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EFFECT OF CINNAMIC ACID ON POLYPHENOLOXIDASE ACTIVITY OF *DIOSCOREA ROTUNDATA*

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ABSTRACT

Spectrophotomeric method was used to determine the effect of cinnamic and on the activity of polyphenoloxidase extracted from *Dioscorea rotundata*. In the control analysis, increasing concentrations of catechol in the order: 0.00075M, 0.0015M, 0.003M, 0.006M and 0.012M were added as substrate to the enzyme extract. In the test sample, 0.04M concentration of cinnamic acid was added to the enzyme assay mixture in addition to the earlier specified concentrations of the enzyme and substrate. The Michealis-Menten constant (Km value) and the maximum velocity (Vmax) were evaluated under the two experimental conditions. The Km value of the control experiment gave 9.09 x 10^{-3}M while the Vmax was 2.34 x 10^{-3} O.D/Sec for both the control and test samples. Further evaluation of the inhibitor binding constant (K_i value) gave 0.039 x 10^{1}M and the presence of cinnamic acid did not alter the maximum enzyme activity (Vmax) of polyphenoloxidase. Therefore this was an indication that cinnamic acid exhibited competitive inhibition kinetics on the activity of polyphenoloxidase.

KEYWORDS: Cinnamic acid, Michealis-Menten constant, maximum velocity, Polyphenoloxidase, *Dioscorea rotundata*.

INTRODUCTION

The genus, *Dioscorea rotundata* also referred to as "white yam" rank as an important staple food for millions of people in tropical countries (Coursey, 1976).

Enzymatic browning requires four different components namely, oxygen, an enzyme, copper and a substrate (Mayer and Harel, 1979). The most important enzyme being polyphenoloxidase (PPO), which is a generic term for the group of enzymes that catalyses the oxidation of phenolic compounds to produce a brown colour on the cut surface of yam. During the operations of peeling and cutting, cell membranes are broken and appropriate substrates come into contact with the oxidizing enzyme. In the presence of oxygen, rapid browning occurs due to the enzymatic oxidation of phenols to orthoquinones, which rapidly polymerize to form brown or black pigments such as melanin (Whitaker and Lee, 1995).

Since yam has to be peeled or cut into slices before processing, these colour changes would invariably be an associated feature of the finished products. Therefore, knowledge of browing process should be clearly understood it its effect is to be countered for aesthetic purposes and nutritive quality of the product (Mbadiwe, 1978, Synge, 1975). For these reasons, it becomes imperative that some means have to be explored and applied to minimize the browning reaction. This study is concerned with the possible application of cinnamic acid, a plant secondary metabolite, as a potent inhibitor of the activity of polyphenoloxidase extracted from *D. rotundata*, which is responsible for the browning process.

MATERIALS AND METHODS

Sample collection

Yam tuber, *D. rotundata* was purchased from Eke Ukwu local market in Owerri West Local Government Area, Owerri, Nigeria.

Preparation of Crude Extract of Polyphenoloxidase

Ten grams (10g) of the peeled yam tuber were washed with distilled water and cut into 10g/l ice cold sodiumsulphite solution and allowed to stand for 20minutes. After soaking the sodiumsulphite solution was decanted and the cut sample was washed with distilled water. The sampie was blended in 20ml phosphate buffer (pH = 7) for 3 minutes and the resulting homogenate was quickly squeezed through two layers of clean cheese cloth into a beaker kept in ice. The crude extract was filtered through Whatman No 1 fitter paper. The filtrate was subsequently centrifuged at 1000g for 5 minutes with the use of 80 – 2 Electric Centrifuge (B. Bran Scientific and Instrument Company, England).

Determination of Polyphenoloxidase Activity

The activity of polyphenoloxidase was determined based on the methods of Ensiminger and Vamos – Vigyazo (1995). Five serial dilutions of 0.012M, 0.006M, 0.003M, 0.0015M and 0.00075M were prepared from a stock solution of 0.024M catechol. To each of the five test tubes, corresponding concentrations of 1ml of catechol solution was added. It was followed by the addition of 1ml of 0.10M phosphate buffer (pH = 7). A 3ml of distilled water was added and the enzyme reaction started by the introduction of 0.5ml of the enzyme extract. The reaction mixture was quickly transferred into a cuvette and the change in absorbance was monitored spectrophotometrically at wavelength (λmax) of 540nm at a regular internal of 30seconds for 180 seconds.

Determination of Effect of Cinnamic Acid on the Activity of Polyphenoloxidase.

