

## Physicochemical properties of mud clam (*Polymesoda erosa*) hydrolysates obtained using different microbial enzymes

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### Article history

Received: 29 April 2014  
Received in revised form:  
19 November 2014  
Accepted: 1 December 2014

### Keywords

Mud clam  
*Polymesoda erosa*  
Alcalase  
Flavourzyme  
Hydrolysate

### Abstract

Physicochemical properties of mud clam (*Polymesoda erosa*) hydrolysate produced using two microbial enzymes; alcalase and flavourzyme were determined. Hydrolysis using alcalase at 20.28% degree of hydrolysis (DH) resulted in 25.06 % yield and 45.37% protein while flavourzyme hydrolysis showed 22.93 % DH, 46.67 % protein and 30.68 % yield. Both hydrolysates were yellowish. Better emulsifying properties, foaming properties and water and oil holding capacity were exhibited by flavourzyme hydrolysate compared to the alcalase hydrolysate. However, in terms of amino acid composition, alcalase hydrolysate contained higher amino acid composition (75.06%) than flavourzyme hydrolysate (62.37%). The study suggested that mud clam hydrolysate had the potential to be used in food formulations for human consumption.

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### Introduction

Mud clam is an edible bivalve species buried in the stiff mud of mangroves (Bayen *et al.*, 2005). It is a non-seasonal species which can be found abundantly and widely distributed throughout the west Indo-pacific region including Peninsular Malaysia and Sarawak (Ingole *et al.*, 2002). The clam is rich in protein and widely consumed for its delicacy.

Proximate composition, functional properties and amino acid composition of protein hydrolysate are important, especially if they are to be used as ingredients in food product. The functional properties of fish protein hydrolysate include solubility, water holding, emulsifying and foaming capacity (Kristinsson and Rasco, 2000). Fish protein hydrolysate has been shown to contain high amount of amino acid (Kristinsson and Rasco, 2000; Aspomo *et al.*, 2005; Nemati *et al.*, 2012).

Commercial enzymes have been extensively studied to improve the quality of protein hydrolysates (Aspomo *et al.*, 2005; Klompong *et al.*, 2007; Bhaskar and Mahendrakar, 2008; Normah *et al.*, 2013). Among these enzymes, proteolytic enzymes from microbial and plant origin are most suitable for the preparation of fish protein hydrolysates (Aspomo *et al.*, 2005; Thiansilakul *et al.*, 2007b). Alcalase, an alkaline enzyme produced from *Bacillus licheniformis* has been found to be the best enzyme for the preparation of functional fish protein hydrolysates by many researchers (Gbououri *et*

*al.*, 2004; Klompong *et al.*, 2008; Chalamaiah *et al.*, 2010). Flavourzyme is a fungal protease or peptidase complex produced by the submerged fermentation of a selected strain of *Aspergillus oryzae*. This enzyme has been used for the hydrolysis of proteins under neutral or slightly acidic conditions to produce a protein hydrolysate with acceptable functional properties (Kristinsson and Rasco, 2000; Klompong *et al.*, 2008). By enhancing the functional properties, protein hydrolysates can be used for various purposes in the food industry which include protein supplement, surimi production, beverage stabilizers, and flavor enhancers (Klompong *et al.*, 2007). Research and reports on commercialization of mud clam are still very limited where most were focused on biodiversity and heavy metal detection (Yap and Mohd Azri, 2009). Furthermore, there was no report on the production of hydrolysate from mud clam. Therefore, in this study, hydrolysates from mud clam were produced using microbial based enzymes; alcalase and flavourzyme and the physicochemical properties of the hydrolysates were compared.

### Materials and Methods

#### Raw materials

Mud clam (*Polymesoda erosa*) was obtained from a supplier in Kuala Selangor, packed in ice and immediately transported to the laboratory. Upon arrival, the flesh was removed manually from the shell and then washed.

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### Enzymes

Alcalase 2.4 L with a declared activity of 2.4 Anson Units (AU) g<sup>-1</sup> and Flavourzyme 500 L with a declared activity of 500 Leucine Amino Peptidase Units per gram (LAPU/g) were purchased from Novozyme Sdn Bhd Malaysia.

### Preparation of mud clam hydrolysate

Mud clam hydrolysate was prepared according to Normah *et al.*, (2013). An amount of 453 g flesh and 619 ml distilled water was mixed and homogenized until fine in a blender. The mixture was then incubated in a circulated water bath. The pH of the mixture was adjusted to 8.5 and maintained constant during hydrolysis using 4.0 N NaOH. Once the pH and temperature (60°C) of the mixture has stabilized, alcalase at enzyme-substrate ratio of 3% was added and the hydrolysis continued for 2 hrs. The mixture was continuously stirred using a stirring propeller throughout the hydrolysis period. At the end of hydrolysis, the enzymatic reaction was terminated by placing the mixture in a water bath set at 90°C for 15 min. This was followed by centrifugation at 10 000 rpm, 4°C for 20 mins. Supernatant obtained was freeze-dried using the Sanyo-Biomedical freeze dryer (Alpha 1-4, Martin Christ). The powdered hydrolysate obtained after freeze drying was stored at -20°C until further analysis. Similar procedure was repeated using Flavourzyme 500L with conditions at 55°C and pH 7.

