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FULL PAPER

Characterization of Asian swamp eel (*Monopterus sp.*) protein hydrolysate functional properties prepared using Alcalase® enzyme

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Abstract

Fish protein hydrolysates have good nutritional properties and thus can be obtained by treatment of fish meat with enzymes under controlled conditions. It is used for texturing, gelling, foaming, emulsification, protein supplements, flavor enhancers, and beverage stabilizers. The aims of this study were to prepare eel protein hydrolysate (EPH) prepared using Alcalase® enzyme and characterize its physicochemical properties. The structural, solubility, emulsifying and foaming properties, water holding and oil binding capacity of EPH were examined. Structural results obtained found that the presence of hydrophilic and hydrophobic amino acids together with the presence of aromatic groups. There were no significant differences ($p>0.05$) between EPH at different pH levels in solubility. However, in terms of emulsifying and foaming properties, EPH showed significant differences ($p<0.05$) at different pH levels, while water holding capacity showed significant differences ($p<0.05$) at different EPH concentrations. There were no significant differences ($p>0.05$) in the oil binding capacities of EPH at different concentrations. The functional properties possessed by EPH showed that it has potential as an emulsifier and stabilizer in food products, playing an important role in the food industry.

1. Introduction

Hydrolysis of fish protein has been widely conducted to obtain a valuable food ingredient with good functional properties for the food industry (Yin *et al.,* 2010). Different techniques such as enzymatic hydrolysis, autolysis and thermal hydrolysis have been used to produce hydrolysates from fish and its by-products (Molla and Hovannisyan, 2011; Prabha *et al.,* 2013; Wang *et al.,* 2013). Among these techniques, enzymatic hydrolysis with various types of enzymes such as Alcalase®, protamex, neutrase, trypsin, pepsin and α-chymotrypsin is the most used by researchers (Chalamaiah *et al.,* 2012). There has been a great deal of research conducted in producing fish protein hydrolysates from different fish sources hydrolyzed using Alcalase®, such as skipjack, tilapia and shortfin scad (Intarasirisawat *et al.,* 2012; Wang *et al.,* 2013; Ishak and Sarbon, 2016).

The use of proteases in the production of protein hydrolysate is frequently an attractive means for improving functional properties of food proteins by modifying their physical and chemical properties while maintaining their nutritional value (Taheri *et al.,* 2013; Elavarasan *et al.,* 2014). Many studies have demonstrated good functional properties in terms of solubility, emulsifying properties, foaming properties, water holding capacity and oil or fat binding capacity for fish hydrolysates prepared using proteolytic enzymes (Nalinanon *et al.,* 2011; Jemil *et al.,* 2014). Besides that, the structural characteristics of fish hydrolysates are also the concern of researchers (Li *et al.,* 2013; Roslan *et al.,* 2014). In addition, Halim *et al.* (2016) had thoroughly reviewed the functional properties as well as structural properties of fish protein hydrolysates.

The Asian swamp eel (*Monopterus sp.*) is a potential source of fish protein hydrolysate due to its nutritional value and benefits in Eastern medicine (Khanh and Ngan, 2010). This species has several characteristics of a good invader - tolerance of a wide range of temperatures, the ability to breathe air, possible dispersal across basin divides during rainy periods, the ability to burrow into sediments to survive drought, a generalized diet, and parental care of eggs (Hill and Watson, 2007). The eel's special characteristics have attracted many researchers to conduct studies on this fish in various fields. For example, a study conducted by Pederson *et al.* (2014), investigated the effects of salinity on osmoregulation, growth, and survival in Asian swamp eel (*Monopterus albus*). The hydrolysis of eel and its properties has also

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been conducted by several researchers (Yanan *et al.,* 2012; Jamil *et al.,* 2016; Baharuddin *et al.,* 2016).

The application of enzyme technology to recover and modify fish proteins may produce a broad spectrum of food ingredients and industrial products for a wide range of applications (Taheri *et al.,* 2013). The incorporation of fish hydrolysates in cereals, fish and meat products, desserts and crackers had been practiced as early as 2000 (Chalamaiah *et al.,* 2012). In order to explore the potential of eel protein hydrolysate (EPH) as a functional ingredient in food production, this study was aimed to prepare EPH and determine its structural properties by Fourier Transform Infrared (FTIR) Spectroscopy as well as functional properties of EPH produced enzymatically using Alcalase®.

