Asian Journal of Aquatic Sciences, April 2024. Vol 7, Issue (1) 156-160
e-issn: 2716-4608
p-issn: 2655-366X

HYDROLYSATE CHARACTERISTICS OF BERUNOK SEA CUCUMBER (*Paracaudina australis*) AND TOTAL AMINO ACID USING PEPSIN ENZYMES

Bella Billiant J Ananta Kembaren[1*], Rahman Karnila[1], Bustari Hasan[1]
[1]Department of Marine Science, Postgraduate, Universitas Riau, Pekanbaru, 28293 Indonesia
*bbilliantj@gmail.com

ABSTRACT

Berunok sea cucumber has a nutritional content that can be utilized as a functional food ingredient. This study determined the characteristics of berunok sea cucumber hydrolysate (*Paracaudina australis*) and the type and level of total amino acids. This research was conducted using experimental methods, with a Non-Factor Complete Randomized Design consisting of 3 levels of enzyme concentration treatment (1%, 2%, and 3%) with three replicates. In this study, the parameters tested were the proximate content of flour and hydrolysate of berunok and the type and content of total amino acids. The results showed that the chemical composition of berunok flour was water 11,40% (dw), ash 10,82% (dw), protein 68,21% (dw), fat 6,53% (dw), and carbohydrate by the difference of 14,43% (dw). The best treatment for preparing berunok protein hydrolysate using pepsin enzyme is 2% pepsin enzyme concentration. The proximate of berunok protein hydrolysate is water 6.87% (dw), ash 8.45% (dw), protein 80,59% (dw), and fat 2,86% (dw). The results of the identification of types and levels of total amino acids found 17 kinds of amino acids with a total of 16.524% in hydrolysate berunok.

Keywords: Berunok, Pepsin, Protein hydrolysate, Total-amino acids

1. INTRODUCTION

Berunok sea cucumber (*Paracaudina australis*) is a fishery biota from the Tanjung Balai Karimun Islands, Riau Islands Province. The utilization could be more optimal due to the lack of information regarding the nutrients contained in this biota. Berunok contains bioactive compounds that have the potential to cure various diseases, such as wound healing and diabetes mellitus[1]. Berunok has an excellent nutritional content of 20.22% protein[2]. With this protein content, berunok has the potential as a functional raw material that has benefits in the biopharmaceutical field, one of which is by making protein hydrolysates[3].

Protein hydrolysis is the breaking of peptide chains, usually using strong acids, bases, and enzymes, producing simpler peptide chains and the formation of free amino acids[3]. In this study, the hydrolysis method was enzymatic and used pepsin.

The pepsin enzyme is an acidic aspartyl protease, where the enzyme's catalytic activity depends on the aspartic acid residues present in the active site of the enzyme[4]. This enzyme belongs to the class of endopeptidases, which cleave protein peptide bonds with the amino-terminal side of aromatic amino acid residues such as tyrosine and tryptophan, thus cleaving long polypeptide chains into smaller peptides and free amino acids in some cases[1]. The research aimed to determine the characteristics of berunok hydrolysate using pepsin enzyme.

Based on the description above, research is needed regarding the potential of berunok hydrolysate as one of the functional food ingredients in the biopharmaceutical field.

Received : 5 February 2024
Accepted : 29 March 2024

2. RESEARCH METHOD

Time and Place

This research was conducted in July 2022 at the Fishery Products Chemistry Laboratory of Universitas Riau and PT Nawa Bogor Laboratory.

Method

This study uses an experimental method that conducts direct experiments to prepare berunok protein hydrolysate. The treatment used was the use of different enzyme concentrations[4], consisting of three levels, namely H1 (1% pepsin enzyme), H2 (2% pepsin enzyme), and H3 (3% pepsin enzyme), with three replicates. The test parameters in this study include proximate analysis of flour and hydrolysate of berunok and total amino acid testing.

