In-vitro assay and inactivation kinetics of polyphenol oxidase of some Nigerian banana and plantain cultivars

Buliyaminu A. Alimi*

Department of Food, Agricultural and Bio-Engineering,
Kwara State University, Malete
PMB 1530, Ilorin, Nigeria
Email: bun5574@yahoo.com
*Corresponding author

Taofik A. Shittu

Department of Food Science and Technology,
Federal University of Agriculture,
PMB 2240, Alabata Road,
Abeokuta, Nigeria
Email: staofeek0904@yahoo.com

Abstract: Activity of polyphenol oxidase (PPO) from the pulp of three banana cultivars grown in Nigeria, namely: plantain, light green-skinned banana and red-skinned banana at unripe and ripe states were investigated. The efficiency of extraction of crude PPO enzyme and in-vitro assay of its activity were determined. Polyethylene glycol detergent gave the highest extraction efficiency. Enzyme activity and rate of browning were found to be highest in plantain and least in light green-skinned banana. Enzymes from all the cultivars were specific towards O-diphenolic compounds and not to monophenolics. The optima pH of PPO of the cultivars tends towards neutrality (6.6–7.0). Enzyme activity was destroyed between 2 and 4 min at 80ºC. Thermal inactivation kinetic parameters revealed plantain's PPO to be the most thermal stable. Sodium metabisulphite was the most effective chemical inhibitor of the PPO. The electrophoretic patterns of banana PPO showed heterogeneity with greater number of bands in ripe.

Keywords: banana and plantain; browning; polyphenol oxidase; PPO; inactivation; kinetics; electrophoretic pattern.

Reference to this paper should be made as follows: Alimi, B.A. and Shittu, T.A. (2016) 'In-vitro assay and inactivation kinetics of polyphenol oxidase of some Nigerian banana and plantain cultivars', *Int. J. Postharvest Technology and Innovation*, Vol. 5, No. 3, pp.200–214.

Biographical notes: Buliyaminu A. Alimi is a Lecturer and Researcher in Food Science and Technology. He joined Kwara State University in 2013. He is currently a J.W Nelson funded postdoctoral scholar in the School of Bioresources Engineering, University of KwaZulu-Natal, Pietermaritzburg, South Africa. His major area of specialty has been postharvest technology with major emphases on food storage and preservation. His other areas of interest include modelling of food properties and development of safety management system of indigenous food products. He has presented output of his works in major local and international conferences and peer reviewed journals.

Taofik Shittu is an Associate Professor of Food Science and Technology at Federal University of Agriculture, Abeokuta, Nigeria. His focus is basically on study of traditional (or indigenous) food processing technologies for upgrading. He has worked with both plant and animal-based foods through application of different processing operations. These were with a view of promoting the utilisation of indigenous crops for food processing.

This paper is a revised and expanded version of a paper entitled 'In-vitro assay of *polyphenol oxidase* activities of some banana cultivars and plantain grown in Nigeria' presented at 2012 CIGR Section VII International Technical Symposium on 'Innovating the Food Value Chain' Postharvest Technology and Agri-Food Processing, Stellenbosch, South Africa, 25–29 November, 2012.

1 Introduction

Browning reaction occur in many fruits and vegetables when the tissues are exposed to atmospheric oxygen through either mechanical damage during harvesting and processing or storage injury. This reaction is due to the conversion by oxidation of phenol compound to quinones by the endogenous enzyme polyphenol oxidase (PPO) (Chararra et al., 2001). PPO, a copper containing enzymes with oxygen as substrate, is primarily located in the plastids of plant. However, it occurs free in cytoplasm during senescence (Wuyts et al., 2006). PPOs have two reaction activities; phenol hydroxylation to *o*-diphenol (cresolase, EC 1.14.18.1), and diphenol oxidation to *o*-quinones (Palma-Orozco et al., 2011). Quinones rapidly undergo polymerisation to impact brown discolouration due to their unstable and highly electrophilic nature (Mayer and Harel, 1991).

The degree of browning depend to a very large extent on the nature and amount of phenolic compounds, presence of oxygen, reducing substances, metal ions, pH, temperature and the activity of PPO (Yoruk and Marshall, 2003). The involvement of PPO in enzymatic browning of edible plants which has been shown to be highly undesirable due to discolouration and off-flavour development (in many plants and vegetable products) marks its importance in the food industry. Enzymatic browning impairs the nutritional and sensory properties of the food product and ultimately affects its marketability. This has been a major concern in food industry.

Plantain and banana are widely distributed and consumed in the tropics (Vuylsteke and Swenne, 1990). They are utilised in baking and fermentation industries (Ogazi and Jones, 1981). They are primarily use to make such products as plantain chips, banana puree and banana wine.

