Soultani, 2016; Merdzhanova et al., 2017). Lower values of both indices indicate high functional properties and a reduced likelihood of cardiovascular disease upon consumption (Soultani, 2016; Di Renzo et al., 2017).

The ash content suggests the amount of minerals in a food sample. The results of the present study revealed that the ash content of the two Philippine mussel species exceeds that of Indian *P. viridis*, which ranged from 0.99–1.42 % (Chakraborty et al. 2016) but within the range of those observed for Irish *Mytilus* sp. at 2.2–3.4 %. In terms of the mineral content, the results agree with the findings by Chakraborty and Joy (2020), indicating high levels of potassium (320 mg.100 g^{-1}) and sodium (286 mg.100 g^{-1}) in *M. edulis*. Generally, seafood is an excellent dietary source of different minerals (Gopakumar, 1997; Reames, 2012). The present study showed that sodium, potassium, iron, and calcium are present in the two mussel species. *Modiolus modulaides* demonstrated a superior iron at 34.6 mg.100 $g^{-1,}$ twice that in *P. viridis* (17.2 mg.100 g^{-1}). According to US FDA guidelines, both mussel species meet the recommended daily intake value for iron, hence, they can be considered a good source of iron. Furthermore, *M modulaides* has a better source of potassium than *P. viridis* but exhibits comparable levels of sodium and calcium contents.

Conclusion

The two mussels from the Philippines, *Perna viridis* and *Modiolus modulaides*, have high protein content, quality and considerable amounts of nutritional lipids. The nutritional composition of the mussels may vary due to differences in environmental factors, sampling sites, spawning, and food availability. However, the influence of these factors on the nutritional quality of the mussels was not elucidated in the present study. Amino acid profiles of these two species showed high amounts of EAA and NEAA content, with EAAI and DIAAS values indicating nutritional claims as a good quality protein source. Among the SFAs, palmitic acid and stearic acid were most abundant in both mussel species, while polyunsaturated fatty acids (PUFAs) were only detected in *P. viridis*. Minerals were also found in both mussel species at levels that could contribute to fulfilling humans' recommended daily dietary intake. Not only does this information on the mussels' biochemical composition and nutritional properties support their utilisation as a nourishing food source for animals and humans, but it also opens avenue for potential applications in nutraceuticals.

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experiments, data analysis, writing-editing. May Flor S. Muegue: Conceptualisation, conducted the experiments, data analysis, writing-editing.

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