Functional and Antioxidant Properties of Angelwing Clam (*Pholas orientalis*) Hydrolysate Produced using Alcalase

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ABSTRACT

In this study, Hydrolysate from angelwing clam (*Pholas orientalis*) was produced at 0, 1, 2 and 3 hrs and E/S ratio of 0.5 and 3% using alcalase where the pH and temperature were kept constant at pH 8.5 and 60°C, respectively. The hydrolysates were analysed for antioxidant and functional properties such as solubility, emulsifying properties and water and oil holding capacity. Degree of hydrolysis (DH), yield, functional and antioxidant properties were influenced by the hydrolysis time and E/S ratio. Higher enzyme concentration (E/S 3%) and longer hydrolysis time increased the DH. Yield was higher at E/S 3% but reduced with hydrolysis time. Longer hydrolysis time produced more soluble hydrolysate and higher metal chelating activity but lower in emulsifying properties and DPPH activity. Higher enzyme concentration resulted in increase only in solubility and metal chelating activity. This study revealed that enzymatic hydrolysis using alcalase should be performed at shorter hydrolysis time using intermediate concentration of enzyme (E/S between 0.5 to 3%) in order to produce angelwing clam hydrolysate with collectively good functional and antioxidant properties.

Keywords: *hydrolysate, angelwing clam, functional properties, alcalase*

INTRODUCTION

Angelwing clam (*Pholas orientalis*) also known as 'mentarang' in Malaysia originated from the family of Pholadidae. It is a bivalve usually found in muddy shore. Its sweet, juicy and tender flesh makes it one of the most highly sought bivalves in most Asian countries such as Malaysia, Thailand and Philippines [1]. In Malaysia, angelwing clams are normally found in Perlis and Selangor during hot and dry season, which is experienced from January to March [2].

Hydrolysate is obtained from high protein food hydrolysed using either acid, alkaline or enzyme. Among these, enzymatic hydrolysis is more frequently studied as it produces hydrolysate with preferable qualities suitable for human consumption [3]. Several factors need to be considered when performing an enzymatic hydrolysis including the types of enzymes it contains and its concentrations, as well as the condition used to hydrolyse the protein such as pH, duration and temperature. Previous studies indicated that alcalase, flavorzyme, bromelain and papain may influence the taste, solubility, emulsifying properties and degree of hydrolysis of a hydrolysate [4, 5, 6, 7, 8]. Alcalase is an endopeptidase produced from a selected strain of *Bacillus licheniformis*. It is one of the best enzymes used for hydrolysate production. Sathivel et al. [9] successfully produced hydrolysate from fresh herring (*Clupea harengus*) using alcalase and obtained hydrolysate with desirable functional properties comparable to established hydrolysates. Additionaly, Muzaifa *et al.* [7] shows that fish protein hydrolysate obtained using alcalase had higher protein content and better functional properties compared to hydrolysate obtained using flavorzyme.

Degree of hydrolysis (DH) plays an important role in determining the functional properties of the hydrolysate. Bing-Lan and Pei-Shiuan [10] stated that protein solubility, emulsifying properties and foaming capacities can be improved with a lower degree of hydrolysis, whereas excessive hydrolysis often causes loss of some of these functionalities. According to Gbogouri et al. [11] and Souissi et al. [5], the increase in the DH increased the solubility of the hydrolysate. While lower DH enhanced the fat holding capacity and also the emulsifying capacity [11]. DH measures the percentage of peptide bonds cleaved. It can be determined by pH-stat, o-phthaldialdehyde (OPA) and trinitrobenenesulfonic acid (TNBS) method [12].

Foods high in fats and oil are prone to rancidity. Determining the antioxidant properties of a hydrolysate is important as it brings benefits when incorporated into food with oil and fat components. Several seafood products that have been processed into hydrolysate had shown some antioxidant effects. Some examples include hydrolysates from yellow stripe trevally, round scad and mussel [3, 13, 14]. By using angelwing clam for the production of hydrolysate, more people will be aware of the existence of angelwing clam as edible seafood. Angelwing clam hydrolysate obtained can be incorporated into food or supplement in order to increase food protein content. A new market for angelwing clam can be established from the commercialisation of angelwing hydrolysate. Furthermore, the antioxidative property of protein hydrolysate makes it eligible for listing as nutraceutical [15]. This study was carried out to determine the effects of hydrolysis time and alcalase concentration on the functional and antioxidant properties of angelwing hydrolysate.

