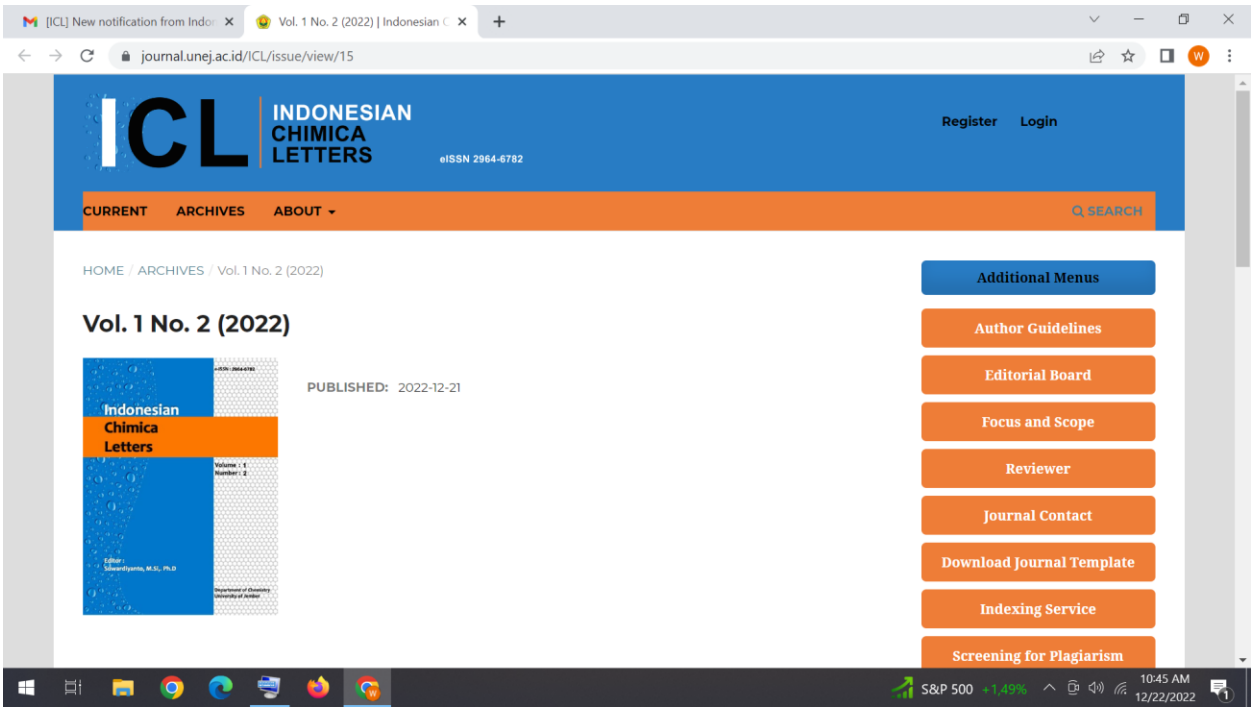
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Optimization of Centrifugation Speed and pH in Extraction of Uricase Enzyme from Goat Liver

Wuryanti Handayani,*[a] Esti Utarti, [b] Riki Juni Krismiadi, [c] and AA. Istri Ratnadewi [a]

Abstract: The human body does not have an enzyme that can break down uric acid, so the accumulation of uric acid can cause disease. This problem can be overcome by uricolytic therapy by utilizing the activity of the uricase enzyme. In this study, the uricase enzyme was extracted from goat liver by optimizing the centrifugation speed and the extraction pH. The purpose of the optimization is to get maximum uricase activity. Uricase extraction to optimize centrifugation speed using borate buffer pH 8.5 then centrifuged at 7.000; 9.000; 11.000; 13.000 and 15.000 rpm with a temperature of 4°C. Furthermore, pH optimization was carried out using pH buffers 6, 7, 8, 9, 10 and 11 by centrifuging the optimum speed obtained. The crude extract obtained was further tested for its enzyme activity. The results showed that the highest uricase activity was achieved if the extraction was carried out at pH 8 using centrifugation at an optimum speed of 13.000 rpm. The higher uricase activity indicates that the extracted uricase concentration is increasing.

Keywords: optimization; uricase; pH; centrifugation speed.

INTRODUCTION

Mammals except primates are able to break down uric acid into simpler products such as allantoin, hydrogen peroxide and carbon dioxide [1]. The formation of allantoin can occur in several organs, such as liver and kidneys. The breakdown of uric acid into allantoin is catalyzed by uricase which has four identical active sites for binding to substrates [2]. Uric acid degradation by uricase occur in the crystalloid core of peroxisomes in the liver [3]. Based on this, in this study uricase extraction from goat liver was carried out. Goats were chosen because they are classified as mammals so it is suspected that goat liver contains uricase which is able to degrade uric acid.

