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Isolation and Characterization of Xanthine Oxidase from Goats Milk

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Abstract: Xanthine oxidase (XO) plays a role in the formation of uric acid and can be obtained from various sources, including goat's milk. This study aims to provide xanthine oxidase enzymes with known purity, characteristics (pH, temperature, and molecular weight), and to determine the effect of the inhibitor allopurinol on xanthine oxidase enzyme activity. Isolation is carried out using NaCl and partial purification through acetone precipitation. Enzyme activity is determined by the amount of uric acid formed in each acetone fraction, while protein levels are analyzed by the Bradford method. Results showed that the 60-90% acetone fraction provided the highest specific activity, with a purity of 29.190%. The enzyme showed optimum activity at pH 7.5 and temperature 33 °C, and a molecular weight of about 65 kDa. The inhibition test showed that the higher the concentration of allopurinol used, the greater the percentage of inhibition produced. The concentration of allopurinol 2 ppm provides the highest inhibition percentage of 90.376%. The xanthine oxidase enzyme produced from the research is expected to provide xanthine oxidase enzyme from goat milk that can be utilized further, both for research on the mechanism of inhibition of enzyme activity, as well as applications in other fields.

Keywords: Acetone Precipitation; Enzyme Characterization; Goat's Milk; Inhibition; Xanthin Oxidase.

Introduction

Xanthine oxidase (XO) is an enzyme that acts as a catalyst in the process of oxidation of hypoxanthine into xanthine and subsequently into acid [1]This process occurs in both the human and animal bodies as part of the final pathway of purine degradation [2]. The high activity of xanthine oxidase can increase the production of uric acid. If this uric acid cannot be eliminated through the kidneys or digestive tract, there will be a buildup of uric acid in the blood, causing hyperuricemia [3]. Therefore, xanthine oxidase can serve as a model for studying the mechanism of inhibition of the xanthine oxidase enzyme performance [4].

Xanthine oxidase can be found in the liver, intestines, kidneys, bacteria, the liver of animals and mammalian milk [5]. Based on research, xanthine oxidase is most commonly found in mammalian milk, especially in the fat globule membrane of mammalian milk containing xanthine oxidase, including cow's milk, buffalo milk, and goat's milk [6]Goat's milk is one of the natural sources that contains xanthine oxidase [7]. This is based on the results of studies that show that the xanthine oxidase enzyme from goat milk has an activity suitable to be used as a model in studying the mechanism of inhibition of xanthine oxidase enzyme performance. Therefore, this study was conducted with the aim of providing xanthine oxidase enzyme from goat milk that can be utilized further, both for the purpose of research on the mechanism of inhibition of enzyme activity, as well as applications in other fields.[8]. The process of purifying the XO enzyme from goat's milk can be carried out using the acetone precipitation method [9]. Acetone precipitation is one of the enzyme purification methods that is often carried out by using organic solvents to precipitate proteins, so that it is expected to increase the purity of the isolated enzymes [10].

Precipitation enzymes using acetone have proven to be an effective method for isolating and purifying various enzymes [11]The research discusses a comparative study of precipitation techniques in the isolation and purification of protease enzymes from the internal organs of Labeo rohita fish. The study compared three precipitation methods, namely ammonium sulfate, ethanol, and acetone, to obtain proteases from fish viscera that are usually considered waste. The results showed that the precipitation method with acetone gave the best results with a percent yield of enzyme protease of 54.4%, compared to ammonium sulfate (18.7%) and ethanol (12%) [12]. In addition, another study discussed partial purification and comparison of precipitation techniques for the enzyme pyruvate decarboxylase (PDC) extracted from Candida tropicalis. The two methods compared were precipitation using ammonium sulfate and cold acetone. The results showed that the precipitation method with cold acetone at a concentration of 20-40% was more effective than ammonium sulfate (40-60%), demonstrated by higher PDC enzyme activity, as well as better purification rate, and had higher specific activity after freeze-drying compared to the ammonium sulfate method. Prior to deposition with acetone, the specific activity of the Pyruvate decarboxylase (PDC) enzyme in the crude extract was 0.98 ± 0.04 U/mg protein. After precipitation using cold acetone at a concentration of 20-40%, the specific activity of PDC increased to 1.62 ± 0.11 U/mg protein [13]So it can be known that there is an increase in enzyme activity after the deposition process with acetone. Increased enzyme activity can also be affected by various factors such as pH and temperature. Therefore, characterization of enzymes such as molecular weight, pH and optimum temperature needs to be carried out [14].

