International Food Research Journal 30(3): 736 - 749 (June 2023)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antioxidant capacity and sequence of peptides derived from oysters and green mussels in Vietnam

[1]Anh, P. T. H., [1]Trang, H. P., [1]Thanh, B. Đ., [1]Trinh, N. T. N., [1]Thang, T. Đ., [2]Phuong, D. L., [1]Tuan, N. N. and [1]*Huyen, T. T.

[1]*Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Vietnam*
[2]*Institute of Natural Product Chemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam*

Article history

Received:
10 July 2022
Received in revised form:
15 October 2022
Accepted:
3 January 2023

Keywords

antioxidant activity,
hydrolysates,
oyster,
green mussel,
bioactive peptides

Abstract

High-nutrition components and antioxidant compounds have been discovered in many marine organisms in recent years, especially oysters and mussels; but, the two bivalves have not been studied extensively in Vietnam. Therefore, both oysters and green mussels from Long Son, Vietnam were collected for investigation. Results showed that the total macronutrient contents in green mussels were greater than those of oysters, with glutamic acid being the most abundant amino acid in both oysters and green mussels at 1.35 and 1.25 g/100 g, respectively. Total relevant mineral content was 521,954 mg/100 g in oyster flesh, while that in green mussels was 496,847 mg/100 g. Specifically, the zinc content was 30.30 mg/100 g in oysters, 21.96 times more than that in green mussels (1.38 mg/100 g). Biological mixtures of peptides were obtained through enzymatic hydrolysis, and their calculated catalytic efficiencies in oysters and green mussels were 0.051 and 0.067 mg/mL/s, respectively. The antioxidant activity of these peptides was determined by IC_{50} values in DPPH, with 6.39 mg/mL for oysters, and 10.4 mg/mL for green mussels, and IC_{50} values in ABTS with 18.0 mg/mL for oysters, and 18.3 mg/mL for green mussels. The sequences of the four most abundant peptide fractions in oyster and green mussel hydrolysates were identified by LC-MS; the two fractions identified in oysters were Asn-Lys-Gln-Ala (F1) and Val-Val-Val-Asp-Val-Gly-Ile (F2), and those in green mussels were Gly-Arg-Thr-Tyr (F3), and Pro-Thr-Gln-Val-Lys-Leu (F4). Tyrosine, a powerful nucleophile, was found in F3. As a result, it is reasonable to believe that green mussels have stronger ABTS free radical scavenging activity than oysters. These results will provide a real insight for research on antioxidant processes involving biological peptides from oysters and green mussels in Vietnam.

Abbreviation

OD: optical density; DPPH: 1,1-diphenyl-2-picrylhydrazy; ABTS: 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); IC_{50}: the half-maximal inhibitory concentration.

DOI
https://doi.org/10.47836/ifrj.30.3.16
© All Rights Reserved

Introduction

Marine organisms are receiving more and more attention in research and development due to their biological activities. Oysters and mussels are examples of marine organisms that have recently been found to have various health and nutritional benefits. Not only these marine organisms have minerals such as zinc, manganese, and copper; and vitamins C, D and E; they are also rich in protein (Chakraborty *et al.*, 2011; Normah *et al.*, 2013; Sreejamole and Radhakrishnan, 2016; Cheong *et al.*, 2017). In Vietnam, oyster production increased quite rapidly from 792 tons in 2002 to 2,743 tons in 2007, and to 25,000 tons in 2014 (Thu *et al.*, 2018). Green mussels have also been cultured in many coastal zones of Vietnam (Bui, 2012). However, there are not many in-depth studies determining the nutrient contents or biological activities of both bivalve groups cultured in Vietnam.

*Corresponding author.
Email: tranthihuyen@iuh.edu.vn

Due to many marine organisms living in more extreme conditions than those found on land, various valuable nutritional components and biological peptides exist in them. Bioactive peptides have been receiving much interest due to their significantly different amino acid contents (Wang *et al.*, 2017; Lordan *et al.*, 2011). For example, several peptides (SCAP1) isolated from oyster (*Saccostrea cucullata*) have been revealed to have anticancer and antioxidant properties; another oyster (*Crassostrea gigas*) has been discovered to be a potent ACE (angiotensin I-converting enzyme) inhibitor containing five special residues of Asp-Leu-Thr-Asp-Tyr (Shiozaki *et al.*, 2010; Umayaparvathi *et al.*, 2014). Apart from that, oyster hydrolysates showed powerful immune stimulatory effects in mice by their anti-tumour properties (Wang *et al.*, 2010). With green mussels, the antioxidant activity in 1 g of powder was found to be 14.5 mg ascorbic acid equivalent (EAA)/g extract, with 36% radical scavenging activity and 76% reducing power as compared to ascorbic acid (Vijaykrishnaraj *et al.*, 2015). Several recent studies have reported that the peptides in oyster have some biological activities, and oyster meat hydrolysates have also been demonstrated to have high antioxidant power; at the same time, the antioxidant nature has been confirmed to depend upon the relationship of concentration, length, and amino acid composition (Dong *et al.*, 2010). Bioactive ingredients contained in mussels have also been shown to have an important role in the development of functional foods (Vijaykrishnaraj *et al.*, 2016). Besides that, many researchers have proposed that peptides containing from 2 - 16 amino acid radicals have strong antioxidant effects brought about by their easy absorption. However, until now the relationship of the length and amino acid sequence with antioxidant properties has not been sufficiently examined (Sarmadi and Ismail, 2010; Tang *et al.*, 2010).

To produce bioactive peptides, a protein mixture can be treated either by acid or alkali. However, this method produces by-products and other harmful substances to the environment, and it is not easy to remove the acid or alkali from the produced mixture (Truc *et al.*, 2015). Enzymatic hydrolysis of proteins is thus more preferred, and has been developed to produce bioactive peptides, which are applied in fields such as nutrition or functional foods (Korhonen, 2009; Cheung and Li Chan, 2010; Normah *et al.*, 2013). Therefore, the main purpose of the present work was to create peptides through hydrolysis, by protease enzyme from powders of oysters and green mussels, in order to determine their catalytic efficiency, followed by assessment of the antioxidant activity, and determination of the mass charge of the hydrolysed peptides. The results obtained from the present work could provide information on useful sources of raw materials for health supplements.

Materials and methods

Materials

Oyster (*Crassostrea gigas*) and green mussel (*Peran viridis*) samples were purchased from a farm in Long Son, Vietnam. They are harvested in the second quarter (from early April to the end of June) of the year. The oysters and green mussels were kept in an ice box at 4°C, and transported to the laboratory. Oysters and green mussels were weighed and evaluated for macronutrient and micronutrient contents before being chopped and dried for hydrolysis.

SEB-neutral PL is a liquid protease enzyme used to break down proteins, thus increasing the solubility and dispersion of proteins. The enzyme was obtained from Hung Thanh Viet Nam Commerce Promotion and Services Investment (Hien *et al.*, 2021).

Methods

Determining the macronutrient and micronutrient compositions in oysters and green mussels

Microwave digestion of the samples was performed with nitric acid using a microwave digestion system as a pre-treatment of samples for mineral analysis. The ion contents of lead (Pb), calcium (Ca), zinc (Zn), copper (Cu), potassium (K), magnesium (Mg), and iron (Fe) were determined by flame atomic absorption spectrophotometry (AAS) (Jorhem and Engman, 2000). The iodine (I) and phosphorus (P) contents was determined by the inductively coupled plasma mass spectrometry method (ICP-MS) (Zywicki and Sullivan, 2015). In addition, high performance liquid chromatography (HPLC) was also used to determine the quantity of vitamins (Devries and Silvera, 2002). Protein was measured by Kjeldahl method (Futagawa *et al.*, 2011).

