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THE EFFECT OF ADDITION DIFFERENT PAPAIN ENZYMES CONSENTRATION ON DEGREE HYDROLYSIS FROM NILEM PROTEIN HYDROLIZATE (*Osteochilus hasselti*)

Nadia Khaerunissa)*, Junianto, Ibnu Dwi Buwono, Rusky Intan Pratama

Mahasiswa Jurusan Perikanan, Fakultas Perikanan Dan Ilmu Kelautan, Universitas Padjadjaran
e-mail: khaerunissanadia@gmail.com

ABSTRACT

Processing of nilem into fish protein hydrolyzate is an alternative diversification of fishery products that is expected to be accepted by the community. One of the protein hydrolysis can be done enzymatically which one or several polypeptide groups (proteins) function as a catalyst and is able to accelerate the process of chemical reactions. The purpose of this research is the effect of adding the best concentration of the papain enzyme to the degree of hydrolysis of the nilem fish protein hydrolysis. Materials and methods to be used are experimental methods with 4 treatments and 4 replications followed by observing the degree of hydrolysis and yield using statistical analysis. The hydrolysis process of the protein of the nilem fish using the papain enzyme produces a degree of hydrolysis with an average of 25.82% - 32.01%, the average value of the smallest degree of hydrolysis is found in treatment D (concentration of papain enzyme 7.5%) of 25.82% . The highest value of hydrolysis degree in treatment C (papain enzyme concentration 5%) is 32.01% as the best result to produce hydrolysis degree of protein hydrolyzate of nilem fish in each treatment does not produce any significant difference and yield results show papain enzyme concentration significant effect on the yield of nilem fish hydrolyzate with a range between 1.10% - 5.12%. The highest yield in protein hydrolyzate of nilem with papain enzyme concentration of 7.5%. The effect of adding the best concentration of papain enzymes to the degree of hydrolysis of the best nilem fish protein hydrolysis was 5% with an optimal time for 6 hours.

Keywords: *Degree, Hidrolyzate, Nilem, Papain Enzyme, Yield*

INTRODUCTION

Fish is the result of resources favored by consumers both in Indonesia and abroad and has a high protein content, no less than 7,000 species of fish are found in Indonesian waters and around 2,000 species are freshwater fish species (Djuhanda, 1981). Nilem is one of the freshwater fish in the family Cyprinidae which has a protein content reaching 38.83%, a calcium content of 0.98% and a moisture content of 30.14%. Nilem fish is considered less economical to be the attraction of consumption fish because nilem has almost the same texture as carp with very small bones. Nilem protein is high enough to be potentially processed into fish protein hydrolyzate which has high water

solubility, good emulsion capacity, ability to expand and is easily absorbed by the body (Fox et al., 1991).

Hydrolysis of a peptide bond is an addition reaction, where proteases act as nucleophilic or react to form a single water molecule. One of the protein hydrolysis that can be done is enzymatically that one or several polypeptide groups (proteins) function as a catalyst and are able to accelerate the process of chemical reactions (Yuanisa, 2017). Papain can be obtained from papaya sap, both in fruit, stems and leaves.

According to Indriani *dkk* (2009) the papain enzyme has advantages over other proteolytic enzymes namely, it is more resistant to temperature processes, has a wide pH range and is purer than bromelin and ficin. The optimum pH range for papain is between 5 - 7.5 and stable at temperatures of 40 - 60 ° C. The use of enzymes in hydrolyzing proteins is considered the safest and most beneficial because the ability of enzymes can produce hydrolyzates that avoid changes and product damage (Kurniawan *et al*., 2012).

Fish Protein Hydrolyzate (HPI) is a product made from fish with the addition of a proteolytic enzyme to accelerate the hydrolysis process under controlled conditions, the final result being a mixture of protein components. indigestion (Utomo *et al*., 2014). Processing of nilem into fish protein hydrolyzate is an alternative diversification of fishery products. Fish protein hydrolyzate is used as a food additive in soups, gravy, sausage flavorings, biscuits, and crackers. In addition, fish protein hydrolyzate can also be included for diets in people with digestive disorders.

The purpose of this research is the effect of adding the best concentration of the papain enzyme to the degree of hydrolysis of the nilem fish protein hydrolysis which is expected to be accepted by the public.

MATERIALS AND METHODS

TOOLS AND MATERIALS

Tools and materials that will be used are plastic basins as nilem fish containers, measuring cups as measuring devices, Analytical balance / Scales for weighing nilem fish samples, pH meters for measuring pH samples, waterbath shakers as a tool to hydrolyze nilem fish samples, centrifuge as a tool centrifuging hydrolysis samples to separate supernatants and pellets, spray dryers to dry samples, beakerglass as a tool for mixing enzymes and fish samples, test tubes as sample containers to be hydrolyzed, knives for filtering and counting nilem fish, cutting boards as a base to assist in cleaning fish , dropper pipette for moving samples, bottles for storing hydrolyzate samples, tissue to help dry the tools, valcon tubes as sample containers to be centrifuged, fresh nilem fish as samples obtained from Kertahayu Village, Banjar, Java West as much as 5 kg, Papain Brand enzymes "Paya" (Commercial) for the addition of the enzyme pad a hydrolysis process is obtained from Super Indo Jatinangor, NaOH / HCl 1 M as an additive to regulate optimizing the pH of the hydrolyzate, and distilled water as a solvent.

