Journal of Bio-Molecule Research and Engineering
Vol. 1 | No. 1 | 7 – 13 | 2022
https://www.e-journal.unair.ac.id/biome

Isolation and Characterization of Uricase Produced from Chicken Liver

Wuryanti Handayani, Nasrul Amaliyatun Naja, Muhamad Kiki Afindia Joenata, Anak Agung Istri Ratnadewi*
Department of Chemistry, Faculty of Mathematic and Natural Science, University of Jember, Jember 68121, Indonesia
*Corresponding author: istri_dewi.fmipa@unej.ac.id

ARTICLE INFO
Article history
Received 09th Dec 2021
Accepted 12th May 2022
Keywords:
characterization
isolation
uricase
chicken liver

ABSTRACT
Uricase is an enzyme that degrades uric acid into allantoin. One of the uricase sources is obtained from chicken species (*Gallus gallus domesticus*) liver which are broiler and native chicken. This study aims to determine the maximum uricase activity in broiler and native chicken liver. The uricase activity was obtained by measuring the uric acid concentration as uricase substrate using spectrophotometric method and wavelength at 291 nm. Uricase isolation was carried out into extraction process, ammonium sulfate fractionation (0-60% saturation of ammonium sulfate), and dialysis. During isolation process, centrifugation speed was also optimized to obtain the maximum uricase crude extract and uricase activity. The molecular weight of uricase was also determined by SDS PAGE. The result showed that the highest uricase activity remained using centrifugation speed of 15,000 rpm. The optimum uricase fraction for broiler chicken liver was obtained at 20-40% saturation of ammonium sulfate with uricase activity was 1.854×10^{-2} U/mg, and the uricase fraction for native chicken liver was obtained at 40-60% saturation of ammonium sulfate with uricase activity was 2.496×10^{-2} U/mg. The optimum fraction for uricase production and isolation is carried out to the dialysis process. The optimum uricase activity of broiler chicken liver crude extract was 4.921×10^{-4} U/mg, the uricase fraction was 3.989×10^{-3} U/mg, and the dialysate was 5.120×10^{-3} U/mg. While the native chicken liver crude extract was 2.980×10^{-4} U/mg, the uricase fraction was 1.415×10^{-2} U/mg, and the dialysate was 1.753×10^{-2} U/mg. The molecular weight of the uricase was around 35 kDa according to the SDS PAGE result.

1. Introduction

Uric acid is the end product in purine metabolism [[1]]. Besides that, purine metabolism also can produce different end products. Some organisms have the ability to break down uric acid into nitrogen compounds that are simpler compound [[2]]. Humans are one of the organisms that do not have the ability to break down uric acid. It is because humans do not have uric acid-degrading enzyme activity called uricase. As a result, uric acid accumulates as stones in the joints and become some diseases such as rheumatism, gout, and hyperuricemia that can occur when uric acid levels exceed normal limits

[[3]]. Something that can be done is to obtain the uricase from other sources by isolating uricase so that it can be applied for the prevention of uric acid in the human body.

Several studies have carried out the isolation of the uricase in plants, microorganisms, and animals. Isolation of uricase from green bean leaves [[4]], *Pseudomonas aeruginosa* [[5]], *Streptomyces exfoliates* UR10 from agricultural waste [[6]], *Bacillus subtilis* strain SP6 [[7]], *Bacillus cereus* SKIII [[8]], *Aspergillus welwitschiae* strain 1-4 [[9]]. Isolation of the uricase from the liver of rainbow fish, mackerel, trout, catfish, shark and tilapia [[10]], Indonesian coelacanth [[11]], camel liver [[12]], and bovine kidney [[13]]. Based on previous studies, this study will isolate and characterize the uricase from chicken (*Gallus gallus domesticus*) liver which are broiler and native chicken liver. The different sources of chicken are due to the differences between the two types of chicken liver caused by the feed and physical activity of that chicken itself, thus are allowing for different enzyme-specific activity values and the long-term prospects are expected to be applied to the prevention of uric acid in the human body.

