Antioxidant Featherback (*Chitala ornata*) Head Protein Hydrolysate with Foaming and Emulsifying Capacities

Tam Dinh Le Vo[1*], Bao Ngoc Nguyen[1], Nhi Ngoc Yen Luong[1], Son Truong Nguyen[2], Quang Thanh Duy Nguyen[3], Nguyen Van Tran[1], Phuong Thi Bich Vu[4], Dung Van Nguyen[2], Tin Phuc Vo[5], Khoa Trong Pham[6], Toan Duc Vu[1], Nhu Thi Quynh Pham[1], Huy Nguyen Nhat Le[1], Thao Phuong Nguyen[1], Nghi Phuong Truong[1], and Bao Chi Vo[1]

[1]*Department of Food Technology, Faculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), VNU-HCM, Ho Chi Minh City 700000, Vietnam*

[2]*Department of Physicochemical & Analytical Engineering, Faculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), VNU-HCM, Ho Chi Minh City 700000, Vietnam*

[3]*Department of Gas and Oil Processing Engineering, Faculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), VNU-HCM, Ho Chi Minh City 700000, Vietnam*

[4]*Ho Chi Minh City University of Technology (HCMUT), VNU-HCM, Ho Chi Minh City 700000, Vietnam*

[5]*Department of Biomedical Engineering, Faculty of Applied Science, Ho Chi Minh City University of Technology (HCMUT), VNU-HCM, Ho Chi Minh City 700000, Vietnam*

[6]*School of Biosciences, University of Sheffield, Sheffield S10 2TN, United Kingdom*

* ***Corresponding author:***

tel: +84-798967699
email: vdlt@hcmut.edu.vn

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Abstract: *Featherback fish (*Chitala ornata*) paste production discards fish heads with a notable protein content of 27.99 ± 0.68% (dry weight basis), showing a potential for using fish heads as a natural source of bioactive hydrolysate generation. This study utilized the fish heads for hydrolysis reaction using Alcalase to obtain a protein hydrolysate with antioxidant activity and foaming and emulsifying capacities. The best Alcalase hydrolysis condition, was determined using a single-factor test, included a fish head to water ratio of 1:4 (w/v), an E:S ratio of 60 U/g protein, and a hydrolysis duration of 3 h. The obtained hydrolysate had a hydrolysis degree (DH) of 38.64 ± 0.65%, 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH$^{\bullet}$) scavenging activity of 88.73 ± 0.38%, and superoxide anion radical ($O_2^{\bullet-}$) scavenging capacity of 70.06 ± 2.74%. The hydrolysate was rich in Glu and Asp, making up approximately 25% of the hydrolysate's total amino acid content. Within a pH range 3–8, the hydrolysate displayed maximum foaming capacity at pH 3, whereas its emulsifying capacity peaked at pH 8. These findings indicated promising applications of featherback head protein hydrolysate in food development, as a natural antioxidant, foaming agent and emulsifier.*

Keywords: *antioxidant activity; featherback head; protein hydrolysate; foaming capacity; emulsifying capacity*

■ INTRODUCTION

Fish processing factories generate a significant amount of by-products, including heads, fins, scales, bone, and trimming meat [1]. The recovery of protein from these sources has attracted significant attention from many scientists due to increased protein demand and the limited availability of natural protein resources [2]. Valorization of fish processing by-products to generate protein hydrolysates with a high safety profile and diverse biological activities is promising [3]. The protein hydrolysate includes protein components,

specifically, amino acids and peptides, resulting from protein hydrolysis catalyzed by different agents [4]. Enzymatic hydrolysis is generally preferred because it not only retains protein nutritional value but also increases bioactivities and functional properties of the resulting products [1,5]. Protein hydrolysates are applied in the food industry, pharmaceuticals, cosmetics, and so on, especially as natural antioxidants [6]. Antioxidants scavenge free radicals, protecting the human body from oxidative stress and its related diseases (cancer, cardiovascular diseases, metabolic disorders, neurological pathology, and immunodeficiency) [7-8]. Singh et al. [8] reported that antioxidant protein hydrolysate can donate either its electrons or hydrogen atoms to quench free radicals. For food products, antioxidants terminate oxidation reaction chains, hindering the generation of potentially toxic substances [9]. Many studies regarding antioxidant protein hydrolysates and peptides from diverse fish processing by-products have been published, including bighead carp viscera and rainbow trout viscera [10], swordfish head [2], snakehead fish head [11], white-cheek shark skin [1], and yellowfin tuna skin [7]. According to Karnila et al. [4], antioxidant peptides from various fish protein hydrolysates are different in amino acid composition and sequence.