2. Materials and methods

2.1 Materials

Asian swamp eels (*Monopterus sp.*) were purchased in Kuala Terengganu, Malaysia. The eels were beheaded, eviscerated, filleted and de-skinned to obtain the flesh. The flesh was frozen at -40°C at least within 1 week until further use. The flesh was frozen at -40°C at least within 1 week until further use. The frozen flesh was thawed in a chiller overnight before thoroughly rinsed with excessive water to remove impurities. Liquid Alcalase® 2.4 L (2.4 AU/g) was purchased from Novo Industry (Denmark). A BLUeye pre-stained protein ladder (10–245 kDa) was purchased from GeneDirex (United Kingdom). All chemicals used were of analytical grade.

2.2 Preparation of eel protein hydrolysate (EPH)

Hydrolysis was performed according to the procedure of Halim and Sarbon (2016) with slight modification. For each batch, about 275 g of eel flesh was added to 253 g of distilled water. The mixture was then heated at 85ºC for 20 mins before hydrolysis for inactivation of the endogenous enzyme. After cooling, 20 g of Alcalase® enzyme solution (prepared by diluting the required enzyme mass to a final weight of 20 grams with distilled water) was mixed into the eel flesh and hydrolysis was initiated immediately. Parameters of hydrolysis were temperature of 50.18°C, time of 84.02 mins, Alcalase® to protein ratio of 2.26% and pH of 7.89. The hydrolysis process was carried out using water bath shaker with manual pH adjustment using 1N NaOH. The hydrolysis process was terminated by heating the sample at 85°C for 20 mins to inactivate the Alcalase® activity. The hydrolysate was centrifuged (GYROZEN 1580R, Korea) for 20 mins at 6000 rpm, filtered and freeze-dried. The eel protein hydrolysate was stored at –40°C prior to analysis.

2.3 Structural properties of eel protein hydrolysate (EPH) by Fourier Transform Infrared Spectroscopy (FTIR)

In order to identify the functional groups obtained in the EPH produced, Fourier Transform Infrared (FTIR) spectroscopy (Bruker Instruments, Billerica, MA) was used. The FTIR analysis was initiated by placing EPH powder on the sample compartment of the spectroscope. The FTIR spectra of samples were obtained using a Golden-gate Diamond single reflectance ATR in a FTS 7000 FTIR spectrophotometer with a DTGS detector (Nicolet 380, USA). The spectra of EPH were recorded at the absorbance mode from 450 to 4000 cm^{-1} with 128 added scans at a resolution of 4 cm^{-1} (Halim *et al.,* 2014).

2.4 Solubility of eel protein hydrolysate (EPH)

The solubility of eel protein hydrolysate (EPH) was determined using the method of Jamil *et al.* (2016). EPH powder was dissolved in distilled water at temperature of 60°C to obtain a final concentration of 2% (w/v). The mixture was then stirred at room temperature until the hydrolysate was completely solubilized. Then, the hydrolysate was adjusted to pH 4, 7, and 10 with 1N HCl and 6N NaOH. The volume of the solution was made up to 10 ml with distilled water previously adjusted to the same pH of hydrolysate solution. The solution was then centrifuged (GYROZEN 1580R, Korea) at 8500 rpm at room temperature for 10 mins. The protein content in the supernatant was determined using the Biuret method. The solubility of EPH was calculated according to the equation below:

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

2.5 Emulsifying properties of eel protein hydrolysate (EPH)

The emulsifying properties were determined according to the method as described by Jamil *et al.* (2016) with some modification. Approximately 10 mL of vegetable oil (Vecorn) was mixed with 30 mL of 1% eel protein hydrolysate (EPH) solution and the pH value was adjusted to pH 4, 7 and 10. Then, the mixture was homogenized (IKA T18 Ultra-Turrax, Malaysia) at the speed of 16,000 rpm for 1 min. About 50 µL aliquot of an emulsion was pipetted from the bottom of the container at 0 and 10 mins after homogenization and mixed with 5 mL of 0.1% sodium dodecyl sulphate (SDS) solution. Using a spectrophotometer, the absorbance of the diluted solution was measured at 500 nm (A_{500}). The absorbance was measured immediately at 0 min (A_0) and 10 mins (A_{10}) after the emulsion formation. The formulas used to calculate the emulsifying activity index (EAI) and the emulsion

stability index (ESI) were as follows:

Emulsifying activity index (EAI) (m^2/g) = (2 x 2.303 x A_{500})/0.25 x protein weight (g)

Emulsion stability index (ESI) (min) = (A_0 x Δt)/ΔA

Where A_{500} is the absorbance at 500 nm, ΔA is $A_0 - A_{10}$ and Δt is time at 10 mins.

