Mongolian Journal of Chemistry
www.mongoliajol.info/index.php/MJC

Production of iron-binding protein hydrolysate with foaming and emulsifying properties from featherback (*Chitala ornata*) skin

Tam Dinh Le Vo[1,2*], Hoai Duc Le[1,2], Anh Thi Hoang Nguyen[1,2], Nhi Ngoc Yen Luong[1,2], Tran Bao Vo[1,2], Van Thi Tuyet Nguyen[1,2], Hieu Trung Ma[1,2], Vy Thuy Pham[1,2], Dzung Nguyen Nam Phan[1,2], Linh Vy Truc Nguyen[1,2], Linh Thi Thuy Tran[1,2], Bao Chi Vo[1,2]

[1]*Department of Food Technology, Faculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City 700000, Vietnam.*
[2]*Vietnam National University Ho Chi Minh City, Linh Trung Ward, Thu Duc City, Ho Chi Minh City 700000, Vietnam.*

* *Corresponding author: vdlt@hcmut.edu.vn;* ORCID: https://orcid.org/0000-0001-8295-1165

Received: 3 June 2024; revised:19 November 2024; accepted: 1 December 2024

ABSTRACT

This study utilized featherback skin to generate a versatile protein hydrolysate having capacities of iron chelation, emulsification, foaming and amino acid supplement. The hydrolysate obtained under the chosen condition (Alcalase, the skin:water ratio of 1:9 (w/v), pH 7.5, 55°C, enzyme:substrate (E:S) ratio of 40 U/g protein, 4 h of hydrolysis) showed an iron-binding capacity (IBC) of 7085.2 ± 4.2 (μg Fe^{2+}/g protein), being equivalent to that of ethylenediaminetetraacetic acid disodium salt (Na_2EDTA). Together with a high content of hydrophobic amino acids (63.34 mg/L), in the pH range 3-8, the emulsifying property of the hydrolysate was remarkable with emulsifying activity index (EAI) of 0.16-0.21 m^2/g protein and emulsifying stability index (ESI) of 35.1-107.1 min, which were 1.7-2.3 folds lower and 1.6-5.0 folds higher than those of sodium caseinate, respectively. Meanwhile, the hydrolysate exhibited mild foaming property with its foaming capacity (FC) and foaming stability (FS) being 3.6-16.6 folds lower than those of albumin.

Keywords: Featherback skin, functional property, iron-binding capacity, protein hydrolysate

INTRODUCTION

Iron is a component of hemoglobin in red blood cells, vital for the transportation of oxygen throughout the human body as myoglobin for storage and usage of oxygen in muscles [1]. Being a part of iron-sulfur complexes in enzymes responsible for the respiratory electron chain inside mitochondria and the citric acid cycle, it plays key roles in cellular growth, DNA and energy synthesis [2]. Iron deficiency is the cause of mood changes, muscle weakness, immunodeficiency, and anemia [3]. The origin of iron deficiency is often attributed to limited absorption of iron (II) ion (Fe^{2+}) from food due to the presence of inhibitors such as phytates, polyphenols, oxalic acid and fiber, by forming insoluble complexes [4]. Furthermore, at physiological pH, Fe^{2+} is easily converted to non-absorbable iron salt [5]. To limit the iron shortage in the human body, food fortification seems to be the most cost-effective intervention [6, 7]. The first generation of iron supplement, including ferrous sulfate, ferrous chloride, and ferrous lactate, is used in the clinical treatment of anemia, but they can cause a series of side effects counting gastrointestinal irritation, stomach ache or diarrhea [4]. Although the second generation, ferrous glycinate (an amino acid–Fe (II) complex) showed 2.5 – 3.4 times more bioavailable than the iron salts, it could initiate the fat oxidation and unfavorable color reaction [8]. To meet the physiological demands for iron and minimize unpleasant side effects, iron-binding protein hydrolysates/peptides have attracted many scientists recently. Protein hydrolysates with IBC were generated from various sources including tilapia skin [9], sturgeon skin [10], salmon skin [11], *etc*. Besides, fish skin hydrolysates from sole [12], tiger puffer [13], and saithe skin [14] also exhibited interesting functional properties such as emulsifying and foaming, which can

improve fortified products' characteristics. It indicated that fish skins could be noteworthy sources for producing iron-chelating protein hydrolysates/peptides with emulsifying and foaming capacity.

EXPERIMENTAL

Materials: Featherback skin obtained from a market from Ho Chi Minh city was ground after washing with cold water and cutting to pieces. The ground skin was stored at -20°C in sealed polyamide bags. The chemical composition of the skin including 63.6 ± 1.7 % moisture, 71.18 ± 1.8 % crude protein, and 19.7 ± 1.6 % ash content (on dry weight basis) was analyzed using the guidelines of Nwachukwu and Aluko (2019) [15]. Alcalase® 2.5L was obtained from Novozymes (Denmark) while other chemicals were purchased from Sigma-Aldrich and Merck. All reagents were analytical grade. Distilled water was used in experiments.

