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Baghdad Science Journal **Vol. 13(2s(Supplement))2016**
The 2nd National Conference of Chemistry

DOI: http://dx.doi.org/10.21123/bsj.2016.13.2.2NCC.0392

Partial Purification and Characterization of Catalase from Banana Peels

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Received 7/10/2015
Accepted 20/12/2015

Abstract:

Catalase (EC 1.11.1.6) is a well known enzyme which exists in almost all living creatures exposing to oxygen (such as plants, bacteria, and animals). It is a very necessary enzyme to protect the cell from oxidative detriment by reactive oxygen species (ROS). The aim of this study is the partial purification and characterization of Catalase enzyme from Banana peels.

In this study, fresh banana peels are treated with 70 % ethanol ,further separated with chloroform ,water and ethyl acetate respectively .The supernatant of the enzymatic sample which is treated with chloroform is loaded into gel filtration column with Sephadex G-100 (1.0 x 90 cm) equilibrated with pH7 buffer media (phosphate buffer 0.1 M). Kinetic studies of the purified enzyme activity are measured and characterized .The maximal activity (26.04 units/mg) of catalase is observed with chloroform buffer extraction. The kinetics of catalase; Michalis constant Km and maximum velocity Vmax is determined using Linweaver- Burk plot, The Km value for catalase (434.7mM), Vmax (100 m mole min^{-1}). Characterization results demonstrate that the optimal pH for activity is (7.6). And the optimal temperature for activity is 30˚C .The present study indicates that Banana peels is a good source of catalase enzyme.

Key words: Catalase, Antioxidants, Banana Peels, Purification, Characterization.

Introduction:

Animal life needs oxygen to be consumed and conversed into water in order to continue living , We can notice the production of outflow from mitochondrial electron transported from free radicals [1].Oxidative phosphorylation and the creation of ATP are considered essential and sources of energy [2].

Free radicals can also be in the form of sulfur, nitrogen, carbonyl and other reactive types. [3,4].

The electrons or hydrogen are passed from one molecule to another during oxidation , and the last molecule helps as an antioxidant. Antioxidants, hence, the creation of free radicals and the chain reactions can be broken. [5],

which would cause cell damage or even death [6].

The enzymatic antioxidants includes glutathione peroxidase (GSH-Px) , catalase , glucose -6-phsphate dehydrogenase , and superoxide dismutase (SOD) [7]. This will provide the plants a complex antioxidant defense system [8]. These enzymes stop the initiation of free radical chain reactions [9].

Catalase is found in virtually all living organisms wide-open to oxygen. It acts by catalyzing the disintegration of hydrogen peroxide to oxygen and water [10].

In the process of reproductive reaction, catalase is a very dynamic enzyme. every second one catalase single molecule decomposes millions of molecules of hydrogen peroxide to oxygen and water. [11]

Catalase is very important in industry, it is used as an oxidizers, whitening agents or purifiers while in the analytical field it is used as a source of hydrogen peroxide [12] . Antioxidants play an intrusting role in human health. and prompted researches in the fields of food sciences and horticulture evaluates fruit and vegetable as antioxidants.

Banana is popular fruit which contains a number of beneficial pharmacological effect and can be distributed all over the world. It can be grown in low-lands wetlands into uplands tropical areas [11].

Banana peel is an unused portion of the banana fruit and they have high contain of antioxidant, both phenolic and mineral content [13]. This peel is biodegradable and it will cause an environmental problem due to nitrogen and phosphorus quantity.As a result, the best solution in order to safeguard human beings, obtaining some profit and creating waste to fortune is by extracting the banana peel. Banana peel has more antioxidant than its pulp. Banana peel can also be commercialized because of antioxidant quality and its quantity. It will not contend with banana pulp in producing end products especially in the food industry. The previous facts ensure that banana can continuously act as the natural antioxidant source [14]. Banana peel contains dietary fibers , poly-unsaturated fatty acids , essential amino acids, proteins and potassium [15,16].

The objective of this study is to describe the partial purification and characterization of catalase enzyme extracted from banana peels.

