

## Physicochemical properties of silver catfish (*Pangasius* sp.) frame hydrolysate

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### Article history

Received: 7 August 2012

Received in revised form:

8 January 2013

Accepted: 12 January 2013

### Abstract

The physicochemical properties of silver catfish frame hydrolysate powder at three different degree of hydrolysis, DH43%, DH 55% and DH 68% were studied. The hydrolysates powder were obtained by hydrolysis using Alcalase®, centrifugation and spray drying of the supernatant. The study found that preparation of these hydrolysates affected the protein, ash and fat content as well as amino acid composition. As for essential amino acids, their values were generally considered as adequate as compared to the suggested essential amino acids profile of FAO/WHO. The results showed that SFHs were rich in lysine and glutamate. Hydrolysate at DH 68% exhibited better peptide solubility and water holding capacity. As degree of hydrolysis increased, emulsifying capacity and foaming capacity of the hydrolysate decreased. It was also found that the lightness in hydrolysate powder decreased with increase in degree of hydrolysis. This study shows that silver catfish frame hydrolysate has good solubility, good foaming properties and light colour profile, thus having high potential as food ingredient.

### Keywords

Silver catfish  
hydrolysate  
frame, foaming  
amino acid composition

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### Introduction

Among the by-products from fish processing plants are fish frames which include bone, heads and tails. Bones constitute a significant part of the fish; approximately 10-15% of total fish biomass is bones from the head and vertebrae and contain considerable amount of proteins and minerals.

High protein content in fishery waste make them more perishable, may bring undesirable effects to environment pollution, as well as high cost in managing waste disposal. Production of fish protein hydrolysate via enzymatic hydrolysis is one way to add value to proteinaceous fish waste (Aspmo *et al.*, 2005). Enzymatic modification of proteins using selected proteolytic enzyme preparations to cleave specific peptide bonds is widely used in the food industry. Degree of hydrolysis (DH) is used to measure the extent of protein hydrolysis. DH is defined as percentage of peptide bonds cleaved compared to the total available peptide bonds in a protein substrate.

Protein hydrolysate can be used to improve or modify the physicochemical, functional properties such as solubility, fat absorption, water holding capacity, foaming properties, emulsifying properties and or sensory properties of proteins without losing its nutritional value (Kristinsson and Rasco, 2000b).

Many processed fish by-products possess good functional properties and can be used in food products as binders, emulsifiers and gelling agents.

By careful control of the hydrolysate process, it is possible to produce hydrolysate with different degrees of hydrolysis and different functional properties. The choice of substrate, protease enzyme employed and degree of hydrolysis can greatly affect the physicochemical properties of hydrolysate. Commercial enzyme, Alcalase has been strongly recommended for fish hydrolysis (Shahidi *et al.*, 1995).

Many studies have been reported on the effects of degree of hydrolysis on physicochemical properties of hydrolysate of 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.

Silver catfish (*Pangasius* sp.) is the second most popular freshwater fish in Malaysia (Malaysian Department of Fisheries, 2010). The edible portion of silver catfish is only 50%, implicating that another

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50% is the waste. Amiza *et al.* (2011) has reported on the optimization of enzymatic hydrolysis of silver catfish frame using response surface methodology. The suggested hydrolysis conditions to obtain optimum degree of hydrolysis for silver catfish frame using Alcalase® were – temperature of 58°C, hydrolysis time of 134 min, pH of substrate at 9.4 and an enzyme concentration of 8.3%. However, until now, no information is available on the effects of degree of hydrolysis on physicochemical properties of silver catfish frame hydrolysate. It is hoped that this study will shed some light on the effect of hydrolysis on the properties of silver catfish frame hydrolysate as food ingredient.

## Materials and Methods

### Raw materials

Whole fresh silver catfish (*Pangasius sp.*) were obtained from local supplier. The enzyme used for the hydrolysis was Alcalase® (2.4 AU/kg and a density of 1.18 g/ml), a bacterial endoproteinase from a strain of *Bacillus licheniformis* (Novo Nordisk, Denmark). All other chemicals used were of analytical grade.

