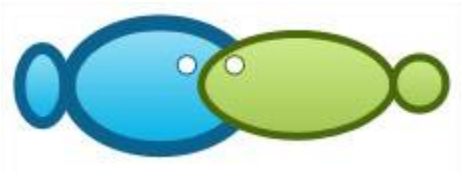

Effects of material types and enzymatic hydrolysis treatments on the production of fish protein hydrolysate powder from snakehead fish (*Channa striata*) head by using endoproteases and exoproteases

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Abstract. The present study investigated the effect of the material type (defatted (DF) or non-defatted (NDF)) combined with alcalase concentration (from 0.2 to 1.2%) and seven enzymatic hydrolysis treatments on the enzymatic hydrolysis of snakehead fish head (SFH) using endoproteases and exoproteases. After enzymatic hydrolysis, the fish protein hydrolysate was spray-dried, and the quality of the hydrolysate (FPH) powder was investigated. The results revealed that the ideal material and alcalase concentration were NDF and 0.8%, respectively. NDF was hydrolyzed using seven enzymatic hydrolysis treatments: single (Alcalase (A), Protamex (P), and Flavourzyme (F)), simultaneous combined (A + F, P + F), and sequential (A > F, P > F) treatments. FPH production using sequential (A > F) treatment resulted in the highest total amino acid content, protein recovery, and degree of hydrolysis among all enzymatic hydrolysis treatments. The FPH powder achieved good nutritional quality and had a high protein content, full essential amino acids, and low lipid content. This, coupled with food safety with no detected pathogenic bacteria and low heavy metal content in the FPH powder, were all below the allowed limit. These results demonstrate that SFH can be used as a raw material to produce FPH powder with high nutritional quality and food safety in the food industry.
Key Words: *Channa striata*, defatted, enzymatic hydrolysis treatments, endoprotease and exoproteases, fish protein powder.

Introduction. Snakehead fish (*Channa striata*), belonging to the Channidae family, are potentially important freshwater fish for both catching and farming in the Southeast Asian region owing to their high protein quality and the presence of essential amino acids (Jais 2007). The strong development of snakehead fish farming in Vietnam has prompted an urgent need to produce fish-based food products, such as dried, fermented, and fish floss (Ngan et al 2017). However, large amounts of fish waste, including head, backbone, skin, scales, fins, and viscera, are discarded without being treated (Benjakul et al 2014). Moreover, many studies have reported that fish by-products contain high amounts of protein (14-20%), good nutritional properties, and good essential amino acid compositions (Nemati et al 2012; Roslan et al 2014; Fallah et al 2015; Prihanto et al 2019). Thus, the production of fish protein hydrolysates from fish waste is the most convenient method conducted by researchers for utilizing fish waste (Ishak & Sarbon 2017).

Protein hydrolysates generated from fish byproducts have received increasing attention in recent years owing to their desirable physicochemical properties and several bioactive peptides. However, the selection of the protease enzyme type and hydrolysis conditions is key for generating protein hydrolysates with the desired potency as bioactive peptides (Chalamaiah et al 2012). Several proteolytic enzymes including

alcalase, protamex, flavourzyme, neutrase, papain, pepsin and trypsin are commonly used to hydrolyze the fish proteins into fish protein hydrolysate (FPH) (Siddik et al 2021). Among these enzymes, alcalase is an endoprotease enzyme produced by *Bacillus licheniformis*, which is widely used in fish protein hydrolysate production for its high extraction ability under moderate pH conditions and its ability to produce FPH with small-sized peptides (Wisuthiphaet et al 2016). Protamex is a bacterial endoprotease isolated from *B. subtilis* strains and is known to produce bitterless hydrolysates (Nguyen et al 2011). Moreover, flavourzyme, an exoprotease preparation obtained from an *Aspergillus oryzae* strain, is a fungal endoprotease and exoprotease mixture with very broad specificity that minimizes the bitterness in protein hydrolysates (Chiang et al 2019). The application of alcalase, protamex and flavourzyme in single, combinational, or sequential hydrolysis treatments to improve hydrolysis efficiency has been previously reported (Klompong et al 2008; Nguyen et al 2011; Wisuthiphaet et al 2016; Vy et al 2018; Chiang et al 2019; Senadheera et al 2021). However, no study has been done on endoprotease and exoprotease to produce FPH powder from snakehead fish head (SFH). Besides, most of the studies reported that total lipid content in hydrolysate should be lower than 0.5% to enhance products stability (Kristinsson & Rasco 2000; Ovissipour et al 2009; Siddik et al 2021). Therefore, in this study, the objective was to investigate the effects of material types (defatted and non-defatted materials) and alcalase concentration on the ammonia content (N_{NH3}), total amino acid content (N_{aa}), protein revovery (PR), degree of hydrolysis (DH) and lipid content in FPH. The effect of enzymatic hydrolysis included single treatment (Alcalase, Protamex and Flavourzyme), simultaneous combined (Alcalase and Flavourzyme, Protamex and Flavourzyme), and sequential (Alcalase > Flavourzyme, Protamex > Flavourzyme) as well as the quality of FPH have been investigated.