The amino acid contents were determined following Futagawa *et al*. (2011). Briefly, the sample was diluted with sample diluent (20 µL, 5 mM

sodium phosphate Na_2HPO_4 buffer, pH 7.4: acetonitrile; 95:5 v/v) before injected into reversed phase binary gradient high-performance liquid chromatography (HPLC), and then detected upon their UV absorbance at wavelength of maximum absorbance (λmax: 254 nm). The mobile phase were eluents A and B; eluent A was 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 was 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 (Chakraborty *et al.*, 2016).

Effect of enzyme concentration, incubation time, and substrate concentration on hydrolysis of oyster and green mussel (Hien et al., 2021)

The effect of enzyme concentration, incubation time, and substrate concentration on hydrolysis of oyster and green mussel was determined following Hien et al. (2021). Mixture reaction was prepared by mixing 2.5 g of oyster or green mussel powders with 100 mL of 0.05 M Tris hydrochloride (Tris-HCl, pH 7.0), and then hydrolysed with a series of concentrations of protease enzyme (0.5, 1.0, 1.5, and 2.0 mg/mL) at room temperature (25°C) for three time periods (360, 488, and 600 min). After that, the hydrolysis reaction was stopped by heating at 90°C for 15 min. Then, the supernatant layer containing water-soluble hydrolysate was collected by centrifugation at 13,000 rpm for 15 min. To test the enzyme's hydrolysis capacity, 2 mL of hydrolysate and 1 mL of 0.5 M Folin and Ciocalteu's reagent solution were mixed in 0.5 M sodium carbonate, and then incubated at 50°C for 30 min. The quantity of the hydrolysed products was measured by spectrophotometer at 660 nm (Sigma-Aldrich).

Degree of hydrolysis (DH%)

The degree of hydrolysis was measured using the ninhydrin colorimetric assay according to Gao *et al*. (2020) with slight modifications. Ninhydrin (1.5 g) was prepared in 10 mL of acetate buffer, followed by the addition of 60 mL of ethylene glycol, 15 mL of *n*-propanol, and 15 mL of *n*-butyl alcohol. The mixture reaction was performed by adding 3 mL of hydrolysate into 1 mL of ninhydrin solution, and the hydrolysis reaction was boiled for 20 min. After the reaction time, the mixture was cooled in an ice bath, and later combined with 1 mL of ethanol solution (40% v/v) to develope the colour reaction. The sum quantity of the hydrolysed product was measured by absorbance at 570 nm, and glycine was used as the standard. The DH% value was calculated by the formula (Saito *et al.*, 2003): $DH = [(A_2 - A_1) / A_0] \times 100\%$; where A_2 was the concentration of free amino nitrogen of hydrolysate (mg/mL), A_1 was the concentration of free amino nitrogen of protein (mg/mL), and A_0 was the concentration of total amino nitrogen of protein (mg/mL).

Catalytic efficiency of protease enzyme on oyster and green mussel

After the proteolytic reaction of oysters and green mussels, the enzyme kinetics determination was carried out based on the refined optimal conditions in the previous results by mixing 2.5 g oyster or 4.5 g green mussel powder with 100 mL of 0.05 M Tris-HCl (pH 7.0). The hydrolysis reaction was performed with the addition of 2 mg/mL of enzymatic protease for each reaction, and immediately incubated at 25°C for 480 min. After 480 min incubation, the hydrolysis reaction was inactivated by heating at 90°C for 15 min. The enzyme catalytic efficiency was determined following the Briggs-Haldane's approach (Schnell and Maini, 2000) using Eq. 1:

$$S+E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_{cat}} E+P \quad \text{(Eq. 1)}$$

where, E = protease (mg/mL); S = oyster or green mussel powders (mg/mL); P = peptides (mg/mL), and K = rate constant.

Enzymes are natural catalysts that speed up chemical reactions. The binding capacity, velocity, and catalytic efficiency of the reaction were calculated based on the Briggs-Haldane equation (Schnell and Maini, 2000) as shown in Eq. 2:

$$v = \frac{V_{max}[S]}{K_m+[S]} \quad \text{(Eq. 2)}$$

where, V_{max} = maximum velocity achieved by the system, at maximum substrate concentrations; K_m (the Michaelis constant) = $(K\text{-}1 + K_{cat}) / K1$; S = concentration of the substrate S.

Finally, the kinetic parameters were calculated using Prism GraphPad software, and the K_{cat}/K_m value was determined to describe the catalytic efficiency of the enzyme.

Content of peptides containing tyrosine from the hydrolysis of oyster and green mussel powders

Firstly, a mixture reaction was prepared by mixing 2.5 g oyster or 4.5 g green mussel powder with 100 mL of 0.05 M Tris-HCl (pH 7.0). The hydrolysis reaction was performed with the addition of 2 mg/mL enzymatic protease for each reaction, and immediately incubated at 25°C for 480 min. After 480 min incubation, the hydrolysis reaction was inactivated by heating at 90°C for 15 min. The precipitate was then removed by centrifugation at 13,000 rpm for 15 min, and each 2 mL of the supernatant was neutralised with 2.5 mL of 0.5 M sodium carbonate and followed by an incubation period at 50°C for 30 min with 1 mL of Folin and Ciocalteu's reagent solution. Subsequently, the absorbance of mixture reaction was measured at 660 nm. Finally, the content of peptides containing tyrosine generated from the hydrolysis of oyster and green mussel meal was calculated based on the standard curve of L-tyrosine (Sigma-Aldrich).

Antioxidant capacity of oyster and green mussel hydrolysates

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity

The DPPH radical scavenging activity was determined following Qian *et al.* (2020) with light modification. Briefly, 1.0 mL of hydrolysate at various concentrations were mixed with 4.0 mL of 0.076 mM DPPH dissolved in methanol. After vortexing, the solution was kept in the dark at 25°C for 30 min, and then the absorbance was measured at 517 nm against methanol as blank. Lower absorbance indicated higher radical-scavenging activity. The DPPH radical-scavenging activity was expressed as (Saito *et al.* 2003): scavenging rate = $[(A_{control} - A_{sample}) / A_{control}] \times 100\%$; where, $A_{control}$ = absorbance of the control (DPPH solution without sample); and A_{sample} = absorbance of the test sample. The standard curve was constructed using a series of concentrations of ascorbic acid to replace the tested samples. Three replicates were completed for each test.

ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical antioxidant activity

The antioxidant activity was determined following Centenaro *et al.* (2011) with slight modifications. The cationic radical ($ABTS^{\bullet+}$) was prepared by mixing two stock solutions (7 mM ABTS dissolved in deionised water and 2.45 mM sodium persulfate) in a 1:1 ratio, and the mixture was incubated for 16 h at 25°C in the dark. Then the stock solution was diluted with deionised water to an absorbance of 0.70 ± 0.02 at 734 nm. The reaction began with 100 µL of hydrolysate at various concentrations mixed with 3 mL of $ABTS^{\bullet+}$ solution, shaken, and incubated for 6 min. Absorbance was measured at 734 nm. The percentage of ABTS radical scavenging activity was calculated by the formula: $[(A_{control} - A_{sample}) / A_{control}] \times 100\%$; where, $A_{control}$ = absorbance of the control ($ABTS^{\bullet+}$ solution without sample); and A_{sample} = absorbance of the test sample. The standard curve was constructed using a series of concentrations of ascorbic acid to replace the tested samples. Three replicates were finished for each test.

