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Evaluation of umaminess in green mussel hydrolysate *(Perna viridis)* produced in the presence of sodium tripolyphosphate and NaCl

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Abstract

This study was conducted to evaluate umami taste in protein hydrolysate produced from green mussel *(Perna viridis)* by hydrolysing with flavorzyme at pH 8, enzyme substrate ratio (E/S) 3% with or without the presence of 0.4% sodium tripolyphosphate (STPP) and 1.5% NaCI. Degree of hydrolysis (DH), yield, amino acid compositions, molecular weight distribution and sensory evaluation were determined. The highest DH (23.18%), darkest color and highest yield (8.34%) were recorded for hydrolysate produced in the presence of both STPP and NaCI. Electrophoresis analysis showed the presence of protein bands between 10 to 70 kDa where hydrolysate with addition of STPP and NaCI had bands with lower intensities. Amino acids which contribute to the umami taste such as glutamic acid, glycine and aspartic acid were higher in hydrolysate produced with STPP and NaCI addition. The hydrolysate has lesser fishy odor and flavor than those produced with only in the presence of flavorzyme and was also rated with highest score for all the five basic tastes including bitterness. However, the score for bitterness was still lower than the reference solutions. Therefore, green mussel hydrolysate produced in this study has a good potential as a food flavorant.

Introduction

Mussels are bivalves categorized under the Mytilidae family. The green mussel *(Perna viridis)* was initially confined in southern Johor in the early 1980s however; its population has expanded to other areas in Peninsular Malaysia due to transplantation (Kamal Zaman *et al*., 2000; Al-Barwani *et al*., 2007). Protein hydrolysates derived from proteins that have been subjected to heating with acid, alkaline or by adding proteolytic enzyme (Clemente, 2000). Previously, proteolytic enzymes such as bromelain and alcalase have been evaluated for its effectiveness to produce protein hydrolysate with desirable characteristics from various seafoods such as cockles, green mussel, oyster and angelwing clam while flavorzyme improved the nutritional and functional properties of protein hydrolysate (Zuzana, 2011; Normah and Nurfazlika Nashrah, 2013; Haslaniza *et al*., 2013; Normah *et al*., 2013; Wang *et al*., 2014).

Umami is one of the five basic tastes that contribute to palatability and savory taste in food (Imamura and Matsushima, 2013). Umami compound which is monosodium glutamate was discovered by Ikeda in 1909 (Ikeda, 2002). Compound which is related to umami taste such as guanosine monophosphate (GMP) and inosine monophosphate (IMP) has a strong synergistic effect with umami flavor and is also widely used in food industry (Coulier, 2011). Reports on the effect of STPP and NaCl addition in improving the hydrolysate properties were unavailable. Therefore, in this study, the effect of using flavorzyme and the addition of STPP and NaCl on the development of umami flavor in green mussel hydrolysate was investigated.

Materials and Methods

Materials

Green mussel *(Perna viridis)* was obtained from Pantai Remis, Jeram, Selangor, Malaysia and stored at -20°C until use. Flavorzyme with a declared activity of 500 LAPU/g and a density of 1.27 g/ml was purchased from Next Gene Sdn. Bhd (Selangor, Malaysia). STPP and NaCl were of food grade quality purchased from Meilun Food Sdn Bhd. Other chemicals used were of analytical grade (Sigma Aldrich, U.K).

Preparation of green mussel hydrolysate

Green mussel hydrolysate was prepared according to Normah *et al.* (2013). An amount of 661.18g green mussel flesh was mixed in 364.13 g distilled water. The mixture was minced in a blender and then poured into a 1 L beaker which was then placed in a thermostatically controlled water bath

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where the mixture was constantly agitated at 200 rpm. The temperature of the mixture was raised to 50°C and the pH was adjusted to 8. Once the pH and temperature stabilized, the hydrolysate was prepared as follows: 1) in the presence of flavorzyme (3% E/S), 2) in the presence of flavorzyme (3% E/S), 0.4% STPP and 3) in the presence of flavorzyme (3% E/S), 0.4% STPP and 1.5% NaCI. STPP and NaCl were added only during half an hour before termination of the hydrolysis process. The reaction was allowed to proceed for 2hr. pH was maintained throughout the hydrolysis by adding 1N NaOH. The amount of NaOH added to maintain the pH during the hydrolysis was recorded and used to calculate the DH. After 2 hr, the reaction vessel was immersed in water bath set at 95°C for 15 mins and constantly stirred in order to ensure inactivation of the enzyme. The resultant slurry was centrifuged (Heraeus, Biofuge pico, Belgium) at 10,000 rpm at 4°C for 20 mins. The supernatant was collected, freeze dried and then stored in a dessicator until further analysis. Hydrolysate was stored in the dessicator in order to keep it in dry environment due to its hygroscopic nature.

