EXTRACTION AND BIOCHEMICAL CHARACTERIZATION OF*BRASSICA OLERACEA*VAR. CAPITATA(L.) METZG.CATALASE

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ABSTRACT

Catalase (EC. 1.11.1.6. oxidoreductase) has been found in animals, plants and all aerobic organisms. In this research, partially purified catalase enzyme was isolated from cabbage by ammonium sulphate precipitation (50 %) method. The partially purified catalase was qualitatively examined by hydrogen peroxide test and catalase activity was confirmed by liberation of oxygen gas bubbles to form foam.Catalase activity was determined by spectrophotometric method using hydrogen peroxide as substrate at 434 nm. Protein content was determined by Biuret method using Bovine Serum Albumin (BSA) as standard at 560 nm. Specific activities, protein recovery and degree of purification were determined in each purification steps.Catalase was purified 5.68 fold over crude extract and protein recovery was found to be39.04 %. Specific activity of catalase in crude extract was0.0753 mmol $min^{-1}mg^{-1}$ after 50 % ammonium sulphate precipitation.The optimum pH of catalase was found to be 7.5 in phosphate buffer and optimum temperature was found to be 35 °C. Enzymic properties such as effect of reaction time and effect of enzyme concentration were also determined.

Keywords :Cabbage, H_2O_2, catalase activity, ammonium sulphate, specific activity

INTRODUCTION

Catalase (CAT, H_2O_2: oxidoreductase; EC 1.11.1.6) by scavenging hydrogen peroxides to water and oxygen is an important enzyme of cell defense mechanisms against oxidative stress in plants. Different organisms have shown different catalase activities. In plants, multiple isoforms of the enzyme are usually present, and they are expressed in different tissues and developmental stages. In green leaves a majority of CAT activity is found in peroxisomes (Foyer and Noctor, 2000).Although the enzyme is present in green plants, but usually it had been extracted from those with higher concentration in their cells.

The aim of this research was to study the extraction and biochemical characterization of *Brassica oleracea*catalase.

MATERIALS AND METHODS

Materials

Cabbage sampleswere purchased from local shop, YangonRegion. Bovine Serum Albumin (BSA) was purchased from Sigma Aldrich, England. All other

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chemicals used were of analytical reagent grade. In all investigations, the recommended standard methods and techniques involving both conventional and modern methods were provided.

Sample Preparation and Extractionof Catalase from Cabbage Leaves

The fresh leaves of cabbage were washed with tap water, chopped and mixed with phosphate buffer pH 7.0 solution. It was stirred in ice for 2 h and filtered. Solid ammonium sulphate was slowly added to this extract to obtain 50 % saturation and stirred for 2 h in ice. After standing overnight, the precipitated protein containing catalase enzyme was collected by centrifugation for 30 min.

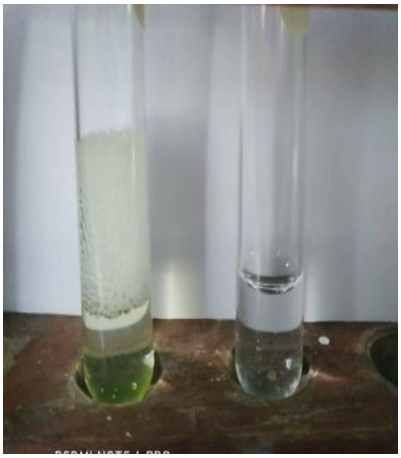
Characterization of Catalase from Cabbage Leaves

The partially purified catalase was qualitatively examined by hydrogen peroxide testfor its catalase activity(Hadwan, 2018). For enzyme assaythe catalase activity of *cabbage*was determined with the carbonato-cobaltate(III) complex method at 434nm using H_2O_2 as a substrate. Protein content was determined by Biuret method using Bovine Serum Albumin (BSA) as standard at 560 nm. Specific activity was calculated by using enzyme activity and protein content. Effect of pH, temperature, reaction time and enzyme concentrationwere determined by spectrophotometric method.

RESULTS AND DISCUSSION

Qualitative Examination of Catalase by Using Hydrogen Peroxide

The partially purified catalase was qualitatively examined by hydrogen peroxide test and catalase activity was confirmed by liberation of oxygen gas bubbles to form foam (Figure 1). In the presence of catalase, formation of foam was observed. However, without catalase, hydrogen peroxide did not liberate oxygen to form foam.