Protein-based foaming and emulsifying agents are widely used in the food industry owing to their amphiphilic characteristics, which facilitate the orientation of the protein molecules at air-water and oil-water interfaces, thereby reducing interfacial tension and forming a film around air bubbles or oil droplets [12]. However, the tight structure of protein with hydrophobic groups being hidden inside and inadequate flexibility hinders the adsorption of intact protein molecules at interfaces, lowering their foaming and emulsifying capacities [13]. Partial enzymatic hydrolysis modifies protein molecules by cleaving the peptide bonds in the protein chain, generating low-molecular-weight peptides and exposing previously hidden hydrophobic and charged residues [14]. These changes improve the peptide's solubility, promoting their diffusion and rearrangement at the phase boundary, thus enhancing their surface activity [14-15]. Fish protein hydrolysates with elevated foaming and emulsifying features have been generated from various sources. For instance, Elgaoud et al. [2] reported that the foaming capacity (FC) and foaming stability (FS) of swordfish head proteins were 21.35 and 7.42%, increasing to 35.07 and 26.42%, respectively, after the fish head proteins were hydrolyzed by Alcalase. In addition, the Alcalase hydrolysate of the swordfish head exhibited an emulsifying activity index (EAI) of 31.75 m^2/g and emulsifying stability index (ESI) of 19.58 min, 1.14 and 1.38 times higher than those of the fish head proteins, respectively [2]. Furthermore, Alcalase hydrolysate of skipjack tuna head demonstrated FC and FS exceeding 60% at pH 2 [16]. Moreover, EAI and ESI of Alcalase hydrolysate of white-cheek shark skin gelatin were 63.8 m^2/g and 20 min, respectively [1]. Additionally, bigeye tuna by-product hydrolysates produced by HCl-catalyzed hydrolysis and *Lactobacillus plantarum* fermentation expressed FC of 50 and 30%, respectively [17]. Besides, tilapia skin protein hydrolysates, prepared by fermentation with *Bacillus subtilis* L4, demonstrated a remarkable EAI, which was beyond 50 m^2/g [18].

In this study, enzymatic hydrolysis was performed to produce an antioxidant protein hydrolysate with foaming and emulsifying capacities from featherback (*Chitala ornata*) head, a by-product from featherback fish cake processing factories. Proximate chemical composition of the fish head was first analyzed. Then, the hydrolysis condition, comprising the fish head to water ratio, E:S ratio, and hydrolysis time, was examined to determine the suitable hydrolysis parameters for obtaining the fish head hydrolysate with the highest antioxidant activity. Subsequently, the gained hydrolysate was evaluated for its DH, amino acid composition, foaming and emulsifying characteristics. The antioxidant activity was measured using two free radical scavenging methods of $DPPH^{\bullet}$ and $O_2^{\bullet-}$, representing for two typical antioxidant mechanisms of single electron transfer and hydrogen atom transfer, respectively [19-20]. This is the very first study on the antioxidant protein hydrolysate from featherback fish heads with foaming and emulsifying capacities, which may be served as an ingredient for functional foods or nutraceuticals development.

■ EXPERIMENTAL SECTION

Materials

Featherback heads, collected from a local market in Ho Chi Minh City, were washed, drained and ground. The minced fish head was stored at −20 °C in sealed polyamide packages until used. Alcalase® 2.5L from Novozymes (Denmark), and analytical grade chemicals (DPPH, pyrogallol, tris(hydroxymethyl)aminomethane, ascorbic acid, albumin, sodium dodecyl sulfate, sodium caseinate) from Sigma-Aldrich, Merck, and Biobasic were utilized in this research.

Instrumentation

An UV-vis spectrometer (UV-VIS 752, China), a water bath (Memmert WB14-Germany), and a freeze-dryer (Alpha 1–2/Ldplus, UK) were used in this study.

Procedure

Analysis of chemical composition of the featherback head

Methods from the Association of Official Analytical Chemists (AOAC International) [21] were used for the determination of moisture (AOAC 950.46B), protein (AOAC 990.03), lipid (AOAC 920.39), and ash (AOAC 920.15) contents of the featherback head.

Alcalase hydrolysis of featherback head

Featherback head was hydrolyzed according to the procedure of Vo et al. [22]. The ground fish head was dispersed in distilled water with a ratio in the range from 1:2 to 1:6 (w/v). Deactivation of endogenous enzymes in the fish head was done by heating the mixture 90 °C for 10 min. Following the adjustment of the mixture's pH to 7.5, Alcalase preparation was added with an E:S ratio of 60 U/g protein and the hydrolysis process was performed at 55 °C for 3 h. Subsequently, the Alcalase hydrolysis was terminated by exposing the reaction mixture to a heat treatment at 90 °C for 10 min. The featherback head protein hydrolysate (FHPH) as the supernatant was collected by centrifugation, then lyophilized, and its antioxidant activity was determined.

