HYDROLYSIS ACTIVITY OF GOLDEN CARP FISH VISCERA PROTEASE EXTRACT (*Cyprinus carpio L.*)

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ABSTRACT

Protease enzymes are a type of enzyme that catalyzes the hydrolysis of proteins by breaking their peptide bonds. This study aims to isolate the protease enzyme from the viscera of golden carp fish (*Cyprinus carpio L.*) and determines the activity of the enzyme to decompose the substrate. This paper reports the protease activity of golden carp fish viscera (*Cyprinus carpio L.*), where the results were obtained by reacting the substrate with the protease enzyme extract with the optimum conditions, namely, incubation time of 25 hours, optimum pH 7, and incubation temperature of 50^{O}C. The measurement of the crude extract activity of the enzyme was determined by the Spectrophotometric method, while the protein content of the enzyme was determined by the Lowry method. Based on the measurements made, the enzyme extract activity gives a value of 0.343 units / g protein, while the protein content of the enzyme is 284.124 g/ml.

1. INTRODUCTION

Enzymes are proteins that can catalyze various chemical reactions with an ability of 1012 - 1020 without the enzyme changing after the reaction is complete. Enzymes are widely used in industrial applications because as biocatalysts, enzymes work specifically and are very efficient. In general, the work of enzymes also does not require heating or pressure treatment like non-biological catalysts (Muchtadi, 1992).

The use of enzymes in the industrial sector, both food, and non-food industries, is growing in Indonesia. The need for enzymes in the food industry such as cheese, dextrin, liquid sugar, fruit juice, milk, meat, beer, oil, and others continues to increase. Enzymes are also widely used in non-food industries such as in leather tanning, toothpaste making, soap making, cosmetics, pharmaceuticals, and so on. (Nurhasanah, 2006).

One of the enzymes that attracts a lot of attention today and has an important role in the development of biotechnology is the prosthetic enzyme. According to Suhartono Thenawidjaja Maggy (2000), protease enzymes are one of three groups of commercial enzymes traded with a value of up to 60% of total enzyme sales.

Until now in Indonesia, fish that have the potential to produce protease are still many and have not been used intensively. Considering that Indonesia is a maritime country that is rich in various types of fish, Indonesia also has a great opportunity to produce and develop this enzyme industry, so that it is one of the breakthroughs in technology transfer (Fatahillah, 1993).

Based on statistical data from the Department of Fisheries and Maritime Affairs of North Sumatra Province in 2001, data obtained for carp production for the city of Medan is around 24.8-tons per year and the marketing level reaches 1.4-tons per month. Seeing the high production and marketing of carp, especially in the city of Medan, it means that the number of carp viscera produced is also relatively high. For most people, generally, the viscera are thrown away. This waste can sometimes cause an unpleasant odor to the surrounding environment. However, on the other hand, the presence of viscera that is thrown away, is still possible to be processed into protease enzyme producers because the protein content in the viscera is still relatively high so it is suspected that the activity of protease enzymes will also be high.

This paper reports the activity of protease enzymes from golden carp fish viscera extract. This research aims to determine the quantitative test of the protease enzyme so that the activity value of the enzyme's work in decomposing the substrate can be known. The results of this study were obtained to provide an understanding of the utilization of golden carp fish viscera which can produce protease activity. In addition, this research can also provide information about natural resources that can be used as producers of industrial enzymes.

2. RESEARCH METHOD

2.1. Tools and Materials

Enzyme preparation was carried out according to the methods of Ritzman (1999) and Abigor (2002). Enzymes were isolated at 4°C for all experiments performed. Golden carp fish viscera were washed with distilled water and homogenized in 0.15 M phosphate buffer solution (5 ml/g wet weight) containing 0.6 M sucrose, 1 mM EDTA, 10 mM KCl, 1 mM MgCl2, while the pH was adjusted to 7 with the addition of KOH using a blender. The blender should not be turned on for more than 30 seconds. The homogenate was precipitated by adding acetone (1:3, v/v) for ± 5 hours. Then it was centrifuged at 14,000 rpm at 4°C. The supernatant layer was obtained in the Freezer dryer at a temperature of -4°C for ± 5 hours to form a fine powder. The white powder obtained was used for testing the hydrolysis activity of the protease and was previously dissolved in a phosphate buffer solution. The lipase enzyme extract was stored at 4°C while waiting for testing.

