Isolation and Determination of Amylase Enzyme Activity from Durian Seed Sprouts

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Abstract: One of the favorite fruits in Medan-Indonesia is durian. The number of fruit is comparable to the most waste of durian seed produced. Seeds are one of the ways to breed through the process of germination. In the process of germination which plays an important role is the enzyme amylase. Based on this, the research aims to isolate the enzyme amylase found in durian seed sprouts on the fifth day through dialysis with ammonium sulfate and then determined its activity against temperature, pH, substrate concentration. Through this research, the enzyme amylase has been isolated from the sprouts of durian beans with the best activity at temperature 40 oC (36.87 U/ml), pH 7 (36.86 U/ml), substrate concentration 1% (26.36 U/ml). The best ammonium sulfate saturation in the dialysis process is 60% acquired enzyme activity of 58.59 U/ml.

1 INTRODUCTION

Enzyme is a biocatalyst which can accelerate the process of a reaction. Currently, the use of enzymes in an industrial field is increasing. Some industries that use enzymes among others are pharmaceutical industry, food and beverage industry, and energy sector (Chapman et al., 2018). One of the most widely used enzymes is the amylase enzyme. Amylase enzyme is an enzyme that hydrolyzes starch to dextrin, maltose and glucose units by cutting glycosidic bonds α- 1,4 and α- 1,6 in starch (Mohanan & Satyanarayana, 2018; Simair et al., 2017). In the food industry, amylase enzymes play an important role in producing syrups and sweeteners, in baking, in cereals and also in beverage production. The wide use of amylase enzymes in industry makes this amylase enzyme take up 25% of the world's market enzymes (Naili et al., 2016; Sindhu et al., 2017). The large enzyme needs make researchers have to find new sources to produce the enzyme amylase.

One of the process which requires amylase enzymes is the germination process, in the germination process the amylase enzyme is needed to produce energy that will be used for the growth process (Joshi, 2018). Based on these reasons, the potential for durian seed sprouts as a source of amylase enzymes is considered to have great potential. In this study the age of durian seed sprouts used as a source of the amylase enzyme was 5 days old. Some factors that determine enzyme activity are enzyme purity, pH, temperature and substrate concentration of the enzyme. Therefore in this study the parameters to be tested are the activity of the amylase enzyme isolated from durian seed sprouts on variation of temperature, pH and substrate concentration. After the optimum conditions are obtained then it will be applied to determine the enzyme activity after the purification process by using several variations of ammonium sulfate.

The success of this research is expected to add new information on the source of the amylase enzyme from durian seed sprouts. The potential of durian is very much in Medan will add another advantage of the waste produced by the durian fruits.

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2 MATERIALS AND METHODS

2.1 Isolation of Amylase Enzyme from Durian Seed Sprouts

Durian seeds are washed with running water and then put into a container for the germination process, prepared by growing media and then waited for durian seed germination for 5 days. 150 grams of 5 days durian seed sprouts obtained were homogenized with 250 ml of 1% Isotonic NaCl solution in a cold state. Then mashed and filtered until the filtrate and pulp separate. The filtrate was centrifuged at 10,000 rpm at 20°C for 10 minutes. The crude of enzyme produced was tested for its activity against temperature variations (30, 35, 40, 45 dan 50°C), pH (3, 5, 7, dan 9) and Substrate concentration (0,5; 1; 1,5; 2; dan 2,5% w/v). The resulting enzyme crude is purified by varying the saturation level of ammonium sulfate (20, 40, 60 dan 80% w/v) then the purification results of each variation were tested their activity back to the optimum temperature, pH and substrate concentrations to determine the activity of the resulting amylase enzyme after purification.

2.2 Crude Amylase Enzyme Activity Test on Variation of Temperature

0.5 mL of 1% starch solution was put into the test tube then added 5 mL of buffer phosphate pH 7. Added 1 mL of crude amylase enzyme extract and 1 ml of 1% NaCl. Then incubated at temperature variations (30, 35, 40, 45 and 50 0C) for 1 hour. After that, 1 mL of 0.1N NaOH was added and centrifuged at 3400 rpm for 20 minutes. 1 ml of the supernatant is taken and then diluted in 10 ml measuring flask and then homogenized. Put 1 ml of dilution results into the test tube then add 1 ml of Nelson's reagent and heated in a water bath for 20 minutes. Then removed and cooled until the temperature 25 0C. 0.5 mL of arsenomolybdate was added and then shaken until all the sediment dissolved. Then added 7 mL of distilled water and then shaken until homogeneous. Its absorption is measured at a wavelength of 645 nm.