The effect of a hydrolysis condition on the antioxidant activity of the FHPH was investigated using a single-factor experimental design. Firstly, the fish head-to-water ratio was varied from 1:2 to 1:6 (w/v), while the E:S ratio and hydrolysis time were fixed at 60 U/g protein and 3 h, respectively. The fish head-to-water ratio that produced the FHPH with the highest antioxidant activity was selected for subsequent experiments. Subsequently, the E:S ratio was used in the range from 30 to 70 U/g protein, while the selected fish head to water ratio from the previous investigation and a hydrolysis time of 3 h were maintained. The E:S ratio, at which the antioxidant activity of the FHPH reached a peak, was chosen for further studies. Finally, the hydrolysis time was prolonged from 1 to 5 h, while the fish head-to-water ratio and E:S ratio were fixed at previously determined values. The hydrolysis time yielding the FHPH with the maximum antioxidant activity was used for subsequent experiments. After that, the hydrolysis was implemented under the chosen condition to gain the FHPH with the highest antioxidant activity. The hydrolysate's characteristics were further evaluated, including DH (based on the formol titration method [23]), amino acid profile, and foaming and emulsifying capacities.

Determination of DPPH$^{\bullet}$ scavenging activity

DPPH$^{\bullet}$ scavenging activity of the FHPH was examined employing the method of Vo et al. [24]. A sample, including 1 mL of the hydrolysate (1 mg/mL in distilled water) and 2 mL of 0.06 mM DPPH solution prepared in 99.5% (v/v) methanol was incubated at ambient temperature in the dark for 30 min. Blank and control (distilled water and 99.5% (v/v) methanol solution replacing the hydrolysate and DPPH solution, respectively) were prepared at the same time as the sample. After the incubation, absorbances at 517 nm of the sample, control and blank were recorded, which were set for variables of A_s, A_c and A_b, in order. DPPH$^{\bullet}$ scavenging activity of the hydrolysate was then calculated via Eq. (1).

$$\text{DPPH}^{\bullet}\text{scavenging activity}(\%) = \frac{A_b - (A_s - A_c)}{A_b} \times 100\% \quad (1)$$

The antioxidant activity of a standard (vitamin C) was determined using the identical method.

Determination of $O_2^{\bullet-}$ scavenging activity

The $O_2^{\bullet-}$ scavenging activity was performed following the method of Vo et al. [24]. After being

incubated at 25 °C for 20 min, Tris-HCl (50 mM)-ethylene diamine tetraacetic acid (1 mM) buffer pH 8.2 was mixed with 0.2 mL of the FHPH (1 mg/mL in distilled water). Pyrogallol solution (0.1 mL, 5 mM) was added to the mixture, whose absorbance at 320 nm was then measured at 30 s intervals throughout 4 min. A blank, in which the hydrolysate was replaced with distilled water, was simultaneously prepared. The hydrolysate's capacity for scavenging $O_2^{\bullet -}$ was determined using Eq. (2):

$$O_2^{\bullet -}\text{scavenging activity}(\%) = \frac{\Delta A_0/\text{min} - \Delta A_s/\text{min}}{\Delta A_0/\text{min}} \times 100\% \quad (2)$$

where ΔA_0/min and ΔA_s/min denote the change in absorbance per min of the blank and the sample, in order. The same procedure was conducted to determine the activity of vitamin C (standard).

Amino acid composition analysis

The procedure of Vo et al. [23] was applied to analyze the amino acid composition of the FHPH. Firstly, protein components in the hydrolysate were completely hydrolyzed into free amino acids using an acidic hydrolysis catalyzed by a 6 M HCl solution at 110 ± 2 °C for 23 h. The resulting mixture was submitted to an ion-exchange chromatography in which individual amino acids were separated. They were then reacted with Ninhydrin and the absorbances of the reaction products were measured at 440 and 570 nm. The absorbance at 440 nm was compared to that of standard proline solutions to quantify its content in the hydrolysate, whereas the absorbances at 570 nm were used to determine concentrations of other amino acids.

Determination of foaming capacity

Foaming capacity of the FHPH was examined according to our published method [25]. pH of the hydrolysate (20 mL, 10 mg/mL in distilled water) was adjusted in the range from 3 to 8 using 1 M HCl solution or 1 M NaOH solution. The hydrolysate was then foamed for 1 min using a handheld whisk-type frother (RoHS, China) and its volume was measured after 30 s. The frothed sample was left at 20 °C for 3 min and its volume was recorded again. FC and FS of the hydrolysate were determined using Eq. (3) and (4):

$$\text{FC}(\%) = \frac{A - B}{B} \times 100\% \quad (3)$$

$$\text{FS}(\%) = \frac{A_t - B}{B} \quad (4)$$

where B (mL), A (mL), and At (mL) denote the initial volume of the hydrolysate, the volume of the sample after frothing, and the volume of the frothed sample after 3 min, leaving at 20 °C, respectively. Albumin was considered a standard whose foaming capacity was estimated using the same protocol.

