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Physicochemical and Functional Properties of Flavourzyme-Mediated Protein Hydrolysate from *Priacanthus hamrur* Skin

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Abstract

Valorization of fish processing waste is critical for enhancing seafood processing and addressing environmental concerns. This study investigated the production of flavourzyme-mediated fish protein hydrolysate (FMPH) from the skin of *Priacanthus hamrur* (bull's eye) under optimized conditions. The degree of hydrolysis and yield of FMPH were reported as 19.94 ± 0.23% and 10.52 ± 0.24%, respectively. Proximate analysis of FMPH indicated a high protein content of 91.07 ± 1.32%, low lipid content of 3.25 ± 0.30%, and ash content of 3.29 ± 0.24%. The bulk density and tapped density of FMPH were determined to be 0.29 ± 0.02 g/cm³ and 0.34 ± 0.03 g/cm³, respectively. The Hausner ratio (1.16 ± 0.15) and Compressibility Index (12.82 ± 0.64) of FMPH fell within the typical ranges of 1.12–1.18 and 11–15, respectively, indicating favourable flowability and moderate compressibility. FMPH exhibited exceptional solubility across various pH levels, with the highest solubility of 83.77 ± 0.90% at pH 7.0. The FMPH demonstrated significant water-holding capacity (0.96 ± 0.06 mL/g) and oil-holding capacity (2.51 ± 0.03 mL/g), along with notable foaming and emulsifying properties. This study demonstrated the feasibility of transforming *P. hamrur* skin into a high-value hydrolysate with superior functional properties, thereby supporting the sustainable utilization of fish by-products. The results highlight the potential of FMPH for application in nutraceutical, functional food, and biopharmaceutical formulations.

Keywords: *Priacanthus hamrur,* flavourzyme, fish protein hydrolysate, physicochemical properties, functional properties

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Introduction

The bull's eye (*Priacanthus hamrur*), a deep-sea fish native to the Indo-Pacific, is preferred for surimi production due to its superior flesh quality and flavour. This species accounted for approximately 8% of Indian maritime landings in 2021–22, with a total catch of 63,534 tons (CMFRI, 2023). Surimi processing generates various by-products such as bones with residual flesh, heads, skin, and intestines. These by-products constitute approximately 60–70% of the total fish weight and contain about 10–30% protein (Yin & Park, 2023; Naseem, Imam, Rayadurga, Ray, & Suman, 2024). However, these nutrient-rich by-products are often disposed of as industrial waste or utilized for low-value purposes, such as animal feed or fertilizers, indicating considerable untapped potential for maximizing resource utilization (Baraiya et al., 2023; Jaies, Qayoom, Saba, & Khan, 2024). Efficient utilization of these by-products can significantly contribute to the environmental and economic sustainability of the seafood processing industry.

Enzymatic hydrolysis is the most efficient method for converting fish by-products into valuable substances with enhanced functional and biological activities. This technique produces protein hydrolysates enriched with peptides that possess balanced amino acid profiles, enhanced digestibility, and diverse functional properties (Baraiya et al., 2024; Chalamaiah, Kumar, Hemalatha, & Jyothirmayi, 2012; Da Rocha et al., 2018). Numerous proteases play essential roles in hydrolysis, providing specificity and efficacy. Flavourzyme, a fungal enzyme produced by *Aspergillus oryzae*, is widely known for its dual endo and exopeptidase activities, making it highly versatile for protein hydrolysis applications. It hydrolyzes peptide bonds to release free amino acids and short peptides, thereby enhancing the bioactive properties, functional attributes, and sensory qualities of the resulting hydrolysate (Alahmad, Noman, Xia, Jiang, & Xu, 2023). Flavourzyme is well-suited for applications in the food, nutraceutical, and pharmaceutical sectors, where improved product functionality and sensory quality are essential. The extent of hydrolysis, peptide molecular weight, and enzyme specificity substantially affect the functional characteristics of hydrolysates, highlighting the significance of enzyme selection and process optimization (Chalamaiah et al., 2012; Halim, Yusof, & Sarbon, 2016). Enzyme-mediated hydrolysis of surimi by-products provides a sustainable method to valorize waste, reduce production costs, and ameliorate environmental effects. Despite its significant potential, limited research exists on enzymatically derived hydrolysates from *P. hamrur* skin. Such studies are critical for optimizing processing parameters and ensuring functional consistency across diverse applications.

