Characterization of Enzyme from Goldfish (*Cyprinus carpio*) Based on pH and Temperature Treatment

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ABSTRACT

Enzymes are produced and can be obtained from all living cells, namely plants, animals and microbes, because the natural function of enzymes is as a biocatalyst in life reactions. One of the enzymes found in animals, especially fish is the protease enzyme. The purpose of this study was the extraction and characterization of enzymes from goldfish (Cyprinus carpio) waters to find out how to extract enzymes from goldfish (Cyprinus carpio) meat and characterize the crude extract based on pH and temperature treatment. The method used is experimental method, the resulting data is analyzed descriptively. The characterization test of the crude extract was carried out based on the pH and temperature treatment. The results of the study can be concluded that the activity of crude extract from goldfish meat is 0.125 units. Enzyme activity is affected by pH and temperature. The highest enzyme activity using casein as a substrate is at pH 5 which is equal to 0.36 Units, at 30^{o}C which is equal to 0.73 Units.

Keyword: - *Enzyme, Cyprinus carpio, characterization, pH value, temperature*

1. INTRODUCTION

Enzymes are a class of proteins that are most abundant in living cells, and have an important function as catalysts for biochemical reactions that collectively form intermediate metabolism of cells[1]. Enzymes work in orderly sequences, they catalyze hundreds of step-by-step reactions that decompose nutrient molecules, reactions that store and convert chemical energy, and that make cellular macromolecules from simple precursors [2].

Based on their production, enzymes are produced and can be obtained from all living cells, namely plants, animals and microbes, because the natural function of enzymes is as a biocatalyst in life reactions [3]. One of the enzymes found in animals, especially fish is the protease enzyme. Protease or proteolytic enzymes are enzymes that have specific and efficient catalytic power against the peptide bonds of a peptide or protein molecule.

Proteolytic enzymes are ubiquitously distributed in all biological fluids and tissues. Digestive proteases are involved in protein hydrolysis and do not play a role in protein cycling in an organism. Very little is known about intracellular tissue proteases, their enzymatic specificities, and their substrate physiology. The list of proteases that are integral components of cells is extensive and largely unexplored. Some of them include all types of lysosomal proteases (catepsins, intermembrane proteases, and proteases from special tissues, such as reproduction, muscle, skin, eye lens, and kidney [4]. Protease enzymes are important enzymes in fishery products, which are present in muscle cells, the extracellular matrix and connective tissue surrounding muscle cells, and in the digestive tract and other organs [5].

The characteristics of proteinases in fish began with digestive proteases, which began to be studied more than one hundred years ago. Some digestive enzymes, such as the pepsin in salmon, were purified more than 50 years ago. In recent years a better understanding has been gained of the multiple molecular forms of digestive enzymes. The presence of lysosomal enzymes in fish muscle has been recognized since about thirty years ago, although cateptic enzymes have been studied more in fish and shellfish [5].

In this research, the extraction of protease enzymes obtained from goldfish (*Cyprinus carpio*) meat was carried out. The resulting enzyme extract is a crude extract. Characterization of the crude extract was carried out based on pH and temperature treatment. The purpose of this study was the extraction and characterization of enzymes from goldfish (*Cyprinus carpio*) waters to find out how to extract enzymes from goldfish (*Cyprinus carpio*) meat and characterize the crude extract based on pH and temperature treatment.

2. MATERIAL AND METHOD

2.1 Materials and tools

The materials used in this study consisted of the main ingredient in the form of goldfish (*Cyprinus carpio*) meat as much as 600 g and the ingredients used to make standard buffers pH 3 to 7 (citric acid, sodium phosphate, and distilled water).

The tools used include icubator (Thermoline), oven (Yamato), cold temperature centrifuge (Kokusan), spectrophotometer (Yamato), micropipette (Pipetman), analytical balance, homogenizer, magnetic stirrer, volumetric pipette, bulb, dropper pipette, test tube, test tube rack, Erlenmeyer, pH meter, tissue, aluminum foil, and beaker glass.

