Universiti Malaysia Terengganu Journal of Undergraduate Research *eISSN: 2637-1138*
Volume 2 Number 3, July 2020: 15-26 *© Penerbit UMT*

PHYSICOCHEMICAL PROPERTIES OF UNDULATED SURF CLAM (*Paphia undulata*) HYDROLYSATE AS AFFECTED BY DEGREE OF HYDROLYSIS

YAP JAA YEE AND AMIZA MAT AMIN*

Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu. Malaysia

**Corresponding author: ama@umt.edu.my*

Abstract: This study aimed to determine the physicochemical properties of undulated surf clam *(Paphia undulata)* hydrolysate as affected by the degree of hydrolysis (DH). Three levels of DH of undulated surf clam hydrolysate were prepared which were DH 36.57% (without any enzymatic hydrolysis), DH 58.25% (*0.5% Alcalase®; 5 min; pH 7.5; 60ºC*) and DH 91.26% (*1% Alcalase®; 30 min; pH 7.5; 60ºC*). After protein hydrolysis, the undulated surf clam hydrolysates were centrifuged, and their supernatants were freeze-dried. This study found that the protein hydrolysate with lower DH (DH 36.57%) gave lower protein content and higher ash and fat contents compared to other samples (DH 58.25% and DH 91.26%). However, the carbohydrate content is similar in all samples (16.56-20.04%). This study also found that foaming properties (29.43-67.50%), emulsifying capacity (11.94-110.52%) and peptide solubility (57.61-94.08%) were affected by the DH. As DH increased, the emulsifying capacity decreased, while peptide solubility increased. While the foaming capacity increased with increasing DH until it reached a maximum value and level off afterwards. For colour parameters, although there were differences between L*, a* and b* values for all three samples, a fluctuating pattern was noted with DH. DH also did not affect the water-holding and oil-holding capacity of undulated surf clam hydrolysate. This study shows that certain physicochemical properties of undulated surf clam hydrolysate can be tailored by adjusting the degree of hydrolysis.
Keywords: undulated surf clam, protein hydrolysate, degree of hydrolysis, physicochemical properties, Paphia undulata.

Introduction

Protein hydrolysis is a process of cleaving the peptide bonds of protein. Protein hydrolysate produces a mixture of amino acids and peptides. Protein hydrolysis can be employed using acid, base or proteases. Enzymatic protein hydrolysis is the most preferable and effective method due to its specificity, easier control of the reaction and less formation of by-products after the reaction (Tavano, 2013). Protein hydrolysis is commonly used in the food industry to modify physicochemical properties of the protein (Chalamaiah *et al.*, 2012) and to prepare essential amino acid and bioactive peptides (Clemente *et al.*, 2000; Chalamaiah *et al.*, 2012). Alcalase® is one of the commercial food-grade enzymes that is widely used in the protein hydrolysis due to its stability towards heat treatment and high optimal pH, which allows extensive protein hydrolysis to occur with minimal microorganism growth (Salwanee *et al.*, 2013). Besides, it is suggested as the best protease in protein hydrolysis of fish and other protein hydrolysates because of its high protein recovery and low lipid composition (Shahidi *et al.*, 1995; Šližytė *et al.*, 2005).

The extent of protein hydrolysis can be measured using an index called the degree of hydrolysis (DH). DH is defined as the proportion of cleaved peptide bonds in a protein hydrolysate (Rutherfurd, 2010). For enzymatic protein hydrolysis, DH is influenced by several factors, such as pH, temperature, hydrolysis time and enzyme concentration (Shu *et al.*, 2016). DH is an important aspect of protein hydrolysis. Many studies reported that DH affected functional properties and nutraceutical properties of protein hydrolysate (Klompong *et al.*, 2007). The solubility of protein hydrolysate increased as the DH increased (Klompong *et al.*, 2007). While Gbogouri *et al.* (2004) reported that a lower DH will contribute to better emulsifying capacity and emulsion stability. Besides, higher DH will increase the antioxidant properties of

fish protein hydrolysate (Thiansilakul *et al.*, 2007). However, a very high DH can have negative effects on the functional properties of hydrolysates (Kristinsson and Rasco, 2000a) and resulted in a bitter taste (Adler-Nissen, 1986).

Undulated surf clam is one of the clam species usually classified into bivalve molluscs in the shellfish group. Few common names of Undulated surf clam are found such as Undulated clam, Short-necked surf, Surf clam, Carpet clam and Venus shell (Chanrachkij, 2013). In Malaysia, it is locally known as 'Lala'. This species is found along the shallow sandy seabed and coastal areas in the Indo-West Pacific, Red Sea region. The previous study indicated that undulated surf clam has high protein content (68.77% crude protein dry basis) (He *et al.*, 2013). However, there is no information is available regarding the effect of DH on the physicochemical properties of undulated surf clam meat hydrolysate. Therefore, there is a need to evaluate the physicochemical properties of undulated surf clam hydrolysate with different DH. Thus, this study aimed to determine the physicochemical properties of undulated surf clam hydrolysate as affected by the degree of hydrolysis.

Materials and Methods

Materials

Whole and fresh undulated surf clams (50 kg) were obtained from a wholesale fish market in Kuala Terengganu. The fresh undulated surf clams were transported in ice to the laboratory. The whole clams were thoroughly cleaned with tap water and a toothbrush to remove the contaminants, mud, and soil on the shells. Next, the clam meat was separated from the shells manually. Finally, the clam meat was homogenized in a food processor, placed in plastic bags and frozen at -20ºC until further use.