(a) (b)

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Hydrogen peroxide — water — oxygen

Figure 1 (a) 6 % H_2O_2 solution with enzyme (control)
(b) 6 % H_2O_2solution without enzyme at room temperature

Calibration Curve for Protein Determination by Biuret Method

In this work, bovine serum albumin (BSA) was used as a standard protein (Savary *et al.,* 1969). The different absorbance values were obtained for various standard protein solutions by using a UV-visible spectrometer. It was found that the nature of the plot of absorbance at 560 nm *vs.* concentration of protein (mg mL^{-1}) (Table 1 and Figure 2), was a straight line passing through the origin showing that Beer's Law was obeyed.

Table 1 Relationship Between Absorbance and Concentration of Bovine Serum Albumin (BSA) Solutions

No.	Protein Concentration (mg mL^{-1})	Absorbance at 560 nm
1	1.0	0.060
2	3.0	0.170
3	5.0	0.265
4	7.0	0.345
5	9.0	0.429

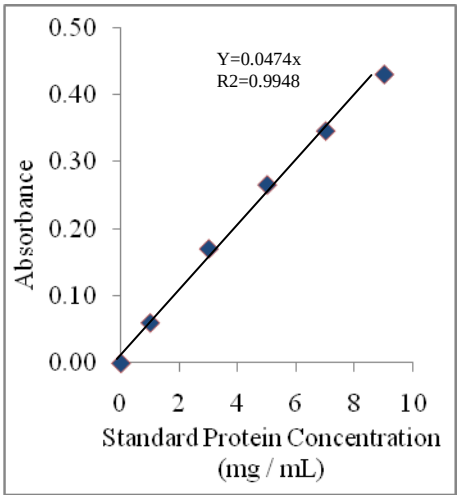


Figure2Calibration curve for standard protein solution

Catalase Activity, Protein Content and Specific Activity of*Brassica oleracea* Var. *Capitata* (L.) Metzg.

Hydrogen peroxide was used as a substrate for catalase activity. Dissociation of hydrogen peroxide is proportional to the activity of catalase enzyme in the used sample.Cobalt-bicarbonate solution can act as a" stop bath" for reactions regulated by the catalase enzyme(Hadwan, 2018). Immediately after mixing the cobalt-bicarbonate reagent with the enzyme reaction solution, its content of cobalt (II) is oxidized to cobalt (III); any unreacted hydrogen peroxide resulting from the catalase activity were oxidized the cobalt (II) to cobalt (III) and then reacted with carbonate to produce a carbonato-cobaltate (III) complex ($[Co(CO_3)_3]Co$), which has an intense olive green color. Catalase activity is always directly proportional to the rate of dissociation of hydrogen peroxide in the used samples. The decrease of color intensity can be used as an index to represent the increase catalase activity.The 434-nm has been used for the assessment of catalase activity.

One unit of catalase activity is defined as the amount of activity required to convert 1 mmol of H_2O_2 to water and oxygen per minute at 25°C. The protein content was determined by Biuret method and it was observed to be274.93 mg mL^{-1}(Table 2).The specific activity was calculatedto be0.1455mmol min^{-1} mg^{-1}. After 50 % ammonium sulphate precipitation, catalase was purified to 5.68 fold over crude extract. Protein recovery percent was 39.04 %.

Table2Enzyme Activity, Protein Content and Specific Activity of the Enzyme Solution

Fraction	Enzyme Activity (mmol min^{-1} mL^{-1})	Protein Content (mg/mL)	Specific Activity (mmol min^{-1} mg^{-1})	Protein Recovery (%)	Degree of Purity (fold)
Crude	18.028	704.27	0.0256	100	1
After purification with 50 % ammonium sulphate	40.000	274.93	0.1455	39.04	5.68

Optimum pH of Catalase Activity

At an optimum pH, an enzyme's activity is the highest. At pH above and below optimum pH, the activity of the enzyme is reduced and reaction rates are slower (Charles, 2007).In this work, different buffers of pH values5.0 to 8.5 were used to determine the activity of the prepared catalase sample. The nature of the activity *vs.* pH curve of the enzyme (Table 3 and Figure 3) was obviously found to be unsymmetrical and the optimum pH was obtained at pH 7.5 with hydrogen peroxide as substrate.