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There are several studies that have been conducted related to the isolation and characterization of xanthine oxidase. Based on the research, it was found that the optimal pH for the coarse extract of xanthine oxidase from goat's milk is 7.2 with an optimal temperature of around 27 °C [8]. Meanwhile, another study used the ammonium sulfate precipitation method to isolate xanthine oxidase from goat's milk, resulting in an enzyme activity of 0.243 mU/mL [15]. There is also research on the purification and characterization of the xanthine oxidase from water buffalo liver. After going through acetone deposition followed by purification using DEAE-cellulose chromatography and Sephacryl S-300, a specific activity of 7.2 units/mg was obtained, with an optimal pH of 7.6 [16] In addition, the purification and characterization of the xanthine oxidase from sheep liver used a similar method, namely acetone deposition and chromatography of DEAE-cellulose and Sephacryl S-300, and found a specific activity of 3.5 U/mg, optimal pH of 7.6 [17]In addition to the optimal pH and temperature, the molecular weight of xanthine oxidase. According to research, the enzyme xanthine oxidase has a homodimeric structure with a total molecular weight of about 190 kDa. Each enzyme-making subunit is identical to each other and has a molecular weight of 95 kDa [18]. Another study using the SDS PAGE technique obtained the results of xanthine oxidase from purified buffalo milk, having a total molecular weight of 270 kDa, and is a homodimer consisting of two identical subunits with a molecular weight of 135 kDa [19] Meanwhile, another study stated that the xanthine Oxidase from buffalo liver had a molecular weight of 67 kDa based on analysis using the SDS-PAGE technique. The molecular weight of enzymes differs depending on the source of the enzyme, the type of enzyme, the extraction technique, and the precision of purification, which can affect the molecular weight of the enzyme [20]

Enzymes that have been purified and characterized can be used in various fields, one of which is medicine [21]The use of xanthine oxidase in this field is carried out by inhibiting the activity of the enzyme using inhibitors [22]. Allopurinol is a medicinal compound that functions as an inhibitor of xanthine oxidase, which is an enzyme that plays a role in the oxidation of hypoxanthine into xanthine and subsequently into uric acid [23]By inhibiting the activity of this enzyme, allopurinol can decrease uric acid production in the body, so it is often used in the treatment of hyperuricemia and gout [24]The inhibition mechanism of allopurinol takes place competitively, in which allopurinol and its active metabolite, oxypurinol, bind to the enzyme's active center and block the binding of natural substrates [25]. Thus, the use of allopurinol is effective in controlling uric acid levels and preventing the formation of uric crystals in body tissues [26] Allopurinol is a competitive inhibitor that works to inhibit the formation of uric acid [27] Based on the research, the percentage of inhibition of XO enzyme activity by allopurinol was 71.17% [28]The study also conducted an inhibition test of XO enzyme activity using allopurinol and obtained a result of 87.47% [29]. In addition, another study stated that at the highest concentration of 10 µg/ml, the inhibition of the activity of the xanthine oxidase by allopurinol was 77.32% with an IC_{50} value of 2.05 ppm[30]

This study differs from previous studies that generally use animal tissues as enzyme sources and conventional purification methods, such as salt fractionation and dialysis. Based on this background, this study was conducted using the acetone precipitation method to purify the xanthine oxidase enzyme from goat milk. This method is still rarely used to purify the xanthine oxidase enzyme, especially from the goat milk source. Furthermore, enzyme characterization was carried out, including pH, temperature, and molecular weight. After that, the purified enzyme was analyzed for its activity through an inhibition test using allopurinol. In addition, the enzyme produced aims to provide xanthine oxidase enzyme as a model in studying the mechanism of enzyme inhibition, and it has the potential to be used in various fields such as biomedical research and other enzymatic studies.

Research Methods

The materials used in the study were goat milk, xanthine substrate (Merck), aquadest, EDTA pro analysis (Chemindo), NaCl pro analysis (Chemindo), Tris base (Chemindo), HCl (Chemindo), NaOH (Chemindo), Bovine Serum Albumin (Merck), acetone (Chemindo), standard solution of uric acid, Bradford Reagent, and Allopurinol (Hexa Pharm). Tests were conducted in triplicate.