The commercial protease used in the hydrolysis was Alcalase® 2.4L (Novo Industry, 1989). Other chemicals used were of analytical grades.

Experimental Design

In this study, undulated surf clam hydrolysates at three different DHs were prepared in duplicates. Then the supernatant of the hydrolysates was lyophilized and subjected to physicochemical analysis. The physicochemical analysis involved were proximate, emulsifying capacity, foaming properties, water-holding capacity, oil-holding capacity, peptide solubility and colour. All analyses were carried out in duplicates.

Preparation of Lyophilized Undulated Surf Clam Hydrolysate (LCH)

Prior to enzymatic protein hydrolysis, the protein content of undulated surf clam meat was determined first using Kjeldahl method (AOAC, 2000). It was found that the undulated surf clam meat contained 3.75% crude protein. The protein content of clam meat will be used in the calculation of the mass of raw material, water and enzyme concentration needed in each run of enzymatic hydrolysis. All calculations were carried out according to Kristinsson and Rasco (2000a).

Three different levels of DH of undulated surf clam hydrolysate were prepared which were DH of 36.57% (without any enzymatic hydrolysis), DH of 58.25% (0.5% Alcalase®; 5 min; pH 7.5; 60ºC) and DH of 91.26% (1% Alcalase®; 30 min; pH 7.5; 60ºC). For undulated surf clam hydrolysate with DH of 36.57% (without any enzymatic hydrolysis), the homogenized meat clam was freeze-dried prior to physicochemical analyses. For higher DH, enzymatic hydrolysis of clam meat was carried out first. The protein hydrolysis of clam meat was carried out as described by Bhaskar and Mahendrakar (2008). The hydrolysates were frozen (-20°C) before freeze-drying. The lyophilized clam meat hydrolysate (LCH) was ground with mortar and pestle and stored in airtight containers at room temperature until further analysis.

Determination of Degree of Hydrolysis (DH)

The degree of hydrolysis (DH) of LCH was determined using the trichloroacetic acid (TCA) method as described by Hoyle and Merritt (1994). DH was calculated as follows:

$$\%DH = \frac{10\%\ TCA\ soluble\ N}{Total\ N} \times 100$$

Determination of The Proximate Composition of LCH

The proximate analysis of LCH was performed according to AOAC method (AOAC, 2000).

Determination of Emulsifying Capacity

Emulsifying capacity (EC) of LCH was measured according to the method of Diniz and Martin (1997). A volume of 30 ml of vegetable oil and 0.5 g of LCH was added to 60 ml of NaCl solution (30 g/l) and mixed using a homogenizer at a speed of 10,000 rpm for 30 min. After 30 min, 30 ml of vegetable oil was added again over 1.5 min and mix for another 30 seconds. The mixture was placed in a centrifuge tube and held in a water bath at 85°C for 15 min. The mixture was then centrifuged at 3000 × g for 30 min. EC of LCH was calculated as follows:

Where V_A = Volume of vegetable oil added to form an emulsion (ml)

V_R = Volume of oil released after centrifugation (ml)

W_S = Weight of the sample (g)

Determination of Foaming Properties

Foaming capacity and stability of LCH were determined using the method as described by Shahidi *et al.* (1995) and Klompong *et al.* (2007). Three grams of LCH were mixed with 100 ml of distilled water. The mixture was then homogenized at 16000 rpm for 2 min. Immediately, the homogenized mixture was poured into a 250 ml graduated cylinder and the total volume was taken and recorded. The foaming capacity was calculated as follows:

$$Foaming\ capacity\ (\%) = \frac{A-B}{B} \times 100$$

Where,

A = Volume of the mixture after homogenization (whipping)

B = Volume of the mixture before homogenization (whipping)

After the foaming capacity had been determined, the foaming stability of LCH was recorded. The foaming stability was calculated as the volumes of foam remained after 0.5, 10, 40 and 60 min quiescent periods.

Determination of Water Holding Capacity

Water holding capacity (WHC) of LCH was determined using the centrifugation method as described by Diniz and Martin (1997).

Determination of Oil-Holding Capacity

Oil holding capacity (OHC) of LCH was determined using the method described by Haque and Mozaffar (1992) and Wasswa *et al.* (2007).

Determination of Peptide Solubility

To determine the solubility of LCH, nitrogen solubility index (NSI) was used as described by Morr *et al.* (1985). LCH sample (0.5 g) was dispersed in 50 ml of 0.1 M sodium chloride (pH 7). The mixture was stirred at room temperature for 1 hour followed by centrifugation at 4000 rpm for 30 min. The supernatant was filtered using Whatman No.1 filter paper. The nitrogen content in the total fraction and in the soluble fraction was analysed by the Kjeldahl method (AOAC, 2000). The NSI was calculated as follows:

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

Determination of Colour Parameter

The measurement of a colour parameter of LCH was determined using Minolta Chromameter CR-310. A total of 5 g of LCH was weighed and placed into the transparent container of Minolta Chromameter.

Data Analysis

All analyses were carried out in duplicates. The data were subjected to a one-way analysis of variance (ANOVA). Duncan's multiple range test was used to determine the significant difference between samples. SPSS package (SPSS 20.0) software was used for data analysis.

