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Research Article
Optimization, Characterization and Antioxidant Activity of Fish Protein Hydrolysate from Payangka (*Ophieleotris aporos*) using Papain and Bromelain

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Abstract

Background and Objective: Payangka fish (*Ophieleotris aporos*), an underutilized endemic species from Lake Tondano, North Sulawesi, Indonesia, represents a potential resource for sustainable food fortification. Despite comprising 35% of the local catch, its biochemical valorization remains unexplored. This study aimed to optimize the enzymatic hydrolysis of Payangka fish muscle protein using papain and bromelain to produce bioactive hydrolysates suitable for functional food applications. **Materials and Methods:** Fish protein hydrolysis was performed at 55°C with a solid-to-liquid ratio of 1:4, using varying concentrations of papain and bromelain. The resulting hydrolysates were characterized for degree of hydrolysis (DH), proximate composition, color, amino acid profile and antioxidant activity. Statistical analysis was conducted to assess the effect of enzyme type and concentration on hydrolysis and bioactivity. Differences were considered statistically significant at $p<0.05$. **Results:** Increasing enzyme concentration positively correlated with DH. Papain achieved optimal DH (86.5±0.01% to 93.13±0.01%) within 2 hrs, whereas bromelain reached 71.94±0.01% to 84.01±0.01% after 8 hrs. Papain-derived hydrolysates (2 hrs, 4% enzyme) showed increased protein content (0.52-0.68%) and the highest antioxidant activity (85.76%). Total essential and non-essential amino acids were 74.47 ppm and 86.30 ppm, respectively, with lysine (26.65 ppm), leucine (15.02 ppm) and valine (11.01 ppm) being dominant essential amino acids and glutamic acid (26.34 ppm), aspartic acid (17.53 ppm) and alanine (9.83 ppm) as dominant non-essential amino acids. **Conclusion:** Fish protein hydrolysate from Payangka, particularly using 4% papain, demonstrates high bioactivity and favorable amino acid composition, highlighting its potential as a functional ingredient for food fortification. Future research could explore its incorporation into food products and long-term bioactivity.

Key words: Amino acids, bromelain, fish functional food, papain, *Ophieleotris aporos*, protein hydrolysis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish protein possesses unique characteristics among aquatic resources, making it a valuable material for various food and nonfood applications. This protein can be sourced from various fish species, including marine and freshwater fish, each exhibiting diverse chemical compositions and functional properties[1]. One promising approach to enhance the value of underutilized fish species is the production of FPH, a bioengineered ingredient obtained through enzymatic hydrolysis. This process breaks down proteins into smaller peptides and amino acids, improving their digestibility and functional properties[2]. The FPH is widely recognized for its nutritional potential, particularly in addressing malnutrition and stunting and is recommended for use in sports nutrition, clinical diets and infant formulas[3]. According to Sánchez and Vázquez[4], FPH is a rich source of bioactive peptides with diverse physiological effects, including antioxidant, antihypertensive, antimicrobial, antithrombotic, anticancer and immunomodulatory activities.

Enzymatic protein hydrolysis offers significant advantages over other hydrolysis methods, particularly in the food industry. This process can produce nutritional and functional products suitable for various food applications[5]. FPH is widely used as a source of essential amino acids and a natural flavor enhancer[6]. It also functions as a nutritional fortifier by increasing the protein content of food products[7]. Utilizing protein-rich fish viscera as raw material for FPH production presents a sustainable and cost-effective strategy with the potential to support public health initiatives, such as reducing stunting[8].

No study has specifically focused on synthesizing FPH from Payangka fish. Therefore, there is a clear need to optimize the enzymatic hydrolysis process for these species, including determining the most effective enzyme type, concentration and incubation time to achieve the highest degree of hydrolysis (DH). The DH is a key indicator of protein breakdown efficiency and is linked to the bioactivity and functionality of the resulting hydrolysate. This study aimed to characterize and optimize the production of Payangka FPH for potential applications in food products.

MATERIALS AND METHODS

Study area: This research was conducted from October, 2024 to August, 2025. Hydrolysis and antioxidant analysis took place at the Chemistry Laboratory of Universitas Negeri Manado, North Sulawesi, Indonesia and proximate analysis and HPLC analysis were carried out at the Chemistry Laboratory of Universitas Gadjah Mada, Yogyakarta, Indonesia. Materials: Payangka fish (*Ophieleotris aporos*) used in this study were obtained from Lake Tondano, located in Minahasa Regency, North Sulawesi Province, Indonesia. Payangka fish of approximately 4 months old and 120-130 g body weight (selected to minimize compositional variability) were collected from Lake Tondano at depths 2-5 m using standard gill nets. Fish were transported in aerated containers to the laboratory within 4 hrs at ambient temperature (28±2°C). Only fish showing normal behavior without visible disease or parasites were selected. Sex was not determined, but the population was assumed 1:1 male:female ratio based on maturation stage analysis.