3. RESULT AND DISCUSSION

Characteristics of the Berunok (*Paracaudina australis*)

The berunok used in this study has an elongated transparent body shape with thin, soft, and slippery skin. This species of sea cucumber is usually found buried in soft intertidal sediments. The body of the yellowtail berunok is cylindrical and has a short tail on the backside. In its fresh state, the maximum length of this biota can reach 230 mm. The body of this type of berunok is smooth and does not have tube feet. When fresh, the berunok's body is translucent pink with a slight ivory-white color around it[2]. The chemical composition of the berunok flour can be seen in the following Table 1.

Table 1. Chemical Composition of Berunok Flour

Parameters	Percentage (%)
Water (ww)	11.40
Ash (dw)	10.82
Protein (dw)	68.21
Fat (dw)	6.53
Carbohydrate (dw)	14.43

Note: 1) ww= wet weight; 2) dw= dry weight

From the results of chemical composition testing on berunok flour, the protein content obtained is 68.21 (dw). In general, protein content in food can determine the quality of the food. The higher the protein contents of food, the better the quality of the food[6]. In addition, protein in the body can be in the form of food reserves, building and regulating substances (enzymes, antibodies, etc.)[7].

Berunok Hydrolysate

Protein hydrolysate is a product that results from breaking the peptide chain using acids, bases, and enzymes1. The hydrolysate produced from this research uses an enzymatic method with different concentrations of pepsin enzyme, and the best concentration is 2% pepsin enzyme. The chemical composition of the hydrolysate produced from this study can be seen in Table 2.

Table 2. Chemical Composition of Berunok Hydrolysate

Parameters	Percentage (%)
Water (ww)	6.87± 0.04
Ash (dw)	8.45 ± 0.19
Protein (dw)	80.59 ± 0.83
Fat (dw)	2.86 ± 0.11
Carbohydrate (dw)	8.10 ± 0.21

Water Content

The water content of berunok hydrolysate obtained from this study was 6,87%. The decrease in water content in the hydrolysate of berunok was due to the evaporation that occurred during the freeze-drying process[1]. The addition of enzymes in making protein hydrolysates also influences the reduction of water content. Free water will bind to amide and carboxyl groups during protein or four-peptide cleavage. So the higher the enzyme has given, the less accessible water[8]. The decrease in water content in food due to evaporation causes the reduction of water, which is four peptides, limiting the growth and life of all microorganisms[9].

Ash Content

Berunok protein hydrolysate has an ash content of 8,45%. The amount of ash in

each food is different because ash is composed of various types of minerals based on the kind of food source[10]. In the process of making hydrolysate using pepsin enzyme, NaOH is added to maintain pH stability during hydrolysis. If there is a mixing of alkaline and acidic compounds in the protein hydrolysate solution, it will cause the formation of salt compounds, increasing the ash content value in protein hydrolysate[11].

Fat Content

From the research that has been done, it is known that the fat content in hydrolysate berunok is 2.86%. The fat content of berunok hydrolysate decreased significantly from that of berunok flour. This happens when hydrolysate berunok is made through a centrifugation process where the fat contained in the hydrolysate berunok protein is dissolved along with the supernatant. Protein hydrolysates with a lower percentage of fat in their composition are more resistant to fat oxidation than fish protein hydrolysates with a higher percentage of fat in their composition[12].

Protein Content

In this study, the protein content of berunok hydrolysate was 80,59%. This is in line with the results of research conducted by Jhon[13], which states that enzyme activity is influenced by several parameters, such as enzyme concentration, temperature, and pH, and each enzyme has a different preference for one of these peptides. The increase in protein content in hydrolysate products is due to the conversion of insoluble protein into soluble nitrogen compounds during the hydrolysis process[14]. These soluble nitrogen compounds then break down into simpler compounds, such as peptides and amino acids, so the body can easily absorb them. This results in an increase in the amount of protein present in the hydrolysate product.