Preparation of featherback skin hydrolysate: The featherback skin hydrolysate was prepared based on our previous protocol with modifications [16]. Distilled water was added to the skin before bringing the mixture to 95°C for 15 min to deactive pre-existing endoenzymes. The mixture was then cooled to 55°C (the working temperature for Alcalase) and its pH was adjusted to 7.5 (the optimal pH for Alcalase) using NaOH 1M and/or HCl 1M solution. Subsequently, Alcalase was added to 7.5 (the optimal pH for Alcalase) using NaOH 1M and/or HCl 1M solution. Subsequently, Alcalase was added to the mixture with the required E:S ratio to start the hydrolysis. After the set hydrolysis time, the Alcalase was deactivated by heating the hydrolysate for 15 min at 95°C. The hydrolysates were centrifuged to separate the upper fat fractions and the obtained supernatants were filtered with Whatman no. 3 filter paper to remove suspended particles. Protein content of the hydrolysate was determined based on Lowry's method [15].

Effects of hydrolysis condition on the IBC of the hydrolysate: The effect of skin:water ratio, E:S ratio and hydrolysis time on IBC of the hydrolysate was examined using a single factor test, performed by varying one factor with different levels while fixing the others. The initial hydrolysis condition was selected at 55°C, pH 7.5, E:S ratio of 40 U/g protein and 4 h of hydrolysis. The skin:water ratio was varied from 1:1 to 1:11 (w/v), the E:S ratio was controlled from 20 to 60 U/g protein, and hydrolysis time ranged from 2 to 6 h.

Determination of IBC of the hydrolysate: The quantification method for IBC of Vo *et al*. (2020) [3] was employed to measure the IBC of the featherback skin hydrolysate. In brief, the mixture of the demineralized hydrolysate (1 mL), sodium acetate buffer (2.5 mL, 0.1 M, pH 5) and 0.2 mM $FeSO_4$ solution (0.6 mL) was placed at room temperature for 30 min. Subsequently, 0.3 mL of Ferrozine (5 mM) was added to the mixture, whose absorbance was then recorded at 562 nm. For the blank sample, 2 mL of the hydrolysate was replaced with 2 mL of distilled water. Na_2EDTA was used as a standard. The IBC of the hydrolysate was calculated using the following formula:

$$\text{IBC } (\mu g\ Fe^{2+}/\ g \text{ protein}) = \frac{A_c - A_s}{A_c} * \frac{m_{Fe^{2+}}}{m_{protein}} \quad (1)$$

Where A_c is the absorbance of the blank; A_s stands for the absorbance of the sample; represents the initial weight of Fe^{2+}, µg; $m_{protein}$ denotes the weight of protein of the hydrolysate, g.

Determination of degree of hydrolysis (DH): The featherback skin protein hydrolysate's DH was determined employing our previously published method [17]. Firstly, o-phthaldialdehyde (OPA) reagent was prepared as follows: 80 mg of OPA and 88 mg of dithiothreitol were dissolved in 2 mL of absolute ethanol, and the resulting solution was diluted to 100 mL using D1 solution containing sodium borate (50.8 g/L) and sodium dodecyl sulfate (1.333 g/L). Then, samples, blanks and standards were prepared by mixing 0.4 mL of the protein hydrolysate, distilled water or 0.9516 milliequivalents/L serine solution with 3 mL of the OPA reagent in 5s. After 2 min, the absorbance of two standards was measured, followed by the absorbance of the blanks, the samples, and two other standards. The DH of the protein hydrolysate was calculated using the equation provided below:

$$\text{DH } (\%) = \left(\frac{A_s - A_b}{A_{st} - A_b} * \frac{0.9516}{P} - \beta\right) * \frac{100}{\alpha * h_{tot}} \quad (2)$$

Where, A_s and A_b orderly indicate the absorbance of the sample and the blank. A_{st} is average absorbance of the 4 serine standards. P (mg/mL) dictates the protein content determined using the Lowry method. α and β are constant which are 1.0 and 0.4, respectively, for fish. h_{tot} is hydrolytic equivalence at complete hydrolysis to amino acids, being 8.6 for fish.

Amino acid composition analysis: Firstly, all peptides in the hydrolysate were converted into free amino acids by the cleavage of 6 M HCl solution for 23 h at 110 ± 2°C. They were then separated by ion-exchange chromatography and reacted with Ninhydrin to form their Ninhydrin-derivatives before being detected. Free amino acids in the hydrolysate were quantified based on the absorbance of standard solutions of amino acids at 440 nm for Pro and 570 nm for other amino acids [15].

Determination of foaming property: Foaming property (FC and FS) of the featherback skin hydrolysate were tested according to the procedures depicted in the study of Vo et al. (2020) [3]. pH of 40 mL of the hydrolysate (soluble protein content of 10 mg/mL) was adjusted to a value within the range from 3 to 8 using either 1 M HCl or 1 M NaOH solution before being homogenized. The whipped specimen was then instantly transferred into a 100 mL cylinder and the total

volume was recorded after 30 s at room temperature and after being left at 20°C for 3 min. For the standard sample, 40 mL of 10 mg/mL albumin solution was used to replace the protein hydrolysate. The FC and FS of the hydrolysate and the standard were determined using the following equations:

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

$$FS\ (\%) = \frac{A_t-B}{B} * 100 \quad (4)$$

Where A indicates the volume of the whipped hydrolysate measured after 30 s (mL), A_t expresses the volume of the whipped hydrolysate measured after being left for 3 min at 20ºC (mL); B depicts the initial volume of the hydrolysate (mL).