All chemicals and reagents used in this study were of analytical grade. Papain and bromelain enzymes (Nanning Pangbo Biological Engineering Co., Ltd., Batch No. 20240815-PA and 20240816-BR). Enzyme activities determined were 25.3±1.2 units/mg for papain and 18.7±0.9 units/mg for bromelain. All enzyme preparations were stored at -20°C in sealed vials and used within 3 months of receipt to ensure consistent activity throughout experiments. Distilled water was obtained from WaterOne (Indonesia). Methanol (CH_3OH; Pro analysis EMSURE® ACS, Reag. Ph Eur; ≥99.8%), sodium hydroxide (NaOH; ACS reagent, ≥97.0%, pellets), hydrochloric acid (HCl; ACS standard; 37% w/w), trichloroacetic acid (TCA, CCl_3COOH; 99%; white crystalline powder), sulfuric acid (H_2SO_4; EMSURE®; 95.0-98.0%), boric acid (H_3BO_3; EMSURE®; 99.5%; crystals), sodium acetate (CH_3COONa; ≥99.0%; crystals) and petroleum ether (C_5H_{12}-C_6H_{14}) were purchased from Merck (Darmstadt, Germany). Tetrahydrofuran (THF, C_4H_8O; ≥99.8-99.9%), o-phthalaldehyde (OPA, $C_8H_6O_2$; ≥99.0%; crystalline powder) and DPPH (2,2-diphenyl-1-picrylhydrazyl, $C_{18}H_{12}N_5O_6$; ≥99.0%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The amino acid standard (18 amino acids; L-form for standardization), fluoraldehyde™ OPA reagent solution and crystals were supplied by Thermo Fisher Scientific (Waltham, MA, USA).

Main tools used are waterbath (Memmert), UV-Vis Spectrophotometer (Carry Multicell by Agilent), chromameter (CR-400 Konica Minolta, Inc.) from the Chemistry Laboratory of Universitas Negeri Manado. HPLC (Thermo Dionex Ultimate 3000) was conducted at the Chemistry Laboratory of Universitas Gadjah Mada.

Fish preparation: Before processing, the fish were transported under controlled conditions to the laboratory to maintain freshness and minimize biochemical degradation that might affect protein integrity. After arrival, the fish were thoroughly washed with clean running water to remove

adhering impurities, descaled and eviscerated to separate edible muscle tissue from inedible parts such as bones, viscera and skin. The muscle tissues, which serve as the primary protein source, were homogenized and subsequently stored at -20°C until further hydrolysis experiments were conducted. The experimental protocol, including fish handling and sampling, was approved by the Ethics Committee of Universitas Negeri Manado. All necessary permits for the collection of Payangka fish samples from Lake Tondano were obtained from the relevant local authorities.

Protein hydrolysis assay: The Payangka fish flesh was homogenized and mixed with distilled water at a 1:4 (w/v) ratio. Enzymatic hydrolysis was conducted using papain and bromelain at 2-10% (w/w) concentrations. Hydrolysis was conducted at a constant 55±1°C (maintained via water bath with ±1°C precision) at pH 7.0±0.2. Incubation times of 2, 4, 6 and 8 hrs were employed. Enzyme activity was terminated by heating, followed by sequential filtration (spinner, micro- and ultrafiltration). The filtrate was centrifuged and the supernatant was filtered through Whatman paper to obtain a clear FPH, which was then analyzed for its DH, proximate and amino acid compositions[9,10].

Degree of hydrolysis measurement: The DH was measured using a modified OPA spectrophotometric method[10,11]. The sample was split into two portions: treated with 6.25% TCA and untreated. After 15 min of incubation, the TCA-treated sample was centrifuged at 8,000 rpm for 15 min. A 20 µL aliquot of the supernatant was mixed with 150 µL of OPA reagent, vortexed and its absorbance was measured at 340 nm (UV-Vis Spectrophotometer Carry Multicell by Agilent). The FPH sample with the highest DH was selected for further analyses, including proximate composition, amino acid profile and antioxidant activity. The DH was calculated as:

$$\text{DH}\ (\%) = \frac{\alpha - \text{amino groups in TCA} - \text{soluble fraction}}{\text{Total } \alpha - \text{amino groups in untreated sample}} \times 100$$

Proximate analysis: Proximate analysis was conducted to determine the protein, fat, moisture and ash content of the FPH, following the standard procedures of the AOAC[12]. The color values for all samples were measured using a Minolta colorimeter (Konica Minolta CR-400 Series) and expressed in terms of L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) values on the Hunter scale.

