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Effects of degree of hydrolysis on physicochemical properties of Cobia *(Rachycentron canadum)* frame hydrolysate

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Abstract: The effect of degree of hydrolysis (DH) on the physicochemical properties of cobia frame hydrolysate was determined. Three levels of degree of hydrolysis of cobia frame hydrolysate were studied, which were 53%, 71% and 96%. After enzymatic hydrolysis using Alcalase®, the samples were spray-dried. Cobia hydrolysate powder samples were analyzed for their proximate analysis and physicochemical properties. The proximate analysis showed significant differences in fat and ash content only. DH96 hydrolysate showed desirable essential amino acid profile for human requirement except for methionine and isoleucine. The study found that cobia frame hydrolysate had good colour, emulsifying capacity and excellent foaming properties. However, there were no significant differences in water-holding capacity, oil-holding capacity and peptide solubility among the hydrolysate samples. This study suggested that cobia frame hydrolysate is a potential ingredient and foaming agent for food industry.

Keywords: Cobia, frame, hydrolysate, emulsifying, foaming

Introduction

Enzymatic hydrolysis has been used for modification of functional and nutritional properties of various proteins. Enzymatic hydrolysis has been shown to increase solubility, modify foaming, emulsifying and gelation properties as well as producing bioactive peptides from certain proteins (Spellman *et al*., 2003). It is possible to produce the desirable functional properties of protein hydrolysate by controlling the hydrolysis parameters such as pH, time, enzyme concentration and temperature. The choice of substrate, protease employed and degree of hydrolysis generally affects the physicochemical properties of the resulting hydrolysates (Mullaly *et al*., 1995). Commercial enzyme, Alcalase® has been strongly recommended for fish hydrolysis (Shahidi *et al*., 1995). The extent of hydrolysis is monitored using degree of hydrolysis (DH). DH is defined as the percentage of the total number of peptide bonds in a protein which have been cleaved during hydrolysis (Adler-Nissen, 1986).

Several studies have been reported on the effects of extent of hydrolysis on the physicochemical properties of fish hydrolysate in grass carp skin, yellow stripe trevally muscle, round scad muscle, shark muscle, salmon muscle and capelin muscle (Shahidi *et al*., 1995; Diniz and Martin, 1997; Kristinsson and Rasco, 2000a; Sathivel *et al*., 2005; Wasswa *et al*., 2007; Klompong *et al*., 2007; Thiansilakul *et al*., 2007). They reported that selective enzymatic hydrolysis improved their functional properties, including solubility, water holding, oil holding, emulsifying and foaming characteristics.

Cobia (*Rachycentron canadum*) is a pelagic fish, living in the open ocean in tropical, subtropical, and temperate waters. Nowadays, cobia has been successfully cultured in marine farm due to increasing demand on it. Several characteristics make cobia suitable for commercialization purpose such as ease of spawning, fast growth and high survival rates through the first year (Benetti, 2007). Cobia global marine-farmed production is estimated at 62 million pounds in 2007 and this amount may reach 100 million pounds by 2012 (Marine Farm Belize, 2008). In addition, Cobia contributes a very high yield for dressed fillets of over 60% total body weight high yield of fillet. Cobia generates about 40% of waste from its total body weight (Benetti, 2007).

Our group has carried out research on the optimization of enzymatic hydrolysis from cobia frame using Alcalase® (Amiza *et al*., 2010). However, no information is available on the effects of degree of hydrolysis on physicochemical properties of cobia frame hydrolysate. The aim of this study was to determine the effect of degree hydrolysis on physicochemical properties of cobia frame hydrolysate.

Materials and Methods

Materials

Whole fresh cobia (*Rachycentron canadum*) were obtained from Langkawi Island, Kedah. Fresh cobia were eviscerated, filleted and decapitated, to obtain its frame. The frame was frozen until further use. The enzyme used for the hydrolysis was Alcalase® 2.4 L (2.4 AU/g and a density of 1.18 g/ml), a bacterial endoproteinase from a strain of *Bacillus licheniformis* (Novozymes, Denmark). All other chemicals used

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were of analytical grade.