Determination of emulsifying property: The emulsifying capacity of the featherback skin hydrolysate was assessed in the pH range of 3-8. 15 mL of the hydrolysate (10 mg soluble protein/mL) was homogenized with 5 mL of vegetable oil to gain the emulsion. Then, 50 µL of the emulsion, pipetted from the bottom of its container at 0 min and 10 min after homogenization, was blended with 4.95 mL of sodium dodecyl sulfate solution (1 mg/mL) prior to measurement of absorbance of the mixture at 500 nm. Sodium caseinate solution (10 mg/mL) was used as standard [3]. The EAI and ESI were estimated by the following equations:

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

$$ESI\ (min) = \frac{A_0*\Delta t}{\Delta A} \quad (6)$$

Where ; ; A_0 and A_{10} are the absorbances of the samples taken at 0 min and 10 min after homogenization, respectively. $2*2.303*A_0/1$ denotes oil-water interface area (m^2); 1: the pathlength of the cuvette (cm); 0.25: volume proportion of oil phase in the emulsion, $m_{protein}$ is the weight of protein of the hydrolysate, g.

Data analysis: All experiments were triplicated. All data were analyzed using Statgraphics 15.1.02 and Microsoft Excel.

RESULTS AND DISCUSSION

Effects of hydrolysis condition on the IBC of the featherback skin hydrolysate: Alcalase was proven to have a broad specificity for various cleavage sites, including aromatic (Phe, Trp, Tyr), acidic (Glu), S-containing (Met), hydrophobic (Leu and Ala), hydroxyl (Ser), and basic (Lys) residues [2, 18], which have been considered as anchoring sites for ferrous ions [3, 10]. The enzyme was also employed to generate iron-binding protein hydrolysates from diverse protein sources like whey protein [2], mung bean [19], and red Tilapia viscera [20]. In this study, the alcalase was used to convert the featherback skin into an iron-chelating protein hydrolysate.

Regarding the effect of skin:water ratio, as shown in Fig. 1, the IBC of the hydrolysate reached a peak at the ratio of 1:9 (w/v). Low IBCs at other skin:water ratios could be due to the fact that there was a negative impact on enzyme-substrate interaction via dilution (in case of too high water) or high viscosity (in case of too low water) of the mixture, lessening the amount of bioactive peptides in the hydrolysates [21]. On the other hand, a sufficient water amount not only resulted in high solubility of protein but also could effectively disperse the products of hydrolysis, preventing the feedback effect, enhancing bioactivity of the protein hydrolysate [21]. In this investigation, the skin:water ratio of 1:9 (w/v) was chosen for all future experiments.

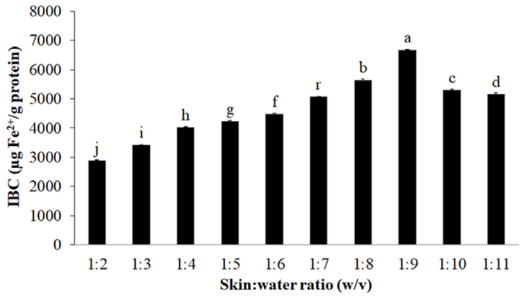

Fig. 1. Effect of skin:water ratio on IBC of the featherback skin protein hydrolysate. Bars with different letters indicate significant differences ($p<0.05$).

In terms of impact of E:S ratio, IBC of the skin hydrolysate was in direct proportion to E:S ratios ranging from 20 to 40 U/g protein, and the relationship was in inverse proportion afterward (Fig. 2). It was reported that an increase in E:S ratio ameliorated the hydrolysis, generating large amounts of high metal-affinity peptides from the featherback skin protein [16]. A further increase in E:S ratio could induce the generation of shorter peptides and amino acids from the early released bioactive peptides, thus decreasing the overall IBC [3]. In this experiment, the E:S ratio of 40 U/g protein was applied for the subsequent experiments.

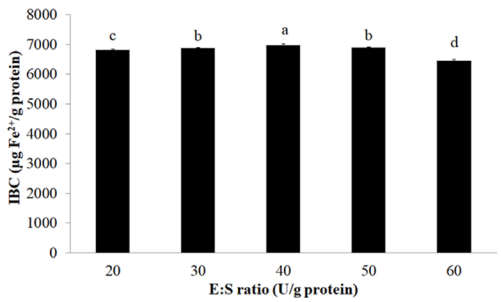

Fig. 2. Effect of E:S ratio on IBC of the featherback skin protein hydrolysate. Bars with different letters indicate significant differences ($p<0.05$).

Fig. 3 showed that the IBC of the hydrolysate reached

a maximal value at 4 h of hydrolysis before decreasing quickly with further increase in hydrolysis time to 6 h. The explanation for this declination may be due to the fact that prolongation in hydrolysis time could lead to the deeper cleavage of the early freed bioactive peptides into smaller inactive fragments, reducing their IBC. Our previous study also observed a similar trend [3]. To this point, 4 h was set as the hydrolysis time for next experiments.