MATERIALS AND METHOD

Materials

Fresh angelwing clam was purchased from a supplier in Kuala Selangor and stored in styrofoam boxes filled with ice. Upon arrival at the laboratory, the clam was cleaned to remove mud followed by flesh removal from the shell.

Alcalase 2.4L purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) was used in the enzymatic hydrolysis process. Alcalase with a declared activity of 2.4AU/g and density of 1.18g/mL is a bacterial protease prepared from *Bacillus licheniformis*. Other chemicals used were of analytical grade.

Preparation of Angelwing Clam Hydrolysate

Angelwing clam hydrolysate was prepared according to Normah and Nurfazlika Nashrah [16] with a slight modification. The hydrolysis using Alcalase 2.4L was done at 0.5% and 3% enzyme-substrate ratio (E/S). The amount of enzyme, angelwing clam and water needed for the hydrolysis process was calculated based on Adler-Nissen [17]. The clam and water mixture were homogenised until fine for about 2-3 minutes using a blender

and then placed in thermal controlled water bath. The temperature of the water bath was raised until the mixture's temperature recorded 60°C while the pH was adjusted to pH 8.5 using 1M NaOH. Once the temperature and pH stabilised, alcalase was added. The temperature and pH were maintained at 60°C, pH 8.5, respectively throughout the hydrolysis period of 0, 60, 120 and 240 minutes. The amount of NaOH added to maintain the pH at 8.5 was recorded. At the end of the hydrolysis, the mixture was removed from the water bath and transferred to another water bath set at 90°C for 15 minutes in order to inactivate the enzyme. The supernatant was collected after centrifugation at 10,000rpm, 4°C for 10 minutes using a centrifuge (Universal 320R, Germany). The supernatant was then freeze dried.

Degree of Hydrolysis

The degree of hydrolysis was determined according to Adler-Nissen [17] using the formula as follows:

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

where β is the amount of NaOH consumed, N_{β} is the normality of the NaOH used, M_p is the mass of substrate, $1/\alpha$ is the calibration factors for pH-stat and h_{tot} is the total number of peptide bonds in the protein.

Yield

The yield was calculated as the percentage of dry weight of freeze-dried hydrolysates with respect to weight of angelwing clam.

$$\text{Yield}\ (\%) = \frac{\text{Weight of freeze-dried hydrolysates}}{\text{Weight of angelwing clam}} \times 100$$

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was used to determine the molecular weight of the samples. Samples were mixed in 0.25 µl of sample buffer and then heated at 70°C for 10 minutes. 10 µl was loaded into each well. Benchmark™ protein ladder was used as a marker. Electrophoresis was performed using the XCell Surelock electrophoresis cell (Bio-Rad laboratories, Hercules, CA, USA) with the running time of 50 minutes at 100-125mA/gel. Subsequently, the gel was washed in 100ml ultrapure water, heated in microwave oven at 180°C for one minute and shaken in an orbital shaker for about two 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 two minutes. Finally the gel was destained using ultrapure water and shaken in an orbital shaker for two minutes to remove the dark background.

Nitrogen Solubility (%)

Nitrogen solubility was determined according to the method outlined by Foh et al. [18] with some modification. 1g hydrolysate was added into 100mL distilled water and adjusted to pH 7 by using 0.5 N HCl and 0.5N NaOH. The mixture was vortexed for 10 minutes at room temperature. 25mL of the aliquot was then centrifuged at 2800 x g for 35 minutes. The nitrogen concentration of the supernatant was analysed using Kjeldahl method [19].

$$\text{Nitrogen solubility (\%)} = \frac{\text{Supernatant nitrogen concentration}}{\text{Hydrolysis mixture nitrogen concentration}} \times 100$$

Emulsifying Properties

The emulsion capacity was determined according to methods described by Foh *et al.* [18]. 0.5g hydrolysate was transferred into a 250mL beaker and dissolved in 50mL of 0.5N NaCl and then 50mL of corn oil was added into the mixture. The mixture was homogenized using high speed homogenizer (CAT X120, CAT Scientific, USA) at 14,000rpm for two minutes to produce the emulsion. The homogenised mixture was transferred into a centrifuge tube and was maintained in water bath at 90°C for 10 minutes to increase the

separation between oil and water. The mixture was then centrifuged again at 2800 × g for 20 minutes. The emulsion capacity was calculated as follows:

$$\text{Emulsion capacity} = \frac{V_a - V_p}{W_s}$$

where V_a = volume of oil added to form emulsion
V_p = volume of oil obtained after centrifugation
W_s = weight of hydrolysate

