International Journal of Research and Review
[Indexed, Peer-Reviewed Journal]
Volume 13; Issue: 5; May 2026
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Characteristics of Hydrolyzed Snakehead (*Channa striata*) Fish Protein Using Pepsin Enzyme

Siti Aisyah[1], Iin Khusnul Khotimah[1], Hafni Rahmawati[1], Purnomo[1], Agustiana[1]

[1]Department of Fish Product Technology, Fisheries and Marine Science Faculty, Lambung Mangkurat University, Banjarbaru, Indonesia.

Corresponding Author: Hafni Rahmawati

DOI: https://doi.org/10.52403/ijrr.20260509

ABSTRACT

Snakehead fish (*Channa striata*) is a high-value fishery commodity with high protein content, especially albumin. However, optimal utilization remains a challenge, especially for the meat, which has so far only been sold whole or as fillets. Meeting the demand for fish protein hydrolysate is highly beneficial as a source of amino acids, functional food ingredients, and dietary supplements, as well as for increasing the added value of snakehead fish into a superior product in the form of HPI using pepsin enzymes. This study aims to obtain the best results from variations in concentration and hydrolysis time on the functional properties of HPI. This study will evaluate the effect of enzyme concentration variations (1 and 2%) and hydrolysis time (4 and 6 hours) on yield, degree of hydrolysis, and protein content. The method used is a laboratory experiment with a completely randomized factorial design. Data analysis showed that yield was not affected by concentration or time factors or the interaction between the two, while the degree of hydrolysis was significantly affected by concentration and the interaction between the two factors but was significantly not affected by time. In contrast, protein content was significantly affected by time but was significantly not affected by concentration and the interaction between concentration and time. The best results based on yield, degree of hydrolysis, and protein content of snakehead fish HPI were obtained using a pepsin enzyme concentration of 2% and a hydrolysis time of 6 hours.

Keywords: amino acids, degree of hydrolysis, molecular weight, proximate analysis, yield

INTRODUCTION

Snakehead fish (*Channa striata*) is a freshwater commodity rich in protein with high albumin content and potential as a source of functional protein (Zakaria *et al*., 2007; Mustafa *et al*., 2012; Dewita *et al*., 2022). Various studies have reported that snakehead fish protein has physiological benefits, such as accelerating wound healing and improving nutritional status, making it a potential candidate for development into high-value-added food or nutraceutical ingredients (Mustafa *et al*., 2012; Suprayitno *et al*., 2020).

The utilization of fish protein in the form of protein hydrolysates has gained increasing attention due to their enhanced functional properties and bioactivities compared to intact proteins (Kristinsson & Rasco, 2000; Klompong *et al*., 2007; Shahidi & Zhong, 2008; Chalamaiah *et al*., 2012). The enzymatic hydrolysis process can produce small peptides that are easily digestible and have potential biological activities, such as antioxidant and antihypertensive properties

(Kristinsson & Rasco, 2000; Chalamaiah *et al*., 2012). One of the most widely used protease enzymes is pepsin, which functions optimally under acidic conditions and is effective in hydrolyzing fish proteins into smaller peptides and amino acids (Benjakul *et al*., 2014, Idowu & Yupanqui, 2025).

However, most studies on fish protein hydrolysates still focus on marine fish, such as tuna, sardines, and mackerel, using alkaline enzymes such as alcalase and papain. Studies on freshwater fish protein hydrolysates, particularly snakehead fish, are still limited, especially those using pepsin enzymes. Additionally, the physicochemical characteristics of snakehead fish protein hydrolysates, such as the degree of hydrolysis, proximate composition, and protein profile, have not been widely studied.

The limited information available indicates a gap in research on the effect of pepsin enzyme use on the characteristics of snakehead fish protein hydrolysate. Therefore, the novelty of this study lies in the use of pepsin enzyme to produce snakehead fish protein hydrolysate and the systematic study of its characteristics, which is expected to enrich scientific data on the use of freshwater fish as a source of functional protein.

This research is important in the context of diversifying the utilization of snakehead fish and increasing the added value of local fishery products. The objectives of this study are to examine the characteristics of snakehead fish protein hydrolysate produced through hydrolysis using pepsin enzymes, including physicochemical properties and protein characteristics, as a basis for developing functional food or nutraceutical products based on local resources.