Peptide sequence determination by liquid chromatography-mass spectrometry (LC-MS)

The oyster hydrolysate was digested with 6 N acid hydrochloric (HCl) solution for 24 h at 110°C, then evaporated to dryness at 40°C in vacuum. Amino acids were separated at 37°C using a reverse phase HPLC with a gradient mobile phase of deionised water, acetonitrile, and eluent A (sodium acetate, phosphoric acid, and triethylamine), and amino acids were detected by a UV detector at 220 nm. The amount of amino acids was calculated by comparing the peak area with standards (Umayaparvathi *et al.*, 2014).

Peptides were separated and sequenced using an LC-MS system (micrOTOF-QII Bruker Daltonic, Germany). Total ion chromatogram was obtained in information dependent acquisition mode with a linear ion trap mass spectrometer (AB Sciex 4000 QTrap) coupled to a UPLC (Waters Acquity), equipped with a C_{18} column (BEH 130 peptide; 1.7 µL; 2.1 × 100 mm). A gradient elution program was used, with 99% eluent A (water containing 0.1% TFA) and 1% eluent B (acetonitrile containing 0.1% TFA); 0 min to 1% eluent A and 99% eluent B in 33 min. Enhanced mass spectra (EMS) and enhanced resolution spectra of the prominent peak eluting at 23.8 min were obtained.

Further enhanced product ion (EPI) was acquired and exported to Mascot software to determine the peptide sequence (Asha *et al.*, 2016).

Results

Protein contents and amino acid compositions of oysters and green mussels

The amino acid compositions of oysters and green mussels in Vietnam are shown in Table 1. The free amino acid contents of Vietnamese and Chinese oysters were similar and significantly lower than that reported previously in Japanese oysters (Futagawa *et al.*, 2011). The protein content of Japanese oysters (7.52 ± 0.05 g/100 g), on the other hand, was lower than that of Vietnamese and Chinese oysters (9.72 ± 0.006 and 10.7 ± 0.49 g/100 g, respectively). This can be explained by the fact that Vietnamese oysters were professionally handled and stored, samples had not been decomposed, and had better quality than Japanese oysters (Erbas *et al.*, 2005). Glutamic acid was the amino acid with the highest content in both oysters and green mussels in Vietnam (1.35 and 1.25 g/100 g meat, respectively), but its content was much lower than in Japanese oysters (5.04 ± 0.43 g/100 g). Total amino acids and total essential amino acids in green mussels were higher than in oysters, but the E/T (essential/total amino acids) and E/N (essential/nonessential amino acids) values of oysters were higher in green mussels, and higher than in oysters cultured in Japan.

Several amino acids such as glutamic acid, histidine, arginine, and lysine have been found to have antioxidant activity (Pérez *et al.*, 2007; Dash and Ghosh, 2017). In addition, phenylalanine's aromatic ring interacts with free radicals to create stable molecules, which may also be why it increases antioxidant activity (Li *et al.*, 2013). Therefore, the fact that oysters and green mussels cultured in Vietnam also contained significant amounts of these amino acids (glutamic acid being the main amino acid) indicated their ability to produce hydrolysed peptides with potential antioxidant activity (Table 1).

Micronutrient compositions of oysters and green mussels

Of the micronutrient, B vitamins play an important role in promoting enzyme activity, growth, and development of the body, especially vitamin B_{12} which is essential for the production of blood cells from bone marrow and formation of the nerve sheath (myelin sheath). Vitamin composition analysis of oysters and green mussels in Vietnam showed that B vitamins had the highest concentration, particularly vitamin B_{12}: 45.7 ± 0.05 µg/100 g in oysters and 18.0 ± 0.33 µg/100 g in green mussels (Table 1). Moreover, oysters also contain high vitamin B_1 content of 3.69 ± 0.02 mg/kg. Vitamin A, also known as retinol, contributes to many important tasks for the human body; vitamin A deficiency will cause dry skin, damage to the synovial membrane, or when phenomenon spreads to the cornea of the eye, it will affect vision and may cause corneal softening. The contents of vitamin A in Vietnamese oysters and green mussels were 0.26 ± 0.01 mg/kg and 712 ± 0.58 IU/100 g, respectively; there are no reported data on vitamin A contents in these species elsewhere in the world.

The essential mineral composition for human health has been proven to be present in oysters and mussels (Özden *et al.*, 2009). Results of the present work revealed that the mineral content of Vietnamese oysters was higher than that of farmed oysters from Japan, but lower than that of those from China, which might have been due to differences in species, seasons, locations, and characteristics (Özden *et al.*, 2009; Futagawa *et al.*, 2011). Potassium (K) is an abundant mineral in oysters and green mussels, participating in water balance and regulating the normal functioning of organs. Results showed that K was the most abundant in oysters (189 ± 0.5 mg/100 g) and green mussels (162 ± 0.33 mg/100 g) in Vietnam, its content being much higher than that in Japanese oysters (20 ± 1.4 mg/100 g) (Futagawa *et al.*, 2011). Secondly, both calcium (Ca) and magnesium (Mg) are extremely important factors in the food field, hence having an imbalance of these minerals can lead to calcification or hardening of the arteries. The requirement of an average person is to have a Ca:Mg ratio of 2:1, so the Ca:Mg ratio in the oysters (1.6:1) and green mussels (2.5:1) in Vietnam seems to meet the need for human health, while the ratio observed in oysters from Japan and China is 0.4:1 and 1.2:1, respectively. Our results revealed that the ratio of Ca to Mg in oyster and green mussel samples in Vietnam was superior for the absorbance of important ions into the human body.

Iron (Fe) is an essential mineral for the formation of haemoglobin and myoglobin, but Fe levels in the body should not exceed 10 mg per day. In comparison to the content of 0.22 ± 25 mg Fe/100 g in Japanese oysters and 131 ± 0.30 mg Fe/100 g in

Table 1. Macronutrients and micronutrients in oysters and green mussels in Vietnam and other countries.