### Preparation of silver catfish frame

Whole silver catfish were degutted, filleted and beheaded to obtain its frame (without head). The frame was washed, drained and chopped into small pieces. The frame was mixed with water in a ratio of 1 kg silver catfish frame to 400 ml water and homogenized using a Waring blender for 60 seconds. The homogenized silver catfish frame was frozen at -40°C until further use.

### Preparation of silver catfish frame hydrolysate powder (SFH)

Before the protein hydrolysis was carried out, the protein content of silver catfish frame was determined using Kjeldahl method (AOAC, 2000). Prior to enzymatic hydrolysis, some calculations have to be carried out to determine the mass of raw material, water and enzyme solution to be used in the experiment. The calculation is necessary because the mass of raw materials depend on the protein content. All calculations were carried out according to Bhaskar *et al.* (2007). The preparation of the hydrolysate was performed according to the procedure of Bhaskar *et al.* (2007) with slight modification. For each batch, about 82.5 g of silver catfish frame was added with 60.5 g of distilled water (including the volume of 1N NaOH added to adjust the pH) and heated at 85°C for 20 min prior to hydrolysis. After cooling, 20 g of diluted Alcalase® enzyme (prepared by diluting the required enzyme mass to a final weight of 20 g with

distilled water) was added into the slurry and mixed using a magnetic stirrer. Hydrolysis condition was set according to previous study (Amiza *et al.*, 2011). The hydrolysis conditions for DH43 hydrolysate were temperature of 50°C for 90 min, 1% Alcalase® to protein ratio and pH of 7.5. The hydrolysis conditions for DH55 hydrolysate were temperature of 40°C for 120 min, 1.5% Alcalase® to protein ratio and pH of 8.5. The hydrolysis conditions for DH68 hydrolysate were temperature of 60°C for 180 min, 2% Alcalase® to protein ratio and pH of 9.5. Hydrolysis was carried out in a water-bath shaker. Throughout the enzymatic hydrolysis reactions, the pH was maintained manually by addition of 1N NaOH. After hydrolysis was completed, the process was terminated by heating the liquid samples to temperature at 85°C for 20 min to inactivate the Alcalase® activity. Then the hydrolysate was centrifuged for 20 min at the speed of 6000 g in order to remove the insoluble particles and oil layer. The supernatant was then frozen prior to spray drying.

For each DH, the 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 SFH powder at inlet and outlet temperature of 185°C and 108°C, respectively. Maltodextrin (5% w/v) was added to the liquid protein hydrolysate to avoid caking of the resultant silver catfish hydrolysate powder. The resulting silver catfish hydrolysate powder was stored in a sealed plastic bag at room temperature until further use. The three samples of silver catfish hydrolysate powder were then analysed for their degree of hydrolysis as well as their physicochemical properties.

### 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, 1995). Kjeldahl method was used to determine nitrogen content. The formula used is as follows:

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

### Determination of physicochemical properties of hydrolysates

Moisture, crude protein, crude fat and ash content of raw material and hydrolysate powder were determined using AOAC methods (AOAC, 2000). Determination of amino acid composition was performed using Dedicated Amino Acid Analyzer L-8800 Hitachi according to the methods by Guo *et al.* (2005). Solubility of silver catfish protein hydrolysate

was determined by using nitrogen solubility index (NSI) according to the method of Morr *et al.* (1985). 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 was determined by using oil titration method (Diniz and Martin, 1997). Foaming capacity was determined according to the method of Shahidi *et al.* (1995). The colour of the hydrolysates were determined using a colorimeter (Minolta Chromameter CR 300).

#### Data analysis

All experiments were carried out in triplicates. All data were stated as mean  $\pm$  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 samples at  $p < 0.05$  level using the SPSS software (SPSS Version 16.0).

