Jurnal Teknologi

Full Paper

CHEMICAL COMPOSITION, AMINO ACIDS PROFILE AND ANTIOXIDANT ACTIVITY OF ENZYMATIC PROTEIN HYDROLYSATE FROM *Bohadschia marmorata* SEA CUCUMBER

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Article history
Received
25 October 2023
Received in revised form
23 November 2023
Accepted
17 January 2024
Published Online
15 April 2024

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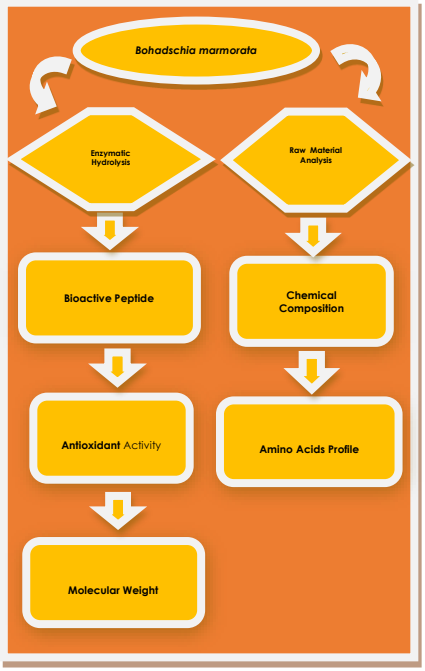
Graphical abstract



Abstract

The sea cucumber is one of the potential sub-exports of fisheries. Sea cucumbers contain highly nutritious and bioactive compounds with antioxidant, antibacterial, anticoagulant, anti-inflammatory, and antiviral activities. Protein hydrolysates from sea cucumbers have the potential as antioxidants with the ability to reduce free radicals. The objective of this research is to examine the chemical characteristics and antioxidant activity of protein hydrolysates from *Bohadschia marmorata* sea cucumber. The research method used in this study was experimental and laboratory analysis. The results showed that the chemical composition of fresh *Bohadschia marmorata* sea cucumber had high moisture content (80.45 %), ash content (4.12 %), low fat content (6.17 %), and low protein content (8.96 %). This could be due to the environmental conditions and nutrient absorption by *Bohadschia marmorata* sea cucumbers. Fifteen types of amino acids with a total of 53.81 % were obtained from this study. The yield obtained for crude papain enzyme concentration was 16.26 %, and for pure papain enzyme concentration, it was 11.23 %. The antioxidant activity with IC_{50} > 200 indicates very weak antioxidant activity, and the resulting molecular weight reached 17.98-32.70 kDa.

Keywords: Sea cucumber, *Bohadschia marmorata*, protein hydrolysate, enzymatic chemical composition, amino acids p, antioxidant

1.0 INTRODUCTION

Sea cucumbers are echinoderms that come in various colors. They have elongated, cylindrical bodies covered in mucus and are commonly found on the seabed in clear, calm waters, particularly in areas rich in seagrass, seaweed, and coral reefs. Sea cucumbers primarily feed on small organisms, detritus, protozoa, and nematodes [1]. Sea cucumbers, in particular, are a potentially valuable export commodity within the fisheries sub-sector. They are highly nutritious, containing significant levels of protein, calcium, and collagen. The *Bohadschia marmorata* species of sea cucumber has the potential for secondary

metabolites and can be used as an antioxidant. Additionally, it is a good source of protein, minerals, omega-3, 6, and 9 fatty acids, amino acids, and vitamins, making it a valuable resource for health and nutrition.

Protein hydrolysate involves the enzymatic degradation of proteins into smaller peptide molecules. Generally, protein hydrolysates comprise compact segments of peptides consisting of 2-20 amino acids. [2]. Protein hydrolysis can utilize protease enzymes. Enzymatic hydrolysis is advantageous because it does not result in damage to peptides and amino acids. Papain is an enzyme belonging to the protease class, which facilitates the cleavage of polypeptide chains within proteins by catalyzing the hydrolysis of their peptide bonds, resulting in the formation of simpler compounds like peptides and amino acids. Numerous studies have indicated that the bioactive compounds discovered in marine organisms have been empirically demonstrated to exhibit functions including antioxidants, antibacterial agents, antihypertensive properties, anticoagulants, anti-inflammatory effects, antimalarial activities, and antiviral properties. [3, 4, 5, 6, 7, 8, 9, 10]. Several investigations conducted in Indonesia have focused on the antioxidant properties of various sea cucumber species include: [11, 12, 13, 14], and several research findings from various countries on the antioxidant properties of sea cucumbers include: [15, 16, 17, 18]. This research is aimed at harnessing the potential of marine resources, specifically the *Bohadschia marmorata* sea cucumber. One of the efforts involved is the production of bioactive compounds from *Bohadschia marmorata* sea cucumber. Therefore, the inception of this research project aimed to generate bioactive compounds displaying antioxidant properties and to assess the chemical composition and amino acid profile of sea cucumbers known as *Bohadschia marmorata*.

2.0 METHODOLOGY

2.1 Materials

The materials used in this research include fresh *Bohadschia marmorata* sea cucumbers from the waters of Suli Village, Central Maluku Regency. The sea cucumber samples are first measured for length and weight before testing. Subsequently, their abdominal contents are extracted, cleaned, and transported to the laboratory.

2.2 Research Stage

This research began with the collection of sea cucumber samples from the waters of Suli Village in Central Maluku Regency. The samples were then subjected to proximate analysis and amino acid profiling of fresh sea cucumber meat. Subsequently, enzymatic protein hydrolysis was conducted, and the yield value was calculated. The research further included antioxidant testing and the determination of peptide molecular weights.