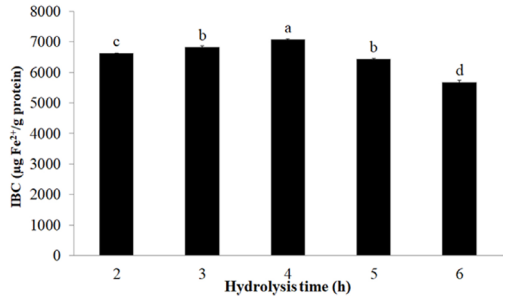

Fig. 3. Effect of hydrolysis time on IBC of the featherback skin protein hydrolysate. Bars with different letters indicate significant differences ($p<0.05$).

As has been demonstrated above, the optimal hydrolysis condition for the featherback skin comprised hydrolysis agent of Alcalase® 2.5L, the skin:water ratio of 1:9 (w/v), pH 7.5, hydrolysis temperature of 55°C, E:S ratio of 40 U/g protein, hydrolysis duration of 4h. The resulting featherback skin protein hydrolysate exhibited an IBC of 7085.2 ± 4.2 (µg Fe^{2+}/g protein) and a DH of 16.9 ± 0.3%. This IBC was 7.23 times better than that of 8% DH shrimp processing by-products hydrolysate [22], while it was 1.33 times lower than that of 43.18 ± 0.61% DH scallop skirts hydrolysate [23]. The variation in IBCs of these hydrolysates might be attributed to the difference of their DH values, as higher DH values indicated a greater abundance of low molecular weight peptides, which usually exerted increased IBC [23, 24]. Generally, small peptides possessed a simple spatial structure and more exposed metal ion binding sites, resulting in higher metal chelating rates compared with large peptides [19]. However, the IBC of the featherback was superior compared to that of higher DH hydrolysates from sole fish skin (IBC = 1160 µg Fe^{2+}/g of protein, DH = 23.60 ± 0.75%) [12], and Pacific cod skin gelatin (IBC = 690 µg Fe^{2+}/g of protein, DH = 24.4 ± 0.5%) [25]. This might be because these hydrolysates contained too many small peptides or free amino acids that were incompetent to chelate the ferrous ions [24]. Furthermore, in addition to molecular weight, the spatial distribution, amino acid composition and sequence of peptides also have decisive influences on their IBC [26].

Amino acid composition of the featherback skin hydrolysate: It is obvious that the amino acid profile greatly contributed to the protein hydrolysate's bioactivity. In this study, as presented in Table 1, the featherback skin hydrolysate contained a high amount of Phe, whose role in chelating ferrous ions has been emphasized in the study of Du et al. (2022) [8]. Also, the combination of Phe and other aliphatic amino acids such as Ile, Leu, Val, and Ala could increase the stability of peptide – Fe^{2+} complexes, while the hydrophobic residues (methyl or methylene groups) were perpendicular to the peptide – metal bonds' plane, shielding the Fe^{2+} from external factors like water molecules [2, 3].

Table 1. Amino acid profile of the featherback skin hydrolysate

Amino acids	Content (mg/L)	Amino acids	Content (mg/L)
His	0.45 ± 0.21	Thr	0.51 ± 0.23
Ile	1.80 ± 0.82	Val	5.26 ± 2.40
Leu	2.43 ± 1.11	Ser	0.72 ± 0.33
Lys	0.49 ± 0.22	Glu	3.17 ± 1.45
Asp	1.88 ± 0.86	Gly	4.45 ± 2.03
Ala	8.68 ± 3.96	Phe	51.17 ± 13.12

As well, some regular metal binding sites consist of O-carboxyl group of Glu and Asp, O-hydroxyl group of Thr and Ser, N-amino group of Lys and N from imidazole ring of His; and the amino acids were found in the featherback skin hydrolysate in the studies of Du et al. (2022) [8], Xu *et al*. (2022) [5], and our study [3]. Another spotlight of the skin hydrolysate was that it provided 7 out of 9 essential amino acids for human nutrition, which made up approximately three-quarters of the total amino acid content.

Functional properties of the featherback skin hydrolysate: Transportation, penetration and rearrangement of peptides in the hydrolysate at the air–water interface were the three predominant factors of foam formation [27]. The peptides needed to be straightforwardly scattered in water and swiftly adsorbed at the water-air interfaces and rearranged their structure to form a film surrounding air bubbles to exhibit high FC [28]. Previous studies had stated that the lower the solubility of a hydrolysate was, the lower the migration speed of protein molecules to the boundary layer between air and water was, resulting in a lower FC [29]. In addition, Daliri *et al*. (2021) [28] suggested that FC was affected by multiple factors such as the hydrophobic/hydrophilic balance, molecular weight, physical flexibility and net surface charge of proteins/peptides.

On the other hand, high FS was achieved if a cohesive and flexible film was created by peptide molecules [3]. The property of the film seemed to be determined by the protein-protein interaction level within the matrix that associates with the ionic repulsion of peptides [30]. Besides, pH could be considered as one of the most important factors that affected both FC and FS via protonation or deprotonation of amino acid side chains of the peptides, altering the peptide's charge, and thus impacting the solubility and interaction of peptide molecules [31]. In this study, the featherback

skin hydrolysate exhibited the highest FC and FS at pH 5 (Fig. 4 A and B).

