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Extraction and Kinetic study on crud catalase from *Solanum tuberosum*

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ABSTRACT

Crud catalase from Solanum tuberosum have been extracted, its activity and kinetics parameters also be determined, the enzyme showed maximum activity at temperature of 40°C, for pH media the enzyme showed to be interrogated to pH value so the optimum pH for enzyme activity was 7 .The enzyme was stable for two days at 5°C, Km,Vmax values were 2.1 X10-4Mol/l, 1.02mol /mg/minμ respectively.

KEYWORDS: Catalase, Plant, kinetics parameters, Solanum tuberosum.

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INTRODUCTION

Catalase have various forms (isozymes) in plants. Over 30 years ago, when Scandalios [1, 2] first demonstrated that the multiple isozymes of catalase in maize were the products of distinct. Catalase in plants have been studied intensified especially the removal of extravagant H2O2 produced within the growth process or by ecological environmental motivators to its products(water and oxygen) in all aerobic organisms [3].Catalase (EC 1.11.1.6) is a heme containing enzyme that decomposes H2O2. In aerobic organisms, H_2O_2 is produced by various enzymatic reactions involving molecular oxygen by oxidases and the disproportion of superoxide by Super oxide dismutase (SOD). H_2O_2 is a toxic molecule as it can both oxidize and reduce organic substrates in cells. Catalase removes this toxicity by catalyzing the disproportion of H_2O_2 into molecular oxygen and water [4]. Plants have several antioxidant enzymes of which catalase is the most efficient [5].Plant cells contain an anti-oxidant system consists of vary small particles depending on the nature and types of oxidative stress found in most types of plants [6]. For the system antioxidant important role in the control of the types of free active oxygen which plays an important role in increased susceptibility to oxidation and show harm in plant cells [7].Antioxidants systems also divided into two parts the first part is the counter system of the antioxidant enzyme and the second section is the counter system and Non-oxidizing non-enzymatic each component of these two sections important and effective role in removing the damage of free radical [8]. Catalase in plants particularly in the storage organs like potato tubers so foregoing previously the aim of this study is to isolate catalase from potato tubers and examine some of the characteristics of the enzyme and appreciation of enzymatic efficiency in local potatoes and optimum temperature, pH of the effectiveness and stability of the enzyme action.

MATERIALS AND METHODS

Potato collected from the local market and diagnosed in the Department of Biology/ College of Education / Al-Iraqia University in Baghdad.

Extraction of catalase: A fresh potato cutter into approximately pea-sized pieces. Weigh out 75 g of tissue.75 ml of chilled ddH_2O (deionized, distilled = dd) and a small handful of crushed ice in a pre-chilled blender homogenize for 30 sec at high speed, then Filter the potato extract. Pour the filtrate into a 250 ml graduated cylinder and add cold ddH_20 to a final volume of 150 ml this extract will be arbitrarily labeled 100 units of enzyme per ml (100 units/ml).

Enzyme assay: The activity of catalase was determined according to [9]in 3ml, of reaction solution , which contained 2ml of phosphate buffer pH 7.0 and 0.3ml of hydrogen peroxide solution (3%) in a test tube , then 0.2ml of extract containing enzyme (supernatant) was added .The blank was composed from : 2.3 ml of phosphate buffer pH 7.0 and 0.2 ml of extract containing

enzyme (supernatant).Using UV-Vis spectrophotometer at wave length of 240 nm after 1 min the absorbance was measured for activity test and blank tubes .

Protein content: Protein concentration of the enzyme extract was determined by the method of Lowry et al., [10].

