Jurnal Kimia Valensi, Vol 7(2), November 2021, 158-167
Available online at Website: http://journal.uinjkt.ac.id/index.php/valensi

Extraction and Characterization of Urease from *Durio zibethinus* L

Zusfahair*, Amin Fatoni, Dian Riana Ningsih, Mardiyah Kurniasih, Puspita Chandra Alviana

Department of Chemistry, Faculty of Mathematics and Natural Sciences Universitas Jenderal Soedirman, Purwokerto. Jl. Soeparno 62, Karangwangkal, Purwokerto, Central Java, Indonesia 53123

**Corresponding author: zusfahair@gmail.com*

Received: July 2021; Revision: Agustus 2021; Accepted: September 2021; Available online: November 2021

Abstract

Urease is a biocatalyst that serves to hydrolyze urea into ammonia and carbon dioxide. Since it is an imported product, the price of urea is still high. Urease can be found in grains. One of the grains that has not been explored for its urease content is durian (*Durio zibethinus* L.) seeds. This study aims to determine the effect of germination time on the activity of urease from durian seeds and its characteristics including the effect of pH, incubation temperature, enzymatic reaction time, addition of EDTA and metals, and storage time on the activity of urease from durian seeds. The first step of this study was seed germination which was carried out in the dark for 0, 3, 5, 7, and 9 days. Durian seed sprouts were extracted by mashing them using a mortar and pestle. They were then homogenized using a stirrer and centrifuged in a cold state. The crude urease extract obtained was then tested for its activity using the Nessler method. The acquired data was tested statistically using ANOVA. The results showed that the activity of urease from durian seeds was optimum at 3-day germination time, pH 7, incubation temperature at 30 °C, and 15-minute enzymatic reaction time with an activity of 163.6 U/mL. Urease is a metalloenzyme with its inhibitor being the Cu^{2+} and Na^{+} metal ions and its activator being Ba^{2+} metal ion. Tukey's test analysis showed that the effect of urease storage time at 4 °C resulted in a stable urease activity for 8 days while at room temperature it decreased its activity significantly to 72.8%.

Keywords: *Durio zibethinus* L., Nessler method, urease.

DOI: *10.15408/jkv.v7i2.21768*

1. INTRODUCTION

Urease is a biocatalyst that serves to hydrolyze urea into ammonia and carbon dioxide. It plays a role, among other things, to improve the quality of alcoholic beverages by lowering the level of urea, the precursor of carcinogenic ethyl carbamate (Liu *et al.*, 2012), to detect heavy metals in milk (Kaur *et al.*, 2014), to be antifungal (Postal *et al.*, 2012), in hemodialysis, and space missions to be life support (Iyer *et al.*, 2018).

It is a protein that can be found in bacteria, molds and several higher plants (El-hefnawy *et al.*, 2014) such as such as cereals and legumes (Modolo *et al.*, 2015). Various seeds have been isolated and produce urease such as *Cicer arietinum* L seeds (Pervin *et al.*, 2013), *Pisum Sativum* L seeds (El-hefnawy *et al.*, 2014), *Vigna unguiculata ssp unguiculata* L. seeds (Zusfahair, *et al.*, 2018), *Vigna unguiculata ssp sesquipedalis* L. seeds (Zusfahair, *et al.*, 2018), and jackfruit seeds (Chouhan *et al.*, 2018). Durian seeds contain nutrients such as protein. Each durian seed contains 2.6 g / 100 mg protein (Nurfiana *et al.*, 2009). This makes durian seeds a potential source of enzymes, including urease. Indonesia's production of durian is abundant, i.e., 1,133,195 tons in 2020 (BPS, 2021).

The large amount of durian production results in increased number of its seeds. To prevent this abundant number of durian seeds from being wasted and polluting the environment, their utilization needs to be increased. One way of doing so is to use durian seeds as a source of urease. To obtain urease from durian seeds, they need to be extracted to figure out their characteristics first. In a previous study, the urease from durian seeds was extracted by grinding it using mortar and

P-ISSN: 2460-6065, E-ISSN: 2548-3013

pestle. It was then homogenized using a stirrer and centrifuged when it was cold to obtain crude urease extract. The crude extract was characterized in terms of the influence of pH, incubation temperature, enzymatic reaction time, addition of EDTA and metals, and enzyme storage time on the urease activity of durian seeds.

2. MATERIALS AND METHODS

Tools and Materials

The tools used in this research are those commonly used in a biochemistry laboratory plus supporting tools such as centrifuge (*Quantum*), UV spectrophotometer (*Shimadzu UV-1800)*, pH meter (*Hanna* Instrument), analytical balance (*Ohaus*), incubator (*Memmert*). As for the materials, they include local durian seeds purchased from durian farmers in Kemranjen District, Banyumas, distilled water, wrapping, urea (*Merck*), sulfuric acid (*SmartLab* Indonesia), citric acid (*Merck*), sodium citrate (*Merck*), NaH_2PO_4 (*Merck)*, Na_2HPO_4 (*Merck*), tris base, HCl, ammonium sulfate (Merck), Na-tungsten, AgCl, $CuCl_2$, $BaCl_2$, EDTA and Nessler's reagent (*Merck*).