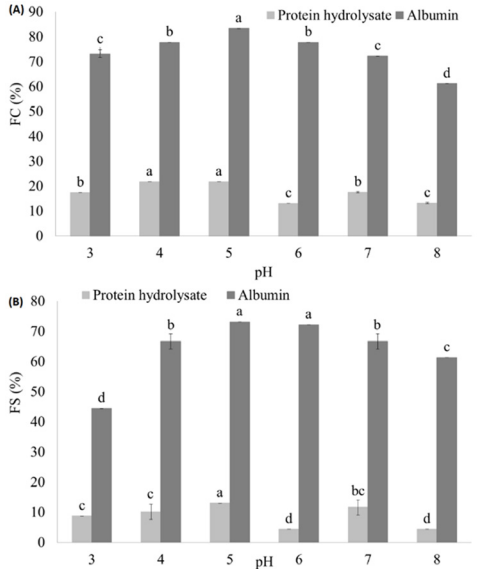

Fig. 4. FC (A) and FS (B) of the featherback skin protein hydrolysate. The same color bars with different letters indicate significant differences (p<0.05).

When compared to the foaming property of albumin, in the tested pH range from 3 to 8, FCs and FSs of the hydrolysate were 2.81 – 6.32 and 3.41 – 16.81 times lower than those of albumin. It could be attributed to the poor balance between the skin hydrolysate's hydrophobic and hydrophilic amino acids, in which the aliphatic amino acids content was 91% of total amino acid, while this value was only 49.5% in albumin (calculated from the result of Goto *et al*. (2021) [32]). In addition, low DH value of 16.9 ± 0.3% suggested that the skin hydrolysate may contain a high amount of large peptides which showed low rate of diffusion and adsorption at the air-water interface, decreasing the hydrolysate's foaming property [33]. Qoms *et al*. (2023) [34] observed a positive correlation between DH and FC of fern (*Azolla pinnata)* protein hydrolysate. While smaller peptides may incorporate more air in the solution, resulting in a faster film formation at the air-water interface and encapsulation of air bubbles, their flexible structures hindered their capacity of stabilizing these air bubbles [35]. Despite this, both the FCs and FSs of the featherback skin protein hydrolysate significantly surpassed those of higher DH hydrolysates from Atlantic mackerel by-products (FC = 5%, FS = 3.33%, DH = 45%) and sardine by-products (FC = 3.33%, FS = 3.33%, DH = 55%) [36]. This might be ascribed to the contribution of other parameters including hydrophilic to hydrophobic ratio and configurational structures of peptides in these hydrolysates [37]. Taken together, the featherback skin hydrolysate could be applied in some food products for moderate enhancement of foaming feature.

As for emulsifying property, in this study, the highest EAI of the skin hydrolysate was observed at an alkaline pH (pH 8) (Fig. 5A), while its ESI reached the peak at an acidic pH (pH 4) (Fig. 5B). Our previous studies also found that the *Acetes* protein hydrolysate exerted the maximum EAI at pH 8 [3, 16]. It could be due to the fact that pH 8 converted peptides into their anionic forms, enhancing the repulsion forces between peptide molecules, benefiting their better orientation at the oil-water boundary, thus, elevating EAI of the hydrolysate [28]. Meanwhile, Tang et al. (2023) [33] unveiled that in the acidic conditions, Glu, Asp and other positive charge residues in the hydrolysate induced the formation of ordered aggregation, ameliorating the hydrolysate's ESI. In comparison to the emulsifying characteristic of sodium caseinate, although EAIs of the skin hydrolysate were 1.7 - 2.3 folds lower than those of sodium caseinate, its ESIs were 1.6 - 5.0 folds higher than those of sodium caseinate in the pH range from 3 to 8. The high ESI of the hydrolysate was possibly contributed by its high content of hydrophobic amino acids, which boosted hydrophobic interactions between the peptides, resulting in the formation of a stable protein film around the oil droplets [33]. In addition, with a low DH value of 16.9 ± 0.3%, it could be predicted that the hydrolysate predominantly contained long-chain peptides, which could form a steady and continuous network between oil droplets [28, 38].

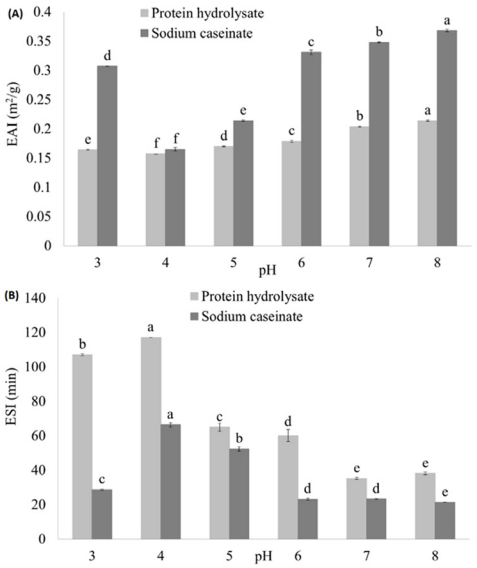

Fig. 5. EAI (A) and ESI (B) of the featherback skin protein hydrolysate. The same color bars with different letters indicate significant differences (p<0.05).

