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Characterization of Fish protein Hydrolysate from Tilapia by-products using acid and enzymatic hydrolysis

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Key words: Fish protein hydrolysate, Acid hydrolysis, Solubility, Enzymatic hydrolysis, Emulsifying properties

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Abstract

Waste management has been a significant problem in the fish processing industry due to environmental and public health impacts. Food products can be developed from the by-products of the aquaculture industry. This study extracted and characterized fish protein hydrolysate (FPH) from tilapia by-products (viscera). It was produced by enzymatic and acid hydrolysis. The degree of hydrolysis (DH), protein pattern, solubility, emulsifying, and foaming properties of the FPH were determined. The yield of the fish protein hydrolysate increased with increasing concentration for acid hydrolysis. Decreasing total protein was observed with the use of increasing HCl concentration. The DH ranged from 12.79-13.95%. The molecular weight distribution of fish protein hydrolysate using acid and enzymatic hydrolysis was analyzed by SDS-PAGE. Limited hydrolysis formed larger peptides which led to improved emulsification and foaming properties of the fish protein hydrolysate. Tilapia intestine crude enzyme hydrolysis produced FPH with higher solubility in water than using acid solutions. The optimum concentration for acid hydrolysis to produce FPH with high emulsifying activity index was found to be 4M acid solution. The Foaming stability for both the acid and enzymatic hydrolysis were low ranging from 9.17% 10.83%. Based on their characteristics and quality, fish protein hydrolysate extracted using acid and enzymatic hydrolysis were within the criteria that can be used as a value-added product in nutraceutical supplements such as sources of small peptides and amino acids in dietetic foods. The improved solubility, emulsifying and foaming capacities of tilapia protein hydrolysate warrant its application in formulated food systems.

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Introduction

Tilapia are prepared by bleeding, gutting, beheading, filleting, skinning, and trimming before being bought by consumers. The potential use of fish by-products should be considered. Increasing focus on the utilization of fisheries by-products in product development and value addition can be explained through waste management efforts and characterization of the raw materials as a potential food protein source and functional foods. Several food products could be obtained from the wastes of the aquaculture by-products industry.

Fish protein hydrolysates are products of hydrolysis reaction by breaking the peptide bonds in proteins resulting in shorter peptides or amino acids which are easier for animals to absorb. Extraction of proteins from by-products and conversion to high value products, such as bioactive peptides is a very promising alternative. Bioactive peptide production from fish by-products has received growing attention due to their physiological activities as antioxidant and antihypertensive suitable for healthcare and nutraceutical applications (He *et al.*, 2013; Je *et al.*, 2005; Jung *et al.*, 2006).

The considerable volume of tilapia produced in the country, aside from the significant requirement for processing before final sale generates a large amount of solid waste or residues and by-products, which account for up to 70% of the total fish weight. These so-called wastes composed of the head, carcass, bones, skin, fins and viscera of tilapia are traditionally considered of low economic value and are disposed in land-based waste disposal system or at sea. Moreover, a large amount of fish is also being discarded each year due to fish kill and disease outbreaks. If not properly discarded or used, they can be an important environmental contamination source since the release of these organic wastes might significantly change the community structure and biodiversity of the benthic assemblages(Caruso, 2015). It is estimated that 32 million tons of waste are produced from the total fish capture and are not used as food (Kristinsson & Rasco, 2000). One of the important waste reduction strategies for the industry is the recovery of marketable by-products from fish wastes (Arvanitoyannis & Kassaveti, 2008). The study was conducted to produce and characterize fish protein hydrolysate from tilapia by-products.

Materials and methods

Collection of Samples

Tilapia (*Oreochromis niloticus* L.) by-products composed of viscera, skin, fins and scales were collected from Science City of Muñoz Public Market, Nueva Ecija and transported in iced condition in plastic containers for processing at the BONP Laboratory. The tilapia by-products were homogenized in a food processor and stored at -20°C.

Preparation of Fish Protein Hydrolysate

The protein residues after extraction of fish oil were used for fish protein hydrolysate production. Fish protein hydrolysates were produced using crude enzymes and acid (HCl) described by Ovissipour *et al.* (2009) and Wisuthiphaet *et al.* (2015).

