Effect of Hydrolysis Time and Bromelain Enzyme Concentration on Protein Levels Of Apple Snail (Pila ampullaceal) Hydrolyzate

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Effect of Hydrolysis Time and Bromelain Enzyme Concentration on Protein Levels Of Apple Snail (*Pila ampullaceal*) Hydrolyzate

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ABSTRACT

A high enough population of snails is considered detrimental to society. Currently, snails have a fairly low economic value, even though the protein content is quite high. In general, animal protein content is higher than vegetable protein, but animal protein tends to be more expensive. The need for protein as a human nutrient is quite high. There needs to be an effort to provide a source of animal protein in sufficient quantities, the price is relatively cheap, and contains high protein. For this reason, it is necessary to use the protein of snails. Apple Snail (*Pila ampullaceal*) is a type of snail that has a high protein content. There needs to be a method to optimize the extract of snail protein. The protein hydrolysis process was one method to optimize the process of separating protein from non-protein content. Snail protein hydrolyzate can be used for umami products, soy sauce, or biscuit mixtures. The process of the hydrolysis of Apple snails using the bromelain enzyme at concentrations: of 1%, 5%, and 10% with variations in hydrolysis time of 3 hours, 6 hours, 9 hours, 12 hours, 15 hours, and 18 hours. The results showed that treatment at 1% bromelain enzyme concentration obtained the highest soluble protein content (4.18%) at 15 hours of hydrolysis time. While in the treatment of 5% bromelain enzyme concentration, the highest dissolved protein content (3.35%) was obtained at 15 hours of hydrolysis time. In the treatment of 10% bromelain enzyme concentration, the highest dissolved protein content (4.13%) was obtained at 12 hours of hydrolysis time. The results of this study can be concluded that the highest protein content was obtained by using the enzyme bromelain 1%-10% with a hydrolysis duration of 12 hours - 15 hours.

Keywords: Apple snail, protein, bromelain, hydrolysis time

Introduction

Indonesia's natural fish biological resources have a high economic and scientific value. Various types of fish are very important capital in national development to improve the standard of living, prosperity, and welfare of the community. Fish are all types of organisms that all or part of their life cycle is in the aquatic environment (Sugianti et al., 2014).

Most people consider molluscs as pests of rice plants. The potential for crop damage by snails, which ranges from 10% to 40%, is still a threat to farmers, especially in the distribution areas such as Java, Sumatra, Kalimantan, NTB, and Bali (Budiyono, 2006). The population of snails, which is quite high when it starts to harvest, is a problem for the agricultural world. The eradication is quite difficult, giving rise to new problems, namely snail production which has increased sharply but its management is not optimal. Besides being detrimental, the high population of snails can be used for food products.

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Currently, snails have a fairly low economic value, this is because the processing is still very simple. In general, animal foods contain more protein than plant foods, although some vegetables, such as soybeans, are high in protein. But in reality, the consumption of animal protein which is classified as high quality and preferred by consumers has an expensive price and its production is not sufficient. This fact, it is necessary to make efforts to provide sources of animal protein in sufficient quantities, the price is relatively cheap, and contains high protein. According to Malayanti (2010) state, golden snails have a fairly high protein content of 16 to 18% and low fat of about 2.4%.

Fermented golden snail meat (koji) added with pineapple extract in making soy sauce resulted in increased protein content with more pineapple extract added. The protein content of golden snail sauce with pineapple extract concentrations of 3:1; 3:2 and 3:3 were 1230.19 mg/g; 1365.89 mg/g and 1475.02 mg/g, respectively (Badraih et al. 2008). The results of research by Apriyani et al. (2015) in making crackers from golden snails with the addition of 50% golden snail and 50% water resulted in crackers with a high protein content of 5.04%, a texture of 1075.6 gf, a swelling power of 80.1%, and fat 7.01%.

Pila ampullacea, is the largest freshwater snail, its shell size can reach 100 mm high with a diameter of ± 100 mm. This snail lives in lowland freshwater, such as swamps, lakes, slow-flowing rivers, and ponds (Djajasasmita 1987). The nutritional content in apple snails from every 100 grams of snail meat contains 654 calories, 12 grams of protein, 1 gram of fat, 6.6 grams of carbohydrates, and 61 milligrams of phosphorus (Pitojo, 1996), so it is unfortunate if it utilization is not optimal.

