THERMAL PROPERTIES OF BANANA POLYPHENOL OXIDASE (PRO)

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Polifenol oksidase pisang (PPO) diekstrak dengan menggunakan larutan deterjen dan 1,5 kali pemurnian diperoleh dari diasilasi. Aktivitas jenis dari diazylate adalah 10,6. Suhu optimum pengamatan untuk reaksi PPO dopamine adalah 35°C. Nilai Z untuk PPO isoenzim labil pisang adalah 4,3° + 0,4°C dan unutk kalor resisten isoenzim adalah 8,3° + 0,8°C.

INTRODUCTION

Polyphenol oxidase (E.C.1.10.3.1) has been called tyrosinase, polyphenolase, cathecoloxidase, cresolase, and catecholase. The enzymes from higher plants and fungi oxidize a great variety of monophenolic and diphenolic compounds. The enzyme are probably present in all plant but it is inparticularly high concentration in mushrooms, potato tubers, peaches, apples, bananas, avocadoes, tea leaves, coffie beans, and tobacco leaves. PPO is of great importance in determining the quality attributes of some fruits and vegetable. Its detrimental effects caused by browning of bruised and broken plant tissues are well-known (Whitaker, 1972; Whistler 1985). The most prevalent naturally occuring subtances in bananas which could potentially be PPO subtrate is dopamine of 3,4 dihydroxyphenylethylamine (Schwimmer, 1981; Zhou et.al., 1993).

A diversity of approaches to prevent or delay PPO Induced discolouration and to retain the original, desirable colours of plant foods have been explored, for instance by chemicals inhibition and irreversible destruction of the enzymes (Schwimmer, 1981; Khan 1985; Soto, 1986; Siddiq, 1992; Pizzocaro et.al., 1993) Several workers have studied chemical inhibition on Banana PPO (Palmer, 1963; Palmer and Roberts 1967), but no such work has been reportted on heat inactivation of the enzymes. Such information is required to design processing condition for production of dehydrated products, such as hot air and freeze-dried products. The present investigation was undertake to study the effect of heat on the inactivation of banana PPO.

MATERIALS AND METHODS

Extraction of PPO. Extraction of enzyme was carried out using a modification of the method of Palmer (1963). All extraction materials were maintained at low temperatur (2 - 5°C) to reduce enzymatic activity during extraction. Ripe banana fruits (Musa paradisiaca, L. Group AAA) were the source of the enzyme. The fruits were obtained from local grocer. The experiment were initiatd with buffered homogonates. Four g of tissue was homogenated in a microblender in 20 ml of a 1% detergent solution (Triton X-100) buffered at pH 7.0 with 0.1 M phosphate buffer. The homogenate was centrifuged at 15.000 x G for 15 min at 0°C. These detergent extracts were appropiately diluted 1 : 100 with 0.02 M potassium phosphate, pH 7. To measure activity of the enzyme 1.0 ml of this diluted extact was added to a cuvette which contain 1.0 ml of 0.1 M phosphate buffer and 1.0 ml dopamine solution which give a final concentration of subtrate of 5×10^{-3} M. The increase in optical density at 470 nm was followed at room temperature with a recording spectrophotometer. From the intial slope of the curve, rates were calculated as optical density per time (OD/time). To calculate specific activity of the enzyme, protein content was determined on the undiluted extract according to the dye binding method of Bradford (1976) with bovine serum albumine as standard.

Partial purification. The detergent extract were dialyzed overnight against 1.0 M phosphate buffer pH 7.0. The dialyzed enzyme solution was assayed as previously described.

Optimum Temperature. The optimum temperature of the PPO-dopamine reaction was determined by adding 0.1 ml of the partially purified enzyme solution to 2.9 ml 0.05 M dopamine solution pH 7.0. Preequilibrate for 5 min at various temperatures ranging from 30° to 80°C prior to addition of the enzyme and measuring the rate of the raction.

Thermal Inactivation. In the thermal inactivation studies, 4 tubes each containing 0.8 ml of partially purified PPO were incubated at various temperatures: 72, 75, 82 and 83° C and then 0,1 ml aliquotts were withdrawn at various time from each tube and rapidly added to 2,9 ml of ice-cold 0,05 M dopamine solution pH 7 and the residual enzyme activity was assayed.