2.6 Foaming properties of eel protein hydrolysate (EPH)

The foaming properties were determined by using a method of Jamil *et al.* (2016) with modification. About 20 mL of 0.5% sample solution was adjusted to pH 4, 7, and 10, followed by homogenization at a speed of 16,000 rpm, using a homogenizer to incorporate air for 2 mins at room temperature. The whipped sample was immediately transferred into a 25-mL cylinder and the total volume was read after 30 s. The foaming capacity was calculated using the following formula:

Foaming capacity (%) = [(A-B)/B] x 100

Where A is the volume after whipping (mL) and B is the volume before whipping (mL).

The whipped sample was allowed to stand at 20°C for 3 mins and the volume of the whipped sample was then recorded. Foam stability was calculated as follows:

Foam stability (%) = [(A-B)/B] x 100

Where A is the volume after standing (mL) and B is the volume before whipping (mL).

2.7 Water holding capacity of eel protein hdyrolysate (EPH)

Water holding properties were examined according to the centrifugation method described by Razali *et al.* (2015) with some modification. EPH samples with concentrations of 0.2%, 0.4%, 0.6%, 0.8% and 10.0% (w/v) were dispersed in 50 mL distilled water and mixed for 2 mins. The mixture was kept at room temperature for 30 mins and then centrifuged at 5000 rpm for 30 mins. The supernatant was filtered through a filter paper and the volume recovered was accurately measured. Water holding capacity was calculated as follows:

$$\text{Water holding capacity (mL/g)} = \frac{\text{Volume of water added (mL)} - \text{Volume of supernatant (mL)}}{\text{Mass of hydrolysate (g)}}$$

2.8 Oil binding capacity of eel protein hydrolysate (EPH)

The oil binding capacity of EPH was determined according to a method by Razali *et al.* (2015) with slight modification. Samples with concentrations of 0.2%, 0.4%, 0.6%, 0.8% and 10.0% (w/v) were put into 50 mL centrifugal tubes and 10 mL of corn oil was added. The sample was thoroughly mixed and kept for 30 mins at 25°C with intermittent mixing every 10 mins, and then centrifuged (GYROZEN 1580R, Korea) at 2560 rpm for 25 mins. Free-oil was then decanted and the oil absorption of the sample determined from the weight difference. Oil binding capacity was calculated as follows:

$$\text{Oil binding capacity (mL/g)} = \frac{\text{Volume of oil added (mL)} - \text{Volume of oil decanted (mL)}}{\text{Mass of hydrolysate (g)}}$$

2.9 Data analysis

All analyses of eel protein hydrolysate (EPH) were conducted in triplicate. The data were presented as mean±SD. One-way ANOVA was carried out with a level of significance of ($p<0.05$).

3. Results and discussion

3.1 Preparation of eel protein hydrolysate (EPH)

The yield of eel protein hydrolysate (EPH) prepared was reported by Halim and Sarbon (2016) as accounting for about 6.97% of the total weight of the eel used. During the enzymatic hydrolysis process, the presence of Alcalase® catalyzed the breakdown of the eel's complex amino acid chains into several numbers of smaller and shorter amino acid chains, in which the number of peptide bonds varies depending on the enzyme concentration, hydrolysis time, pH value of the mixture and temperature used for hydrolysis (Srichanun *et al.,* 2014; Jamil *et al.,* 2016). The yield of hydrolysate produced is dependent on the number of broken peptide bonds, which is measured by the number of peptide bonds in protein mass (degree of hydrolysis DH), during the entire hydrolysis process (Hamid *et al.,* 2015; Halim and Sarbon, 2016). This means that higher DH results in a higher amount of protein hydrolysate yield. The yield of EPH produced was quite similar to the yield of freshwater carp (*Catla catla*) hydrolysate (6.60%) (Elavarasan *et al.,* 2014). However, the recorded yield was lower than the eel (*Monopterus albus*) protein hydrolysate produced by Jamil *et al.* (2016) at 9.45%. This finding was also lower than golden apple snail (*Pomacea canaliculata*) hydrolysate (9.72%) as reported by Hamid *et al.* (2015).