Yield

Yield was determined based on the ratio of hydrolysate mass to the total weight of raw green mussel and calculated as follows:

$$\text{Yield (\%)} = \frac{\text{weight of freeze dried hydrolysate}}{\text{weight of green mussel}} \times 100$$

Degree of hydrolysis

Degree of hydrolysis was calculated by using the pH-stat method according to Adler-Nissen (1986). Degree of hydrolysis was calculated as follows:

$$\text{DH (\%)} = \frac{(\beta \times N_\beta)}{(\alpha \times M_P \times h_{tot})} \times 100$$

where:

β = volume of NaOH

N_β = molarity of NaOH

α = average degree of dissociation of NH_2 released during hydrolysis

MP = mass of substrate

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

Color measurement

Hydrolysate samples were packed in a clear plastic bag and the color was measured by using Hunterlab Ultrascan Sphere Spectrocolorimeter (Model Minolta CR-400, Malaysia). L^*, a^* and b^* parameters indicate lightness, redness and yellowness, respectively. Blank used for calibration was white tile CM-A101.

Chemical analysis

Moisture, protein, ash and fat content were determined according to AOAC (2005). Moisture content was determined from weight difference before and after drying. Protein content was determined by using Kjeldahl method; ash content by charring a pre-dried sample in a crucible at 550°C until constant weight of white ash was obtained while fat content was determined by Soxhlet extraction method.

Determination of molecular weight distribution using SDS-PAGE

SDS-PAGE was prepared according to Normah *et al.* (2013). Hydrolysate was mixed with sample buffer and reducing agent and then heated at 70ºC for 10 mins. Low molecular weight markers ranging from 10 to 220 kDa was used. Accurately, 10.0 µL sample was loaded into each well of the gel comprising of 12% resolving and 4% stacking gel and electrophoresis was run at 25 mA for 1.5 hr. The gel was stained in Coomassie brilliant blue and destained in ultrapure water.

Amino acid analysis

Sample was digested for 24 hr in 15 ml of 6N HCI at 110°C prior to determination of the amino acid composition through a reverse phase HPLC AccQ Tag column (3.9 x 150 mm) at 36°C with flow rate of 1 mL/min (Normah and Nurul Fasihah, 2017). An aliquot of 5 µL was injected into the AccQ Tag HPLC (Model 1525, Binary HPLC pump) system equipped with refractive index and multi fluorescence detectors (Waters, U.S.A). The mobile phases used were AccQ Tag Eluent A (concentrate) and 60% acetonitrile as Eluent B. Amino acid composition was determined based on the peak comparison with the standard.

Sensory evaluation

Flavor intensity of green mussel hydrolysate

Nine panelists were trained to be able to detect bitter, sweet, salty, umami and sour taste by using different concentration of reference solutions such as caffeine, sugar, salt, monosodium glutamate and citric acid. Panelists were served with caffeine solution and asked to taste and identify the intensity of the taste. They were then marked a point along a 15 cm line scale anchored from for example 'no bitter' to 'very bitter' that best reflect their perception. After being able to detect the lowest level of bitterness, training proceeded with the determination of intensity for sweetness, saltiness, umaminess and sourness.

Panelists were trained until they are able to detect the lowest intensity for each taste. The solutions with lowest intensity (umami, bitterness, salty, sweet and sour) were used as reference solution.

Sensory evaluation for green mussel hydrolysate was carried out according to Nilsang *et al.* (2005). Panelists were served with 20 ml of 3% (w/v) hydrolysate and the intensity of the five basic tastes were identified by comparing with the reference solutions. They were asked to mark on the 15 cm line that best reflect their perception.

The intensity of fishy odor and fishy flavor were also evaluated by using a 15 cm line scale anchored with 'none' to 'very strong'. Every sample coded with random number was evaluated by panelists. They were asked to taste and sniff every sample and mark on the line.

Degree of acceptability

Degree of acceptability was evaluated according to Normah *et al.* (2013). The sensory evaluation involved thirty panelists using 9 point hedonic scale. Hydrolysate (0.3% w/v) was mixed with 50g plain rice porridge. A commercial hydrolysate was prepared with the same procedure for comparison. Panelists were asked to evaluate their acceptability for color, odor and taste.

