e-ISSN 2581-0405
Asian Journal of Applied Research for Community Development and Empowerment
Journal home page: http://ajarcde-safe-network.org ISSN 2581-0405

Study of the Hydrolysis Process Using Bromelain Enzymes on the Characteristics of Hydrolyzed Protein from Jengki Anchovies (*Stolephorus indicus*)

Dalilah Edenya Zata Yumni [1], *Dedin Finatsiyatull Rosida* [2*]

[1] *Food Technology Department, Faculty of Engineering and Science, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya, Indonesia*
[2] *Innovation Center of Appropriate Food Technology for Lowland and Coastal Area, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya 60294, Indonesia*

ARTICLE INFO

Article History:
Received: 27 April 2025
Final Revision: 01 June 2025
Accepted: 01 June 2025
Online Publication: 03 June 2025

KEYWORDS

Bromelain, protein hydrolysate, anchovy.

CORRESPONDING AUTHOR

*E-mail: dedin.tp@upnjatim.ac.id

A B S T R A C T

Fish protein hydrolysates have great potential in various fields, particularly in food. Researchers developed fish hydrolysates enzymatically to utilize anchovies and produce functional fish-based food products that meet standards. This study aims to determine how different amounts of bromelain enzyme and how long the hydrolysis process lasts affect the chemical properties of anchovy protein hydrolysate. The study employed a completely randomized design (CRD) factorial design with two factors and two replications. The first factor was bromelain enzyme concentration (6%, 7%, 8%). The second factor was hydrolysis time (4 hours, 5 hours, 6 hours). Data were analyzed using Analysis of Variance (ANOVA) at a 5% significance level. If we observed significant interactions between the two treatments, we conducted a post-hoc DMRT test at 5%. The best treatment used 8% enzyme concentration and took 6 hours, leading to a moisture content of 82.91%, ash content of 2.46%, soluble protein content of 8.12%, and fat content of 3.27%.

Contribution to Sustainable Development Goals (SDGs)
SDG 2: Zero Hunger
SDG 3: Good Health and Well-being
SDG 12: Responsible Consumption and Production

1. INTRODUCTION

1.1. Research Background

Protein is one of the most important macronutrients for the body because it plays a role in tissue formation and repair and serves as a source of energy. However, the protein needs of the Indonesian population are still not optimally met, especially from high-quality and affordable animal sources [1]. One potential animal protein source that has not yet been fully utilized is the jengki anchovy (*Stolephorus indicus*). This fish has a relatively high protein content, is widely distributed in Indonesian waters, and has a relatively low price [2].

Protein hydrolysate is the result of breaking down proteins into peptides and amino acids through hydrolysis, which is generally carried out with the help of enzymes. This product has high biological value, is easily digestible, and has potential as a functional food ingredient [3]. One enzyme that is widely used in the protein hydrolysis process is bromelain, a protease enzyme obtained from pineapple fruit that has high proteolytic activity [4]. The use of bromelain enzyme in the hydrolysis of anchovy protein has the potential to produce hydrolysates with a good degree of hydrolysis and nutritional quality.

The development of nutritious instant products, such as instant rice, is one solution to meet the nutritional needs of modern society, which desires practical, fast-cooking foods that are still highly nutritious. The addition of fish protein hydrolysate to instant rice can increase the protein content while providing functional added value [5]. Therefore, this study was conducted to determine the characteristics of fish protein hydrolysate from anchovy fish produced through bromelain enzyme hydrolysis.

Based on the above description, this study involved the production of anchovy protein hydrolysate with varying concentrations of bromelain enzyme and hydrolysis times. The finished product will be tested for moisture, ash, soluble protein, and fat content.

1.2. Literature Review

Protein hydrolysate is the result of protein breakdown that produces bioactive peptides and free amino acids. Protein hydrolysate products have the potential as functional foods and flavor enhancers. Protein hydrolysate can be produced from protein-rich food ingredients, one of which is anchovies. Anchovies contain 16% protein [6].

