STUDY ON KINETIC PROPERTIES OF CATALASE ISOLATED FROM *CORIANDRUM SATIVUM* L. LEAVES

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Abstract

Isolation and purification of catalase enzyme from *Coriandrum sativum* L. leaves were performed by ammonium sulphate precipitation followed by dialysis and gel filtration chromatography on Sephadex G-100. The catalase activity of the final purification steps was measured by following the dismutation of H_2O_2 spectrophotometrically using an extinction coefficient for H_2O_2 at 439 nm of 0.0113 mM^{-1} cm^{-1}. The effect of substrate concentration and enzyme concentration on the catalase-catalyzed reaction was studied. The K_m value was calculated to be 5.1420×10^{-3} M H_2O_2, and the V_{max} value was found to be 0.1308 M min^{-1} using the plot of Lineweaver-Burk. The activation energy (E_a) of the catalase-catalyzed reaction was calculated to be 2.978 kcal mol^{-1}. The reaction order (n) of the catalase-catalyzed reaction was found to be the first-order reaction. The catalase activity decreased with each hour of incubation in buffers with different pH values and temperatures. After 3 h of incubation at 40 ºC, the catalase activity was almost completely lost.

Keywords: catalase enzyme, ammonium sulphate precipitation, *gel filtration chromatography*, K_m, V_{max}, E_a, reaction order

Introduction

Antioxidants found in nature are enzymatic and non-enzymatic antioxidants. They all work together in collaboration to equalize oxidative stress. In all living cells, enzymes were found to be very necessary. They impact the rate at which a biochemical reaction achieves equilibrium (Susmitha *et al*., 2016). Catalase (EC 1.11.1.6) is the first discovered and potent antioxidant enzyme that catalyzes the disintegration of hydrogen peroxide into water and dioxygen (Demir *et al.*, 2008). It is broadly spread among a diversity of life forms, consisting of plants, animals, and microbes, and is usually away from anaerobic organisms. They play crucial roles in promoting health by forming part of our bodies' primary system of defence against free radical damage (Dumen and Kaya, 2013). Catalase holds an important place in the enzymatic world because of its use in various industries and medicines. The main role of catalase in cells is to protect against the accumulation of hydrogen peroxide (H_2O_2) up to a toxic level that forms as a by-product of the metabolic process that occurs in a living system. It also finds diverse industrial applications in textiles, waste treatment, cosmetics, and as a disinfectant agent, while in the analytical field it is used as a source of H_2O_2 (Farhan *et al.*, 2016).

Since ancient times, there has been a growing interest in plants. *Coriandrum sativum* has a very effective antioxidant profile, showing radical scavenging activity, hydroxyl radical scavenging activity, superoxide dismutation, etc. The present study aimed to study the kinetic behaviour of the purified catalase enzyme isolated from *Coriandrum sativum* L. leaves.

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Materials and Methods

Sample Collection

Coriander leaf samples were collected from Hledan Market, Kamayut Township, Yangon Region. Then, identification of the sample was done at the Department of Botany, University of Yangon. Sample extraction and purification were performed at the Analytical Chemistry Research Laboratory, Department of Chemistry, University of Yangon.

Materials

The chemicals required and catalase assay reagents were purchased from Sigma-Aldrich, England. All the chemicals used in this work were analytical grade. In all examinations, the recommended standard methods and techniques involving both conventional and modern methods were provided.

Sample Preparation, Extraction, and Purification of Catalase Enzyme from *Coriandrum sativum* L. Leaves