Statistical analysis

Statistical analysis was carried out using statistical analysis system (SAS) windows version 9.3.2 (SAS Institute Inc., USA. 2004). ANOVA was used in order to compare differences between means using least significant difference (LSD). The test was conducted at significantly different of 95% confidence interval ($p < 0.05$).

Results and Discussion

Yield and organoleptic characteristics

Yield was calculated as the percentage of hydrolysate to the wet weight of raw green mussel. Green mussel hydrolysate prepared with the addition of STPP and NaCl showed significantly ($p < 0.05$) higher yield (8.34%) followed by green mussel hydrolysate with the addition of STPP (6.34%) and green mussel hydrolysate prepared only in the presence of flavorzyme (4.17%). Addition of small amount of NaCl (1.5%) accelerated the breakdown of fish meat which results in the increase of soluble protein and subsequently the yield of the dried hydrolysate upon centrifugation and freeze-drying of the supernatant. However, studies have shown that in the presence of NaCl above 15%, this will slow down the hydrolysis process due to the effect on the proteolytic activity (Klomklao *et al.*, 2006). The hydrolysate color ranged from light to dark brown in the increasing order from green mussel + flavorzyme hydrolysate (L^* 64.71, a^* -0.89, b^* 21.83) > green mussel + flavorzyme + STPP hydrolysate (L^* 60.52, a^* 2.88, b^* 30.22) > green mussel +flavorzyme + STPP + NaCl (L^* 57.30, a^* 1.82, b^* 25.94) hydrolysate in which hydrolysate produced in the presence of STPP + NaCl exhibited the darkest color. It has been suggested that discoloration occurs during the hydrolysis of muscle protein was due to lipid oxidation via Maillard reaction (Yarnpakdee *et al.*, 2012). The carbonyl group of oxidation products such as ketone and aldehyde react with free amino acid generated during hydrolysis process and led to yellowish hydrolysate (Yarnpakdee *et al.*, 2014). Darker green mussel hydrolysate was probably related to its higher in fat content.

Table 1. Chemical compositions of green mussel hydrolysate produced in the presence of STPP and NaCl

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
GM	$81.50^{a} \pm 0.78$	$13.05^{c} \pm 0.07$	$10.32^{a} \pm 0.48$	$2.36^{c} \pm 0.89$
GM + F	$8.97^{b} \pm 0.12$	$64.52^{a} \pm 0.24$	$3.79^{c} \pm 0.69$	$9.42^{c} \pm 0.75$
GM+ F+ STPP	$7.47^{c} \pm 0.50$	$54.65^{b} \pm 0.29$	$9.39^{ab} \pm 0.88$	$18.88^{b} \pm 0.99$
GM + F + STPP + NaCl	$6.80^{c} \pm 0.34$	$45.94^{c} \pm 0.71$	$8.24^{b} \pm 1.21$	$29.57^{a} \pm 0.66$

Means within column followed by superscript a, b or c is significantly different at $p < 0.05$. Values are means of triplicate determination.

GM: raw green mussel; GM + F: green mussel + flavorzyme; GM + F + STPP: green mussel + flavorzyme + STPP; GM + F + STPP + NaCl: green mussel + flavorzyme + STPP + NaCl

Degree of hydrolysis (DH) was calculated according to the pH stat method. Green mussel hydrolysate with the addition of STPP and NaCI shows highest DH (23.18%) followed by only flavorzyme hydrolysed green mussel hydrolysate and green mussel hydrolysate with addition of STPP. Enzyme type and percentage used during the hydrolysis process affects the DH (Cheung and Li- Chan, 2010; Li *et al.*, 2010). For example, alkaline proteases including alcalase exhibited higher hydrolytic activities than acid or neutral protease such as flavorzyme (Sugiyama *et al.*, 1991). In a comparative study involving alcalase and flavorzyme where alcalase at pH 8, 60ºC and flavorzyme at pH 7, 50ºC were used, higher DH was exhibited by alcalase over the entire hydrolysis period which is 360 min (Dong *et al.*, 2008). DH also depends on the availability of hydrolysis sites, enzyme auto digestion or product inhibition and protein conformation