2.3 Crude Amylase Enzyme Activity Test on Variation of pH

0.5 mL of 1% starch solution was put into the test tube then added 5 mL of buffer phosphate pH 3, 5, 7 and 9. Added 1 mL of crude amylase enzyme extract and 1 ml of 1% NaCl. Then incubated at optimum temperature for 1 hour. After that, 1 mL of 0.1N NaOH was added and centrifuged at 3400 rpm for 20 minutes. 1 ml of the supernatant is taken and then diluted in 10 ml measuring flask and then homogenized. Put 1 ml of dilution results into the test tube then add 1 ml of Nelson's reagent and heated in a water bath for 20 minutes. Then removed and cooled until the temperature 25°C. 0.5 mL of arsenomolybdate was added and then shaken until all the sediment dissolved. Then added 7 mL of distilled water and then shaken until homogeneous. Its absorption is measured at a wavelength of 645 nm.

2.4 Crude Amylase Enzyme Activity Test on Variation of Substrate Concentrations

0.5 mL of starch solution variation (0,5; 1; 1,5; 2; dan 2,5%) was put into the test tube then added 5 mL of buffer phosphate optimum pH. Added 1 mL of crude amylase enzyme extract and 1 ml of 1% NaCl. Then incubated at optimum temperature for 1 hour. After that, 1 mL of 0.1N NaOH was added and centrifuged at 3400 rpm for 20 minutes. 1 ml of the supernatant is taken and then diluted in 10 ml measuring flask and then homogenized. Put 1 ml of dilution results into the test tube then add 1 ml of Nelson's reagent and heated in a water bath for 20 minutes. Then removed and cooled until the temperature 25°C. 0.5 mL of arsenomolybdate was added and then shaken until all the sediment dissolved. Then added 7 mL of distilled water and then shaken until homogeneous. Its absorption is measured at a wavelength of 645 nm.

2.5 Crude Amylase Enzyme Activity Test after Purification with Ammonium Sulfate

0.5 mL of starch solution optimum variation was put into the test tube then added 5 mL of buffer phosphate optimum pH. Added 1 mL of crude amylase enzyme extract after purification with variations in the saturation level of ammonium sulfate (20, 40, 60 dan 80%) and 1 ml of 1% NaCl. Then incubated at optimum temperature for 1 hour. After that, 1 mL of 0.1N NaOH was added and centrifuged at 3400 rpm for 20 minutes. 1 ml of the supernatant is taken and then diluted in 10 ml measuring flask and then homogenized. Put 1 ml of dilution results into the test tube then add 1 ml of

Nelson's reagent and heated in a water bath for 20 minutes. Then removed and cooled until the temperature 25°C. 0.5 mL of arsenomolybdate was added and then shaken until all the sediment dissolved. Then added 7 mL of distilled water and then shaken until homogeneous. Its absorption is measured at a wavelength of 645 nm.

3 RESULTS AND DISCUSSIONS

Durian seeds used in this study were obtained from durian traders around Medan, North Sumatera, Indonesia. Durian seeds that have been cleaned are then germinated, after 5 days the resulting sprouts will then be processed as a source of the amylase enzyme. Germination carried out in this study after 5 days as in Figure 1 below.

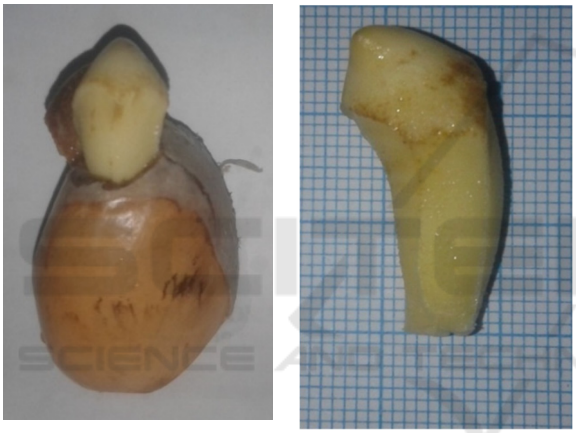

Figure 1: Durian Seed Sprouts.

Based on Figure 3.1 above durian seed sprouts after 5 days have an average height of 2.5 cm - 2.8 cm. Some factors that can affect the process of germination development are temperature, water potential, nutrition, light and humidity (Joshi, 2018; Shaban, 2013). These factors must be conditioned to obtain sprouts with a uniform height.

Amylase enzyme isolation process carried out on 150 grams of durian seed sprouts, at this stage obtained an orange solution, which will then be tested for its activity against variations on temperature, pH and substrate concentration before and after purification.