In this research, optimization of centrifugation speed and pH during extraction was carried out. Previous studies performed uricase extraction from bovine kidneys using borate buffer pH 10 with a centrifugation speed of 3,000 rpm and obtained crude enzyme activity of 53 U/mg [4]. Uricase extraction was also carried out from leek seeds using borate buffer pH 8.5 with a centrifugation speed of 8,000 rpm and obtained a specific crude enzyme activity of 0.057 U/mg [5]. The difference in the use of pH and centrifugation speed during extraction is thought to affect the concentration of the enzyme obtained, this is based on the stability of uricase where uricase is stable in the pH range of 5 to 11 [6]. This study used centrifugation speeds of 7,000, 9,000, 11,000, 13,000 and 15,000 rpm, while the extraction pH was 6, 7, 8 (carbonate buffer) 9, 10 and 11 (borate buffer).

Buffer solutions with low concentrations can play a role in the process of cell lysis. The salt in the buffer solution will affect the salt concentration outside the cell. The low salt content in the buffer solution allows water to osmosis and enter the cell. The cell will secrete cell organelles and other materials including the target protein which in this study is uricase on peroxisome organelles. Based on this, the buffer solution apart from maintaining the stability of the target protein due to the pH used, also functions in the process of cell lysis [7].

Centrifugation plays a role in the separation of cell components by centrifugal force so that centrifugation is also the initial stage of enzyme purification. Particles that have a higher density will settle more easily than particles with a lower density. Centrifugation speed plays an important role in this deposition process. A high centrifugation speed will be able to precipitate cell components which are relatively small in size and density. at a centrifugation speed of 600 g only the cell nucleus and cytoskeleton were deposited on the pellet. Mitochondria, lysosomes, peroxisomes and chloroplasts (in plant cells) precipitate at a centrifugation speed of 15,000 g. Other organelles that have a very low density precipitate after 100,000 g [8].

The stability of an enzyme is strongly influenced by pH, this is related to the isoelectric point of the enzyme. The isoelectric point is theoretically the sum of all the charges from the primary structure and each amino acid in the enzyme structure. The total charge at the isoelectric point is zero and the isoelectric point for uricase is at pH 7.5. The total charge of uricase will change if the surrounding pH changes. At a pH above the isoelectric point, the total uricase charge tends to be negative, while at a pH below the isoelectric point it tends to be positive. This change in total charge creates an electrostatic repulsion force which can cause changes in the structure of uricase and has an impact on changes in the active site of the enzyme so that it has an impact on uricase performance [9].

METHODS

Uricase Extraction

Uricase extraction refers to the method [4], 10 grams of goat liver mashed then extracted with borate buffer pH 8.5 and filtered. The filtrate was centrifuged for 30 minutes (4 °C, 7.000, 9.000, 11.000, 13.000, 15.000 rpm).

[a] W. Handayani, AAI. Ratnadewi
Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Jember.
*e-mail: 196008221985032002@mail.unej.ac.id
[b] E. Utarti
Department of Biology, Faculty of Mathematics and Natural Sciences, University of Jember.
[c] RJ. Krismiadi
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The supernatant obtained (crude uricase enzyme) will be tested for activity and protein content. The same method was used to optimize the extraction pH, but the pH buffers used were 6, 7, 8, 9, 10 and 11 and the optimum centrifugation speed was 13,000 rpm.

Analysis of Uricase Activity

0.1 mL of crude uricase enzyme was incubated in 0.6 mL sodium borate buffer (0.1 M) containing 2 mM uric acid, 0.15 mL 4-aminoantipyrine (30 mM), 0.1 mL phenol (1, 5%) and 0.05 mL peroxidase (15 U/mL). The mixture was incubated at 37°C for 20 minutes. The enzymatic reaction was stopped by adding 1 mL of ethanol. The sample was then measured for its absorbance at a wavelength of 490 nm. One U of activity is equivalent to the amount of enzyme that produces 1.0 µL H_2O_2 per minute [5].

Analysis of Protein Concentration

0.1 mL of crude uricase enzyme was added with 5 mL of Bradford reagent and then incubated for 5 minutes. After that, the absorbance was measured at a wavelength of 595 nm using a UV/Vis spectrophotometer [10].