Determination of emulsifying capacity

The emulsifying capacity of the FHPH was evaluated using EAI and ESI, which were determined according to the method described in our previous study [25]. pH of a mixture of the hydrolysate (15 mL, 10 mg/mL in distilled water) and vegetable oil (5 mL) was adjusted to a required value between 3 and 8 using 0.1 M HCl or 0.1 M NaOH solution. The mixture was subsequently homogenized for 1 min to gain an emulsion. From the bottom of the emulsion, an aliquot of 50 µL was taken at 0 and 10 min after the homogenization. The aliquot was dispersed in 4.95 mL of 1 mg/mL sodium dodecyl sulfate solution and absorbance at 500 nm of the dispersion was measured. EAI and ESI of the hydrolysate were calculated in Eq. (5) and (6):

$$\text{EAI}(\text{m}^2/\text{g}) = \frac{2 \times 2.303 \times A_0}{0.25 \times \text{protein weight(g)}} \quad (5)$$

$$\text{ESI}(\text{min}) = \frac{A_0 \times 10}{A_0 - A_{10}} \quad (6)$$

where A_0 and A_{10} represent the absorbances of the samples collected at 0 and 10 min after homogenization, respectively. EAI and ESI of the standard, sodium caseinate, was also evaluated using the identical procedure.

Data analysis

Data were processed using the Microsoft Excel 2024 application, and the results were presented as the average of triplicate experiments ± standard deviation. Statistically significant differences were determined using the Tukey method ($p < 0.05$) in Statgraphics Centurion 18.

■ RESULTS AND DISCUSSION

Proximate Chemical Composition of the Featherback Head

The featherback head is composed of 45.33 ± 1.93% moisture, 27.99 ± 0.68% protein, 11.52 ± 2.22% lipid, and

9.40 ± 1.48% ash (on a dry weight basis). Fish processing by-products with protein content from 14.29 to 71.90% (on a dry weight basis) have been exploited to produce protein hydrolysates with antioxidant activity [2,11,26]. The fish head in this research was enzymatically hydrolyzed to obtain an antioxidant protein hydrolysate.

Effect of Hydrolysis Condition on Antioxidant Activity of the FHPH

Bioactivities of protein hydrolysates are affected by molecular weight, amino acid composition and sequence of their peptides, which in turn depends on protease type and condition employed in the hydrolysis process [9,27]. Alcalase, a serine endopeptidase obtained from *B. subtilis*, cleaves peptide bonds inside protein molecules, generating hydrolysates with a great content of small peptides [28]. As reported by Singh et al. [8] and Wardani et al. [7], small peptides highly expose electron and hydrogen atom donating residues, easily quenching free radicals, thus improving the antioxidant activity of the containing hydrolysates. In addition, the Alcalase typically releases hydrophobic peptides because of its high specificity towards hydrophobic residues, particularly Phe, Val, Leu, and Ile [29]. The effect of these hydrophobic amino acids on the antioxidant activity of peptides was published by Hamed et al. [9] and Hashem et al. [3]. Specifically, among ten peptide fractions separated from annular seabream muscle protein hydrolysate, the fraction F4, containing 408 peptides with their hydrophobic amino acid to total amino acid ratios exceeding 50%, exhibited the highest DPPH$^{\bullet}$ scavenging activity of 97.28% [9]. The findings of Hashem et al. [3] indicated that hydrophobic amino acids (Leu, Gly, Pro, Ala, Met), comprising of 42.85 to 60.00% of the total of two antioxidant peptides identified from red-bellied pacu meat protein hydrolysate, contribute to the peptide's antioxidant activity by enhancing the peptide interactions with hydrophobic free radicals. Besides, various protein sources were converted into protein hydrolysates with elevated antioxidant activity using Alcalase hydrolysis. In particular, Dhanabalan et al. [30] tested the DPPH$^{\bullet}$ scavenging activity of small shrimp (*Acetes* sp.) protein hydrolysates with various DH ranging from 5 to 25%, prepared using four distinct enzymes, including Alcalase, pepsin, trypsin, and papain. Among them, all Alcalase hydrolysates demonstrated the highest DPPH$^{\bullet}$ scavenging activity. Moreover, the studies of Hamed et al. [9] and Tadesse et al. [26] showed that Alcalase protein hydrolysates (derived from annular seabream muscle and ray-finned fish)'s DPPH$^{\bullet}$ scavenging activity increased by approximately 2 and 5 times, respectively, compared to the activity of corresponding unhydrolyzed proteins. Furthermore, mudskipper fish meat protein hydrolysates with three different Alcalase concentrations (0.5, 1.5, and 2.5% of the fish meat weight) expressed DPPH$^{\bullet}$ scavenging activity beyond 80% [4]. In addition, as reported by Singh et al. [8], shrimp shell hydrolysate (using Alcalase) with a higher DH (30%) resulted in a 2.1-fold increase in DPPH$^{\bullet}$ scavenging activity, compared with that of the hydrolysate with a lower DH (20%). Therefore, Alcalase hydrolysis was also employed in this study.