Preparation of cobia frame

Thawed cobia frame were rinsed to remove the water-soluble compounds, minerals, enzymes and pigments (Yanez *et al*., 1976). After cleaning, the frame were chopped into small pieces. Then, the frame were homogenized using a Waring blender (model HGB2WTS3) at high speed for 60 seconds, with the addition of water (400 g water for 1 kg of cobia frame) to help the homogenization process. Minced cobia frame was sealed in plastic packs and stored in freezer at -40ºC until further use.

Preparation of cobia frame hydrolysate powder (CPH)

Before the protein hydrolysis was carried out, the proximate analysis of the cobia frame was carried out (AOAC, 2002). Some calculations have to be carried out to determine the mass of raw material, distilled water and enzyme solution to be used in the hydrolysis experiment. The calculation is necessary because the mass of raw materials depends on the protein content. All calculations were carried out according to Hordur and Barbara (2000). The hydrolysis was performed according to the procedure of Bhaskar and Mahendrakar (2007) with slight modification. For each batch, about 70 g of cobia frame was added with 51.3 g of distilled water (including the volume of 1 N NaOH used to adjust to required pH) and heated at 85°C for 20 min prior to hydrolysis. After cooling, 20 g of Alcalase enzyme solution (prepared by diluting the required enzyme mass to a final weight of 20 g with distilled water) was mixed into the cobia frame and the hydrolysis was initiated immediately. Parameters of hydrolysis conditions of low, medium and high DH were selected based our preliminary study (Amiza *et al*., 2010). The hydrolysis conditions for DH53 hydrolysate were temperature of 40°C, hydrolysis time of 120 min, Alcalase® to protein ratio of 1.5% (w/w) and pH of 8.5. The hydrolysis conditions for DH71 hydrolysate were temperature of 60°C, hydrolysis time of 180 min, 2% Alcalase® to protein ratio (w/w) and pH of 9.5. The hydrolysis conditions for DH96 hydrolysate were temperature of 60°C, hydrolysis time of 300 min, 20% Alcalase® to protein ratio (w/w) and pH of 10.5. Hydrolysis can be carried out either by using a bioreactor (automatic pH adjustment with 1N NaOH) or water bath shaker (manual pH adjustment using 1N NaOH).

After hydrolysis was completed, the process was terminated by heating the hydrolysate samples at 85°C for 20 min to inactivate the *Alcalase®* activity. Next, the hydrolysate was centrifuged for 20 min at the speed of 6000 g in order to remove the insoluble particles and oil layer. For each DH, the soluble fraction of hydrolysate prepared from several batches of hydrolysis were mixed together prior to drying to ensure homogenous sample. The liquid protein hydrolysate was then spray-dried using a spray drier to produce dry CPH powder at inlet and outlet temperature of 185ºC and 108ºC, respectively. Maltodextrin (5% w/v) was added to the liquid protein hydrolysate to avoid caking in the hydrolysate powder. The cobia hydrolysate powder was stored in a sealed plastic bag at room temperature prior to physicochemical analysis.

Determination of degree of hydrolysis (DH)

Nitrogen solubility index was used to determine the DH by using trichloroacetic (TCA) acid as precipitating agent (Hoyle and Merritt, 1994). The formula used is:

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

Determination of amino acid composition

Determination of amino acid composition was performed using a dedicated Amino Acid Analyzer (L-8800 Hitachi) according to the methods by Guo *et al*. (2005).

Peptide solubility

Solubility of cobia protein hydrolysate was determined by using nitrogen solubility index (NSI) according to the method of Morr *et al*. (1985).

Determination of water-holding capacity (WHC) and oil-holding capacity (OHC)

Water-holding capacity was determined using the centrifugation method (Diniz and Martin, 1997). Oil-holding capacity was determined by measure the volume of edible oil held by 1.0 g of material (Haque and Mozaffar, 1992).

Emulsifying capacity (EC)

Emulsifying capacity was determined by using oil titration method (Diniz and Martin, 1997).

Foaming capacity and foaming stability

Foaming capacity and stability was determined according to the method of Shahidi *et al.* (1995). CPH (3 g) was dispersed in 100 ml of distilled water and the mixture was homogenized for 1 min using a homogenizer at high speed. The mixture was then poured into a 250-ml graduated cylinder and the total volume was read. Foaming capability or whippability

was expressed as percentage of volume increase upon whipping. To determine foaming stability, foam volume was measured after 0.5, 10, 40 and 60 min quiescent periods.