## Results and Discussion

#### Fat, protein and ash content

The moisture, crude fat, crude protein and ash content of silver catfish frame hydrolysate (SFH) are shown in Table 1. Moisture content of SFH did not show remarkable variation between three different levels of DH. However, the ash content in the hydrolysates increased with increasing degree of hydrolysis and the values were statistically different for all samples. In this study, coincidentally hydrolysate at higher DH was prepared using higher pH, and this pH was maintained throughout the hydrolysis process by adding NaOH. The proportional increase in ash content with degree of hydrolysis, was probably caused by the increased addition of NaOH to maintain a higher pH at higher degree of hydrolysis (Liceaga and Li-Chan, 1999; Kristinsson and Rasco, 2000c; Severin and Xia, 2005). Choi *et al.* (2009) have reported similar trend of ash contents which was between 3.7 - 4.7% for small croacker protein hydrolysates. However, higher ash content were reported in Pacific whiting muscle hydrolysate (11.7 - 11.9%) and sardinella byproduct hydrolysate (12.1 - 14.8%) (Souissi *et al.*, 2007; Pacheco-Aguilar *et al.*, 2008).

The fat content was less than 0.7% in all hydrolysate samples. The fat content of SFH at DH 43% sample is not significantly different from DH 55% but is significantly lower than that of DH 68%. This may due to the fact that, as the hydrolysis proceed, the muscle cell membranes tended to round

**Table 1.** Moisture, crude fat, crude protein and ash content of silver catfish frame hydrolysate at different degree of hydrolysis

Composition	DH 43	DH 55	DH 68
Moisture	3.99 $\pm$ 0.05 <sup>a</sup>	5.93 $\pm$ 1.6 <sup>a</sup>	5.61 $\pm$ 0.14 <sup>a</sup>
Ash	3.35 $\pm$ 0.28 <sup>c</sup>	3.96 $\pm$ 0.09 <sup>b</sup>	4.45 $\pm$ 0.25 <sup>a</sup>
Crude Fat	0.58 $\pm$ 0.01 <sup>b</sup>	0.63 $\pm$ 0.02 <sup>ab</sup>	0.68 $\pm$ 0.04 <sup>a</sup>
Crude Protein	32.9 $\pm$ 0.99 <sup>b</sup>	35.17 $\pm$ 0.29 <sup>a</sup>	35.6 $\pm$ 0.28 <sup>a</sup>

<sup>a</sup>Different letters (a, b, and c) indicate significant different ( $p \leq 0.05$ ).

up and form insoluble vesicles, leading to the removal of membrane structured lipid (Shahidi *et al.*, 1995). The insoluble fraction (vesicles) was separated from the soluble hydrolysate in the form of a pellet by centrifugation and removed prior to spray-drying. Thus higher DH will lead to higher fat removal. A similar result of low lipid content ranged from 0.06 - 0.8% was reported in the FPH from herring (Liceaga and Li-Chan, 1999), herring body (Sathivel *et al.*, 2003), salmon head (Gbogouri *et al.*, 2004), round scad (Thiansilakul *et al.*, 2007) and Pacific whiting muscle (Pacheco-Aguilar *et al.*, 2008).

DH 55% and DH 68% samples gave similar protein content, whereas DH 43% sample showed a significantly lower value (32.9%). This study shows that at higher DH (55% DH and 65% DH), the protein content increased. Although each batches of enzymatic hydrolysis used the same amount of protein (silver catfish frame), the difference occurs because of the centrifugation step used, whereby only the supernatant was spray dried, not the whole hydrolysis slurry. The high protein content at higher DH was due to the fact that more protein was solubilised and thus contained in the supernatant layer compared to lower DH. At low DH, some of the protein is still attached to the fish bone, resulting in less protein to be recovered in the supernatant after centrifugation. This leads to lower protein content in the resulting spray-dried hydrolysate at low DH. The protein content of silver catfish hydrolysate in this study is in similar range with that of spray dried black Tilapia hydrolysates with 10% maltodextrin (37.7%) (Abdul-Hamid *et al.*, 2002). However, the protein content in this study is lower compared to other investigators whom reported protein content ranging from 70% to 88% for lyophilized hydrolysate samples of herring protein (Liceaga and Li-Chan, 1999), salmon frames (Kristinsson and Rasco, 2000c), herring byproduct (Sathivel *et al.*, 2003), round scad (Thiansilakul *et al.*, 2007) and Pacific whiting muscle (Pacheco-Aguilar, 2008) The low protein content in the silver catfish hydrolysate may be due to the addition of 5% maltodextrin (carrier agent) during spray drying to reduce the stickiness and wall deposition in spray-drying (Truong *et al.*, 2005).