Several studies have been done on the PPO activity of banana fruits of different origin (Palmer, 1963; Thomas and Nair, 1971; Montgomery and Sgarbieri, 1975; Galeazzi and Sgarbieri, 1981; Galeazzi et al., 1981; Cano et al., 1997; Sojo et al., 1998; Yang et al., 2000, 2001; Gooding et al., 2001; Umit Unal, 2007). However, published data on the PPO activity of Nigerian banana and plantains are very scarce. It is important to understand PPO activity in these materials to effectively control its activities in food processing for optimal product quality. Therefore, the objective of this work was to analyse the PPO activities of some banana cultivars and plantain grown in Nigeria.

2 Materials and method

2.1 Materials

Plantain and banana cultivars (light green skinned and red skinned varieties) were obtained from local farmers at Ikire, Osun State of Nigeria. The fresh wholesome fruits samples were procured at their pre-climacteric stages and transported to the laboratory in open top plastic containers within two hours of harvest. The chemicals used and their suppliers are as follows: Catechol by Catechol Fisher Scientific Co. UK; polyethylene glycol (PEG) (Carbowax 6000); Pyrogallol; M-cresol; sodium chloride; and Quinic acid by BDH Chemicals, England; Citric acid, ascorbic acid and cysteine by Hopkins and Williams, England; sodium metabisulphite by May and Baker Ltd, Nigeria.

2.2 Enzyme extraction

PPO was extracted from plantain and banana pulps at two physiological stages namely mature green and table ripe corresponding to stage 6 of the USA banana ripening chart (Von Loesecke, 1950) using three different extraction methods as described by Palmer (1963).

2.2.1 Buffered homogenates

Fresh fruit pulp (2 g) was homogenised in 20 ml of 0.1 M potassium hydrogen phosphate, pH 7 and centrifuged at 4,000 rpm for 20 min using Beckman centrifuge (model J 12B). The supernatant was taken as the crude enzyme extract.

2.2.2 Detergent extract

Two grams of fresh pulp was homogenised with 18 ml of 1-5% PEG buffered at pH 7with 0.1M potassium phosphate. The slurry was centrifuged at 4,000 rpm for 20 min at 18ºC in each case. The resultant supernatant was used as the enzyme extract.

2.2.3 Acetone precipitate

The supernatant collected in the preparation under method (b) above was freed of excess detergent by precipitating with 1.7 ml of cold acetone (–12ºC). It was allowed to stay overnight and thereafter centrifuged at 4,000 rpm for 15 min to remove residue.

2.3 Measurement of browning rate

Fresh fruit pulp of each sample was homogenised in a warring blender (HR2001, Philips, China) for 2 min with 0.1 M potassium phosphate buffer, pH 7.0 in a 1:9 (w/v) ratio. After allowing staying for 5 min at 25ºC, the homogenate was quickly filtered through a layer of acid asbestors on No. 41 Whatman filter paper in a Buchner funnel. The rate of browning was calculated by dividing the absorbance with the weight of pulp used. The absorbance was measured at 470 nm after 5 min incubation as described by Sciancalepore and Longone (1984).

2.4 Assay of plantain and banana PPO activity

Enzyme activity was determined by measuring the initial rate of the increase in absorbance at 470 nm with CECIL UV spectrophotometer (model CE 202 series 2) as described by Palmer (1963). The assay was done in triplicate. The reaction mixture contained 1 ml of 0.33 M potassium phosphate (pH 7.0), 1 ml of 0.05 M substrate (catechol) solution and quantity of enzyme to give total reaction volume of 3 ml. The reference contained the reaction mixture with distilled water in place of enzyme.

One unit of PPO activity was defined as potency of enzyme that increased in absorbance of 0.01 per minute.

2.5 Substrate specificity and Michaelis constant

Substrate specificity was determined using six different substrates (catechol, pyrogallol, chlorogenic acid, m-cresol and tyrosine). Substrate solutions were prepared in 0.33 M potassium phosphate (pH 7.0). Activity was monitored by following maximum absorbance wavelength for each of the substrates.

2.6 Determination of pH optima of PPO activity

The optima pH of plantain and banana cultivars' pulp PPO were determined using method of Palmer (1963) by measuring the activity as described in enzyme assay using 0.33 M phosphate buffers pH 6-8 and standard pH buffer pH 9.2. The reaction mixture contained the substrate (1 ml), buffer solutions (1 ml) at different pHs and enzyme solution (1 ml) to give total of 3 ml.

2.7 Thermal inactivation

The thermal denaturation of the enzyme was determined using the method of Park et al. (1980). 5 ml portion of enzyme solution were sealed in test tubes and placed in water-bath heated to 50–80ºC. 1 ml of the samples were withdrawn every minute and immediately cooled by immersion in ice-water. The PPO activity was measured as described in enzyme assay.