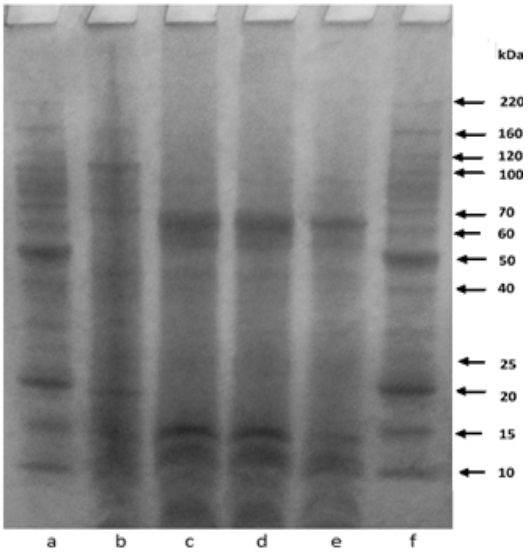

Figure 1. Electrophoresis pattern of green mussel (Perna viridis). From left ; (a) protein marker, (b) raw green mussel, (c) green mussel + flavorzyme, (d) green mussel + flavorzyme + STPP, (e) green mussel + flavorzyme + STPP plus NaCI, (f) protein marker.

(Krinstinsson and Rasco, 2000; Li *et al.*, 2010). The addition of NaCl creates an alkaline environment which in its presence assists in the degradation of the fish protein, thus leads to higher DH compared to other samples.

Chemical analysis

Protein, fat, ash and moisture content of green mussel hydrolysate are tabulated in Table 1. Hydrolysate produced in the presence of STPP and NaCI had the least moisture content (6.80%) compared to others. Salt is commonly used as preservative to reduce the water activity in order to prevent microbial growth (Kurlanski, 2002). The least moisture content was probably due to the presence of salt which drives away water from the hydrolysate. Raw green mussel contained 13.05% protein. Meanwhile, green mussel hydrolysate without the addition of STPP and NaCI shows the highest protein content which is 64.52% followed by green mussel with the addition of STPP (54.65%) and green mussel hydrolysate with the addition of STPP and NaCI (45.94%). NaCI affects the electrostatic and hydrophobic interactions thus influence protein solubility of hydrolysate (Melander and Horvath, 1977).

Hydrolysate prepared without STPP and NaCl had the least amount of fat. Low fat content in hydrolysate may be due to centrifugation process where some fat was separated or trapped in the pellet and discarded (Normah and Nurfazlika, 2013). However, low fat content is desirable since it enhances storage stability of the hydrolysate (Kristinsson and Rasco, 2000). Sodium chloride is able to maintain quality characteristics including moisture level, fat content as it is able to bind with protein and fats (Dotsch *et al.*, 2009). Hydrolysate produced in the presence of STPP and NaCI contain the highest ash content (29.57%). This could be due to the addition of salt during the hydrolysate preparation.

Table 2. Amino acid composition of green mussel and green mussel hydrolysates

Amino acid (g/100g)	Raw GM	GM + F	GM + F +STPP	GM +F +STPP+NaCl
Essential Amino Acids				
Cysteine	0.658^{a} ± 0.11	0.468^{cd} ± 0.05	0.365^{d} ± 0.13	0.657^{a} ± 0.01
Tyrosine	0.248^{b} ± 0.20	0.439^{ab} ± 0.02	0.369^{ab} ± 0.12	0.499^{a} ± 0.01
Threonine	0.150^{b} ± 0.04	0.382^{b} ± 0.02	0.325^{b} ± 0.12	0.523^{a} ± 0.00
Valine	0.553^{a} ± 0.24	0.410^{a} ± 0.04	0.375^{a} ± 0.14	0.531^{a} ± 0.01
Methionine	0.552^{a} ± 0.17	0.683^{a} ± 0.22	0.374^{a} ± 0.22	0.549^{a} ± 0.02
Isoleucine	0.128^{b} ± 0.19	0.279^{ab} ± 0.08	0.287^{ab} ± 0.10	0.361^{a} ± 0.01
Leucine	0.422^{a} ± 0.32	0.372^{a} ± 0.21	0.170^{a} ± 0.08	0.490^{a} ± 0.27
Lysine	0.452^{ab} ± 0.05	0.733^{a} ± 0.66	0.053^{b} ± 0.00	0.301^{ab} ± 0.01
Histidine	0.502^{b} ± 0.18	1.117^{a} ± 0.02	1.051^{a} ± 0.38	1.067^{a} ± 0.02
Phenylalanine	5.746^{a} ± 4.76	16.388^{a} ± 2.60	12.590^{a} ± 3.64	19.800^{a} ± 6.49
Total	9.411	21.271	15.959	24.778
Non Essential Amino Acids				
Serine	1.080^{c} ± 1.06	6.200^{a} ± 0.07	2.386^{bc} ± 0.89	2.996^{b} ± 0.23
Glycine	0.158^{c} ± 0.02	0.298^{b} ± 0.01	0.256^{b} ± 0.09	0.432^{a} ± 0.02
Alanine	0.388^{a} ± 0.13	0.382^{a} ± 0.01	0.340^{a} ± 0.12	0.448^{a} ± 0.01
Proline	1.391^{a} ± 1.18	0.504^{ab} ± 0.01	0.215^{b} ± 0.08	0.879^{ab} ± 0.04
Aspartic acid	0.120^{b} ± 0.04	0.113^{b} ± 0.01	0.088^{b} ± 0.03	0.183^{a} ± 0.01
Arginine	0.621^{ab} ± 0.27	0.673^{a} ± 0.05	0.655^{ab} ± 0.01	0.606^{b} ± 0.16
Glutamic acid	0.640^{a} ± 0.25	0.761^{a} ± 0.01	0.886^{a} ± 0.33	0.851^{a} ± 0.03
Total	4.398	8.931	4.826	6.395