MATERIALS & METHODS

Materials and Tools

The main ingredient used is fresh snakehead fish obtained from local markets and cleaned of scales, heads, bones, and entrails. Pepsin enzyme (activity ≥250 U/mg protein) is used as a hydrolysis agent. The chemicals used include distilled water, 1 N HCl and 1 N NaOH for pH adjustment, as well as proximate and protein analysis reagents. The equipment used includes a blender, water bath shaker, pH meter, analytical balance, centrifuge, oven, UV-Vis spectrophotometer, and laboratory glassware.

Preparation of Raw Materials

The snakehead fish meat is washed with running water, drained, then blended until a homogeneous paste is obtained. The fish paste is then stored at a temperature of ±4°C before being used for hydrolysis to prevent protein degradation (Kristinsson & Rasco, 2000).

Protein Hydrolysis Process

Catfish paste is mixed with distilled water at a ratio of 1:2 (w/v) (Saputra *et al*., 2000), then the pH of the mixture is adjusted to pH 2 using 1 N HCl (Agustiari, *et al*., 2019). Pepsin enzyme is added at concentrations of 1 and 2% w/w protein. Hydrolysis is carried out at 37°C for 4 and 6 hours using a water bath shaker. After hydrolysis was complete, the enzyme was inactivated by heating at 90°C for 10 minutes. The hydrolysate was then cooled and centrifuged at 4,000 rpm for 15 minutes to separate the supernatant. The supernatant obtained was the snakehead fish protein hydrolysate and was used for further analysis.

Analysis of Hydrolysate Characteristics

Yield

Analysis of fish protein hydrolysate yield generally refers to the methods used by Kristinsson & Rasco (2000) and Chalamaiah *et al*. (2012), which state that hydrolysate yield is determined based on the ratio of dry hydrolysate weight to initial raw material weight or initial protein weight.

Hydrolysis Degree

The degree of hydrolysis (DH) is determined using the trinitrobenzenesulfonic acid (TNBS) or o-phthaldialdehyde (OPA) method to measure

the amount of free amino groups formed during the hydrolysis process (Nielsen *et al*., 2001).

Protein Content

Protein content was determined using the Kjeldahl method in accordance with AOAC (2016). This method is the international standard method for total protein analysis in foodstuffs, including fishery products and protein hydrolysates.

Statistical Analysis

The study was conducted using a completely randomized design (CRD) factorial with two factors, namely pepsin enzyme concentration and hydrolysis time. The pepsin enzymes used were 1 and 2%, while the hydrolysis times were 4 and 6 hours. Each treatment was performed in three replicates (Montgomery, 2017). The data obtained were statistically analyzed using analysis of variance (ANOVA) at a 95% confidence level. If there were significant differences, they were followed up with Duncan or Tukey post hoc tests (Granato *et al*., 2014).

RESULT

Based on the research data, the average protein hydrolysate of snakehead fish is presented in Table 1.

Table 1 Test Results of Snakehead Fish Protein Hydrolysate Characteristics

Parameters	Treatment (Percentage: Hours)			
	1%: 4	1%: 6	2%: 4	2%: 6
Yield (%)	9.53 ± 0.36[a]	10.87 ± 0.50[a]	8.34 ± 0.58[a]	11.22 ± 0.40[a]
Protein content (%)	78.05 ± 2.17[a]	80,32 ± 2,19[a]	73.16 ± 1.80[b]	86.74 ± 1.96[a]
Hydrolysis degree (%)	16.89 ± 0.98[a]	17.40 ± 0.94[a]	15.28 ± 1.40[a]	22.05 ± 1.38[b]

The same superscript letter on the same line indicates no difference.

DISCUSSION

The results showed that the yield of hydrolyzed snakehead fish protein ranged from 8.34 to 11.22%. The difference in yield values between treatments shows that hydrolysis conditions, such as enzyme concentration or hydrolysis time, affect protein breakdown efficiency. The highest yield was obtained in the fourth treatment (11.22 ± 0.40%), while the lowest yield was found in the third treatment (8.34 ± 0.58%). The increase in yield is generally related to the increased effectiveness of pepsin in breaking down the peptide bonds of fish protein into water-soluble peptide fractions, thereby increasing the amount of hydrolysate that can be separated. These results are in line with the report by Kristinsson and Rasco (2000), which states that the yield of protein hydrolysate is greatly influenced by the intensity of the hydrolysis process.