The present study aimed to produce flavourzyme-mediated fish protein hydrolysates (FMPH) from surimi by-products and evaluate their physico-chemical and functional properties, to promote sustainability and innovation in seafood processing.

Materials and Methods

Flavourzyme 500 L, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and 2,22 -azino-bis (3-ethylbenzothiazolin)-6-sulfonic acid (ABTS) were acquired from Sigma–Aldrich. All remaining chemicals and reagents were of analytical grade and obtained from Sisco Research Laboratories (SRL), India.

Priacanthus hamrur fish were obtained from Munambam fishing harbour, Kerala, India, and transported to the processing laboratory in an insulated box with ice (4 ± 1°C). The raw material was washed twice with cold water (4 ± 1°C), and the skin was segregated. The fish skin was subsequently preserved at -18 ± 2°C for further experiments. The skin was cut into small pieces and crushed in a household blender to achieve uniformity.

The *P. hamrur* skin was homogenized with distilled water at a 1:2 ratio. The reaction mixture was subjected to a temperature of 90 ± 2°C for 20 min to inactivate the internal enzymes. The skin was hydrolyzed under the optimal conditions for flavourzyme using an enzyme-to-substrate ratio of 1:33 (v/w), pH 7.5, temperature 52°C for 120 min. The pH of the mixture was adjusted by adding 0.1 N NaOH as needed. After completion of the reaction period, the enzymes were deactivated by heating the samples at 95°C for 20 min. The samples were centrifuged at 10,000 rpm at 4°C for 20 min, and supernatant was filtered, lyophilized, and stored at -18 ± 2°C for further evaluation.

The degree of hydrolysis (DH) was assessed by formal titration, utilizing the methodology outlined by Noman et al. (2018). The yield was calculated by dividing the mass of the lyophilized powder by the mass of skin that underwent hydrolysis.

The nutritional composition of the FMPH was assessed according to standard protocols (AOAC, 2023). Moisture content was determined by drying the sample at 105 ± 2°C until a stable weight was obtained. Total protein concentrations of the samples were assessed using the Kjeldahl method. Lipids were extracted from the samples utilizing petroleum ether in a Soxhlet system to determine crude lipid content. The ash composition was determined by combusting the samples in a muffle furnace at 600°C for 6 h.

The colour of FMPH was assessed using a colourimeter (LabScan XE, Hunter Lab, Reston, VA, USA) and recorded using the CIE system, L*(lightness), a*(+redness/ -greenness), and b*(+yellowness/ -blueness).

The hygroscopicity of samples was determined by placing pre-weighed freeze-dried FMPH samples in a closed desiccator with a saturated sodium chloride solution (RH 76.00%) at 25°C for a week. The samples were weighed again, and hygroscopicity was quantified as the percentage of moisture absorbed relative to the initial weight (Cai & Corke, 2000).

For the analysis of bulk density and tapped density, a specified amount of dry sample powder was introduced into a graduated cylinder using a funnel, tapping gently to avoid compaction. The measured unsettled apparent volume was recorded to the nearest graded unit, and the bulk density (ρB) was computed from the sample weight relative to the volume it occupied. Similarly, mechanical tapping was employed to pour the protein hydrolysate powder into the cylinder until a consistent volume was achieved for the measurement of the tapped density (ρT) (Chinta et al., 2009). The Hausner ratio and Carr Index, indicative of flow characteristics, were calculated using the bulk and tapped densities as follows:

$$\text{Hausner ratio} = \rho T/\rho B$$

$$\text{Carr index} = 100(1 - \rho B/\rho T)$$

Turbidity was analyzed by dissolving 0.5 g of FMPH powder in 5 mL of distilled water and measuring the absorbance at 600 nm using a UV–visible spectrophotometer (Shimadzu, UV-1800, Japan)

To analyze the browning intensity, FMPH powder (0.1 g) was dispersed in 5 mL deionized water, and the solution was subsequently filtered through a syringe filter (0.2 μm). The absorbance of the filtrate was measured at 420 nm using a UV-Visible spectrophotometer (Shimadzu, UV-1800, Japan) (Elavarasan & Shamasundar, 2016).