Material and Method

Collection of snakehead fish head samples. Snakehead fish heads (SFH) were purchased from a local dried snakehead fish processing plant in An Giang Province, Vietnam. Samples were collected and immediately transported on ice at a fish/ice ratio of 1:2 (w w^{-1}) to Can Tho University within 4 h. Samples were washed with cold water (≤ 10°C), packed in polyethylene (PE) bags, and stored at -20±2°C (less than 1 month).

Preparation of defatted and non-defatted materials. Defatted (DF) material was prepared using the method developed by Riyadi et al (2019b) with slight modifications. Briefly, 500 g of minced SFH and 500 mL of distilled water were homogenized and heated at 95°C for 60 min. Samples were then centrifuged at 5800 rpm, 10°C for 20 min to remove fat. The solid insoluble mixture (protein-rich material) was added to ethanol 20^{o} solution at a ratio of 1:1 (w v^{-1}) and used as the DF material for the hydrolysis process.

For non-defatted (NDF) material preparation, 500 g minced SFH and 500 mL of ethanol 20^{o} solution were homogenized and used as the NDF material for the hydrolysis process.

Hydrolysis process. . Hydrolysis of DF and NDF was performed following the process described by Kechaou et al (2009) with slight modifications. A 50 g sample was used for each hydrolysis reaction. All hydrolysis reactions, optimal activities of alcalase were performed at pH 8, whereas those of Protamex and Flavourzyme were at pH 7.0. The hydrolysis was performed at 50°C for 30 h. Before hydrolysis, the DF and NDF materials were prepared, and the pH was adjusted with 1 M NaOH and pre-incubated at 50°C for 10 min prior to the addition of enzymes. After hydrolysis, samples were immersed in a water bath (model WNB 22, Memmert, Schwabach, Germany) at 95°C for 10 min to inactivate the proteolytic enzyme (Ovissipour et al 2010). The mixture was then cooled and centrifuged at 7000 rpm, at 4°C for 30 min using a Hettich Universal 320R centrifuge, and the supernatant (hydrolysate) was collected using filter paper and mass weighted. Fat was decanted from the aqueous phase after centrifugation. To study the effects of material type and enzyme–substrate (E/S) ratio, alcalase hydrolysis treatment

was conducted at enzyme/substance ratios of 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, and 1.2% for both DF and NDF materials. The N_{NH3}, N_{aa}, PR, DH, and lipid content of the hydrolysate were calculated to determine the optimum material type and E/S ratio for Alcalase. Seven enzymatic hydrolysis treatments - single (Alcalase-A, Protamex-P and Flavourzyme-F), simultaneous combinational (A + F, P + F), and sequential (A → F, P → F) - were used to investigate the effects of the enzyme systems on N_{aa}, PR, and DH in the hydrolysates obtained from SFH. A 1:3 ratio of endoprotease (Alcalase, Protamex) and exoprotease (Flavourzyme) was used in the simultaneous combination and sequential treatments.

Degree of hydrolysis (DH). In this study, the o-phthalaldehyde (OPA) method was used to measure DH, as described by Nielsen et al (2001) with slight modifications. The OPA method involves a reaction between free amino groups and OPA in the presence of dithiothreitol or β-mercaptoethanol. Color changes in the solutions were detected using a spectrophotometer at 340 nm. DH was calculated as follows:

$$DH = h/h_{tot} \times 100 \quad (1)$$

where: h_{tot} is the total number of peptide bonds in the substrate, and h is the number of hydrolyzed bonds. h = (serine-NH_2-β)/α meqv/g protein; β, α and h_{tot} for fish are 0.4, 1.0 and 8.6, respectively.