2. Materials and methods

Fresh chicken liver was taken from the chicken slaughterhouse at Jl. Danau Toba, Tegal Gede, Sumber Sari, Jember. Chicken liver was crushed and meshed using a blender and added sodium borate buffer (0.1 M, pH 10) in a ratio of 1:10 as the crude extract. The chicken liver crude extract was centrifuged at 13,000 rpm at 4 °C for 30 minutes. The pellet that was obtained was resuspended using the same solvent. The pellet and supernatant were centrifuged at 15,000 rpm at 4 °C for 30 minutes to obtain the combined supernatant.

The obtained supernatant was followed by parallel fractionation using ammonium sulfate (0–20%, 20–40%, and 40–60% saturation of ammonium sulfate). Solid $(NH_4)_2SO_4$ is added slowly to the supernatant at 4 °C under gentle stirring. Then the mixture was centrifuged at 12,000 rpm for 30 minutes at 4 °C. The uricase activity and protein content of pellet and supernatant were determined. Then the ammonium sulfate residue in the optimum fraction was removed by dialysis overnight at 4 °C. The fraction was placed into cellophane bags and immersed in the same buffer. The buffer was changed every 3 hours so that the diffusion process could continue.

The uricase assay was done by adding 2 ml of borate buffer (0.1 M, pH 8.5) into 0.02 ml of uricase suspension and uric acid solution (3.57 mM, 0.01 mL), then incubated for 25 minutes at 37 °C. The enzyme reaction is stopped using the temperature of boiled water. The uricase activity can be determined from the amount of uric acid residue that is not degraded by uricase enzymatic reaction using UV-Vis spectrophotometer method at 291 nm wavelength. A unit of uricase activity is equal to 1 μmol degraded uric acid per minute under certain temperature conditions.

The protein content was determined using the Bradford method with Bovine Serum Albumin as standard. The measurement was done by adding Bradford's reagent (1 mL) to the protein solution (0.1 mL) then being vortexed and allowed to stand for a while. After that, the absorbance was determined using a UV-Vis spectrophotometer at 595 nm. The protein content was obtained from absorbance conversion using standard curves [[14]]. The molecular weight of uricase was determined using SDS-PAGE.

3. Results and discussion

Uricase is an enzyme that can degrade uric acid into allantoin and H_2O_2 for secondary products. Uricase isolation was done in several stages like extraction and purification using fractionation and dialysis. The determination of uricase molecular weight was also carried out by SDS-PAGE. The extraction stage was carried out using centrifugation speed 13,000 rpm and 15,000 rpm. The activity of the crude extract can be seen in **Table 1**.

Based on the extraction results, the highest uricase activity was obtained using centrifugation speed of 15,000 rpm. The uricase activity in pellets was not used for the purification process because the uricase activity in pellets was lower than in supernatants. The crude extract activity showed that the centrifugation speed affected the uricase specific activity values. A greater centrifugation speed causes many proteins other than the target protein to settle, so the other proteins are left in the supernatant in a small amount and do not really interfere with the uricase activity. The extract with the highest uricase activity was used in the fractionation optimization process. The uricase activity from the fractionation optimization process can be shown in **Table 2**.

The uricase activity in pellets was not used for the purification process because uricase activity in pellets was smaller than that of supernatants. The optimum fraction of both types of chicken liver lies in the supernatant. This is because the size of the other proteins is larger than the target protein, so when the ammonium sulfate is added, the protein with the larger size has been precipitated and leaves the target protein in the supernatant.

The (20–40%) fraction was determined as the optimum fraction in broiler chicken liver with uricase specific activity of 1.854×10^{-2} U/mg. This is consistent with the previous research which has been done, the isolation of the uricase enzyme from *Chlamydomonas reinhardtii* using 25% saturation of ammonium sulfate [[15]]. Uricase was isolated from soybean root nodules using 35% saturation [[16]]. The fraction (40-60% saturation of ammonium sulfate) was determined as the optimum fraction in native chicken liver with uricase specific activity of 2.496×10^{-2} U/mg. This is in accordance with the previous study using 60% saturation of ammonium sulfate in isolation of uricase from *C. utilis* [[17]].