The featherback skin hydrolysate displayed a superior ESIs compared to higher DH protein hydrolysates originated from Atlantic mackerel by-products (DH= 45%, ESI = 1.56 min) [36] and *Acetes japonicus* (DH = 69.7%, ESI = 15.35 – 83.19 min) [24]. On the other hand, moderate EAIs of the featherback skin hydrolysate could be ascribed to its low DH value. As reported by Daliri *et al*. (2021) [28] and Feng *et al*. (2024) [39], the longer and bigger peptides diffused slowly into the oil-water interface and exerted poorer activity

in creating the emulsion phase compared to shorter and smaller peptides. However, our previous study [24] and the study of Vásquez et al. (2022) [40] have observed a negative relationship between DH and EAI of *Acetes japonicus* and rainbow trout viscera protein hydrolysates, respectively. EAIs of the featherback skin hydrolysate in this study were considerably lower compared to a higher DH hydrolysate from Atlantic mackerel by-products (DH= 45%, EAI = 2.12 m^2/g) [36] or a lower DH hydrolysate from soy protein (DH = 2%, EAI = 3 – 12 m^2/g) [41]. As revealed by Tang *et al*. (2023) [33], in addition to DH, emulsifying property of peptides were influenced by other factors including its hydrophilicity, hydrophobicity, structure, charge and amino acid composition. As a whole, the featherback skin hydrolysate in this study might be applied to fortify some food products to boost their emulsion property.

CONCLUSION

This study demonstrated the potential of featherback skin to produce iron-binding hydrolysate, which can be considered as a natural iron supplement and emulsifier or foaming agent for food product improvement. This could enhance the economic value of the by-product and support for the discarding the waste from the featherback fish cake producers. However, further research should be done on identifying iron-binding peptides in the hydrolysate as well as their capacity to improve iron absorption in the human body.

ACKNOWLEDGMENTS

We acknowledge Ho Chi Minh City University of Technology (HCMUT), VNU-HCM for supporting this study.

AUTHOR CONTRIBUTIONS

The contributions of all the authors to a manuscript must be described in the following format: TDLV author designed the research; TDLV, HDL, ATHN, NNYL, TBV, VTTN, HTM, VTP, DNNP, LVTN, LTTT and VHN authors performed the experiments; TDLV and BCV authors analyzed the data; TDLV and BCV authors wrote the manuscript draft; TDLV author revised the manuscript. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Zhang Y.Y., Stockmann R., Ng K., Broadbent J.A., Stockwell S., *et al.* (2023) Characterization of Fe(III)-binding peptides from pea protein hydrolysates targeting enhanced iron bioavailability. *Food Chem.,* **405**, 134887. https://doi.org/10.1016/j.foodchem.2022.134887
2. Athira S., Mann B., Sharma R., Pothuraju R., and Bajaj R.K. (2021) Preparation and characterization of iron-chelating peptides from whey protein: An alternative approach for chemical iron fortification. *Food Res. Int.,* **141**, 110133. https://doi.org/10.1016/j.foodres.2021.110133
3. Vo T.D.L., Pham K.T., Le V.M.V., Lam H.H., Huynh O.N., *et al.* (2020) Evaluation of iron-binding capacity, amino acid composition, functional properties of *Acetes japonicus* proteolysate and identification of iron-binding peptides. *Process Biochem.,* **91**, 374-386. https://doi.org/10.1016/j.procbio.2020.01.007
4. Fan C., Ge X., Hao J., Wu T., Liu R., *et al.* (2023) Identification of high iron–chelating peptides with unusual antioxidant effect from sea cucumbers and the possible binding mode. *Food Chem.,* **399**, 133912. https://doi.org/10.1016/j.foodchem.2022.133912
5. Xu B., Wang X., Zheng Y., Shi P., Zhang Y., *et al.* (2022) Millet bran globulin hydrolysate derived tetrapeptide-ferrous chelate: Preparation, structural characterization, security prediction *in silico*, and stability against different food processing conditions. *LWT-Food Sci. Technol.,* **165**, 113673. https://doi.org/10.1016/j.lwt.2022.113673
6. Ying L., Kaihua W., Xiaoguang M., Yajuan W., Tuo Z., *et al.* (2017) Separation and Identification of Iron-chelating Peptides from Defatted Walnut Flake by NanoLC-ESI-MS/MS and *De novo* Sequencing. *Process Biochem.,* **59**, 223-228. https://doi.org/10.1016/j.procbio.2017.05.010
7. Hajj S.E., Irankunda R., Echavarría J.A.C., Arnoux P., Paris C., *et al.* (2023) Metal-chelating activity of soy and pea protein hydrolysates obtained after different enzymatic treatments from protein isolates. *Food Chem.,* **405**, 134788. https://doi.org/10.1016/j.foodchem.2022.134788
8. Du Y.-N., Hong J.-N., Xu S.-Q., Wang Y.-Q., Wang X.-C., *et al.* (2022) Iron-chelating activity of large yellow croaker (*Pseudosciaena crocea*) roe hydrolysates. *J. Food Process. Preserv.,* **46**, e17080. https://doi.org/10.1111/jfpp.17080
9. Lin S., Hu X., Li L., Yang X., Chen S., *et al.* (2021) Preparation, purification and identification of iron-chelating peptides derived from tilapia (*Oreochromis niloticus*) skin collagen and characterization of the peptide-iron complexes. *LWT-Food Sci. Technol.,* **149**, 111796. https://doi.org/10.1016/j.lwt.2021.111796
10. Gui M., Gao L., Rao L., Li P., Zhang Y., *et al.* (2022) Bioactive peptides identified from enzymatic hydrolysates of sturgeon skin. *J. Sci. Food Agric.,* **102**, 1948-1957. https://doi.org/10.1002/jsfa.11532
11. Zhang X., Dai Z., Zhang Y., Dong Y., and Hu X. (2022) Structural characteristics and stability of salmon skin protein hydrolysates obtained with different proteases. *LWT - Food Sci. Technol.,* **153**, 112460. https://doi.org/10.1016/j.lwt.2021.112460