Measurement of Total Enzyme Activity using equation 1.

$$\text{Activity}(\text{U/mL}) = \frac{([S]-[C])\times f_p \times \left(\frac{V_{total}}{V_{enzim}}\right)}{t \times Mr\ H_2O_2} \quad \text{..... (1),}$$

which are

[S] = Sample concentration (mg/mL), [C] = Control concentration (mg/mL), Fp = Dilution factor, total V = total volume (mL), enzyme V = enzyme volume (mL), Mr H2O2 = Molecular weight of H2O2 (34.0147 gr/mol), t = Incubation time (minutes)

$$\text{Proteine Concentration (mg/mL)} = \frac{Abs \pm C \times fp}{m} \quad \text{..... (2),}$$

which are

Abs = Solution Absorbance Value, c = Intercept value from the BSA standard curve equation, Fp = Dilution factor, m = Slope value from the BSA standard curve equation.

RESULT AND DISCUSSION

Effect of Centrifugation Speed on Uricase Crude Extract Activity

In this study, it is expected that the uricase protein will concentrate in the supernatant phase and the impurity components will precipitate in the pellet phase. Based on this, the optimum centrifugation speed is the centrifugation speed that can separate the uricase protein (in the supernatant) from other unwanted components. The effect of centrifugation speed on the total activity of the uricase crude extract can be seen in Figure 1.

Based on the total activity data in Figure 1, at a centrifugation speed of 7,000 rpm the smallest total activity was obtained. The low total activity is due to the fact that at 7,000 rpm the separation of protein from the remaining impurities is not optimal. Increasing the speed further increases the separation and at a speed of 15000 rpm the most perfect separation occurs even though it is not too significant at a speed of 13000 rpm. This was proven by the protein content of the sample at 7,000 rpm which was also lower than other centrifugation speeds. The total activity at 9,000 rpm is greater than the total activity at 7,000 rpm, this is because uricase is more concentrated in the supernatant phase so that the total activity increases. Increasing the concentration of the enzyme in the sample can increase its activity.

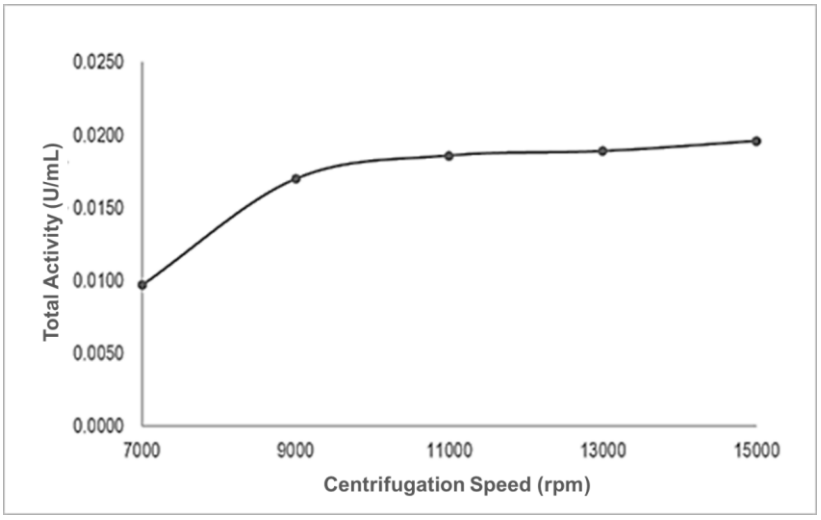

Figure 1. Total uricase activity at various centrifugation speeds

The increase in centrifugation speed is expected to further precipitate cell components other than uricase. According to research [12], the higher the centrifugation speed, the less protein other than the target enzyme contained in the supernatant. Protein concentration due to centrifugation speed are shown in Figure 2.

Figure 2 shows the protein levels in the sample do not match the trend in the study [12]. This is presumably because the protein content in each sample at several centrifugation speeds has an inhomogeneous density. The non-homogeneous density of proteins causes the precipitation of proteins other than uricase to be independent of centrifugation speed so that at speeds of 7.000 to 11.000 rpm the protein content increases while at 13.000 and 15.000 rpm the protein content is the same. The same protein content at 13.000 and 15.000 rpm was due to the cell components deposited on the same pellet at speeds ranging from 11.588 rpm to 29.921 rpm [8]. As a result, the protein in the supernatant at 13.000 and 15.000 rpm has the same concentration because it has the same composition.