3.2 Structural properties of eel protein hydrolysate (EPH) by Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of eel flesh and eel protein hydrolysate (EPH) was shown in Table 1. Based on Table 1, it can be seen that there were shifts on C – H stretching bands indicating the amide group from 2926.49 cm^{-1} found in eel flesh to a range of 2933.76 cm^{-1} – 2960.62 cm^{-1} in EPH. Besides that, the eel flesh and EPH contained proteins with secondary and tertiary

Table 1. Fourier transform infrared (FTIR) spectrum of eel flesh and eel protein hydrolysate (EPH)

Assignment	Structural group	Wavenumbers (cm^{-1})	
		Eel flesh	Eel protein hydrolysate (EPH)
C – H stretching (2970-2850)	Amide	2926.49	2960.62 – 2933.76
C = O (1760-1690)	2º and 3º amide	1696.57 – 1637.05	1684.67 – 1647.76
C = C (1600-1500)	Aromatic	1577.38 – 1508.15	1577.15 – 1508.26
C – H out-of-plane bend (910-660)	Aromatic	668.91	681.39 – 656.72

2º (secondary); 3º (tertiary)

amides at 1696.57 cm^{-1} – 1637.05 cm^{-1} and 1684.67 cm^{-1} – 1647.76 cm^{-1}, respectively. The C = C found in both eel flesh and EPH at 1577 cm^{-1} to 1508 cm^{-1} was functional groups with aromatic rings, while the aromatic compound of the carbonyl group (C – H) in eel flesh was absorbed by infrared spectra at 669.91 cm^{-1}, whereas in EPH, the group was found at wavenumbers 720.08 cm^{-1} and 681.39 cm^{-1} – 656.72 cm^{-1}, which correspond to the aromatic amino acids (Milewski, 2001; Skoog *et al.,* 2007).

During the hydrolysis process, the complex eel proteins were broken down into shorter peptide chains, exposing the amide, carbonyl, carboxyl and aromatic groups in hydrolysate produced. In addition, the exposure of polar amino acids was also increased (Chalamaiah *et al.,* 2015). According to Stuart (2004), the strongest band in the spectra is the C – H stretching mode at 2920 cm^{-1}, while the wavenumber of the band is 'conformative-sensitive' and responds to the changes of the trans /gauche ratio in the acyl chains. Besides that, Gunzler and Gremlich (2002) mentioned that the absorption band at the region between 1800 cm^{-1} and 1650 cm^{-1} is made up of carbonyl compounds, namely ketones, aldehydes, carboxylic acids, acid chlorides, esters, anhydrides, amide I, amide II, amide III and carboxylic acid derivatives.

The presence of polar bonds in polar functional groups of amines (-NH_2) and alcohol (-OH) helps the polar molecules dissolve in polar solvents such as water and alcohols which increase the solubility of proteins (Taniguchi, 2010). As mentioned, polar amino acids such as Asn and Gln are found as secondary and tertiary amides at (1684.67 cm^{-1} – 1647.76 cm^{-1}) in EPH which will affect the solubility of EPH produced (Halim and Sarbon, 2016). Examples of polar (hydrophilic) amino acids include Asp, Glu, Lys, His, Arg, Asn, Gln, Ser and Thr. In contrast, the presence of apolar (hydrophobic) amino acids such as Gly, Ala, Val, Leu, Ile, Met, Pro, Phe, Trp and Tyr would decrease the solubility of proteins in polar solvents.

The secondary and tertiary amides found in both eel flesh and EPH might indicate the presence of asparagine (Asn) and glutamine (Gln), as reported by Halim and Sarbon (2016) in the amino acid composition of EPH. The finding was also in agreement with the previous study on amino acid composition of eel flesh and eel protein hydrolysate in which the aromatic amino acids contained in eel flesh and EPH were tyrosine (2.15%, 2.61%), phenylalanine (2.49%, 3.47%) and tryptophan (0.21%, 0.74%), respectively (Halim and Sarbon, 2016).