The optimum fraction was used for the production stage or the isolation stage using a larger volume with the same isolation technique. The uricase purity at the production stage is shown in **Table 3**. **Table 3** shows that the uricase purity from the extraction process to dialysate has been increasing. The increased uricase specific activity can be seen from the increase in purification fold. The uricase specific activity of broiler chicken liver extract was 4.921×10^{-4} U/mg and increased to 3.989×10^{-3} U/mg in the fractionation process. The dialysate also increased to 5.120×10^{-2} U/mg. The uricase specific activity of native chicken liver extract was 2.980×10^{-4} U/mg and increased during the fractionation process by 1.415×10^{-2} U/mg. The dialysate also increased to 1.753×10^{-2} U/mg.

Table 1. Extraction result

Sample	Volume (ml)	Enzyme Activity (U/ml)	Total Activity (U)	Protein Content (mg/ml)	Total Protein (mg)	Enzyme Specific Activity (U/mg)
Extract A/ 13,000 rpm	8	7.005×10^{-3}	5.60×10^{-2}	30.325	3.791	2.310×10^{-4}
Extract B/ 13,000 rpm	8	3.914×10^{-3}	3.13×10^{-2}	29.300	3.663	1.336×10^{-4}
Extract A/ 15,000 rpm	6	4.636×10^{-3}	2.78×10^{-2}	0.290	0.048	1.599×10^{-2}
Extract B/ 15,000 rpm	6	4.430×10^{-3}	2.66×10^{-2}	0.687	0.114	6.452×10^{-3}

A: Broiler chicken liver; B: Native chicken liver

Table 2. Optimization of fractionation

Sample	Fraction	Volume (ml)	Enzyme Activity (U/ml)	Total Activity (U)	Protein Content (mg/ml)	Total Protein (mg)	Enzyme Specific Activity (U/mg)
Supernatant A	0-20	1.5	1.349×10^{-2}	2.024×10^{-2}	0.886	1.329	1.524×10^{-2}
	20-40	1.3	1.607×10^{-2}	2.089×10^{-2}	0.867	1.127	1.854×10^{-2}
	40-60	1.05	5.872×10^{-3}	6.166×10^{-3}	0.674	0.708	8.717×10^{-3}
Supernatant B	0-20	1.5	6.490×10^{-3}	9.735×10^{-3}	1.214	1.821	5.344×10^{-3}
	20-40	1.3	8.550×10^{-3}	1.112×10^{-2}	1.352	1.758	6.326×10^{-3}
	40-60	1.05	1.628×10^{-2}	1.709×10^{-2}	0.652	0.685	2.496×10^{-2}
Pellet A	0-20	1.5	6.902×10^{-3}	4.601×10^{-3}	1.207	1.811	5.717×10^{-3}
	20-40	1.5	4.430×10^{-3}	2.953×10^{-3}	2.448	3.672	1.810×10^{-3}
	40-60	1.5	8.035×10^{-3}	5.357×10^{-3}	0.991	1.487	8.109×10^{-3}
Pellet B	0-20	1.5	3.502×10^{-3}	2.335×10^{-3}	1.337	2.006	2.619×10^{-3}
	20-40	1.5	1.751×10^{-3}	1.167×10^{-3}	1.921	2.882	9.115×10^{-4}
	40-60	1.5	5.666×10^{-3}	3.777×10^{-3}	1.063	1.595	5.330×10^{-3}

A: Broiler chicken liver; B: Native chicken liver

Table 3. Production

Sample	Volume (ml)	Enzyme Activity (U/ml)	Total Activity (U)	Protein Content (mg/ml)	Total Protein (mg)	Enzyme Specific Activity (U/mg)	Purification (fold)
Extract A/15,000 rpm	32.3	1.545×10^{-3}	4.990×10^{-2}	3.140	101.416	4.921×10^{-4}	1.00
Fraction A 40%	16.5	5.872×10^{-3}	9.689×10^{-2}	1.472	24.285	3.989×10^{-3}	8.11
Dialysate A	5	1.236×10^{-3}	6.180×10^{-3}	0.242	1.208	5.120×10^{-3}	10.40
Extract B/15,000 rpm	30	9.271×10^{-4}	2.781×10^{-2}	3.108	93.225	2.980×10^{-4}	1.00
Fraction B 60%	16.1	2.987×10^{-3}	4.809×10^{-2}	0.211	3.400	1.415×10^{-2}	47.48
Dialysate B	5	3.090×10^{-3}	1.545×10^{-2}	0.176	0.882	1.753×10^{-2}	58.83