2.8 Thermal inactivation kinetics

First order inactivation kinetics was calculated from the slope of inactivation rate with time. Rake constants (k values) were calculated using regression analysis of the straight lines for each heating curve. The half life ($t_{1/2}$) and decimal reduction time (D-values) were determined using the following respective equations;

$$t_{1/2} = 0.693 / k \tag{1}$$

$$D = 2.303 / k \tag{2}$$

The activation energies of denaturation (E_a values) were determined using the Arrhenius equation. The slope of the Arrhenius plots (i.e. natural logarithm of *k* values (*Ink*) against the reciprocal of absolute heating temperatures (1/T)) were multiplied by the universal

gas constant: 8.314 $Jmol^{-1}k^{-1}$. The z-values were calculated from the plot of $\log_{10}D$ against temperature. Z value is equal to the reciprocal of the slope of the graph.

2.9 Chemical inhibition

Investigation on chemical inhibition of the enzyme was determined by measuring activity using 1 ml of 0.05 M substrate solution. 1 ml of sodium metabisulphite, ascorbic acid, cysteine, citric acid and sodium chloride from concentration of 0.01mM to 0.1mM were reacted separately with 1 ml of 0.33 M phosphate buffer (pH 7.0) and 1 ml enzyme solution (Park et al., 1980).

2.10 Electrophoresis assay

Characterisation of plantain and banana cultivars' PPO was done by polyacrylamide gel electrophoresis (PAGE) using the method described by Whitaker (1972). Part of the gel was developed by staining with 1% Coomassie brilliant blue in 7% acetic acid for at least 15 min followed by washing with 7% acetic acid for 15 min. Bromophenol blue was used as reference marker.

2.11 Data analyses

Data were analysed using SPSS version 15.0. Statistically significant differences ($p < 0.05$) in the data were determined by one way analysis of variance (ANOVA) and means were compared using Duncan multiple range test.

Table 1 Extraction of crude soluble PPO from pulps of banana cultivars

	Buffer homogenate	*PEG detergent*	*Acetone precipitation*
UPL	0.25 ± 0.020^{b}	0.550 ± 0.023^{c}	0.340 ± 0.017^{c}
UGB	0.138 ± 0.012^{a}	0.400 ± 0.014^{a}	0.250 ± 0.022^{a}
URB	0.140 ± 0.015^{a}	0.453 ± 0.032^{b}	0.270 ± 0.011^{b}
RPL	0.490 ± 0.022^{e}	0.735 ± 0.041^{e}	0.610 ± 0.032^{f}
RGB	0.385 ± 0.018^{c}	0.670 ± 0.012^{d}	$0.4400.054^{d}$
RRB	0.400 ± 0.031^{d}	0.680 ± 0.051^{d}	0.470 ± 0.052^{e}

Notes: Mean values with different letter superscripts in the same column are significantly different at $P < 0.05$.
UPL: unripe plantain; UGB: unripe light green skinned banana; URP: unripe red skinned banana; RPL: ripe plantain; RGB: ripe light green skinned banana; RRB: ripe red skinned banana

3 Results and discussion

Activity of crude PPO extracts from pulps of plantain, light green skinned and red skinned banana cultivars at unripe and ripe stages using three different extractants namely: buffer homogenate, PEG detergent solution and acetone is presented in Table 1. PPO had been reported to be absorbed or structurally associated with cell wall (Mayer and Harel, 1979). The low extraction efficiency with buffer homogenate could be due to

its inability to release the enzyme into solution. PEG detergent had the highest extraction efficiency because of the solubilisation and activation of membrane bound and latent PPO, respectively (Wuyts et al., 2006). Time lag for inactive residue to be precipitated and destruction of activity in the course of re-preparation presumably contribute to the lower activity of acetone precipitate.

Significant ($p \leq 0.05$) correlation was observed between total PPO activity and rate of browning with coefficient of determination of 0.906. The total activity and hence the rate of browning were higher at ripe stage for all the cultivars (Table 2). Plantain had the highest activity at the two physiological stages studied.

Table 2 PPO activity and rate of browning of the cultivars

	Activity (U/mL min)	*Browning ratio (U/g)*
UPL	94 ± 3.23^{c}	24 ± 1.10^{a}
UGB	70 ± 2.45^{a}	20 ± 2.12^{a}
URB	88 ± 2.14^{b}	23 ± 3.20^{a}
RPL	110 ± 6.15^{e}	40 ± 2.46^{b}
RGB	98 ± 5.24^{c}	30 ± 1.88^{a}
RRB	105 ± 4.31^{d}	37 ± 3.23^{b}

Notes: Mean values with different letter superscripts in the same column are significantly different at $P < 0.05$.
UPL: unripe plantain; UGB: unripe light green skinned banana; URP: unripe red skinned banana; RPL: ripe plantain; RGB: ripe light green skinned banana; RRB: ripe red skinned banana

3.1 Substrate specificity and Michaelis-Menten kinetics

PPO activity of all the cultivars at the two physiological stages showed activity towards o-diphenolic compounds but not towards monophenols (Table 3). This has been similarly reported for bananas of other origins (Palmer, 1963; Galeazzi and Sgarbieri, 1981; Yang et al., 2001; Wuyts et al., 2006; Umit Unal, 2007).