Colour

The colour of cobia hydrolysate powders was determined in triplicate using a colorimeter (Minolta Chroma Meter CR 300).

Data analysis

All experiments were carried out in triplicates. All data were stated as mean ± standard deviation. The data obtained were subjected to one way analysis of variance (ANOVA), followed by the Duncan's multiple range test to determine the significant difference between sample at $p<0.05$ level using the SPSS programme (SPSS Version 16.0).

Result and Discussion

Proximate composition

The proximate composition of cobia hydrolysate at three different DH are listed in Table 1. In general, there were significant differences in proximate analysis for all DH except for protein content. The ash content of CPH at DH53 was significantly different than those of DH71 and DH96. CPH possessed high ash content, which is in the range of 4.73-22.35 %. The high ash content of samples was due to the addition of alkali required for pH adjustment and its control during the hydrolytic process. According to Severin and Xia (2005), as DH increase, the pH of the hydrolysis process increase and subsequently the volume of NaOH used will also increase. The ash content in Pacific whiting muscle hydrolysate was in the range of 11.7-11.9 % and that of sardinella byproduct hydrolysate was in the range of 12.1-14.8% (Souissi *et al*., 2007; Pacheco-Aguilar *et al*., 2008).

Table 1. Proximate composition of protein hydrolysates produced from cobia frame

Degree of hydrolysis (%)	53.42	70.81	95.63
Moisture content (%)	6.25 ± 0.1 ^{a}	4.83 ± 0.1 ^{c}	5.64 ± 0.1 ^{b}
Ash (%)	4.73 ± 0.1 ^{b}	22.25 ± 1.7 ^{a}	22.35 ± 2.0 ^{a}
Protein (%)	41.83 ± 1.6 ^{a}	40.43 ± 0.9 ^{a}	43.11 ± 1.6 ^{a}
Fat (%)	0.54 ± 0.1 ^{a}	0.39 ± 0.1 ^{ab}	0.26 ± 0.1 ^{b}
Carbohydrate (%)	46.65± 0.5^{b}	32.1± 0.7^{a}	28.64± 0.9^{a}

Values are mean ± SD of three replicates. Values with different letter are statistically different between samples ($p \leq 0.05$).

There was no significant difference in protein content for all CPH samples. Similar trend was reported in the spray-dried hydrolysate from sardinella byproducts (Souissi *et al*., 2007) as well as freeze-dried Pacific whiting muscle (Pacheco-Aguilar *et al*., 2008). The amount of protein content in spray-dried sardinella byproducts hydrolysate without maltodextrin addition and freeze-dried Pacific whiting muscles hydrolysate were within the range of 72 – 75% and 85 – 88%, respectively. The high protein content was due to the solubilization of protein during hydrolysis, the removal of insoluble undigested non-protein substances and the partial removal of lipid after hydrolysis (Benjakul and Morrissey, 1997). However, the protein content of CPH in this study was lower (40 – 43%), due to the addition of 5% maltodextrin as well as loss of protein during spray drying process. Abdul-Hamid *et al*. (2002) also reported that the spray-dried black Tilapia muscle hydrolysate with added 10% maltodextrin contained 37.7-49.6% protein content.

CPH samples contained 0.26 - 0.54% fat content, and the difference were significance between all samples. The fat content was found to decrease with increase in DH. This result was in contrary to the study of Pacific whiting muscles and sardinella byproducts hydrolysates, which reported no significant different in fat content between different DH (Souissi *et al*., 2007; Pacheco-Aguilar *et al*., 2008). The fat content of CPH is in similar range with that of Pacific whiting muscle hydrolysate (0.1-0.3 %). However, the fat content for sardinella byproduct was higher than CPH, which accounted for 8 – 11%. This difference in fat content may be attributed to the difference in raw materials and the processes involved in preparing the hydrolysate. The lower content of carbohydrate content at DH71 and at DH96 as compared to DH53 was due to the high content of ash content in both samples at higher degree of hydrolysis. Since carbohydrate content is calculated by difference, it is directly affected by other proximate composition of CPH.