Materials and Methods:

Preparation of Extracts:

Banana peels (250 g) are cut into parts, heated in 1 L of distilled water for 2 minutes at 37^{o}C, and mixed with 70 % acetone ,filtered and concentrated on oven at 25 ºC for 2 hours to a final volume of 200 ml. Followed by partitioned into chloroform and distilled water then extracted with 75 % ethyl acetate . Ethyl acetate, distilled water and chloroform extracts are collected and used for the determination of catalase specific activity.

Activity Assays:

Catalase activity is measured spectrophotometrically by monitoring the reduction in absorbance at 240 nm caused by the evanescence of H_2O_2 [17].

(ε_{H2O2}=3.94 M^{-1} cm^{-1} at 240 nm [18]. The mixture of standard reaction for the assay contains 75 mM H_2O_2, 0.1 M phosphate buffer [pH 7] in a total volume of 3 ml.

The amount of enzyme that catalyzes oxidation of 1mM hydrogen peroxide per minute under the test conditions is called enzyme unit. The specific activities are conveyed in term of enzyme units per 1 mg of protein.

Determination of Protein Content: Lowry *et al* determined the protein

concentrations in crude and purified enzyme extracts. [19], using bovine serum albumin as standard protein.

Purification of Catalase from Banana Peels : The extract is loaded to the Sephadex G-100 gel filtration column (1.0 x 90 cm) and equilibrated with phosphate buffer (0.1M , pH 7.0) .The enzyme elution is carried out with the phosphate buffer (0.1 M, pH 7.0) at a flow rate of 0.5 ml/min. Fractions of 3 ml are collected and catalase activity assayed.

Kinetic Parameters (Km and Vmax):

Effect of pH :

Buffers with different pH [5.8, 6.4 , 7 ,7.6 , 8.2] are used to estimate the enzymatic reaction of catalase and plot the relationship between the pH values versus the enzyme activities .

Effect of the Temperature :

Different temperatures [10, 20, 30, 40 ,50^{0}C] are used to estimate the enzymatic reaction of catalase and plot the relationship between the temperature values against the activities of the enzyme .

Effect of Concentration Of Substrate:

Different concentrations of substrate [55 , 65 , 75 , 85, 95 mM], are used to estimate the enzymatic reaction of catalase and plot the relationship of enzyme activity against temperature values **.** Then the values of V max and Km for catalase toward substrate are estimated using the Line weaver-Burk plot [the relationship between 1\V agains 1\[S].

Results and Discussion:

Maximum catalase specific activity is obtained when it is extracted by chloroform (26.04 units/mg) . When compared to the Specific activity of Catalase which is obtained from the extraction with ethyl acetate and water are (14.3 units/mg ,7 units/mg)respectively as shown in Figure 1.

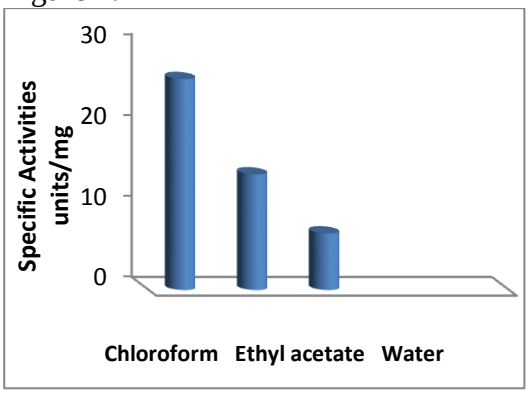

Fig (1): Specific Activities of CAT in Banana Peels Extracts Using Different Solvents.

The supernatant of chloroform extract is used in the purification of enzyme. The eluted solution of enzyme from Sephadex G-100 columns is shown in Figure 2 and Table 1.The enzyme was purified about 6.85 - fold, with a final specific activity of 178.5 U/mg. The overall recovery of the purification is 40 %.

Table (1): Purification outline of Catalse From Banana PeelsS.

