The Kinetic Parameters of Polyphenol Oxidase Enzyme from *Ipomoea batatas* (L.) Lamk (White Sweet Potato)

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Abstract

Polyphenol oxidase (EC 1.10.3.1), a Cu containing metalloprotein, catalyzes the oxidation of several phenols to *o*-quinones, which produce brown pigments (melanins). In this research, polyphenol oxidase was extracted from *Ipomoea batatas* (L.) Lamk (white sweet potato) sample using phosphate buffer solution at pH 6.8. And then, partially purified polyphenol oxidase enzyme was isolated by using ammonium sulphate precipitation (35 % - 85 %) method. The polyphenol oxidase activity was determined by spectrophotometric assay method using catechol as substrate at 420 nm. The enzyme kinetic parameters of polyphenol oxidase were studied. From the data obtained, the value of maximum velocity (V_{max}), Michaelis-Menten constant (K_m) and reaction order (n) were calculated.

Keywords: *Ipomoea batatas* (L.) Lamk, polyphenol oxidase, V_{max}, K_m, n

Introduction

Ipomoea batabas (L.) lamk (sweet potatoes) are native to the tropical parts of the Americas and were domesticated there at least 5000 years ago. The sweet potato is one of the world's most significant food crops with both the tubers and foliage finding their way into the traditional dishes of many countries. This root is susceptible to browning reactions that affect quality and consumer acceptance. Sweet potatoes discolor when cut or sliced, peeled and heat-processed and the tissue damage caused by these processes results in activation of polyphenol oxidase and leads to discoloration of the product (Arthur and McLemore, 1956).

Polyphenol oxidases are a group of Cu-containing enzymes that catalyze the oxidation of several phenols to *o*-quinones (Oliveira *et al*., 2011). In turn, *o*-quinones are highly reactive molecules that can undergo non-enzymatic secondary reactions to form brown complex polymers known as melanins and cross-linked polymers with protein functional groups (Friedman, 1996). The conversion of phenolic substrates to *o*-quinones by polyphenol oxidases occurs by means of two oxidation steps (Vamos-Vigyazo, 1981). The first is the hydroxylation of the *ortho*-position adjacent to an existing hydroxyl group ("monophenol oxidase" activity). The second is the oxidation of *o*-dihydroxybenzenes to *o*-benzoquinones ("diphenol oxidase" activity) (Yourk and Marshall, 2003).

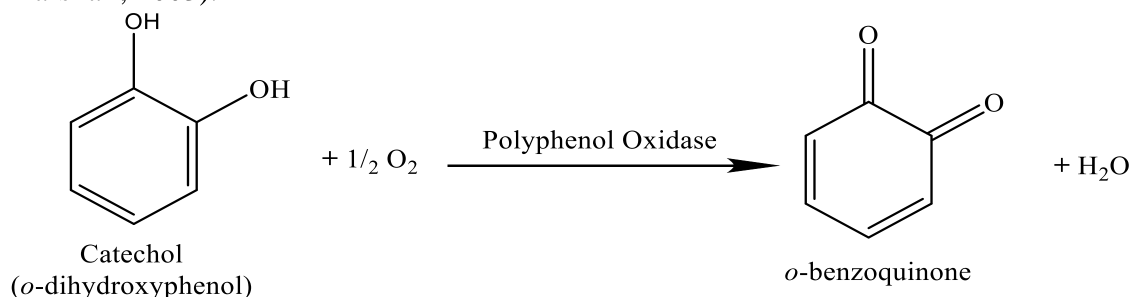

Figure 1. Enzymatic oxidation of *o*-dihydroxyphenol to the *o*-benzoquinone

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Materials and Methods

Sample Collection

Ipomoea batatas (L.) Lamk (white sweet potato) sample was collected from Pathein Myoma market, Ayeyarwady Region.

(a) (b)

Figure 2. White sweet potato (a) plant and (b) tuber

Sample Preparation and Extraction of Polyphenol Oxidase Enzyme

The sample was washed and stored in refrigerator at 4˚C for overnight. After overnight-cooled, white sweet potato sample was peeled and cut into small pieces. Rapidly weighted white sweet potato sample was blended and mixed with 1:1 sodium chloride and phosphate buffer solution at pH 6.8 and then filtered. Filtration was centrifuged for 60 min by using centrifugation method. Crude polyphenol oxidase enzyme was partially purified by using ammonium sulphate precipitation method. Solid ammonium sulphate was added to the crude enzyme to give 35 % saturation. After standing for 1 hour, the precipitated was removed by centrifugation for 30 min and was discarded. Additional ammonium sulphate was then added to 85 % saturation. After standing overnight, complete precipitate was collected by centrifugation for 30 min. Finally, partial purified polyphenol oxidase enzyme was obtained.