### Amino acid composition

Amino acid composition of SFH at different DH are shown in Table 2. Total amino acids increased with increasing protein hydrolysis at DH 43% ( $25.01 \pm 1.6\%$ ) followed by DH 55% ( $25.65 \pm 0.8\%$ ) and then at DH 68% ( $39.60 \pm 1.6\%$ ). It was found that the hydrophobic amino acids, leucine and isoleucine had 2 to 3-fold increase as DH increased, whereas typtophan had 10-fold increase in hydrolysate samples. The increase in hydrophobic amino acids is important due to the effects that these have on the functional properties of food proteins. An increase in hydrophobic amino acids content also reported to enhance the antioxidative activity of protein hydrolysate (Dong *et al.*, 2008).

**Table 2.** Amino acid compositions of silver catfish frame hydrolysates

Amino acid	DH 43 (%)	DH 55 (%)	DH 68 (%)	EAA <sup>a</sup>
Histidine	$0.96 \pm 0.0$	$0.44 \pm 0.0$	$0.67 \pm 0.0$	1.6
Threonine	$0.88 \pm 0.0$	$1.2 \pm 0.0$	$1.7 \pm 0.0$	0.9
Valine	$0.9 \pm 0.0$	$0.87 \pm 0.0$	$1.4 \pm 0.1$	1.3
Methionine	$1.3 \pm 0.0$	$1.25 \pm 0.1$	$2.1 \pm 0.1$	1.7
Tryptophan	$0.2 \pm 0.0$	$0.9 \pm 0.0$	$2.5 \pm 0.3$	-
Phenylalanine	$0.87 \pm 0.0$	$0.81 \pm 0.1$	$1.2 \pm 0.0$	-
Isoleucine	$0.6 \pm 0.0$	$0.58 \pm 0.0$	$0.93 \pm 0.0$	1.3
Leusine	$1.35 \pm 0.0$	$1.4 \pm 0.0$	$2.4 \pm 0.0$	1.9
Lysine	$1.5 \pm 0.3$	$4.8 \pm 0.0$	$4.95 \pm 0.2$	1.6
Tyrosine	$1.2 \pm 0.1$	$1.15 \pm 0.2$	$1.95 \pm 0.1$	-
Arginine	$1.35 \pm 0.0$	$1.4 \pm 0.0$	$2.3 \pm 0.1$	-
Aspartate	$2.35 \pm 0.1$	$1.85 \pm 0.1$	$3.1 \pm 0.1$	
Glutamate	$5.1 \pm 0.3$	$3.5 \pm 0.1$	$5.8 \pm 0.2$	
Serine	$1.9 \pm 0.4$	$1.4 \pm 0.0$	$2.15 \pm 0.2$	
Glycine	$1.95 \pm 0.1$	$1.75 \pm 0.1$	$2.65 \pm 0.1$	
Alanine	$2.6 \pm 0.3$	$2.35 \pm 0.1$	$3.8 \pm 0.1$	
Total amino acids	$25.01 \pm 1.6$	$25.65 \pm 0.8$	$39.60 \pm 1.6$	

<sup>a</sup>Suggested profile of essential amino acid requirements for adults (FAO/WHO, 1990).

However, amino acid composition of SFH at three different DH was low compared to freeze dried hydrolysates for grass carp skin (Wasswa *et al.*, 2007) and silver carp muscle (Dong *et al.*, 2008). This could be due to the exposure to high temperature (inlet and outlet temperature is 185°C and outlet 108°C respectively) during spray drying of silver catfish hydrolysate which could significantly decrease the total amino acid content. Abdul-Hamid *et al.* (2002) reported that high temperature significantly decreased the content of all amino acids tested for black Tilapia muscle hydrolysate.