A: Broiler chicken liver; B: Native chicken liver

The results of uricase isolation using broiler chicken liver and native chicken liver have a smaller specific activity value compared to the previous study which used aves family (*Gallus gallus*) liver as an uricase source with the uricase specific activity of 0.030 U/mg [[18]]. This is due to differences in centrifugation techniques. Isolation of uricase using *Gallus gallus* as a sample was carried out by ultracentrifugation technique. The ultracentrifugation technique has more specific sample preparation in separating proteins based on the layer of the cells compared to ordinary centrifugation techniques, so that the protein from the uricase obtained is purer. The molecular weight of each isolation stage was determined using SDS-PAGE. The electrophoregram is shown in **Figure 1**. Based on the electrophoregram in **Figure 1**, the target protein is around 35 kDa. This is consistent with the previous study which reported that the molecular weight of the avian uricase was 35 kDa [[5]]. Another study also reported that the molecular weight of the uricase from Pseudomonas aeruginosa was 33 kDa [[19]] and uricase from bovine kidney was 70 kDa [[13]]. The molecular weight difference of uricases is due to the different species that they come from.

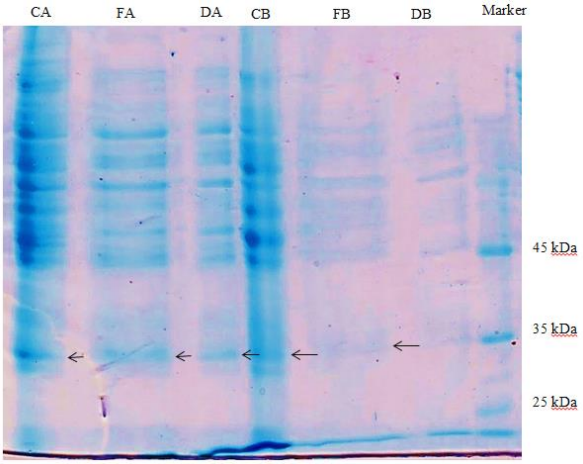

Figure 1. SDS-PAGE result (C: extract; F: fraction; D: dialysate; A: broiler chicken liver; B: native chicken liver)

4. Conclusions

Centrifugation speed with maximum uricase activity was 15,000 rpm with a value of 1.599×10^{-2} U/mg for broiler liver and 6.452×10^{-3} U/mg for native chicken liver. The optimum fraction for broiler chicken liver was in the fraction (20–40%) with a value of 1.854×10^{-2} U/mg, while the native chicken liver was in the fraction (40–60%) with a value of 2.496×10^{-2} U/mg. The dialysis process had a maximum uricase activity of 5.120×10^{-3} U/mg for broiler liver and 1.753×10^{-2} U/mg for native chicken liver. The uricase molecular weight was obtained for broiler and native chicken livers and was around 35 kDa. The uricase produced from broiler and native chicken liver can not be applied to the prevention of gout because it has a lower enzyme specific activity value than the uricase source from *Gallus gallus* liver which is 0.030 U/mg.

References

[1] R. M. Kong, A. Yang, Q. Wang, Y. Wang, L. Ma, and F. Qu. Uricase Based Fluorometric Determination of Uric Acid Based on the Use of Graphene Quantum Dot@Silver Core-Shell Nanocomposites. *Microchimica Acta*. pp. 2–8, 2017.

[2] E. Diantari and A. Candra. Pengaruh Asupan Purin dan Cairan terhadap Kadar Asam Urat Wanita Usia 50-60 Tahun di Kecamatan Gajah Mungkur, Semarang. Journal of Nutrition College. 2(1):44–49, 2013.

