OPEN ACCESS International Journal of Applied Biology

ISSN : 2580-2410
eISSN : 2580-2119

Protein Hydrolyzate of Grouper Viscera : Effects of Crude Bromelain Extract Concentration and Hydrolysis Time on Yield and Degree of Hydrolysis

Andi Nur Faiziah Palla[1*], Metusalach[2], Nursinah Amir[2]

[1] Fisheries Science Study Program, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia.
[2] Department of Fishery, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia

Abstract

Grouper is a fishery commodity that has high demand for both domestic and export markets. For export market, the grouper is commonly sold in a fillet form. However, production of the grouper fillet generates substantial amount of by-products, such as skin, scales, bones and viscera. The viscera contains high amount of protein and can be converted into protein hydrolyzate. The enzymatic processing of the protein hydrolyzate requires proteolytic enzymes, such as bromelain. This study aimed to analyze the effect of concentration of the bromelain crude extract and hydrolysis time on yield and degree of hydrolysis of grouper viscera protein hydrolyzate. The experimental design used was a completely randomized factorial design with different enzyme concentrations (4, 6, 8, 10 and 12%) and different length of hydrolysis time (4, 6, 8 and 10 h). The results showed that the concentration of the bromelain enzyme with the length of time in the waterbath had an effect on the yield and degree of hydrolysis of the grouper viscera ($P<0.05$). The highest yield was 18.17%, while the highest degree of hydrolysis was 89.29%, indicating the potential use of the grouper viscera and the crude extract of bromelain in the production of the fish protein hydrolyzate.

Article History
Received November 19, 2022
Accepted December 14, 2022

Keyword
By-product;
degree of hydrolysis;
fish viscera;
protein hydrolyzate;
proteolytic enzyme

Introduction

In fish processing industry, demersal fish like grouper is usually processed into fish fillet which produces high amount of by-product such as head, scales, bones, frames, skin, fins, and viscera. This by-product may account up to 45-65% of the total weight of the fish. Fish viscera is one of the fish processing by-product that is still unutilized or underutilized despite of its high protein content (Mardina et al., 2018). The fish viscera contains high level of protein (Atma, 2016), reaching up to 31.20% of protein on a dry basis (Aditya, 2018), and making it a highly potential raw material for the production of certain or specified fish product such as fish protein hydrolyzate. Fish protein is highly digestible and has an excellent essential

CONTACT : ANDI NUR FAIZIAH PALLA andinurfaiziah15@gmail.com

amino acid (EAA) profile that suits the needs of the human diet as defined by the World Health Organization (WHO) (Egerton et al., 2017).

Fish protein hydrolyzate can be produced using chemical or enzymatic hydrolysis. For the enzymatic hydrolysis, a proteolytic enzyme is into the fish or fish parts raw materials to accelerate the hydrolysis process under controlled conditions with the end result being a mixture of protein components (Piggot and Tucker 1990). Proteolytic enzymes that are often used in the food industry include bromelain and papain. Of the two enzymes, the papain is more commonly used in the production of the protein hydrolyzates. However, previous research has shown that the papain enzyme leaves a bitter taste (Somanjaya, 2013). So the bromelain enzyme is used which is a protease that has considerable potential for application in the food industry. The bromelain is relatively inexpensive (Wijayanti et al., 2016), but it causes no bitter taste to the protein hydrolyzate. Bromelain can be obtained from the pineapple plant (*Ananas comosus*) including the stalks, skin, leaves, fruit and stems in varying amounts (Masniar et al., 2016).

Research on fish protein hydrolyzate from grouper viscera with bromelain enzymes has not been carried out. This research utilized the grouper viscera for the production of the fish protein hydrolyzate and examined the effects of the concentration of the bromelain crude extract on the yield and degree of hydrolysis of the grouper viscera protein. It is hoped that this research may offer help to produce an added value product for the grouper processing industry.

Materials and Methods

This research was carried out from July to November 2022 at the Animal Microbiology Laboratory, Faculty of Veterinary Science, Hasanuddin University. Materials used in this study were the viscera of grouper, pineapple, K_2SO_4, HgO, H_2SO_4, H_3BO_3, HCl, CH_3COOH, NaOH, aquadest. The equipment used included commercial blender, calico cloth, measuring cup, erlenmeyer, hotplate with temperature control, centrifuge and oven, a set of laboratory equipment for chemical analysis of fish hydrolyzate such as Kjeldahl flasks, digestion and distillation units, soxhlet, oven, desiccator, porcelain cup, ashing furnace, filter paper, pH meter, stirrer.