SFH at DH 68% was found to be abundant in lysine (4.95%) and tryptophan (2.5%) but low in

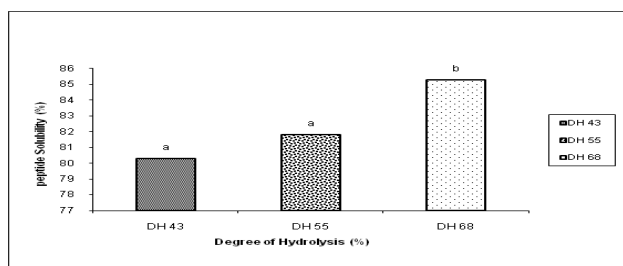
histidine (0.67%). Dong *et al.* (2008) and Thiansilakul *et al.* (2007) reported that several amino acids such as tyrosine, methionine, histidine, lysine, and tryptophan were generally accepted as antioxidants. Nevertheless, tryptophan and histidine showed high antioxidative activity in comparison with methionine, lysine and alanine. Histidine or histidine-containing peptide may attribute to the chelating and fat radical-trapping ability of the imidazole residues while tyrosine residues may act as potent hydrogen donor.

In the case of essential amino acids, the results show that the amino acid profiles of the SFHs at DH 68% were generally higher in essential amino acid profiles compared to the suggested pattern of requirement by FAO/WHO for adult humans except for histidine and isoleucine (FAO/WHO, 1990). The amino acids composition of the SFH fulfilled this requirement and by this standard, it can be suggested as a good source of essential amino acids for nutritional value (Vidotti *et al.*, 2003). From the result, it can be suggested that SFH may have antioxidative activity and it does not lose its nutritional value with increase in DH.

### Peptide solubility

Peptide solubility is one of the most important physicochemical and functional properties of hydrolysates such as foaming and emulsifying properties because rapid migration and adsorption of the peptides at the interface are critical (Kinsella, 1976). In this study, the relationship between solubility and DH values was observed. Solubility was measured as nitrogen solubility index (NSI). As shown in Fig. 1, hydrolysates with high DH values gave higher solubility than those of low DH values. This is in agreement with findings of Shahidi *et al.* (1995), Diniz and Martin (1997), Gbogouri *et al.* (2004), and Klompong *et al.* (2007) whom reported that fish hydrolysates at higher DH values exhibited better solubility. According to Gbogouri *et al.* (2004), the smaller peptides from myofibrillar proteins are expected to have proportionally more polar residues, with the ability to form hydrogen bonds with water and enhance solubility.

In this study, protein solubility was studied at pH 7 only. This is slightly different with other studies which examined solubility over a wide range pH. At pH 7.0, the peptide solubility of all SFHs samples were in the range of 80 - 85% and increased in DH value resulted in significant NSI values between hydrolysate sample DH 55 ( $81.8 \pm 0.8\%$ ) and that of DH 68 ( $85.3 \pm 0.3\%$ ) ( $P < 0.05$ ). No significant difference was observed between hydrolysate at DH 43 ( $80.3 \pm 1.0\%$ ) and DH 55.



**Figure 1.** Peptide solubility of silver catfish frame hydrolysate at different degree of hydrolysis

The high solubility of SFHs was probably due to the reduction of secondary structure and generation of low molecular weight peptides by hydrolysis, which are expected to have more polar residues than intact proteins, with the ability to form more hydrogen bonds with water and increase solubility (Gbogouri *et al.*, 2004). In addition, insoluble protein fractions present in liquid hydrolysate form were removed by centrifugation after hydrolysis and therefore are not present in the spray-dried hydrolysate powder.

The solubility of SFH at pH 7 was in agreement with hydrolysates from capelin muscle (Shahidi *et al.*, 1995), shark muscle (Diniz and Martin, 1997), salmon by-products (Gbogouri *et al.*, 2004), yellow stripe trevally muscle (Klompong *et al.*, 2007) and silver carp muscle hydrolysate (Dong *et al.*, 2008). However, several studies of fish protein hydrolysates have been reported to have high solubility over 95-99% at pH 7 (Thiansilakul *et al.*, 2007). The pH of protein solution should be taken into account as this factor affects the charge on the weakly acidic and basis side-chain groups and hydrolysates generally show low solubility at their isoelectric points and the highest when maximally charged (Linder *et al.*, 1996).

