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OPTIMIZATION OF BROMELAIN-ASSISTED HYDROLYSIS FOR ANTIOXIDANT PEPTIDE PRODUCTION FROM BAGRID CATFISH (*Mystus nigriceps*) VISCERA

Matthew Arian[1], Sulistyo Emantoko Dwi Putra[1], Yayon Pamula Mukti[2], Dicky Harwanto[3,4], Gabriel Tirtawijaya[5*]
[1]Department of Biotechnology, Faculty of Biotechnology, University of Surabaya, Surabaya, Indonesia
[2]Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung, Taiwan
[3]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Semarang, Indonesia
[4]Program Study of Doctoral Aquatic Resources Management, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Semarang, Indonesia
[5]Department of Biology, Faculty of Biotechnology, University of Surabaya, Surabaya, Indonesia
Email: gabrieltirtawijaya@staff.ubaya.ac.id

ABSTRACT

Bagrid catfish (*Mystus nigriceps*) is among the commonly processed species in traditional smoked fish production, which generates waste approximately 35% of total fish weight. This waste is typically discarded without treatment and may lead to environmental degradation. However, these by-products are rich in protein with antioxidant potential, presenting an opportunity for sustainable valorization. This study aimed to optimize the enzymatic hydrolysis of bagrid catfish viscera using bromelain to produce antioxidant peptides. Hydrolysis was carried out using viscera waste sourced from a smoked fish production center, as a representative model of fishery waste. Optimization parameters included hydrolysis time (0–6 hours) and bromelain concentration (1–5%). Bagrid fish protein hydrolysate (BVPH) was analyzed for antioxidant activity using DPPH *(2,2-Diphenyl-1-picrylhydrazyl)* radical scavenging assay, degree of hydrolysis, soluble protein content, and yield. The highest antioxidant activity was observed at 3% bromelain concentration and 1-hour hydrolysis, which was 66.0 ± 0.7%. The best-performing BVPH exhibited 6.1 ± 0.2% degree of hydrolysis, 1207.4 ± 9.8 µg/mL soluble protein content, and 16.1 ± 0.1% yield. The peptides produced show strong potential for application as bioactive ingredients in food, nutraceutical, and cosmetic products. This approach supports circular bioeconomy strategies and highlights the practical potential of transforming fishery waste into high-value functional compounds.

Keywords: Antioxidant Peptide, Bromelain Assisted Hydrolysis, Bioactive Compound; Bioconversion; Fish Protein Hydrolysate

INTRODUCTION

Indonesia is the world's number two seafood producer with an incredibly diverse system (Surbakti *et al*., 2026). Surabaya, as one of Indonesia's active urban fisheries hubs, includes the Kenjeran Smoked Fish Center (KSFC)—a site that illustrates typical waste generation from smoked fish processing. Management of the waste is a priority for many fisheries hubs because the active urban fisheries hubs. Due to the large amounts of fish waste discarded into the environment without further processing, leading to environmental pollution (Merliza & Mirwan, 2022). The fish waste is mainly composed of 7% viscera, 18% head, and 10% backbone frames. The waste often represents about 20 – 50% of the total fish weight (Fouda, 2018). The crude protein content of fish waste ranges from 8% to 35%, depending on the type of waste and the fish species (Rana *et al*., 2023), making it a promising raw material for bioconversion into functional ingredients. To mitigate the environmental impact caused by the disposal of smoked fish waste, it is crucial to explore sustainable utilization and processing strategies. Fish waste valorization can be accomplished by utilizing by-product biomasses through hydrolysis, particularly enzymatic hydrolysis of fish waste into fish protein hydrolysate (FPH). The type of fish that used in this study is bagrid fish, since it is one of the commonly processed into smoke fish product in the KSFC.