Methods

This research used an experimental method, employing the experimental design used was a completely randomized factorial design. The factors used were concentration of the bromelain (crude extract of the pineapple) and hydrolysis time. The bromelain concentration consisted of five levels (4, 6, 8, 10, and 12% of the homogenized viscera weight) while hydrolysis time consisted of three levels (4, 6, 8, and 10 h). All experimental treatments were carried out in three replicates. A certain amount of pre-homogenized grouper viscera was thoroughly mixed with the crude extract of bromelain at a proposed concentration. The mixture was the hydrolyzed in a waterbath at 55°C for a period of time according to the proposed hydrolysis time. At the end of the hydrolysis, the hydrolyzate was recovered by filtration, enzyme-inactivated at 90°C, centrifuged at 3500 rpm, and then the solid hydrolyzate was dried in an oven. After drying, the yield of the protein hydrolyzate was calculated and the degree of hydrolysis was measured.

Preparation of Pineapple Crude Extract

The pineapple fruit used was a ripe one, peeled, the peeled fruit was splitted and chopped into small pieces and then homogenized using a commercial blender. To recover the crude extract of the bromelain, the homogenized pineapple was then squeezed and filtered to separate suspended fine particles. The filtrate was then transferred into a tight-capped plastic bottle until used for the experiment.

Production of Fish Protein Hydrolyzate

An approximately 250 g of the pre-homogenized grouper viscera were transferred into a 500 ml glass beaker, added with either 4, 6, 8, 10, or 12% of the bromelain extract and then mixed thoroughly. The beakers containing experimental samples were arranged properly in a waterbath, and then the hydrolysis process was carried out at 55°C for either 4, 6, 8, or 10 hours. The pH of the samples was maintained at 7 during the hydrolysis process, using either CH_3COOH or NaOH solutions. At the end of each of the proposed hydrolysis time, each sample was filtered through a 160 mesh filter cloth then inactivated the enzyme at 90°C for 20 minutes, and centrifuged at 3500 rpm for 15 minutes. The solid (protein hydrolyzate) was recovered, spresded evenly in a drying pan and dried in an oven at a temperature of 65°C for 24 hours. The dried protein hydrolyzate was then finely grinded to obtain the protein hydrolyzate flour.

The parameters tested in this study were the yield and degree of hydrolysis.

a. Yield

The yield of the protein hydrolyzate was calculated by dividing the weight of the hydrolyzate powder with the initial sample weight, and multiplied by 100%.

$$Yield\ (\%) = \frac{hydrolyzate\ powder\ weight\ (g)}{initial\ sample\ weight\ (g)}$$

b. Hydrolysis Degree

The degree of hydrolysis was analyzed following Amiza et al. (2012) and Nurilmala et al. (2018). As much as 20 mL of protein hydrolyzate was thoroughly dissolved in 20 mL of 20% TCA (v/v). The mixture was then allowed to stand for 30 minutes for precipitation to occur, then centrifuged (7,800 x g) for 15 minutes. The supernatant was analyzed for nitrogen content using the Kjeldahl method (AOAC 2005). The degree of hydrolysis is calculated using the following formula (Hoyle and Merritt, 1994).

$$Degree\ of\ hydrolysis = \frac{Number\ of\ soluble\ nitrogen\ in\ 20\%\ TCA}{Total\ nitrogen\ in\ initial\ sample}\ X\ 100$$

Data Analysis

The experimental design used in this study was a completely randomized factorial design with different concentrations of the bromelain enzyme (4, 6, 8,10 and 12%) and different lengths of hydrolysis (4, 6, 8 and 10 hours). The parameters measured in this study were the yield and degree of hydrolysis. Data were analyzed using analysis of variance

(ANOVA) and if the treatment showed a significant effect, a Tukey test was performed. Significant difference was determined at 95% level of probability (α = 0.05).