The protein solubility of FMPH was evaluated following the methodology outlined by Choi, Hur, Choi, Konno, and Park (2009). The samples were dissolved at 10 mg/mL in distilled water, and the pH was adjusted to 3.0, 5.0, 7.0, 9.0, and 11.0 using appropriate buffer systems: citrate buffer (pH 3.0 and 5.0), phosphate buffer (pH 7.0), and glycine–NaOH buffer (pH 9.0 and 11.0). The solutions were centrifuged at 4000 rpm for 20 min, and the supernatant was collected. The protein content in the supernatant was quantified using the Lowry method, and solubility was expressed as a percentage of the total protein content in the sample (Peterson, 1977).

$$\text{Solubility (\%)} = \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \times 100$$

For the determination of water-holding capacity, 0.1 g of the sample was mixed with 1 mL of distilled water and centrifuged at 2000 rpm for 20 min at 25°C. The supernatant was discarded, and the retained water was quantified using the following formula:

$$\text{WHC (mL/g)} = \frac{\text{The volume of absorbed water (mL)}}{\text{Weight of sample (g)}}$$

To analyze the oil-holding capacity, FMPH (0.5 g) was added to 10 mL of sunflower oil and vortexed for 30 s. The oil mixture was then centrifuged at 3000 rpm for 30 min. The amount of oil extracted from the hydrolysate was quantified, and the OAC was calculated according to Wasswa, Tang, Gu, and Yuan (2007).

The foaming capacity and stability of the hydrolyzed samples were assessed according to the method described by Sathe and Salunkhe (1981), with slight modifications. An FMPH concentration of 10 mg/mL was used, and the pH was adjusted to 4.0, 7.0, and 10.0. Subsequently, 50 mL of the FMPH solution was placed into a 100 mL graduated cylinder and homogenized at 18,000 rpm for 1 min. The volumes before and after whipping were recorded, and the foaming capacity was calculated. The foam was left for 10 min, and the foaming stability was assessed.

$$\text{Foaming capacity (\%)} = \frac{\text{Volume after whipping(mL) - Volume before whipping(mL)}}{\text{Volume before whipping(mL)}} \times 100$$

$$\text{Foaming stability (\%)} = \frac{\text{Volume after standing(mL) - Volume before whipping(mL)}}{\text{Volume before whipping(mL)}} \times 100$$

The emulsifying activity index (EAI) and emulsion stability index (ESI) were quantified using the methodology described by Liu et al. (2014). The pH of the FMPH solution was adjusted to 4.0, 7.0, and 10.0. Subsequently, 45 mL of the sample was combined with 15 mL of sunflower oil and dispersed at 18,000 rpm for 1 min. An aliquot of the emulsion (20 μL) was collected at 0 and 10 min and diluted with 2 mL of a 0.1% SDS solution. The absorbance of each sample was recorded at 500 nm using a Shimadzu UV-1800 spectrophotometer.

$$\text{EAI } (m^2/g) = \frac{2 \times 2.303 \times A0}{0.25 \times \text{protein weight (g)}} \times 100$$

$$\text{ESI (min)} = A_0 \times 10/\ A_0 - A_{10}$$

Results and Discussion

The degree of hydrolysis (DH) quantifies the amount of peptide bond breakage during protein hydrolysis and is a critical determinant of protein recovery, biological activity, and functional properties (Yarnpakdee, Benjakul, Kristinsson, & Kishimura, 2015; Noman et al., 2018) . The DH of FMPH was reported as 19.94 ± 0.23% under optimal conditions (Table 1). These results correspond with previously established DH ranges for enzymatically hydrolyzed fish proteins (Bui et al., 2021; Nguyen et al., 2022; Alahmad et al., 2023; Nurdiani et al., 2024). The production of fish protein hydrolysates from bighead carp (*Hypophthalmichthys nobilis*) under optimal conditions yielded a maximum degree of hydrolysis (DH) of 20.15% (Alahmad et al., 2023). Nguyen et al. (2022) reported that the dark muscle of Tra catfish exhibited a degree of hydrolysis of 22.50 ± 1.30%. The protein recovery yield for FMPH was 10.52 ± 0.24%. This finding aligns with previously established ranges of 10–35% for fish protein hydrolysates, which vary depending on the type of raw material and hydrolysis conditions (Kristinsson & Rasco, 2000). The yield and degree of hydrolysis are significantly influenced by parameters such as the enzyme type, substrate properties, and processing conditions, underscoring the need to optimize hydrolysis methods to improve product recovery and functioning.