METHODS

The research method used was an experimental method with a Completely Randomized Design (CRD) consisting of 4 treatments and 4 replications. The concentration of the papain enzyme was calculated based on the weight of the nilem fillet sample. The treatment of the addition of papain concentration consisted of (A) papain concentration 0% as a control, (B) papain concentration 2.5%, (C) papain concentration 5%, and (D) papain concentration 7.5%.

OBSERVATION PARAMETERS AND DATA ANALYSIS

The statistical analysis used in the study observed from the yield and degree of hydrolysis is to use analysis of variance (ANOVA Table) which aims to determine the "significant" effect of adding papain enzymes to the yield and degree of hydrolysis. If there is an influence then the test is continued by using the Duncan Test in order to find out the differences between treatments of the observed parameters (Steel and Torie, 1993).

The yield according to Widadi (2011) is the ratio between the weight of parts that can be utilized against the total weight with the following formula:

$$\frac{\text{Weight of nilem protein hydrolizate (g)}}{\text{Weight of nilem meat (g)}} \text{ x } 100\%$$

The degree of hydrolysis is calculated based on the percentage ratio of trichloroacetic acid (TCA) which is analyzed its nitrogen content using the Kjeldahl method (AOAC, 2005) with the following formula:

$$\frac{\text{Nitrogen dissolved in TCA 20\% } (b/v)}{\text{Total nitrogen sample}} \text{ x } 100\%$$

RESULTS AND DISSCUSSIONS

THE YIELD OF FISH PROTEIN HYDROLYZATE NILEM

The yield is the ratio of the presentation of raw material parts that can be utilized with whole material, the higher the yield value of a raw material, the higher the economic value of a material (Widadi, 2011).

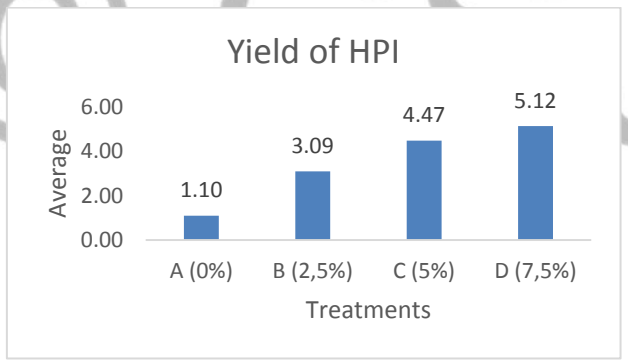

Figure 1. Persentase yield HPI

Nilem minced meat weight used was 50 grams at different concentrations of the papain enzyme with the yield of protein nilem hydrolyzate ranged from 1.10% - 5.12%. Figure 1 shows the smallest change in value, namely the addition of 5% to 7.5% enzymes by 3% which means that the change is not too significant for the addition of different papain enzymes. Treatment D with the addition of the enzyme papain as much as 7.5% is the best result because it produces the highest average so that nilem fish meat can be used as a protein fish hydrolyzate.

Tucker (1995) explains that protein hydrolysis involves adding water so that the amount of water in the process is greater than the amount of substrate used. Giving water in the process of hydrolysis aims to stabilize the pH value in the process of protein hydrolysis, facilitate the stirring and homogenization between enzymes and existing substrates and affect the rate of enzymatic reaction. The use of water is able to expand the contact area between enzymes and substrates, so that over a certain period of time a larger fish protein hydrolyzate product can be produced. The type and

concentration of enzymes is one factor that influences the rate of degradation of proteolytic enzymes (Shahidi and Botta, 1994).

Tabel 1. Anova yield of HPI

SK	db	JK	KT	Fhit	F tab	
					0.05	0.01
PERLAKUAN	3	37.9592	12.6531			
GALAT	12	0.9148	0.076234	165.98	3.49	5.95
TOTAL	15	38.8740				

The results of the analysis showed that the concentration of the enzyme significantly affected the yield of nilem fish protein hydrolyzate. The highest yield in protein hydrolyzate of nilem with papain enzyme concentration of 7.5%. Table 1 shows the increase in yield value of nilem fish protein hydrolyzate products with the addition of the enzyme papain.