2.2 Research Stages

Preparation of reagents for the analysis of protease enzymes

There are several kinds of reagents needed in research on enzymes from goldfish (*Cyprinus carpio*), namely:

1. Buffer Tris-HCl 0,1 M pH 7,4
   A total of 9.6912 grams of Tris base was dissolved in 800 ml of distilled water and added with HCl, the pH was adjusted to pH 7.4.
2. Trichloroacetic Acid
   Dissolve 20.1 grams of TCA in 400 ml of distilled water
3. Tyrosine solution (5 mM)
   Dissolve as much as 0.018 gram into 20 ml of distilled water
4. Casein solution (1%)
   Casein is dissolved in Tris-HCl because when dissolved in distilled water casein cannot be dissolved. Casein is used as a substrate to test cathepsin enzyme activity which is suspected to be present in fish tissue samples. 0.1 gram of casein was dissolved in 10 ml of Tris buffer.

2.3 Enzyme Extraction from Goldfish

First of all, sample preparation was carried out to obtain crude enzyme extracts by killing the fish, then the fish meat was taken quickly and washed to remove blood. Meat tissue taken from the back of the fish suspended in distilled water with a ratio of fish meat and distilled water of 1:1. In this study, 600 grams of carp meat was obtained, so it was suspended in 600 ml of distilled water, then homogenized at 4^{o}C. Once homogeneous, it was centrifuged at 600g for 10 minutes to obtain the supernatant.

The resulting supernatant was centrifuged at 10,000g for 30 minutes at 4oC. The resulting pellets from the centrifuge were then dissolved in 600 ml of 0.1 M Tris-HCl buffer pH 7.4. Enzyme crude extract was stored at 4^{o}C and tested for its activity.

2.4 Enzyme Crude Extract Characterization

The characterization of the crude extract of enzymes obtained from carp meat was carried out on several components, namely:

- The substrate used is casein
- Crude extract activity at 37^{o}C
- pH is carried out in the range of pH 3 to 7
- Temperature : at a temperature of 10^{o}C to 40^{o}C

2.5 Activity Testing of Crude Extracts

1 ml of 0.1 M tris HCl buffer pH 7.4 and 0.5 ml of casein substrate solution were incubated with 0.2 ml of enzyme solution at 37^{o}C for 10 minutes. The reaction was stopped by adding 2 ml of 5% TCA. The mixture was filtered and 2 ml of the filtrate was added with 2 ml of folin's reagent, then incubated at 37^{o}C for 20 minutes. Then measured with a spectrophotometer at a wavelength of 750 nm. In addition, measurements were also made for the blank solution and standard solution with the same procedure as the sample solution, only for the standard solution the enzyme was replaced with tyrosine, while for the enzyme blank it was replaced with distilled water. Enzyme activity can be calculated by the following formula:

$$UA = \frac{Absorbance\ of\ sample - Absorbance\ of\ blank}{Absorbance\ of\ standard - Absorbance\ of\ blank}\ x\ P\ x\ T$$

Note: P = dilution factor
T = Incubation time

2.6 Activity Testing at Different pH

Enzyme activity testing at various pHs, a standard pH of 3,4,5,6, and 7 must be made beforehand. The way to make a pH solution is:

- Acid solution: 0.1 M citric acid as much as 2.10 grams dissolved in 100 ml of distilled water.
- Sodium phosphate solution: 3.8 grams of sodium phosphate is dissolved in 50 ml of distilled water

The pH was prepared by pipetting 20 ml of sodium phosphate, then citric acid was added, the pH was measured as desired.

The procedure for testing enzymes at various pHs is as follows:

1) Put 1 ml of 0.1 M tris HCl buffer pH 7.4 and 0.5 ml of casein substrate solution into test tubes (5 test tubes for pH 3,4,5,6 and 7 treatments)
2) Added 0.2 ml of pH solution (3,4,5,6, and 7) into each test tube for each pH treatment
3) Added 0.2 ml of enzyme solution
4) Incubated at 37^{o}C for 10 minutes. The reaction was stopped by adding 2 ml of 5% TCA and the mixture was filtered
5) Pipette 2 ml of the filtrate and add 2 ml of foline reagent
6) Incubated at 37^{o}C for 20 minutes
7) Measured with a spectrophotometer at a wavelength of 750 nm.