2.3 Procedural Analysis

2.3.1 Moisture

Porcelain crucibles are dried in an oven at 100 3°C for 1 h and then cooled in a desiccator. The porcelain crucible is then weighed. Two grams of the sample are placed in a dry porcelain crucible and dried in an oven at 100 - 102 °C until a constant weight is achieved. The crucible containing the sample is cooled in a desiccator. The next step is to weigh the crucible containing the sample after drying [19].

2.3.2 Fat

5 g sample is placed inside a filter paper. Both ends of the filter paper are sealed with grease-free cotton, then wrapped and placed inside a fat tube. The wrapped sample is placed inside a pre-weighed fat flask and connected to a Soxhlet tube, rinsed with a fat solvent, and refluxed for 6 h. The fat-soluble substance in the fat flask is subjected to distillation until all the fat-soluble material evaporates. Throughout the distillation process, the solvent will be accumulated in the extractor chamber. Subsequently, the fat flask is desiccated in an oven at a temperature of 105 °C and subsequently allowed to cool in a desiccator [19].

2.3.3 Protein

A 0.1 g sample is placed in a Kjeldahl flask, and then 2.5 mL of concentrated H_2SO_4, 1 g of catalyst, and a few boiling chips are added. The solution is digested until it becomes clear and is then allowed to cool. The resulting digestion solution is transferred to a distillation apparatus, and 15 mL of 50 % NaOH is introduced. An Erlenmeyer flask containing 25 mL of 0.02 N HCl and 2-4 drops of a mixed indicator (a combination of 0.02 % methyl red in alcohol and 0.02 % methyl blue in alcohol in a 2 : 1 ratio) is positioned under the condenser, ensuring that the tip of the condenser is submerged in the HCl solution. Distillation is conducted until the solution in the Erlenmeyer flask reaches twice its initial volume. The condenser's tip is rinsed with distilled water, and this rinse is collected in the Erlenmeyer flask. The solution in the Erlenmeyer flask is titrated with 0.02 N NaOH until a color change from green to purple is achieved. Additionally, a blank determination is carried out afterward [19].

2.3.4 Ash

A porcelain crucible is first dried in an oven at 105 °C for 1 hour and subsequently allowed to cool for 15 minutes in a desiccator. The crucible is then weighed to obtain its initial weight. A 2 g sample is carefully placed into the crucible and gently heated over an open flame until no smoke is generated. The sample is then transferred to a muffle furnace set at 600 °C for a

duration of 6 h. Following this, the crucible, now containing the sample, is allowed to cool in a desiccator. After the cooling process, the crucible is weighed once again to determine the final weight [19].

2.3.5 Amino Acid Profile

The analysis of the amino acid profile was carried out utilizing the following [19] method. The sample solution, which had been hydrolyzed in 10 mL of 0.01 N HCl, was then filtered using Millipore paper. Pre-column sample analysis was performed by adding potassium borate buffer at pH 10.4 in a 1 : 1 ratio to the filtered sample. Five microliters (5 µL) of the sample were placed into an empty vial, and 25 µL of o-phthalaldehyde (OPA) reagent was added. The mixture was left for 1 min to ensure complete derivatization. It was then injected into the HPLC (Hight Performace Liquid Chromatography) column, and the separation of all amino acids was allowed to complete. Pre-column sample analysis involved injecting 1 mL of the sample into a clean empty vial, which was then injected using an automatic sampler. The concentration of amino acids was expressed in µmol amino acids in the sample The HPLC instrument conditions for pre-column analysis were as follows: Column: Thermo Scientific Octadecyl Silane (ODS2) Hypersil, Flow rate of mobile phase: 1 mL/min, and detector: Fluorescence.

2.3.6 Antioxidant Activity

The assessment of radical scavenging activity was conducted using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) method as outlined in the procedure. [20]. Isolated peptides from sea cucumbers were dissolved in 4 mL of distilled water at different concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL). In each solution, 1 mL of 0.5 mM DPPH was introduced. The blend was left to incubate for 30 minutes at room temperature in darkness, followed by the measurement of absorbance at a wavelength of 517 nm utilizing a UV-visible spectrophotometer. A control sample was prepared using distilled water.

2.3.7 Molecular Weight Analysis

The assessment of molecular weight is carried out through the utilization of Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) as per the method developed by. [21]. The gel consists of two types: an 8 % separating gel and a 4 % stacking gel. Silver staining is employed for visualization, and the staining process is outlined as follows: The gel is immersed in a fixation solution (25 % methanol and 12 % acetic acid) for 1 h. After fixation, the gel is soaked in 50 % ethanol for 20 min. The ethanol is subsequently replaced with 30 % ethanol for two cycles of 20 min each. The solution is then substituted with an enhancer solution (containing $Na_2S_2O_3 \cdot 5H_2O$), and the gel is rinsed with distilled water. Following rinsing, the gel is immersed in a silver nitrate solution for 30 min. Subsequently, the gel is briefly washed with distilled water for 2 x 20 seconds. A developer solution (a mixture of Na_2CO_3 and formaldehyde) is added to the gel. Finally, the gel is fixed with a fixation solution.

2.4 Treatmen

The experimental design involves two treatment factors: enzyme concentration and hydrolysis time, using both crude papain enzyme and pure papain enzyme.