Content	Green mussels in Vietnam	Oysters in Vietnam	Oysters in Japan (Futagawa *et al.*, 2011)	Oysters in China (Chen *et al.*, 2012)
Macronutrient (g/100 g)				
		Amino acid		
Histidine*	0.18 ± 0.005	0.19 ± 0.017	0.62 ± 0.06	0.19 ± 0.00
Arginine**	0.67 ± 0.012	0.54 ± 0.006	1.92 ± 0.13	0.50 ± 0.06
Threonine*	0.40 ± 0.023	0.37 ± 0.012	1.47 ± 0.13	0.37 ± 0.06
Valine*	0.39 ± 0.009	0.25 ± 0.012	1.50 ± 0.15	0.37 ± 0.06
Methionine*	0.22 ± 0.007	0.28 ± 0.003	0.59 ± 0.05	0.16 ± 0.12
Isoleucine*	0.39 ± 0.003	0.23 ± 0.017	1.35 ± 0.14	0.31 ± 0.12
Leucine*	0.61 ± 0.011	0.56 ± 0.003	2.13 ± 0.21	0.55 ± 0.12
Phenylalanine*	0.36 ± 0.006	0.28 ± 0.006	1.26 ± 0.12	0.29± 0.06
Lysine*	0.70 ± 0.003	0.52 ± 0.003	2.00 ± 0.23	0.60 ± 0.17
Glutamic acid**	1.35 ± 0.012	1.25 ± 0.003	5.04 ± 0.43	1.19 ± 0.50
Proline**	0.42 ± 0.014	0.38 ± 0.003	1.91 ± 0.15	0.34 ± 1.25
Glycine**	1.08 ± 0.014	0.63 ± 0.012	2.43 ± 0.19	0.59 ± 0.32
Alanine**	0.52 ± 0.015	0.48 ± 0.006	1.62 ± 0.14	0.47 ± 0.46
Cystine**	0.20 ± 0.008	0.14 ± 0.003	-	0.09 ± 0.06
Aspartic acid**	0.94 ± 0.009	0.88 ± 0.007	3.47 ± 0.25	0.79 ± 0.93
Tyrosine**	0.38 ± 0.003	0.30 ± 0.003	0.68 ± 0.18	0.29 ± 0.06
Serine**	0.45 ± 0.009	-	1.49 ± 0.12	0.38 ± 0.06
Tryptophan*	-	-	-	0.12 ± 0.06
Taurine	-	-	3.92 ± 0.32	-
Citrulline	-	-	0.06 ± 0.03	-
TAA	9.26	7.28	33.5	7.62
TEAA	3.25	2.68	10.9	2.96
E/T (Saito *et al.*, 2003)	35.1	36.8	32.6	38.8
E/N (Saito *et al.*, 2003)	54.1	58.3	48.2	63.5
Protein	11.4 ± 0.088	9.72 ± 0.006	7.52 ± 0.05	10.7 ± 0.49
Micronutrient (g/100 g)				
Potassium (K)	162 ± 0.33[a]	189 ± 0.5[a]	20.0 ± 1.4[a]	-
Calcium (Ca)	117 ± 0.33[a]	105 ± 0.5[a]	2.60 ± 0.39[a]	445 ± 0.59[a]
Iron (Fe)	1.82 ± 0.01[a]	7.47 ± 0.01[a]	0.22 ± 25[a]	131 ± 0.30[a]
Magnesium (Mg)	46.3 ± 0.03[a]	65.4 ± 0.1[a]	6.39 ± 0.3[a]	371 ± 0.12[a]
Zinc (Zn)	1.38 ± 0.003[a]	30.3 ± 0.15[a]	0.53 ± 39[a]	124. ± 0.06[a]
Phosphorus (P)	168 ± 0.58[a]	121 ± 0.5[a]	12.8 ± 3.1[a]	-
Iodine (I)	117 ± 0.33[b]	94.6 ± 0.05[b]	-	-
Copper (Cu)	0.23 ± 0.01[a]	3.69 ± 0.02[a]	0.24 ± 15[a]	26.0 ± 0.01[a]
Lead (Pb)	0.10 ± 0.003[c]	< 9.10[c]	-	-
Vitamin A	712 ± 0.58[d]	0.26 ± 0.01[c]	-	-
Vitamin B_1	N.D.	3.69 ± 0.02[c]	-	-
Vitamin B_{12}	18.0 ± 0.33[b]	45.7 ± 0.05[b]	-	-
Vitamin D	< 48.0[d]	N.D.	-	-

*essential amino acids; **non-essential amino acids; TAA: total amino acids; and TEAA: total essential amino acids. E/T: ratio of TEAA and TAA. E/N: ratio of TEAA and non-essential amino acid. [a]mg/100 g; [b]µg/100 g; [c]mg/kg; and [d]IU/100 g. N.D.: not detected.

Chinese oysters, the Fe concentration in Vietnamese oysters of 7.47 ± 0.01 mg/100 g was sufficient for the body's needs. Another mineral, zinc (Zn), is one of the indispensable micronutrients for the human body due to its role in the biochemical processes taking place inside our body. However, too much Zn can increase the risk of cardiovascular disease. That is the reason for the recommended Zn intake being only 11 mg/day, and the advice not to use more than 40 mg per day. Vietnamese oysters yielded Zn content of 30.3 ± 0.15 mg/100 g, enough for the body's requirement, while the content of Japanese oysters was 10-fold lower (0.53 ± 39 mg/100 g); in contrast, that of Chinese oysters was 10-fold higher (124 ± 0.06 mg/100 g). Similarly, based on the recommended copper (Cu) intake of around 1 to 5 mg per day, the calculated Cu content of 3.69 ± 0.02 mg/100 g in Vietnamese oyster meat was in the average range of the body's requirement, and was higher than that in Japanese oysters of 0.24 ± 15 mg/100 g. Finally, the safety level of heavy metals such as lead (Pb), is of great concern to human health. Based on the maximum permissible Pb content in bivalve molluscs being 1.5 mg/kg (QCVN 8-2:2011/BYT), the Pb content of both oyster (9×10^{-2} mg/kg) and green mussel meat (0.10 ± 0.003 mg/kg) met the safe threshold of the Ministry of Health. In conclusion, the total mineral contents in oysters were higher than those of mussel meat assessed in the present work.

Effect of enzyme concentration, incubation time, and substrate concentration on hydrolysis of oysters and green mussels

The effects of enzyme concentration on hydrolysis were demonstrated by different results from four reactions carried out with a series of protease enzyme concentrations (0.5, 1.0, 1.5, and 2.0 mg/mL) to hydrolyse 15 mg of oyster powder at 25°C for three different time periods. The higher enzyme concentrations and longer reaction times created a higher number of products, with an enzyme concentration of 2 mg/mL yielding the maximum number of products in the three time periods. In each period, the created product rate increased gradually in reaction periods of both 480 and 600 min; in contrast to that, the rate in 360 min was lower and almost remained at the minimum level. Furthermore, the three curves of the hydrolysis reactions reached steady state at an enzyme concentration of 2 mg/mL, hence the optimum hydrolysis time of oyster powders was 480 min, and the optimum enzyme concentration was 2 mg/mL (Figure 1).

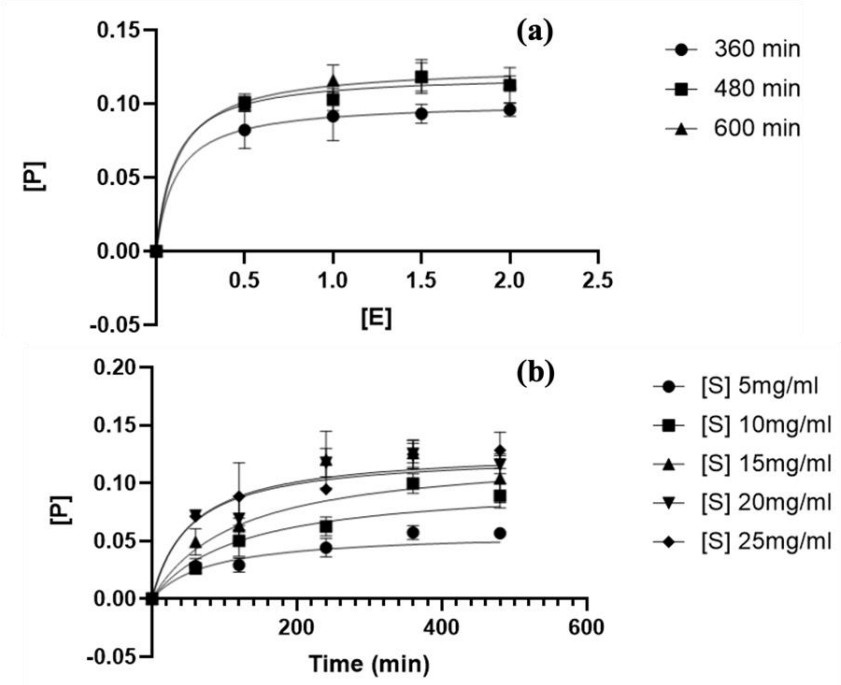

Figure 1. Effect of enzyme concentration, incubation time,and substrate concentration on hydrolysis reaction.