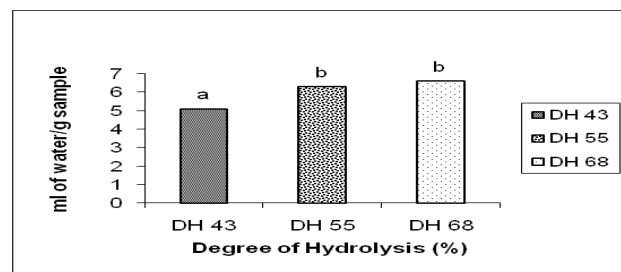
Due to the high solubility of Silver catfish hydrolysate at DH 68 ( $85.4 \pm 0.3\%$ ), it was suggested that hydrolysate DH 68 possess potential application in many functional ingredients, especially emulsions, foams and gels.

#### Water-holding capacity

Several studies have shown that fish protein hydrolysates have excellent water holding capacity and increase the cooking yield when added to mince meat (Shahidi *et al.*, 1995; Kristinsson and Rasco, 2000).

The relationship of WHC and DH of SFHs was determined and the result shown in Figure 2. In this study, the WHC of SFHs increased with increasing DH. This may be attributed to the high solubility and thus, dissociation of proteins into smaller subunits, which increase the functional groups especially hydrophilic groups with more water binding sites

as a result of enzymatic hydrolysis. The WHC of hydrolysate at DH 43% was significantly lower compared to those of DH 55% and DH 68%. However, there was no significant difference in the WHC between hydrolysate at DH 55% and DH 68%.



**Figure 2.** Water-holding capacity of silver catfish frame hydrolysate at different degree of hydrolysis

At higher hydrolysis, increased polar groups such as COOH and NH<sub>2</sub> had significant effects on water absorption and tend to exert better water-holding capacity (Kristinsson and Rasco, 2000b). Low-molecular weight peptides from high extent of enzymatic hydrolysis also appeared to be more effective in water-holding capacity than larger size peptides as smaller fragments of peptides are possibly more hydrophilic (Cumby *et al.*, 2007).

A similar trend in WHC was observed for grass carp skin hydrolysate (Wasswa *et al.*, 2007). However, the WHC result of SFH also contradicted with other fish protein hydrolysates study. Diniz and Martin (1997) observed that WHC was negatively influenced by extensive hydrolysis for shark muscle hydrolysates. The decreased in WHC might have been due to the hydrolytic degradation of the protein structure where physical entrapment plays an important role in the adsorption of water and oil. This result is in agreement with Orban *et al.* (1992) whom observed that high solubility led to decreasing WHC of fish protein.

This study shows that the WHC for SFHs ranged from 5.1 ml/g sample to 6.6 ml/g sample. This value is lower than that of shark muscle hydrolysates which ranged from 8.30 ml/g to 14.61 ml/g (Diniz and Martin, 1997). This may be due to the raw material used in preparing hydrolysate, i.e. muscle, skin, visceral or bone. Slizyte *et al.* (2005) reported that fish protein hydrolysate powders made from fish viscera without backbones showed 0.5 - 9% increase in WHC, while fish protein hydrolysate powder made from viscera plus backbones exhibited low WHC.

With respect to water-holding capacity, hydrolysate at DH 68% with good solubility exhibited good WHC compared to another two samples with lower DH values. This enables SFH at DH 68% to act

as water-binding agents and may improve cooking-yield by reducing drip loss especially in comminuted meat and baked dough.

#### Oil-holding capacity

It was found that OHC for SFH was 3.5 ml/g protein, 3.2 ml/g protein and 2.9 ml/g protein at DH 43%, DH 55% and DH 68% respectively. However, no significant difference was found between hydrolysate samples.