Amino acids analysis: Amino acid analysis was performed using the HPLC method[10,12]. 60 mg of dried FPH were hydrolyzed with 4 mL of 6 N HCl at 110°C for 24 hrs. After diluted with Aquadest to a final volume of 10 mL and filtered using a 0.2 µm Whatman filter. A volume of 50 µL of the filtrate was mixed with 300 µL of derivatizing reagent and 10 µL of the mixture was injected into the HPLC for amino acid analysis. A standard chromatogram was prepared using an amino acid standard. HPLC conditions: column: LiChrospher 100 RP-18 (5 µm); mobile phase: CH_3OH:50 mM CH_3COONa: tetrahydrofuran (2:96:2) pH 6.8; flow rate: 1.5 mL/min; temperature: Room temperature (27°C); pressure: 3000 psi; detector: Fluorescence (λ = 350-450 nm). The amino acid content (ppm) was calculated using a linear regression equation derived from the amino acid standard curve.

Antioxidant analysis: The antioxidant activity of the hydrolysate was evaluated using the DPPH radical scavenging assay[13]. Sample solutions were prepared at various concentrations in methanol. For each assay, 1 mL of the sample solution was mixed with 1 mL of 0.1 mM DPPH solution and 2 mL of methanol. The mixture was incubated in the dark and the absorbance was measured at 517 nm (Agilent UV-Vis spectrophotometer). A blank solution was prepared by replacing the sample with methanol (1 mL). Antioxidant capacity was assessed by calculating the percentage of DPPH inhibition.

Statistical analysis: Data were analyzed using analysis of variance (ANOVA) one-way in a completely randomized design, with Duncan's Multiple Range Test used for *post-hoc* comparisons. Each determination was performed in triplicate to ensure reliability and the results were expressed as the Mean±Standard Deviation. Differences were considered statistically significant at $p<0.05$. IBM SPSS Statistics 27 Program (International Business Machines Corporation, New York, USA) is used for statistical analysis.

RESULTS

Degree of hydrolysis of Payangka fish protein hydrolysate: The degree of hydrolysis (DH) was used as the main parameter to evaluate the efficiency of Payangka fish protein hydrolysis using papain and bromelain. Optimization was carried out by varying enzyme concentrations (2, 4, 6, 8 and 10%) and incubation times (2, 4, 6 and 8 hrs). The DH values obtained from each treatment combination are presented in Fig. 1.

Figure 1 shows that papain and bromelain exhibited distinct hydrolytic behaviors on Payangka fish protein. Papain demonstrated rapid and concentration-dependent hydrolysis, with DH values at 2 hrs incubation ranging from 86.5±0.01% (2% enzyme) to a maximum 93.13±0.01% (4% enzyme). Further increase in papain concentration to 6-10% did not