For enzymatic hydrolysis, tilapia intestine crude enzymes extracted following the procedure of El-Beltagy *et al.* (2004) regarded as EH1 and Kim *et al.* (2003) as EH2 were used to digest the homogenized tilapia by-products, 2%(EH1 2%), 4%(EH1 4%), 6% (EH1 6%), 2% (EH2 2%), 4% (EH2 4%)and 6% (EH2 6%)(w/w) of crude enzyme was added to the residue from fish oil extraction. After adjusting pH to 5, hydrolysis reaction was carried out in a shaking water bath at 40˚C, 200 rpm for 5 hours. Enzyme was inactivated at 90˚C for 30 minutes. After the termination of reaction, the mixture was centrifuged at 6700 g at 10°C for 20 min to collect the supernatant. The collected supernatant was oven-dried at 70°C for 48 hrs. The collected dry sample was made into powder using mortar and pestle. The powdered sample was referred to as the fish protein hydrolysate.

For acid hydrolysis, protein residue was homogenized and then mixed with distilled water at a ratio of 2:1 (protein residue: distilled water). Four M (4M AH), 6 M (6M AH) and 8 M (8M AH) of HCl was added to 50 g fish oil residue. Acid hydrolysis was performed under high pressure (15 psi) at 121˚C for 90 minutes.

Hydrolysis reaction was terminated by adjusting pH value to 5 by 6 M NaOH then filtered to separate some pieces of bones. The collected supernatant was oven-dried at 70°C for 48 hrs(Wisuthiphaet *et al.*, 2015). The collected dry sample was made into powder using mortar and pestle. The powdered sample was referred to as the fish protein hydrolysate. All experiments were done in triplicate.

Characterization of Fish Protein Hydrolysate

Degree of Hydrolysis (%DH)

Formol titration method adapted from Navarrete was used to determine the degree of hydrolysis of the sample. Two and a half milliliters (2.5mL) of sample in pH 8 (adjusted using 0.1 NaOH solution) was added with 1mL of 35% formaldehyde solution, pH 8.1. The mixture was incubated at room temperature for 1 minute. The solution was titrated with 0.25 N NaOH solution until it reaches the potentiometric point of 8.1. The volume of the utilized NaOH solution was recorded. The degree of hydrolysis (%DH) was calculated using equation 1:

$$\% \text{ DH} = \frac{B \times N_b \times 1.5}{M_p \times h_{tot}} \times 100 \qquad (1)$$

Where B refers to the volume of NaOH solution utilized to reached the pH of 8.1, N_b is the normality of the NaOH solution, h_{tot} is the number of peptides bond per unit: 8.6 meq/g (Fish protein concentrate), and Mp is the amount of protein in grams.

Protein Pattern Determination by SDS-PAGE

Five µLs of 1% sample solution was prepared and mixed with 5 µL of sample buffer and heated for 2 min at 50^0C. A total of 10 µL of the mixture was loaded to each well in a 15% polyacrylamide gel. The loaded gel was subjected to electrophoresis at 180 V, 40 A for 1 ½ hours (Labnet, 2017). Following the electrophoresis, the gels were stained using 100mL of 0.2% Coomassie Blue G-250, added with 100mL 2N H_2SO_4 and incubated overnight. Afterwards, the solution was filtered and added with 22.2mL 10N KOH and 28.7g TCA. The solution was allowed to stand for at least 3 hours before filtration (Stoyanov *et al.*, 2001).

Functional Properties of Fish Protein Hydrolysates

Fish protein hydrolysate functional properties such as solubility, emulsifying, foaming and were determined. Solubility and emulsifying properties were determined using Klompong *et al.* (2007) method. Protein content was determined using Biuret test. Biuret test was done by adding 2mL of biuret solution to a 1mL sample. The solution was mixed with a vortex and left to stand for 15 minutes. The solution was read using UV-Vis spectrophotometer (T60 UV-Vis Spectrophotometer, PG Instruments Limited, FB, UK) set at 500nm.

Solubility Properties

Protein solubility was calculated using the formula (Equation 2):

$$\text{Solubility (\%)} = \frac{\text{protein content of the supernatant}}{\text{protein content of the sample}} \times 100 \qquad (2)$$

where protein content of the supernatant and protein content of the sample were obtained through Biuret method.

One percent (1% w/v) fish protein hydrolysate solution was prepared by dispersing 0.05 g of dried fish protein hydrolysate in 5mL distilled water. The solution was prepared at pH 7 adjusted using 0.1 N HCl or 0.1 N NaOH and stirred at room temperature for 30 mins. The samples were centrifuged at 7500g for 15 minutes. The supernatant collected were measured using Biuret method. For the total protein content of the sample, 5mL of 1% (w/v) fish protein hydrolysate was mixed with 0.25N NaOH then treated with Biuret reagent.