### Degree of hydrolysis

Degree of hydrolysis (DH) was determined according to the method described by Hoyle and Merrit (1994) and Liceaga-Gesualdo and Li-Chan (1999). Ten mL supernatant was mixed with 10 mL of 20% trichloroacetic acid (TCA) to obtain soluble and insoluble fraction in 10% TCA solution and then centrifuged at 3000 × g for 15 min (Model 5420, Kubota, Japan). The soluble protein content of the supernatant was determined by Lowry method modified by Hartree (1972). Bovine serum albumin was used as standard. The degree of hydrolysis was calculated according to the equation below:

$$\text{DH \%} = \frac{\text{Soluble protein content in 10 \% TCA}}{\text{Total protein content in the supernatant}} \times 100$$

### Yield

Yield was determined by the ratio of hydrolysate mass to the wet weight of mud clam flesh. The yield obtained was calculated as follows:

$$\text{Yield (\%)}: \frac{\text{Weight of powdered hydrolysate}}{\text{Wet weight of mud clam flesh}} \times 100$$

### Color measurement

The color of powdered hydrolysate was measured by chromameter CR400 (Konica Minolta, Japan). L\*, a\* and b\* parameters indicate lightness, redness and yellowness, respectively. The white calibration plate was used as reference. Measurement was performed in triplicate.

### Proximate composition

Moisture, fat and ash contents were determined according to AOAC (2005). The moisture content was determined by placing approximately 2 g of sample into a pre-weight aluminium dish. Samples were dried in an oven at 105°C until constant weight was obtained. Total lipid was determined by soxhlet extraction. Ash content was determined by charring a pre-dried sample in a crucible at 600°C until constant weight of white ash was obtained. Protein concentration was determined using the Lowry method based on the modified procedure of Hartree (1972).

### Determination of functional properties

#### Solubility

Solubility was determined according to Klompong *et al.*, (2007) with slight modification. Two hundred mg hydrolysate was dispersed in 20 ml deionized water and the pH was adjusted to 2 to 10. The mixture was stirred at room temperature for 30 mins and then centrifuged at 7, 500 x g for 15 mins (Model 5420, Kubota, Japan). Protein content of the supernatant was determined using Lowry method based on the modified procedure of Hartree (1972). Protein solubility was calculated as:

$$\text{Solubility} = \frac{\text{Protein content in supernatant}}{\text{Total protein content in the sample}} \times 100$$

#### Emulsifying properties

The emulsifying activity index (EAI) and the emulsion stability index (ESI) were determined using the method described by Klompong *et al.*, (2007). Three hundred mg hydrolysate was dissolved in 30 ml deionized water. The solution was then mixed with 10 ml sunflower oil and the pH was adjusted to 2 to 10. The mixture was homogenized at a speed of 14, 000 rpm for 1 minute using a homogenizer (IKA T25 digital Ultra-Turrax). Fifteen µl of aliquot emulsion was pipetted from the bottom of the container at 0 and 10 min after homogenization. The sample was then mixed with 5 ml of 0.1% sodium dodecyl sulfate solution. The absorbance was measured at 500 nm using a UV-Vis spectrophotometer. This was

used to calculate the EAI and ESI using the method suggested by Pearce and Kinsella (1978) computed by the equation below:

$$\text{EAI (m}^2/\text{g)} = (2 \times 2.303 \times A_{500}) / (0.25 \times \text{protein weight (g)})$$

$A_{500}$  is the absorbance at 500 nm

$$\text{ESI (min)} = A_0 \times \Delta t / \Delta A$$

$\Delta A = A_0 - A_{10}$ ,  $\Delta t = 10\text{min}$ , where  $A_0$  is the absorbance at zero min and  $A_{10}$  is the absorbance at 10 min, at 500nm

#### *Foaming properties*

Foaming capacity and stability was measured using the method of Sathe and Salunkhe (1981) with slight modification. Three g sample was dissolved in 100 ml distilled water and the pH was adjusted to 2 to 10. The protein solution was whipped at 16, 000 rpm for 3 mins with a homogenizer (IKA T25 digital Ultra-Turrax) and poured into a 100 ml graduated cylinder. The total sample volume was taken at 0 min for foam capacity and up to 60 mins for foam stability. Foam capacity and stability were then calculated as in the equation below:

$$\text{Foam capacity (\%)} = [(A-B) / B] \times 100$$

A = the volume after whipping (mL) at 0 min

B = the volume before whipping (mL)

$$\text{Foam stability (\%)} = [(A-B) / B] \times 100$$

A = the volume after standing (mL) at 60 min

B = the volume before whipping (mL)