FPH consists of short-chain peptides with various functional properties. FPH is a source of short-chain bioactive peptides (2 – 20 amino acids) possessing several health-related properties, including antihypertensive, antithrombotic, antimicrobial, immunomodulatory, and antioxidant activities (Medhe *et al.*, 2025). Antioxidants are essential because an imbalance in free radicals can trigger oxidative damage, including protein degradation, phospholipid membrane oxidation, modification of low-density lipoproteins, and DNA mutations that can lead to cancer and diabetes mellitus (Chaudhary *et al.*, 2023). Consequently, many studies have focused on identifying natural antioxidants, including products derived from animals and plants. Natural product sources offer benefits such as being safe for consumption, having low production costs, being available in the environment, and contributing to reducing environmental waste (Prado & Rostagno, 2022). A significant amount of antioxidant compounds is present in the FPH. Leiva-Portilla *et al.* (2023) showed an antioxidant activity of 52.4% for FPH from Chilean nylon shrimp (*Heterocarpus reedi*), while Elavarasan & Shamasundar (2022) reported an antioxidant activity up to 92% for FPH from freshwater carps (*Catla catla*). Peptides with free radical-stabilizing properties serve as the source of these antioxidants (Mardani *et al.*, 2023; Liu *et al.*, 2024)

The FPH can be produced through protein hydrolysis assisted by bromelain enzymes. Bromelain showed higher protein solubility, as well as higher content of hydrophobic and

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flavor-contributing amino acids than another commercial protease, PROTEASE FP51® (Selamassakul *et al*., 2016). This enzyme is a thiol endopeptidase derived from pineapple fruit and stem, whose activity depends on the presence of thiol groups in cysteine residues (Alnajar, 2023). Bromelain contains cysteine thiol groups that confer antioxidant activity by donating hydrogen atoms from their thiol groups, thereby neutralizing free radicals (Nikoo *et al.*, 2023). Through enzymatic hydrolysis, peptides gain enhanced bioactivity, including mitigating DNA damage and acting as radical scavengers against reactive oxygen species (ROS) and related oxidants, thereby boosting antioxidant potential (Habinshuti *et al.*, 2023). Hydrolysis enhances antioxidant potential by exposing amino acids, which increases hydrogen ion availability and carboxylic acid concentration (Yasar *et al.*, 2024). Consequently, these peptides can have promising therapeutic potential in disease prevention and treatment (Ishak & Sarbon, 2018). Based on its source and functionality, bromelain is safe to use and is widely applied in food and pharmaceutical industries.

Several studies demonstrated that FPH from bromelain hydrolysis have antioxidant activities. A study by Noman (2022) demonstrated that FPH from hybrid sturgeon (*Huso dauricus* × *Acipenser schrenckii*) muscle tissue, hydrolyzed for 3 hours with 1% bromelain, yielded an antioxidant activity of 29.15%. When the bromelain concentration was increased to 5%, the antioxidant activity of the hybrid sturgeon FPH increased to 65.17%. This indicates that enzyme concentration significantly influences the resulting antioxidant activity, necessitating optimization of the enzyme concentration. Mohammad *et al.* (2023) added that the release of bioactive compounds during hydrolysis is dependent on the type of protease enzyme used. Meanwhile, research conducted by Sholahuddin *et al.* (2024) on tilapia viscera FPH showed that FPH produced from hydrolysis with varying concentrations of bromelain and hydrolysis times yielded hydrolysates with various IC_{50} values (0.089 - 0.131 mg/mL). This states the importance of optimizing hydrolysis parameters to produce FPH of optimal quality. However, the application of bromelain for hydrolyzing *Mystus nigriceps* viscera remains unexplored, and the specific relationship between hydrolysis parameters (enzyme concentration and hydrolysis time) and the resulting antioxidant activity of the hydrolysate is not well established.

This study focused on generating antioxidant peptides from bagrid fish viscera through enzymatic hydrolysis with bromelain. The resulting bagrid viscera protein hydrolysate (BVPH) was characterized through proximate analysis to analyze its moisture, ash, fat, protein, and carbohydrate content (Nielsen, 2024). Furthermore, hydrolysis conditions were optimized based on 4 parameters: antioxidant activity, degree of hydrolysis (DH), soluble protein, and BVPH yield.

RESEARCH METHODS

The research was conducted from October 2023 to June 2024. Bagrid fish viscera waste was obtained from the Kenjeran Smoked Fish Center (Surabaya, Indonesia). Bromelain was purchased from Shaanxi Rainwood Biotech Co., Ltd. with the enzyme activity of 50,000 GDU/g. The experiments and analyses were carried out at the Laboratory of the Faculty of Biotechnology, University of Surabaya.