The influence of five oyster powder concentrations from 5 to 25 mg/mL within the periods of 60, 120, 240, 360, and 480 min, was investigated. In the same condition of 2 mg/mL enzyme protease, the concentration of 25 mg/mL oyster powder, and a reaction period of 480 min provided the best conditions for the hydrolysis of oyster powder. With these optimum conditions, the hydrolysis of green mussel powder was also performed with the same procedure; the optimal substrate concentration was found to be 45 mg/mL. Based on these results, we decided to hydrolyse oysters (25 mg/mL) and green mussels (45 mg/mL) for 480 min using protease enzyme (2 mg/mL) to investigate the catalytic efficiency of protease enzyme (Figure 2).

Degree of hydrolysis (DH%)

The DH% for oysters and green mussels was 52.34 ± 0.27 and 29.40 ± 0.43% (Figure 3), respectively, higher than the value obtained for hydrolysis of oysters (*Crassostrea madrasensis*) by papain and pepsin (27.3 and 22.4%, respectively) (Asha *et al.*, 2016), or of green mussels (*Perna canaliculus*) by pepsin and Alcalase (7 and 10%, respectively) Jayaprakash and Perera, 2020). These indicated that protease and other optimum parameters such as reaction temperature, incubation time, and substrate concentration in the present work were more appropriate for hydrolysing oysters and green mussels.

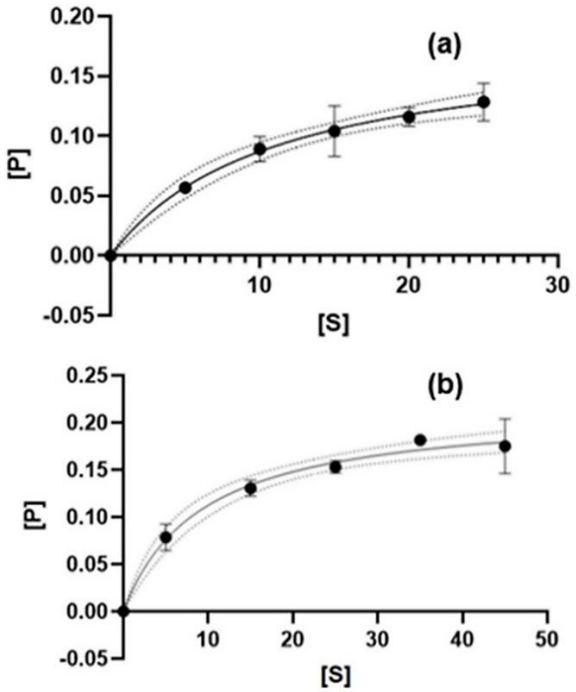

Figure 2. Kinetic of protease enzyme on oyster **(a)** and green mussel **(b)**.

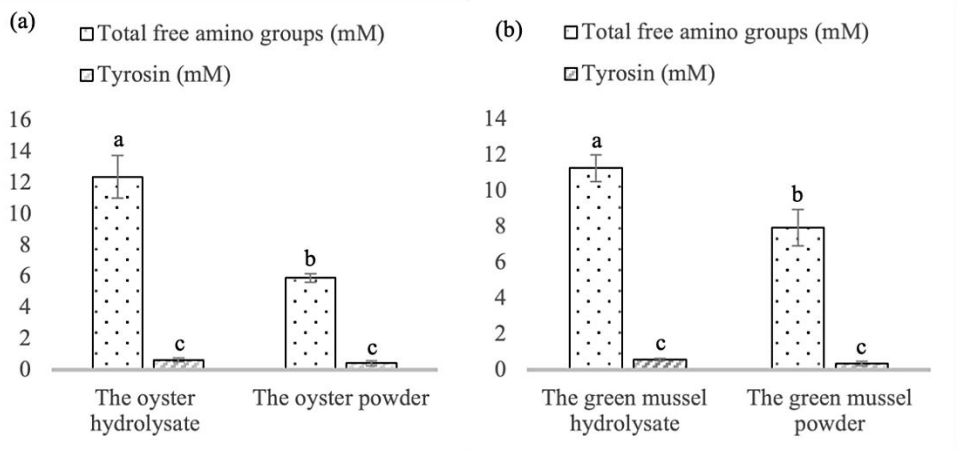

Figure 3. Tyrosine content in oyster **(a)** and green mussel **(b)** hydrolysates. Values are mean ± standard deviation of three replicates (n = 3). Different lowercase letters indicate significant difference at $p < 0.05$.

Catalytic efficiency of protease enzyme on oyster and green mussel powders

After finding the optimal hydrolysis conditions, protease enzyme kinetics were established on the substrate of oyster and green mussel powders following the Briggs-Haldane approach. The constant K_m is an important property of an enzyme-catalysed reaction, and has implications for its biological function. The Km in oysters and green mussels were determined to be 10.93 and 9.238 mg/mL, respectively. V_{max} is the maximum velocity that can only be achieved when all enzymes are bound to the substrate. In the present work, the hydrolysis reaction of oysters and green mussels had a V_{max} of 0.1821 and 0.2163 mM/s, respectively. Table 2 shows the K_{cat}/K_m ratio of the oyster hydrolysis reaction to be 0.051 mg/ml/s while that for green mussels to be 0.067 mg/ml/s, thus showing that the affinity of the protease enzyme for green mussels was higher than that for oysters.

The antioxidant activity of tyrosine has been demonstrated by the presence of phenolic groups, which have a special ability to donate hydrogen, which in turn is a mechanism to prevent radical-mediated peroxidation chain reactions (Ren *et al.*, 2008). Analysis of the content of peptides involving tyrosine in the hydrolysate (Figure 3) showed that the hydrolysed product in green mussel contained 5.15%, an increase of 0.62% as compared to the starting material (4.53%), while that in oyster decreased from 7.44 to 5.16%. This indicated that protease hydrolysis of green mussel powder produced peptides containing tyrosine more than that of oysters, which may affect their antioxidant capacity.

Table 2. Summary of the bioactivities of hydrolysed products from oyster and green mussel proteins.

		Kcat/Km (mg/ml. s^{-1})	**DPPH (Saito *et al.*, 2003)**	**ABTS (Saito *et al.*, 2003)**	**Peptide**	**Retention time (min)**
Oyster	F1	0.051	82.92	61.15	Asn-Lys-Gln-Ala	18.6
	F2				Val-Val-Val-Asp-Val-Gly-Ile	15.1
Green mussel	F3	0.067	79.98	81.61	Gly-Arg-Thr-Tyr	20.6
	F4				Pro-Thr-Gln-Val-Lys-Leu	22.3

Antioxidant capacity of oyster and green mussel hydrolysates

1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity

DPPH is a stable free radical with strong absorption at 517 nm. When DPPH encounters a proton-donating substance, the radical is scavenged by changing colour from purple to yellow; radical scavenging activity is measured by a decrease in its absorption (Srivastava *et al.*, 2006). The DPPH radical scavenging activity of oyster and green mussel hydrolysates at different concentrations is shown in Table 2. The results showed that the scavenging effect increased with increasing hydrolysate concentration, meaning the DPPH radical scavenging capacity was dose-dependent. The DPPH radical scavenging capacity of oyster and green mussel hydrolysates (IC_{50} values of 6.39 and 10.4 mg/mL, respectively) was found to be significantly lower than that of vitamin C (IC_{50} values of 3.56×10^{-3} mg/mL). The hydrolysate from oysters exhibited the highest DPPH radical scavenging activity of 82.92 ± 0.27% at a concentration of 25 mg/mL; green mussel hydrolysate exhibited the highest DPPH radical scavenging activity of 79.98 ± 0.40% at 45 mg/mL. This showed that the free radical scavenging ability of Vietnamese oyster hydrolysate was higher than that of green mussel hydrolysate, and the scavenging ability of both these hydrolysates was higher than that obtained for oyster hydrolysate (*Crassostrea talienwhannensis*) using Alcalase in China (64.52%) (Dong *et al.*, 2010) and that of green mussels (*Perna viridis*) from the Southwestern coast of India (23.7-29%) (Chakraborty *et al.*, 2016).