The activity of polyphenoloxidase was determine in the presence of 0.04M cinnamic acid. To each of the five test tubes, 1ml of 0.012M, 0.006M, 0.003M, 0.0015M and 0.00075M catechol, was added respectively. It was followed by the addition of 1ml of 0.1M phosphate buffer (pH =7). A 3Ml of 0.04M cinnamic acid was added and the enzyme reaction started by the introduction of 0.5Ml of the enzyme extract. The reaction mixture was quickly transferred into a cuvette and the change in absorbance was monitored spectrophotometrically at wavelength of 540nm at a regular internal of 30 seconds for 180 seconds.

RESULTS

The change in absorbance of the reaction mixture which reflected polyphenoloxidase activity is presented in Table 1. The enzyme activity in the presence of cinnamic acid is shown in Table 2.

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Table 1: Change in absorbance during the determination of polyphenoloxidase activity of *D. rotundata*.

Time (sec)	ABSORBANCE VALUES AT DIFFERENT CONCENTRATIONS OF CATECHOL 0.012M	0.006M	0.003M	0.0015M	0.00075M
0	0.000	0.000	0.000	0.000	0.000
30	0.015	0.016	0.020	0.010	0.007
60	0.025	0.046	0.040	0.020	0.007
90	0.025	0.096	0.060	0.030	0.012
120	0.035	0.126	0.080	0.040	0.012
150	0.035	0.156	0.100	0.050	0.012
180	0.035	0.186	0.120	0.060	0.017
V_I (0.D/Sec)	$2.92x10^{-4}$	$1.00x10^{-4}$	$6.67x10^{-4}$	$3.33x10^{-4}$	$2.00x10^{-4}$
$\frac{1}{V_I}$(O.D/Sec^{-1})	$3.425x10^{3}$	$1.000x10^{3}$	$1.499x10^{3}$	$3.000x10^{3}$	$5.000x10^{3}$
$\frac{1}{[S]}$(M^{-1})	$0.083x10^{3}$	$0.167x10^{3}$	$0.333x10^{3}$	$0.667x10^{3}$	$1.333x10^{3}$

Table 2: Change in absorbance during the determination of polyphenoloxidase activity of *D. rotundata* in the presence of cinnamic acid.

Time (sec)	ABSORBANCE VALUES AT DIFFERENT CONCENTRATIONS OF CATECHOL 0.012M	0.006M	0.003M	0.0015M	0.00075M
0	0.000	0.000	0.000	0.000	0.000
30	0.010	0.030	0.018	0.013	0.008
60	0.010	0.060	0.038	0.028	0.013
90	0.020	0.100	0.048	0.033	0.018
120	0.020	0.120	0.068	0.038	0.023
150	0.030	0.150	0.088	0.043	0.023
180	0.030	0.190	0.098	0.043	0.023
V_I (0.D/Sec)	$16.00x10^{-4}$	$10.00x10^{-4}$	$6.40x10^{-4}$	$3.47x10^{-4}$	$1.53x10^{-4}$
$\frac{1}{V_I}$(O.D/Sec^{-1})	$0.593x10^{3}$	$1.000x10^{3}$	$1.563x10^{3}$	$2.725x10^{3}$	$6.536x10^{3}$
$\frac{1}{[S]}$(M^{-1})	$0.083x10^{3}$	$0.167x10^{3}$	$0.333x10^{3}$	$0.667x10^{3}$	$1.333x10^{3}$

The results showed a general increase in absorbance with time for both the control enzyme assay (Table 1) and the test enzyme assay (Table 2). However, the numerical reciprocal values of enzyme velocity (I/V_I O.D/Sec^{-1}) were less in the control enzyme assay than the test enzyme assay owing to the inhibitory effect of cinnamic acid on polyphenoloxidase activity.

Evaluation of Some Kinetic Constants for Polyphenoloxidase Activity.

The kinetic constant were evaluated with the aid of the double reciprocal plot of initial velocity (I/V) versus substrate concentrations (catechol) (I/[s]. Superimposition of the double reciprocal plot derived from results of the control enzyme assay (Table 1) on the enzyme assay in the presence of cinnamic acid (Table 2,) gave the characteristic plot above.