Oxidation and polymerisation of phenolic compounds occurs in intact tissue as a result of mechanical injury, wounding and senescence. With regards to this study, the PPO activity of the banana cultivars was influenced by their physiological stage. It has been established that enzyme activity in a preferred substrate enhances browning. In this study, PPO activity was higher at ripe stage than unripe stage. Also, irrespective of the substrate, Plantain PPO had the highest activity at the two physiological stages. This is consistent with the observation of Palma-Orozco et al. (2011). Beside the degree of ripeness, Yoruk and Marshall (2003) reported that the nature of side chain in the substrate, number of hydroxyl groups, and their position in the side chain have an enhanced effect of PPO catalytic activity.

The numerical value of Michaelis constant is universally proportional to the affinity of the enzyme for its substrate. A smaller value of the Michaelis constant indicates a greater affinity of the enzyme for its substrate (Wuyts et al., 2006). Irrespective of the cultivars and their physiological stages, all the PPO enzymes had highest affinity for pyrogallol and least for catechol. This confirmed the report of Wuyts et al. (2006) and Umit Unal (2007) that catechol is a poor substrate of banana PPO. Differences

in cultivars' PPO affinity to the same substrate have been similarly observed by Palma-Orozco et al. (2011).

Table 3 Substrate specificity and Michaelis constant of plantains and bananas

Substrate	*Sample*	*Activity (U/mL min)*	*Km (M)*
Catechol	UPL	345	2.40×10^{-3}
	UGB	280	2.75×10^{-3}
	URB	305	2.60×10^{-3}
	RPL	475	4.00×10^{-4}
	RGB	420	8.00×10^{-3}
	RRB	453	1.00×10^{-3}
Pyrogallol	UPL	580	1.80×10^{-3}
	UGB	390	2.10×10^{-3}
	URB	543	1.90×10^{-3}
	RPL	760	6.00×10^{-4}
	RGB	620	1.20×10^{-3}
	RRB	710	1.00×10^{-3}
Chlorogenic acid	UPL	420	1.50×10^{-3}
	UGB	295	2.50×10^{-3}
	URB	378	2.10×10^{-3}
	RPL	530	8.00×10^{-4}
	RGB	460	1.40×10^{-3}
	RRB	490	9.00×10^{-3}
m-cresol	UPL	-	-
	UGB	-	-
	URB	-	-
	RPL	-	-
	RGB	-	-
	RRB	-	-
Tyrosine	UPL	-	-
	UGB	-	-
	URB	-	-
	RPL	-	-
	RGB	-	-
	RRB	-	-

Notes: UPL: unripe plantain; UGB: unripe light green skinned banana; URP: unripe red skinned banana; RPL: ripe plantain; RGB: ripe light green skinned banana; RRB: ripe red skinned banana.

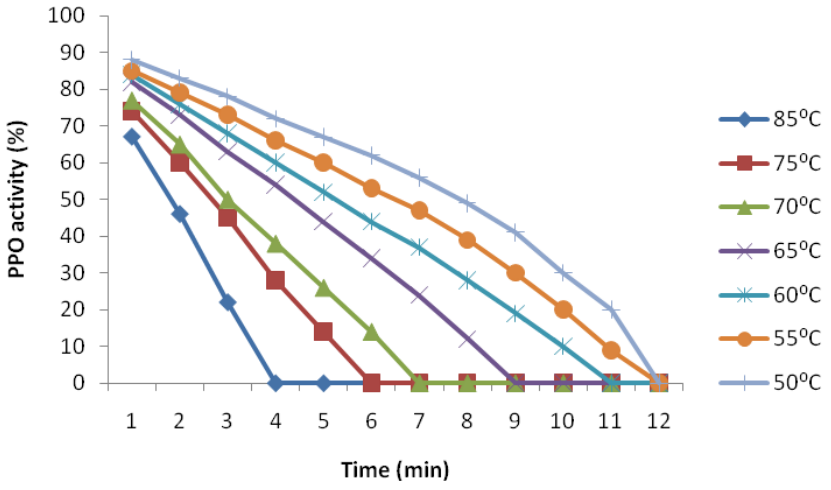
3.2 Optimum pH

pH was reported to be an important determinant of enzymatic activity (Arslan et al., 2004). Activities were at maximum at pH 6.8 and 6.6, 5.9 and 6.8, and 7.0 and 6.8, respectively, for PPO from ripe and unripe plantain, light green skinned banana and red skinned banana. The optima pH of maximum PPO activities varied with enzyme source. PPO activities of the studied banana cultivars tend toward neutrality. This has been reported to be the case with PPO system of most fruits (Vamos-Vigyazo, 1981; Das et al., 1997; Kumar et al., 2008).