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS free radical scavenging activity

The ABTS radical assay is a common method for measuring antioxidant activity. By reducing the colour of the ABTS radical, hydrolysates have been

identified as potential antioxidants (Centenaro *et al.*, 2011). In the present work, the peptides hydrolysed from oysters and green mussels exhibited free radical scavenging activity, and the results are shown in Table 2. Oyster and green mussel hydrolysates had significantly lower ABTS radical scavenging activity (IC_{50} values of 18.0 and 18.3 mg/mL, respectively) than vitamin C (IC_{50} values of 18.3×10^{-3} mg/mL). The hydrolysate from oysters exhibited the highest ABTS radical scavenging activity of 61.15 ± 0.17% at a concentration of 25 mg/mL; green mussel hydrolysate exhibited the highest ABTS radical scavenging activity of 81.61 ± 0.48% at 45 mg/mL. These showed that the hydrolysed products from green mussel powder had higher ABTS radical scavenging capacity than those from oyster powder. These in turn indicated that the peptides or free amino acids in the hydrolysates had the ability to donate hydrogen atoms to free radicals, and possibly interfere with the spread of lipid peroxidation (Faithong *et al.*, 2010). More specifically, the ABTS results for Vietnamese green mussel hydrolysate of 81.61 ± 0.48% were better than the radical scavenging activity in green-lipped mussel (*Perna canaliculus*) grown in New Zealand of around 77% (Jayaprakash and Perera, 2020).

Liquid chromatography-mass spectrometry (LC-MS)

An accurate molecular mass and amino acid sequence of the purified peptides were determined using a quadrupole time-of-flight (Q-Tof) mass spectrometer (Q-Tof-premier, Waters, Milford, MS) coupled with an electrospray ionisation source in positive mode (Tira-Picos *et al.*, 2009). Molecular mass was determined by a doubly charged $(M + 2H)^{2+}$ state in the mass spectrum. Spectra were recorded over the mass/charge (*m/z*) range 100 - 1,400. Following determination of molecular mass, the peptides were fragmented using low energy collision-induced dissociation to reveal peptide fragments for *de novo* sequencing. Sequence information was obtained by processing the raw spectra with Biolynx 4.1 (subroutine "peptide sequencing") (Umayaparvathi *et al.*, 2014).

Amino acid sequences and molecular weights of hydrolysed peptides from oysters and mussels were determined by LC-MS. The peptide mass list (mass H^+) generated from the major peaks of the spectra was submitted to a database search (SWISS-PROT) using MS-Fit (protein prospector), thereby predicting peptide sequences (Nesvizhskii, 2007). The amino acid sequences and molecular weights of the representative peptides were determined as Asn-Lys-Gln-Ala (F1, 587.33 Da) and Val-Val-Val-Asp-Val-Gly-Ile (F2, 699.42 Da) in oysters, and Gly-Arg-Thr-Tyr (F3, 638.26 Da) and Pro-Thr-Gln-Val-Lys-Leu (F4, 684.43 Da) in green mussels (Figure 4). The molecular weights of the detected peptides were in good agreement with the theoretical masses calculated from the sequence (Table 2).

Hydrolysed peptides from oysters and mussels were identified by peaks in the chromatogram showing the highest levels of the four peptides in the hydrolysate. The greater antioxidant activity of a hydrolysate might have been due to the composition and amino acid position of the peptides. Hydrophobic amino acids have a significant scavenging effect on radicals, and a high amount of hydrophobic amino acids in peptides can boost their antioxidant potential (Dong *et al.*, 2008). Besides that, several amino acids including Tyr, Met, His, Lys, Gly, and Trp are known to be antioxidative; if integrated into peptides, they may increase their antioxidant activity (Saito *et al.*, 2003; Memarpoor-Yazdi *et al*., 2013). In the present work, fractions of F2 and F4 were determined to contain mainly hydrophobic residues such as Val, Gly, Ile, Pro, and Leu, while most of the amino acids in fractions F1 and F3 were hydrophilic residues such as Asn, Lys, Tyr, Gln, and Arg. Theoretically, by the presence of individual H atoms of Gly residue or the NH_2 group of Asn, Arg, and Lys amino acids within the F1 and F3 peptide sequences, they could donate protons to neutralise electron-deficient radicals and then improve the radical scavenging ability of the peptides following the DPPH mechanism. In addition, since the hydroxyl group of Tyr also behaves as a hydrogen donor, a peptide with a Tyr residue at the C-terminus (F3) was found to have substantial free radical scavenging action (Saito *et al.*, 2003). Furthermore, because tyrosine's functional side chain is a strong nucleophile, it can donate electrons to positively charged compounds like $ABTS^{\bullet+}$, thus improving the peptides' radical scavenging capacity (Bischoff and Schlüter, 2012). Therefore, the presence of a Tyr residue at the C-terminal end of the peptide was considered to be the cause of the higher $ABTS^{\bullet+}$ free radical scavenging capacity in mussel hydrolysates than in oyster hydrolysates.