Procedure

Determining The Standard Curve of Ammonium Sulfate

The calibration curves were made using standard solutions of ammonium sulfate at 20, 30, 40, 50, and 60 ppm concentrations. A total of 1.5 mL of standard solution and 1.5 mL of distilled water as blanks were put into different cuvettes and 0.25 mL of Nessler's reagent were added to each of them. Finally, the absorption was measured using a UV-Vis spectrophotometer at 500 nm wavelength (Zusfahair, *et al.,* 2018).

Determining Durian Seeds

The durian seeds were determined at the Biology Faculty of Unsoed under an acceptance test letter number: 127/HP.LL/V/2019.

Extraction of Urease from Durian Seeds

The extraction of urease from durian seeds began with a germination process as follows: The cleaned durian seeds were germinated by putting them into a container which contained wet cotton. The container was put into previously perforated black plastics and the germination was carried out in the dark for 0, 3, 5, 7, and 9 days. Next, the urease was extracted with the following steps: 20 g of germinated durian seeds were weighed and crushed using a mortar and pestle in a cold state. The finely crushed durian seeds were soaked in 80 mL of cold 0.2 M phosphate buffer solution pH 7 and homogenized using a stirrer for 3 hours. Using the stirrer, they were mixed in cold condition. The results were then centrifuged at 4 ^oC for 15 minutes at 12000 rpm. The supernatant obtained was a crude extract of urease from durian seeds to be used for activity and characterization tests. The data from activity and characterization tests were collected three times (Zusfahair, *et al.,* 2018).

Activity Test of Urease from Durian Seeds

The activity test of urease from durian seeds was carried out by pipetting 1.95 mL of 0.2 M phosphate buffer pH 7, 50 µL of crude extract of urease from durian seeds, and 1 mL of 1000 ppm urea solution as a substrate which was put into a test tube and homogenized. The solution was then incubated at 35 ^oC for 15 minutes and then cooled down. Once it cooled down, 1 mL of H_2SO_4 2/3 N solution and 1 mL of 10% Na-tungsten solution were added to the solution to stop the enzyme activity. For the control treatment, the same order of solution sampling was repeated, i.e., by pipetting 2 mL of 0.2 M phosphate buffer pH 7, 1 mL of H_2SO_4 2/3 N solution, 1 mL of 10% Na-tungsten solution, and 1 mL of 1000 ppm urea solution as substrates were put into a test tube. Both the sample and control solutions were centrifuged to separate the precipitate. 1.5 mL of the supernatant obtained was taken and 250 L of Nessler's reagent was added and its absorption was immediately measured at 500 nm wavelength using a UV-Vis spectrophotometer. The unit of urease enzyme activity was determined based on the equation;

$$\frac{([s] - [k]) \text{ x } 5}{1.5 \text{ x } 0.05 \text{ x } 15} \qquad (1)$$

Where: [s] = ammonia concentration in the sample; [k] = ammonia concentration in the control; 5 = total solution volume (mL); 1.5 = solution volume from hydrolysis whose absorbance was measured (mL); 0.05 = enzyme volume used (mL) and 15 = enzymatic reaction time (minutes).

The ammonia concentration was calculated using the equation formula for determining the standard curve of ammonium sulfate. One unit of urease enzyme activity is the amount of enzyme needed to release 1 mmol of NH_3 from urea per minute under standard condition (Zusfahair, *et al.,* 2018).

Characterization of Urease from Durian Seeds

The urease from durian seeds was characterized by determining the optimum condition of an enzyme which include the influence of pH, incubation temperature, enzymatic reaction time, influence of EDTA and metal additions, and enzymatic storage time on the activity of urease from durian seeds.

The urease activity was determined using the same mechanism as in the activity test. Each optimum condition obtained was used for further research. The influence of pH was figured out by varying 0.2 M pH buffer using citrate buffer pH 5, phosphate buffer pH 6, phosphate buffer pH 7, phosphate buffer pH 8, and tris HCl buffer pH 9; the temperatures were varied at 25, 30, 35, 40, and 45 ^oC; and the incubation times were varied for 5, 15, 25, 35, 45, and 55 minutes. The influence of metal ($CuCl_2$, NaCl, dan $BaCl_2$) and EDTA variations on the activity of urease from durian seeds was figured out by adding the metal and EDTA concentration variations until the last concentration of 10^{-3}-10^{-8} M.

Influence of Enzyme Storage on Activity of Urease from Durian Seeds

The urease was stored at 4 ^oC and room temperature for 0-10 days. Its activity was then determined under optimum condition and analyzed using Tukey Test (Bedan, 2020).

Data Analysis

The obtained research data were analyzed using one-way ANOVA statistically using IBM SPSS Statistics Version 25 to distinguish the repetition variations and differences between treatments. The fixed variables in this study were pH, incubation temperature, enzymatic reaction time, EDTA and metal addition, and storage time. The independent variable in this study was the urease enzyme activity. Each variable was analyzed using one factor ANOVA separately and in stages. The ANOVA results which showed significant differences were followed with Tukey test at 95% confidence.