Emulsifying Properties

Three milliliters (3mL) of 1% of fish protein hydrolysate solution was mixed with 1mL vegetable oil. The pH of the mixture was adjusted into different pH. The sample was homogenized for 1 min using a vortex mixer. A total of 50 microliter was collected at the bottom of the container. Another 50 microliter was collected from the sample after 10 mins. The sample was added with 5mL of 0.1% sodium dodecyl sulfate

(SDS) solution and was read at 500nm. The emulsion activity index was calculated using (Equation 3):

$$EAI\left(\frac{m^2}{g}\right)=\frac{(2\times 2.303\times Abs_{500})}{(0.25\times \text{protein weight (g)})} \quad (3)$$

The emulsifying stability index was calculated as follows (Equation 4):

$$ESI\ (min)=\frac{A_0\times \Delta t}{\Delta A} \quad (4)$$

Where ΔA refers to absorbance of blank at 500nm minus to the absorbance of sample in 10 min, while Δt refers to 10 mins reaction time.

Foaming Properties

A total of 20mL of 0.5% fish protein hydrolysate was prepared at pH 7. The solution was homogenized at 16000 rpm for mins at room temperature. The whipped sample was transferred in a graduated cylinder and the volume recorded after 30 sec. The foaming capacity was calculated using the formula (Equation 5):

$$\text{Foaming capacity (\%)}=\frac{(A-B)}{(B\times 100)} \quad (5)$$

Where A refers to recorded volume after whipping (mL), B is the volume before whipping (mL).

The whipped sample were left to stand at 20°C for 3 mins; the volume of the sample after 3 mins were recorded. Foaming stability was calculated using the formula (Equation 6):

$$\text{Foaming stability(\%)}=\frac{(A-B)}{B\times 100} \quad (6)$$

Where A refers to the recorded volume after 3 mins (mL), and B refers to the volume before whipping (mL)

Data Analysis

All experiments were carried out in triplicates. Means and standard deviations of the data were reported. Analysis of variance (ANOVA) comparison was performed at the significant level of $P < 0.05$ using SPSS version 23. Tukey's tests were performed to determine differences among the treatment means.

Results and discussion

Fish Protein Hydrolysate Yield

Fish protein hydrolysate (FPH) is a breakdown product of protein containing smaller peptides and amino acids. It is obtained by treatment of fish protein with chemical agents or enzymes under controlled conditions of pH and temperature (Fig. 1). The fish protein hydrolysate powder from tilapia viscera has a dark brown color.

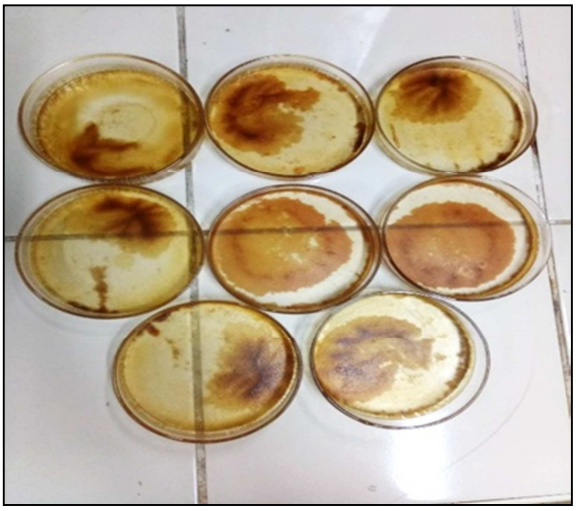

Fig. 1. Oven-dried tilapia protein hydrolysate (photo by MJ Rafael).

The yield of fish protein hydrolysates produced using acid and enzymatic hydrolysis from protein residues after oil extraction is presented in Fig. 2.

The amount recovered using acid hydrolysis was significantly higher ranging from 19.09±0.23 to 32.73±0.58% compared to enzymatic hydrolysis which ranged from 3.99±0.25 to 4.84±0.15%. It can be noted that the yield of the protein hydrolysates increases with increasing concentration for acid and enzymatic hydrolysis. Significant differences were recorded in the yield of protein hydrolysates with increasing concentration used.