Crude Papain Enzyme:
Factor A (Enzyme Concentration: - 3 % (A_1)
- 5 % (A_2)

Factor B (Hydrolysis Time): - 4 hours (B_1)
- 5 hours (B_2)
- 6 hours (B_3)

Pure Papain Enzyme:
Factor A (Enzyme Concentration: - 0.3 % (A_1)
- 0.5 % (A_2)

Factor B (Hydrolysis Time): - 1 hours (B_1)
- 1.5 hours (B_2)
- 2 hours (B_3)

2.5 Data Analysis

Proximate analysis and amino acid profile analysis are conducted descriptively, while yield is analyzed using a Full Factorial Design (FFD) and Analysis of Variance (ANOVA). Data are calculated for mean values and standard deviations. Meanwhile, antioxidant activity is assessed using linear regression to determine IC_{50} and the T-test.

3.0 RESULTS AND DISCUSSION

3.1 Sampling Location

The sea cucumbers (*Bohadschia marmorata*) were sampled for our research, which is from the waters of Suli Village in Central Maluku Regency.

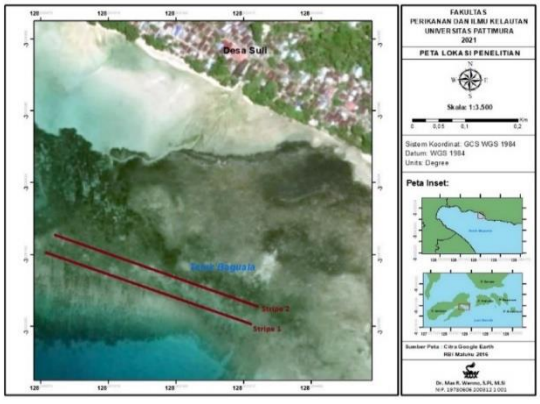


Figure 1 Research Location Map

The *Bohadschia marmorata* (Figure 2), sea cucumber is round in shape, with a length and a back that is grayish or blackish with white or yellowish spots. The entire surface of the body is covered with a layer of chalk. The sea cucumber's body is firm, heavily muscled, and spotted. The average length of the *Bohadschia marmorata* sea cucumber in this study ranges from 12 to 14 cm with a weight between 210 to 240 g per individual.

Figure 2 *Bohadschia marmorata* Sea Cucumber (*Source:* Personal Documentation)

3.2 Chemical Composition

The results of the nutritional content analysis (proximate analysis) of fresh *Bohadschia marmorata* sea cucumber meat are presented in Table 1.

Table 1 Chemical Composition of Fresh Sea Cucumber Meat

Chemical Composition (%)	Content (wb)
Moisture	80.45 ± 0.45
Ash	4.12 ± 0.16
Fat	6.17 ± 0.10
Protein	8.96 ± 0.06

n = 2

3.2.1 Moisture Content

Based on the analysis, the moisture content obtained is 80.45% (on a dry weight basis). Moisture content is a crucial component in food products as it can influence their appearance, texture, and taste. Additionally, moisture content affects the shelf life of food products by influencing their susceptibility to microbial growth [22]. The high moisture content in sea cucumbers is attributed to the fact that they are aquatic organisms, and their entire life cycle occurs in water. Variations in moisture content in different sea cucumber species can be influenced by factors such as seasonal variations and feeding habits, and it may also be related to differences in their body structures [23]. The moisture content of 80.45% in Bohadschia marmorata sea cucumber is somewhat consistent with the findings of previous studies. [24, 25] reported moisture content of around 93.36% for sea cucumber species like *S. Variegatus and S. Horens.* [26] also found a moisture content of approximately 93.55% in sea cucumbers of the T. ananas species. These comparisons highlight the variability in moisture content among different sea cucumber species and provide valuable information about the composition of Bohadschia marmorata sea cucumbers.

3.2.2 Ash Content

The ash content found in *Bohadschia marmorata* sea cucumber meat is 4.12% (on a dry weight basis). The ash content in this study is lower than the ash content reported for sea cucumbers of the *Actinopyga mauritiana* species, which was found to be 15.4% by [27]. Other studies have reported varying ash content for different sea cucumber species, with [28 reporting 34.43% and [29] reporting 48.3%. On the other hand, the ash content obtained in this study is higher than the findings of [30], who reported an ash content of 2.42%, and the research by [26], which found an ash content of 1.22% for the *B. vitiensis* sea cucumber species. The variations in ash content can be attributed to differences in habitat and environmental factors. Each marine region can provide different mineral intake for the organisms inhabiting it. Additionally, different organisms may have varying abilities to absorb minerals into their bodies, which can affect the ash content values of different materials [31]. These findings illustrate the diversity in ash content among sea cucumber species and emphasize the influence of habitat and species-specific characteristics on ash content measurements.

3.2.3 Fat Content

The fat content in *Bohadschia marmorata* sea cucumber meat is 6.17% (on a dry weight basis). This fat content is notably higher than the fat content reported in studies on other sea cucumber species. For instance, [32] found a fat content of 0.55% in sea cucumbers of the *Holothuria polii* species, and [33] reported sea cucumber fat content ranging from approximately 0.1% to 0.9%. Similarly, in the research by [26], sea cucumbers of the T. ananas species were found to contain only 0.36% fat in their fresh meat. The variations in fat content among different sea cucumber species can be attributed to several factors, including environmental conditions, seasons, the food sources consumed by sea cucumbers, and the behavior of the sea cucumbers themselves [30]. It's also worth noting that fat content tends to increase with age, as animals require more energy stored in the form of fat for reproduction. Fat is a crucial component in food as it serves as a source of essential energy and contains essential fatty acids.