The Total Amino Acid Content of Berunok Hydrolysate with Buntings

The protein hydrolysate of berunok produced from this study was then analyzed for type and total amino acid content, as seen in Table 3.

Table 3. Types and Levels of Total Amino Acids of Berunok Hydrolysate.

No.	Types of amino acids	Content (%)
Essential Amino Acids		
1	Valin	0.374
2	Phenylalanine	0.276
3	Isoleucine	0.766
4	Treonin	0.718
5	Histidine	0.883
6	Lisin	1.308
7	Methionine	0.773
8	Leucine	1.203
		6.301
Non-Essential Amino Acids		
1	Glycine	0.760
2	Cysteine	0.486
3	Arginine	0.496
4	Proline	0.850
5	Alanin	0.720
6	Serin	0.625
7	Tyrosine	0.626
8	Aspartic Acid	2.163
9	Glutamic Acid	3.497
	Total	16.524

From Table 3, it can be seen that the number of amino acids obtained from berunok protein hydrolysate is 17 types. Amino acids contained in protein molecules are almost not all that can be made in our bodies, so when viewed in terms of the formation of amino acids, they are divided into two groups, namely endogenous amino acids and exogenous amino acids. Exogenous amino acids are also called essential amino acids, and endogenous amino acids are also called non-essential amino acids[15].

According to Cholifah[16], overall hydrolysis will produce hydrolysates consisting of a combination of 18-20 types of amino acids. Hydrolysis of berunok protein is close to ideal because it produces

17 kinds of amino acids. The amino acid groups that can stimulate insulin secretion in the blood are leucine, isoleucine, lysine, arginine, methionine, alanine, and phenylalanine, which have different functions and benefits for each type.

Lysine is a fundamental component of many growth factors, and it plays an essential role in cell division and the overall development of the organism. Lysine deficiency can lead to anemia, problems with fatty acid metabolism, slow wound healing, decreased muscle mass, and faulty connective tissue development. On the other hand, high amounts can lead to neurological abnormalities[17.]

Leucine increases the synthesis of glucosamine, an inhibitor of endothelial NO synthesis, by increasing rapamycin signaling and the expression of fructose-6-phosphate aminotransferase (the rate-limiting enzyme of glucosamine synthesis), thereby inhibiting NO synthesis in endothelial cells and causing vascular endothelial dysfunction[18].

4. CONCLUSION

The berunok flour produced from this study has a pretty good chemical composition, especially the protein content, which is 68,21% (dw), ash 10,82% (dw), water 11,40% (dw), fat 6,53% (dw) and carbohydrate by difference 14,43% (dw). For the preparation of protein hydrolysate using pepsin enzyme, the best concentration is 2% with the composition of berunok protein hydrolysate as water 6.87% (dw), ash 8.45% (dw), protein 80,59% (dw), and fat 2,86% (dw). The analysis results of the types and levels of total amino acids in the hydrolysate of berunok found 17 types of amino acid compounds with a total of 16.524%. So, the hydrolysate of berunok has good nutritional content that can be utilized as a functional food raw material.

The research that has been conducted obtained nutritional information from the berunok, which can be utilized as a functional food raw material. However, before its use, further research must be conducted on the nutritional content of berunok hydrolysate in vitro.