3.3 Solubility of eel protein hydrolysate (EPH)

The solubility of eel protein hydrolysate at different pH was presented in Figure 1. The highest solubility was recorded at pH 10 (81.17%), followed by EPH at pH 4, actual EPH and EPH at pH 7 (79.15%, 76.10% and 75.06%, respectively). However, there were no significant differences ($p>0.05$) on the solubility of EPH from pH 4 to pH 10. The actual pH value of EPH was pH of 8.3. The results showed EPH was soluble in broad pH range which was desired for protein hydrolysates as it contributes to the emulsifying and foaming properties in a food system (Pacheco-Aguilar *et al.,* 2008). pH values influence the solubility properties of fish protein hydrolysate via the ability of peptide bond being cleavage in broad pH range. Thus, a variety of PH range will result in different properties of protein hydrolysate which causes by different level of PH, especially in terms of its solubility.

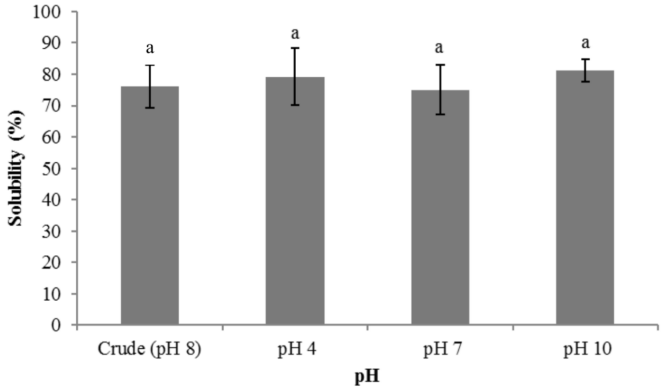

Figure 1. Solubility of eel protein hydrolysate (EPH) at pH 4, pH 7 and pH 10 as compared to crude (pH 8)
Same alphabet superscript indicated no significant differences ($p>0.05$)

The high solubility of hydrolysate at PH 10 can be explained by the protein cleavage into smaller peptide units during hydrolysis (Yin *et al.,* 2010). High solubility is also related to high degree of hydrolysis (DH) of the protein hydrolysate, in which more peptide bonds are broken down and expose the hydrophilic site of protein (Jamil *et al.,* 2016). Besides that, the presence of

hydrophilic amino acids has the potential to form more hydrogen bonds with water and increase hydrolysate solubility (Milewski, 2001). Furthermore, a previous study on the amino acid composition of EPH found the presence of hydrophilic amino acids, including glycine, proline, tyrosine, serine, threonine, glutamine, lysine, arginine and histidine (Halim and Sarbon, 2016).

The solubility of EPH extracted from eel flesh at pH 10 was in agreement to the solubility of hydrolysate from pink perch muscle hydrolyzed at pH 8.8 (Naqash and Nazeer, 2013). In addition, the reported result was also similar with striped catfish muscle hydrolyzed at pH 7.0 (Tanuja *et al.,* 2012), zebra blenny muscle hydrolyzed by fermentation at pH 8.0 (Jemil *et al.,* 2014) and eel protein hydrolysate hydrolyzed at pH 9 by Jamil *et al.* (2016). In food production, an attractive appearance and 'smooth' mouth-feel of food products are from the incorporation of hydrolysate with high solubility in which EPH has the targeted property (Tanuja *et al.,* 2012). Therefore, EPH has potential as an ingredient in human and animal food production.

3.4 Emulsifying properties of eel protein hydrolysate (EPH)

The emulsifying activity index (EAI) and emulsifying stability index (ESI) showed no significant differences ($p>0.05$) of EPH at all pH levels except for pH 4 with the highest EAI was at pH 10 (89.65 m^2/g) followed by EAI of actual EPH (88.54 m^2/g), EAI at pH 7 (72.39 m^2/g) and pH 4 (4.28 m^2/g), while the highest ESI was at pH 4 (61.57 min) followed by ESI at pH 10 (13.93 min), pH 7 (11.84 min) and ESI of actual EPH (11.30 min) as presented in Table 2.

