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# Bitterness of green mussel (*Perna viridis*) hydrolysate as influenced by the degree of hydrolysis

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**Abstract**

Green mussel (*Perna viridis*) was hydrolysed with alcalase under two different conditions consisting of pH7, E/S5% or pH 9, E/S 3% at 60°C for two hours. Hydrolysis at pH 9, E/S3% resulted in a higher degree of hydrolysis (DH) than pH7, E/S5% with degree of hydrolysis of 37.00% and 28.33%, respectively. The green mussel hydrolysates were characterized by molecular weight of <38 kDa and <18 kDa, respectively. Hydrophobic group analysis by using FTIR detected the presence of amine group which contributed to the bitterness of hydrolysates produced under both conditions. Sensory evaluation indicated that both hydrolysates were slightly bitter but did not exceed the bitterness of the standard caffeine solutions. Further analysis should be done on the hydrolysis process of green mussel to produce hydrolysate with improved sensorial properties thus make it more applicable as flavoring agent in food product.



## Introduction

Hydrolysis can be achieved by treatment with enzymes, acids or alkali (Jia *et al*., 2010; Nalinanon *et al*., 2011, Adler-Nissen, 1986). However, enzymatic hydrolysis is preferred due to faster reaction rates, mild conditions and high specificity. The product of hydrolysis is hydrolysate (Ovissipour *et al.*, 2009; Xia *et al.*, 2007; Bhaskar and Mahendrakar, 2008). Hydrolysates refer to proteins that have been broken down into peptides of various sizes by chemical or enzymatic hydrolysis. They are used in food industries as milk replacers, protein supplement, beverage stabilizers and flavor enhancer (Li *et al*., 2010). Protein hydrolysates have found increased use in the food industry recently because of their improved nutritive value, enhanced functional properties and potential biological activity (Cheung and Li-Chan, 2010).

Mussels are bivalves belonging to the Mytilidae family. They are commercially valuable species, easy to cultivate or collect in coastal areas. They are important for marine ecology and for human diet since they are an important source of nutrients (Fuentes *et al*., 2009). World productions of all types of mussel increased at an average of 5% per year during 1950-2003, reaching about 1.6 million tonnes in 2003 constituting 13% of the 12.3 million tonnes total mollusk supply (FAO, 2005). The blue mussel (*Mytilus edulis*), Mediterranean mussel and green mussel form bulk of the total world production (Sallih, 2005). The green mussel in the tropics is mainly *Perna viridis*, which is cultivated in India, Indonesia, Philippines, Singapore, Thailand and Malaysia (Sallih, 2005). They are also excessively distributed in the Indo-Pacific region which includes the coastline of Malaysia (Siddall, 1980). Based on the statistic issued by Food and Agriculture Organisation (2005), the production of green mussel in the year of 2009 was reported at 281 941 tonnes in India, Malaysia, Philippines, Singapore and Thailand. Mussel is an aquaculture species which serves as a low cost alternative source of protein. Consumption of these bivalve mollusks provides an inexpensive source of protein with a high biological value, essential minerals and vitamins (Fuentes *et al*., 2009).

The production of seafood flavors from bivalve species, using protein hydrolysis is very challenging in order to ensure a high organoleptic quality. Enzymatic processes using specific proteases have been developed to produce flavorants from seafood by-products. However, the hydrolysis of protein is often accompanied with flavor defects such as bitterness and off-flavor which will affect the sensory acceptability of protein hydrolysate (Nilsang *et al*., 2005; Spellman *et al*., 2009). The degree of bitterness that develops during hydrolysis is associated with the level of hydrophobic amino acids and the release of bitter tasting peptides (Nilsang *et al*., 2005; FitzGerald and O'Cuinn, 2006). The choice of substrate, the protease enzyme applied

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and the degree of hydrolysis play significant roles in determining the physico-chemical properties of hydrolysate (Wasswa *et al.*, 2007; Normah *et al.*, 2005). Several attempts have been made to limit the formation of bitterness through controlling the degree of hydrolysis including using plastein reaction or specific enzyme such as exopeptidase (Raksukulthai and Haard, 2003; Nilsang *et al*., 2005). This study was conducted to evaluate the degree of bitterness of green mussel (*Perna viridis*) hydrolysate obtained from enzymatic hydrolysis by manipulating the pH and enzyme-substrate ratio (E/S).