Results and Discussion

Yield

The yield of the protein hydrolyzate of the grouper viscera in this study ranged from 8.00 to 18.17%, where the lowest yield was at 4% bromelain enzyme concentration with 4 hours lengths of hydrolysis time, whereas the highest yield was obtained at 10% bromelain enzyme concentration with 8 hours lengths of hydrolysis time (Table 1).

Table 1. The yield of the grouper viscera protein hydrolyzate produced at various concentration of bromelain and length of hydrolysis time.

Bromelain enzyme concentration (%)	Yield (%)			
	4 Hours	6 Hours	8 Hours	10 Hours
4%	8.00 ± 0.24^{ay}	15.22 ± 0.82^{abw}	11.39 ± 0.50^{ax}	9.97 ± 0.50^{az}
6%	11.93 ± 0.54^{bz}	14.86 ± 0.01^{ay}	15.86 ± 012^{bx}	11.40 ± 0.47^{bz}
8%	14.80 ± 1.27^{cy}	16.60 ± 1.33^{bcy}	14.78 ± 0.09^{cy}	13.59 ± 0.22^{cz}
10%	14.92 ± 0.34^{cy}	15.29 ± 0.14^{by}	18.17 ± 0.54^{dx}	10.68 ± 0.23^{az}
12%	14.16 ± 0.78^{cy}	17.97 ± 0.18^{cx}	14.19 ± 0.19^{ey}	10.76 ± 0.40^{abz}

Values followed by the same letter in the same row (x,y,z..) or in the same column (a,b...) indicating no difference at 95% confidence level (p>0.05)

The results of a two-way Anova showed that the concentration of bromelain enzyme and hydrolysis time had a significant ($p<0.01$) effect on the yield. Based on the hydrolysis time, Tuckey test showed that at 4 hours hydrolysis time, the yield of the 8, 10, and 12% bromelain concentration was similar ($p>0.05$) but was significantly higher ($p<0.05$) as compared those of the 4 and 6% bromelain concentrations. At 6 hours hydrolysis time, the 12% bromelain concentration produced a significantly higher yield than those of other concentration, except with the 8% bromelain concentration. However, significant difference ($p<0.05$) of the yield existed between all the concentration treatments for the 8 hours hydrolysis time, being highest at 10% and lowest at 4% bromelain concentrations. For the 10 hours hydrolysis time, only the concentration of 8% bromelain produced a significantly higher yield ($p<0.05$), while the other concentration were relatively similar in their yields ($p>0.05$). For any concentration tested, the highest yield produced was obtain at either 6 or 8 hours of hydrolysis time.

The highest yield of the combination of 10% bromelain enzyme concentration and 8 hours lengths of hydrolysis time may indicate the optimum combination for the production of the protein hydrolyzate from the grouper viscera. Wijayanti et al (2016) showed that the increase in the yield protein hydrolyzate of the milkfish meat was primarily due to the increase in the concentration of the bromelain enzyme. Similarly, Harahap (2022) reported that the increase in the yield was due to the contribution and activity of the bromelain enzyme in the process of protein hydrolysis. The yield of the protein hydrolyzate of using the bromelain enzyme has been reported by several authors, such as Wijayanti et al (2016) for

milkfish meat (11.41%); Purbasari (2008) for golden clams (17%); Amelia et al (2021) for the viscera of bombay duck fish (12.55%), and Widadi (2011) using papain enzyme for African catfish (25%).

The yield in this study was much higher compared to the results of Anissa et al (2017) for tilapia (5.64%), for milkfish (2.73%) and for sharks (2.83%), and Kamini et al. (2016) for Siamese catfish viscera (2.77%). However, it may worth to note that these researchers used different concentrations of enzymes and hydrolysis times.

Degree of Hydrolysis

The degree of hydrolysis of the grouper viscera in this study ranged from 55.18 to 89.29%, being lowest at 8% bromelain concentration with 10 hours hydrolysis time and highest at 10% bromelain concentration with 8 hours hydrolysis time (Table 2).