3.2.4 Protein Content

The protein content in *Bohadschia marmorata* sea cucumber meat is 8.96% (on a dry weight basis). This protein content is notably higher than the protein content reported in studies on other sea cucumber species. For example, [33] found protein contents of 1.78% for Stichopus horens and 2.69% for *Stichopus variegatus* sea cucumbers. Studies by [34] reported a wide range of protein content in different sea cucumber species, ranging from 3.14% to 10%. Among the species studied, S. naso had the lowest protein content at 3.00 %, followed by *T. anax* at 3.14 %, *H. scabra* at 4.96 %, *H. spinifera* at 8.22 %, and *H. leucospilota* at 10.06 %. These variations in protein content among different sea cucumber species highlight the diversity of nutritional composition. Protein is a vital component in the diet as it provides essential amino acids required for various bodily functions, including muscle formation and hormone synthesis. Protein is also sensitive to physical and chemical factors, making it susceptible to changes in its structure. The higher protein content in *Bohadschia marmorata* sea cucumbers suggests their potential as a source of essential nutrients, including amino acids. These amino acids play important roles in muscle development and the production of endorphins, which act as natural painkillers.

3.3 Amino Acid Profil

The results of the amino acids profile analysis of *Bohadschia marmorata* sea cucumber meat can be seen in Table 2.

Table 2 Amino Acid Profile of *Bohadschia marmorata* Sea Cucumber

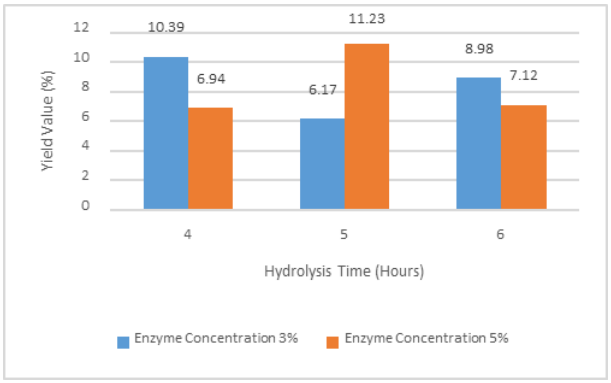
No	Amino Acid Types	Content (%)
1	Methionine*	5.41
2	Threonine*	9.03
3	Valine *	5.41
4	Phenilalanine*	1.55
5	Ileusine*	1.47
6	Lysine*	2.87
7	Histidine*	1.68
8	Glysine **	10.97
9	Serine **	2.19
10	Tyrosine**	2.26
11	Arginine**	1.20
12	Alanine**	2.56
13	Leusine**	0.70
14	Asam glutamate **	0.46
15	Asam aspartate**	6.06

*Essential Amino Acids, **Non Essential Amino Acids

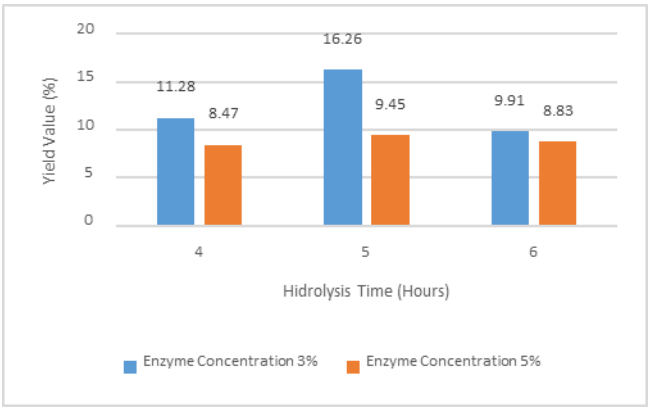
Based on the research findings, there are 15 types of amino acids present in *Bohadschia marmorata* sea cucumber protein, with a total amino acid content of 53.81 %. Sea cucumber protein contains a complete range of amino acids, including both essential and non-essential amino acids. According to the research by [35], the average amount of amino acids found in sea cucumbers ranges from 33.32 to 54.13 g/100 g of body weight, with the most abundant amino acids being glycine, aspartic acid, alanine, and arginine. Essential amino acids are of particular importance. In the amino acid composition of *Bohadschia marmorata* sea cucumbers, which function as antioxidants, tyrosine and phenylalanine are present at levels of 2.26 % and 1.55 %, respectively. [36] It has been mentioned that antioxidant activity is associated with the concentration of total hydrophobic amino acids. Tyrosine, phenylalanine, and tryptophan are hydrophobic amino acids that possess aromatic rings. The stable electrons of peptides ensure that any lost electrons do not convert the peptide into other free radicals. These findings suggest that the presence of specific amino acids, particularly tyrosine and phenylalanine, in *Bohadschia marmorata* sea cucumber protein may contribute to its antioxidant activity. These amino acids play a role in stabilizing electrons and preventing the formation of free radicals, which is a valuable attribute in terms of antioxidant function.

3.4 Yield of Protein Hydrolysate

Protein hydrolysate is a substance derived from the enzymatic, acidic, or alkaline breakdown of proteins into simpler peptides and individual amino acids through the process of hydrolysis. Generally, protein hydrolysate comprises small fragments of peptides, each containing 2 to 20 amino acids. Factors that influence the rate and specificity of the resulting hydrolysate include temperature, time, enzyme concentration, hydrolyzing agents, and the ratio of acid to protein. This research aims to obtain the highest yield based on the treatment of enzyme type, enzyme concentration, and hydrolysate duration. The hydrolysis process in this study was carried out using both crude papain enzyme and pure papain enzyme. Yield, in this context, refers to the percentage of a material's weight that is obtained through a processing or extraction process. Yield is the percentage comparison between the weight of the extracted part of the material and the total weight of the material. The results of the analysis of variance (ANOVA) indicate that both the concentration of the papain enzyme and the duration of hydrolysis significantly affect the yield. The yield values for both treatments can be observed in Figure 3.