Preparation of Bagrid Fish Viscera Waste

Bagrid fish viscera waste was transported in an icebox, equipped with ice packs. The waste was cut into smaller pieces using a kitchen knife. Approximately 700 g of the waste was homogenized with 700 mL of sterile distilled water (pH 7.7) using a blender until a uniform slurry was obtained. The homogenate was then centrifuged at 6,000 rpm for 10 min at 4°C to separate the floating lipid layer and aqueous phase from the insoluble tissue fraction. The upper lipid layer and supernatant were discarded, and the pellet was collected as a standardized tissue substrate for subsequent enzymatic hydrolysis. The pellet was collected in a sterile plastic container and stored in a freezer (-20°C) until further processing.

Hydrolysis of Bagrid Fish Viscera

Briefly, 50 g of bagrid fish viscera was mixed with 100 mL bromelain solution at various concentrations (0, 1, 3, and 5%) (w/v). The final pH of the mixtures was 7.00 ± 0.10. Hydrolysis was performed at 60°C with orbital shaking (225 rpm) for various times (0, 1, 2, 4, and 6 hours). After the hydrolysis process, samples were centrifuged at 6,000 rpm for 5 minutes to collect the supernatant hydrolysate. The supernatant was collected into a new centrifuge tube, and enzyme inactivation was done by heating (95°C, 15 min) in a water bath. Bagrid catfish viscera protein hydrolysate (BVPH) was stored at -20°C until further use (Borges *et al.*, 2025).

Antioxidant Activity of BVPH

Antioxidant activity was calculated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Hashem *et al.*, 2023). Briefly, 100 µL of a 5% BVPH solution was mixed with 100 µL of DPPH solution in a 96-well microplate. The samples were kept in the dark for 30 min before measuring the absorbance at 517 nm using a microplate reader (FLUOstar Omega Plate Reader, BMG LABTECH, Jerman). The sample blank consisted of 100 µL of BVPH and 100 µL of 96% methanol. The control consisted of 100 µL of distilled water and 100 µL of DPPH. The control blank consisted of 100 µL of distilled water and 100 µL of 96% methanol. The antioxidant activity was calculated using the following formula:

$$\text{Radical scavenging activity (\%)} = \frac{(Ac - Acb) - (As - Asb)}{(Ac - Acb)} \times 100 \quad (1)$$

Where: Ac = absorbance of control; As = absorbance of samples; Acb = absorbance of control blank; Asb = absorbance of sample blank.

Serial dilutions of BVPH were prepared with a of concentrations between 0.1– 2 mg/mL. A regression curve from the serial dilutions was made to determine the half maximal inhibitory concentration (IC_{50}) of the BVPH.

Degree of hydrolysis (DH)

The DH, which quantifies peptide bonds cleaved during hydrolysis, was calculated according to the SN-TCA method described by Korkmaz & Tokur (2022) with some modifications. DH was calculated by dividing soluble protein in 10% TCA by total protein in the bagrid catfish viscera sample. The calculation formula is as follows:

$$\text{DH (\%)} = \frac{Soluble\ protein\ in\ 10\%\ TCA}{Total\ protein\ in\ sample} \times 100 \quad \text{.....(2)}$$

Soluble Protein Content

Soluble protein formed in the hydrolysate was measured using the Bradford assay (Ishak & Sarbon, 2018). A standard curve generated with bovine serum albumin (BSA) was used for the quantification. The total soluble protein content was measured at a wavelength of 595 nm.

Yield

The gravimetric method was used to determine the yield of BVPH to assess the mass of BVPH recovered from the hydrolysis (dry weight) against the wet mass of the bagrid fish viscera. The hydrolysate samples were dried at 50°C until they reached a constant dry weight, then weighed. The yield of the BVPH was calculated using the following equation:

$$\text{Yield (\%)} = \frac{A - B}{A - C} \times 100 \quad \text{……..……(3)}$$

Where: A = initial mass (sample + crucible) (g); B = final mass of the sample (g); C = mass of the empty crucible (g)

Proximate Analysis

Indonesian National Standards Procedure is used for several proximate analysis, including: moisture (SNI 2354.2:2015), ash (SNI 01-2354.1-2006), and protein (SNI 01-2354.4-2006). Fat content of BVPH was analyzed using the Bligh and Dyer method (Bligh & Dyer, 1959), while carbohydrate content of BVPH was analyzed using the by difference method (Abd El-Rady *et al.*, 2023).

