

Optimum pH Buffer of Phosphate and Carbonate on The Crude Extraction of Uricase Enzyme from Goat Liver

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Abstract: Uricase enzyme (urate oxidase) is an enzyme that catalyze the oxidation of uric acid in the presence of oxygen to produce allantoin, carbon dioxide (CO₂) and hydrogen peroxide (H₂O₂). Human and primates do not have this enzyme while other mammals have it in the liver therefore the uricase enzymes are extracted from goat liver use a buffer that is compatible with the human buffer system. The type of buffer selected adjusted at the appropriate pH. The optimum uricase pH ranged from 7.5 to 9.5. The selection of buffer type is adjusted to the human buffer system. The purpose of the study was to determine the effect of the type and pH of the extraction buffer on the total activity, protein total and specific activity of crude uricase. The types of buffer selected are phosphate buffer

and carbonate buffer, while the selected pH is 7.5; 8.5; and 9.5. The method used is enzyme extraction, then determination of enzyme activity and protein content to determine the specific activity of the enzyme. The results obtained the highest total enzyme activity at pH 8.5 both in carbonate buffer (0.0481 U/mL) and phosphate buffer (0.0383 U/mL). The highest protein total in carbonate buffer was at pH 9.5 (4.55 mg/mL) while the highest value was in phosphate buffer pH 8.5 (4.1 mg/mL). The specific activity of uricase pH 8.5 was carbonate buffer (0.0114 U/mg) and phosphate buffer (0.0094 U/mg). The highest uricase specific activity value was at pH 8.5 for both carbonate and phosphate buffer types and in the long term it is used as a gout therapy.

Keywords: Uricase enzyme, goat liver, pH buffer, buffer type.

INTRODUCTION

The uricase enzyme catalyzes the oxidation reaction of uric acid to produce allantoin, carbon dioxide and hydrogen peroxide [1]. Uricase enzymes are widely applied as uricolitic therapy in patients with gout and hyperuricemia [2]. Another application is diagnosis of uric acid levels in blood and urine [3].

Uric acid levels in humans and primates tend to be higher because they do not have the enzyme uricase. The uricase enzyme in non-primate mammals is found in the liver and kidneys [4]. The process of uric acid degradation in the liver of mammals is in the peroxisome organelles [5]. The uricase enzyme in this study will be isolated from goat liver as a renewable sample source.

The effect of pH is a factor that can affect enzyme activity [6]. The stability of uricase enzyme at pH 7 to 10 and the optimum pH of uricase ranged from 8.5 to 9.5 [7-8]. The choice of buffer type is adjusted to the human buffer system so that a standard buffer suitable for consumption (food grade buffer) is needed [9].

This study used the type and pH of the buffer used the same pH, 7.5; 8.5; and 9.5. The research objective was to determine the effect of the type and pH of the extraction buffer on the total activity, protein content and specific activity of crude uricase. The results of the study are expected to provide information regarding the effect of the type and pH of the extraction buffer in characterizing the uricase enzyme isolated from goat liver and in the long term used as a gout therapy.

METHODS

Material and Equipment

The Materials Used were Goat Liver, K₂HPO₄ (Merck, Germany), KH₂PO₄ (Merck, Germany), H₂CO₃ (Merck, Germany), NaHCO₃ (Merck, Germany), Na₂CO₃ (Merck,

Germany), Bradford Reagent (Sigma-Aldrich, USA), 95% Ethanol (Merck, Germany), H₂O₂ (Merck, Germany), 4-Aminoantipyrine (Sigma-Aldrich, USA), Phenol (Merck, Germany), Uric Acid (Sigma-Aldrich, USA), horse Radish Peroxidase (Pierce™, Singapore). The Equipment used was a Visible Spectrophotometer (Genesys 10s Uv-Vis Spectrophotometer Thermo Scientific, Singapore), Magnetic Stirrer (Labnet, USA), Mortar and Pestle (Sigma-Aldrich, USA), Centrifuge (Thermo Scientific, Singapore), pH Meter (Sigma-Aldrich, USA), Micropipette (Biologix, USA), Tip (Biologix, USA), Eppendorf Tube (Biologix, USA), Volumetric Flask (IWAKI, Indonesia), Beaker Glass (IWAKI, Indonesia), Glass Funnel (IWAKI, Indonesia), Pipette (IWAKI, Indonesia), Water Bath (Biovopeak, China).

Uricase Extraction

Goat liver was homogenized for 15 min in mortar with 25 ml of 0.1 M phosphate buffer (pH 7.5) and centrifuged for 30 min (4°C, 13000 rpm). Repeated with a different type of buffer. Pellet and supernatant were separated. The pellet contains cells while the supernatant contains the uricase enzyme. The supernatant was combined and protein in the enzyme preparation was quantified by the Bradford method [10] using bovine serum albumin as the standard.

Uricase Assay

The activity of uricase crude extract was measured on the basis of liberated hydrogen peroxide from uric acid (2 mM) using peroxidase in treatment buffer phosphate and buffer carbonate. The mixture was incubated at 37°C for 240 minutes.