#### *Water holding capacity*

The water holding capacity (WHC) was determined according to Diniz and Martin (1997). An amount of 0.5 g hydrolysate was dissolved in 10 ml distilled water in a centrifuge tube and then vortexed for 30 seconds. The dispersions were allowed to stand at room temperature for 30 mins and then centrifuged at 2,800 x g for 25 mins (Model 5420, Kubota, Japan). The supernatant was filtered and the volume was accurately measured. The difference between initial volume of the distilled water and hydrolysate mixture and the supernatant volume was retrieved. The result was reported as mL of water absorbed per gram of protein hydrolysate.

#### *Oil holding capacity*

The oil holding capacity (OHC) was determined using the method of Shahidi *et al.*, (1995) with slight modification. Ten ml soybean oil was added into 500 mg hydrolysate in a centrifuge tube. The mixture was centrifuged for 25 mins in 3800 x g (Model 5420, Kubota, Japan). Oil absorption was determined by

decanting the supernatant oil and the remaining oil was reported by weight difference. The analysis was done in triplicate.

$$\text{OHC (mL/g)} = \text{volume before centrifuge (mL/g)} - \text{volume of supernatant (mL/g)}$$

#### *Amino acid analysis*

Samples were hydrolyzed in 6N HCl at 110°C for 24 hrs and derivatised using phenyl isothiocyanate prior to analysis. The total amino acid was analysed using the AccQ Tag HPLC equipped with fluorescence detector, an AccQ Tag column (3.9 x 150 mm) at a flow rate of 1 ml/ min. The mobile phase used was AccQ Tag Eluent A consists of 100 ml Eluent A and 1000 ml deionized water, while AccQ Tag Eluent B consists of 60% acetonitrile and 40% deionized water or 60% acetonitrile. The total running time was 50 mins.

#### *Statistical analysis*

The data obtained was analysed using the Analysis of Variance (ANOVA) to determine significance at 5% level. Duncan Multiple Range Test (DMRT) was used to identify differences between means. Statistical analysis was performed using the Statistical Package for Social Science (SPSS for windows: SPSS Inc. Chicago, IL, USA, 2006).

## **Results and Discussion**

#### *Degree of hydrolysis*

Degree of Hydrolysis (DH) is one of the basic parameters that describe the properties of protein hydrolysate which indicate the percentage of peptide bond cleaved (Adler-Nissen, 1986). Degree of hydrolysis of mud clam hydrolysate production is shown in Table 1. At the given hydrolysis conditions, flavourzyme produced a slightly higher degree of hydrolysis (22.93%) than alcalase (20.28%). Flavourzyme also produced higher DH when compared with neutrase (Slizyte *et al.*, 2005a). The higher DH for hydrolysate production could be due to the higher percentage of peptide bonds cleaved during the hydrolysis (Adler-Nissen, 1986). It could also be due to the conformation of protein which leads to the difference in the number of peptide bond to alter the hydrolysis rate.

#### *Yield and color*

The yield of mud clam hydrolysate was significantly higher ( $p < 0.05$ ) when the hydrolysate was produced using flavourzyme (30.68%) compared to alcalase (25.06%) (Table 1). Higher yield

Table 1. Degree of hydrolysis (%), yield and color of mud clam (*Polymesoda erosa*) hydrolysate produced using alcalase and flavourzyme

|           | Alcalase                 | Flavourzyme              |
|-----------|--------------------------|--------------------------|
|           | hydrolysate              | hydrolysate              |
| DH (%)    | 20.28 <sup>a</sup> ±0.17 | 22.93 <sup>a</sup> ±0.06 |
| Yield (%) | 25.06 <sup>b</sup> ±0.52 | 30.68 <sup>a</sup> ±0.74 |
| Color     |                          |                          |
| L*        | 70.03 <sup>b</sup> ±0.10 | 74.53 <sup>a</sup> ±0.14 |
| a*        | 0.13 <sup>a</sup> ±0.01  | -0.66 <sup>b</sup> ±0.05 |
| b*        | 21.82 <sup>a</sup> ±0.08 | 21.77 <sup>a</sup> ±0.16 |

Values are expressed as means ±standard deviation. Different letters within rows indicate significant different at  $p < 0.05$

produced by flavourzyme was due to its ability to solubilise more protein (Thiansilakul *et al.*, 2007a). Type of enzyme, substrate and hydrolysis conditions including pH, temperature, incubation time and enzyme concentration affect the yield of soluble materials (Adler-Nissen, 1986). The studies on the effect of hydrolysis times at 1 to 4 hr hydrolysis on the protein yield from different parts of mackerel using different alcalase concentrations (0.5, 1 and 2%) showed that protein yield was lowest after 1 hr at 0.5% enzyme concentration and highest after 4 hr of hydrolysis using 2% enzyme concentration (Ramakrishnan *et al.*, (2013).