2.2.1.2. Preparation of reagents for measurement of protease enzyme activity

a. Solution A: A total of 2 g of Na2CO3 is dissolved in 100 ml of 0.1 N NaOH.

b. Solution B: 5 ml of 1% CuSO4, 5H2O was added to 5 ml of 2% Na-K-tartrate (C4H4KNaO6.4H2O) solution.

c. Solution C: A total of 2 ml of solution B is added to 100 ml of solution A.

d. Solution D: Ciocalteu Folin Phenol Reagent is diluted with distilled water (1:1, v/v).

e. Standard solution Tyrosine concentration 0-300 ppm.

2.2.1.3. Lowry's Reagent Production

a. Solution A : 2 g of Na_2CO_3 dissolved in 100 ml of NaOH 0,1 N.

b. Solution B : 5 ml of CuSO4. 5H2O 1% added to 5 ml of 2% Na-K-tartrate ($C_4H_4KNaO_6.4H_2O$) solution.

c. Solution C : A total of 2 ml of solution B added to 100 ml of solution A

d. Solution D : Folin-CiocalteuPhenol Reagent diluted with distilled water (1:1, v/v)

e. Standard Solution of Bovine Serum Albumin (BSA) concentration 0-300 ppm

2.2.2. Optimization of the Protease Hydrolysis Reaction of Golden Carp Fish Viscera Extract

In the required number of Erlenmeyer flasks filled with Bovine serum albumin (BSA) solution of 3 ml each, added protease extract of carp viscera extract (1 ml) and 7 ml of cysteine phosphate buffer solution Na-EDT A. Optimum conditions were determined by

varying pH, temperature, and incubation time. The variation of pH is 3, 3,5; 4; 4.5; 5; 5.5; 6; 6.5; 7; 7.5; 8; 8.5; and 9. The incubation temperature variations were 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C and 70°C. While the incubation time was carried out for 40 hours with observation intervals of 5, 10, 15, 20, 25, 30, 35 and 40 hours. The hydrolysis reaction was carried out under these optimization conditions.

2.2.3. Protease Hydrolysis Reaction Goldfish Viscera Extract

The hydrolysis reaction was carried out by adding a protease extract of carp viscera extract (1 ml) into 3 ml of Bovine Serum Albumin (BSA) solution and 7 ml of Na-EDTA cysteine phosphate buffer solution. Subsequently, incubation was carried out in a shaker at a speed of 3400 rpm at the optimum temperature, pH, and incubation time. The solution that has been incubated is then tested for the levels of amino acids that can be liberated.

2.2.4. Determination of Enzyme Protein Levels

Protein content was determined using the Lowry method, in which 1 ml of the enzyme solution was put into a test tube and 5 ml of C solution was added, then the mixture was allowed to stand at room temperature for 10 minutes. Then 1 ml of D solution was added and stirred, then allowed to stand at room temperature for 30 minutes. The blue color formed is read for absorption at a wavelength of 750 nm with a spectrophotometer. The amount of protein was determined based on the standard curve of Bovine Serum Albumin (BSA) with a concentration of 0-300 ppm.

2.2.5. Protease activity testing

To determine the activity of the protease enzyme, the Lowry method was used, in which 2 ml of the protease hydrolyzed solution of golden carp fish viscera extract was added to a 5 ml Erlenmeyer. The enzyme-substrate was inactivated by adding 1 ml of CCl3COOH (Trichloro Acetate: TCA 30%) then added 5 ml of rectifier C, shaken, and allowed to stand for 10 minutes at room temperature, added 1 ml of solution D, then allowed to stand at room temperature for 30 minutes. The blue color formed is read for absorption at a wavelength of 750 nm with a spectrophotometer. The amount of activity was determined based on the Tyrosine Solution curve with a concentration of 0-300 ppm.