Determination of Polyphenol Oxidase Activity and Substrate Concentration

Polyphenol oxidase enzyme activity was determined with the spectrophotometric assay method by monitoring the increase in absorbance (420 nm) at 15 seconds time intervals with UV-visible spectrophotometer. The enzyme kinetic parameters such as maximum velocity (V_{max}), Michaelis-Menten constant (K_m) and reaction order (n) of polyphenol oxidase-catalyzed reaction were also determined by using various graphical and statistical methods.

$$\text{Polyphenol oxidase activity} = \frac{\Delta A \text{ at } 420 \text{ nm}}{\text{volume of enzyme solution} \times \Delta T \text{ (min)} \times 0.001}$$

ΔA = absorbance at 420 nm

ΔT = time

Results and Discussion

Polyphenol Oxidase Activity

Polyphenol oxidases are copper containing oxidoreductases that catalyze the hydroxylation and oxidation of phenolic compounds in the presence of molecular oxygen (Whitaker, 1972).

In this research, crude polyphenol oxidase enzyme was extracted from white sweet potato sample by centrifugation method. The supernatant liquid 80 mL was collected. Ammonium sulphate precipitation method was chosen for partial purification of crude polyphenol oxidase enzyme. Solid ammonium sulphate was added to the supernatant to give 35 % and 85 % saturation. Therefore, the partial purified polyphenol oxidase enzyme from white sweet potato sample was obtained 40 mL. Polyphenol oxidase enzyme activity was determined by spectrophotometric assay method. The polyphenol oxidase activity from white sweet potato sample was found to be 207.5 EU. The optimum pH and temperature were obtained 6.5 and 37˚C, respectively.

Effect of Substrate Concentration on Polyphenol Oxidase-Catalyzed Reaction

The rate of any enzyme-catalyzed process depends upon the concentration of the enzyme and its substrate (Tucker, 1991).

In this work, The K_m and V_{max} values of polyphenol oxidase-catalyzed reaction were carried out with substrate concentration values ranging from 0.05 to 0.5 M. The Michaelis-Menten plot, Lineweaver-Burk plot, Eadie-Hofstee plot, Hanes-Wilkinson plot and Eisenthal-Cornish Bowden plot for polyphenol oxidase-catalyzed reaction were drawn (Table 1 and Figures 3-5).

The comparison of different methods (statistical treatment using linear regression method and various graphical methods) for reaction kinetic parameters of polyphenol oxidase from white sweet potato sample was shown in Table 2. It can be seen from the Table 2, clearly that the results (K_m and V_{max}) from all methods except Michaelis-Menten plot are in agreement with each other. Michaelis-Menten plot is approximately method and it gives apparent K_m and V_{max} values.

Table 1. Relationship between Initial Substrate Concentration and Velocity of Polyphenol Oxidase-Catalyzed Reaction

[S] (M)	-[S] (M)	1/[S] (M^{-1})	$V\times10^5$ (M min^{-1})	$1/V\times10^{-5}$ (M^{-1} min)	$V/[S]\times10^5$ (min^{-1})	$[S]/V\times10^{-5}$ (min)
0.05	-0.05	20.0	1.075	0.930	21.500	0.047
0.10	-0.10	10.0	1.431	0.699	14.310	0.070
0.20	-0.20	5.0	1.647	0.607	8.235	0.121
0.30	-0.30	3.3	1.761	0.568	5.870	0.170
0.40	-0.40	2.5	1.831	0.546	4.578	0.218
0.50	-0.50	2.0	1.881	0.532	3.762	0.266

