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Study of enzyme kinetics for urease extracted from *Glycine max*

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ABSTRACT: Urease is an enzyme that has a key role in nitrogen metabolism in plant germination. Grains are produced more amount of urease than other plants. Glycine max is also called soya bean, a yearly vegetable of the pea family Fabaceae and it's an eatable seed. Glycine max is mostly used as protein rich legume only, but it has a lot of medical and industrial applications too. Glycine max is used as a source of urease. The goal of this task is to study the kinetic activities of urease enzyme in Glycine max. The first enzyme was extracted from soya beans and partially purified. The enzyme activity was determined in both crude and partially purified using standard procedure. Further, enzyme kinetics (effect of temperature, pH, and substrate concentration) was studied using urease enzyme extracted from soya beans. The purification fold was 1.8 with a yield of 75%. The factors accustomed for its action was pH 8, temperature 50°C, Vmax and Km in Michaelis-Menten plot is 100 units and $8x10^{-4}$ moles/litre and LB Plot is 0.25units and $20x10^{-4}$ moles/litre.

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Keyword Urease; acetone precipitation; temperature; substrate concentration; *Glycine max*

Urease is an enzyme that catalyzes the hydrolysis of urea forming ammonia and carbon dioxide. Ureases (urea amidohydrolases, EC 3.5.1.5) are one of the known highly efficient enzymes that belong to amidohydrolase and phosphotriesterase superfamily (Krajewska, 2009). It is found in large quantities of jack beans, soya beans, horse gram and other plant seeds. Several reports have been published on the extraction of urease from various bacteria (Clemens et al., 1995; Hirayama et al., 2000) and plants (Das et al., 2002; Balasubramanian and Ponnuraj,2008; Balasubramanian et al., 2009; Krishna et al., 2011; Tekiner et al., 2014). Urease is also important in human bodies due to the fact that many urinary tracts and gastroduodenal diseases (Burne and Chen, 2000; Iwona Konieczna et al., 2012) including cancer (Sekichi et al., 2009) are related in some ways to this enzyme. The increased need in finding proper ways to remove urea from different environments brought great attractions in the biotechnology field (Qin and Cabral, 2002). Some of the urease's applications include the treatment of industrial waste (George et al., 1997), the industry of alcoholic beverages (Kobashi, 1988). With the increasing use of urease enzymes, there is a need to find sources of urease enzyme from readily available raw materials. One ingredient that has the potential to be explored as a source of urease enzymes is the soya bean. Soybean, (*Glycine max*), also called soja bean or soya bean, yearly leguminous plant of the pea family (Fabaceae) and its eatable seed. Soya bean is mostly used as protein rich legume only. In this study, soya beans will be used as a source of urease. The use of *Glycine max* as a source of urease enzyme is projected to increase the economic significance of soya beans.

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MATERIALS AND METHODS

Urease extraction [*all of the following procedures were done at 4°C.*]: 10 grams of germinated seeds of soybean, (Glycine max), also called soya bean were

pasted in a mortar and pestle and then suspended in a 40ml of 20% chilled acetone. Occasional stirring for ten minutes. Double-layer Muslin cloth was used for removing the suspension. After 15 minutes, the filtrate was centrifuged and the supernatant was isolated and used as a "crude extract".

*Enzyme assay***:** Pipette out 1ml of substrate solution (3%). Urea solution buffered with 0.2M phosphate buffer (pH 7)) and add 1ml of enzyme extract and incubate at 55˚C for 15 minutes. At the end of incubation time immediately keep the tubes in the ice. To stop the reaction, 1ml of 0.66N sulfuric acid was added and 1ml of 1M sodium tungstate solution was added to precipitate the protein. The precipitate was filtered or removed and aliquots of supernatant are assayed for ammonia and the enzyme activity is determined. The urease assay was carried out using the standard curve of ammonium sulphate.

*Standardization of ammonium sulphate***:** A pure ammonium sulphate solution (20mg/100ml) was prepared and different aliquots (0.2-1.0ml) were taken and the volume is made up to 3ml with distilled water. One ml of Nessler's reagent was added and mixed well. The color intensity is measured at 500nm. A standard graph was drawn.

*Standardization of Pro*tein ***(****Lowry et al., 1951)***:** Different concentrations of Bovine Serum Albumin (BSA) were prepared ranging from (0.2 to 1ml) 40-200mg. The standardization curve was drawn to calculate the amount of protein in the urease enzyme. The urease was partially refined to homogeneousness by the subsequent sequential phases which are carried out at 4°C.