Anchovy (*Stolephorus indicus*) is known as a nutrient-rich food source, particularly protein. Anchovy has a high protein content, making it a high-quality human protein source. Anchovy contains a complete set of essential amino acids in balanced proportions, crucial for growth and body maintenance [7]. Additionally, anchovies contain fats, minerals, and vitamins that contribute to overall health [8]. The protein in anchovies also has high bioavailability, making it easy for the body to digest and absorb [9].

The protein hydrolysate of Jengki anchovy produced through enzymatic hydrolysis is known to have a higher protein content than anchovy meal. Enzymatic hydrolysis breaks down protein molecules into smaller peptides and amino acids, increasing the final product's availability and protein content [10]. Anchovy protein hydrolysate has a more complete amino acid profile and is more easily digested by the body than conventional anchovy meal [11], thereby offering advantages in protein content and nutritional value over anchovy meal [12].

1.3. Research Objective

To determine the chemical characteristics of anchovy protein hydrolysate, with treatment of bromelain enzyme concentration and hydrolysis time.

2. MATERIALS AND METHODS

2.1 Materials

The material used in this study was anchovy (Stolephorus sp) obtained from the Pabean Fish Market in Surabaya, East Java. Additional materials used included natural protease enzymes from young pineapples. Chemicals used were NaOH (Merck), HCl (Merck), K_2SO_4 (Merck), MgO (Merck), H_2SO_4 (Merck), H_3PO_3 (Merck), $MgCO_3$ (Merck), CH_3COOH (Merck), $Na_2S_2O_3$ (Merck), Na_2CO_3 (Merck), $AgNO_3$ (Merck), $BaCl_2$ (Merck), Folin-Ciocalteu reagent, and Bovine Serum Albumin solution.

2.2 Research Procedure

This study used a completely randomized design (CRD) with a factorial pattern, consisting of two factors, where each treatment was repeated twice. The first factor (Factor I) was the bromelain enzyme concentration of 6% (A1), 7% (A2), and 8% (A3). The second factor (Factor II) is the hydrolysis time of 4 hours (B1), 5 hours (B2), and 6 hours (B3). The collected data were analyzed using Analysis of Variance (ANOVA) to determine the significance of the treatments. If significant differences were found, further analysis was conducted using Duncan's Multiple Range Test (DMRT) at a 5% significance level to compare the average differences between treatments.

2.3 Production of Anchovy Protein Hydrolysate

The anchovies were separated from their heads and tails, then the meat was washed thoroughly with running water. After that, distilled water was added to the anchovy meat at a ratio of 1:4, then the mixture was blended using a blender. Bromelain enzymes with concentrations of 6%, 7%, and 8% are added to the mixture and hydrolyzed at 55°C for 4 hours, 5 hours, and 6 hours. The enzyme inactivation process is carried out at 90°C for 20 minutes. The inactivated liquid was filtered using filter paper. Subsequently, the liquid and supernatant were separated by centrifugation at 3500 rpm for 20 minutes. The supernatant obtained was collected and used as the hydrolysate.

3. RESULT AND DISCUSSION

3.1. Chemical Characteristics of Anchovy Protein Hydrolysate

The chemical characteristics of anchovy (Stolephorus indicus) protein hydrolysate were determined by proximate characteristics, namely moisture content, ash content, soluble protein, and fat content, as shown in Table 1. The analysis of variance showed that enzyme concentration had a significant effect on moisture content, ash content, soluble protein, and fat content ($P<0.05$). The best treatment for anchovy protein hydrolysate was obtained at an enzyme concentration of 8% bromelain and a hydrolysis time of 6 hours.