Emulsion stability was determined by the methods performed by Gbogouri *et al.* [11]. 500mg of hydrolysate was added into 50mL of distilled water followed by the addition of 50mL corn oil. The emulsion was stirred at 14,000rpm for 2 minutes and then transferred into 100mL measuring cylinder. The emulsion was allowed to stand for 15 minutes before the volume of the aqueous phase was measured. The emulsion stability was calculated as:

$$\text{Emulsifying stability (\%)} = \frac{\text{total volume of mixture - aqueous volume after standing}}{\text{total volume of mixture}} \times 100$$

Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

Water holding capacity (WHC) and oil holding capacity (OHC) was determined according to methods developed by dos Santos *et al.* [20]. 0.5g hydrolysate was placed in a previously weighed centrifuge tube. 15ml of water was added into the tube. For oil holding capacity, instead of water 15mL of corn oil was added. The mixture was vortexed and let to stand for 30 minutes at room temperature before being centrifuged at 5000 × g for 25 minutes. The excess water/oil was removed from the tube by inverting the tube over a tissue paper. The difference in the weight of sample before and after water/oil absorption represents the amount of water/oil absorbed.

Scavenging Effect on DPPH Free Radical

The antioxidant properties of angelwing hydrolysate were determined according to method outlined by Souissi *et al.* [5]. 1.5g hydrolysate was added into 1.5mL of 0.1mM α,α-di-phenyl-β-picryhydrazyl (DPPH) in 95% ethanol. The mixture was placed in an amber bottle covered with aluminium foil followed by vigorous shaking. The mixture was stored at room temperature for 30 minutes. The absorbance of the mixture was measured at wavelength of 517nm. The scavenging effect was calculated as follows:

$$\text{Scavenging effect (\%)} = \frac{\text{blank absorbance - sample absorbance}}{\text{blank absorbance}} \times 100$$

Reducing Power

Reducing power was determined according to the methods of Mohamed *et al.* [21]. 1.5mg hydrolysate was added into 2.5mL of 0.2M phosphate buffer (pH 6.6) and 2.5mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. Then, 2.5mL of 10% trichloroacetic acid was added into the mixture. 2.5mL from each reaction mixture was transferred into test tubes containing 2.5mL distilled water and 0.5mL of 0.1 % ferric chloride. The mixture was left to stand for 10 minutes before the absorbance of the mixture was measured at wavelength of 700nm. The reducing power activity of the hydrolysate was determined from the absorbance reading obtained.

Metal Chelating Activity

Metal chelating activity was measured according to Mohamed *et al.* [21]. 50mg hydrolysate was added into 10ml distilled water. 1mL of the solution was mixed with 3.7mL distilled water. 0.1mL of 2mM ferric (II) chloride and 0.2mL of 5mM ferrozine was then added into the solution and mixed. The mixture was allowed to stand at room temperature for 10 minutes followed by the measurement of absorbance at 562nm.

$$\text{Metal chelating ability (\%)} = 1 - \frac{\text{absorbance of sample}}{\text{absorbance of blank}} \times 100$$

Effect of Storage on Antioxidant Activity of Angelwing Hydrolysate

Angelwing hydrolysate was stored for eight days to evaluate the antioxidant activity upon storage. Observation on the antioxidant activity was made daily.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences [22]. Data was subjected to analysis of variance (ANOVA) and mean comparisons was carried out using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Degree of Hydrolysis

Enzymatic hydrolysis using different percentages of enzyme at E/S of 0.5 and 3% resulted in significantly higher ($p \leq 0.05$) DH up to 1hr of hydrolysis (Table 1). Degree of hydrolysis which increased with hydrolysis time ranged between 5.44 to 10.75%. Similar effects of hydrolysis time have been reported by several authors [23, 24, 25, 26] although other conditions such as pH may play a role in influencing the DH [27]. At high enzyme concentration, peptides are converted into shorter peptides and amino acids due to enzymatic hydrolysis [28].