Results and Discussion

Effect of DH on Proximate Composition

Table 1 shows the proximate composition of LCH at different DH in wet and dry basis.

Table 1: Proximate composition of lyophilized undulated surf clam hydrolysate prepared at different degree of hydrolysis

	Wet basis			Dry basis		
Degree of hydrolysis (%)	**36.57**	**58.25**	**91.26**	**36.57**	**58.25**	**91.26**
Moisture (%)	8.98 ± 0.25^{c}	13.31 ± 0.18^{a}	12.59 ± 0.11^{b}	-	-	-
Ash (%)	12.12 ± 0.07^{a}	10.35 ± 0.60^{b}	10.07 ± 0.62^{b}	13.31 ± 0.79^{A}	11.93 ± 0.69^{B}	11.51 ± 0.71^{B}
Fat (%)	7.34 ± 0.21^{a}	0.32 ± 0.03^{b}	0.16 ± 0.20^{b}	8.07 ± 0.23^{A}	0.37 ± 0.03^{B}	0.19 ± 0.03^{B}
Protein (%)	51.52 ± 3.69^{b}	56.59 ± 0.60^{a}	60.62 ± 1.30^{a}	56.61 ± 4.05^{B}	65.28 ± 0.69^{A}	69.35 ± 1.49^{A}
***Carbohydrate (%)**	20.04 ± 4.00^{a}	19.28 ± 1.09^{a}	16.56 ± 1.49^{a}	22.01 ± 4.23^{A}	22.43 ± 1.05^{A}	18.95 ± 1.71^{A}

Values are expressed in mean ± SD of two replicates (n=2). Value with a different letter is significantly different between samples ($p < 0.05$). *Carbohydrate was determined by difference. Statistical analysis was carried out separately for a wet basis and dry basis composition

Table 1 shows that there was a significant difference ($p > 0.05$) in all samples for moisture content. The moisture content of protein hydrolysate is related to the type of sample and the efficiency of the freeze-drying process. Besides, the hydrolysates may absorb moisture from the surrounding. However, the moisture content in this study was higher than previous studies reported for freeze-dried Pacific whiting muscle hydrolysate (2.8-3.6%) (Pacheco-Aguilar *et al.*, 2008) and spray dried fish hydrolysates (sardine byproduct, cobia muscle, cobia frame, silver catfish frame) (1.35-6.25%) (Soussi *et al.*, 2008; Farah, 2010; Amiza *et al.*, 2012; Amiza *et al.*, 2013). Higher moisture content in this study could be due to inefficient drying during freeze-drying. Low moisture content is desirable to increase the stability during storage.

For ash content, this study showed that the ash content of LCH at DH 36.57% was higher than other samples. Pacheco-Aguilar *et al.* (2008) have reported a similar range of ash content (11.7% to 11.9%) for Pacific whiting muscle hydrolysate. However, small croaker protein hydrolysate (3.7% to 4.7%) cobia muscle hydrolysate (7.65% to 18.63%), cobia frame hydrolysate (4.73 to 22.35%) gave a different range of ash content compared to this present study (Choi *et al.*, 2009; Farah, 2010; Amiza *et al.*, 2012). Besides the contribution of ash content in the raw material, the alkali used during pH adjustment also contributes to

ash content (Liceaga-Gesualdo and Li-Chan, 1999; Kristinsson and Rasco, 2000b; Severin and Xia, 2005). Furthermore, centrifugation of hydrolysate before supernatant collection may eliminate some raw materials containing minerals.

For fat content, this study showed that fat content of LCH at DH 36.57% was higher than other samples. During the hydrolysis process, some structural lipid may be removed (Shahidi *et al.*, 1995). Apart from this, the centrifugation step before freeze-drying also helped to remove the lipid layer on the top supernatant fraction (Benjakul and Morrisey, 2007). Thus, hydrolysates with higher DH will contain a lower level of fat compared to lower DH (DH 36.57%). A similar result was obtained for spray-dried cobia frame (Amiza *et al.*, 2012) and spray dried cobia muscle hydrolysate (Farah, 2010), whereby their crude fat content decreased as the DH increased. However, contrasting results have been reported for lyophilized Pacific whiting muscle and spray dried silver catfish frame hydrolysates, whereby fat content increased with increasing DH (Pacheco-Aguillar *et al.*, 2008; Amiza *et al.*, 2013). The fat content in freeze-dried Pacific whiting muscle hydrolysate (Pacheco-Aguillar *et al.*, 2008), spray-dried cobia muscle hydrolysate (Farah, 2010), spray-dried cobia frame (Amiza *et al.*, 2012) and spray dried silver catfish frame hydrolysate (Amiza *et al.*, 2013) were below 1%. However, higher fat content was reported in sardinella by-product hydrolysate (8.53% to 10.29%). This may be due to the different amount of fat in raw material of hydrolysate and the variation in the processing involved in the preparation of hydrolysate.