The tools used are a pH meter (Eutech), magnetic stirrer (D-LAB), centrifuge (Eppendorf 5810), centrifuge (Thermo Scientific), Magnetic stirrer (Heidolp MR Hei-Standard), vortex mixer (Labnet VX-200), a glass jar, micropipette (D-LAB), spectrophotometer UV-Vis (Shimadzu spec. 1800), incubator, and glassware (Pyrex).

Xanthine oxidase isolation

100 mL of fresh goat milk was added with NaCl (pro analysis) as much as 32,032 grams and then homogenized. After homogeneousness, the solution is centrifuged for 30 minutes at a speed of 3000 rpm. The supernatant produced is a crude extract of xanthine oxidase.

Precipitation with Acetone

Crude extract of xanthine oxidase is precipitated using acetone with various fractional variations. The first precipitation stage is carried out with variations of acetone fractions of 0-20, 20-40, and 40-60%. Furthermore, in the second stage, it is carried out with variations of the fraction of 0-30, 30-60, 60-90%. The crude extract of xanthine oxidase used was 20 mL, while the acetone added to each fraction was carried out according to the acetone precipitation table. After the crude extract is added, acetone is centrifuged at a rate of 12,100 xg at 4°C for 15 minutes. The resulting sediment is then stored to measure its activity, while the resulting supernatant is taken, and the next fraction is added with the same treatment. The deposits obtained from each fraction were dissolved using a Tris-HCl buffer solution of 0.02 M, pH 7.5, containing EDTA 0.1 mM.

Uric Acid Standard Solution

Uric acid solution was diluted to a volume of 10 mL with a concentration of 1,3,5,7,9 ppm, and then its absorbance was measured three times using a UV-Vis spectrophotometer at λ 291.7.

Enzyme Activity

The enzyme resulting from the acetone precipitation was taken as much as 0.6 mL and mixed with 3.9 mL of Tris-HCl buffer, 0.02 M, pH 7.5. After that, 2 mL of xanthine substrate 0.15 mM is poured into the test tube. The solution is then pre-incubated for 10 minutes at 25 °C in an incubator. 1 mL of HCl 0.1 N was added to stop the reaction, and its absorbance was measured using a UV-Vis spectrophotometer at λ 291.7. The absorbance obtained is substituted into the linear regression equation obtained from the standard curve of uric acid, then the Enzyme Activity (EA) can be calculated using the formula:

$$\text{EA (mU/mL)} = \frac{\text{uric acid(µmol)x enzyme volume}}{\text{incubation time (minute) x total volume}} \text{x1000}$$

Protein Levels

Protein levels were determined using the Bradford method with Bovine Serum Albumin (BSA) of 25, 50, 100, 200, 400 ppm as the standard solution. Each standard solution and sample was put into a different test tube of 0.4 mL, and then 4 mL of Bradford reagent was added. After that, it was incubated at the temperature of the lobe for 10 minutes, and then its absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 595 nm.

Enzyme-specific activity

Enzyme-specific activity can be determined by dividing the enzyme activity by the protein content of the enzyme.

$$\text{Specific activity (mU/mg)} = \frac{\text{Enzyme activity}}{\text{Protein levels}}$$

Enzyme Purity Level

The enzyme purity level is the ratio between the enzyme-specific activity in the advanced stage of the enzyme and the enzyme-specific activity of the previous stage. To determine the level of purity of an enzyme, the formula:

$$\text{Enzyme purity} = \frac{\text{Specific activity of the refining enzyme}}{\text{Specific activity of enzyme crude extract}}$$

Characterization of Temperatures

Tris-HCl buffer 0.02 M, pH 7.5, as much as 3.9 mL is put into the test tube, then 2 mL of xanthine substrate 0.15 mM is added, then pre-incubated for 10 minutes at 25°C in an incubator, then 0.1 mL of enzyme is added. The mixture is incubated for 25 minutes at various temperatures, 25, 29, 33, 37, 41°C. After incubation is complete, to stop the reaction, 1 mL of HCl 0.1 N is added, then its absorbance is measured at λ 291.7 using a UV-Vis spectrophotometer.