Table 1: Degree of hydrolysis of angelwing clam hydrolysate obtained during the 3 hrs of hydrolysis at E/S ratios of 0.5 and 3%

Hydrolysis time (hr)	Degree of hydrolysis (%)	
	E/S 0.5%	E/S 3.0%
0	5.44^{Db}±0.33	6.38^{Ca}±0.27
1	7.35^{Cb}±0.51	9.10^{Ba}±0.29
2	8.55^{Bb}±0.47	9.35^{Ba}±0.30
3	10.40^{Aa}±0.71	10.75^{Aa}±0.23

Values within the same column followed by different uppercase superscripts are significantly different ($p \leq 0.05$)
Values within the same rows followed by different lowercase superscripts are significantly different ($p \leq 0.05$)

Yield of Angelwing Hydrolysate

Angelwing hydrolysates obtained from the enzymatic hydrolysis using alcalase were brown in color. At E/S 3%, significantly higher ($p \leq 0.05$) yields than E/S 0.5% were recorded at all hydrolysis times studied (Table 2). Higher enzyme concentration allows more enzymatic activity to take place during the hydrolysis therefore resulted in an increase in yield. In contrary, yield significantly decreased ($p \leq 0.05$) beginning from 1hr of hydrolysis at both enzyme concentrations. Hydrolysate yield can be improved by properly maintaining the pH and enzyme concentration throughout the hydrolysis [16].

Table 2: Yield of angelwing clam hydrolysate obtained during the 3hrs of hydrolysis at E/S ratios of 0.5 and 3%

Hydrolysis time (hr)	Yield (%)	
	E/S 0.5%	E/S 3.0%
0	$5.95^{Bb}\pm1.82$	$11.37^{Aa}\pm0.37$
1	$6.37^{Ab}\pm0.32$	$7.81^{Ba}\pm0.22$
2	$5.47^{Bb}\pm0.38$	$7.01^{Ca}\pm0.15$
3	$4.87^{Cb}\pm0.28$	$6.77^{Da}\pm0.62$

Values within the same column followed by different uppercase superscripts are significantly different ($p<0.05$)
Values within the same rows followed by different lowercase superscripts are significantly different ($p<0.05$)

Molecular Weight Distribution

Electrophoresis pattern shows the presence of six peptide bands in angelwing flesh with molecular weight ranging from 8 to 48kDa (Figure 1). Only a single peptide band is observed in E/S 0.5% hydrolysate at 0, 1, 2hrs hydrolysis time characterised with 60, 49 and 12kDa, respectively. This suggested that the longer the hydrolysis time the shorter is the peptide. Such observation is in compliance with previous findings by Normah *et al.* [26] on the hydrolysis of threadfin bream using alcalase. The absence of any bands in the 3hrs hydrolysate suggested that it contains only very short peptides and free amino acids with lower molecular weight undetectable by the gel. All the bands for hydrolysates produced at E/S 3% had lower molecular weight than those produced at E/S 0.5%. The molecular weight also decreased with hydrolysis time showing bands at 40, 39 and 5kDa for 0, 1, and 2hrs of hydrolysis, respectively. Similarly, no band was exhibited at 3hrs of hydrolysis. These observations suggested that protein degradation increased with the increase in hydrolysis time and enzyme concentration.