Table 1 shows that higher DH gave higher protein content of LCH ($p < 0.05$). Theoretically, protein content should not change due to enzymatic hydrolysis alone. However, protein content showed changes due to other processing steps in LCH preparation. In this study, higher DH was prepared by manipulating hydrolysis time and concentration of enzyme. The high protein content in LCH could be due to protein solubilization during hydrolysis, the removal of insoluble intact non-protein substances and the partial removal of lipid after hydrolysis (Benjakul & Morrissey, 1997). Thus, higher DH gave to higher soluble protein in the supernatant of LCH. Higher protein content was reported in spray-dried sardinella byproducts hydrolysate (72 – 75%) and freeze-dried Pacific whiting muscles hydrolysate (85 – 88%) (Souissi *et al.*, 2008; Pacheco-Aguillar *et al.*, 2008). However, lower protein content was reported for spray-dried cobia muscle hydrolysate (30.31% to 43.27%) and blood cockle hydrolysate (9.10% to 12.76%) (Farah, 2010; Amiza & Masitah, 2012). This could be due to the utilization of high temperature during the spray drying process cause loss of protein (Abdul Hamid *et al.*, 2002) and higher carbohydrate content from the addition of maltodextrin during spray drying.

Carbohydrate content in LCH was determined by difference. Table 1 shows that there were no significant differences between all samples ($p < 0.05$). A similar finding was reported by protein hydrolysates from cobia muscle (Farah, 2010) and cobia frame (Amiza *et al.*, 2012).

Effect of DH on Emulsifying Capacity

Table 2 shows emulsifying capacity, foaming capacity, water holding capacity, oil holding capacity and peptide solubility of LCH as affected by the degree of hydrolysis. Table 2 shows that the emulsifying capacity of LCH decreased significantly with increasing DH ($p<0.05$). Similar trend has been reported in sardinella by-product hydrolysate (10.8 ml/g to 20 ml/g) (DH range of 6.62% - 10.16%) and cobia muscle hydrolysate (6.77 ml/g to 18.29 ml/g) (DH range of 54.76% - 29.07%) (Souissi *et a.l*, 2007; Farah, 2010).

Table 2: Emulsifying capacity, foaming capacity, water holding capacity, oil holding capacity and peptide solubility of undulated surf clam hydrolysate as affected by the degree of hydrolysis

Degree of hydrolysis	Emulsifying capacity (%)	Foaming capacity (%)	Water holding capacity (ml/g)	Oil holding capacity (ml/g)	Peptide solubility (%)
36.57	110.52±2.88^{a}	29.43±4.34^{b}	7.63±0.08^{a}	2.48±0.26^{a}	57.61±4.62^{c}
58.25	18.13±1.13^{b}	59.50±5.00^{a}	7.08±1.05^{a}	2.26±0.24^{a}	75.10±1.50^{b}
91.26	11.94±0.27^{c}	67.50±11.59^{a}	6.64±1.06^{a}	2.22±0.11^{a}	94.08±1.48^{a}

Different superscript letter (a, b and c) within the same column indicate significant difference between means of the sample ($p < 0.05$)

Protein hydrolysates are surface-active materials and promote oil-in-water emulsions because they are water-soluble and contain hydrophilic and hydrophobic functional groups (Gbogouri *et al.*, 2004).

It was noted that LCH with the lowest DH (36.57%) exhibited the highest emulsifying capacity (110±2.88 ml/g). Peptide size is an important factor in emulsifying capacity (Bernard, *et al.*, 2011; Morales, *et al.*, 2015). According to Puski (1975), the larger the availability of large peptide units at the oil-water interface, resulting in a larger surface area and give greater emulsion formation. Choi *et al.* (2008) stated that the hydrolysate had less surface hydrophobicity with increasing DH. Besides, emulsifying capacity was influenced by the specificity of enzyme too (Gauthier *et al.*, 1993).

The emulsifying capacity of LCH (11.94 ml/g to 18.13 ml/g) was higher compared to cobia frame hydrolysate (3.33 ml/g to 11.99 ml/g) (DH range of 53.42% - 95.63%) (Amiza *et al.*, 2012), but in a similar range with sardinella byproduct hydrolysate (10.8 ml/g to 20 ml/g). However, LCH gave lower emulsifying capacity compared to other fish hydrolysates such as shark muscle hydrolysates (23.09 ml/g to 51.2 ml/0.5g) (DH range of 6.50% -18.77%) and grass carp skin hydrolysates (20.00 ml/g to 38.00 ml/g) (DH range of 5.02% -14.90%) (Diniz & Martin, 1997; Wasswa *et al.*, 2007).

Effect of DH on Foaming Properties

Table 2 shows that for foaming capacity, LCH with the lowest DH (DH 36.57%) was significantly lower than other samples. However, the foaming capacity of LCH of DH 58.25% and DH 91.26% was similar ($p>0.05$). This study shows that the foaming capacity increased when DH increased until it reached a maximum level, and then level off afterwards.

A protein should be evaluated for both foaming capacity and foaming stability (Wilde and Clark, 1996). Protein in dispersions causes a lowering of the surface tension at the water-air interface, thus creating foaming capacity. Low DH may improve foaming capacity but generally, high DH led to a decrease of the foam stability (Zhou *et al.*, 2011). This is because although smaller peptides can incorporate air into the solution, they do not have enough strength to give stable foam (Kristinsson & Rasco, 2000a; Jamdar *et al.*, 2010). It can be concluded that limited protein hydrolysis may improve its functional properties. So, a compromise must be reached between DH and functional properties.