In the case of enzymatic hydrolysis, increasing concentration of enzyme produced no significant differences in the yield of fish protein hydrolysate. The use of 8M acid solution in the hydrolysis process yielded the highest, 32.73±0.58 while the lowest was 3.99±0.25 hydrolyzed with 2% Enzyme 1(EH1).

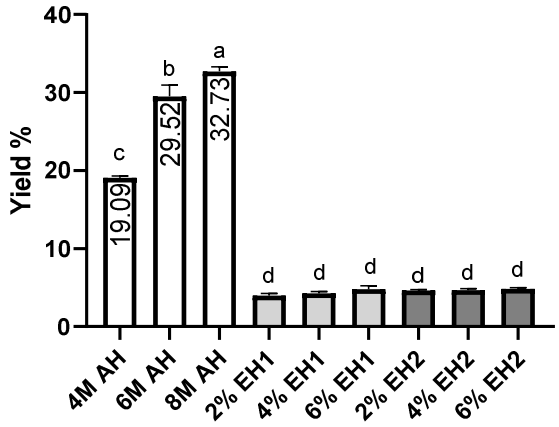

Fig. 2. Percent yield of Tilapia Protein Hydrolysate. Mean ± standard deviation with different superscript letters is significantly different ($p < 0.05$) based on one-way ANOVA followed by Tukey's test.

Šližyte *et al.* (2005) reported the FPH yield for cod (*Gadus morhua*) viscera using commercial enzymes at 5.7% (*Flavourzyme*), 7.3% (*Neutrase*) and 5.2% (*Alcalase*) and 2.6% (Lecitase). Results from the enzymatic hydrolysis of black tilapia (*Oreochromis mossambicus*) flesh was 9.6% yield using Alcalase(Abdul-Hamid *et al.*, 2002).Bhaskar & Mahendrakar, (2008) reported the enzymatic hydrolysis of Catla carp (*Catla catla*) with yield at 6.04% (*Alcalase*) and 5.84% (*Neutral Protease*). The lower yields obtained in the study are due to the fact that only the soluble fractions are dried. Yields of fish protein hydrolysate were also consistent with the degree of hydrolysis (Fig. 18), since lower degree of hydrolysis gave a lower yield(Hoyle & Merritt, 1994).

Hydrolysis reactions may contribute in improvement of the nutritional, functional, immunological, and biological activity of proteins (Cheison *et al.*, 2009). It is an important parameter to understand and interpret the effects and extent of protein hydrolysis and is useful to establish the relationship between proteolysis and improvement of the functional, bioactivity, and sensory properties of these biomolecules.

Fish Protein Hydrolysate Characterization

Total Protein Concentration

Total Protein of fish protein hydrolysate produced from acid and enzymatic hydrolysis is presented in Fig. 3. For acid hydrolysis, decreasing total protein was observed with the use of increasing HCl concentration ranging from 10.40 (8M HCl) to 18.53 (4M HCl). The amount of protein contained in the fish protein hydrolysates hydrolyzed with acid solutions are significantly different from each other. As for the enzymatic hydrolysis, a slight increase in total protein was noted with an increase in concentration of enzyme used. The highest with comparable total protein content was the FPH hydrolyzed with 4% and 6% EH2, at 13.83-14.22mg/ml, respectively. Similar observation was also noted on the FPH produced by EH1 using the same concentration but has significantly lower total protein content at 12.16-12.42 mg/ml.Bhaskar & Mahendrakar, (2008)reported a protein content from visceral waste of Catla carp (*Catla catla*) with enzymatic hydrolysis at 14.25% (*Alcalase*) and 13.85% (*Neutral Protease*). Similar results are reported by Wisuthiphaet *et al.* (2015)for yellow striped trevally (*Selaroides leptolipis*) and mackerel (*Decapterus maruadsi*).

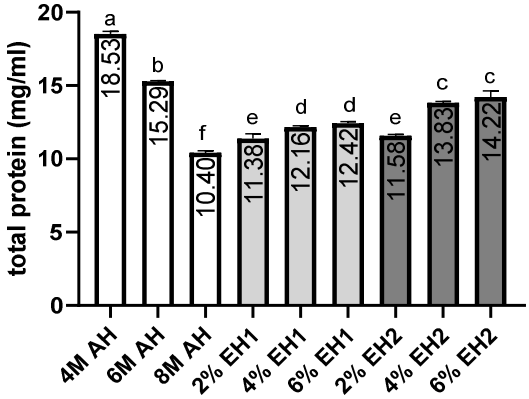

Fig. 3. Total protein of fish protein hydrolysate. Mean ± standard deviation with different superscript letters are significantly different ($p < 0.05$) based on one-way ANOVA followed by Tukey's test.