The emulsifying properties of hydrolyzed compounds are directly related to the effectiveness of hydrolysate compound in reducing the interfacial tension between the hydrophobic and hydrolytic components in food products (Cho *et al.,* 2008). Environmental pH could significantly affect the emulsifying properties of hydrolysate by changing the surface hydrophobicity and charge of the protective layer surrounding the lipid globules (Taheri *et al.,* 2013).

Besides the pH level, the amino acids composition of the eel protein hydrolysate (EPH) also contributes to its emulsifying properties. The presence of hydrophobic amino acids could influence the emulsifying properties of hydrolysates produced (Cho *et al.,* 2008). A previous study on amino acid composition of EPH found the presence of hydrophobic amino acids such as leucine (6.52%), isoleucine (4.35%), alanine (5.01%) and hydrophilic amino acids such as histidine (2.25%), serine (3.46%) and glutamine (13.28%) (Halim and Sarbon, 2016). The amino acid determination for EPH showed that this hydrolysate contains hydrophobic and hydrophilic amino acids involved in the oil-in-water interaction of EPH. Studies have shown that the EAI of fish protein hydrolysates was the highest at pH 6 – pH 10, while the lowest at pH 4 (Pacheco-Aguilar *et al.,* 2008; Naqash and Nazeer, 2013) which was in agreement with the results obtained in this study.

3.5 Foaming properties of eel protein hydrolysate (EPH)

Table 2 shows that the pH significantly ($p<0.05$) affect the foaming capacity and stability of eel protein hydrolysate (EPH) in which EPH at pH 7 had the highest foaming capacity and stability (92.50% and 61.67%), respectively. The foaming properties were the least at pH 4 and it can be seen that the foaming properties decreased as the pH increases from pH 7 to pH 10. The molecules of EPH at pH 7 may have a well-ordered orientation, in which the hydrophilic head is located in the aqueous phase and hydrophobic tail faces non-polar components, resulting in the most stable foam among all pH levels.

Generally, foaming properties of protein hydrolysates were related to the transportation, penetration and rearrangement of molecules at the hair-water interface (Elavarasan *et al.,* 2014). Thus, good foaming properties depend on the ability of hydrolysates to rapidly absorb into the air-water interface and rearrange their structures, as well as having several molecular characteristics such as good surface balance, charge distribution, and molecular hydrophobicity (Pacheco-Aguilar *et al.,* 2008).

A foam was formed when protein dispersed in low water tension at the air-water interface (Tanuja *et al.,* 2012). Besides that, foaming properties were also affected by low molecular weight (MW) of the

Table 2. Emulsifying properties and foaming properties of eel protein hydrolysate (EPH) at different pH

Functional Properties	pH			
	Crude (pH 8)	pH 4	pH 7	pH 10
Emulsifying Activity Index (EAI) (m^2/g)	88.54±13.82^a	4.28±0.15^b	72.39±6.07^a	89.65±4.19^a
Emulsifying Stability Index (ESI) (min)	11.30±0.51^b	61.57±14.17^a	11.84±0.31^b	13.93±1.28^b
Foaming Capacity (%)	92.50±2.17^a	85.83±3.82^b	92.50±2.50^a	91.08±0.14^{ab}
Foaming Stability (%)	54.17±6.29^{ab}	42.08±0.72^b	61.67±4.02^a	47.83±2.02^b

Different alphabet superscripts indicated significant differences ($p<0.05$)

Table 3. Water holding capacity and oil binding capacity of eel protein hydrolysate (EPH) at different concentration

Functional Properties	Concentration				
	0.20%	0.40%	0.60%	0.80%	1.00%
Water Holding Capacity (WHC)(mL/g)	37.53±5.96[a]	20.82±13.72[ab]	7.90±1.54[b]	6.65±1.89[b]	6.46±2.09[b]
Oil Binding Capacity (OBC)(mL/g)	0.44±0.24[a]	0.35±0.04[a]	0.50±0.08[a]	0.45±0.02[a]	0.56±0.13[a]

Different alphabet superscripts indicated significant differences ($p<0.05$)

hydrolysate, as low MW hydrolysate was unable to maintain well-ordered, interface orientation of the molecule (Nalinanon *et al.,* 2011). Based on Table 2, the foaming stability of EPH at all pH levels was lower than the foaming capacity. The findings in this study are similar to those of a study by Naqash and Nazeer (2013) on pink perch muscle hydrolysate in which the foaming properties decreased at pH 4 and increased as the pH increases. In contrast, the results were higher than the foaming capacity of Spanish mackerel hydrolysate (65.0%) as reported by Chi *et al.* (2014).