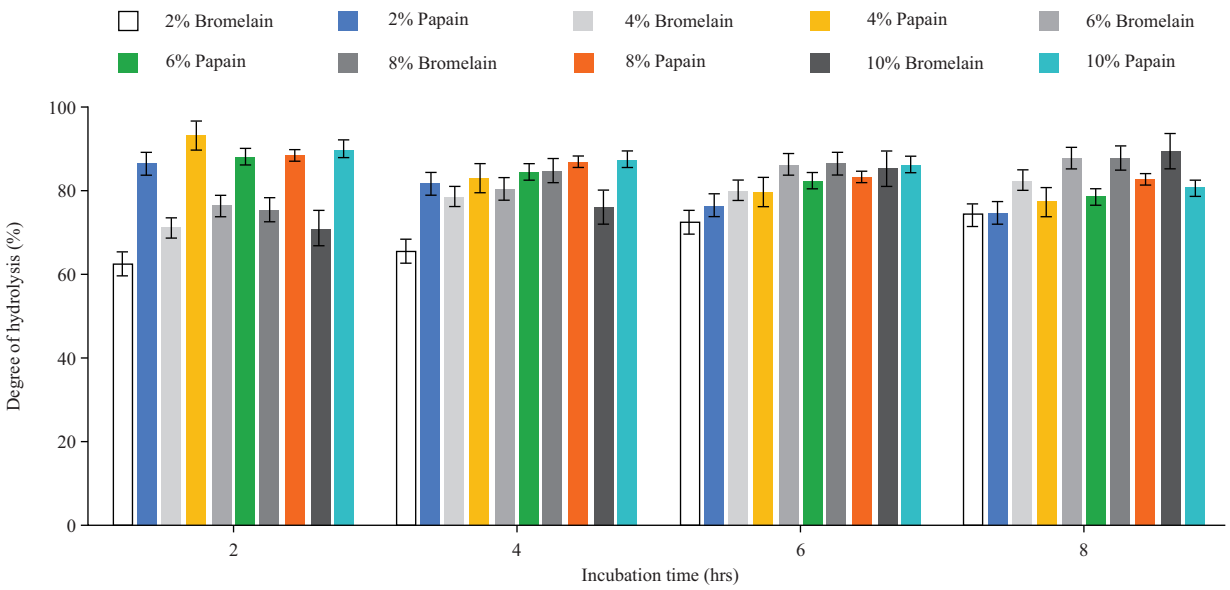

Fig. 1: Degree of hydrolysis of Payangka fish protein hydrolysate at various incubation times and different concentrations of papain and bromelain enzymes

Table 1: Proximate Payangka fish flesh, its protein hydrolysate with papain enzyme and its color analysis

		Payangka fish protein hydrolysate				
		Papain enzyme concentration/Degree of hydrolysis (%)				
Proximate (%)	Payangka fish flesh	2% (DH = 87.14)	4% (DH = 93.13)	6% (DH = 87.92)	8% (DH = 88.29)	10% (DH = 89.98)
Protein	16.56±1.2	3.33±0.01^{e}	3.89±0.02^{a}	3.47±0.01^{d}	3.61±0.015^{c}	3.71±0.01^{b}
Fat	2.81±0.07	0.52±0.01^{cd}	0.68±0.03^{a}	0.47±0.02^{e}	0.53±0.01^{c}	0.67±0.03^{ab}
Moisture	78.05±1.4	90.67±1.2^{a}	90.75±1.3^{a}	90.63±1.1^{a}	90.80±1.1^{a}	90.75±2.0^{a}
Ash	0.87±0.01	0.02±0.01^{d}	0.08±0.015^{a}	0.04±0.01^{b}	0.03±0.01^{bc}	0.08±0.02^{a}
Crude fiber	0.1±0.0	0.05±0.005^{c}	0.09±0.003^{a}	0.08±0.002^{b}	0.09±0.005^{a}	0.09±0.007^{a}
Hunter color parameters						
L*	54.25±0.93^{de}	58.51±1.27^{a}	55.22±0.13^{d}	55.92±0.52^{c}	56.79±0.18^{b}	
a*	-0.32±0.04^{a}	-0.73±0.03^{e}	-0.44±0.05^{b}	-0.45±0.06^{bc}	-0.58±0.03^{d}	
b*	3.14±0.29^{e}	7.64±0.05^{a}	4.86±0.32^{cd}	5.12±0.04^{c}	5.56±0.14^{b}	

Values are expressed as Mean±Standard Deviation (n = 3). L: Indicates lightness (0 = black, 100 = white), a: Represents redness (+) to greenness (-) and b: Represents yellowness (+) to blueness (-). Values are expressed as Mean±Standard Deviation. Different superscript letters (a-e) within the same row indicate significant differences among treatments (p<0.05)

significantly enhance DH (89.98±0.23% at 10%), suggesting optimal hydrolysis at 4% enzyme concentration. In contrast, bromelain showed progressive, time-dependent hydrolysis with maximum DH achieved only at 8 h incubation, peaking at 84.01±0.18% with 10% enzyme concentration.

Based on these findings, papain treatment for 2 hrs was identified as the most effective condition for protein hydrolysis in Payangka fish. Therefore, hydrolysates obtained using papain at enzyme concentrations of 2, 4, 6, 8 and 10% with a fixed incubation time of 2 hrs were selected for subsequent proximate analysis.