Degree of Hydrolysis

Fig. 4 shows the degree of hydrolysis (DH) in the production of fish protein hydrolysates using acid and enzymatic hydrolysis. For acid hydrolysis, DH ranges from 12.79-13.95 percent. There was no significant difference on the degree of hydrolysis using the different HCl concentrations. Moreover, no significant difference was also noted on the degree of hydrolysis in the production of fish protein

hydrolysates by both enzymes where the values ranged from 7.99-8.72%.

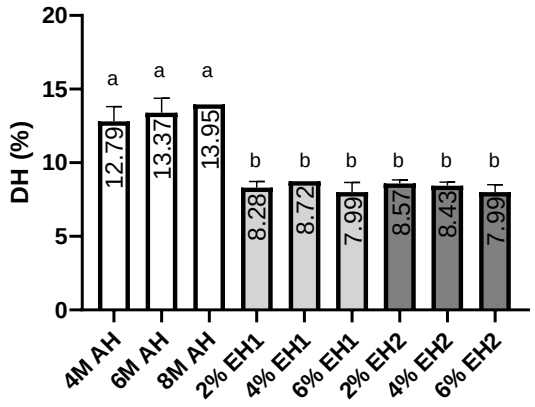

Fig. 4. Degree of hydrolysis of fish protein hydrolysate. Mean ± standard deviation with different superscript letters is significantly different ($p < 0.05$) based on one-way ANOVA followed by Tukey's test.

For enzymatic hydrolysis, DH ranged from 7.99-8.72%. Nevertheless, there is no significant difference between the DH of all the concentrations by enzymatic hydrolysis. Comparing the DH of acid and enzyme treatments, significant differences in DH were recorded. Acid hydrolysis had higher DH than enzymatic hydrolysis. Degree of hydrolysis is defined by Rutherford (2010) as the proportion of cleaved peptide bonds in a protein hydrolysate. It is a measure of evaluating the effectiveness of hydrolyzing a protein molecule using chemical or enzyme treatment process. A higher DH means a greater number of short chain peptides and amino acids in the hydrolysate (Yang *et al.*, 2019). Based on the results of this study, acid hydrolysis was found more effective in hydrolyzing the protein compared with the enzyme used. The enzymes extracted using the two methods elicited similar hydrolyzing effect on the protein since they exhibited similar degree of hydrolysis. Thus, these enzymes are having the same catalytic function. However, the results of the study is lower than the results reported by Wisuthiphaet *et al.* (2015). Shahidi, (2007) reported that fish proteins were completely hydrolyzed with 6M HCl at 110˚C for 20 - 24 hours. An increase in reaction time can raise the production cost and completely hydrolyzed fish protein hydrolysates but with low nutritional quality due to the loss of some essential amino acids and more derivatives. Similar results are reported bySilva *et al.* (2014) for Nile tilapia (*Oreochromis niloticus* L.) using intestine enzyme; and Wisuthiphaet *et al.* (2015) with 9-10% DH using papain. Limitations of formol titration are also observed on its reproducibility and consistency due to its potentiometric technique. Use of spectrophotometric techniques such as ninhydrin reaction technique and trinitrobenzene sulfonic acid (TNBS) reaction technique are recommended due to its ease of use and reproducibility.

Protein Patterns of Fish Protein Hydrolysate by SDS-PAGE

The molecular weight distribution of fish protein hydrolysate using acid and enzymatic hydrolysis was analyzed by SDS-PAGE (Fig. 5). Different protein patterns were observed between the treatments.