Molecular Weight Distribution

The molecular weight of BVPH was analyzed using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method, according to the procedure of Wang *et al.* (2023) with some modifications. The SDS-PAGE analysis utilized a 12% separating gel and a 3% stacking gel using Pre-stained Protein Ladder, 10 – 180 kDa, Biocomma. A 20 µL aliquot of the FPH-loading buffer mixture was loaded onto the SDS-PAGE gel and electrophoresed at 110 V for 90 min. The resulting SDS-PAGE gel was stained with a staining solution containing 0.2 mg Coomassie Brilliant Blue R-250, 75 mL methanol, 75 mL glacial acetic acid, and 50 mL distilled water. The stained gel was subsequently destained using a destaining solution containing the same composition of compounds without Coomassie Brilliant Blue R-250.

Statistical Analysis

Two-way ANOVA was used to evaluate the influence of bromelain concentration and hydrolysis time as the main effects and their interaction effects on the four dependent variables, including antioxidant activity, DH, soluble protein, and yield. All the variables were measured in triplicate. Post hoc comparison using Tukey's Honestly Significant Difference (HSD) test was performed to explore significant main effects and interactions further. Furthermore, Pearson's correlation test was used to analyze the correlation between antioxidant activity and soluble protein content of the BVPH. All statistical analyses were performed using Minitab software version 19.0 at the significance level of 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

The viscera waste of bagrid catfish contains protein with antioxidant activity. The breakdown of protein into simple peptides is necessary to obtain these antioxidants. Enzymatic hydrolysis can be an alternative approach to producing antioxidant peptides from bagrid catfish viscera, referred to as fish protein hydrolysate (BVPH). Hydrolysis was carried out at 60°C and pH 7, in accordance with the optimal conditions for bromelain, while the bromelain concentration and hydrolysis time were optimized in this study. Bromelain was selected based on its efficiency in catalyzing the hydrolysis of fishery products and producing good-quality protein hydrolysate (Borges *et al.*, 2025). The presence of cysteine residues containing active thiol groups is important for the proteolytic activity of bromelain. Several analyses were conducted, including antioxidant activity, degree of hydrolysis, total soluble protein, and yield of BVPH to determine the optimum conditions of bromelain concentration and hydrolysis time.

The Effect of Bromelain Concentration and Hydrolysis Time on Antioxidant Activity of BVPH

The antioxidant activity of BVPH was measured using the DPPH method. Table 1 shows the antioxidant activity of BVPH under various hydrolysis conditions, which affect free radical scavenging. The antioxidant activity values ranged from 24.1 to 66.0%. Based on Table 1, the concentration of bromelain and hydrolysis time significantly increased antioxidant activity ($p < 0.05$).

Table 1. Antioxidant Activity (%) of Bagrid Catfish Viscera Protein Hydrolysate (5% Concentration) at Various Bromelain Concentrations and Hydrolysis Times by the DPPH Method

Hydrolysis Time (h)	Bromelain Concentration (%)			
	0	1	3	5
0	32.4 ± 2.1^{ab}	24.1 ± 2.5^{a}	33.4 ± 2.5^{abc}	55.8 ± 2.7^{defgh}
1	48.2 ± 4.3^{bcdefg}	53.7 ± 3.1^{defgh}	66.0 ± 0.7^{h}	62.6 ± 1.6^{fgh}
2	41.5 ± 2.9^{bcde}	45.8 ± 4.9^{bcde}	63.7 ± 1.2^{gh}	56.6 ± 0.3^{efgh}
4	39.3 ± 6.5^{abcd}	43.8 ± 2.0^{bcde}	45.8 ± 0.6^{bcdef}	53.8 ± 3.8^{defgh}
6	49.4 ± 2.1^{cdefgh}	41.1 ± 0.0^{bcde}	45.3 ± 2.0^{bcde}	43.0 ± 3.8^{bcde}

Note: Statistical significance ($p < 0.05$) between group means is denoted by the absence of a shared letter in multiple comparison notations from Tukey's post-hoc tests.