[a]



[b]

Figure 3 Yield Values for Crude Papain Enzyme Treatment (a) and Pure Papain Enzyme Treatment (b).

Based on the above Figure 3, it can be observed that the highest yield value for the use of crude papain enzyme is at a 3 % enzyme concentration with a hydrolysis duration of 5 h. For the use of pure papain enzyme, the highest yield is achieved at a 0.5 % enzyme concentration with a hydrolysis duration of 1.5 hours. Both of these treatments were used for testing the antioxidant activity of *Bohadschia marmorata* protein hydrolysate. The research results indicate that increasing the enzyme concentration does not lead to higher yield values. Similarly, extending the hydrolysis duration up to 6 h does not result in higher yields.

3.5 Antioxidant Activity

The degree of antioxidant activity is quantified by the IC50 value, which represents the concentration of the sample solution needed to inhibit 50% of the DPPH free radicals. The outcomes of the antioxidant activity evaluation, with IC50 values expressed in parts per million (ppm), are presented in Table 3.

Table 3 Antioxidant Activity Test

Papain Enzyme	IC_{50} (ppm)
Crude	319.6325
Pure	396.7524

In general, all hydrolysates containing peptides or proteins can donate protons and react with radical compounds to transform them into more stable compounds. A compound is considered to have antioxidant activity if, when tested, it can capture free radicals from DPPH. The DPPH solution is purple, but when reacted with the hydrolysate of *Bohadschia marmorata* sea cucumber protein as an antioxidant, the solution changes to bright yellow. This color change indicates that the unpaired electrons in the DPPH free radical have paired up. There is an interaction between the hydrolysate of *Bohadschia marmorata* sea cucumber protein sample, which donates protons to the DPPH free radical, making the radical neutral and no longer reactive. This change demonstrates that the hydrolysate of *Bohadschia marmorata* sea cucumber protein possesses antioxidant properties. The research results from [15, 18] indicate that bioactive peptides from sea cucumbers have the potential to serve as antioxidants.

IC50 (Inhibition Concentration) is a metric that denotes the concentration of an extract required to inhibit oxidation processes by 50 %. Smaller IC50 values indicate stronger antioxidant activity. Specifically, a compound is categorized as a potent antioxidant if its IC50 value is below 50 ppm, effective if IC50 falls within the range of 50 - 100 ppm, moderate if IC50 ranges from 100 - 150 ppm, and relatively weak if IC50 falls between 151 - 200 ppm. [37]. This suggests that the antioxidant activity of the *Bohadschia marmorata* sea cucumber protein hydrolysate using both crude and pure papain enzymes is relatively weak, as the IC50 values exceed 50 ppm, measuring 319.6325 and 396.7524 ppm, respectively. The differences in enzyme type, enzyme concentration, and hydrolysis duration resulted in varying levels of free radical DPPH inhibition. This indicates a relationship between antioxidant activity and the percentage of enzyme used in the hydrolysis process.

The antioxidant activity obtained in this study is considered weak because the peptides produced have not undergone a purification process and are still categorized as crude peptides. Consequently, the peptides generated are relatively long-chain peptides. In cases where a peptide exhibits high activity, there are typically only 2-3 peptide chains within the same peptide, but if the peptide chains are still long, they are classified as crude peptides and may need to be further broken down into shorter peptides. Antioxidant activity is mediated by hydrophobic amino acids, including tyrosine. The antioxidant activity in the *Bohadschia marmorata* sea

cucumber protein hydrolysate is attributed to amino acids because the protein hydrolysate is produced from sea cucumbers containing bioactive peptides. The activity of ROO groups in the tyrosine amino acid peptides indicates the presence of a proton donor. The ROO radical receives a proton from the hydroxy group of tyrosine, forming a neutral molecule, ROOH. Tyrosine then becomes a new radical but can resonate to form a stable ketone group [38].

3.6 Molecular Weight

The most widely employed technique for protein separation involves electrophoresis, which utilizes a non-continuous polyacrylamide gel as the buffer medium and employs sodium dodecyl sulfate (SDS) to denature proteins. This method is commonly referred to as Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). In this study, electrophoretic profiles of the molecular weight of each selected sample are presented in Figure 4 and Table 4, using a separating gel of 15 %, a stacking gel of 5%, a Protein Marker (with 6 molecular weight standards), and an 8 cm gel running distance.

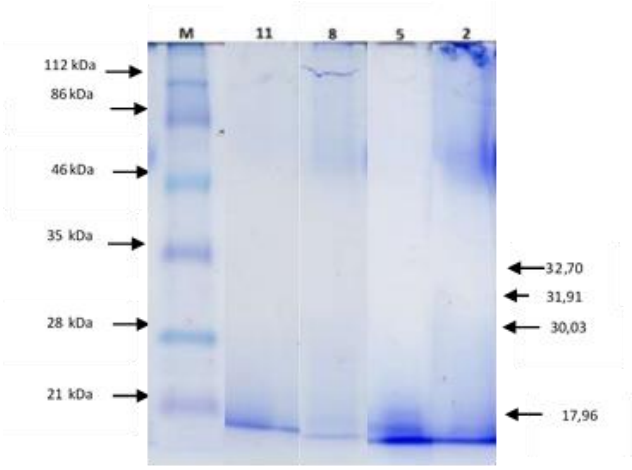


Figure 4 SDS-PAGE Results (Gel subjected to 5 washes/destaining cycles) Caption: M: Protein Marker, 2 & 8: Protein Hydrolysate of Crude Enzyme, 5 & 11: Protein Hydrolysate Pure Enzyme