Hydrolysate color is influenced by several factors such as the process, species difference, fat content, moisture and the presence of hemoglobin and myoglobin (Taheri *et al.*, 2013). The color of mud clam flesh and hydrolysate is shown in Table 1. Mud clam hydrolysate was brownish yellow. Flavourzyme hydrolysate ( $L^* = 74.53$ ,  $a^* = -0.06$ ,  $b^* = 21.77$ ) was significantly ( $p < 0.05$ ) lighter than alcalase hydrolysate ( $L^* = 70.03$ ,  $a^* = 0.13$ ,  $b^* = 21.82$ ). Herring gonad hydrolysate was darker and yellower than whole herring hydrolysate (Sathivel *et al.*, (2003). In addition, anglewing (*Pholas orientalis*) hydrolysate produced using alcalase was light yellow ( $L^* = 72.98$ ,  $a^* = 0.42$ ,  $b^* = 15.15$ ) (Normah and Nurfazlika Nashrah, 2013). Therefore, the color of fish protein hydrolysates depends on the raw materials composition as well as the type of enzyme used.

#### Proximate composition

The protein content of mud clam hydrolysate was significantly higher ( $p < 0.05$ ) than the flesh (Table 2). The percentage of solubilised protein depends on the amount of lipids in the raw materials (Thiansilakul *et al.*, 2007a). During the hydrolysis process, the

Table 2. Proximate compositions of mud clam (*Polymesoda erosa*) hydrolysates produced using alcalase and flavourzyme

| Composition | Mud                       | Alcalase                 | Flavourzyme              |
|-------------|---------------------------|--------------------------|--------------------------|
| (%)         | flesh                     | hydrolysate              | hydrolysate              |
| Protein     | 10.68 <sup>b</sup> ± 0.30 | 45.37 <sup>a</sup> ±0.08 | 46.67 <sup>a</sup> ±0.28 |
| Fat         | 3.09 <sup>a</sup> ± 0.21  | 0.81 <sup>c</sup> ± 0.65 | 1.06 <sup>b</sup> ± 0.26 |
| Moisture    | 72.71 <sup>a</sup> ±0.41  | 6.98 <sup>c</sup> ±0.11  | 8.13 <sup>b</sup> ±0.23  |
| Ash         | 10.50 <sup>b</sup> ±0.31  | 22.45 <sup>a</sup> ±1.29 | 21.09 <sup>a</sup> ±0.28 |

Values are expressed as means ±standard deviation. Different letters within rows indicate significant different at  $p < 0.05$

insoluble undigested non protein substance and lipid from raw sample was partially removed to give higher soluble protein content in the hydrolysate (Thiansilakul *et al.*, 2007b). Raw materials containing the highest amount of lipids gave the lowest percentage of solubilised protein (Slizyte *et al.*, 2005a). Higher protein content than the raw materials have been reported in several hydrolysates (Benjakul and Morrissey 1997; Gbogouri *et al.*, 2004; Wasswa *et al.*, 2007; Normah *et al.*, 2013). Flavourzyme hydrolysate contains higher amount of protein than the alcalase hydrolysate (Table 2). Previous study showed that at 60% DH, flavourzyme produced hydrolysate from round scad containing 69% protein (Thiansilakul *et al.*, 2007a). Flavourzyme hydrolysate when produced at similar DH exhibited higher molecular weight peptides than alcalase hydrolysate (Klompong *et al.*, 2008). In addition, increased in DH resulted in increased in protein recovery (Gbogouri *et al.*, 2004).