The protein content of the hydrolysate of snakehead fish protein ranged from 73.16 to 86.74%, indicating that the product was classified as a high-protein hydrolysate. The fourth treatment showed the highest protein content (86.74±1.96%) and was significantly different from the third treatment (73.16±1.80%), but not significantly different from the first and second treatments. The high protein content indicates that the hydrolysis process using pepsin enzyme was able to effectively dissolve the fish protein fraction, while the non-protein components were relatively less. According to Chalamaiah *et al*, (2012), fish protein hydrolysates generally have high protein content due to the release of small, water-soluble peptides during the enzymatic hydrolysis process.

The degree of hydrolysis (DH) of snakehead fish protein hydrolysate ranged from 15.28 to 22.05%, with the highest value obtained in the fourth treatment (22.05 ± 1.38%), which was significantly different from the other treatments. The DH value reflects the degree of peptide bond cleavage during the hydrolysis process; a higher DH indicates that more peptide bonds have been degraded into peptides and free amino acids. An

increase in DH in certain treatments indicates that the hydrolysis conditions are more optimal for pepsin activity, which works optimally at acidic pH and is capable of specifically hydrolyzing fish protein. These results are consistent with Adler-Nissen (1986), who stated that the degree of hydrolysis is influenced by the type of enzyme, enzyme concentration, and hydrolysis duration.

Overall, the relationship between yield, protein content, and degree of hydrolysis shows an interrelated trend. Treatment with a higher degree of hydrolysis tends to produce higher yields and protein content of the hydrolysate, although not always in direct proportion. This is because an excessively high DH can potentially produce very small peptides that may be lost during the separation process. However, in this study, the use of pepsin enzyme is still considered appropriate.

The results of this study confirm that pepsin enzyme has the potential to be used in the production of high-quality hydrolysed snakehead fish protein, as indicated by high protein content and moderate to high degree of hydrolysis. The hydrolysate produced has the potential to be further developed as a functional food ingredient or nutraceutical raw material based on local freshwater fishery resources.

CONCLUSION

The conclusions from this research activity are:

Based on the results of the study, the hydrolysate of snakehead fish protein produced through hydrolysis using pepsin enzyme had a yield of 8.34–11.22%, a protein content of 73.16–86.74%, and a degree of hydrolysis of 15.28–22.05%. These differences indicate that hydrolysis conditions affect the characteristics of the resulting protein hydrolysate.

Treatment with 2% pepsin enzyme and a hydrolysis time of 6 hours showed the best results with the highest yield (11.22%), the highest protein content (86.74%), and the highest degree of hydrolysis (22.05%), so it can be concluded that this treatment is the most optimal hydrolysis condition in this study. The high protein content and degree of hydrolysis indicate the effectiveness of pepsin enzyme in breaking down snakehead fish protein into water-soluble peptides.

The Suggestion from this research activity are:

Overall, the use of pepsin enzyme has been proven effective in producing hydrolyzed snakehead fish protein with good characteristics and has the potential to be developed as a functional food ingredient or value-added product based on freshwater fishery resources.

Declaration by Authors

Acknowledgement: Thank you to the laboratory technicians, laboratory assistants and students who have helped in carrying out the research.

Source of Funding: The researchers would like to express our deepest gratitude to LPPM ULM for providing research funding through DIPA Lambung Mangkurat University for Fiscal Year 2024 for the PDWM Internal program with decree number 962/UN8/PG/2025 and contract number 1668/UN8/LT/2025.

Conflict of Interest: No conflicts of interest declared.

REFERENCES

1. Adler-Nissen, J. 1986. Enzymic Hydrolysis of Food Proteins. Elsevier Applied Science Publishers Ltd., London.
2. Agustiari, N. M., Anggrahini, S., & Ningrum, A. (2019). Influences of palm sugar, fermentation time and enzyme hydrolysis on activities of angiotensin converting enzyme inhibitory (ACE-I) in joruk oci fish (*Rastrelliger kanagurta*) hydrolyzate. *Pakistan Journal of Nutrition,* 18(12):1094-1100. DOI: 10.3923/pjn.2019.1094.1100.
3. AOAC. (2016). Official Methods of Analysis of the Association of Official Analytical Chemistry International 20th Edition.n *Arlington: AOAC Inc*.
4. Benjakul, S., S. Yarnpakdee, T. Senphan, S. M. Halldorsdottir, & H. G. Kristinsson.