3. RESULTS AND DISCUSSION

This research uses durian seeds as the source of urease. These durian seeds are determined first to ensure that its species is correct. The determination result showed that the durian seeds used belonged to the *Bombacaceae* family and *Durio* genus. The species name of the durian seeds used in this research was *Durio zibethinus* L.

Making The Standard Curve of Ammonium Sulfate

The standard curve was made to discover the correlation between standard solution concentration and its absorbance value to figure out the concentration of a sample. The standard used was ammonium sulfate solution. The ammonium sulfate solution was used as the standard solution since when solved in water ammonium sulfate would be ionized and produce two ammonium ions. The result of ammonium sulfate reaction in water was similar to the result of urea hydrolysis by urease which produced two ammonia molecules (Singh *et al.,* 2017). The two ammonia molecules formed when they were in water would be ionized into two ammonium ions (NH_4^+). Below is the occurring equilibrium reaction (Ambarsari *et al.,* 2020):

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^-$$

The produced ammonium ions were detected using Nessler method and their absorbance was measured using spectrophotometer at a maximum wavelength of 500 nm. The Nessler method was proposed by J. Nessler in 1856 using a basic solution of mercury (II) iodide in potassium iodide (K_2HgI_4) as a reactant for determining ammonia colorimetrically (Patri, 2018). The principle of this method is based on the Nessler reactant (K_2HgI_4) which, when reacting with ammonia, would produce a yellow-brown colloidal dispersion. The intensity of the resulting color is directly proportional to the concentration of ammonium in the sample (Jeong *et al.,* 2013). The data on standard curve calculation can be seen in **Figure 1.**

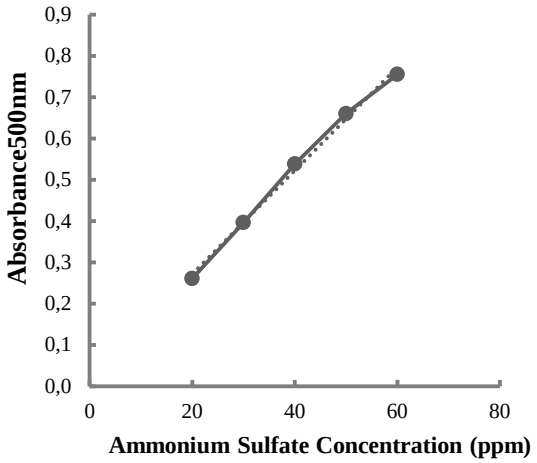

Figure 1. Standard curve of ammonium sulfate.

The line equation obtained from the standard curve measurement is y = 0.0125x + 0.0212 where a = 0.0212; b = 0.0125; and R^2 = 0.9947. The linear standard curve is expressed with correlation coefficient (r), r value is close to 1, indicating that the resulting regression equation is linear. Based on R^2 value, the obtained r value is 0.9973, meaning that the standard curve of ammonium sulfate produces a strong linear response where every increase in concentration is directly proportional to an increase in absorbance.

Influence of Germination Time on Activity of Urease from Durian Seeds

Germination is the beginning of plant growth. It might be influenced by several factors, both internal and external ones. The internal factors include level of seed maturity, seed size, dormancy, and germination inhibition. The external factors include water, temperature, oxygen, and light (Sutopo, 2004). This germination process will produce urease enzyme which will be used to degrade the urea formed in the urea cycle. This urea cycle occurs because of the ammonia resulting from the degradation of amino acids (Modolo *et al.*, 2015).

The germination began with placing cleaned durian seeds on half-wet cotton. This is to prevent the durian seeds from drying out, rotting and undergoing an imbibition process. The germination durian seeds took place in the dark for 0, 3, 5, 7, and 9 days to prevent the performance of the auxin hormone from being disrupted by sunlight. The influence of durian seed germination time on the activity of urease from durian seeds can be seen in **Figure 2.**

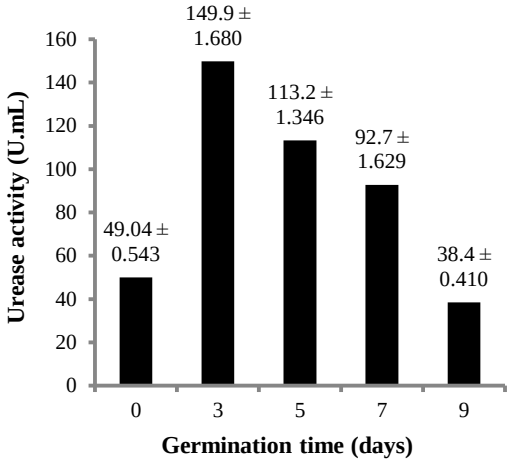

Figure 2. Influence of germination time of durian seeds on urease activity.