Table 3 Relationship between Catalase Activity and pH ofBuffer Solution

No.	pH	Catalase activity (mmol min^{-1} mL^{-1})
1	5.0	6.1776
2	5.5	6.5824
3	6.0	7.0886
4	6.5	7.2912
5	7.0	7.4936
6	7.5	8.1016
7	8.0	7.7976
8	8.5	6.9872

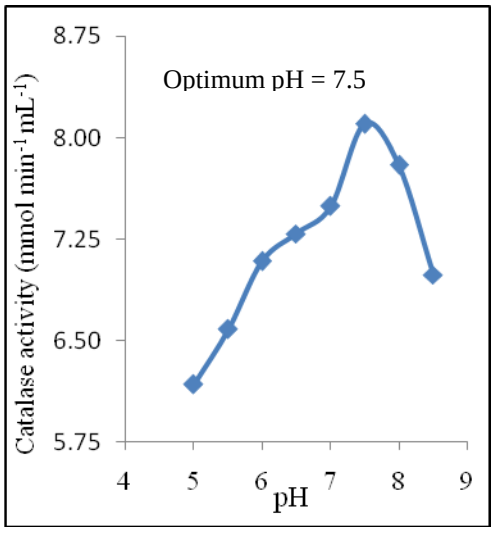


Figure 3 Plot of catalase as a function of pH solutions

Optimum Temperature of Catalase Activity

In this study, the effect of the temperature on the catalase activity was investigated in the temperature range between10 to 70 ºC with 10 ºC intervals. The optimum temperature for catalase was found to be 35 ºC in phosphate buffer pH 7.5(Table 4and Figure 4).

Table 4 Relationship between Catalase Activity and Temperature of the Solution at pH 7.5

Temperature (°C)	Catalase Activity (mmol $min^{-1}mL^{-1}$)
10	4.1512
20	5.2656
25	6.3800
30	7.5952
35	8.3032
40	8.0000
50	7.0888
60	2.0256

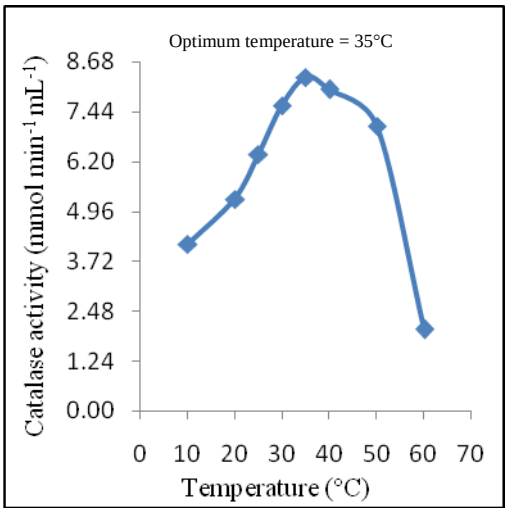


Figure 4 Plot of catalase activity as a function of temperature of enzyme reaction at pH 7.5

Effect of Reaction Time on Catalase-catalyzed Reaction

In this work, the action of the catalase on hydrogen peroxide was studied in phosphate buffer of pH 7.0. The amount of carbonato-cobaltate (III) complexproduced during the various reaction times of 1, 3, 5, 8, 11, 14 and 17 min were determined by spectrophotometric method.At the beginning of the reaction (during8 min), the reaction is very fast. Then, velocity decreased steadily(Table 5 and Figure 5). Therefore, in sequence studies, reaction time of 2 min was chosen for initial velocity measured in enzyme kinetic.

