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Enzymatic hydrolysis of proteins from snakehead-fish (*Channa striata*)

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Abstract. The purpose of this research is to study the characteristics and bioactive components of the production of snakehead fish (*Channa striata*) protein hydrolysates through enzymatic methods. The enzymes used in this study were the papain enzyme and without the enzyme as a comparison. Substrate used is fresh snakehead fish (*Channa striata*) obtained from fish reservoirs. Analysis carried out in the form of total degrees of hydrolysis and nitrogen content in protein. The results showed that the use of the papain enzyme was excellent in producing snakehead fish (*Channa striata*) protein hydrolyzate. The papain enzyme helps break down fish protein into peptides or amino acids.

1. Introduction

Fisheries resources as one of the marine products potential in Indonesia which have long been used by the community. Community needs for fishery products continue to increase every year. Abundant fishery products are an opportunity to be utilized as processed products to meet people's food needs. One type of fishery product in South Sulawesi that is favored by the community is snakehead fish. It is known that snakehead fish has a high protein content [1]. The amount of fresh snakehead fish protein reached 21.98% [2], and after further processing, it became a protein concentrate using additional solvents, the protein content increased to 62.9% [3].

Snakehead fish, which has a high protein content, has the opportunity to be used as protein hydrolyzate. Fish protein hydrolyzate is a product produced from the breakdown of proteins into peptides and amino acids through the hydrolysis process by acids, bases, enzymes, or fermentation. Protein hydrolyzate can be obtained by the hydrolysis process, where the protein is broken down by the enzyme protease. The enzyme that will be used is called the papain enzyme[4]. Papain is a commercially available proteolytic enzyme that can be isolated from the sap of the papaya plant (*Carica papaya*).

The use of enzymes in producing fish protein hydrolyzate is expected to produce quality hydrolyzate. Protein hydrolyzate has functional properties as a nutritional supplement that acts as a bioactive compound [5]. Therefore this research was conducted to find out more about the nature and characteristics of snakehead fish protein hydrolysates using the papain enzyme.

2. Material and Methods

2.1. Preparation of samples

Snakehead fish are used in research this is a fresh snakehead fish that is obtained from the reservoirs District of Antang, South Sulawesi. Snakehead fish unit weight is used around 1.4kg per fish, and then it is weeded to remove from the attached dirt. Next, the fish is fillet to taken about 100g of meat. The Meat is then washed to clean it with flowing water. Furthermore, the enzyme that is used is the papain enzyme at a concentration of 5% (w/v) brands PAYA with the activity of the enzyme specific for 1.0593 u/g.

2.2. Production of protein hydrolyzate

The procedures were performed, namely the snakehead fish meat substrate as much as 100g homogenization with solvent distilled water 1:1 (w/v) and heated with a hotplate stirrer (Stuart) for 2 minutes. Furthermore, the papain enzyme is added as much as 5% to achieve a pH of 7. The sample was hydrolyzed using the tool water bath for 6 hours at a temperature of 55ºC and inactivated for 20 minutes at a temperature of 90ºC. Furthermore, the sample centrifuged (Heraeus Labofuge A) at a temperature of -4 ° C at the speed of 5000 rpm to separate the supernatant and natan. The supernatant obtained from the centrifuge is then dried using a freeze dryer. Furthermore, the hydrolyzate in the form of a supernatant was further analyzed.

2.3. Analysis of samples

In this study, several analyzes were conducted to determine the effect of the papain enzyme in the production of snakehead fish protein. Analysis was conducted, which analyzes the content of nitrogen in proteins and the degree of hydrolyzate in the protein hydrolyzate.

2.3.1. Determination of nitrogen content in protein. Nitrogen content was analyzed by the Kjeldahl standard method using protein analyzer (KjelMaster K-375) [6].

2.3.2. Determination of degree of hydrolyzate in protein hydrolyzate. The degree of hydrolysis was calculated according to percent of trichloroacetic acid (TCA) ratio method [5,7]. After hydrolysis, 20 ml of protein hydrolysate was added to 20 ml of 20% (w/v) TCA to produce 10% TCA soluble material. The mixtures were left to stand for 30 min to allow precipitation, followed by centrifugation (7800 x g for 15 min) (High-Speed Refrigerated Micro Centrifuge, TOMY, MX-305). The supernatant was analyzed for protein content by Kjeldahl method. Sample from the hydrolysate was also analyzed for protein content.

3. Results and discussion

In the research, measurements are done to determine the degree of hydrolysis and nitrogen in the protein hydrolyzate. Material that is used is a substrate of snakehead fish100g and the papain enzyme 5% (w/v), these things are done to see the influence of the degree of hydrolysis to produce the hydrolyzate protein. The results of the study can be seen in Figure 1 for nitrogen levels in proteins and Figure 2 for the degree of hydrolysis.

In Figure 1 shows the levels of nitrogen in the protein for the 5% papain enzyme in the amount of 0.081% and without the enzyme in the amount of 0.008%. It is shown that the addition of the enzyme is very influential to the results of the analysis of nitrogen in protein. In addition to the time of incubation are over 6 hours will increase the levels of nitrogen and the degree of hydrolysis. The longer the incubation time, the easier it is for enzymes to break the peptide bonds of proteins into simple compounds.