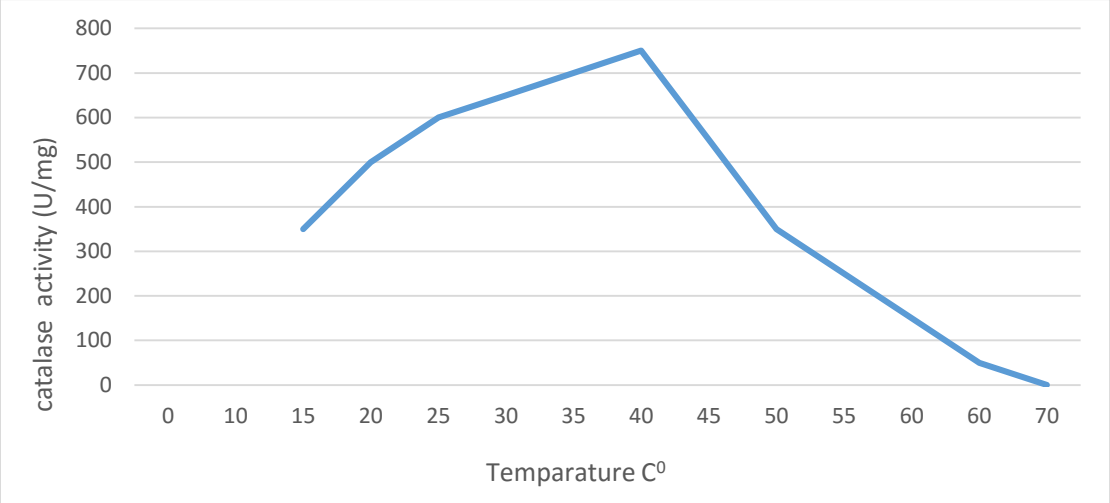
Measurement of the optimum temperature of enzyme activity: To determine the optimum temperature of the enzyme the enzymatic effective estimated by mixing 10 microliter of enzyme solution with 0.99 mL of phosphate buffer concentration (50 mM) pH=7 in test tubes and then incubate the enzyme for one hour at a range of temperatures (20-80) C °. taken 20 microliter of the solution (in the previous step) to all the tubes and add him (1) mL of [(10mM) H2O2] and [(50 mM) Na2HPO4 pH=7] and incubate at a temperature of 25 ° C to stop the work of the enzyme, then measured with a spectrophotometer at the wavelength 240nm [9], and then the relationship between temperature and the effectiveness of the enzyme draw to set the optimum temperature of the enzyme activity.

Measurement of Optimum pH of the enzyme activity: Buffer acetate of concentration (50 mM) with PH from 4-5 and then prepared buffer phosphate of concentration (50 mM with PH from 6 - 8 and buffer gear basal of concentration (50 mM) with PH from9-10 mixing 10 microliter of enzyme solution with 0.99 mL of each of the solutions buffer prepared in test tubes and incubate for one hour at a temperature of 25 ° C .Taken 20 microliter of the solution (prepared in previous step) to all the tubes and add him (1) mL of [(10mM) H_2O_2 and (50mM) Na2HPO4 pH = 7] and incubate at a temperature of 25 ° C and then measured to a spectrophotometer at the wavelength of 240nm, then draw the relationship between the enzyme activity and optimum pH of the enzyme activity.

RESULTS AND DISCUSSION

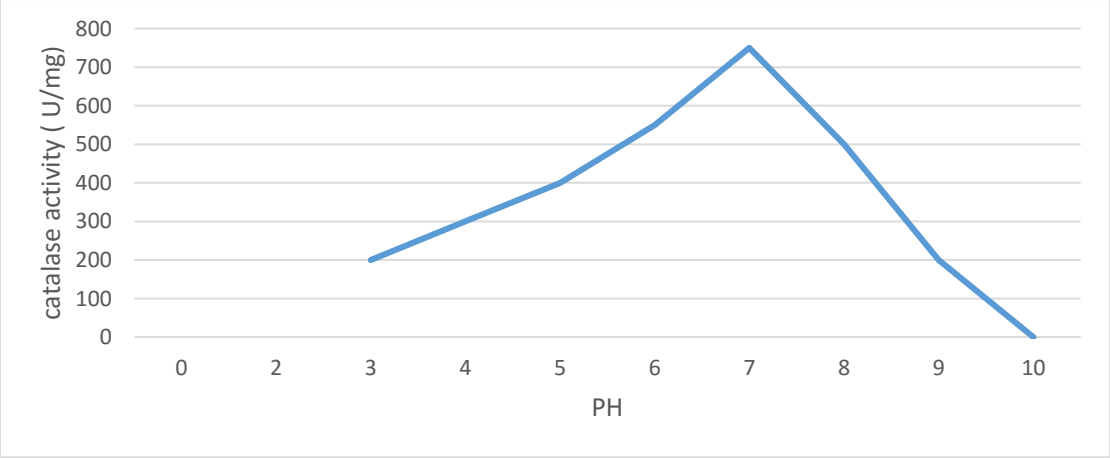
The optimum temperature of enzyme activity and stability: Shown in Figure (1) The effectiveness of enzymatic hydrolysis increases with the temperature of the lap until it reached a maximum of 256 units / ml at a temperature at 40 C ° and is due to increase the speed of interaction with the high temperature of the lap to increase the kinetic energy of the molecules of the enzyme [11] then It began to decline and decrease in the effectiveness of the enzyme at higher temperatures of 50 ° C to the role of heat by breaking bonds with the acids amino at the secondary level and tertiary to the composition of the protein which changes the effective form of the protein to form ineffective because of metamorphosis proteins [12] and this result is agree with [13].