Table 4 Data of Molecular Weight

Sample	Molecular Weight Marker (kDa)
2	30.03
5	17.96
8	31.91
11	32.70

Image 4 shows differences in protein and peptide bands that appear among the various samples. These differences manifest in terms of quantity, thickness, and molecular weight positions. The calculated molecular weights appearing in the electrophoresis are presented in Table 4. The experiment results indicate the formation of thin bands. The thin bands are a result of the low protein concentration, which affects the thickness of the protein bands in the SDS-PAGE results. The thinness of the protein bands in the SDS-PAGE results indicates the presence of proteins with similar molecular weights, situated in the same band position. This aligns with the principle of charged molecule movement, where charged molecules can freely move under the influence of an electric field. Molecules with the same charge and size tend to accumulate in the same or neighboring zones or bands [39]. The peptides in this study have molecular weights ranging from 17.92 kDa to 32.70 kDa.

According to [40], during the process of protein hydrolysis by proteolytic enzymes, proteins are broken down into smaller protein fractions. [32] stated that enzymatic protein hydrolysis, typically using protease enzymes, produces protein hydrolysates containing low molecular weight peptides composed of several amino acids. The use of crude papain enzyme results in higher molecular weights compared to the use of pure papain enzyme. This suggests that crude papain enzyme has an effective capability to break peptide-peptide and amino acid bonds with higher molecular weights.

4.0 CONCLUSION

The chemical composition of the *Bohadschia marmorata* sea cucumber includes a moisture content of 80.45%, ash content of 4.12%, fat content of 6.17%, protein content of 8.96%, and a total of 15 amino acids with a total amino acid content of 53.81%. The most optimal hydrolysis of *Bohadschia marmorata* sea cucumber protein using pure papain enzyme was achieved at a concentration of 0.5% with a hydrolysis time of 1.5 hours, resulting in a yield of 11.23%. Crude papain enzyme achieved the best hydrolysis at a concentration of 3% with a hydrolysis time of 5 hours, resulting in a yield of 16.26%. The protein content and percentage of free radical inhibition were relatively low, with IC50 values exceeding 200 ppm. The molecular weight of *Bohadschia marmorata* sea cucumber protein hydrolysates, using both crude and pure papain enzymes, ranged from 13.86 kDa to 73.93 kDa.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

This study receives complete support from the Faculty of Fishery and Marine Science at Pattimura University through approved funding, which greatly contributes to the feasibility and effectiveness of this significant research.

References

[1] Kustiariyah. 2007. Sea Cucumbers as a Source of Food and Bioactive Compounds. *Indonesian Journal of Aquatic Product Technology*. 10(1): 1-8. Doi: https://doi.org/10.17844/jphpi.v10i1.963.

[2] Witono, Y., Maryanto M., Taruna I., Musahid A. D., Cahyaningati K. 2020. Antioxidant Activity of Protein Hydrolysates Common Barb Fish (*Rasbora jacobsoni*) from Hydrolysis by Calotropin and Papain Enzymes. *Agroteknologi Journal*. 14(1): 46-57. Doi: https://doi.org/10.19184/j-agt.v14i01.14817.

[3] Thenu, J. L. 2016. Functional Characteristics and Antioxidant Activity of Enzymatic Hydrolyzed Protein from Dolphin Fish (*Coryphaena hippurus*) Roes. Thesis. Brawijaya University. URI: http://repository.ub.ac.id/id/eprint/156001.

[4] Wattimena, M. L., Thenu J. L., Wenno M. R., Nendissa D. M., and Soukotta D. 2019. Phisico-Chemical and Microbial Characteristics and Antibacterial Activities of the Fermented Viscera Fish Sauce. *Journal of Food Processing and Technology*. 11(818): 1-6. Doi: http://doi.org/10.35248/2157-7110.20.11.818.

[5] Wenno, M. R., Suprayitno E., Aulani"am A., and Hardoko. 2016. The Physicochemical Characteristics and Angiotensin Converting Enzyme (ACE) Inhibitory Activity of Skipjack Tuna (*Katsuwonus pelamis*) "Bakasang". Jurnal Teknologi (Sciences and engineering). 78(4-2): 11-124. Doi: https://doi.org/10.11113/jt.v78.8191.

[6] Wenno, M. R., Thenu J. L., and M. L. Wattimena. 2020. Chemical Composition, Amino Acids Profile and Angiotensin Converting Enzyme (ACE) Inhibitory Activities of Skipjack (*Katsuwonus pelamisi*) Roe Hydrolysate. *Asian Food Science Journal*. 17(6): 50-57. Doi: https://doi.org/10.9734/afsj/2020/v17i430200.

[7] Fakih, T. M., and Dewi M. L. 2020. Molecular Modelling of Marine Bioactive Peptides as Natural Anticoagulants against Cytochrome P450 (CYP) 2C9 Enzymes. *Galenika Journal of Pharmacy*. 6(2): 347-355. Doi: https://doi.org/10.22487/j24428744.2020.v6.i2.15041.

[8] Hafiludin, Andayani S., Kartikaningsih H., and Firdaus M. 2021.Utilization of *Paromoionchis tumidus* Sea Snail Extract as an Anti-Inflammatory Agent on Lipopolysaccharide-Induced Mouse Lymphocytes through the Inhibition of the TRL4 and NF-kB Pathways. Thesis. Universitas Brawijaya. URI: http://repository.ub.ac.id/id/eprint/188166.