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Control contains inactive enzymes. Sample and control absorbance measurements were performed using a visible spectrophotometer (Genesys 10S UV-Vis Spectrophotometer Thermo Scientific, Singapore) at a wavelength of 490 nm. One U (unit) of uricase is equivalent to the amount of enzyme that produces 1.0 μmol H₂O₂ per minute.

$$Uricase\ Assay\ \left(\frac{U}{ml}\right) = \frac{([s]-[k]) \times f_p \times \left(\frac{V_{total}}{V_{enzyme}}\right)}{t \times MW\ H_2O_2} \dots\dots\dots (1)$$

Protein determination

Protein content of the crude enzyme was determined by Bradford method [10]. Bovine serum albumin was used as the standard. All the experiments were carried out three times in duplicates under the same conditions and mean and standard deviation were calculated. Uricase crude extract added Bradford reagent. The mixture incubated for 5 minutes at room temperature. The blank contains Bradford reagent. Absorbance measurements were carried out using a visible spectrophotometer (Genesys 10S UV-Vis Spectrophotometer Thermo Scientific, Singapore) at a wavelength of 595 nm.

$$Protein\ \left(\frac{mg}{ml}\right) = \frac{Abs \pm C \times f_p}{m} \dots\dots\dots(2).$$

RESULT AND DISCUSSION

Effect of pH on the Crude Extraction of Uricase Enzyme from Goat Liver

Crude enzymes are enzymes produced during extraction. The type and pH of the extraction buffer is one of the factors in the enzyme extraction. The buffer solution plays a role in maintaining pH solution. pH is important environmental factors which affect the activity of the uricase enzyme.

Effect of the type and pH of extraction buffer on total crude uricase activity were carried out at pH variations of 7.5, 8.5, and 9.5 with carbonate buffer and phosphate buffer. The results of type and pH of extraction buffer on total activity crude uricase can be seen in Figure 1. The optimum pH for uricase at pH 8.5 between 0.1 M carbonate buffer and 0.1 M phosphate buffer.

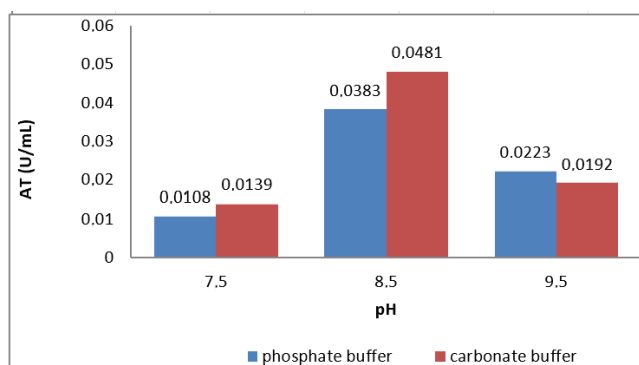


Figure 1. Total Uricase Activity Effect of Type and pH of Extraction Buffer

The total activity uricase at pH 7.5 and 9.5 was decreased. High or low pH than the optimum pH causes the enzymes to lose their total activity due to changes in the structure of the enzymes [11]. The lower pH than the optimum pH causes the active side of the enzyme disrupted by the presence of excess H⁺ ions, so that the proportion of H⁺ ions increase [12]. Excess H⁺ causes the

active site of uricase to become protonated, so that the binding of the active site with the substrate is not maximized. The higher pH than the optimum pH causes the active side of the enzyme to be disrupted by the presence of excess OH⁻ ions, so that the proportion of H⁺ ions decrease. Increase of OH⁻ ions cause deprotonation so that the bond of the enzyme-substrate complex on the active site breaks and cannot bind to the substrate.

At low pH and high pH proportions H⁺ ions and OH⁻ ions are not balanced, so it affects the binding of the enzyme with the substrate. This causes of enzymatic reactions in producing enzyme to decrease, which results in a decrease in total activity. Changes in pH cause changes in charge or structural changes in enzyme molecules so that they can affect enzyme activity [13].

Effect of Phosphate Buffer and Carbonate Buffer on the Crude Extraction of Uricase Enzyme

The results of type and pH of extraction buffer on crude uricase protein content can be seen in Figure 2. The highest protein content was 0.1 M carbonate buffer at pH 9.5, while the highest protein content was 0.1 M phosphate buffer at pH 8.5.

The results obtained in Figure 2 was different from the total uricase activity in Figure 1. The differences are due to the fact that the amount of uricase produced is proportional to the value of the total activity, while the protein levels are not necessarily uricase. This means that not all of the proteins extracted at pH 9.5 are uricase, even though the protein content is high, the total activity is low.