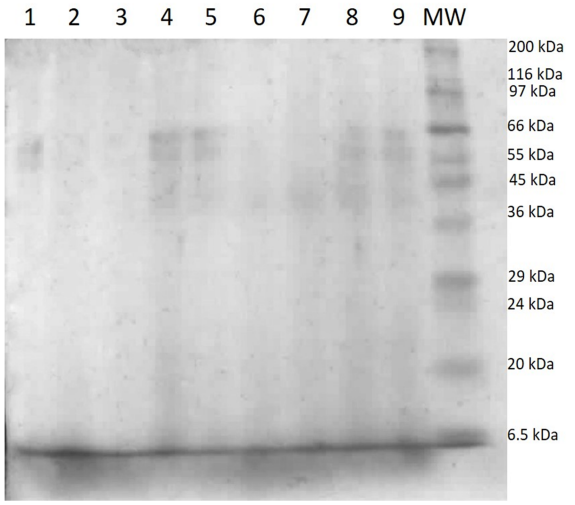

Fig. 5. SDS-PAGE of fish protein hydrolysate. Lane 1- 4M AH; Lane 2- 6M AH; Lane 3- 8M AH; Lane 4- 2% EH1; Lane 5- 4% EH1; Lane 6- 6% EH1; Lane 7- 2% EH2; Lane 8- 4% EH2; Lane 9- 6% EH2; Lane 10 molecular weight standard.

Lanes 1, 4, 5, 8, and 9 showed faint bands from 66 kDa down to less than 6.5 kDa. Majority of the peptides are less than 6.5 kDa, but due to limited molecular weight markers was not able to fully separate the protein bands lower than 6.5 kDa. Limited hydrolysis (larger peptides) leads to improved emulsification and foaming properties of fish protein hydrolysate, while extensive hydrolysis (small peptides) reduces these properties (Jeon *et al.*,

2000; Kristinsson & Rasco, 2000; Liceaga-Gesualdo & Li-Chan, 1999; Quaglia & Orban, 1990). Very small peptides do not have the ability to form a good stable cohesive protein network around oil droplets or air pockets. There is also evidence that as%DH increases (i.e. higher level of small peptides), FPH exhibits less oil binding (Kristinsson & Rasco, 2000b).

Solubility Properties

Among the various properties of the protein hydrolysate, solubility is recognized as one of the most influential characteristics which can significantly affect other properties (de Castro & Sato, 2014; Kristinsson & Rasco, 2000). The solubility property of tilapia protein hydrolysate was recorded at pH 7 (Fig. 6). The solubility of FPH hydrolyzed with acid solutions ranged from 32.96-51.76%. On the other hand, the solubility of the FPH hydrolyzed with enzymes ranged from 68.66- 89.36%. The solubility of FPH due to enzymatic hydrolysis were significantly higher than the solubility of FPH produced by acid hydrolysis. Thus, enzyme hydrolysis produces FPH with higher solubility in water than FPH hydrolyzed with acid solutions. The result indicates that enzyme hydrolysis produced smaller peptides and amino acids in FPH which are soluble to water than acid hydrolysis. Furthermore, the use of increasing concentration for both acid and enzymatic hydrolysis increases the solubility properties of tilapia protein hydrolysates (Kristinsson & Rasco, 2000).

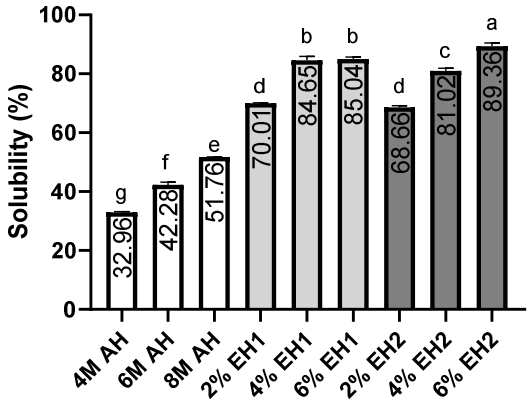

Fig. 6. Solubility of fish protein hydrolysate at pH 7. Mean ± standard deviation with different superscript letters is significantly different ($p < 0.05$) based on one-way ANOVA followed by Tukey's test.

Protein hydrolysates with higher solubility can be obtained by increasing the time of the hydrolysis reaction resulting in smaller peptides with lower molecular weight. Smaller peptides have consequently more ionizable polar groups on their surface, which are more able to form hydrogen bonds with water molecules (de Castro & Sato, 2014; He *et al.*, 2013). The improved solubility enables tilapia protein hydrolysates to be applied readily to formulated food systems (Thiansilakul *et al.*, 2007). Although increased solubility has a positive relationship to the extent of hydrolysis, care has to be taken that the substrate is not too extensively hydrolyzed. Higher degree of hydrolysis may lead to higher solubility, but this can have very negative effects on the rest of the functional properties. To maintain or improve functionality, typically lower degrees of hydrolysis are necessary (Kristinsson & Rasco, 2000).