Free amino groups (serine-NH_2) were quantified using the OPA method. The OPA reagent and serine standard solutions were prepared as described by Nielsen et al (2001). The FPH samples were diluted with distilled water to obtain a suitable concentration with a serine standard. A total of 400 µL of serine standard solution (standard), FPH samples (samples), or deionized water (blank) was added to 3 mL of the OPA reagent and mixed for 5 s. The mixtures were incubated for 2 min at ambient temperature (25±2°C), and the absorbance at 340 nm was measured using a spectrophotometer to obtain the $OD_{standard}$, OD_{sample} and OD_{blank}, which were used to calculate the concentration of free serine-NH_2.

Chemical and microorganism analysis. The lipid content in FPH was determined using the Folch method (Folch et al 1957). Briefly, ammonia and total amino acid content were analyzed using the Vietnam standards TCVN 3706:1990 and TCVN 12620:2019, respectively. The crude protein content in FPH was measured by Kjeldahl digestion (AOAC 2016), and PR in the FPH was calculated using the following formula (Liaset et al 2002):

$$\text{Protein recovery (\%)} = \frac{\text{Protein in hydrolysate (\%) x Mass of hydrolysate (g)}}{\text{Protein in snakehead head (\%) x Mass of snakehead head (g)}} \text{ x } 100$$

The moisture, protein, lipid, and ash contents of the FPH powder were analyzed according to AOAC (2016). The moisture content was determined using the thermogravimetric method in an oven at 105 ± 2°C until the sample reached a constant weight. The crude protein content was determined by Kjeldahl digestion using 6.25 as a conversion factor, and the crude fat content was analyzed using the Soxhlet extraction method. The crude ash was analyzed by combusting the sample in a muffle furnace at 550°C for 24 h.

FPH powder was sent to a commercial service provider (WARRANTEK Joint Stock Company, Can Tho City, Vietnam) for microorganism and heavy metal analysis, where standard ISO methods for coliforms (ISO 4832:2006), *Escherichia coli* (ISO 16649-2:2001), *Staphylococcus aureus* (ISO 6888-1:1999/Amd 1:2003), *Salmonella* spp. (ISO 6579-1:2017), total spores of yeast and mold (ISO 21527-1:2008), and for heavy metals such as arsenic, cadmium, lead, copper (AOAC 2013.06).

Amino acid analysis. The amino acid composition of the FPH powder was determined according to the method described by Tawali et al (2018). The FPH powder was hydrolyzed with 6 N HCl at 110°C for 22 h and the hydrolysates were analyzed using a dedicated amino acid analyzer (Biochrom 32+, USA).

Statistical analysis. Experiments were performed in triplicate and the mean and standard deviation were calculated. Statgraphics Centurion XV Version 15.1.02 (StatPoint, Inc., USA) was used for one way analysis of variance (ANOVA), followed by the LSD multiple range test at 95% probability to determine significant variance of measurements.

Results

Effects of material types and alcalase concentration on the hydrolysis process. The effects of material types (defatted and non-defatted materials) and alcalase concentration on the ammonia content (N_{NH3}), total amino acid content (N_{aa}), protein recovery (PR), degree of hydrolysis (DH) and lipid content in FPH are presented in Figure 1.