Colour

Table 2 showed the result for the colour analysis of CPH at different DH. Hydrolysis of cobia frame produced protein powders that were white to light yellow in colour. L^{*} values of CPH at DH53 was significantly different than that of DH96. However, there was no significant different between L^{*} values of DH 53 and DH 71 samples. Meanwhile, the yellowness of the sample was significantly increased with an increase of DH. Similar trend was observed in the studies of shark muscle hydrolysate and grass carp skin hydrolysate (Diniz and Martin, 1997; Wasswa *et al*., 2007). DH96 sample gave the darkest and most yellowish colour whereas DH53 sample gave the lightest and the least yellowish colour. For

the L* values, both the shark muscle hydrolysate and grass carp skin hydrolysate were in the range of 86-91 and 59-69, respectively. Meanwhile, the b* values in the both studies were 7-11 and 18-27, respectively (Diniz and Martin, 1997; Wasswa *et al*., 2007). The L* values and b* values for shark muscle hydrolysate powder and grass carp skin hydrolysate powder were higher than those of CPH. Increased time of hydrolysis resulted in increased enzymatic browning reactions. Enzymatic browning reactions are assumed to have contributed to reduction in the luminosity, giving a darker appearance at high DH (Wasswa *et al*., 2007) in grass carp skin hydrolysate. The results show that the colour of CPH is positively influenced by DH.

Table 2. Colorimeter parameter values of hydrolysed cobia frame waste

Degree of hydrolysis	Colour parameters		
(%)	*L**	*a**	*b**
53	61.7 ± 1.2 ^{a}	-0.42 ± 0.1 ^{a}	4.57 ± 0.2 ^{c}
71	60.6 ± 0.8 ^{ab}	-0.59 ± 0.0 ^{b}	5.33 ± 0.1 ^{b}
96	59.7 ± 0.8 ^{b}	-0.48 ± 0.0 ^{a}	5.72 ± 0.1 ^{a}

L*: measure of lightness, a*: chronic scale from green (-a) to red (+a), b*: chronic scale from blue (-b) to yellow (+b). Values are mean ± SD of three replicates. Values with different letter are statistically different between samples ($p \leq 0.05$).

Water and oil holding capacity

There was no significant difference in WHC and OHC in all samples. The WHC of CPH was within the range of 0.8-1.1 ml/g. Diniz and Martin (1997) also reported that DH did not affected WHC in shark muscle hydrolysate (for DH range of 6.5-18.8%). However, grass carp skin hydrolysate (DH of 5.02-14.9%) and silver catfish (DH of 43-68%) showed increased WHC as DH increased (Wasswa *et al*., 2007; Amiza *et al*., 2010). WHC values for CPH were also lower compared to grass carp skin hydrolysate (2.0-4.9 ml/g) and shark muscle hydrolysate (4-15 ml/g) (Diniz and Martin, 1997; Wasswa *et al*., 2007).

The OHC of CPH was within the range of 2.4-2.8 ml/g. Diniz and Martin (1997) also reported that DH did not affected the OHC of shark muscle hydrolysate (for DH range of 6.5-18.8%). However, grass carp skin hydrolysate (DH of 5.02-14.9%) gave decreased OHC as DH increased (Wasswa *et al*., 2007). OHC of CPH was in the similar range with grass carp skin hydrolysate (2.4-3.6 ml/g) (Wasswa *et al*., 2007), but higher than those of shark muscle hydrolysate (0.3-0.5 ml/g) (Diniz and Martin, 1997) and whey protein hydrolysate (0.16-0.34 ml/g) (Sinha *et al*., 2007). Decrease in OHC could be due to an extensive hydrolysis that contributed to the hydrolytic degradation of protein structures (Wasswa *et al*., 2007) and decrease in hydrophobic interactions (Haque and Mozaffar, 1992; Liceaga-Gesualdo and Li-Chan, 1999).