12. Viji P., Phannendra T.S., Jesmi D., Madhusudana Rao B., Dhiju Das P.H., *et al.* (2019) Functional and antioxidant properties of gelatin hydrolysates prepared from skin and scale of sole fish. *J. Aquat. Food Prod.,* **28**, 976-986. https://doi.org/10.1080/10498850.2019.1672845
13. Pan J., Jia H., Shang M., Li Q., Xu C., *et al.* (2018) Effects of deodorization by powdered activated carbon, β-cyclodextrin and yeast on odor and functional properties of tiger puffer (*Takifugu rubripes*) skin gelatin. *Int. J. Biol. Macromol.,* **118**, 116-123. https://doi.org/10.1016/j.ijbiomac.2018.06.023
14. Casanova F., Mohammadifar M.A., Jahromi M., Petersen H.O., Sloth J.J., *et al.* (2020) Physico-chemical, structural and techno-functional properties of gelatin from saithe (*Pollachius virens*) skin. *Int. J. Biol. Macromol.,* **156**, 918-927. https://doi.org/10.1016/j.ijbiomac.2020.04.047
15. Nwachukwu I.D. and Aluko R.E. (2019) A systematic evaluation of various methods for quantifying food protein hydrolysate peptides. *Food Chem.,* **270**, 25-31. https://doi.org/10.1016/j.foodchem.2018.07.054
16. Vo T.D.L., Pham K.T., and Doan K.T. (2021) Identification of Copper-Binding Peptides and Investigation of Functional Properties of *Acetes japonicus* Proteolysate. *Waste Biomass. Valor.,* **12**, 1565-1579. https://doi.org/10.1007/s12649-020-01112-3
17. Vo T.D.L., Duong K.L., Nguyen A.N.Q., Vo B.C., Tran M.C., *et al.* (2023) Featherback (*Chitala ornata*) skin protein hydrolysate: Antioxidant activity, amino acid composition, foaming and emulsifying properties. *Chem. Eng. Trans.,* **106**, 877-882. https://doi.org/10.3303/CET23106147
18. Fu Y., Liu J., Hansen E.T., Bredie W.L.P., and Lametsch R. (2018) Structural characteristics of low bitter and high umami protein hydrolysates prepared from bovine muscle and porcine plasma. *Food Chem.,* **257**, 163-171. https://doi.org/10.1016/j.foodchem.2018.02.159
19. Zhang Y., Ding X., and Li M. (2021) Preparation, characterization and *in vitro* stability of iron-chelating peptides from mung beans. *Food Chem.,* **349**, 129101. https://doi.org/10.1016/j.foodchem.2021.129101
20. Gómez L.J., Gómez N.A., Zapata J.E., López-García G., Cilla A., *et al.* (2020) Optimization of the Red Tilapia (*Oreochromis spp.*) viscera hydrolysis for obtaining iron-binding peptides and evaluation of *in vitro* iron bioavailability. *Foods,* **9**, 883. https://doi.org/10.3390/foods9070883
21. Shu G., Zhang B., Zhang Q., Wan H., and Li H. (2017) Effect of temperature, pH, enzyme to substrate ratio, substrate concentration and time on the antioxidative activity of hydrolysates from goat milk casein by alcalase. *Acta Univ. Cibiniensis, Ser. E: Food Technol.,* **20**, 29-38. https://doi.org/10.1515/aucft-2016-0013
22. Huang G., Ren Z., and Jiang J. (2011) Separation of iron-binding peptides from shrimp processing by-products hydrolysates. *Food Bioprocess Tech.,* **4**, 1527-1532. https://doi.org/10.1007/s11947-010-0416-3
23. Yan X., Yue Y., Guo B., Zhang S., Ji C., *et al.* (2024) Novel microbial fermentation for the preparation of iron-chelating scallop skirts peptides-its profile, identification, and possible binding mode. *Food Chem.,* **451**, 139493. https://dx.doi.org/10.1016/j.foodchem.2024.139493
24. Vo T.D.L., Nguyen V.K., Nguyen T.T.P., Vo B.C., and Nguyen T.T.T. (2022) Effect of hydrolytic degree on antioxidant activity and functional properties of *Acetes japonicus* proteolysate. *Acta Aliment.,* **51**, 360-370. https://doi.org/10.1556/066.2022.00048
25. Wu W., Li B., Hou H., Zhang H., and Zhao X. (2017) Identification of iron-chelating peptides from Pacific cod skin gelatin and the possible binding mode. *J. Funct. Foods,* **35**, 418-427. https://doi.org/10.1016/j.jff.2017.06.013
26. Gómez-Grimaldos N.A., Gómez-Sampedro L.J., Zapata-Montoya J.E., López-García G., Cilla A., *et al.* (2020) Bovine plasma hydrolysates' iron chelating capacity and its potentiating effect on ferritin synthesis in Caco-2 cells. *Food Funct.,* **11**, 10907-10912. https://doi.org/10.1039/D0FO02502J
27. Arteaga V.G., Guardia M.A., Muranyi I., Eisner P., Schweiggert-Weisz U. (2020) Effect of enzymatic hydrolysis on molecular weight distribution, technofunctional properties and sensory perception of pea protein isolates. *Innov. Food Sci. Emerg. Technol.,* **65**, 102449. https://doi.org/10.1016/j.ifset.2020.102449
28. Daliri H., Ahmadi R., Pezeshki A., Hamishehkar H., Mohammadi M., *et al.* (2021) Quinoa bioactive protein hydrolysate produced by pancreatin enzyme-functional and antioxidant properties. *LWT-Food Sci. Technol.,* **150**, 111853. https://doi.org/10.1016/j.lwt.2021.111853
29. Putra S.N.K.M., Ishak N.H., Sarbon N.M. (2018) Preparation and characterization of physicochemical properties of golden apple snail (*Pomacea canaliculata*) protein hydrolysate as affected by different proteases. *Biocatal. Agric. Biotechnol.,* **13**, 123-128. https://doi.org/10.1016/j.bcab.2017.12.002
30. de Souza T.S.P., Dias F.F.G., Koblitz M.G.B., Bell J.M.L.N.M. (2020) Effects of enzymatic extraction of oil and protein from almond cake on the physicochemical and functional properties of protein extracts. *Food Bioprod. Process.,* **122**, 280-290. https://doi.org/10.1016/j.fbp.2020.06.002
31. Gao J., Tang Z.-S., He S., Powelle W., Brennan C.S. (2023) The foaming properties of sweet