A 30 g of fresh coriander leaves were washed with tap water, air dried, cut, and homogenized with a 150 mL phosphate buffer (pH 7.0) solution. It was blended with an electrical blender and filtered. The suspensions were centrifuged for about 30 min at 5500 rpm. And then the precipitate was discarded, and the supernatant was collected. Solid ammonium sulphate was slowly added to this supernatant to obtain 20 % saturation. To homogenize it for complete dissolution, it was stirred for 2 h in ice. After 2 h, the mixture was centrifuged again for about 30 min and the precipitate was discarded. Solid ammonium sulphate was slowly added to this filtrate to obtain 20-70 % saturation, and after standing overnight, the precipitated protein-containing catalase enzyme was collected by centrifugation for about 30 min. The precipitate was dissolved in phosphate buffer (pH 7.0), and then the catalase enzyme was dialyzed through a dialysis bag in the same buffer for 5 h, with two changes of the buffer during dialysis. For further purification of catalase, the dialyzed enzyme sample was put onto the column filled with Sephadex G-100 equilibrated with the phosphate buffer at a flow rate of 1.5 mL/7 min. Eluates were collected in 1.5 mL tubes (Gholamhoseinian *et al*., 2006).

Catalase Enzyme Assay

For the enzyme assay, the catalase activity of the purified sample was measured spectrophotometrically by monitoring the reduction in absorbance at 439 nm. To stop the reaction, a cobalt bicarbonate solution was used. Although other methods of measuring catalase activity have been developed, this method is free from the interference that results from the presence of amino acids, proteins, sugars, and fats in the studied sample. Catalase was assayed by its depletion of hydrogen peroxide under slight modification. One unit is the amount of enzyme that decomposes one micromole of H_2O_2 per minute under the assay conditions (Hadwan, 2018).

Kinetic Studies of Catalase Enzyme

The values of K_m and V_{max} were determined using different concentrations of hydrogen peroxide (2.5 mM – 35 mM) as a substrate. The reaction order and activation energy of the catalase-catalyzed reaction were also evaluated.

Determination of pH Stability and Thermostability of Catalase Enzyme

Firstly, 0.5 mL of purified catalase with phosphate buffer pH 7 was placed in a water bath at temperatures of 20, 30, and 40 ℃. For pH stability, the enzyme solution was mixed with a

pH (6, 7, and 8) phosphate buffer solution at room temperature. After the above treatment, enzyme activity was examined at incubation times of 0, 1, 2, and 3 h under the assay conditions.

Effect of Enzyme Concentration

To study the effect of enzyme concentration, the activities of the catalase enzyme were examined using different concentrations of enzyme solution in a 10 mM phosphate buffer at pH 7.

Results and Discussion

Catalase enzyme from *Coriandrum sativum* L. leaves was extracted by (20-70) % ammonium sulphate precipitation method. Purification was achieved by dialysis and gel filtration chromatography on Sephadex G-100. Each 1.5 mL fraction after purification with Sephadex G-100 was estimated for enzyme activity and protein content. Fractions with high catalase activity were pooled and stored at 4℃. Catalase activity was found to be 31221 μmol min^{-1} in a total volume of purified enzyme of 15 mL. The total protein content was determined by the Biuret method, and it was observed to be 85.59 mg. The specific activity was calculated as 364.77 μmol min^{-1} mg^{-1}.

Effect of Substrate Concentration on Catalase Enzyme-catalyzed Reaction

In the present study, the velocities of enzyme reactions measured at varying levels of hydrogen peroxide concentration and their reciprocal values are shown in Table 1. The Michaelis-Menten plot of V vs. [S] is shown in Figure 1. The catalase enzyme reaction was followed by a hyperbolic curve. The concentration of hydrogen peroxide was increased, and the rate of reaction also increased, until a point was reached where the enzyme was working as fast as it could, that is, transforming its maximum number of hydrogen peroxide molecules each minute. At this point, the enzyme is said to be saturated with substrate, and further increases in the concentration of hydrogen peroxide would not increase the rate of reaction. The enzyme could not have worked faster. For a more accurate estimation, K_m and V_{max} values were computed using statistical and various graphical methods (Figures 2, 3, 4, and 5), and the values are presented in Table 2.