RESULTS AND DISCUSSION

Extraction and Purification of PPO. Asummary of banana PPO extraction and purification in shown in Table 1. Around 1,5 fold purification was obtained by dialyzing the crude extract.

Table 1. Summary of the extraction and purification of banana PPO

Purification Step	Specific Activity Unit/mg
Crude extract	7,4
dialyzed extract (partially purified)	10,6

Table 1. also show that the data obtained are in reasonable agreement with results obtained by Palmer (1963) and Connie Weaner and Helen Charly (1974).

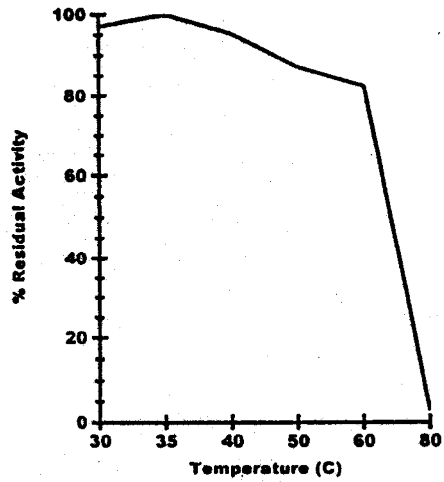

Figure 1. Temperature optimum of Banana PPO

Profile of variation in the activity of banana PPO with temperatures are shown in Figure 1. The optimum temperatures for the PPO - dopamine reaction was 35°C. However the enzyme retained most its activity, i,e. 80% of the maximum over a wide temperature range 30 - 60 °C. Above 60 °C, the PPO activity declined rapidly as the temperature increased but the enzyme was not completely inactivated even at 80 °C. Cash et. al. (1976) and Nakamura et.al. (1983) reported optimum temperature of 25 °C and 30 °C, respectively for grape PPO.

Thermal Inactivation of PPO, One of the thermal inactivation parameter is D value, or decimal reduction value, define as the time required to inactivate 90% of the original enzyme activity at a constant temperature. If an activation reaction follows first order kinetics, the D value equals $\frac{2{,}303}{K}$, then the general equation for 1^{st} order reaction.

$$\log [E] = -\frac{kt}{2.303} + \text{constant}$$

where E is active enzyme and in the current study E is representing by residual activity. k is the specific rate constant for the inactivation process. t is the time for the process.

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$$\log [E] = \frac{-t}{D} + \underline{\text{constant}}$$

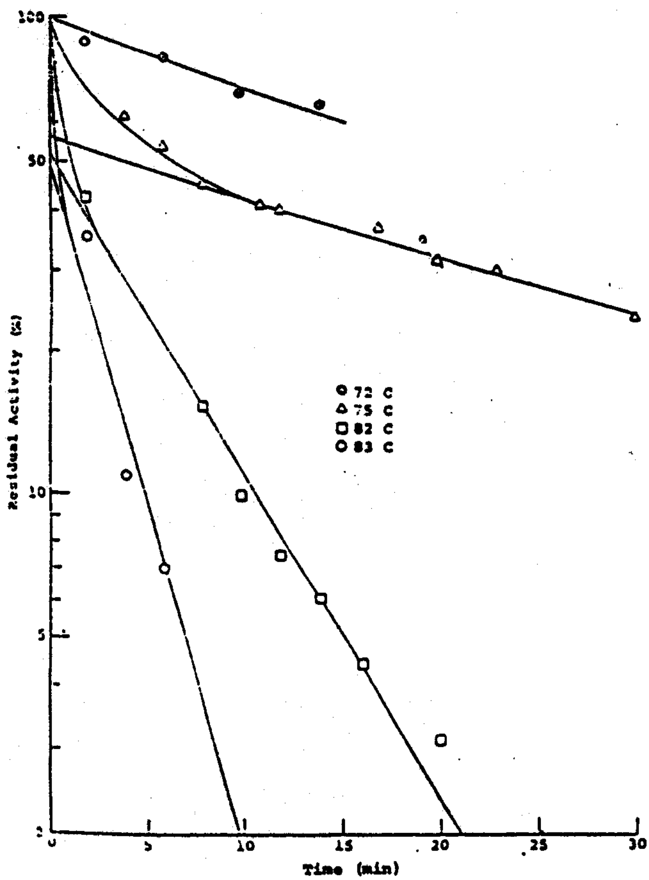
By plotting log [E] against time (t), the slope is equal to $\frac{-1}{D}$.