Table 2. The degree of hydrolysis of the grouper viscera at different concentration of bromelain and length of hydrolysis time

Bromelain enzyme concentration (%)	Hydrolysis Degree (%)			
	4 Hours	6 Hours	8 Hours	10 Hours
4%	70.19 ± 4.50^{ay}	80.50 ± 5.75^{ax}	80.72 ± 6.84^{ax}	84.39 ± 1.98^{acx}
6%	63.56 ± 4.03^{abz}	83.80 ± 3.87^{ay}	87.18 ± 2.13^{abx}	79.76 ± 3.57^{ay}
8%	67.73 ± 3.55^{aby}	79.18 ± 3.00^{ax}	83.74 ± 4.19^{ax}	55.18 ± 9.54^{bz}
10%	58.08 ± 9.38^{bz}	74.03 ± 7.48^{ay}	89.29 ± 1.12^{bx}	77.31 ± 5.25^{ay}
12%	67.23 ± 7.65^{abz}	64.39 ± 2.69^{by}	88.02 ± 4.26^{abx}	87.44 ± 2.02^{cx}

Values followed by the same letter in the same row (x,y,z..) or in the same column (a,b…) indicating no difference at 95% confidence level ($p>0.05$)

The results of a two-way Anova showed that the concentration of the bromelain and length of hydrolysis time significant affected ($p<0.01$) the degree of protein hydrolysis of the grouper viscera. The Tuckey test showed that some significant differences ($p<0.05$) existed among the bromelain concentration as well as the hydrolysis time treatments regarding the degree of protein hydrolysis of the grouper viscera. In line with the yield, the highest degree of hydrolysis was also observed for the 10% of the bromelain at 8 hours hydrolysis time. Also, the 8 hours hydrolysis time tended to produce higher degree of hydrolysis at any concentration of the bromelain enzyme.

The highest degree of protein hydrolysis of the grouper viscera at 10% bromelain with 8 hours hydrolysis time may indicate the optimum conditions for the hydrolysis process. When considering the whole concentration of the bromelain used, the hydrolysis time of 8 hours seems to be the best to obtain a maximum degree of hydrolysis the high degree of hydrolysis in this study may confirm Harahap (2022) that the bromelain enzyme is more active in hydrolyzing protein in animals. The high degree of hydrolysis in this study indicated that the protein hydrolysis process of the protein took place efficiently. The degree of hydrolysis obtained in this study was far greater than that of Fakhrija (2021) for black sea cucumbers (10.11%), and Harahap (2022) for eel (6.46%).

The availability of the enzyme in sufficient quantity can increase the rate of the hydrolysis. According to Hasnaliza et al. (2010), enzyme concentration greatly determines the degree of protein hydrolysis. However, too much of the enzyme does not help in maximizing not only the yield but also the degree of the protein hydrolysis as shown by this study. The concentration of the enzyme required is proportional to the number of peptide bonds that need to be hydrolyzed. The increase in protein content requires an increasing concentration of the added enzyme, but to a certain extent the addition of excess enzyme will produce a constant or even decrease amount of hydrolyzate due to the short of substrate to act on. Where the substrate is abundant, the higher the concentration of the enzyme used the greater the amount of amino acid protein hydrolyzate produced as reported by Wijaya and Yunianta (2015).

Conclusions

The yield and degree of hydrolysis of the grouper viscera protein hydrolyzate were affected by the concentration the bromelain enzyme and the length of the hydrolysis time. The best condition for producing the protein hydrolyzate using viscera of the grouper fish is the bromelain concentrations of 10% with a length of hydrolysis time of 8 hours.

References

Aditya, R.P. 2018. Aktivitas antioksidan dan antibakteri hidrolisat protein hasil fermentasi telur ikan cakalang [tesis]. Bogor (ID): Institut Pertanian Bogor.

Amelia, G. 2021. Pembuatan hidrolisat protein ikan lemok *Harpondo nehereus* menggunakan enzim papain dengan waktu hidrolisis berbeda. *Jurnal Universitas Riau*

Amiza, M.A., Kong, Y.L and Faazaz, A.L. 2021. Effect of hydrolysis on physicochemical properties of cobia (*Rachycentron canadum*) frame hydrolysate. *Journal International Food Research,* 19(1); 199-206

Annisa, S., Y.S. Darmanto, dan Amalia, U. 2017. Pengaruh perbedaan spesies ikan terhadap hidrolisat protein ikan dengan penambahan enzim papain. *Saintek Perikanan*. Vol. 13 No. 1: 24-30. ISSN: 1858-4748.

AOAC. 2005. Official methods of analysis. The association of official analytical chemists. Arlington, Virginia USA: AOAC Inc.