The data in **Figure 2** shows that urease activity is still low before the third day. This is because the seeds' main food reserves are carbohydrates, fats and proteins. The first food reserves that can be broken down first into a source of energy is carbohydrate. Breaking down carbohydrate requires enzymes that can catalyze the breakdown, thus when the germination time is less than three days, not much urease is produced. When no carbohydrate is left, then fat and then protein will be used. Assisted by protease, protein will then be broken down into amino acids. The amino acids are degraded to produce ammonia and it can activate the urea cycle to form urea. This will lead to urease activity which serves to hydrolyze the urea (Bahri & Mirzan, 2012). This stage makes urease production increase as characterized by the magnitude of the activity of urease from durian seeds on the 3rd day of germination at 149.9 U/mL. A decrease in urease activity occurred on the 5th, 7th, and 9th days of germination. This is because the amount of urease produced is decreasing and the food reserves in the cotyledons are slowly starting to run out.

Characterization of Urease from Durian Seeds

Influence of pH Variations on Activity of Urease from Durian Seeds

Data on influence of pH on urease activity can be seen in **Figure 3.**

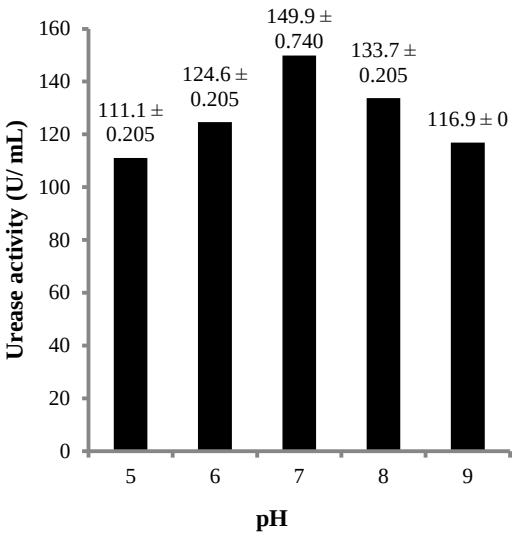

Figure 3. Influence of pH on activity of urease from durian seeds

The data in **Figure 3** indicates that the highest activity of urease from durian seeds is at pH 7. The urease activity value at pH 7 is 149.9 unit/mL. The highest activity means that pH 7 is the optimum pH for urease from durian seeds. This optimum pH makes the enzyme conformation in a condition which suits the substrate, hence the enzyme and the substrate interact maximally to turn a enzyme-substrate complex into a product (Habibie *et al.,* 2014). The change in enzyme activity at varied pH is caused by the change in enzyme ionization, substrate or enzyme-substrate complex (Akhtaruzzaman *et al.,* 2012).

The activity of urease from durian seeds is neutral at pH 7. Similar result is also obtained in the urease from some grains such as from *Pisum Sativum* L seeds which has 7.5 pH optimum with 190 U/mL activity (El-hefnawy *et al.,* 2014). The urease from *Vigna unguiculata ssp sesquipedalis* L. seeds has 7 pH optimum with 407.62 U/mL activity value (Zusfahair, *et al.,* 2018). This pH optimum makes the enzyme conformation in a condition which suits the substrate, hence the enzyme and the substrate interact maximally to turn the enzyme-substrate complex into a product (Habibie *et al.,* 2014).

The fact that some pH is below the neutral might disrupt the urease conformation, hence its activity decreases. The amino acid in the urease's active site could be affected by the changes in reaction media pH which changes the active site's amino acid ionization (Singh *et al.,* 2017).

Influence of Temperature Variation on Activity of Urease from Durian Seeds

The data on the influence of temperature on the activity of urease from durian seeds can be seen in **Figure 4.**

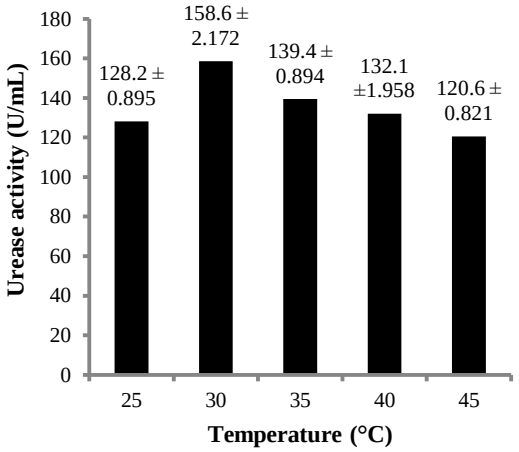

Figure 4. Influence of temperature on activity of urease from durian seeds

Based on the data in **Figure 4**, the enzyme activity increases from 25 °C and reaches the optimum at 30 °C, i.e., 158.6 U/mL. An increase in temperature from 25 °C to 30 °C (optimum temperature) will increase the enzyme reaction speed as the kinetic energy increases. The increased kinetic energy will increase the molecule movement, making the collision between enzyme and substrate molecules faster, thus resulting in equally faster reaction rate. Once the reaction rate is fast, the enzyme activity actively works and products increase. Meanwhile, any incubation temperature exceeding the optimum limit makes the enzyme molecule exceed the energy inhibitor and the hydrophobic and hydrogen bonds which plays a role in maintaining the enzyme functional structure break, leading to decreased activity (Singh *et al.,* 2017). According to Bano *et al.,* (2011) any enzyme activity at 30 °C and above decreases, maybe because when the temperature increases the progressive enzyme inactivation occurs because of the protein thermal inactivation in its structure, or maybe because of the incorrect protein conformation, peptide chain hydrolysis, amino acid breakdown, or aggregation.