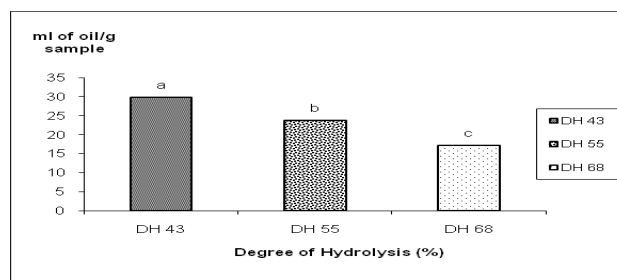
This is contradictory to several studies who reported OHC of fish protein hydrolysate decreased as DH increased, including shark muscle hydrolysates (Diniz and Martin, 1997), red salmon head hydrolysates (Sathivel *et al.*, 2005) and grass carp skin hydrolysates (Wasswa *et al.*, 2007). However, they had studied the OHC at DH lower than this study, i.e. DH 5 - 18%. The mechanism of oil-holding capacity is mainly due to physical entrapment of oil.

The OHC of SFH ranged from 2.9 to 3.5 ml/g protein was lower than that of shark muscle hydrolysate (4.8 to 6.8 ml/g protein) (Diniz and Martin, 1997), herring head (6.1 ml/g protein) and whole herring hydrolysate (7.3 ml/g protein) (Sathivel *et al.*, 2003) and red salmon head hydrolysate (3.9 to 5.0 ml/g protein) (Sathivel *et al.*, 2005). This may be due to the difference in the raw material used in preparation of hydrolysate. Slizyte *et al.* (2005) reported that cod protein hydrolysate powder made from visceral plus backbone had the lowest fat absorption (2.2 ml/g protein) while hydrolysate sample made from cod viscera without digestive tract had the highest fat absorption ability (5.0 ml/g protein).

#### Emulsifying capacity

The emulsifying capacities of SFHs decreased with increasing DH as shown in Figure 3. In this study, the hydrolysates at DH 43% had significantly higher emulsifying value (29.8 ml/g protein) followed by that of DH 55% (23.8 ml/g protein) and then that of DH 68% (17.2 ml/g protein). This could be due to the low level of degradation of protein molecules by Alcalase®. The increase in the availability of large peptide units at the oil-water interface, resulting in larger surface area and greater emulsion formulation (Puski, 1975). With the increase of enzymatic hydrolysis, the extensive degradation of protein resulted in the decrease of emulsifying capacity.

A similar trend between emulsifying capacity and DH has been reported for fish hydrolysates (without maltodextrin) for shark muscle (60 - 47.8 ml/g protein) (Diniz and Martin, 1997) and grass carp skin (76.0 - 41.6 ml/g protein) (Wasswa *et al.*, 2007) at DH 6.5% to DH 18.8%. A decrease in emulsifying



**Figure 3.** Emulsifying capacity of silver catfish frame hydrolysate at different degree of hydrolysis

capacity of casein with increased DH, in the range of 25-65%, using porcine pancreatin also has been reported (Mahmoud *et al.*, 1992).

However, the emulsifying capacity of spray dried SFH powder was lower than those of freeze dried grass carp skin and shark muscle hydrolysate (Diniz and Martin, 1997; Wasswa *et al.*, 2007) without maltodextrin.

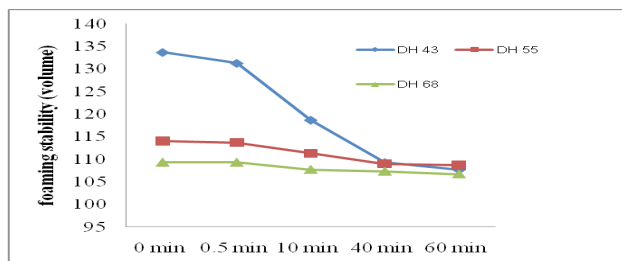
#### Foaming capacity and Stability

Figure 4 showed a significant decrease in the foaming capacity for the DH 43% sample (133.7% volume) followed by DH 55% sample (114% volume) and then DH 68% sample (109.3% volume). The high foaming capacity observed at DH 43% (133.7% volume) is similar with shark muscle hydrolysate at DH 6.5% with 136.3% volume (Diniz and Martin, 1997). The high foaming capacity suggested an increase in surface activity, due to the initially greater number of polypeptide chains that arose from partial proteolysis, which allowed more air to be incorporated. An improvement in foaming capacity for enzymatically modified food protein has been reported (Adler-Nissen, 1996).