Table 5 Relationship between Reaction Time and Velocity of Catalase -catalyzed Reaction

No	Reaction Time (min)	Concentration (mM)	Velocity (mM min^{-1})
1	1	69.872	69.872
2	2	78.984	39.492
3	5	99.240	19.848
4	8	113.424	14.178
5	11	128.608	11.692
6	14	143.800	10.271
7	17	157.976	9.2930

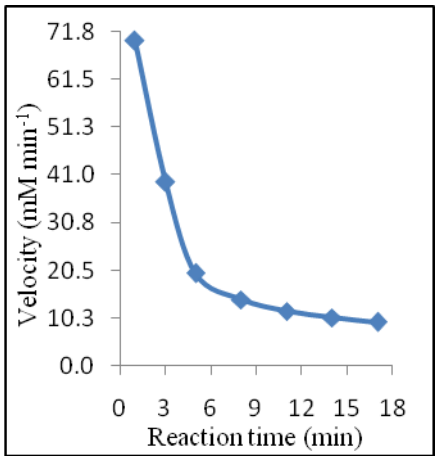


Figure 5Plot of velocity of catalase-catalyzed reaction as a function of reaction time

Effect of Enzyme Concentration on Catalasae-catalyzed Reaction

The activity of an enzyme is determined by the enzyme concentration (Walsh, 1968). As the enzyme concentration increases the rate of reaction increases linearly, because there are more enzyme molecules available to catalyze reaction.The validity of enzyme assay method was tested using different volumes of enzyme. The enzyme activity was found to have a linear relationship with different volumes of enzyme solution ranging between 0.03 to 0.50 mL of enzyme (Table 6 and Figure 6).

Table 6 Relationship between Catalase Activity and Enzyme Concentration

No.	Enzyme solution (mL)	Catalase activity (mmol min^{-1} mL^{-1})
1	0.03	0.5063
2	0.06	1.2151
3	0.13	2.6329
4	0.25	4.7595
5	0.50	8.3038

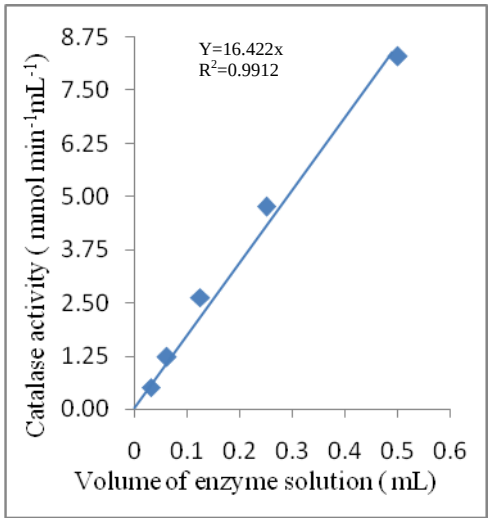


Figure 6 Plot of catalase activity as a function of volume of enzyme solution

CONCLUSION

In this research, partially purified catalase enzyme was isolated from matured green cabbage by ammonium sulphate precipitation (50 %) method. The partially purified catalase was qualitatively examined by hydrogen peroxide test and catalase activity was confirmed by liberation of oxygen gas bubbles to form foam.Catalase was purified 5.68 fold over crude extract and protein recovery was found to be39.04 %. Specific activity of catalase in crude extract was 0.0753 mmol $min^{-1}mg^{-1}$ after 50 % ammonium sulphate precipitation.The optimum pH of catalase was found to be 7.5 in phosphate buffer and optimum temperature was found to be 35 °C.Reaction time of 2 min was chosen for initial velocity measured in enzyme kinetic.The catalase activity was found to have a linear relationship with different enzyme concentrations.

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REFERENCES

Charles, H. H., Larry,C. B. and Norman, W. H. (2007). *A Laboratory for General, Organic, and Biochemistry*. New York: 5th Edition, McGraw Hill Co., pp.357-378

Foyer, C. andNoctor , G. (2000). Oxygen Processing in photosynthesis: Regulation and Signaling. *New Phytologist*, 146(3), pp. 359-388

Hadwan, M. H. (2018) "Simple Spectrophotometric Assay for the Measuring Catalase Activity in Biological Tissues". *BMC Biochemistry,* **19**(7), pp.1–8

Savary, J., Pu,P. H.and Sunderman, F. W. (1969). "A Biuret Method for Determination of Protein in Normal Urine". *Clinical Chemistry*,vol.14(12), pp.1160-1171

Walsh, E. O. F. (1968). An Introduction to Biochemistry. London: The English Press Ltd., pp.135-147