Proximate Payangka fish protein hydrolysate: The proximate composition of raw Payangka fish (*Ophieleotris aporos*) and its protein hydrolysates (FPH) obtained using papain enzyme is presented in Table 1. The flesh of Payangka fish contained 16.56±1.2% protein, indicating a promising nutritional value compared to other freshwater species. Proximate analysis of FPH produced with varying papain concentrations (2, 4, 6, 8 and 10%) at a constant incubation time of 2 hrs showed consistent increases in protein content (3.33±0.01% to 3.89±0.02%) with increasing enzyme concentration and hydrolysis degree (DH). Fat content also increased with higher enzyme concentration and DH (0.52±0.01% to 0.68±0.03%). Moisture content remained relatively stable (90.67±1.2% to 90.75±2.0%), while ash content ranged from 0.02±0.01% to 0.09±0.01%. Crude fiber content showed no clear trend (0.05±0.005% to 0.09±0.007%).

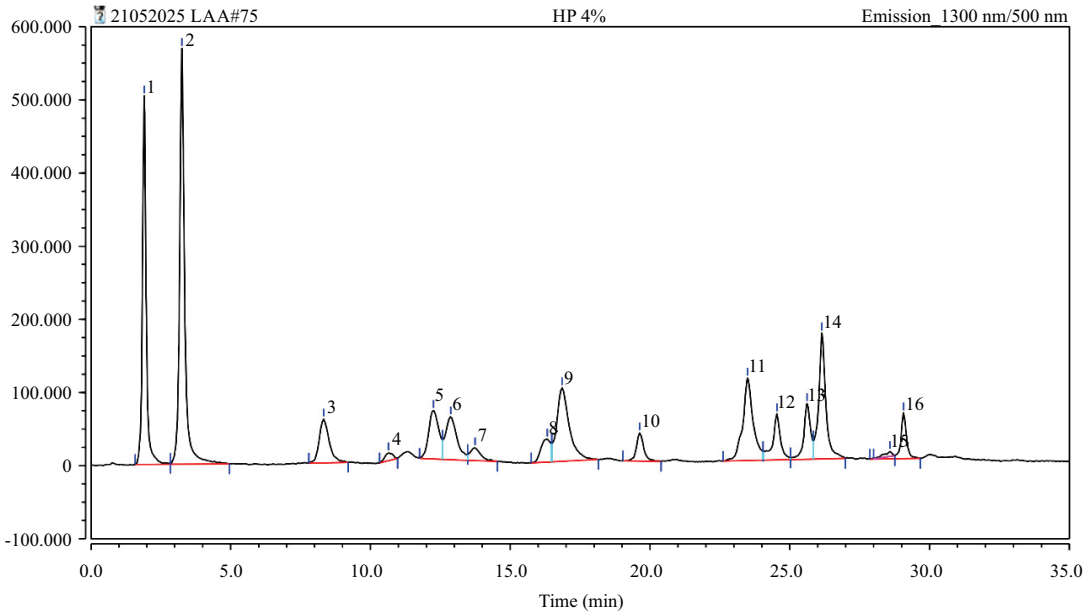

Fig. 2: Amino acid chromatogram of Payangka fish protein hydrolysate with 4% papain enzyme

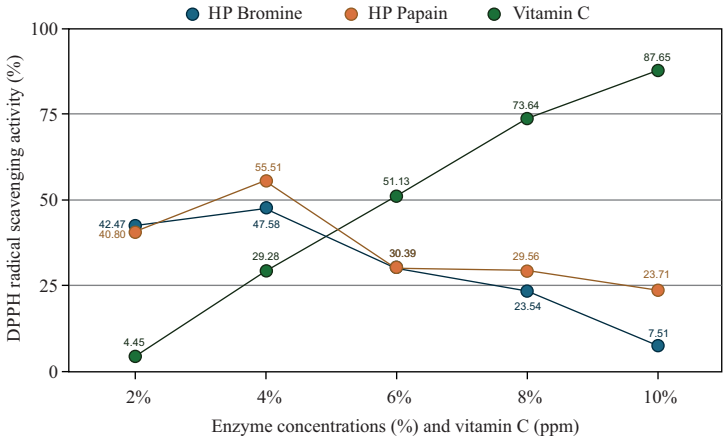

Fig. 3: DPPH radical scavenging activity of FPH Payangka at various enzyme concentrations compared to vitamin C

Color analysis using the Hunter Color Notation System revealed that both enzyme concentration and DH influenced the lightness (L*), redness (a*) and yellowness (b*) values of the FPH. The highest L* value, indicating the brightest hydrolysate, was observed at 4% papain concentration, which also corresponded to the highest DH (93.13%).