3.2 Moisture Content

The analysis of variance showed that the concentration of bromelain enzyme had a significant effect on the moisture content of anchovy protein hydrolysate ($P<0.05$). The highest moisture content was achieved by anchovy protein hydrolysate with a bromelain enzyme concentration of 8% and a hydrolysis time of 6 hours ($82.91 \pm 0.03\%$), while the lowest was achieved with a bromelain enzyme concentration of 6% and a hydrolysis time of 4 hours ($77.79 \pm 0.06\%$).

Moisture content increases with increasing enzyme concentration, as higher bromelain enzyme concentrations enhance enzymatic activity, accelerating the breakdown of proteins into peptides and soluble compounds [13]. Water content increases with higher enzyme concentrations because increased enzymatic activity produces more hydrophilic active compounds in the hydrolysate solution. Additionally, the increase in water content can be attributed to adding bromelain enzyme. The hydrolysis reaction of protein compounds into simpler compounds causes an increase in the volume of the hydrolysate solution, thereby increasing the product's water content. The hydrolysis of lemuru soy sauce resulted in a 17% increase in solution volume when the bromelain enzyme concentration was increased from 6% to 8% [14].

3.3 Ash Content

Most foodstuffs consist of 96% organic matter and water composed of mineral elements. Burning foodstuffs at a temperature of 500°C will cause the organic matter to burn, but the inorganic matter will not burn and will remain in the form of ash consisting of various mineral elements such as Ca, Mg, Na, P, K, Fe, Mn, and Cu.

https://doi.org/10.29165/ajarcde.v9i2.667

The analysis of variance showed that the concentration of bromelain enzyme had a significant effect on the ash content of anchovy protein hydrolysate (P<0.05). The highest ash content was achieved by anchovy protein hydrolysate with a bromelain enzyme concentration of 6% and a hydrolysis time of 4 hours (4.93 ± 0.03%), while the lowest was achieved with a bromelain enzyme concentration of 8% and a hydrolysis time of 6 hours (2.46 ± 0.06%). A longer hydrolysis time can break down more protein into soluble fractions, thereby increasing the soluble organic fraction more rapidly than the inorganic fraction. The soluble organic fraction increases more rapidly than the insoluble inorganic fraction. Insoluble minerals are not filtered out in the filtrate, resulting in a lower ash content relative to the product volume. The decrease in ash content is caused by protease enzymes that break peptide bonds in proteins into smaller peptides and amino acids as hydrolysis time increases [15].

3.4 Soluble Protein Content

The analysis of variance showed that the concentration of bromelain enzyme had a significant effect on the soluble protein content of anchovy protein hydrolysate (P<0.05). The highest soluble protein content was achieved by anchovy protein hydrolysate with a bromelain enzyme concentration of 8% and a hydrolysis time of 6 hours, amounting to (8.13 ± 0.11 mg/mL), while the lowest was achieved with a bromelain enzyme concentration of 6% and a hydrolysis time of 4 hours, amounting to (4.59 ± 0.10 mg/mL).

Bromelain enzyme acts as a catalyst in breaking peptide bonds in proteins into smaller molecules such as short peptides and amino acids. This is because a high enzyme concentration allows more substrate molecules (proteins) to interact simultaneously with the enzyme, thereby increasing the hydrolysis rate and producing more soluble protein. Hydrolysis can break the peptide bonds within proteins, allowing them to break down and release amino acids. Hydrophilic amino acids can bind water, enabling proteins to dissolve in the hydrolysate. Protein hydrolysis can cause proteins that were originally insoluble to become soluble. Long hydrolysis times allow enzymes to hydrolyze proteins for longer periods, hydrolysing more proteins into amino acids [16].

3.5 Fat Content

The results of the analysis of variance showed that the concentration of bromelain enzyme had a significant effect on the fat content of anchovy protein hydrolysate (P<0.05). The highest fat content was achieved by anchovy protein hydrolysate with 6% bromelain enzyme and a hydrolysis time of 4 hours (4.58 ± 0.07%), while the lowest was achieved at a bromelain enzyme concentration of 8% and a hydrolysis time of 6 hours (3.27 ± 0.04%).