The proximate composition analysis of FMPH demonstrated exceptional nutritional characteristics (Table 1). The FMPH exhibited protein, fat, ash, and moisture levels of 91.07 ± 1.32%, 3.25 ± 0.30%, 3.29 ± 0.24%, and 2.39 ± 1.22%, respectively. Previously, in another study, the bighead carp, hydrolyzed by flavourzyme demonstrated protein, fat, ash, and moisture percentages of 88.19 ± 2.02%, 2.04 ± 0.05%, 4.85 ± 0.23%, and 3.77 ± 0.09%, respectively (Alahmad, Xia, Jiang, & Xu, 2022). The nutritional composition of tuna hydrolysate in another study was 7.5% moisture, 85.0% protein, 0.12% lipid, and 4.7% ash (Bui et al., 2021). Elevated protein level in hydrolysates is typically associated with the dissociation of smaller peptides and amino acid chains, which augments the protein proportion of the final product (Mora & Toldrá, 2023). The hydrolysate's substantial protein level, along with its notably low fat and moisture content, indicates that it can be extensively utilized in food processing industries to facilitate large-scale production (Chalamaiah et al., 2013; Noman et al., 2018).

Table 1. Proximate composition, degree of hydrolysis, and yield of FMPH

Parameter	Value (%)
Crude protein	91.07 ± 1.32
Crude fat	3.25 ± 0.30
Ash	3.29 ± 0.24
Moisture	2.39 ± 1.22
Yield	10.52 ± 0.24
Degree of hydrolysis	19.94 ± 0.23

*Values (% on dry weight basis) are presented as mean ± SD of three replicates

FMPH: Flavourzyme-mediated protein hydrolysate

Table 2. Colour measurement of FMPH

Parameter	Value
L*	84.03 ± 0.46
a*	2.37 ± 0.28
b*	16.80 ± 0.26
c*	16.97 ± 0.23
h*	82.03 ± 1.07
ÄE*	23.30 ± 0.17

*Values are represented as mean ± SD of three replicates

Colour is a crucial sensory attribute that influences the acceptance of protein hydrolysates (Table 2). The colour properties of FMPH exhibited lightness (L*), redness (a*), and yellowness (b*) values of 84.04 ± 0.46, 2.37 ± 0.29, and 16.80 ± 0.26, respectively. Chinese sturgeon hydrolysate exhibited lightness values of L* = 85.25, 86.96, and 84.40, and yellowness values of b* = 14.78, 14.28, and 14.65 for DH levels of 13.80%, 16.70%, and 19.10%, respectively (Noman et al., 2018). The hydrolysate of bighead carp demonstrated increased lightness (L* = 89.23, 88.82, 89.55) and mild yellowness (b* = 10.96, 12.41, 12.68) across various levels of hydrolysis (DH: 13.36, 17.09, and 20.15%) (Alahmad et al., 2022). Investigation by Unnikrishnan et al. (2021) revealed that Yellowfin Tuna protein hydrolysates exhibited a creamish-white colouration, with L*, a*, and b* values of 90.62 ± 0.05, -0.61 ± 0.01, and 17.16 ± 0.11, respectively. The colour characteristics of protein hydrolysates are largely influenced by the biochemical composition of the fish and the specific conditions used during enzymatic hydrolysis, both of which impact the final appearance of the powder.

Hygroscopicity is a critical parameter for evaluating the stability and handling of hydrolysates during processing and storage. The ability of a hydrolysate to absorb moisture influences both its shelf-life and powder flowability. FMPH demonstrated a hygroscopicity of 3.89 ± 0.87% (Table 3). The composition of hydrolysate is essential for defining their hygroscopic properties. The elevated protein content in the hydrolysate yields more polar groups, including amino and carboxyl groups, which attract water molecules, resulting in enhanced hygroscopicity.