The fat content of mud clam hydrolysate was significantly lower ( $p < 0.05$ ) than the flesh (Table 2). The reduction of fat content in the hydrolysate was associated with fat separation during the centrifugation process (Thiansilakul *et al.*, 2007a). This is in agreement with the previous report where the fat content in Caspian shad (*Alosa caspia*) by-product (8.26%) were higher than its hydrolysate from hydrolysis using alcalase and flavourzyme where the fat contents were only 0.92% and 1.02%, respectively (Nemati *et al.*, 2012). Decreasing the fat content in a protein hydrolysate contributes significantly to the increase in the stability of the hydrolysate towards lipid oxidation (Kristinsson and Rasco, 2000). The higher fat content in flavourzyme hydrolysate than alcalase may be due to higher fat release during enzymatic hydrolysis. Furthermore, the retention of fat in hydrolysates is also accounted

Table 3. Functional properties of mud clam (*Polymesoda erosa*) hydrolysate produced using alcalase and flavourzyme

| Solubility (%)                                    |    | Alcalase hydrolysate        | Flavourzyme hydrolysate    |
|---|----|-----------------------------|----------------------------|
| pH  | 2  | 98.76 <sup>aA</sup> ± 0.12  | 87.86 <sup>dB</sup> ± 0.39 |
|   | 4  | 95.53 <sup>dA</sup> ± 0.22  | 85.72 <sup>eB</sup> ± 0.20 |
|   | 6  | 96.19 <sup>cA</sup> ± 0.10  | 89.52 <sup>cB</sup> ± 0.23 |
|   | 8  | 98.80 <sup>aA</sup> ± 0.18  | 91.96 <sup>bB</sup> ± 0.24 |
|   | 10 | 96.58 <sup>bA</sup> ± 0.34  | 94.52 <sup>aB</sup> ± 0.20 |
| Emulsion Activity Index (EAI) (m <sup>2</sup> /g) |    |                             |                            |
| pH  | 2  | 0.39 <sup>eB</sup> ± 0.04   | 1.77 <sup>eA</sup> ± 0.01  |
|   | 4  | 0.74 <sup>dB</sup> ± 0.21   | 2.01 <sup>dA</sup> ± 0.04  |
|   | 6  | 1.86 <sup>cB</sup> ± 0.04   | 2.35 <sup>cA</sup> ± 0.04  |
|   | 8  | 2.04 <sup>bB</sup> ± 0.07   | 2.60 <sup>bA</sup> ± 0.04  |
|   | 10 | 2.19 <sup>aB</sup> ± 0.04   | 2.96 <sup>aA</sup> ± 0.02  |
| Emulsion Stability Index (ESI)(min)               |    |                             |                            |
| pH  | 2  | 5.49 <sup>eB</sup> ± 1.03   | 62.77 <sup>cA</sup> ± 0.46 |
|   | 4  | 7.37 <sup>dB</sup> ± 0.15   | 100.5 <sup>aA</sup> ± 0.87 |
|   | 6  | 11.67 <sup>cB</sup> ± 0.41  | 44.76 <sup>eA</sup> ± 0.82 |
|   | 8  | 33.57 <sup>bB</sup> ± 1.85  | 95.33 <sup>bA</sup> ± 0.58 |
|   | 10 | 108.89 <sup>aA</sup> ± 1.92 | 50.17 <sup>dB</sup> ± 0.29 |
| Foam Capacity (%)                                 |    |                             |                            |
| pH  | 2  | 20.50 <sup>eB</sup> ± 0.50  | 25.50 <sup>eA</sup> ± 0.05 |
|   | 4  | 90.50 <sup>aB</sup> ± 0.76  | 98.12 <sup>aA</sup> ± 0.67 |
|   | 6  | 80.50 <sup>bB</sup> ± 1.61  | 85.17 <sup>bA</sup> ± 0.29 |
|   | 8  | 40.50 <sup>dB</sup> ± 0.50  | 60.83 <sup>dA</sup> ± 1.04 |
|   | 10 | 65.20 <sup>cB</sup> ± 0.26  | 71.83 <sup>cA</sup> ± 2.75 |
| Foam Stability (%)                                |    |                             |                            |
| pH  | 2  | 6.80 <sup>eB</sup> ± 0.20   | 22.50 <sup>eA</sup> ± 0.50 |
|   | 4  | 86.57 <sup>bA</sup> ± 1.38  | 90.50 <sup>aB</sup> ± 0.50 |
|   | 6  | 98.1 <sup>aA</sup> ± 0.23   | 80.50 <sup>bB</sup> ± 0.50 |
|   | 8  | 71.47 <sup>cA</sup> ± 0.50  | 40.50 <sup>dB</sup> ± 0.50 |
|   | 10 | 30.33 <sup>dB</sup> ± 0.58  | 55.33 <sup>cA</sup> ± 1.53 |
| Water Holding Capacity (WHC) (mL/g)               |    | 3.17 <sup>b</sup> ± 0.29    | 4.73 <sup>a</sup> ± 0.25   |
| Oil Holding Capacity (OHC) (mL/g)                 |    | 3.40 <sup>b</sup> ± 0.10    | 6.23 <sup>a</sup> ± 0.25   |

Values are expressed as means ± standard deviation. Different letters indicate significant different at  $p < 0.05$

by the emulsion formed in conjunction with protein and peptides during the hydrolysis (Jamdar *et al.*, 2010).