## Materials and method

### *Materials*

Green mussel (*Perna viridis*) was bought from mussel supplier in Pantai Bagan Lalang, Sepang, Malaysia and immediately placed in ice before transported to the laboratory. The flesh was separated manually, washed, and minced by using a blender. The minced green mussel was stored at -21°C before subjected to further treatment.

Alcalase with a declared activity of 2.4 AU/g and a density of 1.18 g/ml is a bacterial endoproteinase from a strain of *Bacillus licheniformis*. The enzyme was purchased from Science Technic Sdn. Bhd., Selangor, Malaysia.

### *Preparation of green mussel (Perna viridis) hydrolysate*

Green mussel hydrolysate was prepared according to the method described by Adler-Nissen (1986) with a slight modification. 661.18 g of minced green mussel was mixed in 364.13 g distilled water and then minced in a blender. All reactions were done in a 1 L reaction vessel in a thermostatically controlled water bath with constant agitation at 200 rpm. The pH of the mixture was adjusted to pH 9 and 7, by adding 4.0 N NaOH. The temperature was maintained at 60°C. The degree of hydrolysis was manipulated by using different pH and enzyme- substrate ratio (E/S) which was pH 9 and enzyme-substrate ratio of 3% for the first treatment. pH 7 and enzyme-substrate ratio of 5% were used for the second treatment.

Once the pH and temperature have stabilized, Alcalase® at E/S of 5% or 3% was added to the mixture at pH 7 or pH 9, respectively. The reaction was allowed to proceed for 2 hours. The amount of NaOH added to keep the pH constant during the hydrolysis was recorded and used to calculate the degree of hydrolysis (DH). At the end of the 2 hours hydrolysis, the reaction was terminated by immersing the reaction vessel in water bath set at 95°C for 15 minutes with constant stirring to ensure the inactivation of the enzyme. The resultant slurry was centrifuged at 5000 rpm, 4°C for 20 minutes. The supernatant was collected and freeze dried. The freeze dried hydrolysates were stored in a desiccator until further analysis.

The hydrolysis was carried out using the pH-stat method which allowed the estimation of degree of hydrolysis based on the consumption of alkali to maintain a constant pH at the desired value (Adler-Nissen, 1986). Degree of hydrolysis (DH) was determined based on the formulation below:

$$DH\ (\%) = \frac{(\beta \times N_b)}{(\alpha \times MP \times h_{tot})} \times 100\%$$

where:

$\beta$ = amount of alkali consumed (ml)

$N_b$ = base normality

MP = mass of the substrate

$\alpha$ = average degree of dissociation of the $\alpha$-$NH_2$ groups released during hydrolysis

$h_{tot}$ = total number of peptide bonds in the protein substrate

### *Protein concentration*

Protein concentration in the green mussel hydrolysate for the determination of molecular weight distribution was determined by Lowry (1951) method based on the modified procedure of Hatree (1972).

### *Gel filtration chromatography*

The proteins of green mussel hydrolysate were separated on a column of Bio-Gel P60 by using gel filtration chromatography to collect the fractions. The column was operated in downward flow at room temperature. Tris-buffer was used to equilibrate the column and to elute the proteins at a flow rate of 5 ml/8 min. 100 ml sample was applied to the column and 5 ml of each fraction were collected. The absorbance of each of the fraction was measured at 214 nm. The fraction which has the highest absorbance value was further analyzed for molecular weight distribution and amino acid composition.