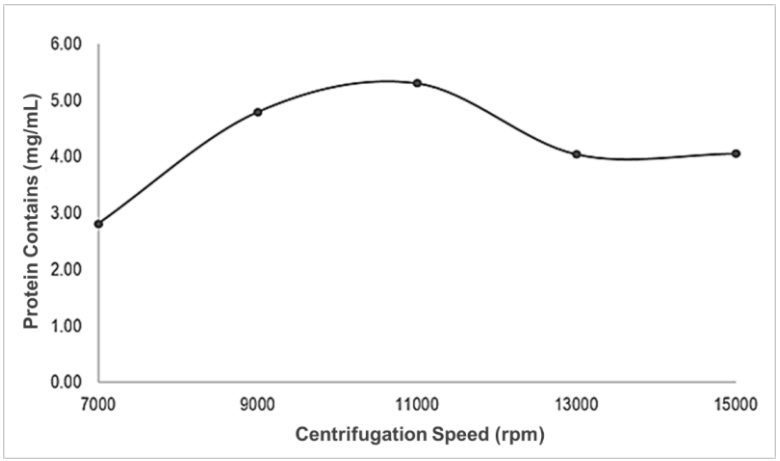

Figure 2. Protein concentration at various centrifugation speeds

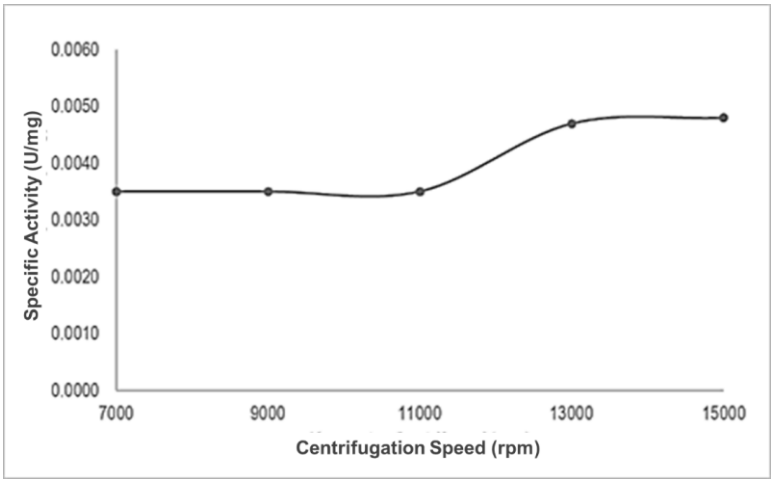

Figure 3. Spesific uricase activity at various centrifugation speeds

This causes the specific activity of crude uricase extract at 13.000 and 15.000 rpm to be highest as shown in Figure 3. Based on this explanation, a centrifugation speed of 13.000 rpm is considered more ideal and is considered the best centrifugation speed. The total and specific activities at 13.000 rpm were 0.0189 U/mL and 0.0047 U/mg respectively.

Effect of Extraction pH on Uricase Crude Extract Activity

Trends in total activity and specific activity of uricase crude extract at various pH can be seen in Figure 4.

Based on Figure 4, uricase produces the highest and optimum total activity at pH 8 and has a fluctuating pattern from pH 6 to pH 11. This proves that uricase tends to be more stable at pH close to its optimum pH. Based on research [4], pH 8.5 is the optimum pH for uricase, therefore in this study pH 8 has the highest total activity because it is close to the optimum pH. Another factor that causes uricase to produce the highest total activity at pH 8 is the isoelectric point of uricase.

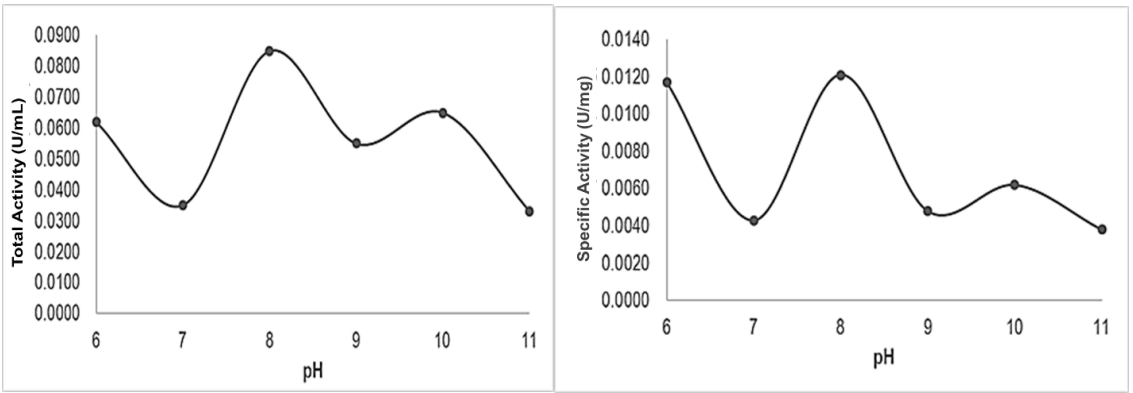

Figure 4. Uricase activity at various pH of Buffer

According to [9], the isoelectric point of uricase is at pH 7.5. The total charge of amino acids in the uricase structure when the isoelectric point is zero, the total charge will change if the pH of the uricase environment changes. At a pH above the isoelectric point, the total charge on uricase tends to be negative, while at a pH below the isoelectric point the total charge tends to be