Emulsifying Properties

Proteins have the ability to stabilize emulsions. The emulsifying activity index (EAI) and the emulsion stability index (ESI) of fish protein hydrolysates (FPH) from tilapia by-products produced by acid and enzyme hydrolysis are presented in Fig. 7. The highest EAI was recorded in FPH from tilapia hydrolyzed using 4M acid solution (85.73 m2/g), while the lowest was the FPH hydrolyzed with 4% Enzyme 2. Significant differences in EAI were exhibited by FPH produced using different concentrations of acids and enzymes. Acid hydrolysis of protein produced significantly higher EAI than enzyme hydrolysis. In acid hydrolysis, a decreasing trend in EAI could be observed with increasing concentration of acid used. The best concentration for acid hydrolysis to produce FPH with high EAI was found to be 4M acid solution. On the other hand, for enzyme hydrolysis, use of 6% enzyme exhibited higher EAI with Enzyme1 producing significantly higher activity compared to Enzyme 2.

For emulsion stability index (ESI) of the FPH, significantly different activities were recorded (Fig. 20). The highest ESI was exhibited by FPH hydrolyzed with 6% EH2 (74.89 min) followed by 2% EH1 (72.53 min), then 8M AH (65.92 min) and the lowest was in 4M AH (35.89 min).

FPH hydrolyzed with 2% EH1 and 6% EH2 have comparable ESI. However, ESI of FPH hydrolyzed with 2% EH1, 6% EH1 and 8M AH was found comparable. This means that FPH hydrolyzed with 6% EH2 and 2% EH1 will produce a more stable emulsion at longer time than the other FPHs.

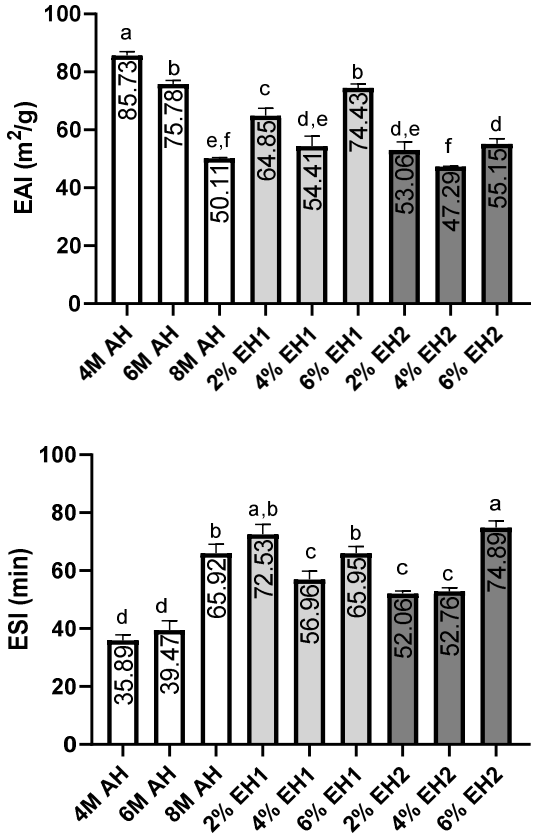

Fig. 7. Emulsifying activity index (EAI) and emulsion stability index (ESI) of fish protein hydrolysate. Mean ± standard deviation with different superscript letters are significantly different ($p < 0.05$) based on one-way ANOVA followed by Tukey's test.

Proteins are interesting biomolecules due to their amphiphilic properties which allow them to reduce the surface tension at the oil and water interface. The emulsifying properties of fish protein hydrolysates are directly connected to their surface properties, or how the hydrolysate effectively lowers the interfacial tension between the hydrophobic and hydrophilic components in food. Proteins adsorb to the surface of freshly formed oil droplets during homogenization and form a protective membrane that prevents droplets from coalescing (Kristinsson & Rasco, 2000).

The incorporation of protein like protein hydrolysates at the oil-water interface are utilized in the formation of emulsions (oil-water or water-oil), a principle applied in food formulation, drugs, and nutrient delivery. Emulsifying properties of proteins are needed to improve the utilization of dietary protein sources in food formulations, an important fact for food scientists and technologists.

Foaming Properties

The underlying foaming properties of protein hydrolysate have many things in common with emulsifying properties which are both relying on the surface properties of protein.