Table 1. Relationship between Substrate Concentration and Velocity of Catalase Enzyme-catalyzed Reaction

[S] (M)	-[S] (M)	1/[S] (M^{-1})	V (M min^{-1})	1/V (M^{-1} min)	V/[S] (min^{-1})	[S]/V (min)
0.0025	-0.0025	400.0000	0.0435	22.9885	17.4000	0.0575
0.0050	-0.0050	200.0000	0.0627	15.9489	12.5400	0.0798
0.0100	-0.0100	100.0000	0.0804	12.4378	8.0400	0.1244
0.0150	-0.0150	66.6667	0.0944	10.5932	6.2933	0.1589
0.0200	-0.0200	50.0000	0.1062	9.4162	5.3100	0.1883
0.0250	-0.0250	40.0000	0.1121	8.9206	4.4840	0.2230
0.0300	-0.0300	33.3333	0.1143	8.7489	3.8100	0.2625
0.0350	-0.0350	28.5714	0.1217	8.2169	3.4771	0.2876

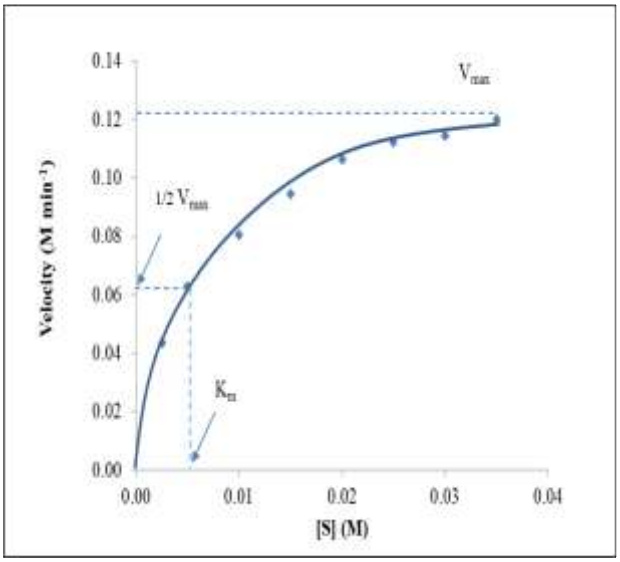

Figure 1. Michaelis-Menten plot of catalase enzyme-catalyzed reaction

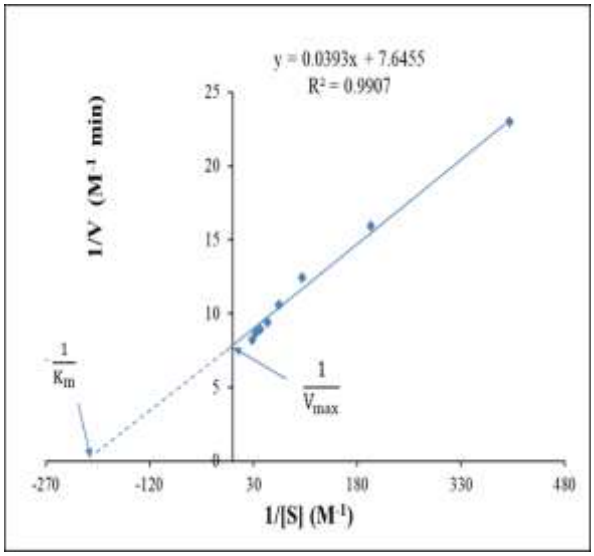

Figure 2. Lineweaver-Burk plot of 1/V vs. 1/[S] for catalase enzyme-catalyzed reaction