REFERENCES

1. Rahman, K. *Potensi Ekstrak, Hidrolisat dan Isolat Protein Teripang Pasir (Holothuria scabra J) untuk Menurunkan Kadar Glukosa Darah dan Memperbaiki Profil Sel Beta Pankreas Tikus Diabetes Melitus*. Laporan Hasil penelitian Hibah bersaing 2010. Universitas Riau. Pekanbaru, 2011.
2. Widianingsih, W., Muhammad, Z., Sutrisno A., Hermin P. Nutritional Value of Sea Cucumber [*Paracaudina australis,* (Semper, 1868)]. *Aquatic Procedia*, 2016; 7:271-276.
3. Elli, M.B., Sofia, M., Konstantinos, M., Dimitris, M., Maria, L. Clean Energy and Resources Recovery. *Biomass Waste Based Biorefineries*, 2021; 1(3): 49-102.
4. Omkar, R., Koli, J.M., Sonavane, A.E., Patange, S.B., Shingare, P.E., Relekar, P.P., Mulye, V.B. Effect of Different Concentrations of Pepsin Enzyme on Extraction of Fish Protein Hydrolysate from Malabar Sole Fish *(Cynoglossus macrostomus)*. *BEPLS*, 2018, 7: 94-103.
5. Vagne, O.M., Bezerra, R.S., Assis, C.R.D. Fish Pepsin: Basic Characteristics, Extraction, Determination, and Biotechnological Applications. *Natural Resources, Aquidabã*, 2014; 4(1):6-14.
6. Maya, L., Musthofa L., Bambang, S. Pengaruh Suhu dan Lama Pengeringan terhadap Mutu Tepung Jamur Tiram Putih (*Plaerotus ostreatus*). *Jurnal Keteknikan Pertanian Tropis dan Biosistem*, 2015; 3(3): 270-279.
7. Chinnathambi, A., Saritha, K. Protease Enzyme: an Eco-Friendly Alternative for Leather Industry. *Indian Journal of Science and Technology*, 2009; 2(12): 29-32.

8. Alfonsina, M., Nanlohy, E.E.E.M., Louhenapessy, J.M. Efek Waktu Pemanasan terhadap Mutu Presto Beberapa Jenis Ikan. *Jurnal Ichthyos,* 2008; 7(2): 65-70.
9. Samaranayaka, A.G.P., Li-Chan, E.C.Y. Food-Derived Peptidic Antioxidants: A Review of Their Production, Assessment, and Potential Applications. *Journal Functional Foods,* 2011l 3: 229–254.
10. Nuri, A., Kusnandar, F., Herawati, D. *Analisis Pangan*. Dian Rakyat. Jakarta, 2011; p328.
11. Roger, V., Riba, J., Ruiz-Capillas, C., Gonzales, A.V., Baeta, M. Amino Acid Composition of Early Stagtacaes of Cephalopods and Effect of amino Acid Dietary Treatments on *Octopus vulgaris* Paralarvae. *Aquaculture*, 2004; 242(4): 455-478.
12. Tatik, N., Salamah, E., Elin, A. Pemanfaatan Kerang Hijau (*Mytilus viridis*) dalam Pembuatan Hidrolisat Protein Menggunakan Enzim Papain. *Akuatik Jurnal Sumberdaya Perairan*, 2011; 5(1): 13-16.
13. John, E. *Factors Affecting Enzyme Activity*. *ESSAI*, 2013; 10: 19.
14. Dian, P. *Produksi dan Karakterisasi Hidrolisat Protein dari Kerang Masngur (Atactodea striata).* Program Studi Teknologi Hasil Perikanan. Fakultas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor. Bogor, 2008.
15. Dwi, W. *Biokimia*. Leppim Mataram, 2017.
16. Cholifah. *Produksi dan Karakterisasi Hidrolisat Jeroan Ikan Kakap Putih (Lates calcarifer).* Departemen Teknologi Hasil Perairan. Fakultas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor. Bogor, 2014.
17. Elena, C., Leon-Constantin, M., Ungureanu, C., Trandafirescu, M.F., Mastaleru, A., Trandafir, M.L., Petrariu, D.F., Badulescu, V.O., Filip, N. Hypolipemiant Actions and Possible Cardioprotective Effects of Valine and Leucine: An Experimental Study. *Medicina*, 2021; 57: 239.
18. Ying, Y., Wu, Z., Meininger, C.J., Wu, G. L-Leucine, and NO-mediated Cardiovascular Function. *Amino Acids*, 2015; 47: 435–447.