Crackers made from 20% wheat substitution and 60% apple snail produced 16.58% protein, 0.38% fat, 63.52% carbohydrates, 25.8 mg phosphorus, 11.5 mg potassium, and 1.88% ash on raw crackers, while the nutritional content of cooked crackers is 14.98% protein, 0.59% fat, 65.82% carbohydrates, 28.4 mg phosphorus, 15.0 mg potassium, and 2.09% ash (Munawaroh, 2014). By the many nutritional contents of this apple snail, the research aims to further optimize the protein content of apple snails. Apple snail meat was optimized for hydrolysis using the bromelain enzyme from pineapple. The resulting hydrolyzate can be used for various food preparations.

Material and Methods

Material

The raw materials used in this study were apple snail (*Pila ampullaceal*) obtained from the Surabaya area and natural protease enzymes from young pineapples (bromelain enzyme) in the traditional market. The chemicals used were: bovine serum albumin (Merck), NaOH (Merck), CuSO4 (Merck), sodium carbonate (Merck), Na.K tartrate (Merck), and Follin Ciocalteu reagent (Merck).

The research on optimizing the hydrolyzate of apple snails used a two-factor design. The first factor was the concentration of the pineapple bromelain enzyme. The second factor was the hydrolysis time. The data obtained from the analysis was processed using the Analysis of Variance.

Research stages

Crude bromelain enzyme production

The young pineapple flesh was mashed using a blender using water in a ratio of 1:1. The crushed pineapple was then wrapped in cloth and then squeezed to get the filtrate. The results were filtered again using Whatman paper to obtain crude bromelain enzyme extract.

The making of apple snail hydrolyzate

The apple snail meat was washed under running water to remove any impurities. Aquadest was added to apple snail meat in a ratio (2:1) and mashed using a blender. Bromelain enzyme concentrations of 1%, 5%, and 10% were added to the mixture and incubated at 54 °C for 6 h, 12

h, and 18 hours. The enzyme was inactivated at 90 °C for 10 minutes. The liquid and supernatant were separated using a centrifuge at 3000 rpm for 30 minutes. The supernatant was taken.

Testing of dissolved protein content (Lowry method)

Determination of dissolved protein content based on the method of Sudarmardji et al. (1997). A total of 0.05 grams of the sample was dissolved to 50 ml. The 1 ml of the solution was taken and then added up to 4 ml. Added 5.5 ml of reagent into each test tube, mixed evenly using a vortex, and incubated for 15 minutes. Added 0.5 ml of reagent into each test tube, mixed evenly using a vortex, and incubated for 30 minutes. Measurement of absorbance at a wavelength of 650 nm using a spectrophotometer. Blanks were made in the same way. The dissolved protein could be calculated by the formula obtained from the standard curve with the formula y = ax + b obtained from the standard curve.

Results and Discussion

The value of soluble protein content obtained from testing the hydrolyzate sample showed how much protein was successfully broken down from the total protein. The higher the value of dissolved protein content, the more optimal the hydrolysis process is carried out.

Figure 1. Effect of hydrolysis time on the use of 1% bromelain enzyme on soluble protein levels of apple snail hydrolyzate

Figure 1 showed that the dissolved protein content of protein hydrolyzate from apple snail was between 2.23% - 4.18% with the use of 1% bromelain enzyme and the hydrolysis duration is 3 h-18 h. The results of the lowest protein content were obtained from the hydrolysis duration of 6 h, while the highest results were obtained from the hydrolysis duration of 15 h. The results of this study showed lower results than Ilfandzahina (2020) on golden snail protein hydrolyzate using the bromelain enzyme with a hydrolysis time of 3 h which was 8.28%. However, these results have higher results than Putra et al., (2018) on golden snail protein hydrolyzate using commercial papain enzymes, which is 1.81%.