positive. Changes in total charge cause electrostatic repulsion on amino acids so that they can change the structure of uricase. Structural changes will affect changes in the conformation of the enzyme so that it affects its catalytic activity. The crude extract enzyme extracted at pH 8 has the highest activity because at that pH the total charge of uricase does not differ much from the charge at its isoelectric point which is around (-2), whereas at pH 11 the total charge of uricase becomes (-35) so it has the lowest total activity because the difference in total charge is too great with the charge at the isoelectric point. The total and specific enzyme activities of the crude extract of uricase at pH 8 were 0.085 U/mL and 0.0121 U/mg, respectively.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that uricase activity from goat liver is optimum when extracted with a pH of 8 and centrifuged at a centrifugation speed of 13.000 rpm. In future research, optimizing the extraction pH should use the same type of buffer or use a different type of buffer with the same pH. Research related to optimizing the type of extraction buffer also needs to be carried out to find out the type of buffer that is suitable and able to maintain uricase activity for quite a long time.

REFERENCES

[1] M. Meraj, K. Ur-Rehman, S. Javed, R. Irfan, and N. Jahan, Hyperproduction of Urate Oxidase by Wild and Mutated Bacillus substilis, *Jounal of Advanced in Chemical Engg*., *Biological Sciences (IJACEBS)*, vol. 1, no. 1, 2014.

[2] S. Hayashi, S. Fujiwara, and T. Noguchi, Evolution of Urate-Degrading Enzymes in Animal Peroxisomes, vol. 32. 2000.

[3] N. Usuda, S. Hayashi, S. Fujiwara, T. Noguchi, T. Nagata T. M. S. Rao, K. Alvares, J. K. Reddy and A. V. Yeldandi, Uric Acid Degrading Enzymes, Urate Oxidase and Alantoinase, are Associated with Different Subcellular Organelles in Frog Liver and Kidney, *Journal of Cell Science*, vol. 107, pp. 1073-1081. 1994.

[4] M. I., K. Ur-Rehman Rajoka, M. Mehraj, M. W. Akhtar, and M. A. Zia, Purification and properties of a bovine uricase, *Protein and Peptide Letters*, vol. 13, pp. 363–368. 2006.

[5] N. S. I. S. Lee, H. M. Khosravi, N. Ibrahim, and S. Shahir, Isolation, Partial Purification and Characterization of Thermophilic Uricase From Pseudomonas otitidis Strain SN4, *Malaysian Journal of Microbiology*, vol. 11, no. 4, pp. 352-357, 2015.

[6] R. K. Yelchuri, Characterization of Uricases of Yeast with Special Emphasis on Kluveromyces Lactis, Selangor: Prasad GS, 2012.

[7] M. S. Islam, A. Aryasomayajula, P. R. Selvaganapathy, A Review on Macroscale and Microscale Cell Lysis Methods, Canada: Micromachines, 2017.

[8] J. Koolman and K. H. Roehm, Color Atlas of Biochemistry, New York: Thieme Stuttgart, 2005.

[9] F. Bonnete, Macromolecular Crystallization Controlled by Colloidal Interactions: The Case of Urate Oxidase, 5, 2014.

[10] M. M. Bradford, A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding, Georgia: Academic Press, Inc., 1976.

[11] N. A. Naja, Isolasi dan Karakterisasi Enzim Urikase Pada Hati Ayam, Skripsi, Program Studi Kimia Fakultas Matematika dan Ilmu Pengetahuan Alam: Universitas Jember, 2020.

[12] S. N. Fauziah, D. Herasari, and A. Laila, Studi Pengaruh Penambahan Gliserol dan Sorbitol Terhadap Aktivitas Enzim Protease dari Actinomycetes Anl4 2b-3, Bandar Lampung: Universitas Lampung, 2012.

[13] H. H. Htay, K. Sato, K. Tamaki, H, Tsutsumit, and Y. Katsumata, Comparative Immunology of Mammalian Uricase Using Anti-Hog Liver Uricase, Nagoya: Pergamon Press plc., 1989.