The study of milkfish protein hydrolyzate yield with the same enzyme concentration yielded a yield of 9.43% (Wijayanti, 2016) and the Widadi (2011) study of African catfish had a yield of around 4-10% at a concentration of 4-6% papain enzyme. Dissolution of nutritional components such as fats, proteins and minerals during the hydrolysis process affects the yield of the hydrolyzate product produced (Shahidi *et al*., 1995). The yield of nilem fish hydrolyzate protein produced in the form of yellowish white powder as follows.

Figure 2. Nilem fish Hydrolizate Protein

DEGREE HYDROLYSIS OF NILEM FISH PROTEIN HYDROLYZATE

The degree of hydrolysis is a parameter that shows the ability of protoase to decompose proteins by comparing amino nitrogen with total nitrogen, the degree of hydrolysis is used to determine the degree of perfection of the hydrolysis process (Hasnaliza *et al*., 2010).

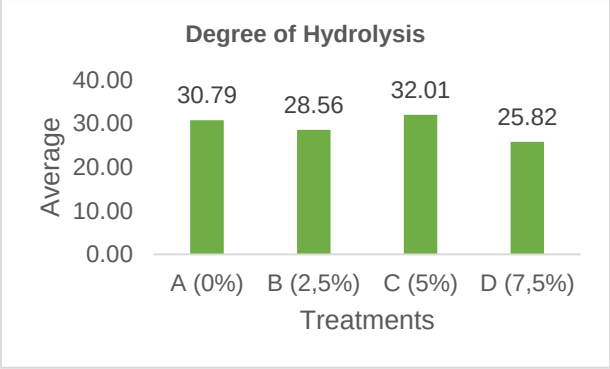

Figure 3. Average Degree of Hydrolysis

The hydrolysis process of the protein of the nilem fish using the papain enzyme produces a degree of hydrolysis with an average of 25.82% - 32.01%, the average value of the smallest degree of hydrolysis is found in treatment D (concentration of papain enzyme 7.5%) of 25.82% . The average value of the highest degree of hydrolysis is in treatment C (papain enzyme concentration 5%) that is equal to 32.01% as the best result to produce hydrolysis degrees of protein hydrolyzate of nilem fish. The increase in the degree of hydrolysis is caused by an increase in peptides and amino acids dissolved in Trichloro Acid (TCA) as a result of breaking bonds during protein hydrolysis. The papain enzyme has the ability to hydrolyze peptide bonds from proetein. The high concentration of enzymes used will affect the amount of transformed susbstrat. However, excessive concentration of the papain enzyme will cause the process to be inefficient.

A decrease in the mean degree of hydrolysis by increasing the concentration of the enzyme papain is caused by the high levels of salt contained in the papain enzyme used. According to Xu *et al*. (2008), the greater the addition of the enzyme papain the greater the levels of salt which can inhibit the process of protein breakdown due to osmotic pressure.

Research Guerard *et al*. (2001) hydrolysis of tuna waste protein with alkalase enzymes decreased the degree of hydrolysis. The presumed tendency to decrease in the degree of hydrolysis can be attributed to the decrease in the concentration of peptides available for hydrolysis. Reducing enzyme activity and product inhibition.

Table 2. Anova Degree Hydrolysis of Nilem Fish Protein Hydrolyzate

SK	db	JK	KT	Fhit	F tab	
					0.05	0.01
PERLAKUAN	3	88.94	29.65			
GALAT	12	639.64	53.30	0.56	3.49	5.95
TOTAL	15	728.59	-			

The variance table (Anova) in Table 2 explains that between treatments A (papain enzyme concentration 0%), B (papain enzyme concentration 2.5%), C (papain enzyme concentration 5%), and D (papain enzyme concentration 7) , 5%) were not significantly different (Fhit <Ftab). Thus it can be seen that the concentration of the papain enzyme used in the process of making protein hydrolyzate of the nilem fish has no significant significant effect because the degree of hydrolysis of the protein of the nilem fish can be influenced by several factors, namely the enzyme concentration, hydrolysis time, and the type of enzymes used. Research by Bernadeta et al (2012), shows that differences in the concentration between the enzyme papain and fish protein hydrolyzate cause no significant difference.

CONCLUSION AND SUGGESTION

CONCLUSION

Determination of the effect of adding the best concentration of the papain enzyme to the degree of hydrolysis of the hydrolyzed protein of the nilem fish resulted in a degree of hydrolysis with an average of 25.82% - 32.01% and the yield of the hydrolyzed protein of the nilem ranged between 1.10% - 5.12%. The effect of adding the best concentration of the papain enzyme to the degree of hydrolysis of the hydrolyzed protein of the nilem is 5% with an optimal time for 6 hours.

SUGGESTION

Suggestions that can be given based on research results are that further research needs to be done on the effect of adding papain enzymes to different types of fish and hydrolysis time at certain doses and the use of pure papain enzymes to produce the best protein hydrolyzate efficiently. The tools used at the time of the study must be examined in advance for the accuracy of the data to be obtained.

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