The optimum temperature of urease from durian seeds has different characteristic from that of urease from *Pissum sativum* L. The urease from *Pissum sativum* L seeds has

40 °C optimum temperature with 190 U/mL activity (El-hefnawy *et al.*, 2014).

Influence of Enzymatic Reaction Time on Activity of Urease from Durian Seeds

The data on the influence of enzymatic reaction time on activity of urease from durian seeds can be seen in **Figure 5.**

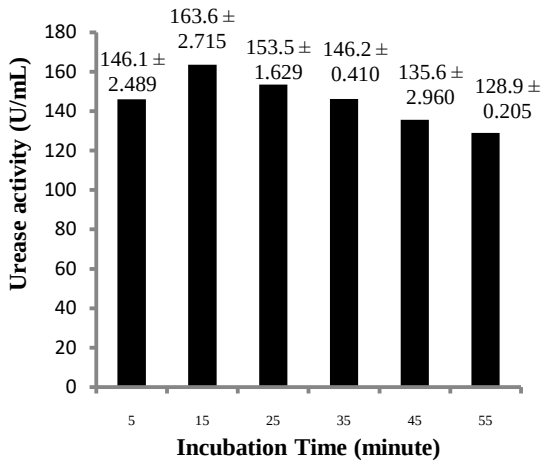

Figure 5. Influence of enzymatic reaction time on activity of urease from durian seeds

The data in **Figure 5** shows that the highest activity is obtained after 15 minute of enzymatic reaction time at 163.6 U/mL. The highest activity shows that the 15-minute enzymatic reaction time is the optimum reaction time of urease enzyme from durian seeds. The urease from durian seeds catalyzes the reaction of urea into ammonia. The longer the enzymatic reaction time, the more ammonia is produced. The resulting ammonia is suspected to increase the reaction pH (Modolo *et al.*, 2015). This pH increase makes the enzyme activity decrease.

Influence of metal and EDTA Ion Variations on Activity of Urease from Durian Seeds

The result of determination of influence of metal and EDTA variations on the activity of urease from durian seeds can be seen in **Figure 6.** The data was calculated in the form of relative activity. The control shows that when no metal nor EDTA ion is added, the urease enzyme has relative activity 100%. The relative activity is enzyme activity when metal or EDTA is added at certain concentrations and divided by the enzyme activity with no metal nor EDTA addition.

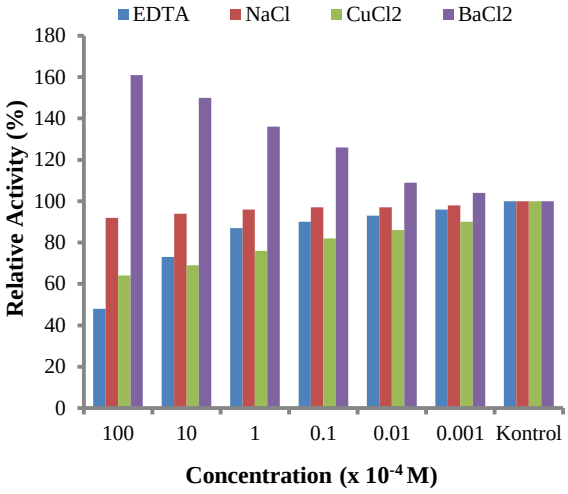

Figure 6. Influence of EDTA and metal on activity of urease from durian seeds

The data in **Figure 6** shows that EDTA addition at 10^{-2}–10^{-7} M concentrations decrease the urease activity. The inhibition effect on the enzyme activity increases parallelly with the increase in EDTA concentration. Pervin *et al.*, (2013) suggest that adding EDTA to urease enzyme from *Cicer arietinum* L. seeds also decreases the urease activity. From the decreased urease activity resulting from EDTA addition, it can be concluded that the urease from durian seeds is a metalloenzyme. Metalloenzyme is an enzyme which has a strong bond with certain metal ions which can activate enzyme (Kumari *et al.*, 2013).

The active site of urease contains a binuclear nickel center (**Figure 7**). The center of the nickel (II) ion is bridged by carbamylated lysine through its atom O, Ni(1) is then coordinated by two histidines through its atom N, and Ni(2) also by two histidines through its atom N and added by aspartic acid through its atom O. In addition, Ni ion is bridged by hydroxide (WB) ion, which together with two terminal water molecules, W1 in Ni(1), W2 in Ni(2), and W3 located the opening of the active site, forming a H-bonded water tetrahedral cluster, fulfill the active site cavity. It is this cluster which is replaced by urea when bonding to the active site for reaction (Krajewska, 2009).