Regarding the selection of hydrolysis conditions (Fig. 1), the greatest scavenging activity against both tested free radicals (DPPH$^{\bullet}$ and $O_2^{\bullet-}$) of the FHPH was observed at the hydrolysis condition including a 1:4 (w/v) ratio of the fish head to water, a 60 U/g protein of E:S ratio, and 3 h of hydrolysis. Suitable hydrolysis conditions promote the release of bioactive peptides in the hydrolysates, elevating the antioxidant activity. Low solubility of protein limits accessibility of enzymes to protein molecules, reducing reaction yield [22,31]. Meanwhile, a sufficient E:S ratio and hydrolysis time can prevent excessive degradation of bioactive peptides, generating inactive fragments, or obstructing enzyme aggregation or competitive inhibition [5,32]. Inadequate enzyme amount lowers the reaction rate, yielding hydrolysates with low antioxidant activity [32]. Previous publications also showed that the antioxidant activity of protein hydrolysates peaked only under suitable hydrolysis conditions. For example, Xiao et al. [6] documented that within the tested material to water ratios of 5 to 25% (w/v), E:S ratios of 1000 to 9000 U/g, and hydrolysis times of 2 to 6 h, DPPH$^{\bullet}$ scavenging activity of sika deer antler tips protein hydrolysates peaked at 15% (w/v) ratio of material to water, 5000 U/g E:S ratio and 3 h of hydrolysis. Similarly, the highest

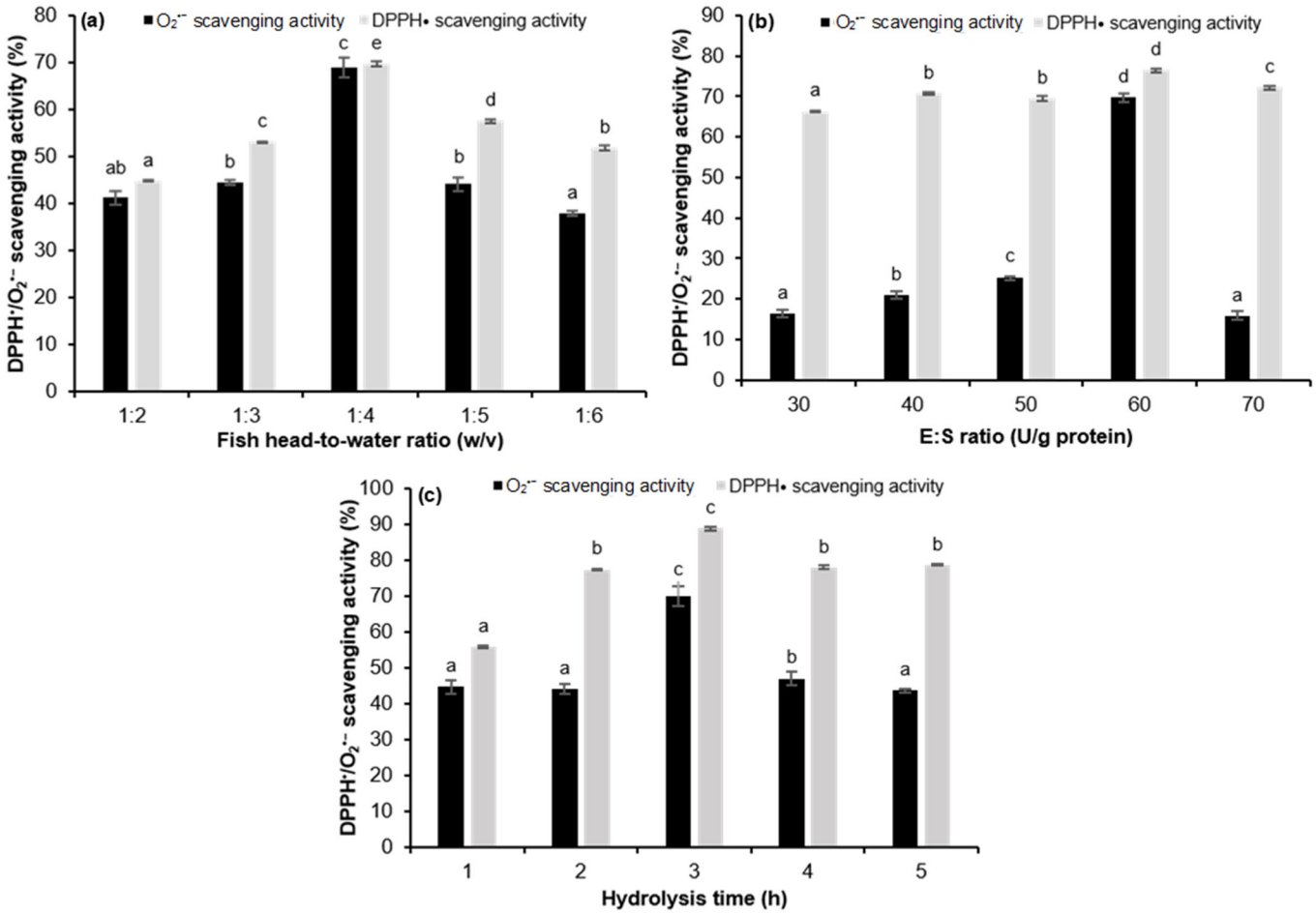

Fig 1. Influence of (a) featherback head to water ratio, (b) E:S ratio, and (c) hydrolysis time on antioxidant activity of the FHPH. Different letters presented on the same color bars denote significant differences (p <0.05)