Amino acids of Payangka fish protein hydroliste: The amino acid chromatogram of Payangka FPH produced with 4% papain is shown in Fig. 2, while the essential and non-essential amino acid compositions of FPH obtained with varying papain concentrations are summarized in Table 2. An increase in the degree of hydrolysis (DH) was accompanied by an increase in essential, non-essential and total amino acid contents. The dominant amino acids detected across all treatments were aspartic acid, glutamic acid, lysine and leucine. Additionally, the total content of essential amino acids was consistently higher than that of non-essential amino acids in all hydrolysates.

Antioxidant activity of Payangka fish protein hydrolysate: Based on Fig. 3, the antioxidant activity of Payangka FPH produced with papain at concentrations ranging from 2% to 10% varied between 23.71% and 55.51%, with the highest activity observed at 4% enzyme concentration. Similarly, FPH produced with bromelain showed antioxidant activity ranging from 7.51 to 47.58%, with peak activity also occurring at a 4% enzyme concentration. The high antioxidant activity of FPH

Table 2: Essential and non-essential amino acid content of Payangka fish protein hydrolysate with papain enzyme

	Amino acids concentration (ppm)				
	Papain enzyme concentration/Degree of hydrolysis (%)				
Amino aids	2% (DH=87.14)	4% (DH=93.13)	6% (DH=87.92)	8% (DH=88.29)	10% (DH=89.98)
Essential					
Histidine	3.24±0.48^{a}	2.24±0.14^{c}	2.43±0.12^{b}	2.09±0.60^{e}	2.15±0.14^{d}
Threonine	6.58±0.47^{a}	6.51±0.20^{a}	3.08±0.48^{c}	6.40±0.57^{a}	4.76±0.10^{b}
Valine	8.05±0.54^{b}	11.01±22^{a}	5.98±0.46^{d}	10.72±0.42^{a}	6.21±0.54^{c}
Phenylalanine	6.01±0.27^{c}	7.80±0.66^{a}	4.52±0.62^{e}	6.13±0.40^{b}	5.34±0.37^{d}
Isoleucine	4.04±0.33^{b}	5.24±0.29^{a}	2.88±0.20^{d}	4.62±0.60^{a}	3.13±0.59^{c}
Leucine	12.79±0.71^{b}	15.02±0.35^{a}	9.04±0.54^{d}	14.54±67^{a}	10.33±0.76^{c}
Lysine	23.85±0.29^{b}	26.65±0.41^{a}	21.48±0.38^{c}	20.31±0.57^{e}	20.46±0.38^{d}
TOTAL	64.56	74.47	49.41	64.81	52.38
Non-Essential					
Aspartic acid	17.53±0.49^{b}	19.71±0.28^{a}	9.40±0.38^{d}	19.45±0.12^{a}	11.4±0.27^{c}
Glutamic acid	26.34±0.33^{c}	29.8±0.18^{a}	17.54±0.69^{e}	28.7±0.40^{b}	19.27±0.34^{d}
Serine	6.13±0.27^{b}	6.60±0.22^{a}	4.78±0.69^{c}	6.54±0.21^{a}	4.48±0.18^{d}
Glycine	4.74±0.47^{a}	4.59±0.43^{a}	3.75±0.57^{b}	4.19±0.82^{a}	3.54±0.43^{c}
Arginine	4.83±0.54^{a}	5.30±0.65^{a}	3.88±0.38^{b}	5.44±0.45^{a}	3.34±0.33^{c}
Alanine	9.83±0.21^{b}	11.03±0.11^{a}	7.20±0.22^{d}	10.99±0.39^{a}	7.76±0.68^{c}
Tyrosine	7.80±0.19^{b}	9.27±0.73^{a}	6.14±0.31^{d}	9.06±0.53^{a}	6.39±0.43^{c}
TOTAL	77.20	86.30	52.69	84.37	56.18

Values are expressed as Mean±Standard Deviation (n = 3). Means with different letters in the same row were significantly different at the p<0.05 level

prepared with 4% papain is associated with its high essential and non-essential amino acid content, as shown in Table 2. Furthermore, based on the linear regression equation of vitamin C antioxidant activity, the DPPH radical scavenging ability of FPH produced using 4% papain, when tested at full concentration, was equivalent to vitamin C at approximately 6.8 ppm.

DISCUSSION

The DH is a key indicator of the extent of protein hydrolysis, as it reflects the proportion of peptide bonds cleaved relative to the total number of bonds in the original protein[14]. Based on Fig. 1, the results of this study demonstrate that papain and bromelain differ in their hydrolytic efficiency and optimal incubation times. Papain exhibited rapid hydrolysis within 2 hrs, whereas bromelain required longer incubation (8 hrs) to achieve its maximum DH. This suggests that papain operates more efficiently at early stages, whereas bromelain exhibits progressive activity over extended incubation periods.