[9] Murtihapsari, M., Roreng, M. K., Parubak, A., and Rahman, A. 2021. In Vivo Antimalarial Activity of Marine Sponge Xestospongia sp. Collected from Yapen Island, Papua. *Tropical Marine Journal*. 24(2): 177-184. Doi: https://doi.org/10.14710/jkt.v24i2.10107.

[10] Mayer, A. M. S., Rodriguez A. D., Berlinck R. G. S., and Fusetani N. 2011. Marine Compounds with Antibacterial, Anticoagulant, Antifungal, Anti- Inflammatory, Antimalarial, Antiprotozoal, Antituberculosis, and Antiviral Activities; Affecting The Immune and Nervous System, and Other Miscellaneous Mechanisms of Action. *Comparative Biochemistry and Physiology Elsevier Journal*. 153(2): 191-222. Doi: https://doi.org/10.1016%2Fj.cbpc.2010.08.008.

[11] Ardiansyah, A. 2016. Extraction and Oral Suspension Formulation of Sea Cucumber *Holothuria scraba* as Source of Antioxidants. *Oseanology and Limnology in Indonesia,* 1(1): 29-37. URI: http://jurnal-oldi.or.id.

[12] Waja, A. A., Adi, T. K., Hanapi A., and Fasya A. G. 2014. The Antioxidant Capacity and Total Phenoloc Compound Content of Sea Cucumber (*Holothuria scraba*) Extract from Kenjeran Surabaya. *Alchemy*. 3(1): 76-83. Doi: https://doi.org/10.18860/al.v0i0.2901.

[13] Nurhamzah, L. Y., Agustini, T. W., and Fahmi S. 2022. Antioxidant Stability of Black Sea Cucumber Extract (*Holothuria atra*) on Temperature and Heating Time. *Nutrition Scientific Journal*. 1(1): 8-20. Doi: https://doi.org/10.37058/nsj.v1i1.5897.

[14] Avigail, Y., Yudianti, E., and Pringgenies, D. 2019. Antioxidant Activity and Total Phenolic Content from Extract Sea Cucumber in Karimunjawa Island, Jepara. *Journal of Marine Research*. 8(4): 346-354. URI: http://ejournal3.undip.ac.id/index.php/jmr.

[15] Safari, R., and Yaghoubzadeh, Z. 2020. Antioxidant Activity of Bioactive Peptides Extracted from Sea Cucumber (*Holothuria leucospitala*). *International Journal of Peptide Research and Therapeutics*. (6): 2393-2398. Doi: https://doi.org/10.1007/s10989-020-10031-9.

[16] Esmat, A. Y., Said, M. M., Soliman, A. A. 2013. Bioactive Compound, Antioxidant Potential, and Hepatoprotective Activity of Sea Cucumber (*holothuria atra*) against Thioacetamide Intoxication in Rats. *Nutrition*. 29: 258-267. Doi: https://doi.org/10.1016/j.nut.2012.06.004.

[17] Althunibat, O., Hashim, R. B., Taher, M., Daud, J. M., Ikeda, M. A., and Zali, B. I. 2009. In Vitro Antioxidant and Antiproliferative Activities of Three Malaysian Sea Cucumber Species. *European Journal of Scientific Research*. 37(3): 376-387. URI: http://www.eurojournals.com/ejsr.htm.

[18] Jin, A. X., Xu, H. P., Li, Y., Zhang, Q. W., and Xie H. 2019. Preparation and Evaluation of Peptides with Potential Antioxidant Activity by Microwave Assisted Enzymatic Hydrolysis of Collagen from Sea Cucumber *Acaudina molpadioides* Obtained from Zhejiang Province in China. *Marine Drug*. 17(169): 1-14. URI: http://www.mdpi.com/journal/marinedrugs.

[19] [AOAC] Association of Official Analytical Chemist. 2005. Official Methods of Analysis of The Association of Official Analytical Chemist 17th Edition. Agriculture Chemicals Contaminant drug. Maryland, AOAC International, USA.

[20] Molyneux, P. 2004. The Use of The Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. *Songklanakarin Journal of Science and Technology*. 26(2): 211-219. URI: http://rdo.psu.ac.th/sjstweb/index.php.

[21] LaemmLi, U. K. 1970. Cleavage of Structural Proteins During the Assembly of The Head of Bacteriophage T4. *Journal of Nature*. 227(5259): 680-685. Doi: https://doi.org/10.1038/227680a0.

[22] Gafari, A. 2011. Fatty Acid Characteristics of *Baby Ionia spirata*, *Meretrix meretrik* and *Pholas dactylus Meat*. Thesis. Bogor Agricultural University. URI: http://repository.ipb.ac.id/handle/123456789/48067.

[23] Dewi, Abdul H. L., and Musdalifah N. 2017. Lipid Contain of Meat Black Sea Cucumber (*Holothruria Edulis*), Sand Sea Cucumber (*Holothruria Scabra*) and Its Implementation as a medium of Learning. *Jurnal Ilmiah Pendidikan Biologi*. 5(2): 20-29. URI: http://jurnal.untad.ac.id/jurnal/index.php/EBiol/article/view/9368.

[24] Ridhowati, S., Chasanah E., Syah D., and Zakaria F. 2018. A Study on the Nutrient Substance of Sea Cucumber *Stichopus variegatus* Flour using Vacuum Drying. *International Food Research Journal*. 25(4): 1419-1426. URI: http://ifrj.upm.edu.my/volume-25-2018.htmL.