DPPH• scavenging activity of red-bellied pacu fish meat protein hydrolysate was obtained at E:S ratio of 1.5% (w/v), while lower activities were observed at both lower (0.5% (w/v)) or higher (2.5% (w/v) E:S ratios [4]. DPPH• scavenging activity of flaxseed meal protein hydrolysate also reached the peak at hydrolysis time of 120 min, then declined with prolonged hydrolysis to 200 min [5]. Moreover, both DPPH• and $O_2^{\bullet-}$ scavenging activity of sheep plasma protein hydrolysate increased along with the augmentation of hydrolysis time from 1 to 4 h, but no significant differences were observed afterward [33].

As seen in Fig. 1, the FHPH's DPPH• scavenging activity was 86.74 ± 0.53% and its $O_2^{\bullet-}$ scavenging activity was 70.06 ± 2.74%. Compared to unhydrolyzed fish head, Alcalase hydrolysis significantly enhanced antioxidant activity of the hydrolysate, with 7.62-fold increase in DPPH• scavenging activity and 6.47-time increase in $O_2^{\bullet-}$ scavenging activity. This could be due to the generation of smaller peptides, exposure of hydrophobic and charged residues [7,14]. The DPPH• scavenging activity of the FHPH in this study was greater than that of hydrolysates derived from mudskipper fish meat (85.57%) [4], yellowfin tuna skin (56.74 ± 2.5%) [7], snakehead fish head (50.70%) [11], and white-cheek shark skin (56.8%) [1]. Similarly, its $O_2^{\bullet-}$ scavenging activity of the FHPH was 3.5, 2.8, and 1.8 times higher than that of deer antler tips hydrolysate [6], sheep plasma hydrolysate [33], and squid pen hydrolysate [34], in order. It could be attributed to the higher DH of the FHPH (DH = 38.64 ± 0.65%), compared to those hydrolysates with lower DH (19.43–27.39%). This might be explained by the fact that the FHPH may contain a greater amount of

small peptides, improving its availability of H-atom donating groups, leading to its higher scavenging activity against $O_2^{\bullet-}$ [8]. Compared to a popular antioxidant used in food products, DPPH$^\bullet$ scavenging activity and $O_2^{\bullet-}$ scavenging activity of the FHPH were 1.07-fold and 1.38-fold lower than those of vitamin C, respectively. The enhanced antioxidant activity highlighted the potential of the FHPH as an effective antioxidant.

Amino Acid Profile of the FHPH

Table 1 shows that the FHPH was abundant in negatively charged amino acids (Glu and Asp), accounting for 24.07% the hydrolysate's total amino acids. These amino acids play a key role in the palatability and digestibility of foods [35]. Chemically, antioxidant peptide's free paired electrons on O-carboxyl in side chains act as electron donors, quenching free radicals [10,36]. Whereas hydrogen atom transfer capacity of the hydrolysate could be ascribed to the presence of His, specifically its imidazole ring, and Tyr, particularly its phenolic hydroxyl groups. His and Tyr, donation of hydrogen atoms, become new stable radicals owing to their resonance structures on which the newly formed unpaired electron was effectively distributed [8,37]. Moreover, the high ratio of hydrophobic amino acids in the hydrolysate may improve the solubility of the peptides in lipids, facilitating interactions between bioactive groups and lipid-soluble free radicals [36]. The hydrophobic amino acid to total amino acid ratio of the FHPH in this study achieved 50.54% (Table 1), which was higher than that of Alcalase hydrolysates derived from *Chlorella* (43.4%) [35], horse bone marrow (40.8%), and mudskipper fish meat (45.7%) [4]. This higher ratio might result in the stronger scavenging activity of the FHPH against DPPH$^\bullet$ (86.74 ± 0.53%), a lipophilic free radical, compared to those hydrolysates whose activities ranged from 55.73 to 85.57% [4,35,38].

Foaming Capacity of the FHPH

Proteins containing both hydrophilic and hydrophobic residues could migrate and be absorbed on the gas-liquid interface, followed by a rearrangement of their structure at the interface, promoting the formation and stabilization of foam [39]. The foaming capacity of