3.3 Thermal inactivation

Plot of relative activity of PPO against time for the cultivars studied followed similar trends. Linear graph with slope dependent on temperature were obtained (Figure 1). From the resulting graph, rate of thermal inactivation was proportional to temperature. This could be due to the fact that the enzymes are proteins and are denatured by heat. Thus the activities of PPO from plantain, light green skinned, and red skinned banana lost their activities at 4 and 12 min, 2 and 12 min, and 3 and 12 min when heated at 80 and 50ºC, respectively. High temperature short time relationship for thermal inactivation of the enzyme obtained in this study had been reported for fruits such as mango (Park et al., 1980) and Anamur banana (Umit Unal, 2007). It had also been reported that PPO from meddler (Dincer et al., 2002), mamey (Palma-Orozco et al., 2011), apple cultivars (Yemenicioglu et al., 1997) and ravat grapes (Wissemann and Lee, 1981) was rapidly inactivated when heated at 80ºC.

Figure 1 Heat inactivation of PPO activity of ripe plantain (see online version for colours)



3.4 Thermal inactivation kinetics

Inactivation kinetics is an important approach use to predict quality change during processing of fruits and vegetables and subsequent storage of the resulting products. Two

procedures are commonly used to characterise the thermal stability of a given enzyme: one is a measure of activation rate at a given temperature expressed either as a rate constant (k), half-life ($t_{1/2}$) or as a D value; while the other is a measure of the rate of change of rate constant with temperature denoted by either an activation energy (E_a) or z value (Anthon and Barrett, 2002). Thermal inactivation of the studied banana cultivars' PPOs followed first order kinetics. Generally, inactivation rate constants (k-values) were increasing with temperature. This is an indication that PPO were less stable at higher temperature since the higher the rate constant the lower the enzyme stability (Marangoni, 2002). Though, the values obtained in this study at a given temperature were higher than those reported by Umit Unal (2007) for Anamur banana, the trends were similar. Comparing the k-values of the studied cultivars' PPO, green-skinned banana had the least while plantain had the most stable PPO activities.

The half-life, $t_{1/2}$, which was the time required for 50% reduction of the initial enzyme activity, was generally decreasing with increased temperature. For a given temperature, plantain had the highest $t_{1/2}$ while green-skinned banana had the least. The $t_{1/2}$ values obtained in this study at a given temperature were lower than those reported by Umit Unal (2007) but within the range reported by Yang et al. (2000) for banana.

Decimal reduction time, D-value, (time to inactivate 90% of the population at a given temperature and pressure) was decreasing with increased temperature for all the cultivars. Comparing the D-value of the studied cultivars, Plantain's PPO was the most thermal stable while green-skinned banana was the least stable since higher D-value implies higher thermal stability (Yemenicioglu et al., 1997). D-values obtained in this study were higher than those reported by Umit Unal (2007) for Anamur banana (286 and 24.3 min at 60 and 75ºC, respectively). Other reported D-values include 30.3 to 56.6 min for different apple cultivars at 73ºC (Yemenicioglu et al., 1997), 0.24 min at 75ºC for apple puree (Strūbi et al., 1975).

The z-value, i.e., the temperature increase required for one log reduction in D-value, for PPO from studied banana cultivars ranged from 37.14ºC (green skinned banana) to 49.26ºC (plantain). Since the higher the z-value (the lower the E_a), the higher the temperature needed to inactivate the enzymes (Yemenicioglu et al., 1997), thus plantain's PPO are more heat stable than PPO from other cultivars. The z-values recorded in this work were higher than those reported by Vamos-vigyazo (1981) for banana (8.5 to 10.1ºC) and Umit Unal (2007) for dwarf turkey anamur banana (14.2ºC). Some of the reported z-values are 7.1 to 10ºC for apple cultivars (Yemenicioglu et al., 1997), 61.5ºC for soft ripe apricot (Heil et al., 1988) and 37.8–49.2 KJ/mol for cherry laurel cultivars (Colak et al., 2005). The E_a for heat inactivation of PPO of the studied cultivars were 44.22 KJ/mol (plantain), 46.86 KJ/mol (red-skinned banana) and 57.42 KJ/mol (green-skinned banana). Our E_a values were lower than those reported for different apple cultivars (226.36 to 323.02 KJ/mol) by Yemenicioglu et al. (1997), banana (355.66 Kj/mol) by Vamos-Vigyazo (1981), dwarf turkey anamur banana (155 KJ/mol) by Umit Unal (2007) and sultan grape by Aguilera et al. (1987) but higher than the values reported by McCord and Kilara (1983) for mushroom (36.402 KJ/mol) and Ansah (1989) for wild rice (20.08 KJ/mol). The observed difference in the E_a and z-values obtained in this work compared with those reported for banana of other regions could be due to the difference in agricultural practices, varietal difference and environmental factors which affect compositions of PPO. However, our results were within the range of reported values.