*Acetone precipitation***:** The "crude extract" was adjusted to fifty percent saturation by the addition of −20°C acetone under constant and gentle stirring. The resulting precipitate was centrifuged. The pellet was collected and it is dissolved in a five ml solution was then centrifuged for ten minutes and the clear supernatant was designated as "crude enzyme solution".

Table1: Yield and purification fold at different steps of purification of urease from germinated *Glycine max* seeds

Step of purification	Total protein (mg/ml)	Total activity (Units)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude extract	20	8000	400	100	1
Acetone precipitation and dialysis	10	7000	700	75	1.8

*Influence of temperature on the urease enzyme***:** The optimum temperature for urease activity was determined over temperatures from 10°C to 80°C using the standard conditions of the assay.

Influence of pH on the activity of Glycine max urease: The pH for the purified urease was determined using urea as a substrate. The pH range used was from 2 to 10 using 0.2M phosphate buffer.

Influence of different concentration of substrates: The influence of substrate concentration on the enzyme was examined. Pipette out different aliquots was taken in test tubes and the enzyme activity was measured. Km and Vmax for urease was calculated using Line weaver-Burk double reciprocal plot.

RESULTS AND DISCUSSION

Urease extraction: Enzyme urease was isolated from the germination of the soya beans. Soya bean sprouts were then extracted and centrifuged at cold temperatures to prevent the occurrence of denaturation due to hot temperatures. The supernatant obtained was a crude extract of the urease enzyme (Figure 1). Further, it undergoes partial purification through acetone precipitation and dialysis which is then tested for its activity**.**

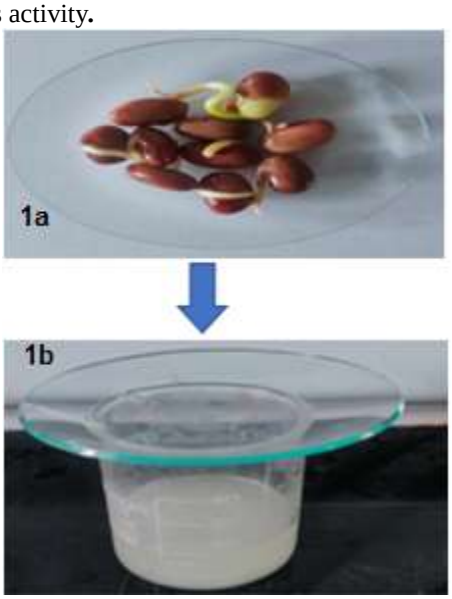

Fig 1: (a) Source (**b)** Crude extract.

Enzyme Assay: The results of the purification activity of the germinated Soya beans seed urease were summarized in Table 1. The total activity which represents the summation of the activity of all proteins in the enzyme extract samples showed a reduction over the period of the purification procedure. Our results showed an increase in the specific activity of enzyme during the purification steps.

The ultimate purification increase was achieved by nearly 1.8. The specific activity after finishing the purification was 700Umg^{-1}. Our results coincide closely near with Mohamed Hefnawy et al., 2014. In comparison with other studies, the purification results of germinated chickpea specific activity were 489.57 and the final purification fold was 45 (Shaela Pervin, 2013). Also, for *Proteus mirabilis* urease, the Specific activity of the extracted enzyme was 22932.86 and the final purification fold was 13.86 (Narjis et al., 2009).

Influence of temperature on the Glycine max Urease enzyme: The complete assays of enzyme were incubated at different temperatures from 10°C to 80°C for 15 minute. A result showed that urease had an optimum temperature at 50°C was shown in Figure 2. The optimum temperature, where the greatest urease activity carries out, is equal to 50°C. Our result is analogous to several studies reported by Srivastava et al., 2001; Das et al., 2002; Mohamed Hefnawy et al., 2014. The kinetic energy of substances raises with a raise in temperature which effects in speeding up the rate of enzyme reaction. When the temperature was further increased, the enzyme molecules go beyond the barrier of energy. This causes the breakage of hydrogen and hydrophobic bonds that are responsible for maintaining the 3D structure of enzyme (Lubbers et al., 1996).