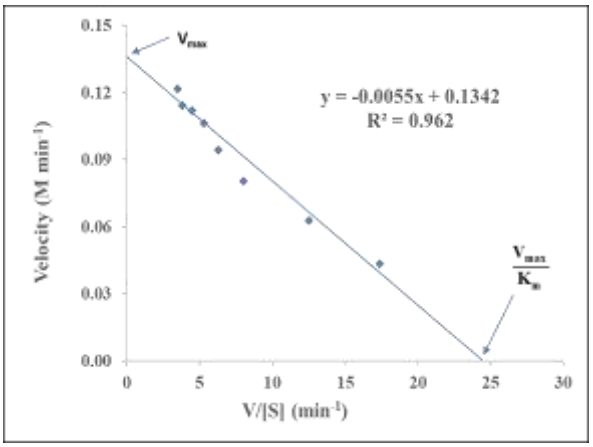

Figure 3. Eadie-Hofstee plot of catalase enzyme-catalyzed reaction

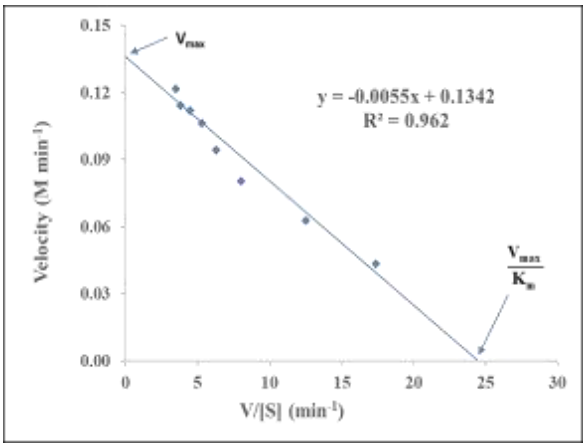

Figure 4. Hanes-Wilkinson plot of catalase enzyme-catalyzed reaction

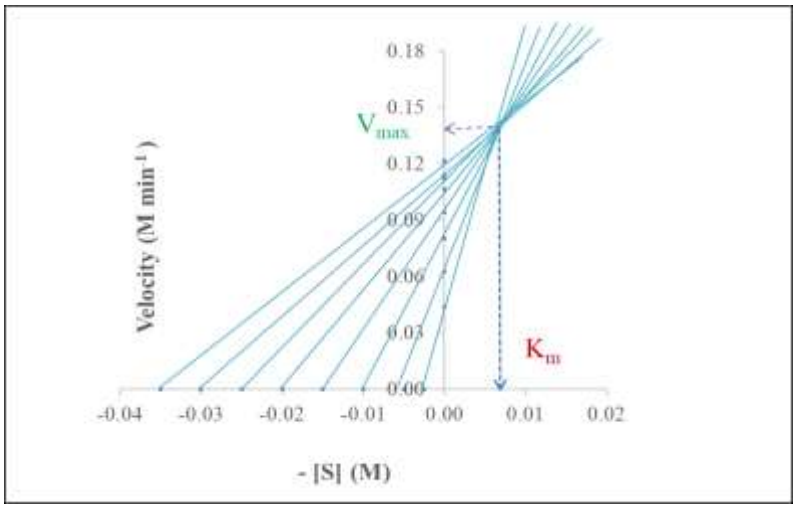

Figure 5. Eisenthal-Cornish Bowden plot of catalase enzyme-catalyzed reaction

Table 2. Representation of V_{max} and K_m Values of Catalase Enzyme Using Statistical and Graphical Methods

Methods	Statistical		Graphical	
	$K_m \times 10^3$ (M)	V_{max} (M min^{-1})	$K_m \times 10^3$ (M)	V_{max} (M min^{-1})
Michaelis-Menten	-	-	5.0000	0.1230
Lineweaver-Burk	5.1420	0.1308	5.1410	0.1308
Eadie-Hofstee	5.5167	0.1342	5.5000	0.1348
Hanes-Wilkinson	6.8160	0.1426	6.6557	0.1416
Eisenthal-Cornish Bowden	-	-	6.3000	0.1360

Reaction Order of Catalase-catalyzed Reaction

A reaction specified by the transformation of one molecule of A to one molecule of B with no impact from any other reactant or solvent is a first-order reaction (Martin *et al*., 1993). In this study, the 'n' value was determined from the plot of Log $V/(V_{max} - V)$ vs. Log [S] for catalase activity using the linear regression method (Table 3 and Figure 6). The reaction order (n) was observed to be a first-order reaction due to the evaluated value being 1.1702.