Table 4 Thermal resistance and heat inactivation kinetics of banana cultivars at various temperatures

Cultivar	*Temperature (°C)*	*Activity*	*K* ($10^{-2}.min^{-1}$)	$t_{1/2}$ *(min)*	*D (min)*	*Z (°C)*	E_a *(kJ/mol)*
Plantain	50	105.48	1.20	57.75	191.88	49.26 ($R^2 = 0.9788$)	44.22 ($R^2 = 0.9715$
	55	104.72	1.40	49.50	164.47		
	60	104.25	1.70	40.76	135.45		
	65	104.37	2.00	34.65	115.13		
	70	103.59	2.80	24.75	82.24		
	75	103.60	3.40	20.38	67.72		
	80	103.33	5.00	13.86	46.05		
Red-skinned banana	50	110.84	1.30	53.31	177.12	46.51 ($R^2 = 0.9849$)	46.86 ($R^2 = 0.9786$)
	55	111.17	1.60	43.31	143.91		
	60	110.92	1.90	36.47	121.19		
	65	110.02	2.40	28.85	95.94		
	70	109.53	3.20	21.66	71.96		
	75	107.31	4.00	17.33	57.56		
	80	104.37	6.00	11.55	38.38		
Green-skinned banana	50	109.20	1.50	46.20	153.51	37.74 ($R^2 = 0.9373$)	57.92 ($R^2 = 0.9390$)
	55	106.88	1.70	40.76	135.45		
	60	107.10	2.10	33.00	109.65		
	65	106.48	2.80	24.75	82.24		
	70	104.44	3.70	18.73	62.23		
	75	101.77	5.00	13.86	46.05		
	80	101.60	10.40	6.66	22.14		

3.5 Chemical inhibition

Effect of chemical inhibitors on plantain and banana fruits PPO activity is presented in Table 4. Inhibitions by various compounds were calculated from the change in absorbance at 470 nm immediately after the induction period with catechol as substrate. The PPO from all the cultivars showed similar behaviour toward the inhibitor used in the study. The percentage inhibition of the enzyme activity was based on change in the absorbance of the reaction measured at 470 nm per absorbance of the reaction solution without chemical inhibitor. All the compounds used in this study inhibited plantain and other banana cultivars' PPO. Inhibition property of most of the chemicals could presumably due to their ability to reduce *o*-benzoquinone back to phenolase substrate as it was formed. They usually inhibit formation of brown pigment by preventing polymerisation of *o*-quinones (Nicolas et al., 1994). The acids could delay or inhibit PPO activity by altering the pH of the buffer (Wuyts et al., 2006). Inhibition for all the enzyme preparation was most effective with sodium metabisulphite and least with sodium chloride. The PPO inhibition results obtained in this study is similar to those on mango (Park et al., 1980), banana (Yang et al., 2000), Anamur banana (Umit Unal, 2007).

Table 4 Chemical inhibitors of PPO activity

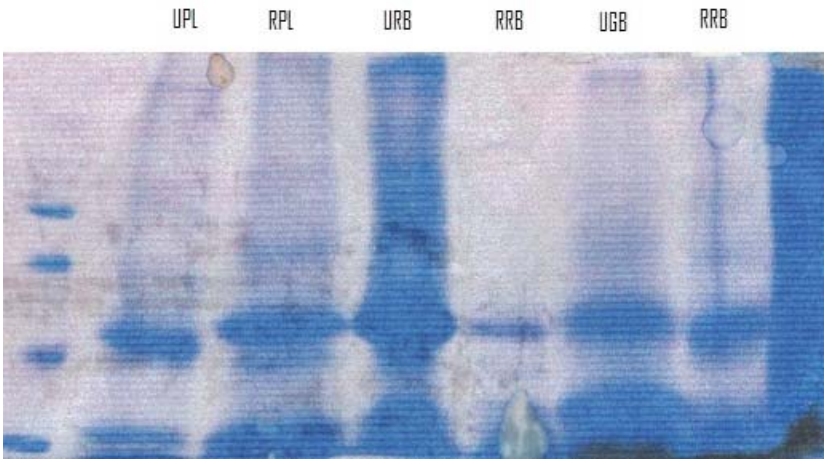
Inhibitor	*Concentration (mM)*	*Inhibition (%)*		
		RPL	*RGB*	*RRB*
Sodium metabisulphite	0.01	45	56	48
	0.05	100	100	100
	0.10	100	100	100
Ascorbic acid	0.01	33	33	32
	0.05	44	52	50
	0.10	100	100	100
Cysteine	0.01	14	16	15
	0.05	34	39	37
	0.10	100	100	100
Citric acid	0.01	22	28	26
	0.05	35	40	37
	0.10	100	100	100
Malic acid	0.01	36	34	35
	0.05	55	60	57
	0.10	100	100	100
Sodium chloride	0.01	0	0	0
	0.05	32	35	35
	0.10	40	53	50