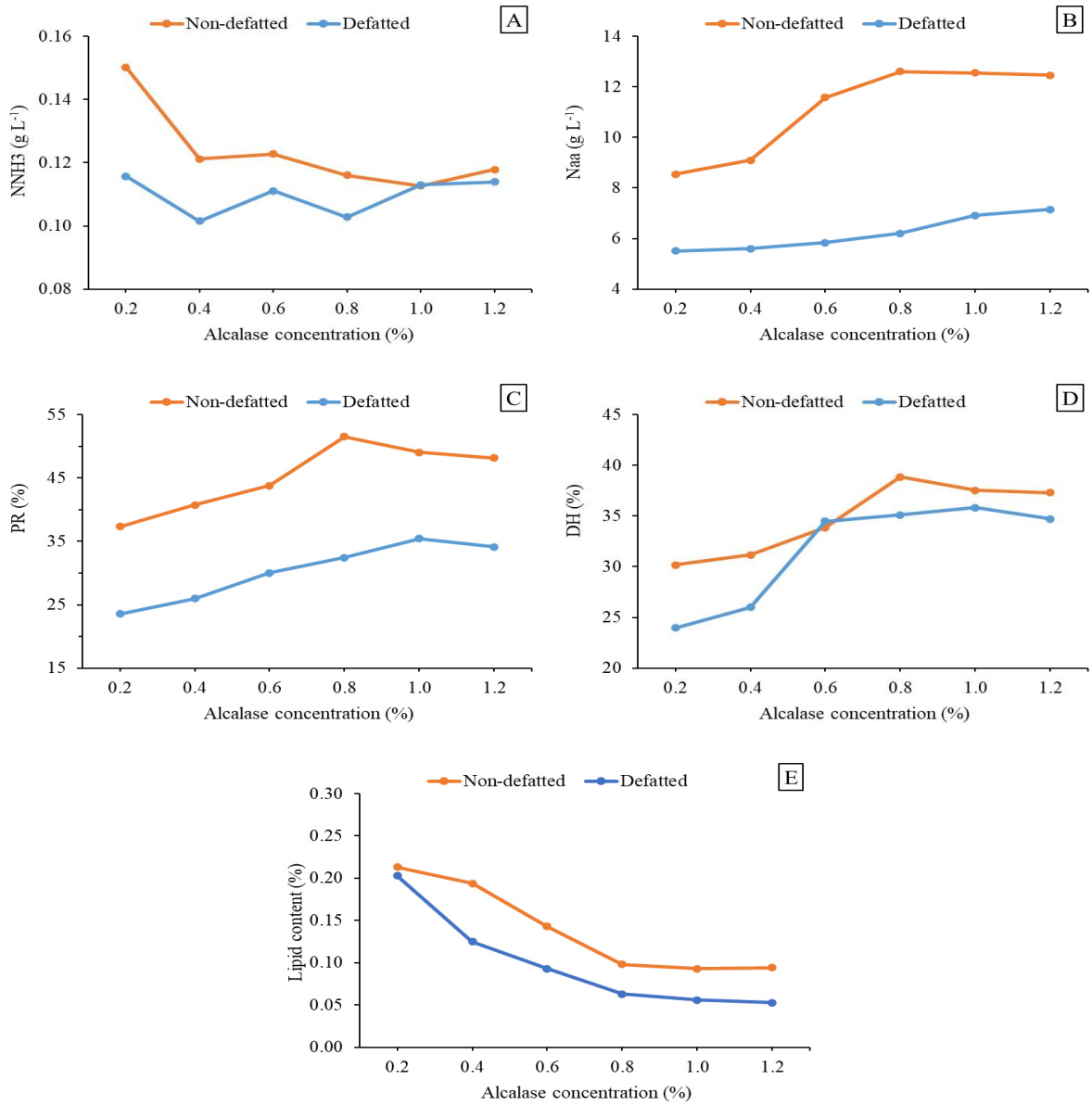

Figure 1. Effects of material types and alcalase concentrations on N_{NH3} (A), N_{aa} (B), PR (C), DH (D), lipid content (E) in FPH (N_{NH3} (g L^{-1}): ammonia content, N_{aa} (g L^{-1}): amino acid content, PR (%): protein recovery, DH (%): degree of hydrolysis, FPH: fish protein hydrolysate).

Figure 1 shows that N_{aa}, PR, and DH in FPH increased, whereas N_{NH3} and lipid content slightly decreased as the Alcalase concentration increased for both NDF and DF materials.

These results may be explained by the increasing interaction between the enzyme and substrate as the enzyme concentration increased, resulting in more peptide cleavage into free amino acids and smaller peptides in FPH, leading to an increase in N_{aa}, PR, and DH in FPH (Salwanee et al 2013). This result is in agreement with those of many previous studies (Normah et al 2005; Salwanee et al 2013; Riyadi et al 2019a). In addition, it favors the release of lipids from the protein matrix, leading to a decrease in lipid content as the enzyme concentration increases (De Oliveira et al 2016). The cell membranes become rounded and form insoluble vesicles during the hydrolysis process, leading to the removal of membrane-structured lipids (Roslan et al 2014). Many studies have reported reduced lipid content of FPH from tilapia by-product (Roslan et al 2014), herring by-product (Sathivel et al 2003) and salmon by-products (Gbogouri et al 2004). NDF with 0.8% alcalase concentration produced the highest N_{aa}, PR and DH value in FPH of 12.6 g L^{-1}, 51.5%, and 38.9%, respectively, whereas N_{NH3} was 0.116 g L^{-1} and lipid contents were 0.098%. However, 1.0% alcalase concentration produced the highest PR, DH and high N_{aa} for DF material. In addition, our results showed no significant increases in the N_{aa}, PR, and DH values at 1.0% and 1.2% for the NDF material and 1.2% for DF material. This result is in accordance with that of Prayudi et al (2020), who reported that when an enzyme is added to a substrate, the enzyme is absorbed into the suspended particles. Rapid hydrolysis in the initial phase indicates that a large number of enzyme-susceptible peptide linkages were hydrolyzed. Thereafter, the hydrolysis rate decreases and enters a stationary phase. At this point, increasing the enzyme concentration may cause substrate saturation, and the degree of hydrolysis may not increase.