The antioxidant activity of BVPH at 0-hour hydrolysis can be attributed to the presence of thiol group in the cysteine residue of bromelain, which can donate its hydrogen atom to neutralize free radicals (Ataide *et al*., 2021). The relatively high

antioxidant activity observed at 0 h in the 5% bromelain treatment is likely attributable to rapid initial hydrolysis immediately after enzyme addition. Since the enzyme was added directly to the viscera maintained at optimal temperature, proteolytic cleavage began immediately. During sample handling and prior to complete thermal inactivation at 95°C, bromelain remained catalytically active, allowing initial peptide release. Meanwhile, the antioxidant activity of BVPH gradually decreased after 1 hour hydrolysis due to the formation of hydrophobic peptide that forms insoluble aggregate that precipitate the pellet upon centrifugation (Mohanty *et al*., 2021; Domínguez *et al*., 2024). Consequently, the antioxidant activity measured in the supernatant fraction gradually decreased over the time of hydrolysis. Meanwhile, the antioxidant activity of BVPH increased after 4 hours of hydrolysis, even in the absence of bromelain. This is due to the natural breakdown of proteins into simple peptides and amino acids over time, including sulfur-containing amino acids such as methionine and cysteine, aromatic amino acids such as phenylalanine, tyrosine, and tryptophan, and valine. Those peptides and amino acids are involved in enhancing antioxidant activity (Bahari *et al.,* 2020). The resulting peptides and amino acids exert antioxidant activity through hydrogen donation, reducing free radical reactions. Phenylalanine and tryptophan can act as free radical scavengers by directly neutralizing free radicals. At the same time, the phenolic group in tyrosine can donate its hydrogen, and the imidazole group of histidine can donate its proton to neutralize free radicals (Zechel *et al.* 2019).

The highest antioxidant activity was obtained from bagrid catfish viscera hydrolyzed with 3% bromelain for 1 hour. BVPH obtained under optimal hydrolysis conditions was analyzed for IC_{50} value (Figure 1). The IC_{50} value of BVPH on DPPH analysis was 1.24 mg/mL. Marinou *et al.* (2025) obtained a higher IC_{50} value on cod backbone protein hydrolysate of 2.1 mg/mL, while Noman (2022) obtained an IC_{50} value of 3.14 mg/mL on *Huso dauricus* and *Acipenser schrenckii* protein hydrolysate. A lower IC_{50} value indicates greater antioxidant strength. Based on this, BVPH exhibits excellent antioxidant potential.

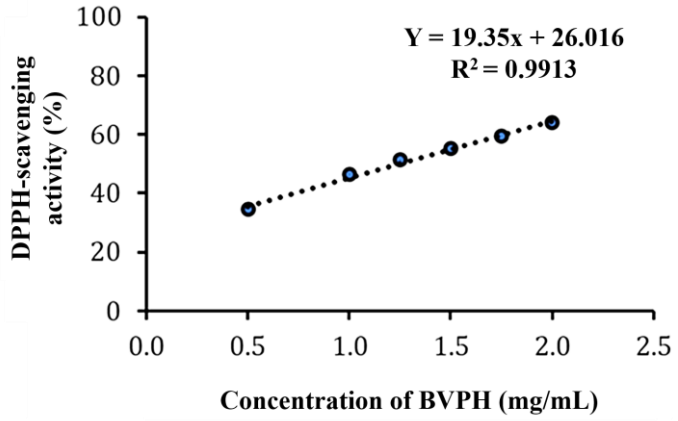

Figure 1. The IC_{50} Value of Bagrid Viscera Protein Hydrolysate (BVPH) at Concentrations of 0.5 – 2 mg/mL. A linear Regression Curve Determines the IC_{50} value (50% Inhibitory Concentration)

The effect of Bromelain Concentration and Hydrolysis Time on Degree of Hydrolysis of BVPH

The degree of hydrolysis (DH) was measured to determine hydrolysis efficiency. A high DH indicates the formation of low-molecular-weight peptides, which can increase the solubility of BVPH. Table 2 shows the DH of BVPH, which ranges from 2.8 to 9.2%.