The higher efficiency of papain at shorter times indicates that it may have a lower activation energy for peptide bond cleavage compared to bromelain. Similar observations were reported by Kaur *et al.*[15], where papain efficiently hydrolyzed milk proteins in shorter reaction times because papain has lower Michaelis-Menten constant (K_M) values than bromelain. The decline or stagnation in DH over time observed with papain is consistent with enzyme kinetics, where the reaction slows down after rapid initial hydrolysis[16]. In contrast, bromelain's progressive increase in DH with longer incubation aligns with the findings of Palla *et al.*[17], who reported that bromelain hydrolysis of grouper viscera protein reached its maximum DH (89.28%) after 8 hrs of incubation.

The DH values obtained in this study are relatively high compared with those reported in previous studies, such as 94%[18] and 77.99%[19], although lower values have also been documented, including 61.33%[10], 27%[20], 9.10-16.14%[21] and 10.11%[22]. These variations indicate that DH outcomes depend on multiple factors, including raw material type, enzyme specificity, concentration, incubation time and the analytical method used for DH determination.

Overall, the present findings highlight papain as the more suitable enzyme for Payangka fish protein hydrolysis, given its ability to achieve higher DH values within shorter incubation times compared to bromelain. This characteristic makes papain a preferred option for producing protein hydrolysates with potentially better functionality and economic efficiency.

The protein content of raw Payangka fish, presented on Table 1, was relatively high (16.56 ±1.2%) compared to other freshwater species inhabiting similar aquatic environments. Ramlah *et al.*[23] reported that fish nutritional composition is strongly influenced by habitat, as demonstrated by Nile tilapia, with protein levels ranging from 12.94% to 16.79%.

In contrast, the protein content of FPH (3.33-3.89%) was considerably lower than that of raw Payangka flesh. This reduction is attributed to the solid-to-liquid ratio of 1:4 applied during hydrolysis, which diluted the protein concentration,

as no post-processing concentration methods (such as evaporation, spray drying or freeze-drying) were employed. Elavarasan and Shamasundar[24] highlighted that additional steps, including centrifugation, filtration, evaporation at 40°C and freeze-drying, substantially increase the protein content of FPH.

Interestingly, the highest protein content in this study (3.89±0.02%) corresponded with the highest DH (89.98%), supporting previous findings by Alahmad *et al.*[25] in bighead carp (Hypophthalmichthys nobilis) hydrolysates. Chalamaiah *et al.*[26] also emphasized that increasing DH reflects enhanced protein solubilization, as insoluble and indigestible fragments are broken down during enzymatic hydrolysis.

Regarding color, the lightness (L*) values of Payangka FPH were consistent with previous studies on protein hydrolysates from silver carp (Hypophthalmichthys molitrix) produced using alcalase, reported by Rostami *et al.*[27]. Color serves as an important indicator of hydrolysate quality and consumer acceptability, as well as potential application suitability in food products. The relatively bright color observed at 4% papain concentration suggests an optimal balance between enzymatic activity and product stability.

The increase in amino acid content with higher DH observed in this study aligns with the findings of Alahmad *et al.*[25], who reported that enzymatic hydrolysis promotes peptide bond cleavage into smaller peptides, thereby enhancing amino acid release. The predominance of aspartic acid, glutamic acid, lysine and leucine is consistent with previous reports on fish protein hydrolysates[25], suggesting that Payangka FPH shares compositional similarities with other fish-derived hydrolysates widely studied for their biofunctional properties.

From a nutritional standpoint, the relatively high levels of lysine and leucine are noteworthy. Lysine is often a limiting amino acid in cereal-based diets and its abundance in Payangka FPH indicates that the hydrolysate has potential as a protein fortificant in food formulations. Leucine, on the other hand, plays a central role in stimulating muscle protein synthesis via activation of the mTOR signaling pathway, making Payangka FPH potentially valuable in sports nutrition and clinical nutrition applications[28]. Similarly, the presence of glutamic acid and aspartic acid contributes to flavor-enhancing properties, as these amino acids are associated with umami taste and can act as natural flavor precursors in food systems[29].

Furthermore, the balance between essential and non-essential amino acids has implications for both digestibility and bioavailability. According to FAO/WHO protein quality evaluation standards, proteins with higher essential amino acid content are considered superior for human nutrition[30]. The predominance of essential amino acids in Payangka FPH indicates a high-quality protein source, supporting its suitability for incorporation into functional foods and nutraceuticals.