Means within rows followed by superscript a, b or c is significantly different at $p< 0.05$. Values are means of triplicate determination
*Raw GM: raw green muscle; GM + F: green mussel + flavorzyme; GM + F + STPP: green mussel + flavorzyme +STPP; GM + F + STPP + NaCl: green mussel + flavorzyme + STPP + NaCl.

Molecular weight distribution

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was used to determine the molecular weight of raw green mussel and the hydrolysate. Protein bands in green mussel flesh ranges from 10 to 100 kDa while in the hydrolysate the bands appeared between 10 to 70 kDa (Figure 1). Enzymatic breakdown during hydrolysis process involved structural changes in which protein is slowly degraded into smaller peptide units (Krinstinsson and Rasco, 2000). Green mussel hydrolysate with

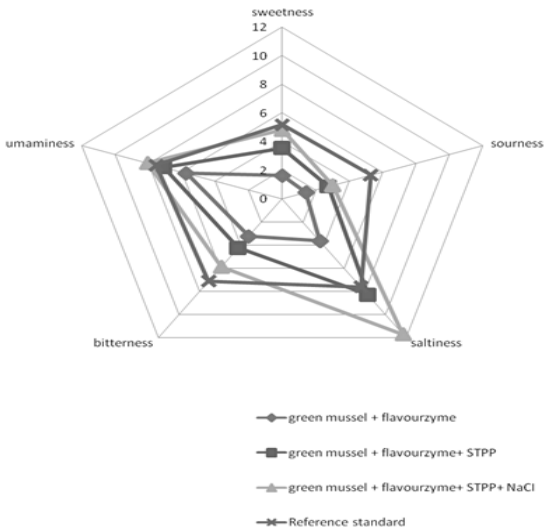

Figure 2. Taste profile of green mussel hydrolysate with or without the addition of STPP and NaCI

addition of STPP and NaCI showed bands with lower intensities especially at 10 to 15 kDa. This result is inline with the protein content where hydrolysate produced in the presence of STPP and NaCl had the lowest amount. Similar finding has been reported previously where the presence of NaCI in duck egg white solution cause a decrease in protein band intensity compared to without the addition of NaCI (Koewmanee *et al*., 2011). Higher NaCI concentration was associated with the aggregation of egg white protein due to induction of hydrophobic interaction (Koewmanee *et al.*, 2011). Surface hydrophobicity decreased with the presence of NaCI (Agyare *et al*., 2009). Salts are able to influence protein solubility by electrostatic and hydrophobic interactions (Melander and Horvath, 1977).

Amino acids composition

Amino acids usually elucidate different tastes (Sarower *et al.*, 2013). Amino acids which are responsible for umaminess and sweetness are glutamic acid, serine, aspartic acid, glycine, threonine, alanine, and proline (Fuke, 1994). Aspartic acid and glutamic acid have a sour taste but give umami taste with the presence of salt (Yamaguchi *et al*., 1971). In scallop, umami taste was due to the presence of glutamic acid, glycine, alanine, arginine, sodium, potassium, and chloride ions (Watanabe *et al.*, 1990). The total amount of aspartic acid, glutamic acid and glycine which contribute to umaminess was higher in green mussel hydrolysate with the addition of STPP and NaCI (1.466 g/100g) followed by green mussel hydrolysate with STPP only (1.23 g/100g), green mussel hydrolysate (1.172g/100g) and raw green mussel (0.918 g/100g) (Table 2). This result is supported by the quantitative descriptive analysis