Table 2. Degree of Hydrolysis (%) of BVPH at Various Bromelain Concentrations and Hydrolysis Times by the SN-TCA Method

Hydrolysis Time (h)	Bromelain Concentration (%)			
	0	1	3	5
0	2.8 ± 0.2^{a}	4.5 ± 0.6^{ab}	5.4 ± 0.4^{abcd}	4.1 ± 0.5^{abcd}
1	6.2 ± 0.0^{abcd}	7.1 ± 0.7^{abcd}	6.1 ± 0.2^{abcd}	5.9 ± 0.3^{abcd}
2	7.2 ± 0.7^{bcd}	5.9 ± 0.0^{abcd}	5.5 ± 0.1^{abcd}	4.7 ± 1.4^{abc}
4	8.8 ± 0.1^{d}	7.6 ± 0.2^{bcd}	5.9 ± 0.5^{abcd}	4.7 ± 0.4^{abc}
6	8.4 ± 0.1^{cd}	9.2 ± 0.5^{d}	6.9 ± 2.1^{abcd}	6.8 ± 1.2^{abcd}

Note: Statistical significance ($p < 0.05$) between group means is denoted by the absence of a shared letter in multiple comparison notations from Tukey's post-hoc tests.

Bromelain concentration did not significantly affect DH ($p > 0.05$). DH from BVPH was lower than DH of *Acipenser sinensis* hydrolyzed with 0.01% bromelain for 3 hours by 19.1% and *Pangasius* sp. hydrolyzed with 0.04% bromelain for 2.8 hours (Noman, 2022; Nurdiani *et al.* 2024). The relatively low DH of BVPH can be attributed to the specificity of bromelain, which cleaves peptide bonds at the carboxyl terminal side of lysine, alanine, and tyrosine residues (Soloviev *et al*., 2003). This specificity resulted in the cleavage of certain peptide bonds rather than all peptide bonds, therby constraining the DH value. Another reason might be caused by an unbalanced enzyme-substrate ratio, as shown in Table 2 when increasing bromelain concentrations beyond 3% actually decreased the resulting DH. Accumulation of too many simple peptides limited bromelain absorption with the substrate, thus reducing DH (Deng *et al.* 2018). Meanwhile, hydrolysis time also did not significantly affect DH ($p > 0.05$). Although bromelain concentration and hydrolysis time did not significantly affect DH, the combination of the two factors (interaction) produced a significant difference ($p < 0.05$). This indicates that the effect of one factor depends on the level of the other. Fluctuating DH values may be due to bromelain's ability to cleave peptide bonds containing glycine, alanine, and leucine residues. At the beginning of hydrolysis, peptides containing glycine, alanine, and leucine residues are still available, increasing the DH. However, over time, the availability of these peptides decreases, resulting in a decrease in the DH value (Feng *et al.,* 2018).

The Effect of Bromelain Concentration and Hydrolysis Time on Total Soluble Protein of BVPH

Enzymatic hydrolysis using bromelain facilitates the breakdown of insoluble protein present in bagrid fish viscera into smaller soluble peptides and amino acids. Increasing enzyme concentration and hydrolysis time promotes the proteolytic degradation, yielding a high amount of soluble peptides in the supernatant. Table 3 demonstrates a significant increase ($p < 0.05$) in soluble protein content up to 2 hours of hydrolysis. The soluble protein content of BVPH is lower than that of *Decapterus macrosoma* (30,800 µg/mL) protein hydrolysates (Ishak & Sarbon, 2018). The comparatively low soluble protein content may be attributed to a limitation of Bradford method, which relies on the colorimetric binding interaction between coomassie brilliant blue G-250 with arginine and lysine residues in the sample (Nasirova *et al*., 2026). Nasirova *et al*. (2026) also reported that short chain peptides with molecular weight below 3 kDa are hard to detected by Bradford assay due to their lack of ability to form stable complexes with coomasie brilliant blue. Accordingly, even though the total peptide content in the supernatant may have increased, the absorbance readings obtained via the Bradford method remained lower.

Pearson correlation analysis was conducted to determine the correlation between antioxidant activity and soluble protein in BVPH. The correlation between soluble protein and antioxidant activity of BVPH was positive, with a correlation coefficient of 0.655 ($p < 0.01$). This indicates that the higher the soluble protein content, the higher the antioxidant activity of BVPH produced. Soluble protein is known to contain antioxidant peptides that can inhibit oxidation through various mechanisms, including inhibition of lipid oxidation, such as hydrogen peroxide reduction, ROS inactivation, free radical scavenging, and binding of pro-oxidative transition metals (Xu *et al.*, 2024). However, non-polar amino acids such as glycine, alanine, valine, and proline have low solubilization, thus reducing soluble protein levels (Pradhan *et al.*, 2023).