Atma, Y. 2016. Pemanfaatan limbah ikan sebagai sumber alternatif produksi gelatin Dan peptida bioaktif: Review. *Seminar Nasional Sains dan Teknologi*. Fakultas Teknik Universitas Muhammadiyah Jakarta.

Egerton, S., Sarah, C., Jason, W., Catherine, S. and Paulros, R. 2017. Characterization of protein hydrolysates from blue whiting (Micromesistius poutassou) and their application in beverage fortification. *Food Chemistry*, S0308-8146 (17) 31744-2.

Fakhrija S. 2021. Hidrolisis Protein teripang hitam (Holothuria edulis) menggunakan bromelin kasar dari batang nanas (Ananas comocus L). Jurusan Kimia Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Tadulako, Palu.

Harahap, M. T., Edison dan Sukmawati, M,. 2022. Pengaruh konsentrasi enzim bromelin terhadap derajat hidrolisis hidrolisat protein belut (*Monopterus albus*). *Jurnal Universitas Riau*. Pekan Baru.

Hasnaliza, H., Maskat, M, Y., Wan,A, M. N dan Mamot, S., 2010. Mamot S. 2010. The effect of enzyme concentration, temperature and incubation time on nitrogen content and degree of hydrolysis of protein precipate from from cockle (Anadara granosa) meat washwater. *International Food Research Journal*. 17(1): 147-152

Hoyle, N. T. and Merritt, J. H. 1994. Quality of fish-protein hydrolysates from herring *(Clupea Harengus)*. *Journal of Food Science* 59(1): 76-79.

Kamini., P. Suptijah., Santoso,J dan Suseno, S.H. 2016. Ekstraksi dry rendering dan karakteristik minyak ikan dari lemak jeroan hasil samping pengolahan salai patin siam *pangasius hypophthalmus.* JPHPI Vol. 19 No. 3.

Mardina, V., Fitirani, Tisna, H. dan Goldha, M. H. 2018. Valorisasi pankreas ikan tongkol *(Eutynnusaffinis)* untuk produksi enzim lipase. Elkawnie: *Journal of Islamic Science and Technology*, Vol. 4 No.2, Desember 2018

Masniar, M., Zainal, A., Muchlisin. Dan Sofyatuddin, K. 2016. Pengaruh penambahan ekstrak batang nanas pada pakan terhadap laju pertumbuhan dan daya cerna protein

pakan ikan betok (*Anabas testudineus*) *Jurnal Ilmiah Mahasiswa Kelautan dan Perikanan Unsyiah Volume 1, Nomor 1: 35-45.*

Nurilmala, M., Hizbullah, H.H., Karnia, E., Kusumaningtyas, E. and Ochiai, Y. 2020. Characterization and antioxidant activity of collagen, gelatin, and the derived peptides from yellowfin tuna (*Thunnus albacares*) Skin. *Marine Drugs.* 18(2); 98.

Piggot, G.M. and Tucker, B.W. 1990. Utility fish flesh effectively while maintaining nutritional qualities. *Seafood Effects of Technology on Nutrition*. NewYork: Marcel Decker, Inc.

Purbasari, D. 2008. Produksi dan karakterisasi hidrolisat protein dari kerang mas ngur (Atactodeastriata). [Skripsi]. Fakultas Perikanan dan Ilmu Kelautan IPB.

Somanjaya, R. 2013. Pengaruh enzim papain terhadap keempukan daging. Jurnal Ilmu Pertanian dan Peternakan Vol.1 No.2

Widadi, I.R. 2011. Pembuatan dan karakteristik hidrolisat protein dan ikan lele dumbo (*Clarias gariepinus*) menggunakan enzim papain. Fakultas Perikanan dan Ilmu Kelautan IPB.

Wijaya J C, dan Yunianta. 2015. Pengaruh penambahan enzim bromelin terhadap sifat kimia dan organoleptik tempe gembus (Kajian konsentrasi dan lama inkubasi dengan enzim). *Jurnal Pangan dan Agroindustri*, 3(1), 96–106

Wijayanti, I., Romadhon, dan Rianingsih, L. 2016. Karakteristik hidrolisat protein ikan bandeng (*Chanos chanos* forsk) dengan konsentrasi enzim bromelin yang berbeda. *Jurnal Saintek Perikanan*. Vol (11). No.2:1229-133.