The decrease in fat content is due to the longer hydrolysis time, which provides sufficient time for the enzyme to break down the protein into a soluble form. The hydrolysis process reduces the number of intact protein particles remaining, decreasing the likelihood of fat fractions being trapped or carried in the suspension. This process causes the fat content in the filtrate to decrease. The anchovy used in the fish protein hydrolysis process has a low-fat content. Enzymatic hydrolysis causes rapid changes in the structure of fish tissue, resulting in a decrease in fat content [17].

Table 1. Results of analysis of moisture content, ash content, soluble protein content, and fat content

Treatment		Moisture Content (%)	Ash Content (%)	Soluble Protein Content (mg/mL)	Fat Content (%)
Concentration (%)	Hydrolysis Time (Hours)				
6	4	77.79 ± 0.06^{a}	4.93 ± 0.03^{g}	4.59 ± 0.10^{a}	4.58 ± 0.07^{g}
	5	78.56 ± 0.07^{b}	4.21 ± 0.02^{f}	5.69 ± 0.09^{b}	4.39 ± 0.04^{f}
	6	80.22 ± 0.01^{c}	3.83 ± 0.04^{e}	7.17 ± 0.06^{d}	4.24 ± 0.04^{e}
7	4	80.12 ± 0.08^{c}	4.14 ± 0.04^{f}	6.53 ± 0.11^{c}	4.24 ± 0.06^{e}
	5	81.10 ± 0.05^{d}	3.83 ± 0.04^{e}	7.29 ± 0.04^{de}	3.64 ± 0.06^{c}
	6	82.68 ± 0.03^{e}	3.37 ± 0.05^{d}	7.50 ± 0.07^{ef}	3.34 ± 0.05^{ab}
8	4	81.68 ± 0.03^{f}	3.23 ± 0.06^{c}	7.42 ± 0.13^{ef}	3.92 ± 0.03^{d}
	5	82.35 ± 0.04^{g}	2.86 ± 0.05^{b}	7.62 ± 0.14^{f}	3.45 ± 0.05^{b}
	6	82.91 ± 0.03^{h}	2.46 ± 0.06^{a}	8.13 ± 0.11^{g}	3.27 ± 0.04^{a}

4. CONCLUSION

Protein hydrolysate is the result of breaking down protein into peptides and amino acids through hydrolysis. This hydrolysate product has high biological value, is easily digestible, and has potential as a functional food ingredient. The optimal treatment for anchovy protein hydrolysate was a combination of 8% bromelain enzyme concentration and a hydrolysis time of 6 hours. The chemical characteristics obtained included moisture content of 82.91%, ash content of 2.46%, soluble protein content of 8.12%, and fat content of 3.27%.

ACKNOWLEDGEMENT

We would like to thank the Innovation Center of Appropriate Food Technology for Lowland and Coastal Area, Universitas Pembangunan Nasional Veteran Jawa Timur, for providing facilities and contributions to this research.

REFERENCE

[1] Hapsari, D. P., & Setyani, S. A. (2020). Aplikasi enzim bromelin dalam industri pangan: Potensi dan tantangannya. *Jurnal Teknologi Hasil Pertanian*, 13(1), 45–52

[2] Kementerian Kesehatan Republik Indonesia. (2020). *Riset Kesehatan Dasar (Riskesdas) 2018: Laporan Nasional*. Badan Penelitian dan Pengembangan Kesehatan.

[3] Kristinawati, E., Wulandari, M., & Nugraheni, R. R. (2018). Karakteristik dan potensi hidrolisat protein ikan sebagai bahan pangan fungsional. *Jurnal Pangan dan Gizi*, 9(3), 180–189.

[4] Sari, N. P., Yuliana, N. D., & Anindyajati, A. (2019). Komposisi nutrisi ikan teri jengki (*Stolephorus indicus*) asal perairan Jawa Tengah. *Jurnal Perikanan dan Kelautan*, 10(2), 95–102.