	Volume[ml]	Total activities [units]	Total protein [mg]	Specific activity[units\mg]	Fold purification	Recovery[%]
Crude enzyme	**150**	**312.5**	**12**	**26.04**	**1**	**100**
Sephadex G-100	**10**	**125.7**	**0.7**	**178.5**	**6.854**	**40**

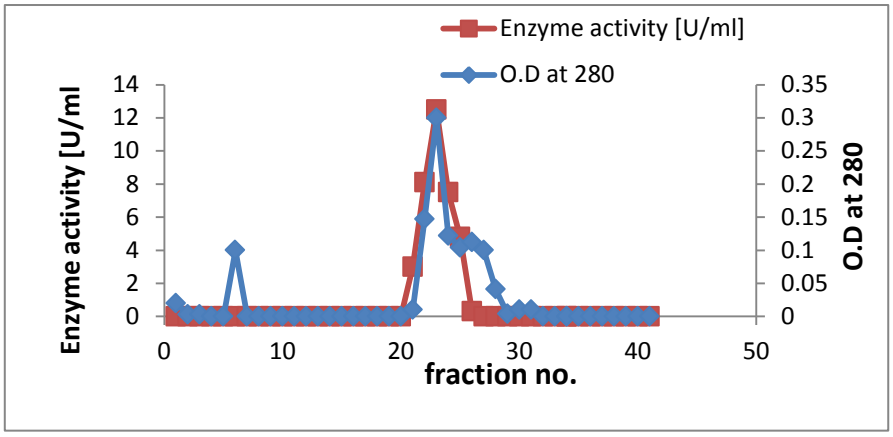

Fig (2) :A Typical Elution Profile For The Chromatography Of Catalase Enzyme Extracted From Banana Peels .

Catalase is partially purified from Van Apple [*golden delicious*], and *Phyllanthusreticulatus* to 8.7 ,8 fold with a yield of 11% , 45% respectively [20-22], and is reported that 12% yield of Catalase from *Rhizobium radiobacter strain 2-1* [23] and about 1.68 % from Chicken Erythrocytes[24].

The increased enzyme activity after purification compared with its activity without purified, can be refered to existence inhibitors which limit the activity of catalase.

Table 1 shows that specific activity of catalase from banana peel is (178.5 U/mg) , and that there is a rise in the specific activity after the purification step.

Our results are consistent with the previous studies [25]. These variation in specific activity values can be described by the different protein composition of various types of catalase. The rigorous conditions used in purification procedures may also cause change of specific activity values .The lack in yield can be the result of denaturation of enzyme during the long time that takes for the purification ,or for some other unknown reasons [26].

The activity of the enzyme is measured after purification at different ranges of pH [from 5.8 to 8.2] at 30°C using hydrogen peroxide as a substrate .The optimum pH was[7.6] , with the activity falling off slowly above this value. (Figure 3).

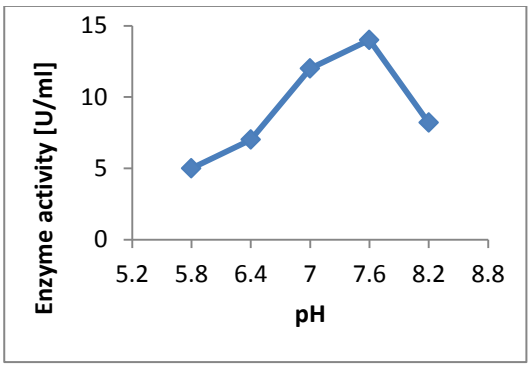

Fig (3): The Effect of pH on the Activity of Partially Purified Catalase from Banana Peels.

Catalase is generally regarded as an enzyme without optimum pH , In the case of other catalases, the enzyme has been shown to exhibit a sharp optimum pH ,or a broad pH optima [27]. The purified enzyme stability in an acidic region is lower than that in a basic region.

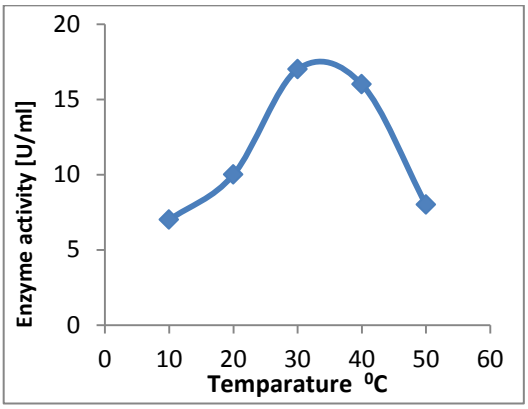

Fig (4): The Effect of Temperature on the Activity of Partially Purified Catalase from Banana Peels.