Table 3. Sensory acceptability of green mussel produced in the presence of STPP and NaCl

Sample	Color	Odor	Taste	Overall acceptability
GM + F	6.50^{a} ±1.77	3.00^{c} ±1.17	3.73^{c} ±0.78	3.97^{c} ±0.61
GM + F + STPP	5.47^{b} ±1.22	5.57^{a}±1.01	4.67^{b} ±1.06	4.77^{a} ±0.63
GM + F + STPP + NaCl	5.03^{b} ±1.04	5.00^{b} ±1.00	4.97^{ab} ±1.08	4.97^{a} ±0.71
Commercial product	3.63^{c} ±0.99	4.97^{b} ±0.85	5.33^{a}±1.03	5.07^{a}±0.64

Means within column followed by superscript a, b or c is significantly different at p< 0.05. Values are means of triplicate determination.
GM: raw green mussel; GM + F: green mussel + flavorzyme; GM + F + STPP: green mussel + flavorzyme + STPP; GM + F + STPP + NaCl: green mussel + flavorzyme + STPP + NaCl

(QDA). Phenylalanine is also found to significantly increase the umami taste (Chen and Zhang, 2007). Phenylalanine and tyrosine are responsible for bitter taste and known as important component for savoury taste in soy sauce with the addition of glutamate (Lioe *et al*., 2004). Sodium ion can intensify saltiness and sweetness and suppress bitterness and sourness (Sarower *et al.,* 2012). QDA results showed that the intensity of saltiness and sweetness was higher in green mussel hydrolysate with the addition of STPP and NaCI. Glycine and aspartic acid which can be found in the flesh contribute to the taste of fish and shellfish (Jung *et al.,* 2005).

Quantitative descriptive analysis and acceptability of green mussel hydrolysate

Quantitative descriptive analysis (QDA) suggested that green mussel hydrolysate produced with the addition of STPP and NaCI had the highest intensity for all the taste attributes (bitter, umami, sweet, sour and salty). This finding contradicted with previous report who stated that the addition of STPP and NaCI can suppress bitterness and enhance saltiness, umaminess and sweetness of hydrolysate (Hayashi *et al.,* 1981; Gillete, 1985). However, the intensity of bitterness for all green mussel hydrolysate was lower than reference caffeine solution (500 ppm) with the score of 7.11. The addition of sodium and potassium can enhance saltiness and umami taste due to their synergistic effect with free amino acids (Lioe *et al*., 2004). The presence of phosphate ion may contribute to the perception of umami taste and saltiness (Hayashi *et al.*, 1981).

Fishy odor and flavor was more intense in green mussel hydrolysate with flavorzyme (10.93 and 11.29)

followed by green mussel hydrolysate with addition of STPP and NaCI (8.31 and 8.8) and green mussel with addition of STPP only (6.13 and 7.1). One factor that contributes to fishy odor is the oxidation of heme protein in the raw material (Raghavan *et al*., 2008). The addition of STPP can be beneficial in terms of retarding the oxidation process and maintaining the flavor characteristics (Isabel, 2010).

Sensory acceptability test showed that there was no significant different ($p>0.05$) in terms of odor, taste and overall acceptability between green mussel hydrolysate produced in the presence of STPP and NaCl with the commercial hydrolysate (Table 3). Odor and taste are two most important attributes that determine consumer acceptability of a product. This finding suggested that green mussel hydrolysate produced in the presence of STPP and NaCl has the potential for commercialization.

Conclusion

Green mussel hydrolysate produced in the presence of STPP and NaCI showed highest yield, DH, ash content and intensity of all the five basic taste attributes (bitter, sweet, sour, salty and umami). The total amount of aspartic acid, glutamic acid and glycine which contribute to umaminess was also highest in this hydrolysate. The hydrolysate was least fishy in terms of odor and flavor and had least amount of protein. Lower protein content could be due to degradation effect in the presence of NaCl which is supported by molecular weight distribution result that indicated the presence of fade protein bands at 10 to 15 kDa. Sensory acceptability data suggested that the hydrolysate is equally acceptable as the commercial hydrolysate in terms of odor and taste. Thus, the addition of STPP and NaCl during the production of green mussel hydrolysate improved the umaminess and fishiness of the hydrolysate which could enhance its acceptance. Most probably, the amount of NaCl added should be reduced so that protein content is not affected.

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