Table 3. Reaction Order for Catalase Enzyme-catalyzed Reaction

No.	Log [S]	Log $V/(V_{max} - V)$
1	-2.6021	-0.3025
2	-2.3010	-0.0359
3	-2.0000	0.2028
4	-1.8239	0.4139
5	-1.6989	0.6352
6	-1.6021	0.7778
7	-1.5229	0.8406
8	-1.4559	1.1263

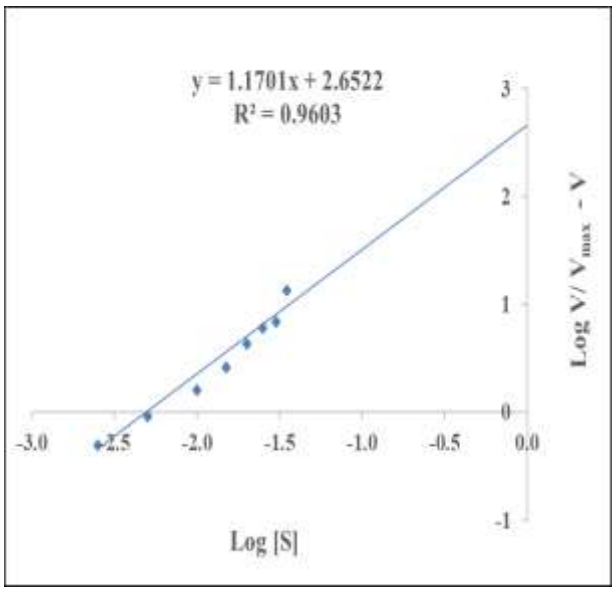

Figure 6. Plot of by log V/V_{max} -V as a function of log [S] of catalase-catalyzed reaction

Activation Energy of Catalase-Catalyzed Reaction

Enzymes lower the activation energy of the chemical reactions that they catalyze, and the reaction proceeds at a faster rate. Activation energy determination was performed using the relation between reaction velocity and temperature (Table 4). There is an increase in temperature and a faster reaction velocity. From the plot of Log V vs 1/T (Figure 7) the evaluated E_a value was found to be 2.978 kcal mol^{-1}.

Table 4. Relationship between Catalase Enzyme Velocity and Temperature of the Solution at pH 7

Temperature (°C)	1/T x 10^3 (K^{-1})	Velocity (M min^{-1})	Log V
10	3.5336	0.0822	-1.0851
20	3.4130	0.0870	-1.0605
25	3.3557	0.0999	-3.3570
30	3.3003	0.1188	-3.0534

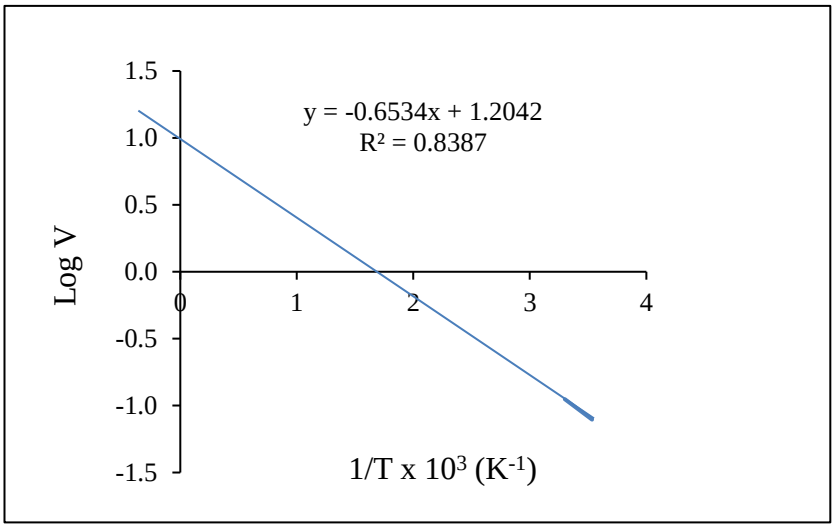

Figure 7. Plot of Log V as a function of 1/T for the determination of E_a

pH Stability and Thermostability of Catalase Enzyme

Stability means the maintenance of a defined functional state (chemical and structural properties that are needed for activity) under extreme conditions. To be a thermostable protein means to be resistant to changes in protein structure due to applied heat (Jaenicke and Bohm, 1998). For the determination of the pH stability of catalase activity in buffers with different pH values, an incubation period of 0–3 h was used. The data results are shown in Table 5 and Figure 8. The catalase activity of each buffer retained over half of its activity after 1 h of incubation. However, activity decreased 24.76 %, 31.11 %, and 28.21 % of its original activity for 3h of incubation.