3.6 Water holding capacity of eel protein hydrolysate (EPH)

The water holding capacity of eel protein hydrolysate (EPH) was significantly decreased ($p<0.05$) with increasing hydrolysate concentration from 0.2% to 1.0% (Table 3). The result showed that at 0.1% EPH had the highest surface area to mass ratio, resulting in the highest water holding capacity. In contrast, EPH with a concentration of 1.0% had the least exposed surface that can be imbibed with water, thus decreasing its holding capacity. During the hydrolysis process, the complex protein is broken down into shorter amino acids chains, exposing the N-terminal (polar groups) which is ready to bind with H-bond of water.

Water holding capacity is referred to as the ability of protein to imbibe water and retain in against a gravitational force within a protein matrix (Foh *et al.,* 2010). A higher water holding capacity shows higher surface area to the mass ratio in hydrolysate which is ready to interact with H-bond of water (Slizyte *et al.,* 2009). The low molecular weight hydrolysate and the increased concentrations of polar groups exposed during the breakdown of amino acid chains were able to increase the water holding capacity of hydrolysate (Taheri *et al.,* 2013). The presence of hydrophilic polar group amino acids in EPH such as serine, threonine, asparagine, and glutamine might also influence the water holding capacity of the hydrolysate, which is important in the increasing of cooking yield (Halim and Sarbon, 2016). The water holding capacity of EPH was higher than those of hydrolysates produced from bluewing searobin (3.75 mL/g), tilapia (1.77 – 2.10 mL/g) and zebra blenny (6.10 mL/g) muscles (dos Santos *et al.,* 2011; Foh *et al.,* 2011; Jemil *et al.,* 2014).

3.7 Oil binding capacity of eel protein hydrolysate (EPH)

Table 3 shows the oil binding capacity of eel protein hydrolysate (EPH) at different concentrations (0.2%, 0.4%, 0.6%, 0.8% and 1.0%). The EPH at 1.0% showed the highest oil binding capacity, followed by EPH at 0.6%, 0.8%, 0.2%, and 0.4%. However, the oil binding capacity of EPH showed no significant differences ($p>0.05$) at different hydrolysate concentrations.

The oil binding capacity of EPH was lower than the water binding capacity. This may be due to the higher amount of polar than non-polar groups at the N-terminal of amino acids chains. Hence, EPH demonstrated lower oil binding as compared to water binding. According to dos Santos *et al.* (2011), oil binding capacity is correlated to the surface's hydrophobicity of protein. Based on these findings, it may be said that EPH at different concentrations showed similar surface hydrophobic properties, making it able to bind with oil. In addition, the bulk density of peptides and enzyme/ substrate specificity also influence the oil binding capacity of protein hydrolysates (Cho *et al.,* 2008).

The ability of hydrolysate to absorb oil is important to influence the taste of products such as meat, salad dressings, bakery products and confectionaries, as well as their functional characteristics (Cho et *al.,* 2008; dos Santos *et al.,* 2011). Therefore, EPH was found to have the ability to binding oil in food products. However, EPH at all concentrations had oil binding capacity of less than 1.00 ml/g, lower than the oil binding capacity of that from striped catfish (1.35 mL/g), and dagaa (3.50 mL/g) muscle hydrolysates (Tanuja *et al.,* 2012; Betty *et al.,* 2014).

4. Conclusion

In conclusion, the hydrolysis process of eel protein using Alcalase® promotes the breakdown of large numbers of shorter amino acid chains exposed to hydrophilic and hydrophobic groups of protein, resulting in changes of structural groups and lower molecular weight proteins with functional properties comparable to other fish protein hydrolysates produced. The eel protein hydrolysate (EPH) had significant functional groups of primary and secondary amides, which indicate the presence of hydrophilic and hydrophobic amino acids. The structural analysis of EPH also found the presence

of aromatic groups related to the existence of amino acids with an aromatic ring in EPH. The EPH also showed significant differences ($p<0.05$) in emulsifying properties and foaming properties at different pH and water holding capacity at different concentration of EPH. The results obtained showed that the EPH has potential as a good source of food emulsifiers and stabilizers in processed food products.

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