Figure 1 shows the foaming stability of LCH after 0.5 min, 10 min, 40 min and 60 min. It was found that clam hydrolysate sample at DH 36.57 formed a stable foam within 60 min. However, the foaming stability reduced with increasing DH. Excessive hydrolysis would reduce the foaming stability of hydrolysates. This is because the small peptides do not have the strength required to sustain stable foam (Shahidi *et al.*, 1995). Low molecular weight contributes rapid foam, but poor in foaming protein-protein interaction to form a stable foam (Farah, 2010).

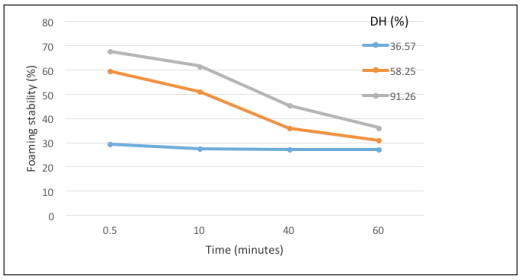

Figure 1: Foaming stability of undulated surf clam hydrolysate at different degree of hydrolysis

Effect of DH on Water Holding Capacity (WHC)

Table 2 shows that water holding capacity (WHC) was similar for all LCH samples ($p<0.05$). A similar trend has been reported for shark muscle hydrolysate (for a lower DH range of 6.5-18.8%) and blood cockle hydrolysate (for a lower DH of 14.48% - 36.12%) (Diniz and Martin 1997; Wong, 2012).

However, a different trend was reported for protein hydrolysates from shark muscle (Diniz & Martin, 1997), cobia muscle (Farah, 2010), cobia frame (Amiza *et al.*, 2012) and silver catfish frame (Amiza *et al.* 2013), whereby they reported with increasing DH, WHC increased, due to the presence of the polar group. Lower DH gave higher values of WHC, presumably due to the hydrophilic polar side chains of the original protein. While at high DH, Don *et al.* (1991) proposed that the protein networks or protein structures were degraded by hydrolysis and consequently this diminished the sample's oil absorption capacity. Fish protein hydrolysates were reported to possess excellent WHC resulting in increased cooking yield when added to minced meat (Shahidi *et al.*, 1995; Kristinsson & Rasco, 2000a).

LCH gave a higher WHC (6.65 ml/g to 7.63 ml/g) compared to cobia muscle hydrolysate (0.60 ml/g to 1.00 ml/g) (DH of 29.07 - 54.76%) (Farah, 2010), cobia frame hydrolysate (0.80 ml/g to 1.10 ml/g) (DH of 53.42 - DH 95.63%) (Amiza *et al.*, 2012) and sardinella by product (2.00 ml/g to 4.90 ml/g) (DH of 5.02 - 14.90%) (Soussi *et al.*, 2007).

Effect of DH on Oil-Holding Capacity (OHC)

Oil-holding capacity (OHC) shows the quantity of oil bound by the protein hydrolysate and it is an important functional characteristic for the meat and confectionery industries (Gbogouri *et al.*, 2004). Table 2 shows that all LHC samples gave similar OHC (2.22-2.48 mg/ml) ($p<0.05$). A similar finding has been reported in Cobia frame hydrolysate (OHC of 2.4- 2.8 ml/g) (DH of 53-96%) and shark muscle hydrolysate (0.3-0.5 ml/g) (for DH of 6.5-18.8%) (Amiza et al., 2012; Diniz & Martin, 1997). OHC of LCH was lower than cobia frame hydrolysate (2.4-2.8 ml/g) and grass carp skin hydrolysate (2.4-3.6 ml/g) (Wasswa et al., 2007). However, it is higher than those of shark muscle hydrolysate (0.3-0.5 ml/g) (Diniz & Martin, 1997) and whey protein hydrolysate (0.16-0.34 ml/g) (Sinha et al., 2007).

However, several studies reported a different trend whereby OHC of fish protein hydrolysate samples decreased with increase in DH for shark muscle (Diniz & Martin, 1997), red salmon head (Sathivel *et al.*, 2005), grass carp skin (Wasswa *et al.*, 2007) and cobia muscle (Farah, 2010).

According to Soussi *et al.* (2007), high OHC is due to the liberation of peptides from the native protein, which would enhance the flexibility of the peptides. The decrease in OHC may be attributed to extensive hydrolysis of protein structures (Wasswa *et al.*, 2007) and reduction in hydrophobic interactions (Haque & Mozaffar, 1992; Liceaga-Gesualdo and Li-Chan, 1999). Moreover, DH values bring a big impact on OHC. Low DH may improve OHC, but high DH may minimize the ability of OHC of the hydrolysate. The flexibility of peptides had been enhanced by the liberation of some peptides from native protein during the hydrolysis process and this would result in a high value of OHC at low DH. Besides, larger peptides size was attributed to the higher fat absorption at the low extent of hydrolysis, therefore, yielding a high value of oil-holding capacity (Kristinnson & Rasco, 2007).

Effect of DH on Peptide Solubility

Table 2 shows that there was a significant increase ($p > 0.05$) in peptide solubility (57.61-94.08%) with increasing DH. Similar trend has been reported in several studies including hydrolysates silver catfish (80-85%) (DH range of 43-68%) (Amiza *et al.*, 2013), Atlantic salmon muscle hydrolysate (40.6 –79.9%.) (DH of 40.6-70.9%) (Kristinsson & Rasco, 2000a) and surimi processing by product (65-90%) (DH 10-30%) (Liu *et al.*, 2014). However, DH did not affect solubility in Pacific whiting muscle hydrolysate (over 97% for all DHs) (DH 10-20%) (Pacheco-Aguilar *et al.*, 2008) and cobia frame hydrolysate (85-86%) (DH of 14.48%-36.12%) (Amiza *et al.,* 2012). This insignificant difference in finding could be due to the smaller range of DH used in both studies as compared to the present study.