Characterization of pH

A buffer solution of 3.9 mL is inserted into the test tube, then 2 mL of xanthin substrate 0.15 mM is added, and then pre-incubated for 10 minutes at the optimum temperature in an incubator. Then, 0.1 mL of enzyme with pH variations from 6, 6.5, 7, 7.5, and 8 is added. The mixture is incubated for 25 minutes at the optimum temperature in the incubator. After that, to stop the reaction, 1 mL of HCl 0.1 N was added, then its absorbance was measured at λ 291.7 using a UV-Vis spectrophotometer.

Characterisation of Molecular Weight

The molecular weight of the xanthine oxidase was determined using the Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method. Protein separation was carried out using 10% polyacrylamide gel and using standard markers from GenScript, namely Pre-stained Protein Standard, Broad Multi Color (Catalog No: M00624-250, Lot No: M2205016). The dye used is Coomassie Brilliant Blue. There were 5 samples tested, namely (1) crude extract of the xanthine oxidase without dilution, (2) crude extract of the enzyme diluted twice, (3) crude extract of the enzyme diluted ten times, (4) xanthine oxidase with a concentration of 1000 ppm, and (5) xanthine oxidase with a concentration of 10.000 ppm.

Xanthine Oxidase Inhibition Test

Preparation of Blank Test Solution

Tris-HCl buffer solution of 0.02 M, optimum pH of 3.9 mL was added with 2 mL of xanthin substrate solution of 0.15 mM and then pre-incubated at the optimum temperature for 10 minutes. After preincubation is complete, 0.1 mL of xanthine oxidase solution is added to the test tube and homogenized using a vortex mixer. The mixed solution is then incubated at the optimum temperature for 30 minutes. The reaction was stopped by the addition of 1 mL of 0.1 N HCl solution, and its absorption was measured using a UV-Vis spectrophotometer at λ 291.7 nm.

Preparation of Allopurinol Test Solution

Allopurinol solution with a concentration of 0.1 ; 0.2 ; 0.5 ; 0.1 ; 2.0 ppm is put 1 mL into different test tubes then in each test tube 2.9 mL is added Tris HCl buffer solution 0.02 M optimum pH then 2 mL of xanthin substrate solution 0.15 mM is added then pre-incubated each at the optimum temperature for 10 minutes. After preincubation is completed then 0.1 mL of xanthine oxidase solution is added to the test tube and homogenized using a vortex mixer. The mixed solution is then incubated at the optimum temperature for 30 minutes. The reaction was stopped by the addition of 1 mL of 0.1 N HCl, and then its absorption was measured using a UV-Vis spectrophotometer at λ 291.7 nm.

Preparation of Allopurinol Control Test Solution

Allopurinol solution with a concentration of 0.1; 0,2 ; 0,5; 0,1; 2.0 ppm 1 mL is put into different test tubes then 3 mL of Tris HCl buffer solution of 0.02 M optimum pH is added to each test tube then 2 mL of xanthin substrate solution 0.15 mM is added then pre-incubated each at the optimum temperature for 10 minutes, then added 1 mL HCl 0,1 N. The mixed solution was then incubated at the optimum temperature for 30 minutes, after which it was absorbed using a UV-Vis spectrophotometer at λ 291.7 nm.

Calculation of enzyme inhibition activity

$$\text{Inhibition (\%)} = \left(\frac{(A - B) - (C - D)}{(A - B)}\right) \text{ x } 100\%$$

Information:
A = Absorbansi larutan uji blanko
B = Absorbance of blank control test solution
C = Absorbance of sample test solution
D = Absorbance of sample control test solution

Results and Discussion

Isolation and precipitation of xanthine oxidase

The process of isolation of the xanthine oxidase is carried out by adding NaCl to fresh goat's milk. The addition of NaCl aims to disrupt the interaction between protein molecules and water, thereby triggering the deposition of key proteins such as casein and breaking down the structure of fat emulsions. After the addition of NaCl, it is then centrifuged to separate the sediment and supernatant. The supernatant obtained is a crude extract of xanthine oxidase, which will be used for further acetone purification processes. The purpose of using acetone is that it can draw water from proteins, thereby reducing their solubility and causing proteins to clump and settle (Achmad et al., 2019). In addition, acetone is volatile, so that the remaining acetone solvent can be removed quickly after completion. Precipitation is carried out using acetone with various fractional variations. The first precipitation was carried out using acetone with fractional variations of 0-20, 20-40, 40-60%. The second precipitation is carried out using acetone with fraction variations of 0-30, 30-60, 60-90%. The purpose of double acetone precipitation is to obtain xanthine oxidase with the highest specific activity and purity. The precipitation results in white deposits from each fraction, which will then be dissolved again using a Tris-HCl buffer solution of 0.02 M, pH 7.5, and the enzyme activity is measured based on the uric acid formed.