In addition to their nutritional role, specific amino acids identified in Payangka FPH may exert physiological benefits beyond basic nutrition. For example, glutamic acid and alanine are known precursors for neurotransmitters, while arginine plays a critical role in nitric oxide synthesis, which regulates vascular function and immune response[31]. These multifunctional properties underscore the potential of Payangka FPH not only as a nutritional supplement but also as a source of bioactive peptides with health-promoting effects.

Taken together, the amino acid composition of Payangka FPH produced with 4% papain indicates that this hydrolysate possesses both high nutritional quality and promising biofunctional attributes. Its richness in hydrophobic amino acids enhances antioxidant activity, while its essential amino acid profile supports its use in improving the nutritional value of food products[32]. These findings highlight the potential of Payangka FPH as a versatile ingredient for the development of functional foods, dietary supplements and therapeutic formulations.

Antioxidant assays of FPH revealed the presence of electron-donating compounds capable of reacting with DPPH free radicals. The results of the DPPH free radical scavenging activity of Payangka FPH are shown in Fig. 3.

The strong antioxidant activity observed in Payangka FPH, particularly with 4% papain, can be attributed to its amino acid composition and degree of hydrolysis. According to Xu *et al.*[33], the 20 amino acids can be classified into two groups based on their antioxidant capacities. Seven amino acids (tryptophan, methionine, histidine, lysine, cysteine, arginine and tyrosine) exhibit higher overall antioxidant capacities compared to the other 13. Among these, four were consistently present at optimal levels in the FPH produced with 4% papain.

This compositional advantage explains why FPH generated with papain at 4% concentration displayed superior antioxidant activity compared to other treatments, including bromelain hydrolysates. Hydrophobic amino acids, such as tryptophan and tyrosine, are particularly effective in donating protons or electrons to free radicals, thereby stabilizing them and preventing oxidative chain reactions in lipid systems.

The relatively high levels of lysine and arginine not only enhance antioxidant activity but also contribute to the nutritional quality of the hydrolysate. In addition, several studies have suggested that peptide size plays an equally important role in determining antioxidant potential. Lower

molecular weight peptides (<3 kDa) generally exhibit higher radical scavenging activity due to their increased mobility and ability to access radical species more efficiently[34]. Since the degree of hydrolysis (DH) was higher in papain-treated hydrolysates, it is reasonable to infer that the presence of shorter peptide chains contributed to the observed strong DPPH radical scavenging activity.

The equivalence of the antioxidant capacity of 4% papain-derived FPH to approximately 6.8 ppm vitamin C underscores its potential as a natural antioxidant source. Unlike synthetic antioxidants, which may pose safety concerns, protein hydrolysates offer multifunctionality-providing both nutritional value and bioactive properties. Furthermore, the results align with previous findings on fish protein hydrolysates from tilapia, sardine and tuna, which demonstrated significant antioxidant effects linked to their amino acid and peptide compositions[35-37]. This highlights the universality of enzymatic hydrolysis as an effective method for generating bioactive compounds from underutilized fish species.

Taken together, the findings suggest that Payangka FPH, particularly when hydrolyzed with 4% papain, represents a promising candidate for functional food applications. Its high antioxidant potential, combined with essential amino acid enrichment, could be leveraged in the development of nutraceuticals or as a natural preservative to improve food stability. Further studies, such as peptide sequencing and *in vivo* antioxidant assays, would be essential to confirm the specific bioactive peptides responsible for the activity and to evaluate their bioavailability and safety in biological systems.

CONCLUSION

The characteristics of Payangka fish protein hydrolysate were markedly influenced by enzyme type, concentration and hydrolysis time. Papain exhibited superior efficiency, with optimal hydrolysis achieved at 4% concentration within 2 hrs. Under these conditions, the hydrolysate showed enhanced nutritional quality and strong antioxidant activity, comparable to 6.8 ppm vitamin C. The enrichment of essential and hydrophobic amino acids contributed significantly to its radical scavenging potential. Overall, Payangka FPH demonstrates strong potential as a functional ingredient for food, nutraceutical and natural preservation applications.

SIGNIFICANCE STATEMENT

This study identifies optimal enzymatic conditions for producing Payangka fish protein hydrolysate with enhanced nutritional quality and antioxidant activity. The findings highlight papain-assisted hydrolysis as an efficient approach to generate a natural antioxidant-rich functional ingredient. The results support the potential application of Payangka FPH in food fortification, nutraceutical formulations and natural preservation systems.

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