The moisture content was significantly lower ( $p < 0.05$ ) in mud clam hydrolysate than the flesh (Table 2). The reduction in moisture content was due to the lost of moisture after freeze drying. The higher moisture content in flavourzyme than alcalase may be due to the higher content of polar groups. Polar groups such as COOH and NH<sub>2</sub> that are present during the hydrolysis process affect the amount of adsorbed water (Kristinsson and Rasco, 2000).

Alcalase and flavourzyme hydrolysate contain significantly ( $p < 0.05$ ) higher ash content than the flesh, respectively (Table 2). High ash content in the hydrolysate has been suggested to be due to the addition of buffer, hydrochloric acid or sodium hydroxide for pH adjustment during hydrolysis (Thiansilakul *et al.*, 2007a). In this study, alcalase hydrolysate had higher ash content because more sodium hydroxide was added during hydrolysis to maintain the constant pH.

### Functional properties

#### Solubility

Solubility is a good indicator of the functionality

of protein hydrolysate. Alcalase hydrolysate showed highest solubility at pH 8 (98.80%) and lowest at pH 4 (95.53%) (Table 3). For flavourzyme hydrolysate, the highest solubility was at pH 10 (94.52%) and lowest at pH 4 (85.72%). Flavourzyme hydrolysate was significantly less soluble ( $p < 0.05$ ) than alcalase hydrolysate. In the hydrolysis of bluewing searobin, the used of alcalase resulted in higher solubility (45%) compared to flavourzyme (35%) at pH 9 (Sarita *et al.*, 2009). High solubility of hydrolysates over a wide pH range is due to the low molecular weight of the peptides which are quite rich in hydrophilic amino acids (Taheri *et al.*, 2013). Different pH gave the variation in solubility to the protein hydrolysate produced by both enzymes (Table 3). Solubility variation could be attributed to both the net charge of peptides that increase as pH moves away from isoelectric point (PI) and surface hydrophobicity which promotes aggregation via hydrophobic interaction (Klompong *et al.*, 2007). Hydrolysate showed low solubility at their isoelectric point (Taheri *et al.*, 2007). The lowest solubility of both hydrolysate was at pH 4 (Table 3). This finding is consistent with Gbogouri *et al.*, (2004) and Klompong *et al.*, (2007) studies. Hydrolysis exposed some hydrophobic groups which were converted into hydrophilic ones by generating two end carbonyl and amino groups

Table 4. Amino acid composition of mud clam (*Polymesoda erosa*) hydrolysate produced using alcalase and flavourzyme

| Amino acid                       |                          | Mud clam<br>flesh         | Alcalase<br>hydrolysate   | Flavourzyme<br>hydrolysate |
|----------------------------------|--------------------------|---------------------------|---------------------------|----------------------------|
| Essential<br>amino acid          | Arg                      | 2.84 <sup>b</sup> ± 0.52  | 5.29 <sup>a</sup> ± 0.83  | 2.09 <sup>b</sup> ± 0.21   |
|                                  | His                      | 4.10 <sup>b</sup> ± 0.96  | 5.69 <sup>a</sup> ± 0.91  | 3.81 <sup>c</sup> ± 0.88   |
|                                  | Ile                      | 1.60 <sup>b</sup> ± 0.50  | 2.45 <sup>a</sup> ± 0.56  | 1.01 <sup>b</sup> ± 0.67   |
|                                  | Leu                      | 1.61 <sup>c</sup> ± 0.52  | 4.89 <sup>b</sup> ± 0.42  | 5.98 <sup>a</sup> ± 0.86   |
|                                  | Lys                      | 2.02 <sup>b</sup> ± 0.91  | 2.97 <sup>a</sup> ± 0.86  | 1.58 <sup>c</sup> ± 0.92   |
|                                  | Met                      | 1.72 <sup>b</sup> ± 0.41  | 3.71 <sup>a</sup> ± 0.32  | 0.68 <sup>c</sup> ± 0.59   |
|                                  | Phe                      | 17.18 <sup>b</sup> ± 0.11 | 21.56 <sup>a</sup> ± 0.41 | 23.12 <sup>a</sup> ± 0.40  |
|                                  | Thr                      | 1.21 <sup>b</sup> ± 0.20  | 2.79 <sup>a</sup> ± 0.47  | 1.41 <sup>b</sup> ± 0.33   |
|                                  | Tyr                      | 1.86 <sup>a</sup> ± 0.97  | 1.25 <sup>a</sup> ± 0.54  | 1.13 <sup>a</sup> ± 0.57   |
| Val                              | 1.35 <sup>b</sup> ± 0.10 | 3.17 <sup>a</sup> ± 0.59  | 1.10 <sup>a</sup> ± 0.02  |                            |
| Total                            |                          | 33.89 <sup>c</sup>        | 53.77 <sup>a</sup>        | 41.91 <sup>b</sup>         |
| Non -<br>Essential<br>amino acid | Asp                      | 0.18 <sup>a</sup> ± 0.13  | 0.79 <sup>a</sup> ± 0.85  | 0.42 <sup>a</sup> ± 0.01   |
|                                  | Cys                      | 0.47 <sup>c</sup> ± 0.56  | 6.54 <sup>b</sup> ± 0.70  | 7.38 <sup>a</sup> ± 0.88   |
|                                  | Glu                      | 3.30 <sup>b</sup> ± 0.40  | 4.10 <sup>a</sup> ± 0.38  | 2.86 <sup>c</sup> ± 0.91   |
|                                  | Ala                      | 2.21 <sup>a</sup> ± 0.93  | 1.86 <sup>b</sup> ± 0.53  | 1.43 <sup>b</sup> ± 0.31   |
|                                  | Gly                      | 2.64 <sup>b</sup> ± 0.94  | 1.71 <sup>a</sup> ± 0.51  | 2.09 <sup>b</sup> ± 0.21   |
|                                  | Pro                      | 3.62 <sup>a</sup> ± 0.39  | 2.72 <sup>a</sup> ± 0.23  | 1.14 <sup>b</sup> ± 0.06   |
| Ser                              | 1.38 <sup>c</sup> ± 0.29 | 3.57 <sup>b</sup> ± 0.14  | 5.14 <sup>a</sup> ± 0.10  |                            |
| Total                            |                          | 13.80 <sup>b</sup>        | 21.29 <sup>a</sup>        | 20.46 <sup>a</sup>         |
| Total amino<br>acid              |                          | 47.69 <sup>c</sup>        | 75.06 <sup>a</sup>        | 62.37 <sup>b</sup>         |