Figure 1 shows that NDF had a higher lipid content in the hydrolysate than DF. This result may be due to the low lipid content of the DF material resulting from the defatting process before hydrolysis (Ghelichi et al 2018). As expected, for both DF and NDF materials, lipid content ranged from 0.053 to 0.213%, which was less than 0.5% and could minimize the risk of lipid oxidation and formation of secondary oxidation products (Ovissipour et al 2009). However, N_{aa}, PR, and DH in the FPH from the NDF material were higher than those in the FPH from the DF material. This can be explained by the fact that during preparation, material defatted by heating may result in the deconformation of the natural features of the protein molecules or complexes, resulting in complex chemical (cross-linking) reactions such as protein interactions and protein denaturation. As a result, protein hydrolysis is reduced, leading to lower N_{aa}, PR, and DH (Nollet & Toldrá 2010).

Subsequently, based on the results obtained for DF or NDF materials and enzyme concentration, an alcalase concentration of 0.8% and NDF material were chosen for the next experiment.

Effects of enzymatic hydrolysis treatments on efficiency hydrolysis. The effects of the enzymatic hydrolysis treatments on N_{aa} content (g L^{-1}), PR (%), and DH (%) were determined (Table 1). As shown in Table 1, N_{aa} was significantly influenced by the enzyme type and differences in enzymatic hydrolysis treatments. When only one type of enzyme was used for hydrolysis in a single hydrolysis treatment, the lowest N_{aa} in FPH was observed when minced SFH was hydrolyzed by flavourzyme. When fish protein samples were hydrolyzed by combining endoprotease and exoprotease, FPH had a higher N_{aa} than fish protein samples hydrolyzed by one enzyme. Furthermore, the FPH produced using sequential (adding alcalase for the first hydrolysis and flavourzyme for the second hydrolysis stage) treatment had the highest N_{aa} among all the enzymatic hydrolysis treatments.

Table 1 shows the effect of enzymatic hydrolysis treatments on the PR of SFH. No significant difference in protein recovery was observed in all samples treated with one type of enzyme in a single treatment (the PR was approximately 49%). As shown in Table 1, PR was significantly influenced by the simultaneous combination or sequential treatment of the two enzymes, and SFH protein treated sequentially (adding Alcalase for first hydrolysis followed by Flavourzyme for secondary hydrolysis) had the highest PR (66.5%).

DH is an important factor in the functional characterization of hydrolyzed proteins (Kristinsson & Rasco 2000). DH is significantly influenced by the enzyme type and process time (Noman et al 2019; Shahi et al 2020). As shown in Table 1, the lowest DH in the FPH was observed when fish protein samples were hydrolyzed by Protamex in a single treatment. Sequential (adding Alcalase for the first hydrolysis followed by Flavourzyme for secondary hydrolysis) treatment led to the highest DH (54.8%) in all enzymatic hydrolysis treatments. This can be explained by the degradation of the molecular weight distribution, and the formation of shorter protein peptides could be observed with increasing DH as a result of the breakage of the peptide bonds and the increase in the amount of free amino acids (Yathisha et al 2022). These results are in agreement with those of Fan et al (2012), Ji et al (2013), Slizyte et al (2018) and Noman et al (2019), who reported the effect of various proteases on the DH of tilapia frame protein (including Properase E, Pepsin, Trypsin, Flavourzyme, Neutrase and Papain), small yellow croakerfish muscle (hydrolyzed by Alcalase, Protamex and Papain), salmon backbones (using Corolase PP, sea-B-zyme, Protamex, Trypsin, Papain and Bromelain) and hydrolysate from Chinese sturgeon (with Alcalase), respectively.