[3] R. M. Hafez, T. M. Abdel-Rahman, and R. M. Naguib. Uric Acid in Plants and Microorganisms: Biological Applications and Genetics - a review. Journal of Advanced Research. 8:475–486, 2017.

[4] P. Montalbini, M. Aguilar, and M. Pineda. Isolation and Characterization of Uricase from Bean Leaves and Its Comparison with Uredospore Enzymes. Plant Science. 147:139–147, 1999.

[5] S. K. Abdullah and M. T. Flayyih. Production , Purification and Characterization of Uricase Produced by Pseudomonas aeruginosa. Iraqi Journal of Science. 56(3):2253–2263, 2015.

[6] A. Magda, S. Tork, S. Al-Garni, and R. Allam. Production and Characterization of Uricase from Streptomyces Exfoliatus Ur10 Isolated from Farm Wastes. Turkish Journal of Biology. 37:520–529, 2013.

[7] S. O. Pustake, P.K. Bhagwat, P.B. Dandge, Statistical media optimization for the production of clinical uricase from Bacillus subtilis strain SP6. Heliyon 5, 5, 2019.

[8] S.E. Tork, M.M. Aly and S.Q. Al-Fattani, A new uricase from Bacillus cereus SKIII: Characterization, gene identification and genetic improvement. International Journal of Biological Macromolecules, 2018.

[9] N.E.A. El-Naggar, S.A. Haroun, E.M. El-Weshy, E.A. Metwally, A.A. Sherief, Mathematical modeling for bioprocess optimization of a protein drug, uricase, production by Aspergillus welwitschiae strain 1–4. Sci. Rep. 9 (1) 1-15, 2019.

[10] J. E. Kinsella, B. German, and J. Shetty. Uricase from Fish Liver: Isolation and Some Properties. Comparative Biochemistry and Physiology -- Part B: Biochemistry And. 82B(4):621–624, 1985.

[11] S. Yainoy, T. Phuadraksa, S. Wichit, M. Sompoppokakul, N. Songtawee, V. Prachayasittikul, C. Isarankura-Na-Ayudhya, Production and Characterization of Recombinant Wild Type Uricase from Indonesian Coelacanth (L. menadoensis) and Improvement of Its Thermostability by In Silico Rational Design and Disulphide Bridges Engineering. Int. J. Mol. Sci. 20 (6): 1269, 2019.

[12] A. Osman, A. Del Corso, P. Ipata, and U. Mura. Liver Uricase in Camelus dromedarius: Purification and Properties. Comp. Biochem. Physiol. 94B(3):469–474, 1989.

[13] M. I. Rajoka, K. Ur-Rehman, M. Mehraj, M. W. Akhtar, and M. A. Zia. Purification, and Properties of a Bovine Uricase. Protein and Peptide Letters. 13:363–368, 2006.

[14] M. M. Bradford. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry.72: 248-254, 1976.

[15] J. M. Alamillo and J. Cfirdenas. Purification and Molecular Properties of Urate Oxidase from Chlamydomonas reinhardtii. Biochimica et Biophysica Acta. 1076:203–208, 1991.
[16] K. Lucas, J. B. Michael, and R. S Karel. Uricase from Soybean Root Nodules: Purification, Properties and Comparison with the Enzymes from Cowpea. Archive of Biochemistry and Biophysics. 226(1):190-197, 1983.
[17] V. Adamek, B. Kralova, M. Schova., O. Valentova and K. Demnerova. Purification of Microbial Uricase. Journal of Chromatography.497:268-275, 1989.
[18] P. J. Scott, L. P. Visentint and J. M. Allen. The Enzymatic Characteristics of Peroxisomes of Amphibian and Avian Liver and Kidney. Ann. N. Y. Acad. Sci. 168:244-264, 1969.
[19] A. Anderson and S. Vijayakumar. Purification and Optimization of Uricase Enzyme Produced by Pseudomonas aeruginosa. Journal of Experimental Sciences. 2(11):5–8, 2011.