3. RESEARCH DISCUSSION AND RESULTS

3.1. Optimization of Protease Hydrolysis Reaction

Optimization of the protease hydrolysis reaction needs to be done to get the best conditions for carrying out the enzyme hydrolysis reaction. It aims to get a good reaction result. The optimum conditions determined in the optimization of this protease hydrolysis reaction include incubation time, incubation pH and incubation temperature.

3.1.1. Period of Incubation

Protease enzyme activity produced from carp viscera homogenate at various incubation times can be seen in Figure 1.

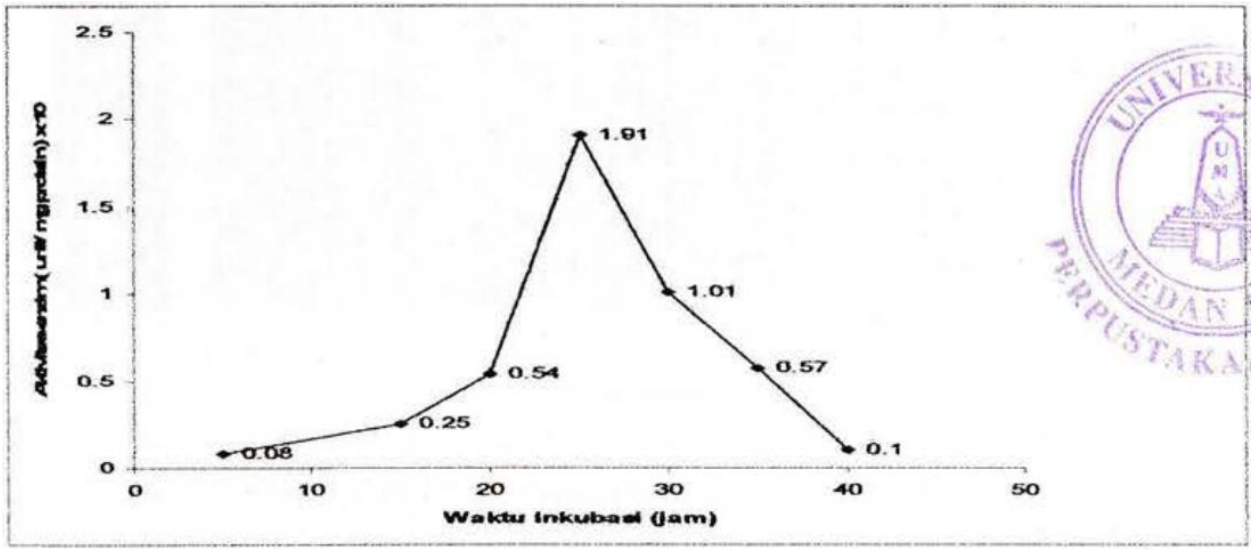

Figure 1: The curve for determining the optimum incubation time for the substrate hydrolysis reaction by the lipase enzyme.

From the picture, we can see that the activity of the protease enzyme produced from the homogenate of carp viscera from the 5th (fifth) hour to the 15th hour has not shown significant activity, this is because the adaptation phase is still going on. The exponential phase lasts from the 15th hour to the 25th hour. In this phase, the protease enzyme has a very sharp increase in activity, the ability of protease to break down protein molecules into amino acid molecules, which is indicated by the amount of amino acid (tyrosine amino acid) produced, which is 15.545 g/ml and reaches the optimum at 15.00 hours. 25th with an activity of 0.0191 unit/mg protein. In this study, the maximum protease activity was reached at the end of the exponential phase, at which time the protease ability to break down protein molecules into amino acid molecules decreased. The stationary phase itself lasts from the 25th hour to the 40th hour. In this phase, it is estimated that the protein contained in the Bovine Serum Albumin (BSA) substrate has decomposed and the protease activity also tends to decrease and continues to decrease until there is no more protease activity.