Table 1. Amino acid composition of the FHPH

Amino acid	Content (mg/100mL)	Ratio (to total amino acids) (%)
Ala	133.02	7.89
Arg	119.31	7.07
Asp	137.56	8.16
Glu	268.36	15.91
Gly	240.82	14.28
His	22.72	1.35
Ile	68.75	4.08
Leu	90.80	5.38
Lys	116.97	6.94
Met	24.09	1.43
Phe	44.27	2.62
Pro	180.08	10.68
Ser	66.58	3.95
Thr	63.73	3.78
Tyr	39.00	2.31
Val	70.55	4.18
Essential amino acids (His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Val)	501.88	29.76
Hydrophobic amino acids (Ala, Gly, Ile, Leu, Met, Phe, Pro, Val)	852.38	50.54
Total amino acids	1686.61	

protein plays an important role in taste, texture and visual of aerated foods such as meringue, milkshakes, baked foods, nougat and ice cream [13,15]. This capacity is typically evaluated via FC (reflecting the protein's ability to diffuse and be absorbed on the air-water interface to form a film surrounding the foam), and FS, denoting the protein's capacity to remain the foam after a specific time [40]. Both the FC and FS strongly depend on the pH of the protein solution, as reported by Zhang et al. [39]. Specifically, a change in environmental pH alters the ionized state of amino acid residues, impacting the protein's solubility, affecting their diffusion rate to the interface of air and water, thus altering the protein's FC [41]. Additionally, pH might unfold protein, exposing their hydrophobic amino acids, facilitating their absorption into gas phase and their hydrophobic interactions, promoting the formation of stable films around air bubbles, boosting the hydrolysate's foaming capacity [42]. In this study, as shown in Fig. 2, the FHPH demonstrated the highest foaming capacity at pH 3, with FC of 60.33 ± 5.39% and FS of 31.67 ± 1.26%, which were 1.59 and 1.18 times better than those of albumin, respectively. These findings suggested that the hydrolysate could serve as a foaming agent in highly acidic foods such as yogurt cake and fruity cake [43].

Emulsifying Capacity of the FHPH

This study found that within the tested pH range from 3 to 8, both EAI and ESI of the FHPH gained their maximum of 8.15 ± 0.23 m^2/g and 41.16 ± 5.63 min, respectively, at pH 8 (Fig. 3). This result was consistent with our previous studies, regarding the emulsifying capacity of hydrolysates produced from small shrimp (*Acetes japonicus*) [24] and featherback skin [44]. It can be assumed that pH 8 generates negative charges on the surface of peptides in the FHPH, thereby enhancing repulsive forces among peptide-derived films covering oil droplets, preventing flocculation and coalescence [14]. In addition to the charge formation, alkaline pH might induce peptide molecules to unfold, favoring the orientation of their hydrophilic and hydrophobic regions at the oil-water interface [45]. These changes resulted in increased emulsifying capacity at pH 8. Compared to sodium caseinate, a common emulsifier used in the food industry, the FHPH's EAIs and ESI were 1.47–8.64 times lower. On the other hand, the FHPH showed a 3.84-fold and 2.72-fold higher EAI than Atlantic mackerel by-product hydrolysate [46] and trypsin hydrolysate from salmon milt [47], respectively. In addition, the ESI of FHPH was 2.74 and 50.19 times greater than those of sardine by-product [46] and abalone [40] hydrolysates, respectively. It might be ascribed to appropriate DH (38.64 ± 0.65%) and hydrophobic amino acid content (50.54% of total amino acids) of the FHPH, allowing its peptides to rapidly diffuse to the oil-water interface, effectively adhere and cover the interface, boosting the hydrolysate's EAI and ESI [14,48]. Hence, the FHPH could be considered as an

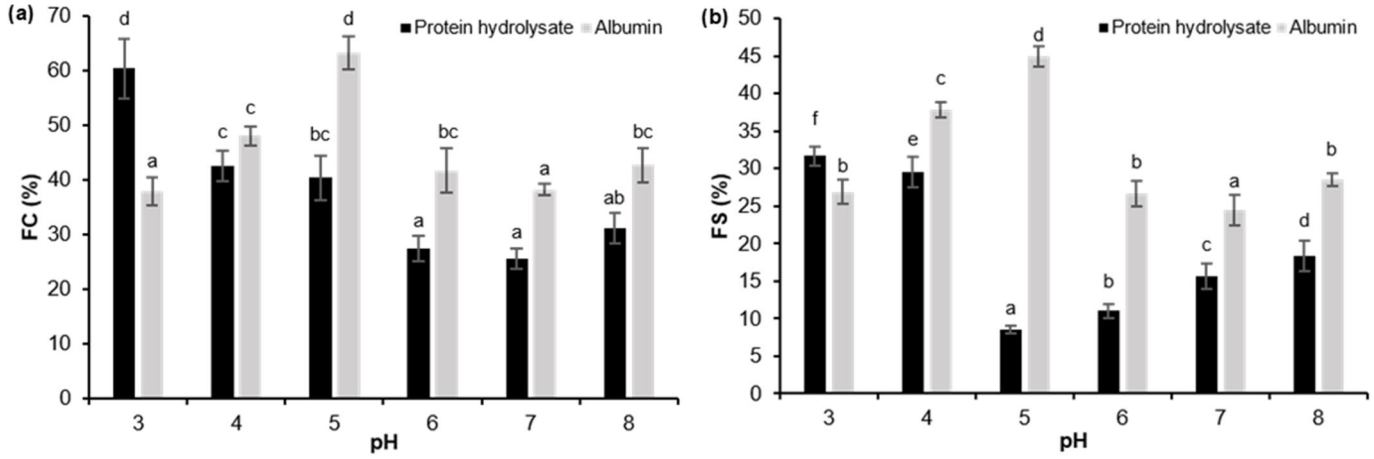

Fig 2. Foaming capacity of the FHPH. Different letters presented on the same color bars denote significant differences ($p < 0.05$)