### *Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)*

SDS-PAGE was used to determine the molecular weight of the raw green mussel, hydrolysate and hydrolysate fraction. Samples were prepared by mixing 0.05 ml of 10% (w/v) hydrolysate solution with 0.25 µl of sample buffer. The sample was heated at 70°C for 10 minutes. 10 µl was loaded into each

well on the gel. The raw green mussel and mussel hydrolysate fractions were prepared similarly as stated above. Benchmark™ protein ladder was used as a standard marker.

Electrophoresis was performed by using the XCell Surelock electrophoresis cell (Bio-Rad laboratories, Hercules, CA, USA) run for 50 minutes at 100-125 mA/gel. Subsequently, the gel was washed in 100 ml ultrapure water, heated in microwave oven at 180°C for one minute and shaken in an orbital shaker for about 2 minutes. The process was repeated twice. The gel was then stained in Coomassie Brilliant Blue solution, heated in microwave oven at 180°C for one minute and again shaken in an orbital shaker for about 2 minutes. Finally the gel was destained using ultrapure water and shaken using orbital shaker for 2 minutes.

*Hydrophobic group analysis in fresh mussel and mussel hydrolysate*

The analysis of hydrophobic group in fresh green mussel and hydrolysate was performed using the Perkin Elmer Spectrum One FTIR spectrometer equipped with a deuterated triglycerine sulphate infrared detector. The mussel sample was initially dried by oven method. Subsequently the mussel or the hydrolysate was ground using agate mortar until the particle size became so small (approximately 2.5 micron) that the surface of the solid appears shiny. 0.08 g of powdered potassium bromide (KBr) was added and the mixture was ground for about 30 seconds. The mixture was scraped into the middle and ground for another 15 seconds. The sample and KBr should be finely ground to avoid the mixture from scattering the infrared radiation excessively. The mixture was then placed in an evacuable mold and subjected to a pressure of 10 to 20MPa (Ewing, 1985). Perkin Elmer Spectrum Software was used to control the spectrometer and data were collected over a wavenumber range of 4000-400 $cm^{-1}$ with resolution of 4 $cm^{-1}$ and collection spectra of 16.

***Sensory Evaluation***

*Quantitative Descriptive Analysis*

Sensory evaluation of the green mussel hydrolysate was conducted according to Nilsang *et al.* (2005) with a slight modification. Ten panelists were trained for two weeks by using caffeine solution as a standard. Different concentrations of caffeine solutions were prepared and presented to the panelists. The lowest concentration that panelists could perceive the bitter taste was identified and this concentration was then used as the reference to evaluate the degree of bitterness of green mussel hydrolysate. A 15 cm line scale anchored from none to strong bitterness was defined and used for the evaluation. 2.5% (w/v) green mussel hydrolysate produced at pH 7, ES5% and 9, ES3% were prepared and given to the well-trained panelist to be evaluated.

*Degree of acceptability*

Hedonic scale was designed to measure the degree of acceptability for the hydrolysate. Category scale ranging from like extremely, through neither like nor dislike, to dislike extremely, with varying numbers of categories, 1 to 9 scale were used. Panelists indicated their degree of liking by choosing the appropriate category.

To test the acceptability of the hydrolysate, rice porridge was used as a carrier. Twenty milliliter of 5% (w/v) hydrolysate solutions were mixed with 100 ml plain rice porridge. A commercial product from seafood species was prepared according to the same procedure for comparison. Panelists evaluated the color, odor, taste and overall acceptability. They were instructed to rinse their mouth with plain water in between tasting and gave their ranking according to the 9 point hedonic scale. This sensory evaluation involved 25 panelists.

***Statistical analysis***

Data was subjected to analysis of variance (ANOVA) and mean comparisons was carried out by using Duncan's multiple range tests. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for windows: SPSS Inc. Chicago, II, USA, 2006).