The high solubility of protein hydrolysates was attributed to the reduction of its secondary structure. Furthermore, the protein hydrolysis had also helped to liberate the smaller polypeptide units from the proteins. The insoluble protein fractions were separated from the hydrolysates by centrifugation before freeze-drying process thus causing it to become more soluble. Besides, it has been reported that the use of Alcalase® in fish protein hydrolysis yielded a highly soluble protein (Quaglia & Orban, 1987; Shahidi *et al.*, 1994). The high solubility of hydrolysate is due to cleavage of proteins into smaller peptides (Shahidi, 1994). Besides, peptide length and the ratio of hydrophilic/hydrophobic peptides could affect the solubility as well. Besides, the increase in peptide solubility is due to increase of soluble peptides from insoluble aggregates or precipitates and the corresponding increase in the ionisable amino and carboxyl groups (Tavano, 2013).

Effect of DH on Colour Parameters

Table 3 shows the colour parameters of LCH at three different DHs. Table 3 shows that the L*, a*and b* values were significantly different for all the samples at different DH ($p < 0.05$).

Table 3: Colorimeter parameter values of undulated surf clam hydrolysate at different degree of hydrolysis

Degree of hydrolysis (%)	Hunter colour parameter		
	L*	a*	b*
36.57	72.21 ± 0.46^{b}	-1.23 ± 0.01^{c}	16.64 ± 0.36^{c}
58.25	68.01 ± 0.72^{c}	4.54 ± 0.96^{a}	26.58 ± 1.01^{a}
91.26	76.24 ± 0.24^{a}	0.15 ± 0.06^{b}	21.96 ± 0.42^{b}

L*: lightness, a*: green (-a) to red (+a), b*: blue (-b) to yellow (+b). Values are means of triplicate determinations. Different alphabets (a, b and c) in a column indicates significant difference between the means of samples at different DH ($p < 0.05$).

By comparing the values for lightness (L* values), LCH gave highest L* value at high DH (DH 91.26%), followed by that of low DH (DH 36.57%) and finally moderate DH (DH 76.24%). Previous studies reported that as DH increased, lightness values decreased (Farah, 2010; Wasswa *et al.*, 2007; Diniz & Martin, 1997). However, in this present study, moderate DH gave the lowest lightness. This is unexpected as hydrolysates from moderate DH (DH 76.24%) and high DH (DH 91.26%) have similar enzymatic hydrolysis condition (pH and temperature), except for hydrolysis time and % Alcalase® used (i.e. 5 min and 0.5% Alcalase® vs 30 min and 1% Alcalase®). The difference in the LCH colour could be contributed to differences in enzymatic hydrolysis conditions during the preparation of the hydrolysates. During enzymatic hydrolysis, hydrolysates were subjected to different chemical changes

including Maillard reaction and enzymatic browning reactions may reduce, giving a darker appearance at high DH (Wasswa *et al.*, 2007).

The lightness of LCH (68.01 to 76.24) (DH of 36.57 - 91.26%) was lighter compared to grass carp skin hydrolysate (59.3 to 68) (DH of 6.5%-18.8%) (Wasswa *et al.*, 2007) but darker compared to cobia muscle hydrolysate (86.4 to 90.5) (DH of 6.5%-18.8%) (Farah, 2010). A quite similar range of lightness was reported for blood cockle hydrolysate (71.85 to 82.49) (DH of 14.48 -36.12%) (Wong, 2012). This difference could be due to the difference in the raw materials, the DH range used as well as the pH, hydrolysis time and temperature used during the preparation of protein hydrolysate.

Both a* and b* values of LCH gave similar trend whereby the highest values of both parameters were given by moderate DH (DH 76.24%), followed by high DH (DH 91.26%) and finally low DH (DH 36.57%).

The yellowness of LCH (16.64 to 26.58) (DH of 36.57 - 91.26%) was in similar range compared to grass carp hydrolysate (26.6 to 22) (DH of 14.9 - 10.4%) (Wasswa et al., 2007) and blood cockle hydrolysate (25.77 to 20.50) (DH of 14.48 - 36.12%) (Wong, 2012). However, the less yellowish colour was reported for cobia muscle hydrolysate (17.37 to 9.97) (DH of 54.76 - 29.07%) (Farah, 2010) and cobia muscle hydrolysate (17.37 to 9.97) (DH of 54.76 - 29.07% (Diniz & Martin, 1997). The a* values at LCH at DH 36.57% was greener (-1.23) compared to a DH 91.26% (0.15). The values are quite similar to shark and cobia muscle hydrolysates (Diniz & Martin, 1997; Farah, 2010).

Conclusion

This study found that DH affected protein, ash, and fat content as well as foaming properties, emulsifying capacity and peptide solubility of undulated surf clam hydrolysate. However, DH did not affect carbohydrate content, water-holding capacity, oil-holding capacity, and colour parameters of undulated surf clam hydrolysate. Thus, this study shows that some physicochemical properties can be tailored using an appropriate degree of hydrolysis.