Peptide solubility

Solubility is one of the most important physicochemical and functional properties of protein hydrolysates (Kinsella, 1976; Kristinsson and Rasco, 2000b). Good solubility of proteins is essential in many functional applications, especially for emulsions, foams and gels purposes. There was no significant difference in the solubility of all CPH samples (in the range of 85-86%). This shows that DH did not affect the solubility of CPH. Similar result has been reported for Pacific whiting muscle hydrolysate and Atlantic Salmon muscle hydrolysate (Kristinsson and Rasco, 2000c; Pacheco-Aguilar *et al*., 2008). However, several studies has reported higher solubility with higher DH including hydrolysate from silver catfish (unpublished data), salmon byproducts (Gbogouri *et al*., 2004) and yellow stripe trevally (Klompong *et al*., 2007). The high peptide solubility of protein hydrolysate indicates potential applications in food industry.

Emulsifying capacity

The emulsifying properties of CPH can be explained based on their surface properties, or how effectively the hydrolysate lowers the interfacial tension between the hydrophobic and hydrophilic components in food. The mechanism of the emulsification process is the absorption of proteins to the surface of freshly formed oil droplets during homogenization and form a protective membrane that prevents droplets from coalescing. 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).

As shown in Figure 1, the emulsifying capacity (EC) of DH53 sample was significantly higher than that of DH96. Similar trend was reported in sardinella byproduct hydrolysate and freeze-dried grass carp skin hydrolysates (Souissi *et al*., 2007; Wasswa *et al*., 2007). This trend is expected because higher DH will lead to the presence of smaller peptides, which are less effective in stabilizing emulsions. The diminishing in emulsifying activity with an extensively hydrolysis process is due to the reduction of hydrophobicity of the hydrolysate and the changes in peptide size during hydrolysis (Souissi *et al*., 2007). According to Diniz and Martin (1997), the low level of degradation of protein molecules by Alcalase® had contributed to the high EC because of the increase of larger peptide units at the oil-water

interface that provide a larger surface area. A peptide is required to have a minimum length of about 20 residues to possess good emulsifying and interfacial properties (Lee *et al*., 1987).

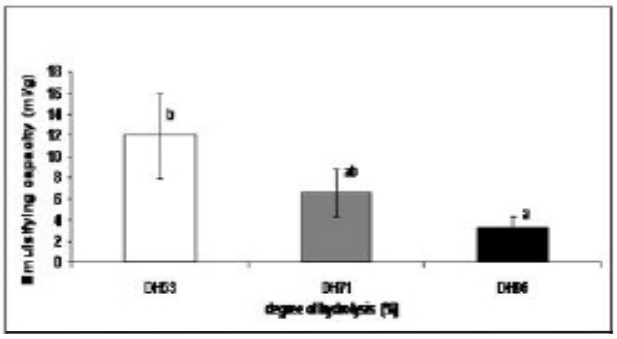

Figure 1. Emulsifying capacity of cobia protein hydrolysate at different degree of hydrolysis.

The EC values obtained for CPH (3-12 mg/g) was lower compared to sardinella byproduct hydrolysates (10-20 ml/g) and grass carp skin hydrolysates (20-38 ml/g) (Souissi *et al*., 2007; Wasswa *et al*., 2007). Peptide molecular characteristics and peptide chain length are mainly responsible for the different emulsification ability of hydrolysates, but there are still many other factors that may account for the differences observed between peptides in the ability to form an emulsion such as degree of hydrolysis (Spinelli *et al*., 1972), acetylation of peptide (Groninger and Miller, 1979), extraction solvent (Dubrow *et al*., 1973), pH, ionic strength, temperature and others (Turgeon *et al.*, 1992).

Foaming capacity and foaming stability

Figure 2 shows the foaming capacity (FC) of CPH samples. The FC of CPH at DH53 showed significant difference with those of DH71 and DH96. Shark muscle hydrolysate and yellow stripe trevally muscle hydrolysate exhibited similar trend of FC (Diniz and Martin, 1997; Klompong *et al*., 2007). In this study, CPH with the lowest DH gave highest high foaming capacity (122.7%). A good foaming capacity might attribute to an increase in the surface activity, which is due to partial proteolysis that produced greater number of polypeptide chain and therefore allowed more air to be incorporated (Kuehler and Stine, 1974). Meanwhile, DH96 had lower foaming capacity (117%). This may be due to the small size of peptides that produce with extensive hydrolysis would lower its surface activity and thus hinder the formation of a stable firm around the gas bubbles, and also by the apparition of hydrophilic peptides during extensive hydrolysis (Kuehler and Stine, 1974). This is in line with previous findings reporting that a good cohesiveness of films is only reached with high molecular mass peptides or partially hydrolysed proteins (Bombara *et al*., 1997).