In the current study the D value was obtained by plotting percent residual activity on logarithmic paper (ordinate) versus time on the arismathic axis (abscissa). The D value is then the time required for the plot to traverse one log cycle. The logarithm of four D values obtained then beplotted againts temperature to obtain a thermal inactivation curve. The slop of thermal inactivation curve is $\frac{-1}{Z}$, where Z represents number of degrees required for the thermal inactivation curve to traverse one log cycle. In this studies results of the thermal inactivation are skown in Table 2 and a plot of log percent residual activity versus time yielded curves shown in Figure 3. The curves exhibited characteristic similiar to those for peroxidase (Vetter et. al., 1959) The non linear curves indicated that banana PPO contained more than one isoenzyme. This is in agreement with the observation of Montgomery and Sgarbieri (1974) that there were more than eight isoenzyme in bananas, while Oba et. al.(1992) from their electrophoresis study indicated that there were 2 isoenzymes in banana bud PPO. In order to analyze the thermal parameter for these isoenzyme in the current study, a simplification was made: it is assumed that the PPO system in banana is composed of several isoenzymes each of which can be catagorized as heat labile and heat resistant, therefore the thermal proporties of the isoenzyme within each group would be considered identical. Through a relatively simple calculation, the Z value for the heat labile isoenzyme was found to be 4.3°C + 0.4°C and for the heat resistent isoenzyme the Z value was 8.3°C + 0.8°C (Figure 4). The Z value for banana PPO isoenzyme fell into Z value ranges commonly found for food enzymes (Esselen and Pflug 1954).

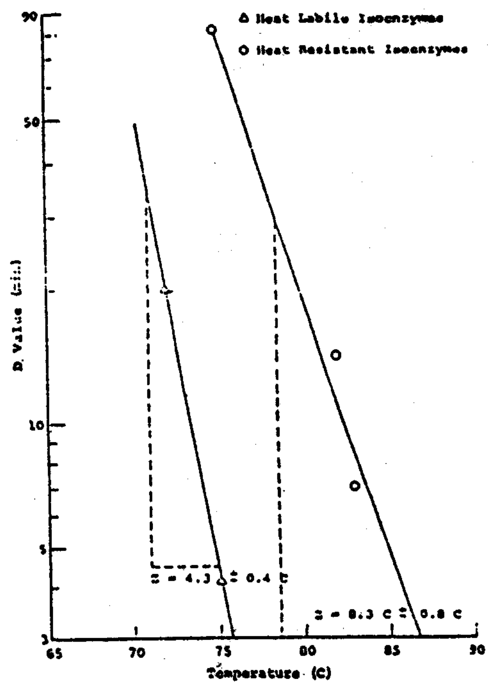
Tabel 2. Thermal Inactivation of Banana PPO Enzyme Activity

Time Minutes)	Temperature			
	72°C	75°C	82°C	83°C
	% Residual Activity			
2	87.93	89.12	42.53	35.55
4	-	62.13	-	10.96
6	82.22	53.84	-	6.94
8	-	45.34	15.24	0.46
10	69.26	- a	10.90	-
11	-	41.11	-	-
12	-	40.38	7.50	-
14	64.90	-	6.09	-
16	-	-	4.45	-
17	-	36.82	-	-
20	-	31.56	3.14	-
23	-	30.09	-	-
30	-	23.71	-	-

Not Determined



Figur 3. Thermal Inactivation of Banana PPO.



Figur 4. The Z-Values of Heat Resistant and Heat Labile Isoenzymes of Banana PPO.

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