EDTA can lower enzyme activity by chelating metals which serve as activators in the enzyme. The activator metals in the enzyme that supposedly activate the active site

of the enzyme is bound by EDTA and produces a new complex. This leads to the removal of metal ions located at or near the active site of the enzyme, resulting in the decrease in enzyme activity (Pervin *et al.*, 2013) (Tallapragada *et al.*, 2017). The magnesium and calcium salts are urease activators from *Vicia faba* L. (Bedan, 2020).

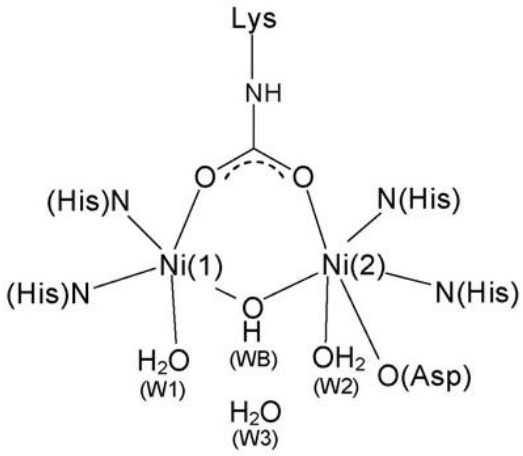

Figure 7. Structure of urease active site (Krajewska, 2009)

Different salts, consisting of NaCl dan $CuCl_2$, were tested to estimate their effect on urease activity. The addition of NaCl and $CuCl_2$ solutions as sources of metal Na^+ and Cu^{2+} ions at 10^{-2}–10^{-7} concentrations decrease the activity of urease from durian seeds. Some studies discuss the causes of decreased enzyme activity resulting from metals addition. 1) Metal ions can bind to catalytic residues at the enzyme's active site, causing enzyme inactivation (Wu *et al.*, 2018). 2) The binding of copper ions disrupts the protein structure (Saboury, 2010). 3) Competition between exogenous cations and enzyme-related cations results in decreased metalloenzyme activity (Kiran *et al.*, 2018). From the decrease in urease enzyme activity resulting from NaCl and $CuCl_2$ addition, it can be concluded that Na^+ and Cu^{2+} the metal ions deriving from the solution are inhibitors.

The addition of $BaCl_2$ solution as a source of Ba^{2+} metal ion at 10^{-2}–10^{-7} M concentration increases the urease enzyme activity. The lowest relative activity at 10^{-7} concentration is 103.9% and highest relative activity at 10^{-2} concentration is 161.0%. (Pervin *et al.*, 2013) suggest that the addition of $BaCl_2$ solution to the enzyme urease from *Cicer arietinum* L. increases the urease enzyme activity. The research finds that the highest relative activity at 3 mM concentration is 113% and the lowest at 1 mM and 5 mM concentrations are 105% and 103%. From the increased urease enzyme activity resulting from the addition of $BaCl_2$ solution, it can be concluded that Ba^{2+} metal ion deriving from the solution is an activator.

Urease is an enzyme that catalyzes the urea hydrolysis reaction. The reaction mechanism is assumed to be similar to that of the active site of urease (**Figure 7**), where urea binds the more electrophilic Ni(1) ion to its carbonyl oxygen atom. As the carbonyl carbon is more electrophilic, it is more susceptible to nucleophilic attack. After replacing the W1-W3 water, the urea is further bound to Ni(2) through from one of its amino clusters. This binding is believed to facilitate the nucleophilic attack of water in the carbonyl carbon, then NH_3 is released (Dixon *et al.*, 1980).

Influence of Enzyme Storage Variations on Activity of Urease from Durian Seeds

The data from the determination of influence of storage time and storage temperature variations on the activity of urease from durian seeds can be seen in **Figure 8**.

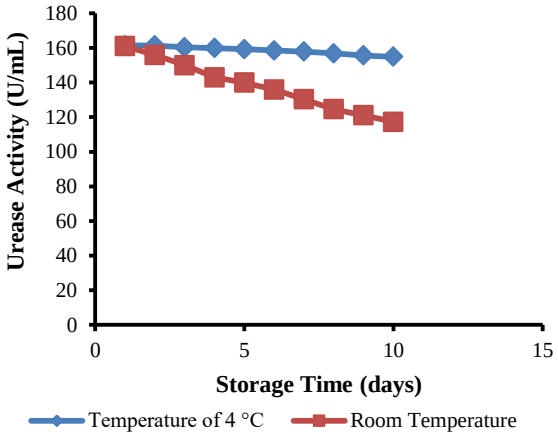

Figure 8. Influence of storage time at 4 °C and room temperature on the activity of urease from durian seeds

The influence of urease's enzymatic storage time at 4 °C and room temperature on the activity of urease from durian seeds shows that the urease's enzymatic storage time at 4

°C on days 1-10 slightly decreases the enzyme activity, and at room temperature the decrease is significant. The data analysis of storage time at 4 °C and room temperature for 10 days is then followed by a test using one-way ANOVA with Tukey HSD Test. It is then found that the storage time at 4 °C and room temperature for 10 days influences the enzyme activity ($p<0.05$).