[25] Gianto, G., Suhandana M., and Putri R. M. S. 2017. Composition of Amino Acid in Golden Sea Cucumber (Stichopus horrens) in Bintan Island, Riau Islands. *FishTech Journal*. 6(2):186-192. Doi: https://doi.org/10.36706/fishtech.v6i2.5850.

[26] One, N. S., Idiawati N., Prayitno D. I., Minsas S., and Fajri M. 2021. Identification and Proximate Analysis of *Thelenota ananas* Sea Cucumber from Kabung Island. *Naturalis Journal*. 10(1): 46-52. Doi: https://doi.org/10.31186/naturalis.10.1.14787.

[27] Wen, J., Hu C., and Fan S. 2010. Chemical Composition and Nutritional Quality of Sea Cucumbers. *Journal of the Science of Food and Agriculture*. 90: 2469-2474. Doi: https://doi.org/10.1002/jsfa.4108.

[28] Dewi, K. H. 2008. Study on the Extraction of Steroid from Sandfish (*Holothuria Scabra*) as Natural Testosterone. Dissertation. Bogor Agricultural University. URI: http://repository.ipb.ac.id/handle/123456789/41026.
[29] Lubis, A. F., Purwaningsih S., and Tarman K. Actioxidant Activity in Tablet Formulation of "Keling" (*Holothuria atra*) Sea Cucumber. *Berkala Perikanan Terubuk.* 44(2): 51-69. URI: https://terubuk.ejournal.unri.ac.id/index.php/JT/article/view/3466/3380.
[30] Oedjoe, M. D. R. 2017. Composition of Nutritional Content of Sea Cucumber (*Holothuroidea*) in Mania Waters, Sabu Raijua Regency, East Nusa Tenggara. *Journal of Aquaculture Research and Development.* 8(7):1-3. DOI: http://dx.doi.org/10.4172/2155-9546.1000502.
[31] Susanto I. 2010. Antioxidant Activity and Bioactive Component in Golden Snail (*Pomacea canaliculata Lamarck*). Thesis. Bogor Agricultural University. URI: http://repository.ipb.ac.id/handle/123456789/62729.
[32] Sicuro, B., Piccinno M., Gai F., Abete M. C., Danieli A., Dapra F., Mioletti S., and Vilella S. 2012. Food Quality and Safety of Mediterranean Sea Cucumber and *Holothuria polii* in Southern Adriatic Sea. *Asian Journal of Animal and Veterinary Advances.* 7(9): 851-859. Doi: http://dx.doi.org/10.3923/ajava.2012.851.859.
[33] Salarzadeh, A., Afkhami M., Bastami K. D., Ehsanpour M. and Khazaali A., and Mokhlesi A. 2012. Proximate Composition of Two Sea Cucumber Species *Holothuria pavra* and *Holothuria arenicola* in Persian Gulf. *Annals of Biology Research Journal.* 3: 1305-1311. URI: https://www.researchgate.net/publication/230750782
[34] Ketharani, U., and Sivashanthini K. 2016. Nutritional Composition of Selected Species of Sea Cucumbers from Waters Around *Jaffna Peninsula*, Sri Lanka. *Ving. Journal of Science.* 13(1): 67-72. URI: https://docslib.org/doc/2427956
[35] Pownall, T. L., Udenigwe C. C., and Aluko R. E. 2010. Amino Acid Composition and Antioxidant Properties of Pea Seed (*Pisum Sativum L.*) Enzymatic Protein Hydrolysate Fractions. *Journal of Agricultural and Food Chemistry*. 58: 4712-4718. Doi: 10.1021/jf904456r.
[36] Hermawati, I. N., Nursape'i N. D., Maharani S., Astriani T., Kusniasih N., and Harun N. 2022. Podcast (Potency of Bidara (*Ziziphus mauritiana)* Special Plants as a Destroyer of Covid-19). *Jurnal Kesehatan.* 9(1): 6-15. URI: https://ojs.stikesmucis.ac.id/index.php/jurkes/article/view/121/83.
[37] Esfandi R., Walters M. E., and Tsopmo A. 2019. Antioxidant Properties and Potential Mechanisms of Hydrolyzed Proteins and Peptides from Cereals. *Heliyon.* 5(4): 2405-2431. Doi: https://doi.org/10.1016%2Fj.heliyon.2019.e01538.
[38] Asadayanti, D. D., Jenie, B. S. L., Kusu aningrum H. D. dan Nurhidayaf N. 2010. Improvement of Lovastatin Angkak Production by Monascus purpureus Strains Co-Cultured with *Endomycopsis burtonii*. *Berita Biologi Jurnal Ilmu-ilmu Hayati.* 10(3): 313-321. DOI: https://e-journal.biologi.lipi.go.id/index.php/berita_biologi/article/view/746/518.
[39] Belkaaloul, K., Chekroun A., Ait-Abdesalam A., Saidi D., and Kheroua O. 2010. Growth, Acidification and Proteolysis Performance of Two Co-cultures (*Lactobacillus plantarum*, *Bifidobacterium longum* and *Streptococcus thermophilus*, *Bifidobacterium longum*). *African Journal of Biotechnology*. 9(10): 1463-1469. Doi: https://doi.org/10.5897/AJB09.1090.
[40] Baehaki A., Lestari S. D., and Romadhoni A. R. 2015. Protein Hydrolysis from Catfish Prepared by Papin Enzyme and Antioxidant Activity of Hydrolyzate. *Indonesian Journal of Aquatic Product Technology*. 18(3): 230-239. Doi: 10.17844/jphpi.2015.18.3.230.