[5] Aryati, E.E., & Dharmayanti A.W.S. (2014). Manfaat ikan teri segar (Stolephorus sp) terhadap pertumbuhan tulang dan gigi. ODONTO Dental Journal, 1(2), 52-56. https://dx.doi.org/10.30659/odj.1.2.52-56

[6] Surono, I. S., et al. (2014). Proximate composition, amino acid and fatty acid profiles of marine fish and shellfish from south sulawesi waters. *Journal of Food Science and Technology.*

[7] Yuliani, S., et al. (2015). Nutritional composition and potensial health benefits of anchovies (*Stolephorus spp*). *Journal of Aquatic Food Product Technology.*

[8] Syah, Y. M., Andayani, R., & Mulyani, S. (2012). kandungan protein dalam hidrolisat protein ikan teri yang dihasilkan melalui proses hidrolisis enzimatik. *Jurnal Teknologi Hasil Perikanan*, 8(1), 45-52

[9] Suhartono, B., Sari, R. A., & Santoso, M. (2011). Profil asam amino dan kecernaan hidrolisat protein ikan teri dibandingkan dengan tepung ikan teri konvensional. *Jurnal Pangan dan Gizi*, 6(2), 99-106

[10] Ahalya, N., Ramachandra, T. V., & Rao, R. M. (2017). Nutritional advantages of fish protein hydrolysates over conventional fish meal. *Journal of Food Science and Nutrition*, 14(4), 341-349

[11] Roslan, M. A., Hadi, M. Y., & Sari, R. (2014). Komposisi kimia dan profil asam amino dari hidrolisat protein limbah ikan nila (oreochromis niloticus). Jurnal Teknologi dan Industri Pangan, 10(3), 212-220.

[12] Harahap, M.T. (2022). Pengaruh konsentrasi enzim bromelin terhadap derajat hidrolisis hidrolisat protein belut (monopterus albus). *Jurnal Perikanan dan Kelautan. Universitas Riau*.

[13] Wijayanti, I., dan Laras Rianingsih Program Studi Teknologi Hasil Perikanan, R., Perikanan Fakultas Perikanan dan Ilmu Kelautan, J., Diponegoro Jl Soedarto, U., & Tembalang, S. (2016). Karakteristik hidrolisat protein ikan bandeng (chanos chanos forsk) dengan konsentrasi enzim bromelin yang *Indonesian Journal of Fisheries Science and Technology (IJFST) 11*(2), 129–133. www.wpi.kkp.go.id

[14] Pandiangan, M., Sihombing, D. R., Tampubolon, S. D. R., & Sidebang, Y. E. (2025). Uji hidrolisis protein dan aktivitas antioksidan hidrolisat protein ikan nila (*Oreochromis niloticus*). *Jurnal Riset Teknologi Pangan dan Hasil Pertanian (RETIPA)*, *5*(2), 84–93

[15] Putra, A. Y. T., Rosida, D. F., & Priyanto, A. D. (2021). Influence of enzyme concentration and hydrolysis time on soluble protein content of protein hydrolysate prepared from apple snail (*pila ampullacea*). *International Journal of Eco-Innovation in Science and Engineering*, Vol. 2 (2): 26-30.

[16] Anggraini, A., & Yunianta. (2015). *Pengaruh Suhu dan Lama Hidrolisis Enzim Papain terhadap Sifat Kimia, Fisika, dan Organoleptik Sari Edamame*. Jurnal Pangan dan Agroindustri, 3(3), 1015–1025

[17] Shahidi, F., Han, X.Q., & Synowiecki, J. (1995). Production and characteristics of protein hydrolysates from capelin (Mallotus villosus). *Food Chemistry*, 53(3), 285–293

 https://doi.org/10.29165/ajarcde.v9i2.667