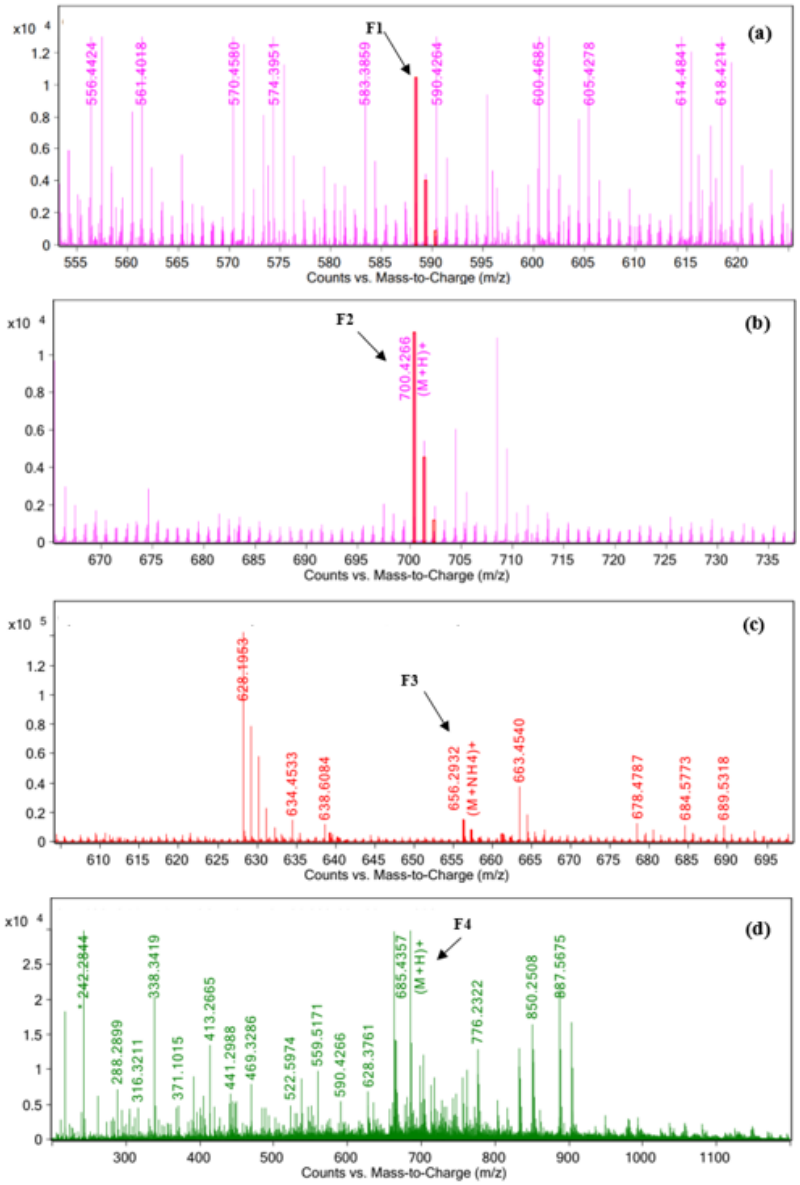

Figure 4. Peptide sequences corresponding to mass peaks obtained from the hydrolysis of oyster powders: **(a)** Asn-Lys-Gln-Ala and **(b)** Val-Val-Val-Asp-Val-Gly-Ile; and green mussel powders: **(c)** Gly-Arg-Thr-Tyr and **(d)** Pro-Thr-Gln-Val-Lys-Leu.

Conclusion

Oysters and green mussels were hydrolysed with protease enzyme to produce bioactive peptides with relevant catalytic efficiencies of 0.051 and 0.067 mg/mL/s, respectively. The antioxidant activity of hydrolysates containing bioactive peptides was evaluated by DPPH and ABTS free radical scavenging assays. ABTS free radical scavenging activity in green mussels was higher than in oysters, while DPPH free radical scavenging activity in oysters was slightly higher than in green mussels. To understand the reason for the enhancement in antioxidant activity, the hydrolysates of oysters and green mussels were analysed through LC-MS, and the sequences of the four most abundant peptides in the hydrolysed peptide mixture were determined. Fraction F1 (Asn-Lys-Gln-Ala) and F2 (Val-Val-Val-Asp-Val-Gly-Ile) were found in oyster hydrolysate, while fraction F3 (Gly-Arg-Thr-Tyr) and F4 (Pro-Thr-Gln-Val-Lys-Leu) were found in green mussel hydrolysate. Furthermore, each amino acid in the fraction was shown to impact the differences in free radical scavenging activity in oyster and green mussel hydrolysates. The amino acid sequences of the four hydrolysed bioactive peptides with antioxidant activity determined in the present work would be useful for further research on the antioxidant mechanisms of bioactive peptides.

Acknowledgement

The authors are thankful to the Ministry of Science and Technology, Vietnam (grant no.: NĐT/CU/22/25) for the financial support received in the completion of the present work.

References

Asha, K., Kumari, K. R., Kumar, K. A., Chatterjee, N. S., Anandan, R. and Mathew, S. 2016. Sequence determination of an antioxidant peptide obtained by enzymatic hydrolysis of oyster *Crassostrea madrasensis* (Preston). International Journal of Peptide Research and Therapeutics 22(3): 421-433.

Bischoff, R. and Schlüter, H. 2012. Amino acids: Chemistry, functionality and selected non-enzymatic post-translational modifications. Journal of Proteomics 75(8): 2275-2296.

Bui, Q. N. 2012. Growth characteristics of green mussel *Perna viridis* (Linnaeus, 1758) in culture area of lobster in Xuan Tu, Van Ninh, Khanh Hoa. Collection of Marine Research Works 18: 98-107.

Centenaro, G. S., Centenaro, M. S. and Hernandez, C. P. 2011. Antioxidant activity of protein hydrolysates of fish and chicken bones. Advance Journal of Food Science and Technology 3(4): 280-288.

Chakraborty, K., Chakkalakal, S. J., Joseph, D., Asokan, P. and Vijayan, K. 2016. Nutritional and antioxidative attributes of green mussel (*Perna viridis* L.) from the southwestern coast of India. Journal of Aquatic Food Product Technology 25(7): 968-985.

Chakraborty, K., Vijayagopal, P., Asokan, P. and Vijayan, K. 2011. Green mussel (*Perna viridis* L.) as healthy food, and as a nutraceutical supplement. Retrieved from website: http://eprints.cmfri.org.in/9763/1/Kajal_Chakraborty_Green_Mussel_Farming.pdf

Chen, D.-W., Su, J., Liu, X.-L., Yan, D.-M., Lin, Y., Jiang, W.-M. and Chen, X.-H. 2012. Amino acid profiles of bivalve mollusks from Beibu Gulf, China. Journal of Aquatic Food Product Technology 21(4): 369-379.

Cheong, K.-L., Xia, L.-X. and Liu, Y. 2017. Isolation and characterization of polysaccharides from oysters (*Crassostrea gigas*) with anti-tumor activities using an aqueous two-phase system. Marine Drugs 15(11): 338.

Cheung, I. W. and Li Chan, E. C. 2010. Angiotensin-I-converting enzyme inhibitory activity and bitterness of enzymatically-produced hydrolysates of shrimp (*Pandalopsis dispar*) processing byproducts investigated by Taguchi design. Food Chemistry 122(4): 1003-1012.

Dash, P. and Ghosh, G. 2017. Amino acid composition, antioxidant and functional properties of protein hydrolysates from Cucurbitaceae seeds. Journal of Food Science and Technology 54(13): 4162-4172.

Devries, J. W. and Silvera, K. R. 2002. Determination of vitamins A (retinol) and E (alpha-tocopherol) in foods by liquid chromatography: collaborative study. Journal of AOAC International 85(2): 424-434.

Dong, S., Zeng, M., Wang, D., Liu, Z., Zhao, Y. and Yang, H. 2008. Antioxidant and biochemical properties of protein hydrolysates prepared from silver carp (*Hypophthalmichthys molitrix*). Food chemistry 107(4): 1485-1493.

Dong, X. P., Zhu, B. W., Zhao, H. X., Zhou, D. Y., Wu, H. T., Yang, J. F., ... and Murata, Y. 2010. Preparation and *in vitro* antioxidant activity of enzymatic hydrolysates from oyster (*Crassostrea talienwhannensis*) meat. International Journal of Food Science and Technology 45(5): 978-984.

Erbas, M., Ertugay, M., Erbas, M. and Certel, M. 2005. The effect of fermentation and storage on free amino acids of tarhana. International Journal of Food Sciences and Nutrition 56(5): 349-358.

Faithong, N., Benjakul, S., Phatcharat, S. and Binsan, W. 2010. Chemical composition and antioxidative activity of Thai traditional fermented shrimp and krill products. Food Chemistry 119(1): 133-140.