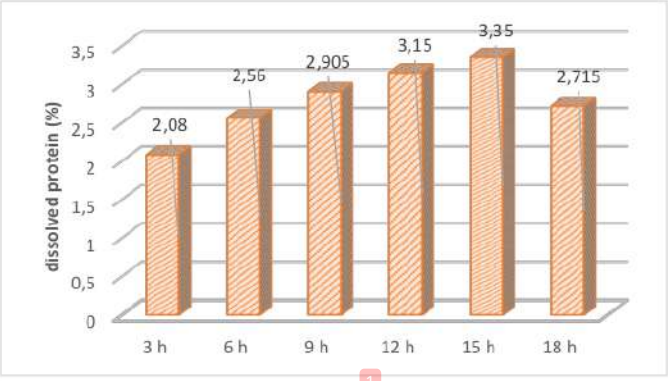

Figure 2. Effect of hydrolysis time on the use of 5% bromelain enzyme on soluble protein levels of apple snail hydrolyzate

Figure 2 showed that the dissolved protein content of protein hydrolyzate from apple snail was between 2.08% - 3.35% with the use of 5% bromelain enzyme and the hydrolysis duration was 3 -18 h. The lowest protein content results were obtained from the 3-h hydrolysis treatment, while the highest yield was obtained from the 15-h hydrolysis time treatment.

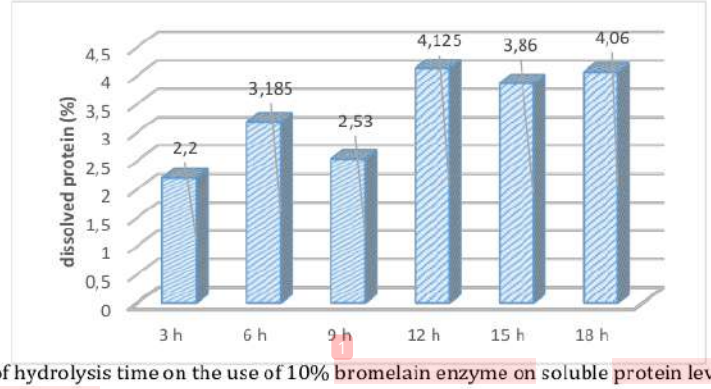

Figure 3. Effect of hydrolysis time on the use of 10% bromelain enzyme on soluble protein levels of apple snail hydrolyzate

Figure 3 showed that the dissolved protein content of protein hydrolyzate from apple snail is between 2.20% - 4.125% when using 10% bromelain enzyme and hydrolysis duration is 3 h -18 h. The lowest protein content results were obtained from the 3-h hydrolysis treatment, while the highest yield was obtained from the 12-h hydrolysis duration treatment.

The more the concentration of enzymes added and the longer the hydrolysis time, the more dissolved protein levels in the apple snail protein hydrolyzate produced tended to increase. However, with the addition of 10% enzyme concentration with a hydrolysis duration of 18 h, the resulting dissolved protein content decreased. This was because the addition of excess enzyme will cause a constant protein content result. After all, the addition of bromelain enzyme in the hydrolysis process has decreased activity.

This is supported by Agustina (2008) that protein levels increase as the concentration of added enzymes increases. This shows that the increase in enzyme concentration will increase the

speed of the hydrolysis reaction, however, to a certain extent the addition of excessive enzymes will result in a constant amount of hydrolyzate because the addition of the enzyme is no longer active. The longer the hydrolysis time, the more peptide bonds are produced. Nafi et al. (2014) stated that during hydrolysis the longer the interaction between the enzyme and the substrate causes an increase in the cleavage of the peptide bond to be simpler so that the solubility of the protein will also increase.

These results showed higher results than those of Putra et al. (2018) on the hydrolyzate of golden snail using papain enzyme which is 1.81% but smaller than the research results of Nurhayati et al. (2007) on the protein hydrolyzate of enzymatic tilapia which is 5.3% and Nurhayati et al. (2011) on green mussel protein hydrolyzate using papain enzyme, which is 11.75% of the initial protein content of 21.9%.

The difference in these results can be caused by the initial protein content of the existing raw materials, the type of enzymes used, and the effectiveness of the enzymes used in this study because the type and effectiveness of the enzymes also determine how much protein can be broken down and dissolved. According to Ovissipour et al. (2012), the dissolved protein content of the hydrolyzate can also be influenced by the degree of hydrolysis (DH) and the solubility of the peptide. The degree of hydrolysis (DH) and the higher solubility of the peptide, will result in a higher concentration of soluble protein.

Conclusion

The protein content in apple snail was quite high so it needs to be utilized optimally. The making of protein hydrolyzate from apple snail was a potential that can be developed into various food products such as soy sauce. The results showed that the higher the bromelain enzyme concentration and the longer the hydrolysis time, the higher the hydrolyzate concentration. At 18 hours of hydrolysis, the hydrolysis process had stagnated so that there was no more protein decomposition.

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