Values are expressed as means ± standard deviation. Different letters within rows indicate significant different at  $p < 0.05$

(Cho *et al.*, 2008). Therefore, the resulting higher solubility after hydrolysis by both enzymes may be due to the increased in the hydrophilicity of the hydrolysate. Protein hydrolysate with high protein solubility can be applied into food formulation by providing attractive appearance and smooth mouth feel to the product (Foh *et al.*, 2011). Solubility also affects other functional properties such as emulsion and foaming properties as shown in Table 3.

#### *Emulsifying activity index (EAI) and emulsion stability index (ESI)*

The formation of good and stable emulsions is important in food system. Therefore the number of peptides and the exposure of hydrophobic amino acid residue should increase in the hydrolysis of protein in order to improve the formation of emulsion (Foh *et al.*, 2011). Emulsifying activity index (EAI) and Emulsion Stability Index (ESI) of mud clam hydrolysate are shown in Table 3. For alcalase hydrolysate, the highest EAI was at pH 10 (2.19 m<sup>2</sup>/g) and lowest at pH 2 (0.39 m<sup>2</sup>/g). The emulsion was most stable at pH 10 (108.89 min) and least stable at pH 2 (5.49 min). For flavourzyme hydrolysate, the highest EAI was at pH 10 (2.96 m<sup>2</sup>/g) and lowest at pH 2 (1.77 m<sup>2</sup>/g). The emulsion was most stable at

pH 4 (100.5 min) and least stable at pH 6 (44.76 min).

The higher EAI value at pH 10 may be due to higher quantities of soluble proteins generated by hydrolysis under alkaline condition. In addition, at highly alkaline pH, polypeptide can be unfolded due to negative charges (Taheri *et al.*, 2013). Repulsion could be resulted from this charge and allowing better orientation at the interface. EAI values also increased with pH. Alkaline condition will affect the structural unfolding of polypeptides where more negative charges were generated causing repulsion and allowing a better orientation at the interface (Aspmo *et al.*, 2005).

Emulsifying properties are influenced by blending speed, temperature, protein source, pH, water content and types of oil added (Linder *et al.*, 1996). Environmental pH changed the surface hydrophobicity and the solubility of the protective layer surrounding the lipid globules (Taheri *et al.*, 2013). In general, the factors that most influence the emulsifying properties are solubility and enzyme specificity (Slizyte *et al.*, 2005a; Klompong *et al.*, 2007). The lower solubility of flavourzyme hydrolysate at every pH range than alcalase hydrolysate resulted in better emulsion properties to flavourzyme hydrolysate (Table 3). Higher emulsion

properties in flavourzyme hydrolysate than alcalase has also been observed in bluewing searobin (Sarita *et al.*, 2009).