**Results and discussion**

***Degree of hydrolysis (DH)***

DH which was calculated according to the pH-stat method is shown in Table 1. Hydrolysis at pH 9, E/S3% produced 43.81% DH compared to only 28.33% at pH 7, E/S5%. DH has been shown to be affected by the percent and type of enzyme used during the hydrolysis (Cheung and Li-Chan, 2010; Li *et al*. (2010). As suggested by Wasswa *et al.* (2007), proteolytic enzymes possibility controlled the degree of cleavage of the protein in the substrate. Ovissipour *et al*. (2009) investigated the progression of hydrolysis of Persian sturgeon viscera using Alcalase® 2.4 L where the DH was found to increase with increasing in incubation time. While Guerard *et al.* (2002) speculated that a reduction in the reaction rate may be due to the limitation of the enzyme activity by formation of reaction products at high degrees of

Table 1: Degree of hydrolysis (%) of green mussel (*Perna viridis*) hydrolysate obtained under different hydrolysis conditions

| Hydrolysis conditions | Degree of hydrolysis (%) |
|---|---|
| pH 7, E/S 5% | 28.33±1.05 |
| pH 9, E/S 3% | 43.81±0.60 |

hydrolysis. However, decrease in hydrolysis rate may also be due to a decrease in the concentration of peptide bonds available for hydrolysis, enzyme inhibition and enzyme deactivation Guerard *et al.* (2002). Another reason for the different in DH could be the conformation of proteins which leads to the difference in the number of accessible peptide bonds, which then alter the hydrolysis rate (Li *et al.*, 2010). Adler-Nissen (1986) reported that a change in pH affects both the substrate and the enzyme by changing the charge distribution and conformation of the molecules.

The method used for calculating the DH may also affect the value of DH. A study conducted by Hoo and Babji (2011) found that the DH for salmon skin was 77.03% by hydrolyzing at pH of 8.39. The value was higher compared to the result achieved in this study. This could probably due to the different method used for the calculation of DH in which trichloroacetic acid (TCA) ratio method was used. Spellman *et al.* (2003) had also conducted a study to determine the degree of hydrolysis using three different methods which were trinitrobenzene sulphonic acid (TNBS), o-phthaldialdehyde (OPA) and pH stat method. Among these three methods, TNBS produced the highest DH with 19.3% followed by OPA with 16.8% and pH-stat with 12.3%. According to Adler-Nissen (1986), pH stat produced lower percentage of DH due to higher pK value for tripeptides, dipeptides and free amino acids than polypeptides. This will indirectly cause the underestimation of the value of degree of dissociation (α) that is used in the calculation.

The amount of sodium hydroxide added during the hydrolysis process to maintain the pH throughout the hydrolysis process might also affect the DH. Green mussel (*Perna viridis*) hydrolysate produced at pH 9, E/S 3% required higher amount of sodium hydroxide than pH 7, E/S 5%. In pH-stat method, the degree of hydrolysis was calculated based on the consumption of base which is essential in controlling pH during hydrolysis, therefore higher addition of sodium hydroxide resulted in higher degree of hydrolysis. In addition, Alcalase is most active at alkaline pH.

### *Protein concentration*

The protein concentration in raw green mussel was 10% while protein concentration in the hydrolysate produced at pH 7, E/S5% and pH 9, E/S3% were 49% and 63%, respectively (Table 2). The results showed that hydrolysis process increased the protein concentration in green mussel hydrolysate. This observation was similar with green mussel hydrolysate fraction where fraction produced at pH 9, E/S3% had 76% protein compared to those produced at pH 7, E/S5% with only 59%. Bhaskar and Mahendrakar (2008) studies indicated that visceral waste of catla had a protein content of 8.52% with a fat content of >12%. Pre-treatment involving heat treatment resulted in removal of fat close to 10% from original material and allowed a recovery of 75% proteins (Ibid). According to Benjakul and Morrissey (1997), the increasing protein content was due to the solubilisation of protein during hydrolysis and the removal of insoluble undigested non-protein substance.