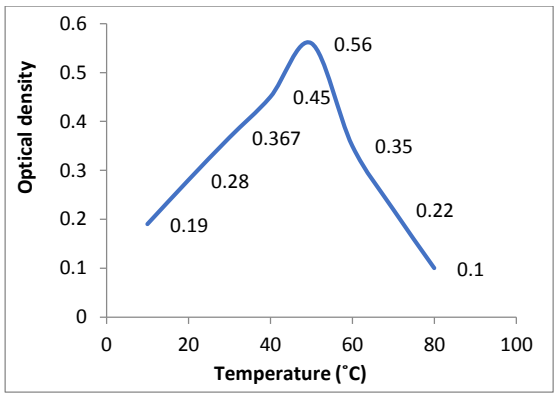

Fig 2: Effect of temperature on urease

Influence of pH on the activity of Glycine max urease enzyme: The pH for the partially purified urease was estimated using urea as a substrate. The pH used in this study, the range was from 3 to 10 with 50mM phosphate buffer. The activity of urease was the highest at pH 8 (Figure 3). Our results coincide with Sung HY *et al.,* 1986. The pH acts a significant role in the enzyme action. The urease isolated from *Pisdium sativum* seed was found to yield maximum activity at pH 7.5 which means that the seeds may belong to the category of basic urease. Despite the fact that Mulberry leaves have shown neutral optimum pH (Hirayama et al., 2000), many other studies reported basic pH as an optimum value for the extracted urease. For example the optimum pH was found to be 8 in jack beans (Sung et al., 1986), pigeon pea (Das *et a*l., 2002).

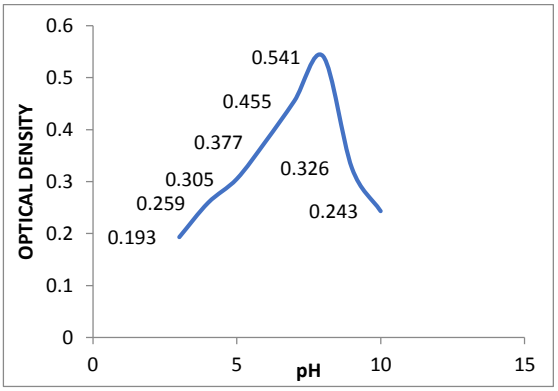

Fig 3: Effect of pH on urease

These results may be elucidated by the detail that acidic pH has a retardation effect on the urease enzyme resulting in reducing its activity. Also, the existence of the active sites in amino acids will be influenced by the change in pH which may alter the ionization of these amino acids (Amin et al., 2010).

Influence of different concentration of substrates on Glycine max Urease enzyme: Influence of substrate concentration on the activity of Glycine max urease by increasing urea concentration, the activity increased until nearly constant maximum activity 100 units / assay at 8x10^{-4} moles/litre of substrate on MM plot. Further increase in urea concentration resulted in a gradual decrease in enzyme activity (Figure 4).

The kinetics constants (Km and Vmax) for the purified urease were determined by incubating a fixed amount of enzyme with varying the concentration of urea solution (urea used as substrate). Km and Vmax for urea was calculated using Line weaver-Burk double reciprocal plot and were found to be 20x10^{-4} moles/litre and 0.25 units respectively. The optimum value of substrate concentration, where the urease activity has the largest value, was found to be 8 x10^{-4} moles/litre. After that the urease enzyme activity starts to progressively reduced.

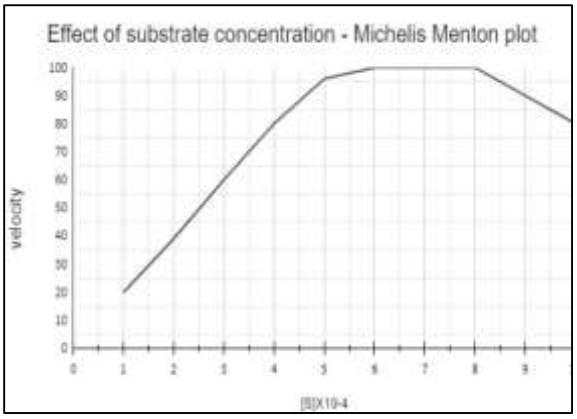

Fig 4: Effect of substrate concentration-MM plot

The enzyme activity reduced could be described by substrate retardation at increased urea concentrations. The enzyme showed the highest activity when incubated for 5 min under standard conditions; temperature is 50°C and pH is 8. The speed of hydrolysis of substrate rises with increasing substrate concentration up to attaining a maximum, beyond that hydrolysis action starts to reduction. Loest, (1979) and Shepard and Lunce ford, (1967) obtained maximum urease activity at 0.25 M and 0.008 M concentration of urea, respectively. The Kinetics constants (Km and Vmax) for urease extracted from germinated Soya beans was calculated using Line weaver-Burk double reciprocal plot and were found to be $20x10^{-4}$ moles/litre and 125 U/g respectively.

Conclusion: Urease was purified from germinating *Glycine max* seeds. The purification fold, yields were determined. On urease activity, the effects of pH, enzyme concentration, temperature, substrate concentration were investigated. This could provide knowledge on the numerous key aspects of the enzyme's properties and its significance in the application of medical fields.

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