In addition, measurements were also made for the blank solution and standard solution with the same procedure as the sample solution, only for the standard solution the enzyme was replaced with tyrosine, while for the enzyme blank it was replaced with distilled water. Enzyme activity can be calculated by converting the spectrophotometer results into a formula.

2.7 Activity Testing at Different Temperatures

Crude enzyme extract from carp meat activity was measured at different incubation temperatures, namely at 10^{o}C, 20^{o}C, 30^{o}C, and 40^{o}C. The activity testing procedure is as follows:

1) Put 1 ml of 0.1 M tris HCl buffer pH 7.4 and 0.5 ml of casein substrate solution into a test tube (consisting of 4 test tubes, namely for the incubation temperature treatment at 10^oC to 40^oC)
2) Added 0.2 ml of enzyme solution
3) Incubated at 10, 20, 30, and 40oC for 10 minutes. The reaction was stopped by adding 2 ml of 5% TCA and the mixture was filtered
4) Pipette 2 ml of the filtrate and add 2 ml of foline reagent
5) Incubated at 10, 2, 30, and 40oC for 20 minutes
6) Measured with a spectrophotometer at a wavelength of 750 nm.

In addition, measurements were also made for the blank solution and standard solution with the same procedure as the sample solution, only for the standard solution the enzyme was replaced with tyrosine, while for the enzyme blank it was replaced with distilled water. Enzyme activity can be calculated by converting the spectrophotometer results into a formula.

3. RESULTS AND DISCUSSION

3.1 Crude Extract Activity

The crude extract was incubated at 37^oC and its absorbance was measured with a spectrophotometer to obtain results to determine its activity. Results of measurement of crude extract from goldfish (*Cyprinus carpio*) presented in Table 1.

Table -1: Results of Measurement of Crude Extract

Measurement Components	Spectrophotometer Results	Crude Extract Activity (Unit)
Sample	0,0295	0,125
Standard	0,0285	
Blank	0,0245	

Crude extract activity from carp meat is 0.125 units. This value indicates that the amount of enzyme that can cause the transformation of one micromole (10^6 mol) of substrate, namely casein per minute at 37^oC in the optimum state of the system is 0.125 units.

3.2 pH Value of Enzyme Activity

Based on the data obtained from the results of the study, it was shown that the highest enzyme activity using casein substrates was at pH 5, which was 0.36 units. This means that the enzymes contained in the crude carp meat extract are enzymes that are active at an acidic pH.

Enzymes at pH 3 did not show any activity, and began to show their activity at pH 4 and continued to increase up to pH 5. However, they showed a decrease in activity starting from pH 6 and slightly constant until pH 7. This can be seen in the curve of the pH value of enzyme activity. An increase in pH causes a decrease in enzyme activity. Thus, the activity of enzymes can be affected by pH and the enzymes present in crude extracts are not able to work optimally at high pH. The pH value of the enzyme activity is presented in Chart -1.