Fig.1: Effect of temperature on catalase activity**.**



The pH of enzyme activity and stability: The enzyme Showed the highest activity when the pH (7) figure 2, then the enzyme activity at acidic and basic PH characterized by lower this return to the changes that occur in the number of electrical charge on each molecule of the enzyme and substrate that lead to a change in susceptibility groups on the surface of the reaction material to interact with each other by ionic and hydrogen bonds [12] and this result agreed with [13**].**

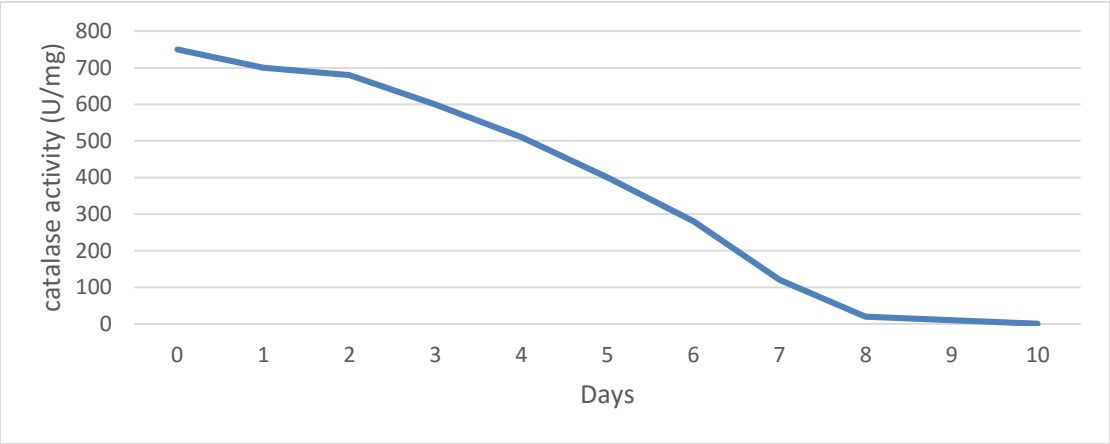
Fig.2: Effect of pH on Catalase activity.



Stability and kinetic studies of catalase: The Stability of catalase activity was studied up to 10 days (fig-3) and the enzyme stored at 5°C. The enzyme activity showed that the enzyme was stable

to the second day and then gradually the activity of enzyme decreased then completely inactivated on last day.

Fig. 3: Stability of catalase activity.

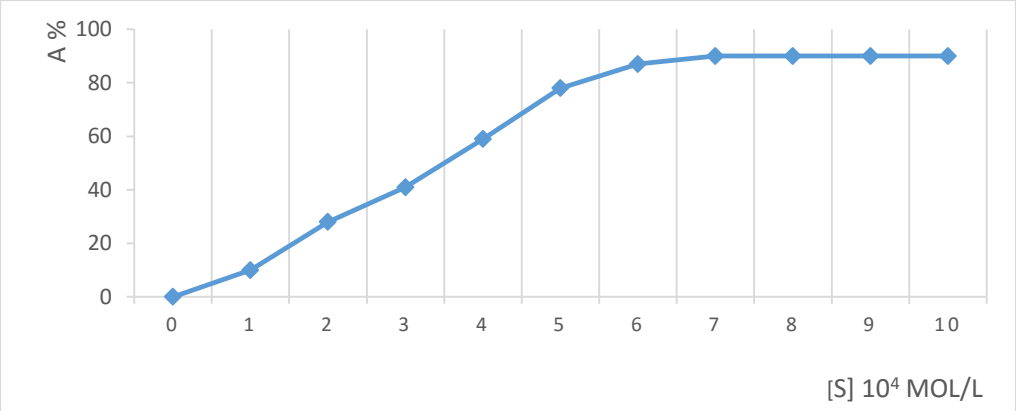


The effect of different concentrations of substrate on the catalase activity was studied using H_2O_2 as substrate in 0.1M phosphate buffer pH 7.0 using 10 mg of enzyme/ml. The results obtained showed in (fig.4).
The kinetic parameters V_{max} and K_m were determined by the lineweaver – Burk transformation of Michaelis-Menten equation

$$\frac{1}{V} = \frac{Km}{V\,max} \cdot \frac{1}{[S]} + \frac{1}{Vmax}$$

K_m,V_{max} values calculated for H_2O_2 as substrate is 2.1 $X10^{-4}$Mol/l, 1.02mol /mg/minμ respectively. This values of K_m,V_{max} Consistent with Michaelis-Menten equation which showe that V_{max} is not characteristic of catalase enzyme but in other side K_m value showed distinguishing characteristic for enzyme because it showed the capacity of the hydrogen peroxide to react with enzyme, also shows the Modification of interaction speed [14].

Fig.4: Effect of H_2O_2 concentration on the catalase activity.



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