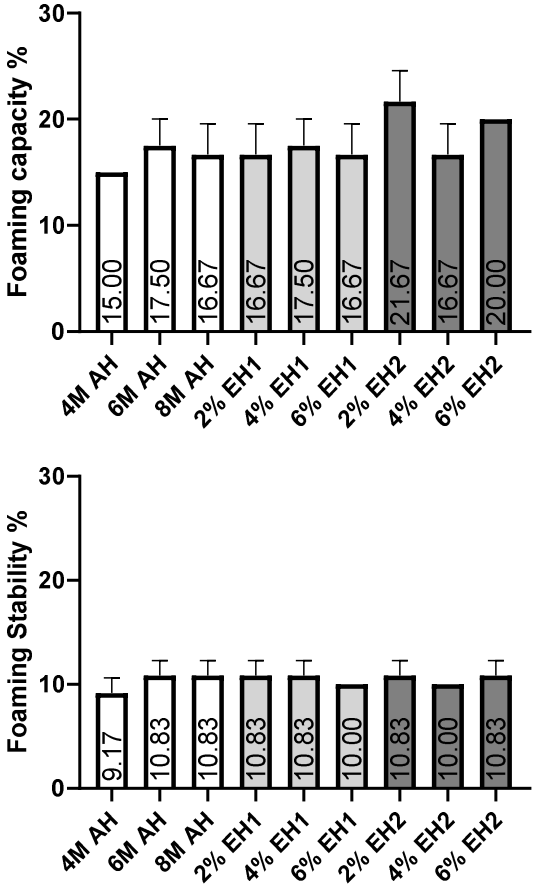

Fig. 8. Foaming capacity (%) and Foaming stability (%) of fish protein hydrolysate. Mean ± standard deviation with different superscript letters are significantly different ($p < 0.05$) based on one-way ANOVA followed by Tukey's test

Food foams consist of air droplets dispersed and enveloped by a liquid containing soluble surfactant

lowering the surface and interfacial tension of the liquid (Kinsella & Melachouris, 1976); Foaming capacity and foaming stability of fish protein hydrolysate (FPH) are presented in Fig. 8.

Foaming capacity of the FPH for both acid hydrolysis and enzymatic hydrolysis are low ranging from 15% to 21.67%. The Foaming stability for both the acid and enzymatic hydrolysis are also low ranging from 9.17% 10.83%. There is no significant difference between the treatments for both foaming capacity and foaming stability. There is a connection between the degree of hydrolysis and foaming properties. The low foaming capacity and stability is due to the high degree of hydrolysis and long hydrolysis time. Foh *et al.* (2011) reported a decrease in foaming capacity and stability in tilapia protein hydrolysate with further hydrolysis. Kuehler and Stine (1974) reported that whey proteins hydrolyzed to a limited degree had increased foaming capacity but reduced foam stability which was attributed to more air being incorporated into solution of smaller peptides, but the smaller polypeptides do not have the strength required to hold a stable foam (Venugopal *et al.*, 1995). Enzymatically hydrolyzed fish protein hydrolysate has generally improved solubility and dispersibility while some other functional properties such as foaming would be reduced (Kristinsson & Rasco, 2000).

Applications of Tilapia Protein Hydrolysate

Fish protein hydrolysates can be used as bioactive compounds in nutritional supplements. A variety of nutraceuticals, health foods or functional foods produced from FPH are commercially-available in the US, Japan and UK. These products have variety of applications including promotion of intestinal health and regulation of bowel function, as anti-stress, sports nutrition, for memory and cognitive function as well as promotion of cardiovascular health (Guérard *et al.*, 2010; Marchbank *et al.*, 2008; Nesse *et al.*, 2011; Parolini *et al.*, 2014). FPH can also be used as a source of small peptides and amino acids in dietetic foods, preparation of fish soups, fish sauce, fish paste, flavoring compounds, artificial crabs and fish sausage, and cereal-based extrusion products such as chips (Halim *et al.*, 2016; Kristinsson & Rasco, 2000; Silva *et al.*, 2014). The improved solubility as well as the emulsifying and foaming capacities of tilapia protein hydrolysates warrants its application as nutraceutical as well as in formulated food systems.

Conclusion

The ideal waste management is to prevent waste generation. This study has shown several chemical methods like extraction by the use of enzymes and acid that can minimize waste and re-use the waste products thereby utilizing resources effectively and sustainably. Overall, this study was able to produce and characterize protein hydrolysate derived from tilapia by-products. Based on their characteristics and quality, these intermediate products could serve as an alternative to mammalian-derived products, hence dictates their application as functional food.

Acknowledgement

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