The thermostability of catalase activity was also investigated using a particular pH of 7 at temperatures of 20, 30, and 40 ºC (Table 6). It can be seen clearly in Figure 9 that the catalase activity at 30 ºC was relatively stable, whereas at 20 ºC and 40 ºC, activity was reduced to 21.21 % and 9.80 % respectively.

Table 5. Relationship between Relative Activity of Catalase Enzyme and Incubation Time at Different pH Values

pH	Incubation time (h)	Catalase activity (μmol min^{-1} mL^{-1})	Relative activity (%)
6.0	0	1239.19	100.00
	1	601.89	48.57
	2	377.66	30.48
	3	306.85	24.76
7.0	0	2124.32	100.00
	1	1428.01	67.22
	2	1227.38	57.78
	3	660.89	31.11
8.0	0	1841.08	100.00
	1	1144.77	62.18
	2	920.54	50.00
	3	519.28	28.21

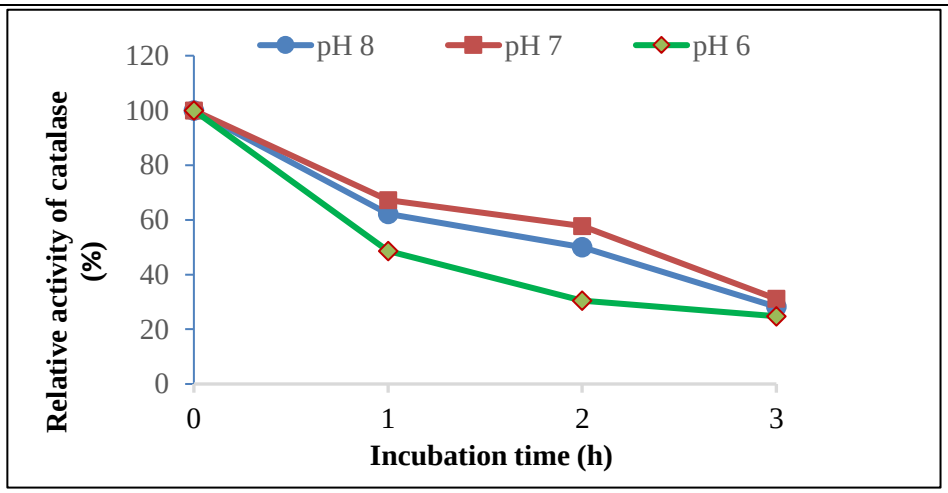

Figure 8. Plot of relative activity of catalase as a function of incubation at different pH values

Table 6. Relationship between Relative Activity of Catalase Enzyme and Incubation Time at Different Temperatures

Temperature (°C)	Incubation time (h)	Catalase activity (μmol min^{-1} mL^{-1})	Relative activity (%)
20	0	1947.29	100.00
	1	778.92	40.00
	2	578.29	29.69
	3	413.06	21.21
30	0	2195.13	100.00
	1	1062.16	48.39
	2	708.11	32.26
	3	625.49	28.49
40	0	1805.67	100.00
	1	613.69	33.99
	2	247.84	13.73
	3	177.03	9.80