Figure 1. Acetone precipitation precipitate from several fractions

Activity and purity of xanthin oxidase

Enzyme purity testing can be performed if the specific activity of the enzyme is known. Enzyme-specific activity can be measured by comparing enzyme activity with enzyme protein levels. Therefore, enzyme activity tests were also carried out to determine the ability of enzymes to degrade xanthin substrates into uric acid. Measurement of enzyme activity is based on the amount of uric acid formed and measured using a UV Vis spectrophotometer at a wavelength of 291.7 nm. In addition, a protein content test was carried out using the Bradford method. The Bradford method measures protein levels by utilising Coomassie Brilliant Blue. When this dye binds to a protein, its color changes from red-brown to blue. This color change was then measured using a spectrophotometer at a wavelength of 595 nm, and the color intensity was proportional to the amount of protein present. The data from protein content and enzyme activity tests can be used to determine enzyme-specific activity and enzyme purity.

Table 1. Purity of xanthine oxidase

Name	Uric acid (µmol/mL)	Protein content (mg/mL)	Enzyme activity (mU/mL)	Specific activities (mU/mL)	Purity (%)
Crude extract	0.011	0.512	0.176	0.344	1
Enzyme 0-20%	0.031	0.315	0.496	1.575	4.581
Enzyme 20-40%	0.008	0.123	0.128	1.041	3.027
Enzyme 40-60%	0,004	0.074	0.064	0.865	2.516
Enzyme 0-30%	0.153	0.243	0.490	2.015	5.861
Enzyme 30-60%	0.144	0.165	0.461	2.793	8.124
Enzyme 60-90%	0.282	0.088	0.902	10.255	29.190

Based on the results in Table 1, it can be seen that in the first precipitation, the specific activity of the xanthine oxidase was highest in the fraction of 0-20%, which is 1.575 mU/mL with a purity level of 4.518%. Therefore, the second stage of acetone precipitation is carried out to obtain a higher enzyme-specific activity. The specific activity of the xanthine oxidase in the second precipitation resulted in a fraction of 60-90% obtained xanthine oxidase with a specific activity of 10.255 mU/mL and a purity of 29.190%. This increase in specific activity showed that the 60-90% fraction contained xanthine oxidase with the highest purity after the refining process using acetone.

Optimum temperature

The xanthine oxidase from the acetone fraction of 60-90%, which has the highest specific activity is then characterized including optimal temperature, optimal pH, and molecular weight. This characterization aims to determine the conditions under which xanthine oxidase can work optimally. The optimum temperature of the xanthine oxidase was characterizsed by measuring the activity of the enzymes incubated at various temperatures, namely 25, 29, 33, 37, and 41°C.