Acknowledgements

The authors acknowledged that this work has been partially funded by Universiti Malaysia Terengganu.

References

Abdul-Hamid, A., Jamiah, B., & Bee, G. H. (2002). Nutritional quality of spray dried protein hydrolysate from Black Tilapia. *Food Chemistry*, *78*, 69-74.

Adler-Nissen, J. (1986). Enzymatic hydrolysis of food proteins. New York: Elsevier Applied Science Publishers Ltd.

Amiza, M. A., & Masitah, M. (2012). Optimization of enzymatic hydrolysis of blood cockle (*Anadara granosa*) using Alcalase. *Borneo Science*, *31*, 1-10.

Amiza, M. A., Kong. Y. L., & Faazaz, A. L. (2012). Effects of degree of hydrolysis on physicochemical properties of cobia (*Rachycentron canadum*) frame hydrolysate. *International Food Research Journal*, *19*(1), 199-206.

Amiza, M. A., Ow, Y. W., & Faazaz, A. L. (2013). Effect of degree of hydrolysis on physicochemical properties of silver catfish (*Pangasuis sp*) frame hydrolysate. *International Food Research Journal*, *20*(3), 1255-1262.

AOAC. (2000). *AOAC Official Methods of Analysis*, 17th edition. Washington: Association of Official Analytical Chemist.

Benjakul, S., & Morrissey, M. T. (1997). Protein hydrolysate from visceral waste protein of Catla (*Catla catla*): Optimization of hydrolysis conditions for a commercials neutral protease. *Bioresource Technology*, *99*, 4105-4111.

Chalamiaiah, M., Kumar, B. D., Hemalatha, R., & Jyothimayi, T. (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chemistry*, *135*(4), 3020-3038.

Chanrachkij, I. (2013). Monitoring the Undulated Surf Clam Resources of Thailand for Sustainable Fisheries Management. *Fish for the People*, *11*(3), 33-44.

Chau, C. F., & Cheung, P. C. K. (1998). Functional properties of flours prepared from three Chinese indigenous legume seeds. *Food Chemistry*, *61*(4), 429–433.

Choi, Y. J., Hur, S., Choi, B.-D., Konno, K., & Park, J. W. (2009). Enzymatic Hydrolysis of Recovered Protein from Frozen Small Croaker and Functional Properties of Its Hydrolysates. *Journal of Food Science*, *74*(1), 17–24.

Clemente, A., Vioque, J., Sánchez-Vioque, R., Pedroche, J., Bautista, J., & Millán, F. (2000). Factors affecting the in vitro protein digestibility of chickpea albumins. *Journal Science Food and Agriculture*, *80*, 79-84.

Dickinson, E. (1997). Properties of Emulsions Stabilized with Milk Proteins: Overview of Some Recent Developments. *Journal of Dairy Science*, *80*(10), 2607–2619.

Diniz, F. M. & Martin, A. M. (1997). Effect of the extent of enzymatic hydrolysis on the functional properties of shark protein hydrolysate. *Lebensmittel-Wissenschaft and Technologies*, *30*, 266-272.

Don, B. L. S., Pilisof, A. M. R., & Bartholoni, G. B. (1991). Enzymatic modification of soy protein concentrates by fungal and bacterials protease. *Journal of the American Oil Chemists Society*, *68*, 102-105.

Farah, I. (2010). Effect of degree of hydrolysis on physicochemical properties of cobia muscle hydrolysate, Unpublished report.

Gauthier, S. F., Paquin, P., Pouliot, Y., & Turgeon, S. (1993). Surface Activity and Related Functional Properties of Peptides Obtained from Whey Proteins. *Journal of Dairy Science*, *76*(1), 321–328.

Gbogouri, G. A., Linder, M., Fanni, J., & Parmetier, M. (2004). Influence of hydrolysis on the functional properties of salmon byproduct hydrolysate. *Journal of Food Science*, *69*, 615-622.

Haque, Z.U., & Mozaffar, Z. (1992). Casein hydrolysate II. Functional properties of peptides. *Food Hydrocolloids*, *5*, 559-571.

He, X. Q., Cao, W. H., Zhao Z. K., & Zhang, C. H. (2013). Analysis of protein composition and antioxidant activity of hydrolysates from Paphia undulate. *Journal of Food and Nutrition Research*, *1*(3), 30-36.

Hoyle, N. & Merritt, J. (1994). Quality of fish protein hydrolysate from herring (*Clupea harengus*). *Food Science*, *59*(1), 76-79.

Jamdar, S. N., Rajalakshmi, V., Pednekar, M. D., Juan, F., Yardi, V., & Sharma, A. (2010). Influence of degree of hydrolysis on functional properties, antioxidant activity and ACE inhibitory activity of peanut protein hydrolysate. *Food Chemistry*, *121*(1), 178–184.

Klompong, V., Benjakul., S., Kantachote, D., & Shahidi, F. (2007). Antioxidative activity and functional properties of protein hydrolysates of yellow stripe travelly (*Selaroides leptolepsis*) as influenced by degree of hydrolysis and enzyme types. *Food Chemistry*, *102*, 1317-1327.

Kristinsson, H. G., & Rasco, B. A. (2000a). Fish protein hydrolysates: production, biochemical, and functional properties. *Critical Review in Food Science and Nutrition*, *40*, 43-81.