Table 3. Physicochemical quality analysis of FMPH

Parameters	FMPH
Hygroscopicity (%)	3.89 ± 0.87
Bulk density (g/cm^3)	0.29 ± 0.02
Tapped density(g/cm^3)	0.34 ± 0.03
Hausner ratio	1.16 ± 0.15
Carr's Compressibility Index	12.82 ± 0.64
Turbidity	0.194 ± 0.003
Browning intensity	0.024 ± 0.001

*Values represent mean ± SD of three replicates

The assessment of bulk density, tapped density, Hausner ratio, and Carr's Compressibility Index (CI) is essential for evaluating the flow and packing properties of hydrolysate powders. The results are presented in Table 3. The bulk density and tapped density of FMPH were measured at 0.29 ± 0.02 g/cm^3 and 0.34 ± 0.03 g/cm^3, respectively. The bulk density of pollock skin hydrolyzed samples was observed to range between 0.12 and 0.14 g/cm^3 (Sathivel, Huang, & Bechtel, 2008). The Hausner ratio, indicative of powder flowability, and the CI, measuring powder compressibility, were determined to be 1.16 ± 0.15 and 12.82 ± 0.64, respectively. These values for FMPH fall within the acceptable range (1.12–1.18 and 11-15), signifying that FMPH exhibits favourable flowability and compressibility (Chaksmithanont, Bangsitthideth, Arunprasert, Patrojanasophon, & Pornpitchanarong, 2024). Their superior flow and packing characteristics are optimal for consistent powders in all formulations, including food, nutraceutical, and pharmaceutical formulations.

Turbidity is a critical measure for assessing the quality of protein hydrolysates, as it indicates the abundance of particles in suspension, dissolved proteins, and other aqueous components. The turbidity value of FMPH was 0.194 ± 0.003 (Table 3). A comparable result was noted with hydrolysates derived from fish by-products (Fuentes, Verdú, Grau, Barat, & Fuentes, 2024). High turbidity may signify the existence of intractable clumps or insufficient hydrolysis, affecting functional and bioactive characteristics. The browning capacity of the hydrolysate is a another important functional attribute that indicates the existence of products of the Maillard reaction generated during processing, which enhances colour and flavour. The browning capacity of FMPH was 0.024 ± 0.001. Elavarasan and Shamasundar (2016) reported that the brown intensity absorbance of freeze-dried mrigal hydrolysate at 420 nm is 0.036. The turbidity and browning capacity of hydrolysates are affected by the level of hydrolysis, protease type, and subsequent processing conditions.

The dissolution of proteins is a vital operational attribute that affects the prospective use of hydrolysates in the nutritional and medicinal sectors. The solubility characteristics of FMPH were assessed over a pH range of 3–11 (Fig. 1). The maximum solubility reported was 83.77 ± 0.90% at pH 7.0. These findings align with previous research on the solubility of fish protein hydrolysates, indicating a significant relationship between the optimum solubility and neutral pH conditions (Halim et al., 2016; Alahmad et al., 2022). In contrast, lowest solubility was noted at pH 5, measuring 58.53 ± 0.65%. This decrease may be ascribed to the agglomeration and sedimentation of larger-molecule peptides that are not completely hydrolyzed, as pH 5 is closely aligned with the isoelectric point (pI) of fish proteins

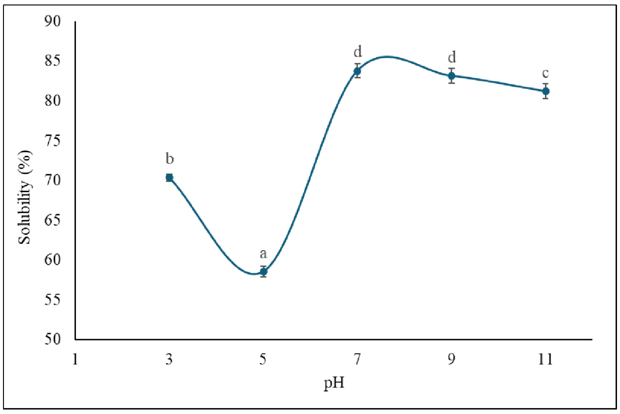

Fig. 1. Solubility of FMPH at various pH

*Values are expressed as mean ± standard deviation (n = 3). Different superscript letters indicate statistically significant differences ($p < 0.05$) in solubility at different pH levels