Table 2: Protein concentration of raw mussel, hydrolysate and hydrolysate fraction of green mussel (*Perna viridis*)

| Sample | Protein concentration (%) |
|---|---|
| Raw mussel | 10 |
| Mussel protein hydrolysate at pH 7 | 49 |
| Mussel protein hydrolysate at pH 9 | 63 |
| Mussel hydrolysate fraction at pH 7 | 59 |
| Mussel hydrolysate fraction at pH 9 | 76 |

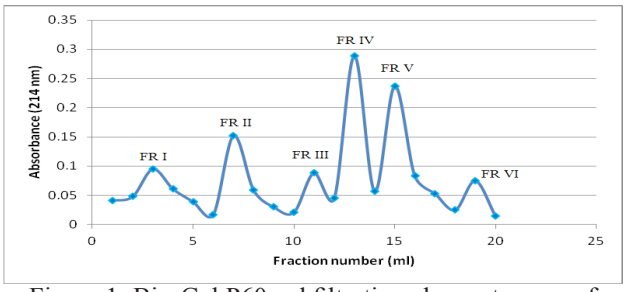


Figure 1: Bio-Gel P60 gel filtration chromatogram of green mussel hydrolysate produced at pH 7, E/S5%

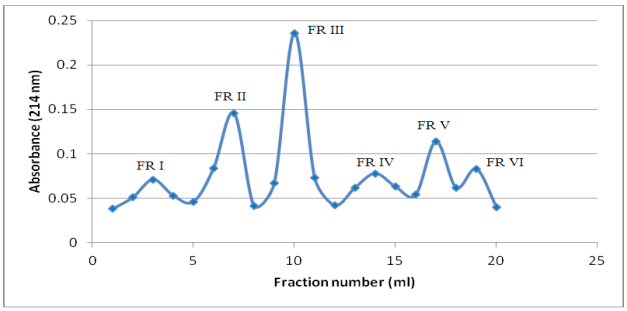


Figure 2: Bio-Gel P60 gel filtration chromatogram of green mussel hydrolysate produced at pH 9, E/S3%

### *Molecular weight distribution*

The hydrolysate produced at pH 7, E/S 5% and pH 9, E/S3% were fractionated using gel filtration chromatography. Figure 1 and 2 show the Bio-Gel P60 gel filtration chromatograms of green mussel

Table 3: Functional groups of different wavenumbers obtained from raw green mussel (*Perna viridis*) and mussel hydrolysates

| Sample | Wavenumber ($cm^{-1}$) | Functional group |
|---|---|---|
| Raw mussel | 3650.65 | Amide N-H Stretch |
| | 1477.64 | Aromatics C-C Stretch |
| | 959.96 | Alkanes C-C Stretch |
| Mussel hydrolysate (pH 7, E/S ratio 5%) | 3409.90 | Amines N-H Stretch |
| | 1654.21 | Alkenes C=C Stretch |
| | 1457.87 | Aromatics C-C Stretch |
| | 1155.67 | Alkanes C-C Stretch |
| Mussel hydrolysate (pH 9, E/S ratio 3%) | 3412.55 | Amines N-H Stretch |
| | 1654.54 | Alkenes C=C Stretch |
| | 1455.61 | Aromatics C-C Stretch |
| | 1155.54 | Alkanes C-C Stretch |

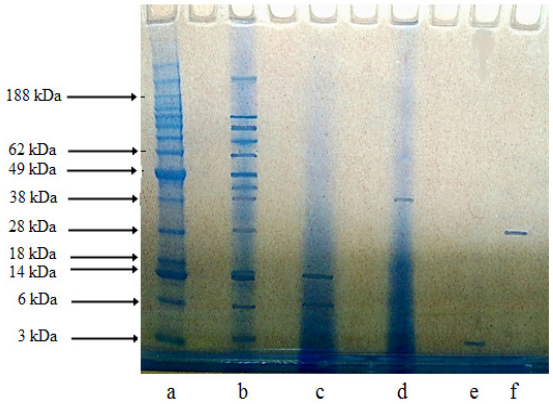


Figure 3: Electrophoresis pattern of green mussel (*Perna viridis*) ; From left: (a) standard protein marker, (b) raw mussel, (c) mussel hydrolysate (pH 7, E/S5%) (d) mussel hydrolysate (pH 9, E/S3%) (e) mussel hydrolysate fraction (pH 7, E/S5%) and (f) mussel hydrolysate fraction (pH 9, E/S3%)