Futagawa, K., Yoshie-Stark, Y. and Ogushi, M. 2011. Monthly variation of biochemical composition of Pacific oysters *Crassostrea gigas* from two main cultivation areas in Japan. Fisheries Science 77(4): 687-696.

Gao, D., Guo, P., Cao, X., Ge, L., Ma, H., Cheng, H., ... and Feng, R. 2020. Improvement of chicken plasma protein hydrolysate angiotensin I-converting enzyme inhibitory activity by optimizing plastein reaction. Food Science and Nutrition 8(6): 2798-2808.

Hien, B. T. T., Huong, T. T., Tung, L. A., Diem, P. T., Que, L. X., Hao, D. V. and Thanh, T. V. 2021. Study of enzymatic hydrolysis from catfish by-products as feed stuff ingredients. Vietnam Journal of Agriculture and Rural Development 4: 80-88.

Jayaprakash, R. and Perera, C. O. 2020. Partial purification and characterization of bioactive peptides from cooked New Zealand green-lipped mussel (*Perna canaliculus*) protein hydrolyzates. Foods 9(7): 879.

Jorhem, L. and Engman, J. 2000. Determination of lead, cadmium, zinc, copper, and iron in foods by atomic absorption spectrometry after microwave digestion: NMKL1 collaborative study. Journal of AOAC International 83(5): 1189-1203.

Korhonen, H. 2009. Milk-derived bioactive peptides: From science to applications. Journal of Functional Foods 1(2): 177-187.

Li, Z., Jiang, A., Yue, T., Wang, J., Wang, Y. and Su, J. 2013. Purification and identification of five novel antioxidant peptides from goat milk casein hydrolysates. Journal of Dairy Science 96(7): 4242-4251.

Lordan, S., Ross, R. P. and Stanton, C. 2011. Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic diseases. Marine Drugs 9(6): 1056-1100.

Memarpoor-Yazdi, M., Mahani, H. and Zare-Zardini H. 2013. Antioxidant activity of protein hydrolysates and purified peptides from *Zizyphus jujuba* fruits. Journal of Functional Foods 5(1): 62-70.

Nesvizhskii, A. I. 2007. Protein identification by tandem mass spectrometry and sequence database searching. Methods in Molecular Biology 367: 87-119.

Normah, I., Hafsah, M. S. and Izzaira, A. N. 2013. Bitterness of green mussel (*Perna viridis*) hydrolysate as influenced by the degree of hydrolysis. International Food Research Journal 20(5): 2261.

Özden, Ö., Erkan, N. and Deval, M. C. 2009. Trace mineral profiles of the bivalve species *Chamelea gallina* and *Donax trunculus*. Food Chemistry 113(1): 222-226.

Pérez, R. A., Iglesias, M. T., Pueyo, E., González, M. and de Lorenzo, C. 2007. Amino acid composition and antioxidant capacity of Spanish honeys. Journal of Agricultural and Food Chemistry 55(2): 360-365.

Qian, B., Zhao, X., Yang, Y. and Tian, C. 2020. Antioxidant and anti-inflammatory peptide fraction from oyster soft tissue by enzymatic hydrolysis. Food Science and Nutrition 8(7): 3947-3956.

Ren, J., Zhao, M., Shi, J., Wang, J., Jiang, Y., Cui, C., … and Xue, S. J. 2008. Purification and identification of antioxidant peptides from grass carp muscle hydrolysates by consecutive chromatography and electrospray ionization-mass spectrometry. Food Chemistry 8(2): 727-736.

Saito, K., Jin, D.-H., Ogawa, T., Muramoto, K., Hatakeyama, E., Yasuhara, T., ... and Chemistry, F. 2003. Antioxidative properties of tripeptide libraries prepared by the combinatorial chemistry. Journal of Agricultural and Food Chemistry 51(12): 3668-3674.

Sarmadi, B. H. and Ismail, A. 2010. Antioxidative peptides from food proteins: A review. Peptides 31(10): 1949-1956.

Schnell, S. and Maini, P. K. 2000. Enzyme kinetics at high enzyme concentration. Bulletin of Mathematical Biology 62(3): 483-499.

Shiozaki, K., Shiozaki, M., Masuda, J., Yamauchi, A., Ohwada, S., Nakano, T., ... and Sato, M. 2010. Identification of oyster-derived hypotensive peptide acting as angiotensin-I-converting enzyme inhibitor. Fisheries Science 76(5): 865-872.

Sreejamole, K. and Radhakrishnan, C. 2016. *In vitro* antioxidant activity of tissue extracts of green mussel *Perna viridis*. Journal of the Marine Biological Association of India 58(2): 94.

Srivastava, A., Harish, S. R. and Shivanandappa, T. 2006. Antioxidant activity of the roots of *Decalepis hamiltonii* (Wight & Arn.). LWT - Food Science and Technology 39(10): 1059-1065.

Tang, X., He, Z., Dai, Y., Xiong, Y. L., Xie, M. and Chen, J. 2010. Peptide fractionation and free radical scavenging activity of zein hydrolysate. Journal of Agricultural and Food Chemistry 58(1): 587-593.

Thu, T. N. T., Minh, T. D., Ngoc, H. T. and Thai, N. H. 2018. Some characteristics of cultured oyster species *Crassostrea belcheri* and

farming system in Ben Tre province. Can Tho University Journal of Science 54(1): 92-100.

Tira-Picos, V., Gbolade, A., Nogueria, J. and Ajibesin, K. 2009. Analysis of leaf essential oil constituents of *Cinnamomum zeylanicum* grown in Nigeria. Journal of Essential Oil Bearing Plants 12(1): 76-80.

Truc, T. T., Tuan, V. N., và Nguyen, V. T. A. M. and Muoi, V. 2015. Study on enzymatic hydrolysis of protein from head meat of black tiger shrimp using intracellular protease. Can Tho University Journal of Science 39-46.

Umayaparvathi, S., Meenakshi, S., Vimalraj, V., Arumugam, M., Sivagami, G. and Balasubramanian, T. 2014. Antioxidant activity and anticancer effect of bioactive peptide from enzymatic hydrolysate of oyster (*Saccostrea cucullata*). Biomedicine and Preventive Nutrition 4(3): 343-353.

Vijaykrishnaraj, M., Kumar, S. B. and Prabhasankar, P. 2015. Green mussel (*Perna canaliculus*) as a marine ingredient to enrich gluten free pasta: Product quality, microstructure and biofunctional evaluation. Journal of Food Measurement and Characterization 9(1): 76-85.

Vijaykrishnaraj, M., Roopa, B. and Prabhasankar, P. 2016. Preparation of gluten free bread enriched with green mussel (*Perna canaliculus*) protein hydrolysates and characterization of peptides responsible for mussel flavour. Food Chemistry 211: 715-725.

Wang, X., Yu, H., Xing, R. and Li, P. 2017. Characterization, preparation, and purification of marine bioactive peptides. BioMed Research International 2017: 9746720.

Wang, Y.-K., He, H.-L., Wang, G.-F., Wu, H., Zhou, B.-C., Chen, X.-L. and Zhang, Y.-Z. 2010. Oyster (*Crassostrea gigas*) hydrolysates produced on a plant scale have antitumor activity and immunostimulating effects in BALB/c mice. Marine Drugs 8(2): 255-268.

Zywicki, R. S. and Sullivan, D. M. 2015. Determination of total iodine in infant formula and adult/pediatric nutritional formula by inductively coupled plasma-mass spectrometry (ICP-MS): Collaborative study, final action 2012.15. Journal of AOAC International 98(5): 1407-1416.