3.6 Electrophoresis patterns

Electrophoresis pattern of the detergent extract of banana cultivars at unripe and ripe stages is shown in Plate 1. PPO of the cultivars at the two physiological stages showed

heterogeneity. Plantain and red skinned banana have five bands each while light green skinned banana has four. The separation of PPO into bands represents presence of isoenzymes which showed that the PPO from the cultivars is not a single enzyme. This has also been established for some fruits like banana (Galeazzi and Sgarbieri, 1981), pear (Gauillard and Richard-Forget, 1997), apple peel (Zhou et al., 1993) and mango (Park et al., 1980). Though the molecular weight of the enzyme was not determined in this study, the values reported for banana cultivars using SDS-PAGE by Galeazzi et al. (1981) and Yang et al. (2000) were 31,000 and 42, 000, respectively. The difference was reported by Yang et al. (2000) to be due to cultivar difference.

Figure 2 Electrophoresis pattern of banana cultivars' PPO at ripe and unripe stage* (see online version for colours)



Notes: UPL: unripe plantain; UGB: unripe light green skinned banana; URP: unripe red skinned banana; RPL: ripe plantain; RGB: ripe light green skinned banana; RRB: ripe red skinned banana
*Molecular weight of the enzyme was not determined; hence, the molecular weight markers were not labelled.

4 Conclusions

PPO enzyme has been found to be responsible for browning in the banana cultivars at the two physiological stages studied. The enzyme was readily extracted using PEG detergent solution. The PPO from ripe plantain had highest activity and the least was light green skinned banana PPO. The soluble enzyme readily catalysed the oxidation of various diphenolic compounds but not monophenols. The optima pH for enzyme activities tends toward neutral. Heat stability study showed that the enzyme was inactivated at high temperature in short time. Thermal inactivation kinetics revealed plantain's PPO to be the most thermal stable and light green skinned banana to be the least. Sodium metabisulphite was the most effective chemical inhibitor of the enzyme out of the chemicals used in the study. The electrophoresis patterns of the cultivars PPO showed heterogeneity with greater number of bands in ripe than unripe.

Based on the findings of this study, it was concluded that the properties exhibited by the PPO in the pulp of the local banana cultivars can be guiding factors in the processing of the pulp especially where browning could be detrimental to the qualities of the final products. Also, substrate specificity of the enzyme can assist the plant breeders to influence the production of varieties low in diphenolic compounds but high in monophenolic compounds.

References

Aguilera, J.M., Oppermann, K. and Sanchez, F. (1987) 'Kinetics of browning of sultana grapes', *Journal of Food Science*, Vol. 52, No. 4, pp.990–993.

Ansah, Y.J.O. (1989) 'Polyphenol oxidase in wild rice (*Zizania palustris*)', *Journal of Agriculture and Food Chemistry*, Vol. 37, No. 4, pp.901–904.

Anthon, G.E. and Barrett, D.M. (2002) 'Kinetic parameters for the thermal inactivation of quality related enzymes in carrots and potatoes', *Journal of Agriculture and Food Chemistry*, Vol. 50, No. 14, pp.4119–4125.

Arslan, O., Erzengin, M., Sinan, S. and Ozensoy, O. (2004) 'Purification of mulberry (*Morus alba* L.) polyphenol oxidase by affinity chromatography and investigation of its kinetic and electrophoretic properties', *Food Chemistry*, Vol. 88, No. 3, pp.479–484.

Cano, M.P., Ancos, B., Lobo, M.G. and Santos, M. (1997) 'Improvement of frozen banana (*Musa cavendishii*, cv. Enana) colour by blanching: relationship between browning, phenols ad polyphenol oxidase and peroxidase activities', *Zeitschrift fur Lebensmitteluntersuchung und Forschung A*, Vol. 204, No. 1, pp.60–65.

Chararra, S., Carcia-carmona, F. and Cabanes, J. (2001) 'Hysteresis and positive cooperativity of iceberg lettuce PPO', *Biochemical and Biophysical Research Communications*, Vol. 289, No. 3, pp.769–775.

Colak, A., Ozen, A., Dincer, B., Guner, S. and Ayaz, F.A. (2005) 'Diphenolases from two cultivars of cherry laurel (*Laurocerasus officinalis* Roem.) fruits at an early stage of maturation', *Food Chemistry*, Vol. 90, No. 4, pp.801–807.

Das, J.R., Bhat, S.G. and Gowda, L.R. (1997) 'Purification and characterization of a polyphenol oxidase from the kew cultivar of Indian pineapple fruit', *Journal of Agricultural and Food Chemistry*, Vol. 45, No. 6, pp.2031–2035.

Dincer, B., Colak, A., Aydin, N., Kadioglu, A. and Guner, S. (2002) 'Characterization of polyphenol oxidase from medlar fruits (*Mespilus germanica* L., Rosaceae)', *Food Chemistry*, Vol. 77, No. 1, pp.1–7.