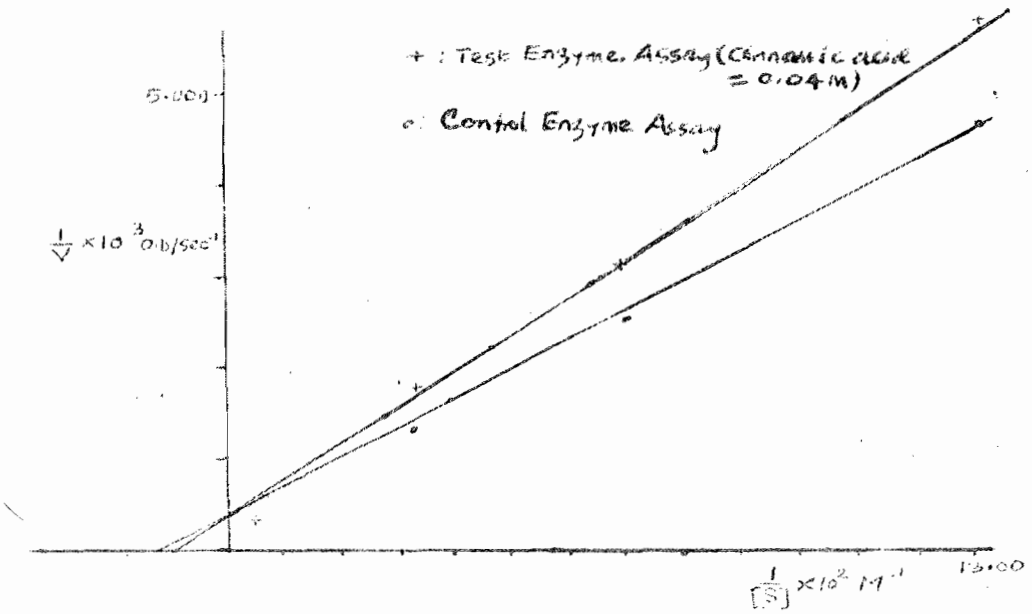

Figure 1: Double Reciprocal Plot of the Control and Test Enzyme Assay of Polyphenol Oxidase Activity of *D-rotundata*.

DISCUSSION

The crude enzyme extract of *D. rotundata* exhibited polyphenoloxidase activity. These results conformed to earlier results by Frinberg (1987), Coseteng and Lee (1987) and Cornwell and Wrolstad (1981), who noted the activity of this enzyme in apple, potato and mushroom respectively.

The Michealis – Menten constant, which is a measure of the affinity of the substrate for the active site of the enzyme, varied with the enzyme inhibitor constant (K_i value). (Km = 9.09 x 10^{-3} M and Ki = 0.0039 x 10M (Figure 1). The comparatively higher K_i value is an indication of lower affinity of the substrate for the enzyme, which is a possible consequence of cinnamic acid being a competitive inhibitor of polyphenoloxidase activity. This is justified by the fact that competitive inhibitors decrease the substrate affinity for the enzyme (Rodwell, 1996). The finding is in agreement with Whitaker and Lee (1995), who noted that polyphenoloxidase extract of pear was competitively inhibited by benzoic acid with Km and K_i values of 1.6 x 10^{-3}M and 0.02 x 10^{1}M respectively. Furthermore, the value of polyphenoloxidase *maximum* enzyme activity (Vmax = 2.43 x 10^{-3} OD/sec) was unaffected during the enzyme assay in the presence of 0.04M cinnamic acid. These results were consistent with classical competitive inhibition enzyme kinetics.

Mayer and Harel (1979) classified the inhibitors, which act directly on polyphenoloxidase into two groups. The first groups, which consist of metal ion chelators such as azide, cyanide, carbon II oxide, halide ions and tropolone, is well documented for inhibition of polyphenoloxidase from various sources. The chloride ion was shown to be non-competitive for apple polyphenoloxidase while other halid ions were observed to have competitive inhibitory effect (Janovitz – klapp, 1990). The second group of inhibitors, which consists of aromatic carboxylic acids of the henzoic and cinnamic series, has been widely studied and reported as well (Janovitz – klapp, 1990). Compounds of this group behave as competitive inhibitors of polyphenoloxidase owing to their structural similarity with phenolic substrate (Martinez and Whitaker, 1995).

In conclusion, the competitive inhibitory effect of cinnamic acid may be due to the structural similarity with phenolic substrate, which was earlier reported by Macrae and Duggleby (1968) who reported that cinnamic acid and its analogues were potent inhibitors of potato and apple polyphenoloxidase. This similarity is enough to accommodate the binding of cinnamic acid to the active site of polyphenoloxidase, but structurally different enough to avoid production of coloured compound, melanin.

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