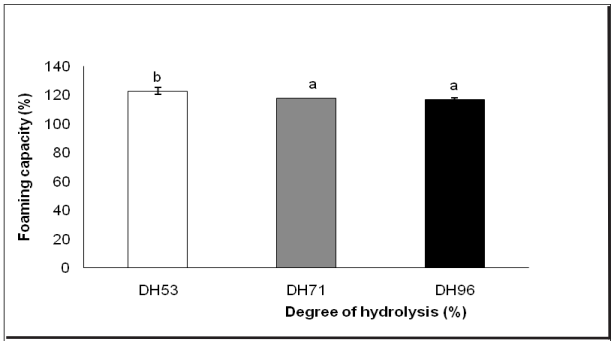

Figure 2. Foaming capacity of cobia hydrolysate samples at different degree of hydrolysis.

Further experiment on foam expansion after whipping was monitored for 60 min to indicate the foam stability of protein hydrolysates. Figure 3 showed the foaming stability of CPH at three DH. Foaming stability decreased significantly with time, with DH96 hydrolysate producing the most stable foam. The foaming capability after 60 min were 105.7%, 114% and 113.3% for DH53, DH71 and DH96, respectively. Similar trend was observed in the study of shark muscle hydrolysate (Diniz and Martin, 1997), herring muscle hydrolysate (Liceaga-Gesualdo and Li-Chan, 1999), and round scad muscle hysrolysate (Thiansilakul *et al*., 2007). According to Shahidi *et al*. (1995), the reduction of foaming stability was due to microscopic peptides did not have strength to hold a stable foam. Foam stability depends on the film's nature and reflects the extent of protein-protein interaction within the matrix (Mutilangi *et al*., 1996). Foam stability can be enhanced by flexible protein domains that increased the viscosity of the aqueous phase, protein concentration and film thickness (Phillips *et al*., 1994).

Shahidi *et al*. (1995) reported capelin protein hydrolysate possessed good foaming properties of 90% at lower DH but the foaming stability is very poor (0% after 60 min). Yellow stripe trevally muscle (Klompong *et al*., 2007) gave the foaming properties up to 200%, but exhibited very poor foaming stability. Shark muscle protein (Diniz and Martin, 1997) gave 50-140% foaming capacity and stability of 45-70% after 60 min. Round scad muscle gave moderate foaming properties (20-70%) with poor stability (94% loss after 10 min) (Thiansilakul *et al*., 2007). Comparing these data with that of cobia hydrolysate indicating that CPH exhibited excellent foaming stability compared to other fish hydrolysate.

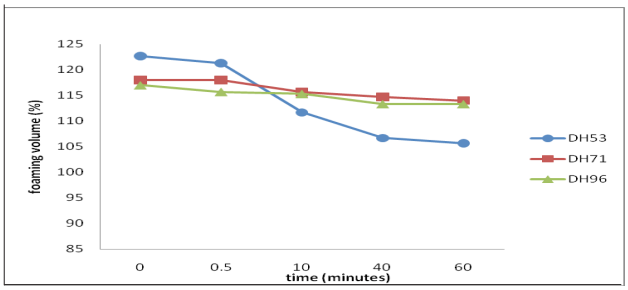

Figure 3. Foaming stability of cobia hydrolysate samples at various degree of hydrolysis with time.

Klompong *et al*. (2007) and Pacheco-Aguilar (2008) suggested that the pH of hydrolysate greatly affect the foaming stability, where pH 4 showed the lowest foaming stability. This is due to the solubility, size and interaction of peptides at the formed film that primarily contributed to the foam stability. However, effect of pH on foaming stability was not studied in this study.