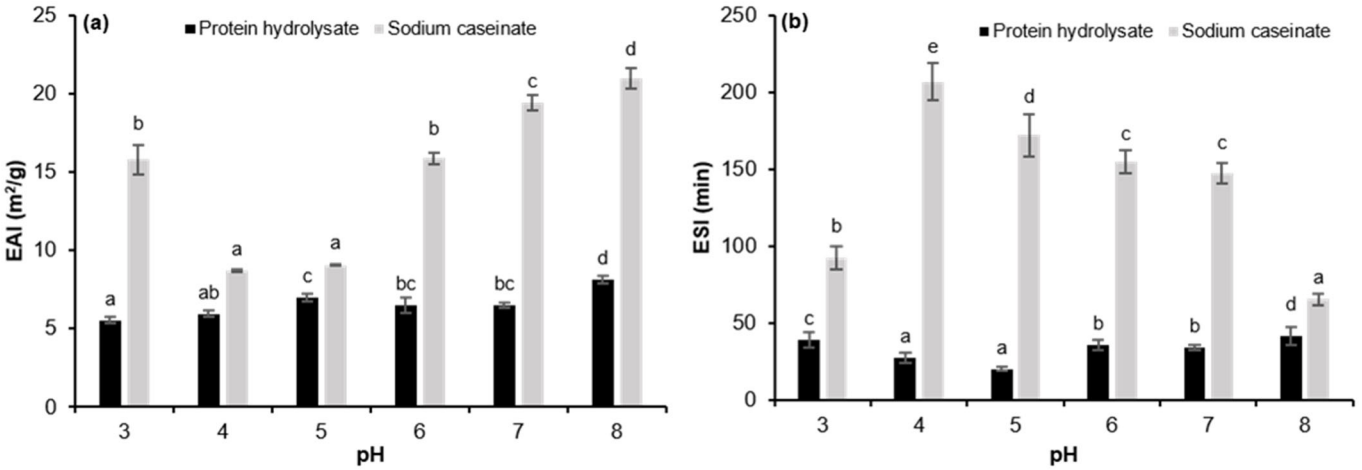

Fig 3. Emulsifying capacity of the FHPH. Different letters presented on the same color bars denote significant differences ($p < 0.05$)

emulsifier, used in mayonnaise, salad dressings, or processed meats.

■ CONCLUSION

This study suggested a promising way for utilizing the featherback head to generate an antioxidant protein hydrolysate with foaming and emulsifying capacities. The obtained FHPH demonstrated 86.74 ± 0.53% and 70.06 ± 2.74% scavenging activity for DPPH$^{\bullet}$ and $O_2^{\bullet-}$, respectively. Besides, its foaming properties at pH 3 were better than those of albumin, a common foaming agent used in foods. Additionally, the hydrolysate's emulsifying properties were greater than those of some marine-derived hydrolysates, such as sardine by-product and abalone hydrolysates. The enhanced antioxidant and interfacial properties of the FHPH could be attributed to its suitable DH (38.64 ± 0.65%), high amount of acidic amino acids (24.07% of total amino acids) and proper ratio of hydrophobic amino acids (50.54% of total amino acids). The hydrolysate could be fortified into some food products to generate functional foods or enhance some foods' properties. Further research could be performed, including amino acid sequencing of bioactive peptides, antioxidant mechanisms or food quality enhancement capacity.

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■ CONFLICT OF INTEREST

The authors have no conflict of interest.

■ AUTHOR CONTRIBUTIONS

Conceptualization, Funding acquisition, Project administration, Supervision, Writing-review and editing: Tam Dinh Le Vo; Visualization, Writing-original draft preparation: Bao Chi Vo; Data curation, Formal analysis: Tam Dinh Le Vo, Bao Chi Vo; Resources, Investigation: Tam Dinh Le Vo, Bao Ngoc Nguyen, Nhi Ngoc Yen Luong, Toan Duc Vu, Nhu Thi Quynh Pham, Huy Nguyen Nhat Le, Thao Phuong Nguyen, Nghi Phuong Truong; Validation: Tam Dinh Le Vo, Bao Chi Vo, Son Truong Quang Thanh Duy Nguyen, Nguyen Van Tran, Phuong Thi Bich Vu; Methodology: Tam Dinh Le Vo, Bao Chi Vo, Dung Van Nguyen, Tin Phuc Vo, Khoa Trong Pham. All authors agreed to the final version of this manuscript.

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