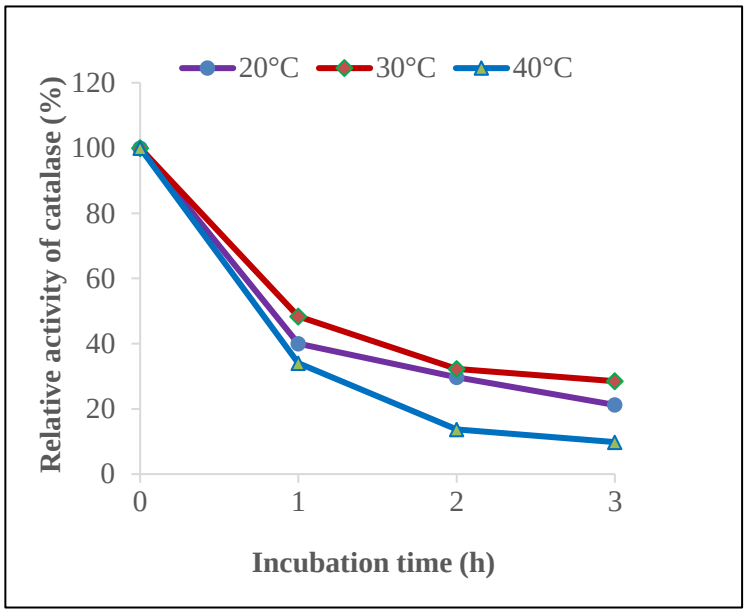

Figure 9. Plot of relative activity of catalase as a function of incubation at different temperature values

Effect of Enzyme Concentration on Catalase-catalyzed Reaction

As the concentration of an enzyme increases, the rate at which the substrate is changed also increases (Henrickson *et al*., 2007). Using different concentrations of enzyme ranging from 5.5 % to 7.5 %, the activities of catalase were examined.

Table 7. Relationship between Catalase Enzyme Activity and Enzyme Concentration

No.	Catalase enzyme concentration (%)	Catalase activity (μmol min^{-1} mL^{-1})
1	5.5	404.96
2	6.0	444.60
3	6.5	478.60
4	7.0	504.08
5	7.5	535.24

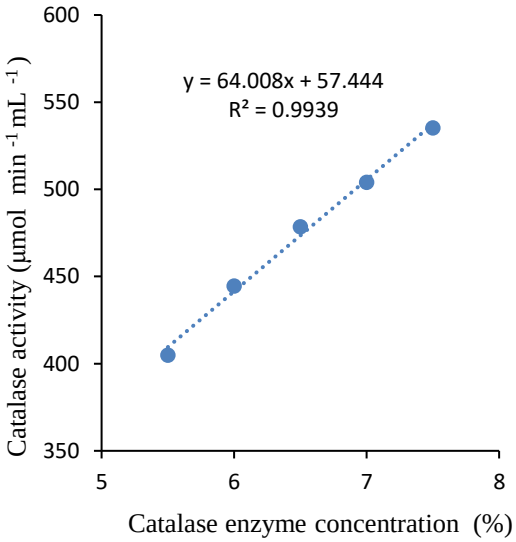

Figure 10. Plot of catalase enzyme activity as a function of enzyme concentration

According to Table 7, it was found that there was a direct relationship between enzyme concentration and catalase activity. The velocity of the reaction increased when the concentration solution was increased. An increase in enzyme concentration causes a decrease in colour intensity due to hydrogen peroxide depletion.

Conclusion

In this research, purified catalase enzyme was isolated from mature green *Coriandrum sativum* L. leaves. The total catalase activity and protein content of purified catalase were computed to be 31221 μmol min^{-1} and 85.59 mg, respectively. The values of K_m and V_{max} were treated statistically using the linear regression method. It was confirmed with various graphical methods: Michaelis-Menten, Lineweaver-Burk, Eadie-Hofstee, Hanes-Wilkinson, and Eisenthal-Cornish Bowden. In various methods of investigation, the values of K_m and V_{max} were seen as nearly the same. For the Lineweaver-Burk plot, the K_m and V_{max} values of catalase were found to be 5.1420×10^{-3} M and 0.1308 M min^{-1}, respectively. The reaction order (n) for catalase was computed to be 1.1702, proving that the reaction order is first order. The activation energy (E_a) of catalase-catalyzed reaction was calculated to be 2.978 kcal mol^{-1}. In the pH stability estimation of catalase activity, activity decreased by 24.76 %, 31.11 %, and 28.21 % of original activity, for pH 6, 7, and 8, respectively, for a 3h incubation at room temperature. The thermostability study on catalase activity revealed that the activity at 30 ºC was relatively stable. After 3 h of incubation at 40 ºC, the catalase enzyme lost nearly all of its original activity. The catalase activity was found to have a linear relationship with different enzyme concentrations.

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