Amino acid composition

Table 3 showed the amino acid composition of CPH. The hydrolysates were rich in glutamic acid, proline, glycine and alanine, where their composition was in a range of 3- 4%. Among CPH samples, DH96 contained the highest total amino acid, which accounted for 33.77% compared with other two samples, which were 28.91 % (DH53) and 28.16 % (DH71), respectively. It was also noted that the hydrophobic amino acids, leucine and isoleucine was higher in DH96 compared to other samples. The increase in hydrophobic amino acids is important due to the effects that these have on the functional properties of food proteins. Besides that, an increase amount of hydrophobic amino acids would also increase the solubility in lipid and therefore enhance the antioxidative activity (Rajapakse *et al*., 2005; Zhu *et al*., 2006).

Table 3 shows that the essential amino acids content for DH96 exceeded the recommended values for a human adult (FAO/WHO 1990) except for methionine and isoleucine. According to Chen *et al.* (1996), amino acids such as tyrosine, histidine, lysine, tryptophan and methionine were generally accepted as antioxidants. In this study, small amount of tyrosine, histidine, and methionine were found in all the samples except for lysine. Based on the research of whey protein isolate fractions (Pihlanto, 2006), the delay of lipid oxidation was found to be related with the presence of histidine and hydrophobic amino acids. Thus, the result showed that the CPH may be a good source of antioxidant to be incorporated into other products as supplement due to the presence of hydrophobic amino acids.

Table 3. Amino acid composition of cobia hydrolysate at different degree of hydrolysis

Amino acid	Composition (%) DH53	DH71	DH96	Reference for human EAA[a]	Chemical score for DH96
Threonine*	**1.11**	**1.12**	**1.33**	**0.9**	**1.48**
Valine*	**1.15**	**1.15**	**1.39**	**1.3**	**1**
Methionine*	**0.31**	**0.23**	**0.41**	**1.7**	**0.24**
Isoleucine*	**0.85**	**0.89**	**1.08**	**1.3**	**0.83**
Leucine*	**1.79**	**1.85**	**2.16**	**1.9**	**1.13**
Tyrosine*	**0.55**	**0.59**	**0.74**	**-**	**-**
Phenylalanine*	**0.87**	**0.87**	**1.06**	**-**	**-**
Lysine*	**2.02**	**1.89**	**2.19**	**1.6**	**1.37**
Histidine*	**0.44**	**0.44**	**0.56**	**1.6**	**0.35**
Arginine	**1.82**	**1.79**	**2.23**	**-**	-
Tryptophan	**-**	**-**	**-**	**-**	-
Aspartic acid	2.37	2.4	2.77		
Serine	1.04	1.06	1.29		
Glutamic acid	4.36	4.3	4.88		
Glycine	3.56	3.16	4.1		
Alanine	2.87	2.69	3.31		
Cysteine	0	0	0		
Proline	3.79	3.71	4.28		
Total	28.91	28.16	33.77		
Total hydrophobic amino acid	11.63	11.39	13.69		

[a]Suggested profile of essential amino acid requirements for adult humans, FAO/WHO (1990).

Several studies has reported that the essential amino acid composition of the fish protein hydrolysate were higher than the recommended value for a human adult.including those of herring (Liceaga-Gesualdo and Li-Chan, 1999), grass carp skin (Wasswa *et al*., 2007), and round scad muscle hydrolysate (Thiansilakul *et al*., 2007).

Conclusion

Proximate composition showed significant difference in ash and fat content, but not in protein content. Cobia hydrolysate at 96% DH fulfilled the normal requirements of all the essential amino acids for an adult human according to FAO/WHO (1990) except for methionine and isoleucine. The colour of CPH was positively influenced by DH. Emulsifying capacity (EC) decreased with increase in DH. Foaming capacity was highest for DH53 sample, but foaming stability is highest for DH96. This study showed that the extent of hydrolysis had greatly influenced the amino acid content, emulsifying capacity, foaming capacity and foaming stability of cobia frame hydrolysate. However, water holding

capacity, oil holding capacity and solubility were not affected by the extent of hydrolysis. The light colour profile of cobia frame hydrolysate, high solubility and excellent foaming properties makes it a good alternative to be used as food ingredients as well as emulsifiers in food industry.

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