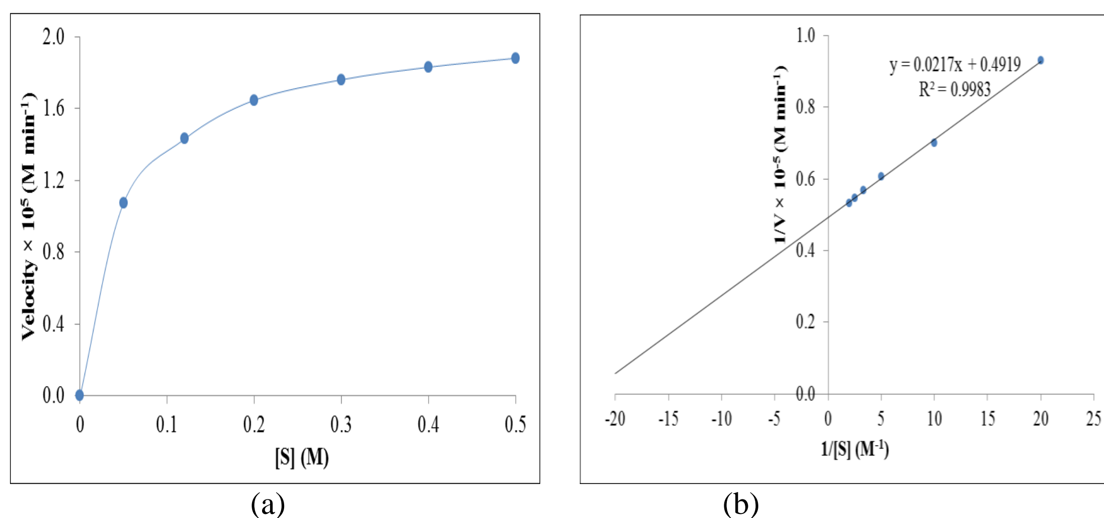

Figure 3. (a) Michaelis-Menten plot of Velocity *vs*. [S] and (b) Lineweaver-Burk plot of 1/V *vs*. 1/[S] used for graphic evaluation of V_{max} and K_m for polyphenol oxidase enzyme

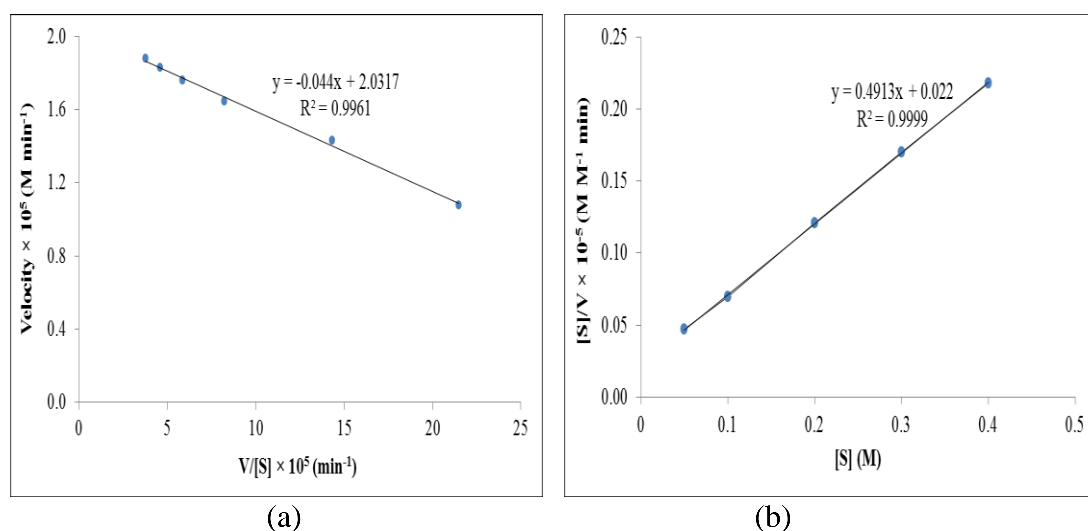

Figure 4. (a) Eadie-Hofstee plot of Velocity *vs*. V/[S] and (b) Hanes-Wilkinson plot of [S]/V *vs*. [S] used for graphic evaluation of V_{max} and K_m for polyphenol oxidase enzyme

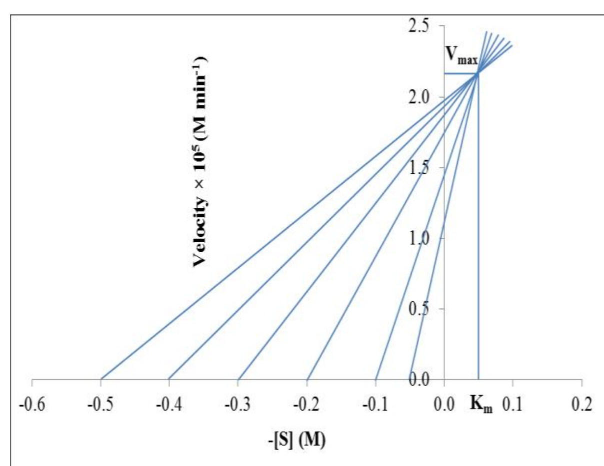

Figure 5. Eisenthal-Cornish Bowden plot used for graphic evaluation of V_{max} and K_m polyphenol oxidase enzyme