(Kristinsson & Rasco, 2000; Liu et al., 2014). This solubility pattern corresponds with other studies, demonstrating a reduction in hydrolysate solubility from pH 4.0 to 5.0, attributed to similar aggregation phenomena (Alahmad et al., 2023; Hassan et al., 2019). The correlation between solubility and hydrolysis rate is fundamental for explaining these data. Elevated DH yields reduced peptide fragments, revealing more ionizable amino and carboxyl groups, augmenting hydrophilic properties, and enhancing solubility (Kristinsson & Rasco, 2000). These results indicate that FMPH has considerable potential for numerous uses owing to its exceptional solubility throughout a broad pH spectrum.

The functional characteristics of proteins in a food system are partially determined by water–protein interactions, and the results are significantly influenced by their capacity to attach to and retain water within the mechanism. The water holding capacity of the FMPH was 0.96 ± 0.06 mL/g. Hassan et al. (2019) reported that the water holding capacity (WHC) of *Pangasianodon hypophthalmus* visceral hydrolysate was 0.80 ± 0.08, 0.84 ± 0.03, 0.71 ± 0.09, and 0.89 ± 0.80 mL/g for hydrolysates derived from papain, pepsin, acid, and alkali, respectively. The variation in the ability to hold water may be attributed to differences in the molecular mass of the peptides. Low molecular weight peptides can retain more water than their larger counterparts. Thus, FMPH may incorporate a hydrophilic polar group which can increase the water-holding capacity of the hydrolysates and is essential for improving cooking yield.

Oil absorption capacity (OAC) is an essential functional property of ingredients used in meat and confectionery products. The hydrophobic nature of protein hydrolysates results from the fragmentation of amino acid chains during hydrolysis, thereby exposing the inherent hydrophobic groups. Physical entrapment of oil significantly influences the efficacy of its intrinsic hydrophobic properties (Kristinsson & Rasco, 2000). The oil absorption capacity (OAC) of FMPH was 2.51 ± 0.03/ mL/g, which falls within the moderate range reported for fish hydrolysates and is considered suitable for incorporation into products such as meat emulsions, bakery items, and flavour-rich formulations requiring lipid retention. The dissolution rate and hydrolysis level of proteins greatly affect their oil absorption capacity, as shorter molecular sizes from hydrolysis generally reduce the oil absorption capacity (Noman et al., 2018). The oil absorption capacity of hydrolysates obtained from grass carp skin varied from 2.0 ± 0.30 to 4.90 ± 0.20 mg oil/g hydrolysate (Wasswa et al., 2007). Hassan et al. (2019) reported OAC values of 1.08 ± 0.12, 1.03 ± 0.04, 1.20 ± 0.12, and 1.39 ± 0.05 mL/g for hydrolysates produced using papain, pepsin, acid, and alkali, respectively. Similarly, sturgeon hydrolysate has an OAC of 2.59 g oil/g protein (Noman et al., 2018).

Foaming capacity and stability are essential characteristics of proteins, which significantly contribute to achieving products with substantially enhanced consistency and sensory value in the dietary supplements and cosmetic sectors. The foaming capability of proteins involves the reorganization of protein molecules at the air-water interface, promot-

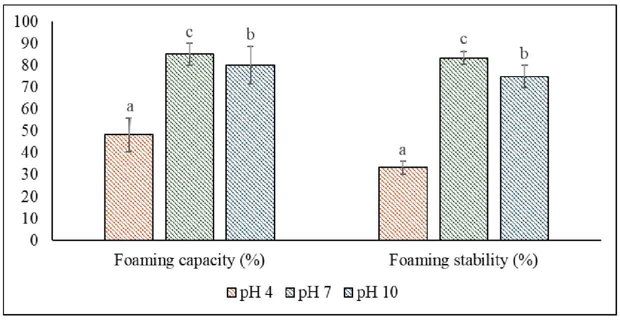

Fig. 2. Foaming ability and foaming stability of FMPH at various pH levels

*Values are presented as mean ± standard deviation (n = 3). Different superscript letters indicate statistically significant differences ($p < 0.05$, Duncan's multiple range test) at various pH levels.