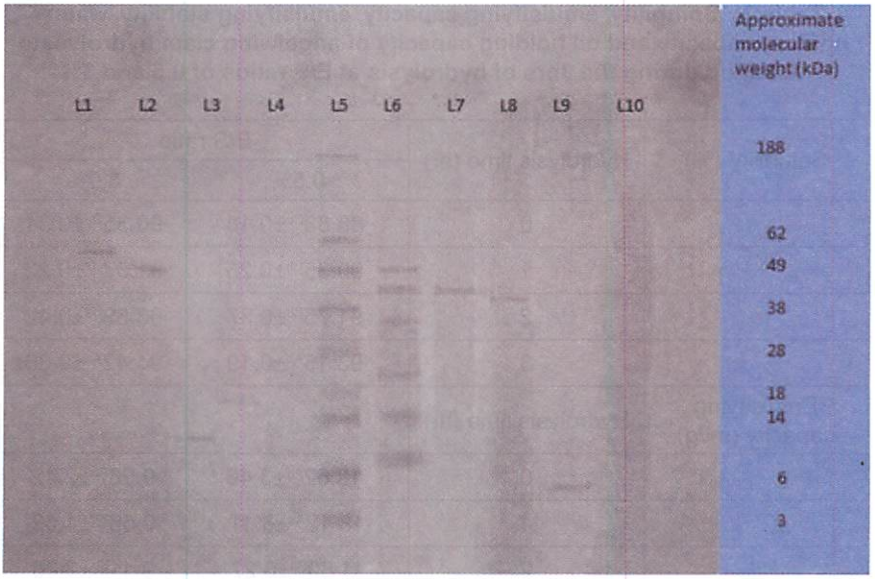

Figure 1: Electrophoresis pattern of angelwing clam hydrolysate produced using alcalase L1) E/S 0.5%, 0hr, L2) E/S 0.5%, 1hr, L3) E/S 0.5% 2hr, L4) E/S 0.5%, 3hr, L5) SeeBlue protein marker, L6) angelwing clam flesh, L7) E/S 3.0% , 0hr, L8) E/S 3.0%, 1hr, L9) E/S 3.0%, 2hr, L10) E/S 3.0%,3hr

Solubility

Angelwing hydrolysate was significantly ($p \leq 0.05$) more soluble with the increase in hydrolysis time at both E/S ratio (Table 3). It is also observed that hydrolysate produced at E/S 3.0% had significantly ($p \leq 0.05$) higher solubility than those produced at E/S 0.5%. Similar trend has also been reported previously [13, 11]. Degradation of proteins into smaller peptides leads to more soluble product [13, 11, 9, 20]. According to Gbogouri *et al.* [11], smaller peptides are expected to have more polar residues with good ability to bind with water. Long hydrolysis time degrades the protein or peptides into shorter peptides with lower molecular weight which then enhance the solubility of the hydrolysate.

Table 3: Solubility, emulsifying capacity, emulsifying stability, water holding capacity and oil holding capacity of angelwing clam hydrolysate obtained during the 3hrs of hydrolysis at E/S ratios of 0.5 and 3%

Solubility (%)	Hydrolysis time (hr)	E/S ratio	
		0.5%	3.0%
	0	88.83^{Db}±0.18	90.35^{Da}±0.31
	1	90.35^{Cb}±0.25	91.57^{Ca}±0.22
	2	91.75^{Bb}±0.37	93.39^{Ba}±0.42
	3	93.15^{Ab}±0.19	94.42^{Aa}±0.38
Emulsifying capacity (ml/g)	Hydrolysis time (hr)		
	0	18.62^{Aa}±3.46	9.96^{Ab}±2.42
	1	18.12^{Aa}±6.11	9.58^{Bb}±1.62
	2	11.83^{Ba}±0.27	9.11^{Bb}±2.06
	3	4.37^{Cb}±3.50	7.17^{Ca}±3.56
Emulsifying stability (%)	Hydrolysis time (hr)		
	0	66.42^{Ab}±3.46	73.72^{Aa}±1.83
	1	65.50^{Ab}±6.11	71.98^{Aa}±1.70
	2	56.00^{Ba}±0.27	54.25^{Ba}±2.82
	3	53.50^{Ba}±3.50	52.33^{Ba}±3.27
Water holding capacity (ml/g)	Hydrolysis time (hr)		
	0	0.15^{Ba}±0.01	0.15^{Ca}±0.01
	1	0.13^{Bb}±0.01	0.19^{Ca}±0.03
	2	0.26^{Aa}±0.07	0.29^{Ba}±0.07
	3	0.29^{Aa}±0.06	0.35^{Aa}±0.05

Oil holding capacity (ml/g)	Hydrolysis time (hr)		
	0	$1.64^{Ca}\pm0.02$	$1.27^{Cb}\pm0.12$
	1	$1.70^{Ba}\pm0.02$	$1.34^{Cb}\pm0.05$
	2	$1.50^{Db}\pm0.05$	$1.59^{Ba}\pm0.03$
	3	$2.03^{Aa}\pm0.03$	$1.81^{Ab}\pm0.11$

Values within the same column followed by different uppercase superscripts are significantly different ($p<0.05$)
Values within the same rows followed by different lowercase superscripts are significantly different ($p<0.05$)