The Tukey test shows that the storage time at 4 °C until the 8th day produces a stable enzyme activity, and at room temperature it produces significantly different enzyme activity. The storage at 4°C for 10 days, the urease from durian seeds can maintain its activity at 96% and at room temperature at 72.8%. The data on 1st day storage is used as the standard (100%). From the fact that the enzyme activity decreases significantly, it can be concluded that the enzyme is better stored at 4 °C than at room temperature. The urease from *Vicia faba* L stored at 4 °C can maintain the activity for 8 days and after that the activity decreases (Bedan, 2020). The urease from *Pisum sativum* L. seeds stored at 4 °C decreases the activity to 80% on the 10th day (El-hefnawy *et al.,* 2014).

4. CONCLUSION

Based on the research results, it can be concluded that: Urease is isolated from the germination process of durian seeds. The urease of durian seeds is characterized by its optimum enzyme activity at pH 7, 30 °C incubation temperature, and 15-minute enzymatic reaction time with activity value of 163.6 U/mL. Urease has the character to be metalloenzyme since it experiences inhibition when added with EDTA. Ba^{2+} ion serves as an activator and Na^{+} and Cu^{2+} ions serve as inhibitors. The enzyme kept at 4°C has a stable activity, i.e., having activity of 97% for 8 days, and at room temperature it significantly decreases, i.e. having activity of 77%.

ACKNOWLEDGMENT

The writers would like to thank LPPM UNSOED which has provided BLU Funds with the "Basic Research" Research Scheme in 2021, so that this research can be completed

REFERENCES

Akhtaruzzaman, M., Mozumder, N. H. M. R., Jamal, R., Rahman, A., & Rahman, T. (2012). Isolation and characterization protease enzyme from leguminous seeds. *Agricultural Science Research Journals*, *2*(8), 434–440.

Ambarsari, H., Asriyani, L., & Ridlo, A. (2020). Isolasi dan produktivitas bakteri ureolitik dari sedimen Muara Sungai Citarum (Isolation and productivity of ureolytic bacteria from Citarum River Estuary sediments). *Jurnal Teknologi Lingkungan*, *21*(2), 147–156.

Bahri, S., & Mirzan, M. (2012). Karakterisasi enzim amilase dari kecambah biji jagung ketan (*Zea mays ceratina* L .) [Characterization of amylase enzyme from glutinous corn seed (*Zea mays ceratina* L.) Sprouts]. *Jurnal Natural Science*, *1*(1), 132–143.

Bano, S., Qader, S. A. U., Aman, A., Syed, M. N., & Azhar, A. (2011). Purification and characterization of novel α-amylase from *Bacillus subtilis* KIBGE HAS. *AAPS PharmSciTech*, *12*(1), 255–261. https://doi.org/10.1208/s12249-011-9586-1

BPS. (2021). *Produksi tanaman buah-buahan 2020* (*Fruit crop production* 2020). Badan Pusat Statistik.

Bedan, D. S. (2020). Extraction, precipitation and characterization of urease from *Vicia faba* L. *Al-Mustansiriyah Journal of Science*, *31*(1), 9. https://doi.org/10.23851/mjs.v31i1.555

Chouhan, S., Vishnu Priya, V., & Gayathri, R. (2018). Extraction and partial purification of urease enzyme from jack fruit. *International Journal of Research in Pharmaceutical Sciences*, *9*(2), 438–441. https://doi.org/10.26452/ijrps.v9i2.1515

Dixon, N. E., Riddles, P. W., Gazzola, C., Blakeley, R. L., & Zerner, B. (1980). Jack bean urease (EC 3.5.1.5). V. On the mechanism of action of urease on urea, formamide, acetamide, N-methylurea, and related compounds. *Canadian Journal of Biochemistry*, *58*(12), 1335–1344. https://doi.org/10.1139/o80-181

El-hefnawy, M. E., Sakran, M., Ismail, A. I., & Aboelfetoh, E. F. (2014). Extraction , purification , kinetic and thermodynamic properties of urease from germinating *Pisum Sativum* L . seeds. *BMC Biochemistry*, *15*(15), 1–8.

Habibie, F. M., Wardani, A. K., & Nurcholis, M. (2014). Isolation and molecular identification of thermophilic microorganism producing xylanase from Hot Mud Disaster Lapindo. *Jurnal Pangan Dan Agroindustri*, *2*(4), 231–238.

Iyer, P. ., Priya, V. V., & Gayathri, R. (2018). Assessment of urease activity in *Pisum sativum* seeds. *Drug Invention Today*, *10*(9), 1810–1813.

Jeong, H., Park, J., & Kim, H. (2013). Determination of NH^+ in environmental water with interfering substances using the modified Nessler method. *Journal of Chemistry*, *2013*. https://doi.org/10.1155/2013/359217

Kaur, H., Kumar, S., & Verma, N. (2014). Enzyme-based colorimetric and potentiometric biosensor for detecting Pb (II) ions in milk. *Brazilian Archives of Biology and Technology*, *57*(4), 613–619. https://doi.org/10.1590/S1516-8913201402160

Kiran, S., Singh, A., Prabha, C., & Kumari, S. (2018). Isolation and characterization of thermostable amylase producing bacteria from Hot Springs of Bihar, India. *International Journal of Pharma Medicine and Biological Sciences*, *7*(2), 28–34.