Table 2. Comparison of K_m and V_{max} Values for Polyphenol Oxidase Enzyme from Different Methods

No.	Method	Graphical Method		Statistical Method	
		$V_{max}\times10^5$ ($M\ min^{-1}$)	$K_m\times10^2$ (M)	$V_{max}\times10^5$ ($M\ min^{-1}$)	$K_m\times10^2$ (M)
1	Michaelis-Menten	1.88	4.00	-	-
2	Lineweaver-Burk	2.03	4.41	2.03	4.43
3	Eadie-Hofstee	2.03	4.40	1.99	4.01
4	Hanes-Wilkinson	2.04	4.48	2.04	4.49
5	Eisenthal-Cornish Bowden	2.15	4.50	-	-

Reaction Order (n) for Polyphenol Oxidase-Catalyzed Reaction

The order of a chemical reaction with respect to the individual components is defined as the power of the component concentration included into the rate of equation (Christensen, 1974).

In the present work, K_m and n values were determined from the plot of log $V/(V_{max}-V)$ *vs.* log [S] for polyphenol oxidase by using the linear regression method (Table 3 and Figure 6). The reaction order (n) for polyphenol oxidase was calculated to be 1.0072 proving that reaction order is first order. The K_m was observed to be 4.362×10^{-2} M.

Table 3. Determination of Reaction Order (n) and K_m Value for Polyphenol Oxidase-Catalyzed Reaction

No.	Log [S]	Log ($V/V_{max}-V$)
1	-1.301	0.050
2	-1.000	0.375
3	-0.699	0.629
4	-0.523	0.810
5	-0.398	0.955
6	-0.301	1.089

Reaction order n = 1.0072 *i.e.*, the first order kinetic is obeyed.
$K_m = 4.36 \times 10^{-2}$ M

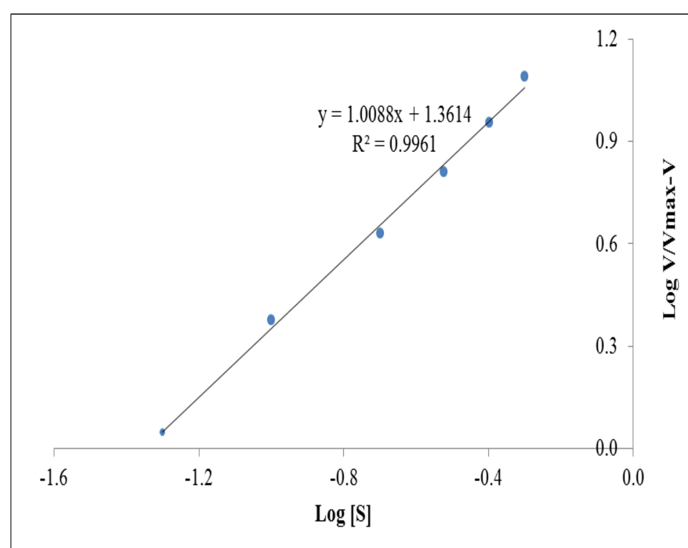

Figure 6. Plot of log $V/(V_{max}-V)$ as a function of log [S] for Polyphenol Oxidase-catalyzed reaction

Conclusion

In this research work, polyphenol oxidase enzyme from white sweet potato sample was extracted by using phosphate buffer solution (pH 6.8). The partially purified polyphenol oxidase enzyme was obtained by using 35% and 85% saturation with ammonium sulphate. The polyphenol oxidase activity was characterized by measuring the absorbance at 420 nm. The values of V_{max} and K_m treated statistically using the linear regression method were compared with various graphical methods (Michaelis-Menten, Lineweaver Burk, Eadie-Hofstee, Hanes-Wilkinson and Eisenthal-Cornish Bowden plots). The calculated V_{max} and K_m values were 4.362×10^{-2} M and 2.0339×10^{-5} $Mmin^{-1}$, respectively. The reaction order (n) of polyphenol oxidase-catalyzed reaction was calculated to be 1.0072. Therefore, the first order is obeyed. Therefore, polyphenol oxidase enzyme is applied for the removal of phenol and related organic compound in industrial wastewater.

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