#### *Foaming capacity and stability*

Foaming capacity and stability of mud clam hydrolysate are shown in Table 3. Foaming properties were lower at acidic pH. For alcalase hydrolysate, the highest foaming capacity was at pH 4 (90.50%) and lowest at pH 2 (20.50%). The foam was most stable at pH 6 (98.1%) and least stable at pH 2 (6.8%). For flavourzyme hydrolysate, the highest foaming capacity was at pH 4 (98.12%) and lowest at pH 2 (25.50%). The foam was most stable at pH 4 (90.50%) and least at pH 2 (22.50%). Protein solubility significantly contributes to the foaming behavior of protein (Gbougouri *et al.*, 2004). The net charge affects the adsorption of the proteins at the air-water interface. Increasing the net charge will enhance the foaming properties (Gbougouri *et al.*, 2004). Flavourzyme hydrolysate had higher foaming capacity at every pH range because the solubility of flavourzyme hydrolysate was lower than that of alcalase (Table 3).

Foam stability depends on the nature of the film and reflects the extent of protein-protein interaction within the sample matrix (Klompong *et al.*, 2007). From the result, both enzymes gave significantly ( $p \leq 0.05$ ) higher foam stability at pH 4 (90.50%) for flavourzyme and pH 6 (98.1%) for alcalase. In addition, foam stability of protein hydrolysate is attributed to the high molecular weight peptides (Klompong *et al.*, 2007).

#### *Water holding capacity (WHC) and oil holding capacity (OHC)*

Protein has both the hydrophilic and hydrophobic properties thereby can interact with water and oil in foods. Variation in water and oil adsorption capacity of protein may be due to differences in protein concentration, their degree of interaction with water and oil and possibly their conformational characteristics (Kristinsson and Rasco, 2000). The water holding capacity of mud clam hydrolysate is shown in Table 3. Flavourzyme hydrolysate had higher water holding capacity (4.73 ml/g) compared to that of alcalase (3.17 ml/g). High solubility will decrease the WHC of fish protein due to the higher concentration of polar group in the hydrolysate that will affect the amount of adsorbed water (Taheri *et al.*, 2013). Therefore, flavourzyme hydrolysate showed higher WHC since it has lower solubility than alcalase hydrolysate as shown in Table 3.

The mechanism of fat absorption is mostly attributed to the physical entrapment of the oil. The higher bulk density of the protein, the higher is the fat absorption. The oil holding capacity obtained from flavourzyme hydrolysate was significantly ( $p < 0.05$ ) higher (6.23 mL/g) than that of alcalase (3.40 mL/g) as shown in Table 3. This is in agreement with previous study where the oil holding capacities for alcalase and flavourzyme hydrolysate of bluewing searobin were 4.50 mL/g and 5.52 mL/g, respectively (Sarita *et al.*, 2011). The good oil holding capacity of flavourzyme hydrolysate could be due to the higher surface hydrophobicity, degree of hydrolysis and enzyme or substrate specificity as suggested by Kristinsson and Rasco, (2000). In contrary, low oil absorption may be due to the presence of large proportion of hydrophilic groups and polar amino acids on the surface of the protein molecules (Sathe *et al.*, 1982).

#### *Amino acid composition*

The amino acid composition of mud clam flesh and the hydrolysates are shown in Table 4. Mud clam hydrolysate had higher amount of essential and total amino acids than the flesh. Enzymatic hydrolysis converts protein in the flesh into sequences of essential amino acids (Klompong *et al.*, 2007). Alcalase hydrolysate contain higher amount of total amino acid than that of flavourzyme. This is in agreement with previous findings when alcalase, protamex and flavourzyme were used to hydrolyse the Caspian shad (*Alosa caspia*) by-product hydrolysate (Nemati *et al.*, 2012). They found that among the three enzymes used, hydrolysis using alcalase resulted in highest total amino acid (95.51%) while the lowest total amino acid was recorded in flavourzyme hydrolysate (83.17%). The essential amino acids to non-essential amino acid ratio were 2.53 and 2.05 for alcalase and flavourzyme hydrolysates, respectively (Table 4). It was stated that adults require essential amino acid to non essential amino acid ratio of not less than 0.6 and essential amino acid to total amino acid ratio should not be less than 40% (Heinrikson and Meredith, 1984). Therefore, mud clam hydrolysate produced by both the alcalase and flavourzyme gave the desirable amino acid value as needed by adults.

#### **Conclusion**

The hydrolysates produced from mud clam differed significantly depending on the type of enzyme used in preparing the hydrolysates. Hydrolysis using flavourzyme gave higher value of protein, ash, fat, moisture content and functional

properties (emulsifying, foaming, water and oil holding capacity) than alcalase hydrolysis. However, higher total amino acid composition was recorded in alcalase hydrolysate. In general, results showed a potential utilization of hydrolysate from mud clam in food formulations for direct human consumption.

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