Emulsifying Capacity and Emulsifying Stability

Emulsifying capacity and stability of angelwing hydrolysate were inversely proportional with hydrolysis time (Table 3). This was evident at both E/S ratio used. However, increase in the concentration of enzyme used (E/S 3%) resulted in lower emulsifying capacity while the emulsions were more stable at E/S 3% than those from E/S 0.5% only up to 2hrs of hydrolysis. The difference in emulsifying properties observed among hydrolysates may have been due to hydrophobicity and length of peptide. Hydrolysates with smaller peptides usually have weaker emulsifying properties [9]. Longer hydrolysis time resulted in the production of large number of small peptides. Smaller peptides increase the absorption of protein onto the oil-water interface and retains for a longer time to prevent the droplets from coalescing. As a result, hydrolysate had less strength to stabilise the emulsion. Emulsifying property at limited hydrolysis time is attributed to the exposure of hydrophobic protein surface which enhances adsorption at the interface, forming a cohesive interfacial film with the hydrophobic residues reacting with oil while the hydrophilic residues reacting with water [29].

Water Holding Capacity (WHC)

The WHC of angelwing clam hydrolysate is within the range of 0.13 to 0.35ml/g (Table 3). Water holding capacity slightly increased with hydrolysis time between 1 to 2hrs of hydrolysis for both E/S ratios. This is in accordance with Wasswa's *et al.* [30] findings on grass carp skin hydrolysate.

At higher DH, polar groups such as COOH and NH_2 which have significant effects on the water absorption increased, hence this improved the WHC of the hydrolysate [31, 32]. Besides, smaller peptides produced at higher DH allows the hydrolysate to have better WHC as it has low molecular weight and are also more hydrophilic.

Oil Holding Capacity (OHC)

In general, OHC of angelwing hydrolysate increased with hydrolysis time (Table 3). Besides, OHC has also been shown to be dependent on DH [33]. The OHC were within the range of 1.27 to 2.03ml/g and was highest at 3hrs of hydrolysis. OHC is influenced by the surface hydrophobicity where an increase in hydrophobicity leads to increase in OHC [34].

Antioxidant Properties

DPPH radical scavenging activity

DPPH activity of hydrolysate produced using both E/S 0.5 and 3% are reduced at hydrolysis time above 1hr (Figure 2). As shown in Table 1, increased in hydrolysis time resulted in an increase in DH while hydrolysate produced at higher enzyme concentration (E/S 3%) showed higher DH than those produced at E/S 0.5% only up to 2hr. For hydrolysate produced at E/S 3%, the DPPH activity was lower than those produced at E/S 0.5% only up to 2hrs of hydrolysis (Figure 2). This observation is in compliance with Klompong *et al.* [13] who stated that as DH increased, DPPH activity decreased. The DPPH activity is excellent in angelwing hydrolysed at 1hr for both E/S ratios. Differences in amino acid sequence in the hydrolysate can influence the radical scavenging activity of the hydrolysate [3]. All hydrolysates still retained the radical scavenging activity until day 8 of storage.

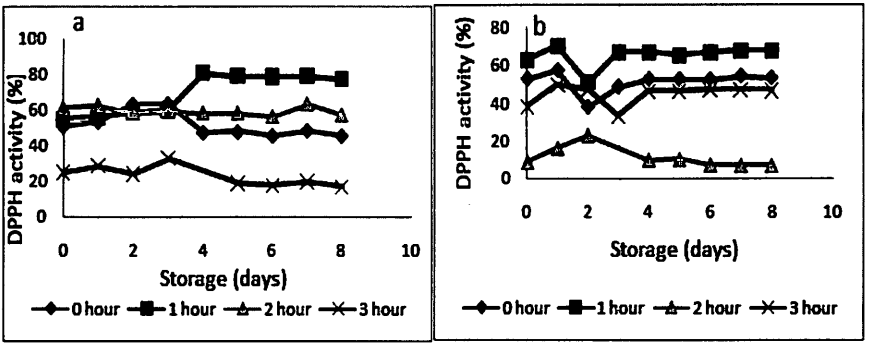

Figure 2: DPPH activity (%) of angelwing clam hydrolysate produced at E/S a) 0.5% or b) 3% during storage