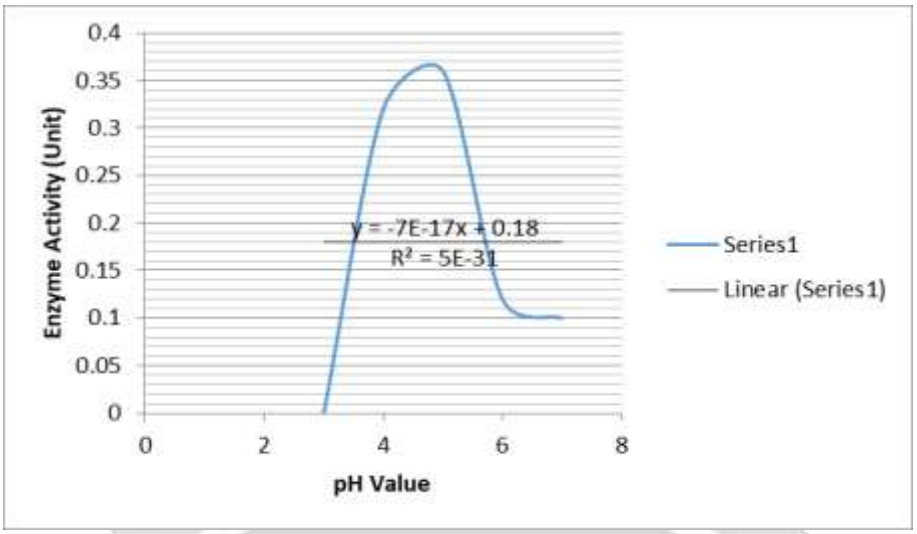

Chart -1: pH value curve for enzyme activity

Acid proteases generally have stability at an acidic pH, which ranges from 2-6. pH stability is related to the low content of basic groups so that at low pH enzymes have great electrostatic repulsion. In addition, the existence of hydrogen bonds between carboxyl groups that are not protonated also affects the stability of the enzyme.

3.3 Incubation Temperature on Enzyme Activity

Enzymes were incubated at 10, 20, 30, and 40^{o}C using casein as a substrate. The purpose of this difference in incubation temperature is to determine the optimum temperature for the activity of the enzymes contained in the crude extract obtained from carp meat. Incubation temperature on enzyme activity is presented in Chart -2.

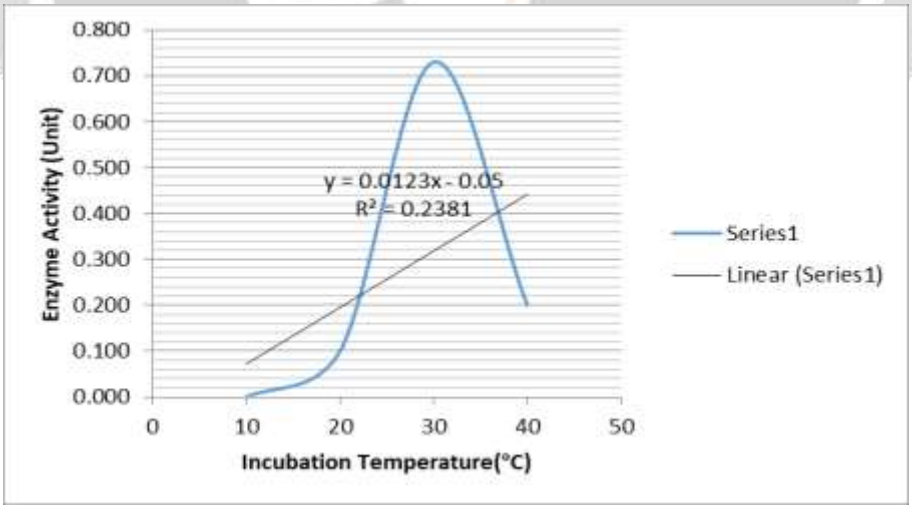

Chart -2: Incubation Temperature Curve for Enzyme Activity

Based on the incubation temperature curve, it can be seen that at 10^{o}C the enzymes are not active. The highest enzyme activity was obtained at 30^{o}C, which was 0.73 units, while at 40^{o}C incubation temperature, the enzyme activity decreased.

Factors that can affect the work of enzymes are enzyme concentration, substrate concentration and temperature. At a certain substrate concentration, the rate of reaction increases with increasing enzyme concentration. Likewise, with a fixed enzyme concentration, an increase in substrate concentration will increase the rate of reaction. but at certain concentration limits there is no increase in reaction rate even though the substrate concentration is increased. Temperature also affects enzymatic reactions. At low temperatures the reaction proceeds slowly, while at higher temperatures the reaction proceeds more rapidly [2].

4. CONCLUSION

From the results of the study it can be concluded that the activity of the crude extract from carp meat is 0.125 units. Enzyme activity is affected by pH and temperature. The highest enzyme activity using casein as a substrate is at pH 5 which is equal to 0.36 Units, at 30^{o}C which is equal to 0.73 Units.

5. REFERENCES

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