The effect of temperature on the activity of the enzyme is studied by assaying the catalase activity at different temperatures extended [from 20°C to 50°C] (Figure 4).

As shown in figure 4 , the activity of catalase increases gradually from 10°C to 30°C and then declines due to the denaturation of enzyme. The results obtained indicate that the catalase is maximally active at 30°C. The catalase of *Agaricusbisporus* also shows the same optimum temperature [25] .

The activity the enzyme is analyzed after purification using different concentrations of hydrogen peroxide as a substrate. The Km value and Vmax for Catalase purified are calculated to be 100 mM , 434.7 m mole min $^{-1}$ respectively as shown in Figure 5.

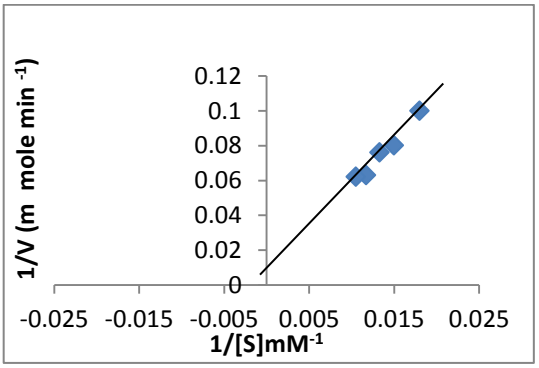

Fig (5): Lineweaver-Burk Plots for the Determination of Km Value of Partially Purified Catalase.

There are many studies that deal with Km and Vmax for catalase from different sources, since report refers to catalase which was taken from cabbage leaves sources it has got Km 25.5 mM and Vmax 31.12 m mole min $^{-1}$ for H_2O_2 [28] , The Km value of Catalase for H_2O_2 has been estimated to be 33 mMfrom chicken erythrocyte [24].

Conclusions:

The present study indicates that banana peels are a good source of catalase enzyme. The current study shows that catalase enzyme extracted from banana peel can be used as a local industry in our country.

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تنقية جزئية وتوصيف لأنزيم الكتلايز من قشور الموز

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الخلاصة

Catalase (EC 1.11.1.6) هومن إلانزيمات المشتركة التي وجدت في جميع الكائنات الحية تقريبا التي تتعرض للأوكسجين (مثل البكتيريا والنباتات والحيوانات). وهو انزيم مهم جدا في حماية الخلايا من الضرر التأكسدي للأنواع الاكسجين التفاعلية (ROS).الهدف من الدراسة هو تنقية جزئيا وتوصيف إلانزيمCatalase من قشور الموز. في هذه الدراسة تم معاملة قشور الموز الطازجة مع 70٪ من الإيثانول، ثم فصل مع الكلوروفورم وخلات الإثيل والماء بالتتابع. مرر محلول الانموذج الانزيمي المعامل بالكلوروفورم بعمود الترشيح الهلامي(1× 90 سم) باستخدام Sephadex-100 وتم معايرتها مع 0.1 M محلول الفوسفات بدرجة الحامضية ,7 تم دراسة الفعالية الانزيمية و الحركيات للانزيم المنقى . وقد لوحظ اعلى فعالية انزيمية في الانموذج المعامل مع الكلورو فورم كان(26.04 units/mg)كانت قيمة Kmللانزيم تساوي (434.7mM) و Vmax تساوي ($100\ m\ mole\ min^{-1}$) ،وكان الحامضية المثلى للانزيم هي 7.6 ودرجة الحرارة المثلى هي(30°C) . وتشير الدراسة الى ان قشور الموز مصدر جيد لانزيمCatalase .

الكلمات المفتاحية : الكتاليز ، مضادات الاكسدة ، قشور الموز ، تنقية ،توصيف .