(*Perna viridis*) hydrolysate. The hydrolysate produced at pH 7, E/S5%, showed three apparent peaks while hydrolysate produced at pH 9, E/S 3% showed only two. According to Chang *et al.* (2007), the peak containing low molecular weight molecules was much larger than those containing high molecular weight molecules. The highest peak (FR IV) on the chromatogram for mussel hydrolysate fraction produced at pH 7, E/S5% and the highest peak (FR III) on the chromatogram for mussel hydrolysate fraction produced at pH 9, E/S3% were further analysed for molecular weight distribution.

The molecular weight distribution of raw green mussel, mussel hydrolysate and hydrolysate fractions are shown in Figure 3. Mussel hydrolysate produced at pH 7, E/S5% had lower molecular weight (<18 kDa) than those produced at pH 9, E/S3% (<38 kDa). Hydrolysate fraction produced at pH 7, E/S5% also had lower molecular weight (<3 kDa) than hydrolysate fraction produced at pH 9, E/S3% (<28 kDa).

The relationship between molecular weight peptides with bitterness properties have been investigated by Matoba and Hata (1972). They reported that intact food proteins or high molecular mass peptides do not display bitterness since most hydrophobic amino acids are oriented towards the interior of the molecule. As hydrolysis continues, more hydrophobic amino acid residues are exposed and therefore hydrolysate bitterness generally increases (Matoba and Hata, 1972). According to FitzGerald and O'Cuinn (2006) and Cheung and Li-Chan (2010), hydrolysates containing peptide of < 6 kDa are likely to be bitter. Hydrolysate produced at pH 7, E/S5% had lower molecular weight than hydrolysate produced at pH 9, E/S3% and sensory evaluation revealed that the hydrolysate had lower acceptability and more bitter than those produced at pH 9, E/S3%. The hydrolysate fraction produced at pH 7, E/S5% had molecular weight of 3 kDa in size (Figure 3). This is in agreement with Cheung and Li-Chan (2010) observation using size exclusion chromatography where fractions from Alcalase-produced hydrolysate were bitter and characterized by the presence of many hydrophobic amino acids and molecular masses of less than 3 kDa. According to Bhaskar and Mahendrakar (2008), fish visceral hydrolysates with low molecular weight peptides (<8 kDa) could be of high nutritive value and can also be used effectively as food flavoring agent. Chain of peptides, which are dependent on degree of hydrolysis (DH) is of special interest because properties such as emulsion capacity and bitterness depend at least in part of molecular size Wasswa *et al.* (2007). Molecular weight distribution and average peptide size of hydrolysate are also affected by enzyme specificity Ovissipour *et al*. (2009).

### *Hydrophobic group analysis*

Hydrophobic group analysis was conducted by using FTIR. The functional groups which represented various wavenumbers are shown in Table 4. In raw mussel, peaks 3695.65 $cm^{-1}$ and 959.10 $cm^{-1}$ represent amide N-H stretch and alkanes C-C stretch, respectively. Whereas, hydrolysate produced at pH 7, E/S5% consist of amines N-H stretch, alkenes C=C stretch, and alkanes C-H stretch at 3409.90 $cm^{-1}$, 1654.21 $cm^{-1}$, and 1040.77 $cm^{-1}$ wavenumbers, respectively. Mussel hydrolysate produced at pH 9, E/S3% shared the similarities of functional group presence with pH 7, E/S5%. In hydrolysate produced at pH 9, E/S3% peak 3412.55 $cm^{-1}$ represents amines N-H stretch, peaks 16544.54 $cm^{-1}$ represents alkenes C=C stretch and finally peak 1125.53 $cm^{-1}$ represents alkanes C-H stretch.