Table 1

N_{aa}, PR and DH of FPH by different enzymatic hydrolysis treatments

Enzyme treatments	*N_{aa} (g L^{-1})*	*PR (%)*	*DH (%)*
Alcalase	12.7 ± 0.17^{c}	49.1 ± 1.30^{d}	36.9 ± 0.90^{cd}
Flavourzyme	6.49 ± 0.08^{d}	49.9 ± 0.92^{d}	39.1 ± 1.10^{d}
Protamex	12.5 ± 0.25^{c}	48.5 ± 0.90^{d}	33.8 ± 0.45^{f}
Alcalase+flavourzyme	13.3 ± 0.14^{b}	64.0 ± 1.08^{b}	49.8 ± 0.98^{b}
Protamex+flavourzyme	13.5 ± 0.32^{b}	57.2 ± 1.02^{c}	41.0 ± 0.90^{c}
Alcalase→flavourzyme	14.7 ± 0.08^{a}	66.5 ± 1.23^{a}	54.8 ± 1.05^{a}
Protamex→flavourzyme	13.1 ± 0.21^{b}	49.3 ± 0.80^{d}	37.1 ± 0.40^{e}

Results are means±standard deviation (n = 3); different superscripts (a-e) within a column are significantly differences among sample ($p < 0.05$).

The quality of fish protein hydrolysate powder. The proximate composition, microorganism and heavy metal content of FPH powder from SFH are shown in Table 2.

Table 2

Proximate composition, microorganism and heavy metal content of fish protein hydrolysate powder from snakedhead head

Parameters	*Value*	*Microorganism*	*Value*
Moisture (%)	4.47	Total aerobic bacteria (cfu g^{-1})	$7.7x10^{2}$
Protein (%)	84.6	Coliforms (cfu g^{-1})	ND
Lipid (%)	2.62	*E. coli* (cfu g^{-1})	ND
Ash (%)	4.61	*Staphylococcus aureus* (cfu g^{-1})	ND
		Salmonella sp.(cfu $25g^{-1}$)	ND
		Total of spore yeasts and moulds (cfu g^{-1})	ND
Lead (Pb) (mg kg^{-1})	0.137		
Cadmium (Cd) (mg kg^{-1})	ND		
Arsenic (As) (mg kg^{-1})	1.084		
Copper (Cu) (mg kg^{-1})	0.472		

ND: not detected.

Table 2 shows that the protein, moisture, ash, and fat contents of the FPH powder were 84.6%, 4.47%, 4.61%, and 2.62%, respectively. The protein content observed in this study was in the range of that of an ideal FPH powder (average of 60–90%) (Siddik et al 2021). The high protein content was due to the solubility of proteins during hydrolysis, the removal of insoluble proteins, and the partial removal of lipids after hydrolysis

(Thiansilakul et al 2007). Moreover, the protein content of FPH powder (84.6%) was relatively high compared to that reported for tilapia by-products, tilapia processing by-products, and catfish frames, which contained protein contents ranging from 44.8 to 65.05% (Amiza et al 2011; Roslan et al 2014; De Paris et al 2016). In addition, the ash content of the FPH powder was low (4.61%). Furthermore, the low fat content of the FPH powder was due to the removal of the fat layer after hydrolysis. FPH powders with lipid content below 5% have been reported by many studies (Kristinsson & Rasco 2000; Bhaskar et al 2008; Wasswa et al 2008; Ovissipour et al 2009). The FPH powder quality may be enhanced by the low lipid content in the FPH powder, which may be due to the stability of the product against lipid oxidation (Tawali et al 2018).

Moisture and lipid content are important for maintaining the quality of FPH powder during storage. The growth of microorganisms and the possibility of lipid hydrolysis may increase with high moisture content in FPH powder, leading to reduced product quality and safety (Tawali et al 2018). Most studies have reported that FPH powders from various fish byproducts have a moisture content of < 10% (Kristinsson & Rasco 2000; Bhaskar et al 2008; Wasswa et al 2008; Ovissipour et al 2009; Chalamaiah et al 2012). The moisture content in the FPH powder from SFH was low (4.47%). Low moisture content can improve the quality and prolong the shelf life of products (Tawali et al 2018).