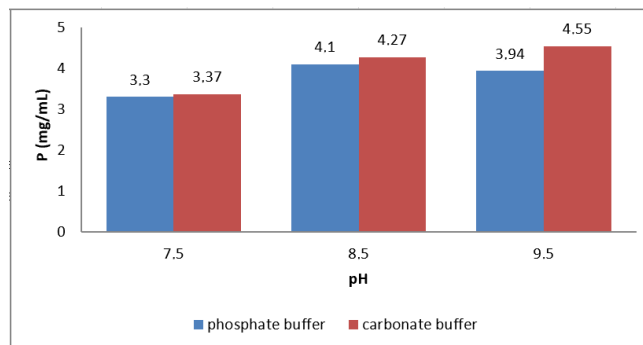


Figure 2. Uricase Protein Levels Effect of Type and pH of Extraction Buffer

Carbonate buffer resulted in higher values than phosphate buffer at pH 7.5 and at pH 8.5. Carbonate buffer had a higher pKa value (10.37), while phosphate buffer had a lower pKa value (7.21). The higher pKa value of a buffer indicates a lower acid strength or a high basicity [14]. The uricase enzyme is stable at an alkaline pH, therefore the pKa factor can determine the solubility of the enzyme in the extraction buffer. In contrast to pH 9.5 in Figure 1, which produced the highest total activity value in phosphate buffer than carbonate buffer. This happens because the higher pKa and pH cause the carbonate buffer to become more alkaline, therefore the enzyme is more soluble in the phosphate buffer.

Proteins can be positively or negatively charged depending on their side chains (the R group). At isoelectric pH, all of the charges on the particles will be lost. The isoelectric point of uricase is 7.5 [15]. The charge of total protein at pH 9.5 in Figure 2 both the phosphate buffer and carbonate buffer are negatively charged because the pH is above the isoelectric point. The charge on the protein can interact with the components buffer in order to

the protein is extracted.

The central atom of carbonate buffer is C (carbon) and the central atom of phosphate buffer is P (phosphorus). The C atom has a smaller radius than the P atom. This causes the C atom to pull the positively charged nucleus stronger to attract negatively charged proteins, therefore the protein is more extracted. At the same pH, carbonate buffer attracts more protein than phosphate buffer. The atomic radius is the distance between the charge of the atomic nucleus and its outer electron shell [16]. The smaller the atomic radius, the stronger attractive force of the core charge on the outer shell electrons, in order that it becomes easier for the atom to attract electrons from the outside.

Specific Activity of Uricase Enzyme

The results of the type and pH buffer on the specific activity of crude uricase can be seen in Figure 3. The optimum pH for uricase at pH 8.5 between 0.1 M carbonate buffer and 0.1 M phosphate buffer. Figure 3 shows the specific activity of crude uricase enzyme, which is proportional to its total activity (Figure 1). Uricase enzymes are optimally produced at an alkaline pH, because the active site of uricase can be ionized in order that enzymes and substrates can bind and react.

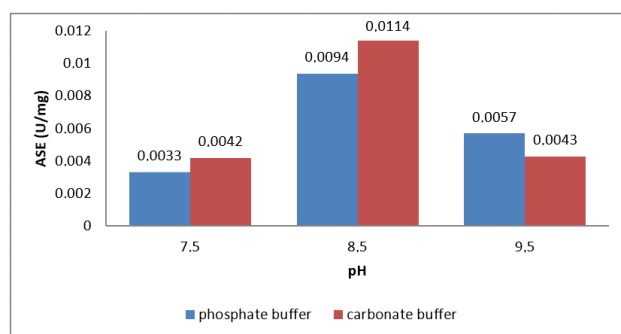


Figure 3. Specific Activity of Uricase Effect of Type and pH of Extraction Buffer

The activity of the uricase enzyme at pH 7.5 between carbonate buffer and phosphate buffer resulted in a small activity. Its because at pH 7.5 does not provide optimal conditions between the substrate and the active site of the enzyme, so the ionization process is not optimal. The specific activity of the enzyme decreased due to changes in pH, which became more alkaline (pH 9.5). Shift in pH from the optimum pH causes a change in the reaction catalyzed by the enzyme due to a change in ionization active site of the enzyme [17].

The activity of the uricase enzyme at pH 8.5 in carbonate buffer and phosphate buffer resulted in the maximum activity. The change in structure and charge on the active site of enzyme at the optimum pH resulted the conformation of the active site enzyme binding to the substrate becomes more effective and produces maximum activity [18]. The folding protein is the process of inserting negatively charged amino acid side groups on the inside of the enzyme and positively charged amino acid side groups on the outside of the enzyme [19]. The environment pH can affect the conformation of the enzyme and the interactions between the charged side chains.

CONCLUSION

The type and pH of the buffer used during uricase extraction affect the total uricase activity. The highest total activity was at

pH 8.5 for both carbonate buffer and phosphate buffer types. The type and pH of the buffer affected protein content, producing a trend that was different from the total activity. The highest protein content was in carbonate buffer at pH 9.5, while the highest protein content was in phosphate buffer at pH 8.5. The highest uricase specific activity value was at pH 8.5 in both carbonate buffer and phosphate buffer.

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