Kristinsson, H. G., & Rasco, B. A. (2000b). Hydrolysis of salmon muscle proteins by an enzyme mixture extracted from Atlantic salmon pyloric caeca. *Journal of Food Biochemistry*, *24*, 177-187.

Liceaga-Gesualdo, A. M., & Li-Chan, E. C. Y. (1999). Functional properties of fish protein hydrolysate from herring. *Journal of Food Chemistry and Toxicology*, *64*, 1001-1004.

Liu, Y., Li, X., Chen, Z., Yu, J. and Wang, J. (2014). Characterization of structural and functional properties of fish protein hydrolysates **from surimi processing by-products** *Food Chemistry*, *151*, 459-465.

Morr, V., German, B., Kinsella, J. E., Regenstein, J. M., Van Buren, J. P., & Kilara, A. (1985). A collaborative study to develop a standardized food protein solubility procedure. *Journal of Food Science*, *50*, 1715–1718.

Pacheco-Aguilar, R., Mazorra-Manzano, M. A., & Ramirez-Suarez, J. C. (2008). Functional properties of fish protein hydrolysates from Pacific whiting (*Merluccius productus*) muscle produced by a commercial protease. *Food Chemistry*, *109*, 782-789.

Puski, G. (1975). Modification of functional properties of soy proteins by proteolytic enzyme treatment. *Cereal Chemistry*, *52*, 655.

Quaglia, G. B., & Orban, E. (1987). Influence of the degree of hydrolysis on the solubility of the protein hydrolysates from sardine (*Sardina pilchardus*). *Journal of the Science of Food and Agriculture*, *38*(3), 271–276.

Rutherfurd, S. M. (2010). Methodology for determining degree of hydrolysis of protein in hydrolysates: A review. *Journal of AOAC International*, *93*(5), 15-22.

Salwanee, S., Wan Aida, W., Mamot, S., Maskat, M.Y., & Ibrahim, S. (2013). Effects of enzyme concentration, temperature, pH and time on the degree of hydrolysis of protein extract from viscera of tuna (*Euthynnus affinis)* by using Alcalase. *Sains Malaysiana*, *42*(3), 279-287.

Sathivel, S., Smiley, S., Prinyawiwatkul, W., & Becthel, P. J. (2005). Functional and nutritional properties of red salmon (*Oncorhynchus nerka*) enzymatic hydrolysates. *Journal of Food Science*, *7*(6), 401-406.

Severin, S., & Xia, W. S. (2005). Enzymatic hydrolysis of whey protein by two different protease and their effect on the functional properties of resulting protein hydrolysates. *Journal of Food Biochemistry*, *30*, 77-97.

Shahidi, F. (1994). Sea Food Processing By-Products. In: *Seafoods: Chemistry, Processing Technology and Quality*, F. Shahidi, J.R. Botta (eds.), Blackie Academic and Professional, London, UK. pp. 321–334.

Shahidi, F., Han, X. Q., & Synowicki, J. (1995). Production and characteristic of protein hydrolysates from capelin (*Mallotus villosus*). *Food Chemistry*, *53*, 285-293.

Shu, G., Zhang, B., Zhang, Q., Wan, H., & Li, H. (2016). Effect of Temperature, pH, Enzyme to Substrate Ratio, Substrate Concentration and Time on the Antioxidative Activity of Hydrolysates from Goat Milk Casein by Alcalase. *The Journal of Lucian Blaga*, *20*(2), 29-38.

Šližytė, R., Dauksas, E., Falch, E., Storro, I., & Rustad, T. (2005). Characteristics of protein fractions generated from hydrolysed cod (*Gadus morhua*) by-products. *Process Biochemistry*, *40*(6), 2021–2033.

Souissi, N., Bougatef, A., Triki-Ellouz, Y. & Nasri, M. (2007). Biochemical and functional properties of sardinella (*Sardinella aurita*) by product hydrolysate. *Food Technology and Biotechnology*, *45*, 187-194.

Tavano, O. L. (2013). Protein hydrolysis using proteases: An important tool for food biotechnology. *Journal of Molecular Catalysis. B: Enzymatic*, *90*, 1–11.

Thiansilakul, Y., Benjakul, S., & Shahidi, F. (2007). Compositions, functional properties and antioxidative activity of protein hydrolysate prepared from round scad (*Decapterus maruadsi*). *Food Chemistry, 103*, 1385-1394.

Wasswa, J., Tang, J., Gu, X. H., & Yuan, X. Q. (2007). Influence of the extent of enzymatic hydrolysis on the functional properties of protein hydrolysate from grass carp skin. *Food Chemistry*, *104*, 1698-1704.

Wilde, P. J., & Clark, D. C. (1996). Foam formation and stability. In: *Methods for Testing Protein Functionality*. Hall, G. M . (Ed.), p. 110-148. Blackie Academic and Professional, London, England.

Wong, C. (2012). Effect of degree of hydrolysis on physicochemical properties of blood cockle (*Anadara granosa*) hydrolysate by using enzymatic hydrolysis. Unpublished reports.

Zhou, H. M., Amza, T., Amadou, I., Kamara, M. T., & Zhu, K. X. (2011). Nutritional and functional characteristics of gingerbread plum (*Neocarya macrophylla*): An underutilized oilseed. *Grasas Y Aceites*, *62*(3), 290-298.