Reducing power

The reducing power of angelwing clam hydrolysate was slightly higher at 2 and 3hrs of hydrolysis for E/S 0.5% while at E/S 3%, there were no difference between hydrolysis time (Figure 3). Hydrolysates produced at E/S 3% beyond 1hr of hydrolysis showed lower reducing power than those produced at E/S 0.5%. Therefore, increase in DH resulted in the decrease of reducing power only for enzyme concentration at 0.5%. Klompong *et al.* [13] obtained lower reducing power as the DH increased during the production of hydrolysate from yellow stripe trivially. This observation was similar to the findings by Normah and Fadilah [14] where the antioxidant activities exhibited an inverse relationship with DH. However, research by Thiansilakul *et al.* [3] showed no major changes in reducing power of round scad hydrolysate at different DH. This variation could be due to higher DH (20, 40 and 60%) used in Thiansilakul's studies compared to Klompong *et al.* [13] with 5, 15 and 25% DH. With extensive hydrolysis perhaps no further differences in the reducing power could be observed. Reducing power of angelwing clam hydrolysate was stable throughout the eight days of storage regardless of hydrolysis time and E/S ratio.

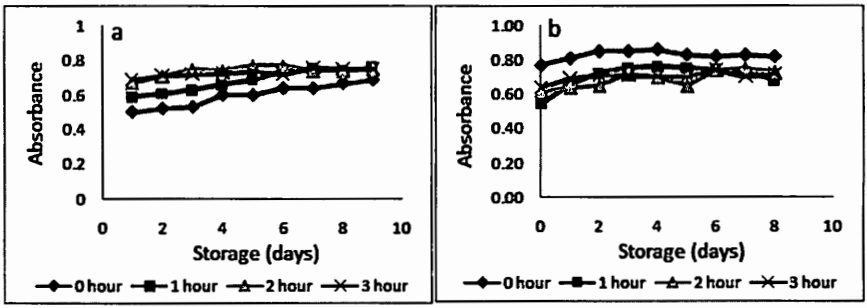

Figure 3: Reducing power of angelwing clam hydrolysate produced at E/S a) 0.5% or b) 3% during storage

Metal chelating activity

Metal chelating ability was higher for both E/S ratios when hydrolysis time was extended to beyond 1hr (Figure 4). The metal chelating ability decreased during storage until no further metal chelating properties was exhibited at day eight onwards for both E/S ratios and at 2 and 3hrs of hydrolysis. However, hydrolysate prepared at E/S 3% had more stable metal chelating ability during storage than those produced at E/S 0.5%.

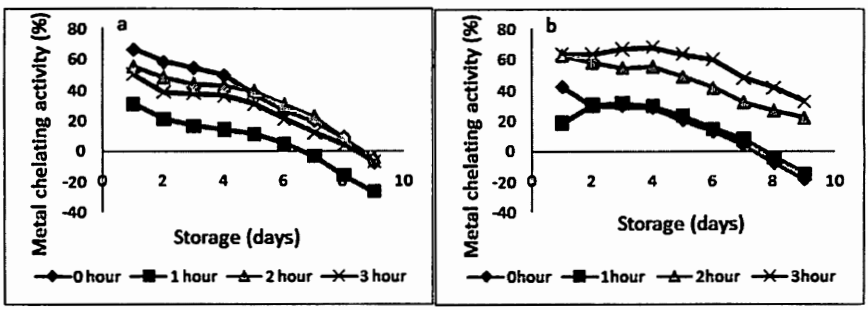

Figure 4: Metal chelating activity of angelwing clam hydrolysate produced at E/S a) 0.5% or b) 3% during storage

Protein hydrolysate from muscle ornate threadfin bream and round scads showed an increase in the percentage of chelating activity with an increase of DH [35, 3]. High metal chelating ability indicates that the angelwing clam hydrolysate is able to chelate the prooxidant iron which results in lower oxidation.

CONCLUSION

The DH, yield, functional and antioxidant properties of protein hydrolysate derived from angelwing clam (*Pholas orientalis*) were influenced by the hydrolysis time and E/S ratio during enzymatic hydrolysis using alcalase. DH increased with hydrolysis time and enzyme concentration. Enzymatic hydrolysis using alcalase should be performed at shorter hydrolysis time using an intermediate enzyme concentration in order to produce angelwing clam hydrolysate with good functional and antioxidant properties.

ACKNOWLEDGEMENT

The authors would like to thank the Research Management Institute (RMI), Universiti Teknologi MARA (UiTM) for financing the project under the Excellence Fund RIF (600-RMI/ST/DANA 5/3/RIF(668/2012).

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