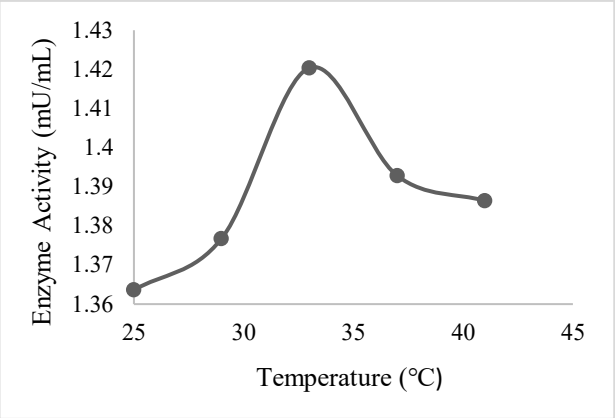

Figure 2. Optimum temperature of xanthine oxidase

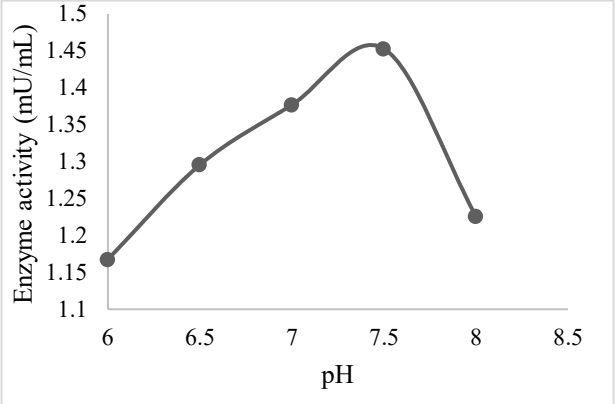

Figure 3. Optimum pH of xanthine oxidase

Based on Figure 2, enzyme activity increases as the temperature rises until it reaches the optimal temperature, then decreases after passing that point. This is due to the fact that as the temperature increases, the kinetic energy increases, the number of effective impacts increases, and the enzyme activity becomes higher until it reaches the optimum temperature of 33°C, with the activity of the xanthine oxidase of 1.4204 mU/mL. Once it passes the optimal temperature, enzyme activity begins to decrease. This decrease is caused by partial denaturation of the enzyme due to higher temperatures, which results in changes in the enzyme's three-dimensional structure, especially at the active site. As a result, the enzyme's ability to bind to the substrate decreases so that its catalytic activity is reduced.

The results of this temperature characterization indicate that the optimum temperature of xanthine oxidase enzyme is 33°C. Compared with other studies, this result is still by the report that found that the optimum temperature of xanthine oxidase enzyme from mammalian milk isolation was 30-37°C [15]. In addition, research showed that the optimum temperature of the xanthine oxidase enzyme from the liver of Notopterus chrysopterus was around 30°C [11].

Optimum pH

Xanthine oxidase at the optimum temperature was then characterized for the optimum pH. In this study, xanthin oxidase was dissolved using buffer with different pH variations, namely, 6, 6.5, 7, 7.5, and 8, then incubated at the optimum temperature.

Based on Figure 3, the activity of the xanthine oxidase from goat's milk showed an increase from pH 6 to reach a maximum point at pH 7.5, then decreased activity at pH 8. An increase in enzyme activity to pH 7.5 with an enzyme activity of 1.4528 mU/mL indicates that the enzyme is in conditions that favour the formation of an active conformation. Under this condition, the ionic groups on the active side of the enzyme and on the substrate are in the right ionisation state, thus allowing the formation of enzyme-substrate complexes optimally. In contrast, a decrease in activity at pH 8 suggests that an overly alkaline environment can lead to partial denaturation or changes in the enzyme's three-dimensional structure, thereby interfering with the active site's ability to recognize and bind substrates.

The results of this temperature characterization indicate that the pH temperature of xanthine oxidase enzyme is 7.5. These results are from research, which states that the optimum pH of the xanthine oxidase enzyme from buffalo liver is pH 7.6 [16]. Likewise, research conducted reported that the xanthine oxidase enzyme produced from the microbe Bacillus pumilus RL-2d showed an optimum pH of 7.5 [18].

Molecular weight

The xanthine oxidase, whose pH and optimum temperature are known, is then determined by molecular weight. Determination of enzyme molecular weight by SDS-PAGE PAGE (Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis) analysis based on the Laemmli procedure. This method was chosen because of its ability to separate proteins based on their molecular size, which is highly effective. Molecular weight testing was carried out with a polyacrylamide gel concentration of 10%. The marker used is GenScript Stained Protein, with a molecular weight range ranging from 30 to 270 kDa. The gel-making process consists of two layers, namely separating gels that separate proteins based on molecular weight and stacking gels as a sample injection site. In this study, there were 5 samples that were tested for molecular weight, namely (1) crude extract of xanthine oxidase without dilution, (2) crude extract of enzyme diluted twice, (3) crude extract of enzyme diluted ten times, (4) xanthine oxidase e with a concentration of 1000 ppm, and (5) xanthine oxidase with a concentration of 10,000 ppm. After the migration is complete, it is stained using Coomassie Brilliant Blue. The staining process aims to clarify and visualise the colour band, which can then be analyzed by molecular weight. The analysis of the SDS-PAGE results begins by determining the Rf value of the marker. The results from the electrophoresis showed that most of the samples had protein bands that appeared at an Rf value of about 0.43 and a molecular weight log of about 1.81, which corresponded to an estimated molecular weight of 65 kDa. This suggests that the dominant protein that was successfully isolated from most of the samples had a size of about 65 kDa. Meanwhile, in wells 6, 7, 8, and 9, no protein bands were detected because the well was not filled with sample.