3.1 Crude Amylase Enzyme Activity Test on Variation of Temperature

One of the main determinants of enzyme activity is temperature. This is because enzymes are part of proteins that are sensitive to extreme changes in temperature (Mohanan & Satyanarayana, 2018). In this study the temperature range used is 30 – 50 ^{0}C with a range of 5 ^{0}C. The amount of crude amylase enzyme activity obtained in accordance with Figure 2

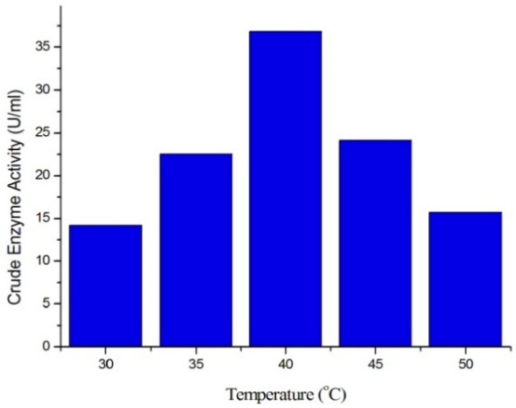

Figure 2: Activity of Crude Enzyme on Variation of Temperature.

Based on Figure 2 above, it can be seen that the activity of crude amylase enzyme produced is influenced by temperature. At temperature of 30-40 ^{0}C the enzyme activity has increased and the optimum temperature is seen at a temperature of 40 ^{0}C with value of activity is 36.87 U/ml and will further decrease its activity until a temperature of 50 ^{0}C. Temparature value of the activity of enzyme is also influenced by the source of the enzyme obtained. Asrat et al 2018 has isolated enzyme amylase from Aspergillus Niger FAB-211, the optimum temperature of enzyme amilase is 45 0C. Several studies have reported that the optimal activity for the amylase enzyme is at temperature 40°C if the enzyme is isolated from H. bacteriophora, A. suum and S. Litorallis and 50°C for α -amylases from C. flavus, S. alluvius ATCC 26074, L. kononenkoae and C. antarctica CBS 667 (Wanderley et al., 2004).

3.2 Crude Amylase Enzyme Activity Test on Variation of pH

Besides temperature, another factor that determines the activity of an enzyme is pH. The crude amylase enzyme activity test results obtained in this study are shown in Figure 3 below.

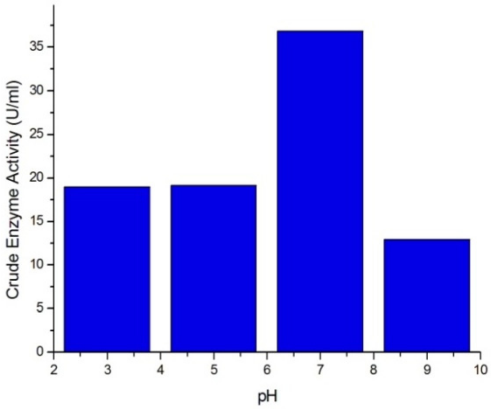

Figure 3: Activity of Crude Enzyme on Variation of pH.

Based on Figure 3 The following shows that at pH 3 and 5 the crude amylase enzyme activity shows no difference, but when pH 7 shows the optimum activity of crude enzyme amylase, the value of activity ezymes is 36,86 U/ml and will decrease at pH 9. Several studies have shown that the optimum pH for several amylase enzymes is also in the pH range of 6-6.5 (Asrat & Girma, 2018; Biazus et al., 2009).

3.3 Crude Amylase Enzyme Activity Test on Variation of Substrate Concentrations

Comparison between enzymes and substrate concentrations also needs to be considered because if a comparison between enzymes and substrate is appropriate a product with maximum hydrolysis results will be obtained. In this study the results of testing the activity of crude enzyme amylase on substrate concentration are shown in Figure 4.

Based on Figure 4 below, it can be concluded that the optimum substrate concentration that can be hydrolyzed by the crude amylase enzyme isolated is at a concentration starch solution 1%. This shows that the ratio between enzymes and substrate is 1 ml of enzyme with 0.5 ml of 1% starch substrate obtained enzyme activity of 26.36 U/ml.

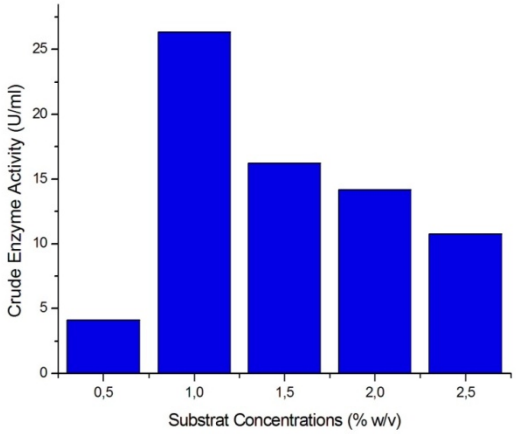

Figure 4: Activity of Crude Enzyme on Variation of Substrat Concentration.