According to Agrawal and Chiddarwar (2010),

Table 4: Colour, aroma, taste and overall acceptability of hydrolysate samples evaluated using 9-point hedonic scale

| Sample | Colour | Aroma | Taste | Overall acceptability |
|---|---|---|---|---|
| Commercial hydrolysate | 4.84$^{c}$±1.0 | 5.00$^{c}$±1.0 | 5.00$^{b}$±0.9 | 5.12$^{b}$±1.5 |
| Mussel protein hydrolysate at pH 7 | 7.36$^{a}$±0.8 | 7.64$^{a}$±1.0 | 5.16$^{b}$±2.0 | 6.76$^{a}$±1.2 |
| Mussel protein hydrolysate at pH 9 | 6.16$^{b}$±1.1 | 5.64$^{b}$±1.2 | 6.88$^{a}$±1.1 | 5.48$^{b}$±1.5 |

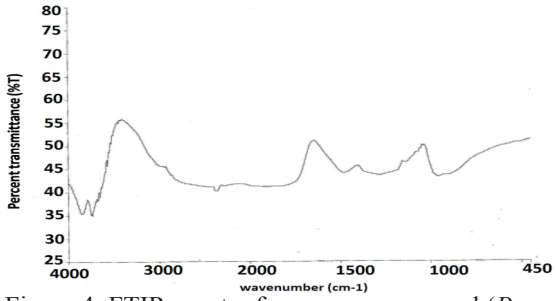


Figure 4: FTIR spectra for raw green mussel (*Perna viridis*)

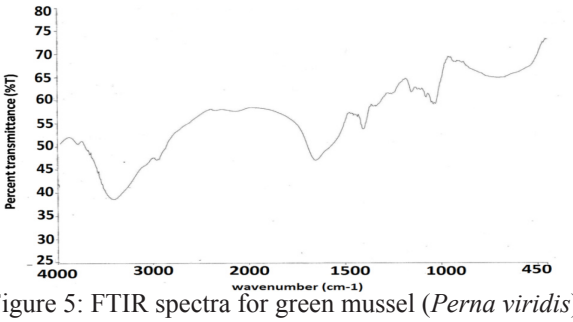


Figure 5: FTIR spectra for green mussel (*Perna viridis*) hydrolysate produced at pH 7, E/S 5%

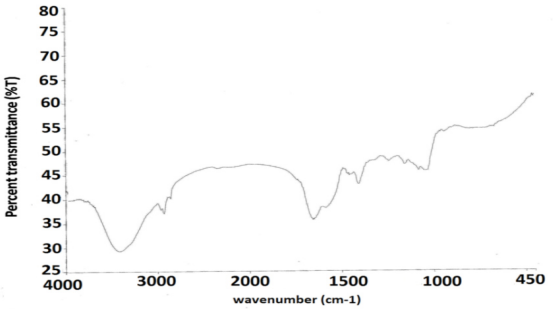


Figure 6: FTIR spectra for green mussel (*Perna viridis*) hydrolysate produced at pH 9, E/S 3%

functional groups that are usually present in the bitter drugs were amine group. The researchers also clarified that the presence of amine group indirectly promoted the obnoxious taste. This theory was in accordance with this study where mussel hydrolysate which commonly associated with bitter taste contained amine N-H stretch for both produced at pH 7, E/S5% and pH 9, E/S3%. However, Birhade *et al*. (2010) has conducted a study on a bitter drug model which was Rizatriptan benzoate by using FTIR. The researchers found that the functional group existed in the drug

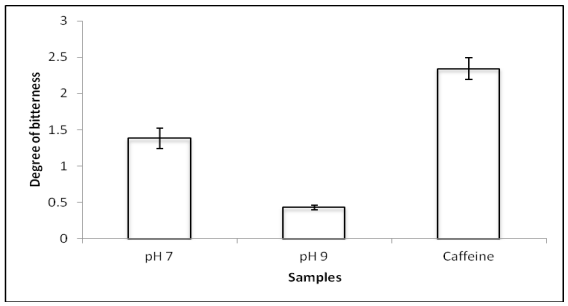


Figure 7: Degree of bitterness of green mussel (*Perna viridis*) hydrolysate produced at pH 7, E/S5%, pH9, E/S3% and caffeine standard

including aromatic C-H stretch, aliphatic C-H stretch, and nitrile C=N stretch. These functional groups might be responsible for the development of bitter taste in the drug. However, none of the functional groups were found in the mussel hydrolysate. Mussel hydrolysate may not develop as much as bitter taste as drug. The FTIR spectra are shown in Figures 4 to 6.