The Effect of Bromelain Concentration and Hydrolysis Time on Yield of BVPH

Table 4 shows the yield of BVPH, which ranged between 1.2 – 21.7%. Based on Table 4, bromelain concentration and hydrolysis time affect the yield of BVPH significantly ($p < 0.05$). Increasing the bromelain concentration resulted in a large number of peptide bonds being cleaved, then generating more small peptides and increasing the yield over time. However, the availability of peptide bonds decreased as hydrolysis progressed (Shafiee *et al.*, 2021), leading to a gradual decrease in the yield of BVPH. During hydrolysis, bromelain continuously cleaves peptide bonds, yielding smaller peptides which exposes the hydrophobic groups of the peptide. These hydrophobic peptides then subsequently accumulate, forming large insoluble aggregate that precipitate upon centrifugation. The study of Mohanty *et al*. (2021) demonstrated that the emulsifying and foaming properties of *Labeo rohita* FPH diminished progressively with the extended of hydrolysis process, indicated the formation of increasing hydrophobic peptides that interact with other hydrophobic peptides. Meanwhile, Domínguez *et al*. (2024) reported that extended hydrolysis could induce structural alterations that affecting both solubility and stability of the FPH, leading to precipitate formation.The yield of BVPH was higher compared to the yield of *Hypophthalmichthys nobilis* FPH (17.83%) and *Thunnus obesus* FPH (11.04%) (Alahmad *et al.*, 2023; Abd El-Rady *et al.*, 2023).

Proximate content of BVPH

Proximate analysis, including water, ash, fat, protein, and carbohydrates, was conducted to determine the nutritional value of BVPH for food product development purposes. Table 5 shows the proximate composition of BVPH hydrolyzed with 3% bromelain for 1 hour. Moisture content determines the shelf-life of BVPH; high moisture content causes BVPH to spoil more quickly or develop mold. The drying process significantly

Table 3. Soluble Protein Content (µg/mL) of Bagrid Catfish Viscera Protein Hydrolysate (BVPH) at Various Bromelain Concentrations and Hydrolysis Times by the Bradford Method

Hydrolysis Time (h)	Bromelain Concentration (%)			
	0	1	3	5
0	521.5 ± 32.7^{ab}	335.6 ± 31.5^{a}	602.0 ± 47.8^{abc}	612.8 ± 6.5^{abc}
1	1015.0 ± 8.7^{cdef}	874.2 ± 45.1^{bcdef}	1207.4 ± 9.8^{f}	1158.0 ± 7.1^{f}
2	1118.3 ± 1.1^{ef}	1050.9 ± 25.0^{def}	1267.2 ± 52.2^{f}	1254.1 ± 45.7^{f}
4	728.0 ± 30.4^{abcde}	876.4 ± 62.5^{bcdef}	1212.8 ± 176.1^{f}	1189.5 ± 39.7^{f}
6	677.0 ± 188.1^{abcd}	736.7 ± 115.2^{abcde}	1122.1 ± 56.0^{ef}	930.2 ± 69.6^{bcdef}

Note: Statistical significance ($p < 0.05$) between group means is denoted by the absence of a shared letter in multiple comparison notations from Tukey's post-hoc tests.

Table 4. Yield (%) of Bagrid Catfish Viscera Protein Hydrolysate (BVPH) at Various Concentrations of Bromelain and Hydrolysis Time by the Gravimetric Method

Hydrolysis Time (h)	Bromelain Concentration (%)			
	0	1	3	5
0	1.2 ± 0.1^{a}	4.7 ± 0.1^{ab}	11.9 ± 0.1^{defg}	18.5 ± 0.8^{ijk}
1	9.5 ± 0.7^{cde}	11.6 ± 1.5^{def}	16.1 ± 0.1^{ghi}	21.7 ± 0.5^{k}
2	10.5 ± 0.3^{cdef}	11.7 ± 0.8^{def}	16.6 ± 1.2^{hij}	20.6 ± 1.1^{jk}
4	8.9 ± 0.5^{bcd}	10.0 ± 0.5^{cdef}	13.9 ± 1.1^{fgh}	17.3 ± 1.3^{hij}
6	6.9 ± 0.2^{bc}	7.8 ± 0.5^{bcd}	10.6 ± 0.7^{cdef}	13.3 ± 0.6^{efgh}