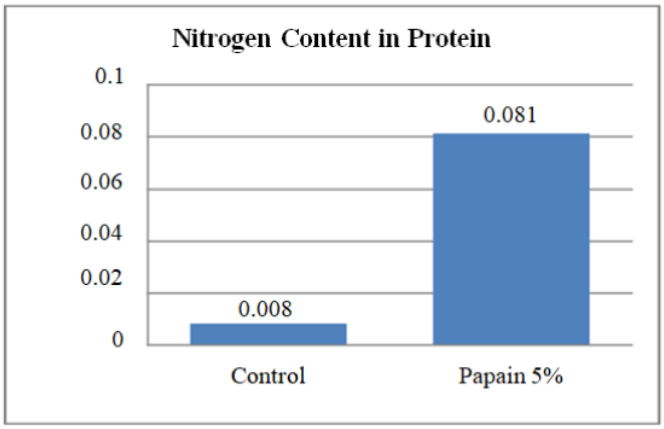

Figure 1. Nitrogen Content in Protein

Hydrolysis Enzymatic with the papain enzyme 5% indicates the percentage that is different by without using enzymes. The results in Figure 1 percentage of 5% papain enzyme with a value of 0.081%, showed the occurrence of the hydrolysis process due to the addition of the enzyme. This increasing shows the reaction of proteolysis to generate the levels of nitrogen dissolved in the hydrolysis of proteins during the process of hydrolysis takes place [8]. Results of nitrogen dissolved in the protein together with the results of the degree of hydrolysis are produced.

The degree of hydrolysis is an important factor in the making of the hydrolyzate of protein by using enzymes. Unknown enzymes greatly determine the magnitude of the degree of hydrolysis. In this study, the papain enzyme was compared with control. The results showed that with the addition of the papain enzyme, as much as 5% indicates the degree of hydrolysis is getting higher as compared with no addition of enzyme. Increased concentrations of the enzyme produce a correlation that is linear to the increase in the degree of hydrolysis [9].

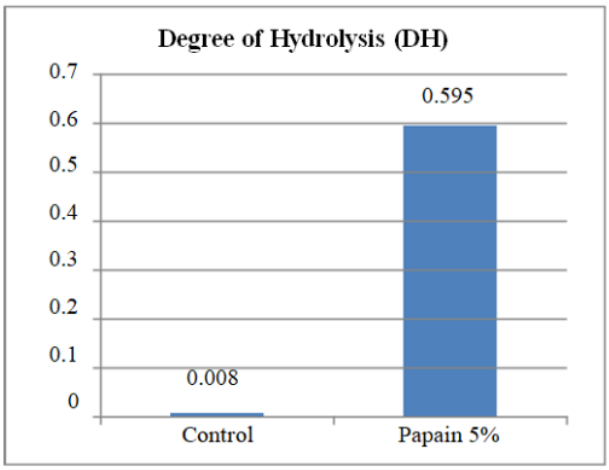

Figure 2. Degree of Hydrolyzate

The value in the degree of hydrolysis is very influential in producing a good protein hydrolyzate. Hydrolyzate will give certain functional properties. It is known during the process of hydrolysis, the value of the

degree of hydrolysis will increase along with the time of hydrolysis. In the study, this time, hydrolysis was carried out for 6 hours at pH 7 at a temperature of 55°C and inactivated for 20 minutes at a temperature of 90ºC.

In Figure 2 the use of the 5% papain enzyme was obtained by the degree of hydrolyzate 0.595% and control 0.008%. It is caused during the process of hydrolysis, the process breaking the bonds of peptides from the protein into compounds more simple. Furthermore, the process of hydrolysis is aided by adding the papain enzyme, which is a class of protease that can break down the molecules of the protein to hydrolyze the bond of the peptide into a compound that is more simple. Furthermore, the value of the degree of hydrolysis as the rate of proteolysis, which refers to the percentage of binding peptides that split [4].

In determining the value of the degree of hydrolysis using the addition of 20% TCA. The use of TCA (*trichloroacetic acid*) as a medium to measure the degree of hydrolyzate. This analysis is known as the SN-TCA method. The advantage of the method is that the process of analysis is relatively more rapid and practical as compared with methods other [10].

The technique of analysis is done by measuring samples that do not precipitate in the centrifugation process, which is then carried out by measuring the levels of nitrogen dissolved in the TCA by using a Kjeldahl. The results of TCA showed the value of the degree of hydrolysis of proteins using the addition of the enzyme papain 5% is better than with no enzyme. The increase in the degree of hydrolysis is caused by an increase in peptides and amino acids dissolved in TCA due to termination of peptide bonds during protein hydrolysis [11]. By because it is with the addition of the enzyme is better to produce a product of hydrolyzed protein.

4. Conclusion

The use of enzymes in a yield of hydrolyzed protein highly influential. The addition of enzymes is better than control. The use of enzymes can produce protein nitrogen and a good degree of hydrolysis to increase the functional value of snakehead fish protein hydrolyzate.

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