3.1.2. Incubation pH

The protease enzyme activity of carp viscera extract at various pH can be seen in Figure 2.

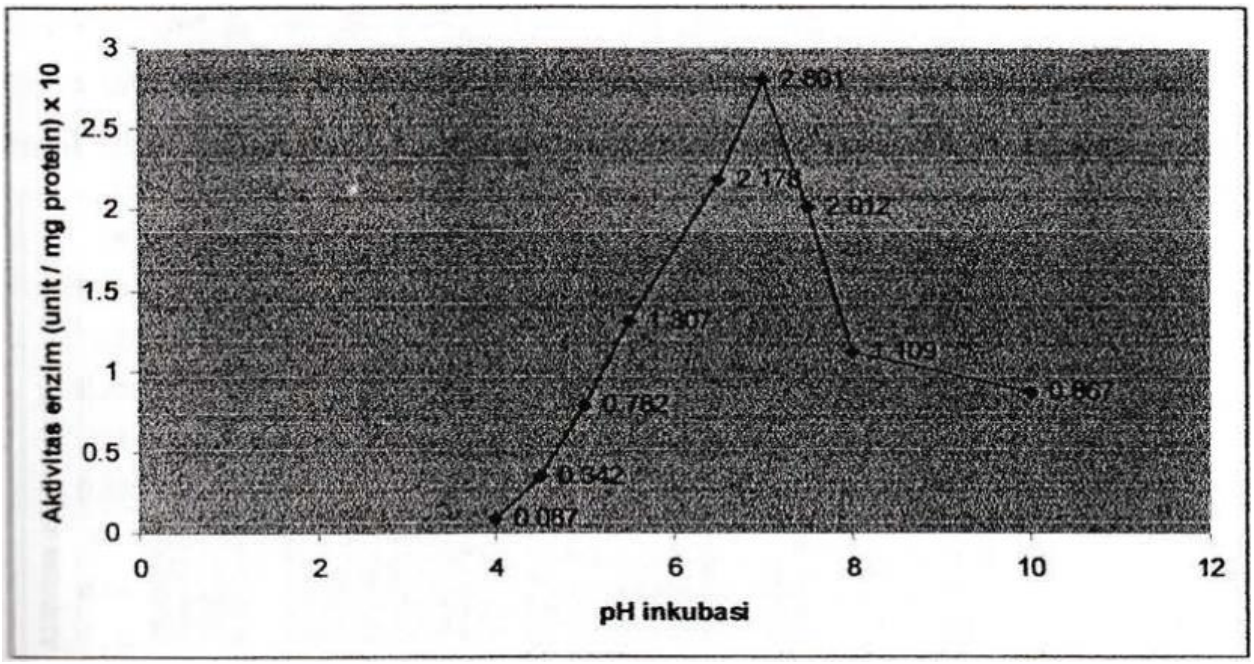

Figure 2: The curve for determining the optimum pH of the substrate hydrolysis reaction by the lipase enzyme.

From the figure above, we can see that the optimum pH for the hydrolysis reaction of the protease enzyme is pH 7 with an activity of 0.2801 unit/mg protein. At conditions below and above pH 7, the activity of the protease hydrolysis reaction decreased. According to Ritzman (1999) changes in pH can also cause denaturation of enzyme molecules. This causes a decrease in the hydrolytic activity of the protease. Enzymes like other molecules have ionizable groups, namely carboxyl groups and amino groups which are easily affected by changes in the surrounding pH. If the group on the active site changes in charge, the catalytic activity of the enzyme will decrease. Changes in pH can also cause the conformation of the enzyme so that the catalytic activity of the enzyme will change.