potato protein hydrolysates produced by Alcalase and Ficin. *J. Sci. Food Agric.,* **103**, 4157-4163. https://doi.org/10.1002/jsfa.12420
32. Goto T., Shimamoto S., Ohtsuka A., Ijiri D. (2021) Analyses of free amino acid and taste sensor traits in egg albumen and yolk revealed potential of value-added eggs in chickens. *Anim. Sci. J.,* **92**, e13510. https://doi.org/10.1111/asj.13510
33. Tang T., Wu N., Tang S., Xiao N., Jiang Y.*, et al.* (2023) Industrial application of protein hydrolysates in food. *J. Agric. Food Chem.,* **71**, 1788-1801. https://doi.org/10.1021/acs.jafc.2c06957
34. Qoms M.S., Arulrajah B., Shamsudin R., Ramli N.S., Ibadullah W.Z.W.*, et al.* (2023) Enzymolysis of *Azolla pinnata* protein concentrate: Effect of protease types and hydrolysis extents on the physicochemical, techno-functional and biological properties. *Food Biosci.,* **53**, 102787. https://doi.org/10.1016/j.fbio.2023.102787
35. Naghdi S., Rezaei M., Tabarsa M., and Abdollahi M. (2023) Parallel extraction of sulfated polysaccharides and protein hydrolysate from skipjack tuna head and their bioactive and functional properties. *Food Bioprocess Technol.,* **16**, 1258-1279. https://doi.org/10.1007/s11947-022-02988-w
36. Fuentes C., Verdú S., Grau R., Barat J.M., and Fuentes A. (2024) Impact of raw material and enzyme type on the physico-chemical and functional properties of fish by-products hydrolysates. *LWT - Food Sci. Technol.,* **201**, 116247. https://doi.org/10.1016/j.lwt.2024.116247
37. Damodaran S. and Parkin K.L. (2017) *Fennema's Food Chemistry*. New York, USA: CRC Press, 1125.
38. Xu Y., Galanopoulos M., Sismour E., Ren S., Mersha Z.*, et al.* (2020) Effect of enzymatic hydrolysis using endo- and exo-proteases on secondary structure, functional, and antioxidant properties of chickpea protein hydrolysates. *J. Food Meas. Charact.,* **14**, 343-352. https://doi.org/10.1007/s11694-019-00296-0
39. Feng L., Wu Y., Han Y., Yao X., Li Q.*, et al.* (2024) Structural characteristics, functional properties and nutritional value of walnut protein by limited enzymatic hydrolysis. *LWT - Food Sci. Technol.,* **197**, 115923. https://doi.org/10.1016/j.lwt.2024.115923
40. Vásquez P., Sepúlveda C.T., Zapata J.E. (2022) Functional properties of rainbow trout (*Oncorhynchus mykiss*) viscera protein hydrolysates. *Biocatal. Agric. Biotechnol.,* **39**, 102268. https://doi.org/10.1016/j.bcab.2021.102268
41. Padial-Domínguez M., Espejo-Carpio F.J., Pérez-Gálvez R., Guadix A., and Guadix E.M. (2020) Optimization of the emulsifying properties of food protein hydrolysates for the production of fish oil-in-water emulsions. *Foods,* **9**, 636. https://doi.org/10.3390/foods9050636