(2014). Fish protein hydrolysates production, bioactivities, and applications. *Wiley*.
5. Chalamaiah, M., Kumar, B. D., Hemalatha, R., & Jyothirmayi, T. (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications. *Food Chemistry,* 135(4):3020-3038. DOI: 10.1016/j.foodchem.2012.06.100.
6. Dewita, Desmelati, Santhy W. Sidauruk, Taufik Hidayat. (2022). Isolation and characterization of snakehead fish meal extract with fresh, boiled, and steamed treatments and its potential for health drinks and immunomodulators. *Pharmacogn J*, 14(5):532-536. DOI: 10.5530/pj.2022.14.131.
7. Granato, D., Calado, V. M. A., & Jarvis, B. (2014). Observations on the use of statistical methods in food science and technology. *Food Research International*, 55:137-149. DOI: 10.1016/j.foodres.2013.10.024.
8. Idowu, O. A., & C. T. Yupanqui. (2025). Enzymatic hydrolysis with pepsin enhanced the nutrient compositions of unfractionated soy protein hydrolysate and its cell viability and nitric oxide activities. *Food Science & Nutrition*, 13(11):1789-1803. DOI: 10.1002/fsn3.71147.
9. Klompong, V., Benjakul, S., Kantachote, D. & Shahidi, F. (2007). Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type. *Food Chemistry*, 102(4):1317-1327. DOI: 10.1016/j.foodchem.2006.07.016.
10. Kristinsson, H. G., & Rasco, B. A. (2000). Fish protein hydrolysates: Production, biochemical, and functional properties. *Critical Reviews in Food Science and Nutrition*, 40(1):43-81. DOI: 10.1080/10408690091189266.
11. Montgomery, D. C. (2017). Design and analysis of experiments (9th ed.). *New York: John Wiley & Sons*.
12. Mustafa, A., Widodo, M. A., & Kristianto, Y. (2012). Albumin and zinc content of snakehead fish (*Channa striata*) extract and its role in health. *International Food Research Journal*, 19(2):1-8.
13. Nielsen, P. M., Petersen, D., & Dambmann, C. (2001). Improved method for determining food protein degree of hydrolysis. *Journal of Food Science*, 66(5):642-646. DOI: 10.1111/j.1365-2621. 2001.tb04614. x.
14. Saputra, D., Nurhayati, T., & Purwaningsih, S. (2020). End post-rigour phase yellowstripe scad fish (*Caranx leptolepis*) peptones and its application for bacteria's growth media. *Food Research*, 4(2):413-420. DOI: 10.26656/fr.2017.4(2).210.
15. Shahidi, F., & Zhong, Y. (2008). Bioactive peptides. *Journal of AOAC International*, 91(4):914-931. DOI: 10.1093/jaoac/91.4.914.
16. Suprayitno, E., Nursyam, H., & Mustafa, A. (2020). Potential of snakehead fish (*Channa striata*) as functional food. *IOP Conference Series: Earth and Environmental Science*, 441: 012041.
17. Zakaria, Z. A., A. M. Mat Jais, Y. M. Goh, M. R. Sulaiman, & M. N. Somchit. (2007). Amino acid and fatty acid composition of an aqueous extract of *Channa striatus* (Haruan) that exhibits antinociceptive activity. *Clinical and Experimental Pharmacology and Physiology*, 34(3):198-204. DOI: 10.1111/j.1440-1681.2007. 04572.x.

How to cite this article: Siti Aisyah, Iin Khusnul Khotimah, Hafni Rahmawati, Purnomo, Agustiana. Characteristics of hydrolyzed snakehead (*Channa striata*) fish protein using pepsin enzyme. *International Journal of Research and Review*. 2026; 13(5): 116-120. DOI: *https://doi.org/10.52403/ijrr.20260509*

******