Krajewska, B. (2009). Ureases I. Functional, catalytic and kinetic properties: A review. *Journal of Molecular Catalysis B: Enzymatic*, *59*(1–3), 9–21. https://doi.org/10.1016/j.molcatb.2009.01.003

Kumari, N., Jain, V., & Malhotra, S. (2013). Purification and characterization of extracellular acidophilic-amylase from *Bacillus cereus* MTCC 10205 isolated from soil. *African Journal of Microbiology Research*, *7*(48), 5440–5448. https://doi.org/10.5897/ajmr12.1371

Liu, J., Xu, Y., Nie, Y., & Zhao, G. A. (2012). Optimization production of acid urease by Enterobacter sp. in an approach to reduce urea in Chinese rice wine. *Bioprocess and Biosystems Engineering*, *35*(4), 651–657. https://doi.org/10.1007/s00449-011-0643-7

Modolo, L. V, Souza, A. X. De, Horta, P., Araujo, D., & Fa´tima, A. de. (2015). An overview on the potential of natural products as ureases inhibitors : A review q. *Journal of Advanced Research*, *6*(1), 35–44. https://doi.org/10.1016/j.jare.2014.09.001

Nurfiana, F., Mukaromah, U., Jeannisa, V. C., & Putra, S. (2009). Pembuatan bioethanol dari biji durian sebagai sumber energi alternatif (The making of bioethanol from durian seeds as an alternative source of energy). *Seminar Nasional V SDM Teknologi Nuklir*, *November*, 669–676.

Patri, M. Y. (2018). Penentuan kadar ammonia (NH_3) pada limbah cair K-36 dalam rangka pengendalian pencemaran lingkungan (Determination of ammonia (NH_3) level in K-36 liquid waste for controlling environmental pollution). *ALKIMIA : Jurnal Ilmu Kimia Dan Terapan*, *2*(2), 32–36. https://doi.org/10.19109/alkimia.v2i2.2998

Pervin, M. S., Jahan, M. G. S., Rana, A. Y. K. M., Sana, N. K., Rahman, M. H., & Shaha, R. K. (2013). Effects of some environmental variables on urease in germinating chickpea (*Cicer arietinum* L .) Seed. *Journal of Stress Physiology & Biochemistry*, *9*(3), 345–356.

Postal, M., Martinelli, A. H. S., Becker-Ritt, A. B., Ligabue-Braun, R., Demartini, D. R., Ribeiro, S. F. F., Pasquali, G., Gomes, V. M., & Carlini, C. R. (2012). Antifungal properties of *Canavalia ensiformis* urease and derived peptides. *Peptides*, *38*(1), 22–32. https://doi.org/10.1016/j.peptides.2012.08.010

Saboury, A. A. (2010). A Thermodynamic Study of the interaction between urease and copper ions. *Journal of Sciences, Islamic Republic of Iran*, *21*(1), 15–20. https://doi.org/10.22059/jsciences.2010.20134

Singh, A. K., Singh, M., & Verma, N. (2017). Extraction, purification, kinetic characterization and immobilization of urease from *Bacillus sphaericus* MTCC 5100. *Biocatalysis and Agricultural Biotechnology*, *12*, 341–347. https://doi.org/10.1016/j.bcab.2017.10.020

Sutopo, L. (2004). *Teknologi Benih* (*Seed Technology*). Rajawali.

Tallapragada, P., Dikshit, R., Jadhav, A., & Sarah, U. (2017). Partial purification and

characterization of amylase enzyme under solid state fermentation from *Monascus sanguineus*. *Journal of Genetic Engineering and Biotechnology*, *15*(1), 95–101. https://doi.org/10.1016/j.jgeb.2017.02.003

Wu, X., Wang, Y., Tong, B., Chen, X., & Chen, J. (2018). Purification and biochemical characterization of a thermostable and acid-stable alpha-amylase from *Bacillus licheniformis* B4-423. *International Journal of Biological Macromolecules*, *109*, 329–337. https://doi.org/10.1016/j.ijbiomac.2017.12.004

Zusfahair, Ningsih, D. R., Fatoni, A., & Pertiwi, D. S. (2018). Determination of urease biochemical properties of asparagus bean (*Vigna unguiculata ssp sesquipedalis* L.). *IOP Conference Series: Materials Science and Engineering*, *349*(1). https://doi.org/10.1088/1757-899X/349/1/012073

Zusfahair, Z., Ningsih, D. R., Putri, D., & Fatoni, A. (2018). Partial purification and characterization of urease from black-eyed pea (*Vigna unguiculata ssp unguiculata* L .). *Malaysian Journal of Fundamental and Applied Sciences*, *14*(1), 20–24.