The results of the molecular weight obtained from this study, when compared with research conducted on xanthine oxidase enzyme isolated from buffalo liver, have a subunit molecular weight of 67 kDa [16]. Another study of xanthine oxidase enzyme from sheep liver milk that has gone through purification with acetone precipitation and DEAE-

cellulose chromatography, and Sephacryl S-300 obtained a molecular weight of 75 kDa [17].

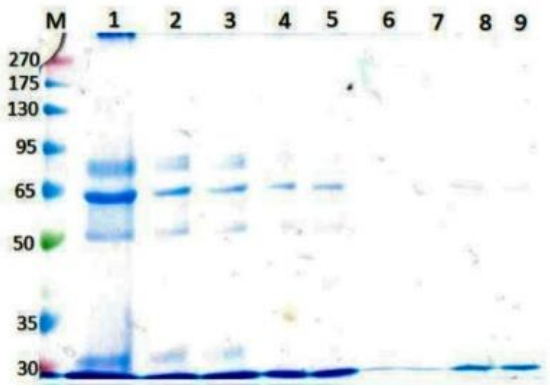

Figure 4 Results of molecular weight determination by SDS PAGE

Allopurinol inhibition

The xanthine oxidase that has been identified as optimal temperature, optimal pH and molecular weight is then inhibited using allopurinol. In this study, the variation in the concentration of allopurinol used was 0.1, 0,2, 0,5, 1 and 2 ppm diluted from a 1000 ppm master solution. Each test sample with a specific allopurinol concentration was compared with a control without the addition of xanthine oxidase. Each solution was measured for absorption at a wavelength of 291.7 nm using a UV-Vis spectrophotometer. The absorbance data obtained from each allopurinol concentration were then used to calculate the inhibition percentage.

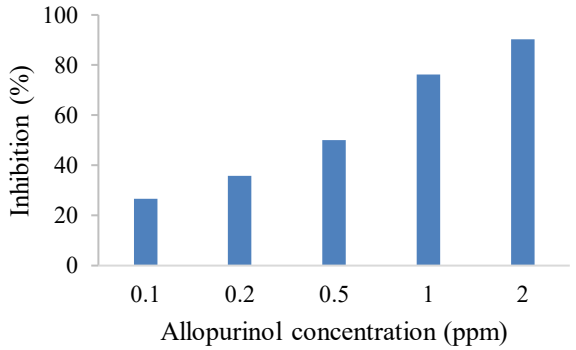

Figure 5 Allopurinol inhibition

Based on Figure 5, it can be seen that the percentage of this inhibition increases gradually along with the increase in allopurinol concentration. An increase in the inhibition percentage was associated with a decrease in the activity of xanthine oxidase. This indicates that allopurinol successfully inhibits the work of xanthine oxidase. Allopurinol works by binding to the active side of xanthine oxidase, thereby inhibiting the binding of natural substrates such as hypoxanthine and xanthine. Allopurinol is then converted by the enzyme into oxypurinol, an active form that lasts longer in the body and acts as a non-competitive inhibitor. In comparison, the absorbance values in the controls and blanks showed uninhibited enzyme activity, where the absorbance value remained high because there was no xanthine oxidase in the solution.

Conclusion

The xanthine oxidase enzyme that has been purified using the acetone precipitation method has a purity level of 29.190%, with an optimum temperature of 33°C, an optimum pH of 7.5, and a molecular weight of 65 kDa. After being characterized, the enzyme was used as a model to study the mechanism of inhibition of enzyme activity. In this study, allopurinol was used as an inhibitor, and the results showed that allopurinol was able to inhibit xanthine oxidase enzyme activity effectively.

Author's Contribution

Maretha Nur Rohma: contributed to the experimental design, sample preparation, laboratory work, and data analysis. Nuniek Herdyastuti: supervised the research process, interpretation of results, and was responsible for manuscript review and editing.

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