### *Sensory evaluation*

*Quantitative Descriptive Analysis (QDA)*

QDA was carried out by ten trained panelists and the result is presented in Figure 7. The scores of bitterness using a 15-cm line scale anchored from none to strong bitterness evaluated by ten trained panelists were 1.38 and 0.43, respectively, for mussel hydrolysate produced at pH 7, E/S5% and pH 9, E/S3%. This indicated that the hydrolysates were weakly bitter. The lowest concentration of caffeine solution which was used as a reference standard has the score of 2.34. This result revealed that the bitterness of mussel hydrolysate for both pH was significantly ($p < 0.05$) lower than that of the reference standard. The result also shows that the hydrolysate produced at pH 7, E/S5% was slightly bitter than those produced at pH 9, E/S3%. The occurrence of bitter peptides is a major obstacle for the utilization of protein hydrolysates for human consumption (FitzGerald and O'Cuinn, 2006). According to Cheung and Li-Chan (2010), besides the amount of peptides, lipid oxidation compounds also contributed to the bitterness of protein hydrolysate. Additionally, peptide size has also been proposed to be a factor that contributes to bitterness besides the hydrophobic oligopeptides and specificity of the enzyme used (Cheung and Li-Chan, 2010; Gildberg *et al.*, 2002; Kristinsson and Rasco, 2000). The choice of enzyme is an important factor, because different enzymes have different specificity and give products of different qualities and the selection criterion can be based on the ability of enzyme to reach a high nitrogen recovery and degree of hydrolysis at a low

concentration of enzymes without producing a bitter taste (Hou *et al*., 2011). However, researchers have various criteria to select suitable enzyme used for hydrolysis of protein (Kristinsson and Rasco, 2000; Nilsang *et al*., 2005). The concentration of enzyme used for hydrolysate produced at pH 9, E/S3% was lower than concentration of hydrolysate produced at pH 7, E/S5%. Higher concentration of Alcalase reacts sufficiently and specifically with the substrate. Hence, results in exposing many low molecular weight peptides which consist mainly of hydrophobic amino acids and thus increases the bitterness of hydrolysate.

*Degree of acceptability*

Data for degree of acceptability were analyzed in one-way analysis of variance (ANOVA). The results observed were tabulated as in Table 4. Overall acceptability shows that hydrolysate produced at pH 7, E/S5% was the most acceptable compared to others. In terms of colour and aroma, green mussel hydrolysate produced at pH 7, E/S5% shows higher acceptability than the other two samples. Based on these parameters, it indicates that panelists preferred hydrolysate produced at pH 7, E/S5% more than those produced at pH 9, E/S3%. However, taste scores indicated that hydrolysate produced at pH 7, E/S5% was less preferred than those produced at pH 9, E/S3%. This probably due to the more bitter taste of the hydrolysate produced at pH 7, E/S5%. This observation was supported by the presence of low molecular weight peptide (Figure 3) and Quantitative Descriptive Analysis (QDA) (Figure 7).

## Conclusions

The hydrolysate derived from green mussel (*Perna viridis*) can be a good source of protein and has the potential to be used as natural flavouring agent in food to substitute synthetic flavouring agent. Unfortunately, problem related to the acceptability of protein hydrolysate which is bitterness was discovered. In order to incorporate these protein hydrolysates as ingredients in functional foods and nutraceutical products, debittering or masking techniques may need to be considered to ensure consumer acceptance. Further analysis should be done in order to achieve more information on the process of producing mussel hydrolysate to improve the sensorial properties thus making it more applicable as flavoring agents in food product.

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