Note: Statistical significance ($p < 0.05$) between group means is denoted by the absence of a shared letter in multiple comparison notations from Tukey's post-hoc tests.

reduces the moisture content of BVPH. The moisture content of the BVPH obtained was higher compared to the mixed FPH made from several marine fish at 8.89% (Niharika *et al.*, 2026); this was due to an incomplete drying process, which caused water molecules to remain trapped within the BVPH. Meanwhile, the ash content reflects the mineral content in the BVPH. The ash content of the BVPH was lower compared to the ash content of the mixed FPH from several marine fish, which was 6.6% (Niharika *et al.*, 2026).

Table 5. Proximate Composition of Bagrid Catfish Viscera and Bagrid Catfish Viscera Protein Hydrolysate (BVPH)

Proximate composition (%)	Bagrid catfish viscera	BVPH
Moisture	83.93 ± 0.01	16.77 ± 0.91
Ash	2.99 ± 0.00	2.19 ± 0.54
Fat	0.20 ± 0.01	0.32 ± 0.05
Protein	7.60 ± 1.91	33.34 ± 0.17
Carbohydrate	5.27 ± 1.94	47.39 ± 0.14

Note: each sample was analyzed in triplicate for all proximate parameters, and the resulting data are reported as mean ± standard deviation.

The fat content of BVPH was higher than the fat content of *Secutor insidator* FPH hydrolyzed with papain (0.5%) and proteinase K (0.01%), which were 0.6% and 1.02%, respectively (Dinakarkumar *et al.*, 2022). Fat can influence the DH of BVPH, as fat can act as an inhibitor of bromelain during the hydrolysis process (Listyaningrum *et al.*, 2022). The protein content of BVPH is higher than the protein content of *Channa striata* FPH, which was 10.82% (Jaya *et al.*, 2024), but lower than the mix FPH from several marine fish, which was 81.93% (Niharika *et al.*, 2026). The lower protein content in BVPH is caused by an incomplete drying process, resulting in some protein molecules being retained in water molecules. Additionally, BVPH also contains a relatively high carbohydrate content. This may be due to the waste from bagrid fish viscera containing carbohydrate substrates.

Molecular Weight Distribution of BVPH

As shown in Figure 2, bands appear in the range of 55 – 180 kDa and 10 – 17 kDa, which become less intense over time with hydrolysis. This indicates that the proteins in bagrid fish viscera waste were successfully hydrolyzed into small peptides.

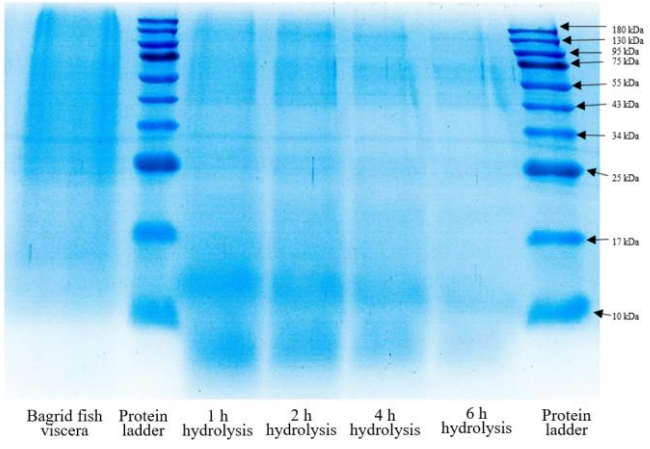

Figure 2. Molecular Weight Profile of Bagrid Catfish Viscera and Its hydrolysates.

The appearance of bands between 10 – 17 kDa is consistent with the high antioxidant activity in BVPH and DH from the hydrolysis of bagrid fish viscera waste. Peptides with smaller molecular weights contain substrates that can donate electrons to neutralize free radicals (Xu *et al.*, 2024).

CONCLUSION

Enzymatic hydrolysis of *Mystus nigriceps* (Valenciennes, 1840) viscera for 1 hour using 3% bromelain produced protein hydrolysates that exhibited antioxidant activity, with an IC_{50} of 1.24 mg/mL. This approach demonstrates the potential valorization of fish viscera into bioactive hydrolysates, which may contribute to more sustainable utilization of fish processing by-products.

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