The decrease in foaming capacity of more extensively hydrolyzed samples, i.e. hydrolysate at DH 55% and DH 68% may be attributed to an opposite effect of surface activity, probably due to the lower surfactant activity of smaller polypeptide chains from extensive hydrolysis (Kong *et al.*, 2007). This is in agreement with Diniz and Martin (1997) (163 - 23.1% volume) and Klompong *et al.* (2007) (130 - 60% volume) whom observed that foaming capacity decreased at higher DH.

For foam stability, it was expressed as the percent of foam remaining at 60 min period. The foaming stability of three SFHs at different DH was in a similar pattern at 60 min quiescent period. DH 43% sample sustaining 107.7% of the initial foam, followed by the DH 55% sample sustaining 108.7% and then DH 68% sustaining 106.7% of the initial foam. However, all changes were not statistically different. All SFHs had higher foam stability than those reported for shark muscle hydrolysate at DH

6.5% (67.3% volume) (Diniz and Martin, 1997) and wheat protein hydrolysate at DH 5% (40.0% volume) (Kong *et al.*, 2007). This phenomenon could also be due to the addition of 5% maltodextrin during spray drying which could act as foam stabilizer (Fennema, 1996).



**Figure 4.** Foaming capacity and stability of silver catfish frame hydrolysate at different DH

This result is contradictory to several studies that observed foam stability decreased as the extent of hydrolysis increased (Diniz and Martin, 1997; Drago and Gonzalez, 2001; Klompong *et al.*, 2007; Kong *et al.*, 2007). Generally, hydrolysates with increased DH are capable of foaming but lack strength to maintain the foam as result of the reduction in peptide size (Shahidi *et al.*, 1995).

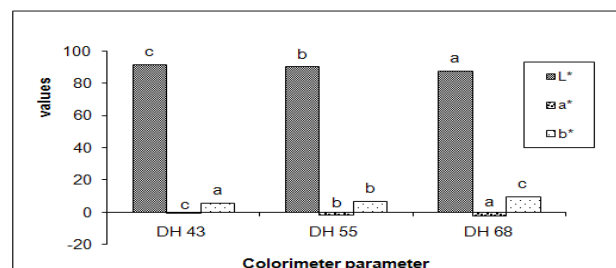
In general, SFHs with limited hydrolysis exhibited good foaming capacity than extensive hydrolysis samples and this result is similar with other studies reported in literature. However, foaming stability of SFH was high at all DH samples which sustained more than 100% of initial volume and enable it to form stable foam compared to other fish protein hydrolysates.

### Colour

Figure 5 shows that DH 68% sample was the darkest and most yellowish, whereas DH 43% sample was the lightest and the least yellowish. According to Wasswa *et al.* (2007), increased time of hydrolysis or extensive hydrolysis resulted in increased enzymatic browning reactions which are assumed to reduce the luminosity, and giving a darker appearance at high degree of hydrolysis. Spray-dried may also contribute to the increased  $L^*$  values obtained in the hydrolysis products (Diniz and Martin, 1997).

As shown in Figure 5,  $L^*$  and  $a^*$  values of SFHs were significantly decreased as DH increased, and  $b^*$  values were markedly ( $P < 0.05$ ) increased as DH increased. A similar trend in  $L^*$  and  $b^*$  values was reported on shark muscle hydrolysates (Diniz and Martin, 1997), grass carp skin hydrolysates (Wasswa *et al.*, 2007) and silver carp muscle hydrolysates (Dong *et al.*, 2008). The result also suggested that SFH powder is suitable to be used in food products

since it obtained high  $L^*$  values and minimal browning colour development during processing.



**Figure 5.** Colorimeter parameters of silver catfish frame hydrolysate at different degree of hydrolysis

### Conclusion

This study showed that the extent of hydrolysis had greatly influenced the physicochemical properties of silver catfish frame hydrolysis. The light colour profile, high solubility and good foaming properties make silver catfish hydrolysate as a good alternative to be used as food ingredients in food industry either for nutritional or functional basis.

### Acknowledgement

The authors gratefully acknowledged the financial support provided by Malaysian Department of Fisheries to carry out this study.

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