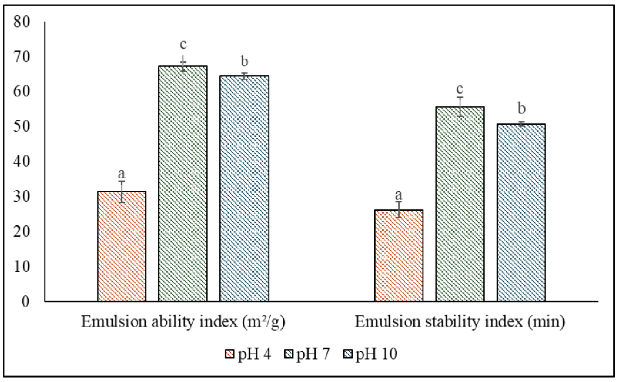

Fig. 3. Emulsion ability index and emulsion stability index of FMPH at various pH

Values are represented as mean ± standard deviation of three replicates (n = 3). Different superscript letters indicate statistically significant differences at various pH levels ($p < 0.05$, Duncan's multiple range test).

ing foam production by improving colloidal stability and preventing bubble amalgamation (Hassan et al., 2019). FMPH exhibited foaming capacity between 48.33 ± 7.63% and 85.00 ± 5.00%, along with stability characteristics that varied from 33.33 ± 2.89% to 83.33 ± 2.89% across various pH levels, as illustrated in Fig. 2. The lowest foaming capability and stability of the derived hydrolysate were observed at pH 5.0, which corresponded to the minimum protein solubility. This may have resulted from the closeness to the isoelectric value of the proteins, leading to a reduction in their net charge (Halim et al., 2016). The foaming characteristics were observed at pH 7.0, yielding FC and FS values of 168.0 ± 8.0% and 86.7 ± 6.3%, respectively (Vásquez, Sepúlveda, & Zapata, 2022). The FC and FS findings remained within the range documented for fish hydrolysates, ranging from 94.61% to 250% and 60.79% to 100%, respectively (Nguyen et al., 2022; Tejpal et al., 2017).

Natural hydrocolloids have gained attention as food emulsifiers due to their wide availability and beneficial biological properties. The ability of peptides to reduce the tension between polar and non-polar components in food is directly related to emulsification characteristics. Despite the generally observed inverse relationship between DH and emulsifying capacity, the FMPH produced in this study with a moderate DH of 19.94% exhibited notably high emulsifying activity (EAI: 67.19 ± 1.24 m^2/g) and stability (ESI: 55.64 ± 2.68 min), indicating that the flavourzyme-generated peptides retained adequate interfacial functionality to stabilize emulsions effectively (Fig. 3). Rainbow trout hydrolysates showed their highest emulsifying activity index (EAI) values at pH 7.0 and 10.0, measuring 45.56 ± 2.02 m^2/g and 45.78 ± 1.71 m^2/g, respectively. The corresponding emulsion stability index (ESI) values were 40.84 ± 3.80 minutes at pH 7.0 and 35.14 ± 3.18 minutes at pH 10.0 (Vásquez et al., 2022). These properties restrict the use of hydrolysates in acidic foods but increase the potential for employing neutral foods.

This study demonstrated that *P. hamrur* (bull's eye) skin can be effectively utilized to produce fish protein hydrolysates using flavourzyme under optimized conditions. The resulting FMPH exhibited a degree of hydrolysis of 19.94 ± 0.23% and a yield of 10.52 ± 0.24%. Proximate analysis of FMPH revealed a high protein content of 91.07 ± 1.32%, with low lipid (3.25 ± 0.30%) and ash (3.29 ± 0.24%) contents. FMPH displayed excellent solubility across a wide pH range, with maximum solubility of 83.77 ± 0.90% at pH 7.0. It also exhibited notable functional properties, including a water-holding capacity of 0.96 ± 0.06 mL/g, oil-holding capacity of 2.51 ± 0.03 mL/g, foaming capacity up to 85 ± 5.00%, and emulsifying activity index (EAI) of 67.19 ± 1.24 m^2/g with an emulsion stability index (ESI) of 55.64 ± 2.68 min. These attributes indicate its potential for use in food formulations, nutraceuticals, and bioactive ingredient development.

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