3.1.3. Temperature of Incubation

The optimum incubation temperature for the protease hydrolysis reaction is the temperature that provides the possibility for the protease enzyme to produce hydrolysis reaction products with maximum results, because under optimum conditions, the protease enzyme has the best activity. The activity of the hydrolysis reaction of the protease produced from the homogenate of carp viscera can be seen that the optimum incubation temperature is 50°C with an activity of 0.359 units/mg protein. At a temperature of more than 50°C the hydrolytic activity of the protease decreased sharply. This is because the protease enzyme produced from carp viscera extract is unstable to heat, so the enzyme is denatured, while below the temperature of so0 c, it is estimated that the kinetic energy of both the substrate molecule and the enzyme molecule is still low, so that the interaction of the enzyme molecule with the substrate is still small. The activity of the lipase enzyme at various incubation temperatures can be seen in Figure 3 below.

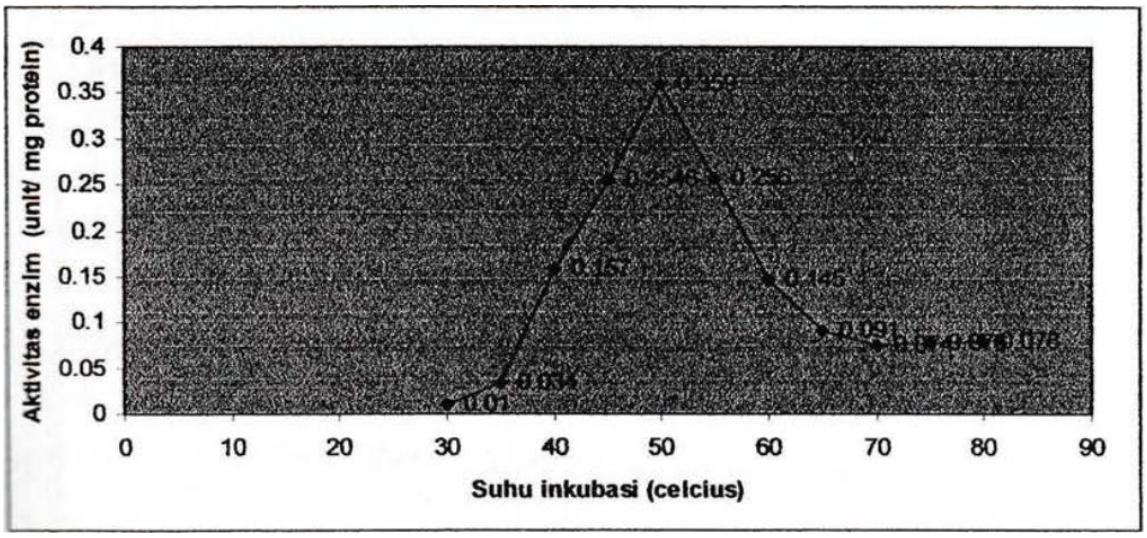

Figure 2: The curve for determining the optimum incubation temperature for the substrate hydrolysis reaction by the lipase enzyme.

4. CONCLUSION AND SUGGESTION

4.1. Conclusion

Based on the results of the research conducted, it can be concluded:

1. The optimum conditions for the hydrolysis reaction of carp viscera extract were at an incubation time of 25 hours, pH 7, incubation temperature of 500C.

2. Crude extract of the protease enzyme of carp viscera gave an activity value at its optimum condition …43 units/µg protein, while the protein content of the enzyme was 284.124 g/ml.

4.2. Suggestion

It is recommended in the following research to carry out the purification stage of the protease extract of carp viscera with salt or purification by chromatography so that the value of the hydrolysis reaction of the protease enzyme is expected to increase.

PROOFREADING

1.	continues	:	continue
2.	amount	:	number
3.	innards	:	viscera
4.	But	:	However
5.	is	:	aims
6.	has the ability to	:	can produce
7.	Goldfish	:	Golden carp fish
8.	by means of	:	using
9.	quite	:	relatively
10.	next	:	following