Microbiological analysis confirmed that the FPH powder reached food safety with no detection of pathogenic bacteria such as coliforms, *E. coli*, *Staphylococcus aureus*, *Salmonella* sp., or total spore yeasts and molds. This result was in agreement with that of De Paris et al (2016), who also found that FPH powder from tilapia processing byproducts did not contain total coliforms, *Salmonella* sp., or *E. coli.* In addition, contents of heavy metal, such as lead, cadmium, and arsenic, in the FPH powder were all below the allowed limit. These analyses followed the adequacy of the parameters employed for foods intended for human consumption as determined according to Vietnamese standards.

The amino acid composition of FPH powder from snakehead fish is shown in Table 3. The FPH powder had a high flavor enhancer content because it was rich in glycine, glutamic acid, alanine, and proline, which accounted for 15.41%, 8.701%, 8.276%, and 6.915% of the total amino acids, respectively. The concentration of amino acids in the FPH powder prepared in this study was higher than that of the protein hydrolysate obtained from the cobia frame and tilapia by-products (Amiza et al 2012; Roslan et al 2014), but still lower than that of the FPH powder from the catla viscera and tuna head (Bhaskar et al 2008; Ovissipour et al 2010). Additionally, the ratio of total essential amino acids to total amino acids (TEAA/TAA) in the FPH powder from SFH was 30.47%, indicating the potential of hydrolysates to serve as a useful source of nutrition (Prihanto et al 2019). Moreover, the essential amino acid content observed in this study was in the range of that of an ideal FPH powder (average of 10-50%) (Amiza et al 2012; Bougatef et al 2012; Prihanto et al 2019). Table 3 shows that most essential amino acids in the FPH powder exceeded the recommended values for human adults (WHO/FAO/UNU 2007). This result agrees with the findings of Bougatef et al (2012), Roslan et al (2014), and Prihanto et al (2019), who reported that the essential amino acid composition of FPH powder was higher than the recommended value for human adults. In addition, the amounts of histidine and isoleucine in 100 g of FPH powder from SFH were higher than the daily recommended intake (DRI) for children, whereas leucine, lysine, methionine, phenylalanine, threonine, and valine contents were lower than the DRI for children. Therefore, FPH powder from SFH can potentially be used as a functional food for protein supplementation in humans because of amino acid content which satisfies human nutritional requirements.

Table 3

Amino acid composition of fish protein hydrolysate (FPH) powder

Amino acids	*FPH powder (%)*	*Daily recommendation WHO/FAO/UNU***	
		Child (g)	*Adult (g)*
Essential			
Histidine*	1.285	1.09	1.60
Isoleucine*	3.104	2.80	1.30
Leucine*	4.780	6.60	1.90
Lysine*	4.727	5.80	1.60
Methionine*	1.643	2.70	1.70
Phenylalanine*	2.307	6.30	1.90
Valine*	3.389	3.50	1.30
Threonine*	3.326	3.40	0.90
Tryptophane*	-		
Non-essential			
Alanine	8.276		
Arginine	4.581		
Aspartic acid	5.555		
Cystine	0.505		
Glutamic acid	8.701		
Proline	6.915		
Serine	4.203		
Glycine	15.41		
Tyrosine	1.880		
TAA	80.587 (g/100g)		
TEAA	24.561 (g/100g)		
TEAA/TAA	30.47%		

TAA: Total acid amin, TEAA: Total essential amino acids; *Essential amino acids, ***WHO/FAO/UNU, 2007.

Conclusions. Fish protein hydrolysate (FPH) from non-defatted material hydrolyzed at 0.8% alcalase concentration had the highest values of N_{aa}, PR, and DH as well as low N_{NH3} and lipid content. Moreover, Alcalase, Protamex, and Flavourzyme single, simultaneous combined, and sequential hydrolysis treatments successfully hydrolyzed snakehead fish head (SFH) into fish protein hydrolysate (FPH). Of the three enzymes used in the single-hydrolysis treatment, Alcalase produced the highest levels of N_{aa}, PR, and DH. Sequential hydrolysis treatments made by adding Alcalase first, followed by Flavourzyme, significantly increased N_{aa}, PR, and DH compared to the single treatment. Hence, sequential hydrolysis treatments involving the addition of Alcalase followed by Flavourzyme were used to hydrolyze SFH into FPH and produce FPH powder. FPH powder from SFH contains high protein content, low fat levels, is rich in essential amino acids, has no detected pathogenic bacteria, and has a low heavy metal content, making it a potential ingredient in food applications. Enzymatic hydrolysis of SFH has the potential to convert low-value materials into high-value functional products.

Conflict of interest. The authors declare that there is no conflict of interest.

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