3.4 Crude Amylase Enzyme Activity Test after Purification with Ammonium Sulfate

The purity of enzymes is important because purer enzymes will have better activity than before purification. In this study crude enzyme amylase was purified through a dialysis process that was previously precipitated with ammonium sulfate at a saturation level of 20% - 80% with range of 20%. At this stage the enzyme activity measured was carried out at the optimum temperature, pH and substrate concentration that had been carried out previously. The results obtained are shown in Figure 5 below.

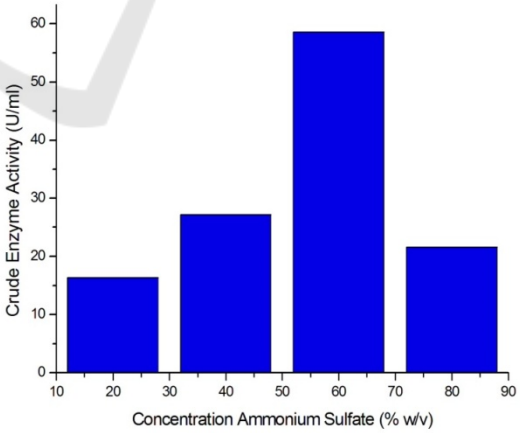

Figure 5: Activity of Crude Enzyme on Variation of Concentration Ammonium Sulfate.

Based on Figure 5 above it can be seen that after the purification process the enzyme amylase activity has increased than before the dialysis process. The optimum activity of the amylase enzyme obtained at

60% ammonium sulfate concentration, the value of activity enzyme is 58.59 U/ml. Based on this it can be concluded that the purification process is one of the important things that must be done to see the activity of an enzyme.

4 CONCLUSIONS

Based on the research that has been done, it can be concluded that durian seed sprouts can be used as a source of amylase enzymes. Crude Activity The amylase enzyme produced in this study before being purified had optimum activity at a temperature of 40 0C, pH 7, and 1% substrate concentration obtained activity values for each of 36.87 U/ml, 36.86 U/ml, and 26.36 U/ml. The optimum conditions obtained were then used to determine the crude activity of the amylase enzyme after purification through dialysis which was previously precipitated with ammonium sulfate and obtained optimum results at a concentration of 60% ammonium sulfate with enzyme activity of 58.59 U/ml.

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REFERENCES

Asrat, B., & Girma, A. (2018). Isolation, Production and Characterization of Amylase Enzyme Using the Isolate Aspergillus niger FAB-211. *International Journal of Biotechnology and Molecular Biology Research*, *9*(2), 7–14.

Biazus, J. P. M., Souza, R. R. de, Márquez, J. E., Franco, T. T., Santana, J. C. C., & Tambourgi, E. B. (2009). Production and characterization of amylases from Zea mays malt. *Braz. Arch. Biol. Technol.*, *52*(4), 991–1000. https://doi.org/https://doi.org/10.1590/S1516-89132009000400024

Chapman, J., Ismail, A. E., & Dinu, C. Z. (2018). Industrial Applications of Enzymes: Recent Advances, Techniques, and Outlooks. *Catalysts*, *8*(6), 1–26. https://doi.org/https://doi.org/10.3390/catal8060238

Joshi, R. (2018). Role of Enzymes in Seed Germination. *International Journal of Creative Research Thoughts*, *6*(2), 1481–1485.

Mohanan, N., & Satyanarayana, T. (2018). *Amylases Reference Module in Life Sciences*. Elsevier Inc.

Naili, B., Sahnoun, M., Bejar, S., & Kammoun, R. (2016). Optimization of submerged Aspergillus oryzae S2 α-amylase production. *Food Science and Biotechnology*, *25*(1), 185–192.

Shaban, M. (2013). Effect of Water and Temperature on Seed Germination and Emergence as A Seed Hydrothermal Time Model. *International Journal of Advanced Biological and Biomedical Research*, *1*(12), 1686–1691.

Simair, A. A., Qureshi, A. S., Khushk, I., Ali, C. H., Lashari, S., Bhutto, M. A., Mangrio, G. S., & Lu, C. (2017). Production and Partial Characterization of α-Amylase Enzyme from Bacillus sp. BCC 01-50 and Potential Applications. *BioMed Research International*, *2017*, 1–9. https://doi.org/https://doi.org/10.1155/2017/9173040

Sindhu, R., Binod, P., Madhavan, A., Beevi, U. S., Mathew, A. K., Abraham, A., Pandey, A., & Kumar, V. (2017). Molecular improvements in microbial α-amylases for enhanced stability and catalytic efficiency. *Bioresour Technol.*, *245*, 1740–1748.

Wanderley, K. J., Torres, F. A. G., Moraes, L. M. P., & Ulhoa, J. C. (2004). Biochemical characterization of α-amylase from the yeast Cryptococcus flavus. *FEMS Microbiology Letters*, *231*(2), 165–169. https://doi.org/https://doi.org/10.1016/S0378-1097(03)00955-8