Galeazzi, M.A.M. and Sgarbieri, V.C. (1981) 'Substrate specificity and inhibition of polyphenol oxidase (PPO) from a dwarf variety of banana (*Musa Cavendishii* L.)', *Journal of Food Science*, Vol. 46, No. 5, pp.1404–1406.

Galeazzi, M.A.M., Sgarbieri, V.C. and Constantinides, S.M. (1981) 'Isolation, purification and physicochemical characterization of polyphenoloxidases (PPO) from a dwarf variety of banana (*Musa cavendishii*, L)', *Journal of Food Science*, Vol. 46, No. 1, pp.150–155.

Gauillard, F. and Richard-Forget, F. (1997) 'PPOs from Williams pear (*Pyrus communis* L. cv Williams): activation, purification and some properties', *Journal of the Science of Food and Agriculture*, Vol. 74, No. 1, pp.49–56.

Gooding, P.S., Bird, C. and Robinson, S.P. (2001) 'Molecular cloning and characterization of banana fruit polyphenol oxidase', *Planta*, Vol. 213, No. 5, pp.748–757.

Heil, J.R., Mccarthy, M.J. and Merson, R.L. (1988) 'Influence of gluconic acid on enzyme inactivation and color retention in canned apricots and peaches', *Journal of Food Science*, Vol. 53, No. 6, pp.1717–1719.

Kumar, V.B.A., Mohan, T.C.K. and Murugan, K. (2008) 'Purification and kinetic characterization of polyphenol oxidase from Barbados cherry (*Malpighia glabra* L.)', *Food Chem*istry, Vol. 110, No. 2, pp.328–333.

Marangoni, A.G. (2002) *Reversible Enzyme Inhibition, Enzyme Kinetics: Modern Approach*, pp.61–65, Wiley, Hoboken, NJ.

Mayer, A.M. and Harel, E. (1979) 'Polyphenol oxidases in plants', *Phytochemistry*, Vol. 18*,* No. 2, pp.193–215.

Mayer, A.M. and Harel, E. (1991) 'Phenoloxidases and their significance in fruit and vegetables', in Fox, P.F. (Ed.): *Food Enzymology*, Vol. 1, pp.373–398, Elsevier, New York.

Mccord, J.D. and Kilara, A. (1983) 'Control of enzymatic browning in processed mushrooms (*Agaricus bisporus*)', *Journal of Food Science*, Vol. 48, No. 5, pp.1479–1484.

Montgomery, M.W. and Sgarbieri, V.C. (1975) 'Isoenzymes of banana polyphenoloxidase', *Phytochemistry*, Vol. 14*,* No. 5, pp.1245–1249.

Nicolas, J.J., Richard-Forget, F.C., Goupy, P.M., Amiot, M.J. and Aubert, S.Y. (1994) 'Enzymatic browning reactions in apple and apple products', *Critical Review in Food Science and Nutrition*, Vol. 34*,* No. 2, pp.109–157.

Ogazi, P.O. and Jones, M.C. (1981) 'Plantain in flour production and potential', *Proceedings of 2nd Conference of International Association for Research on Plantain and Cooking Bananas*, pp.50–55, IITA, Ibadan.

Palma-Orozco, G., Ortis-Moreno, Dorantes-Alvarez, L., Sampedro, J.G. and Najera, H. (2011) 'Purification and partial biochemical characterization of polyphenol oxidase from mamey (*Pouteria sapota*)', *Phytochemistry*, Vol. 72, No. 1, pp.82–88.

Palmer, J.K. (1963) 'Banana polyphenoloxidase. Preperation and properties', *Plant Physiology*, Vol. 38, No. 5, pp.508–513.

Park, Y.K., Sato, H.H., Almeida, T.D. and Moretti, R.H. (1980) 'Polyphenol oxidase of mango *(Magnifera indicavar)*', *Journal of Food Science*, Vol. 45, No. 6, pp.1619–1621.

Sciancalepore, V. and Longone, V. (1984) 'Polyphenol oxidase activity and browning in green olives', *Journal of Agricultural and Food Chemistry*, Vol. 32, No. 2, pp.320–321.

Sojo, M.M., Nuñez-Delicado, E., García-Carmona, F. and Sánchez-ferrer, A. (1998) 'Partial purification of a banana polyphenol oxidase using Triton X-114 and PEG 8000 for removal of polyphenols', *Journal of Agricultural and Food Chemistry*, Vol. 46, No. 12, pp.4924–4930.

Strūbi, P., Escher, F. and Neukom, H. (1975) 'Neuere Arbeiten u¨ ber die Technologie der Apfelnektar', *Herstellung. Ind. Obst-Gemu¨ severwert*, Vol. 60, No. 13, pp.349–351.

Thomas, P. and Nair, P.M. (1971) 'Effect of gamma irradiation on polyphenol oxidase activity and its relation to skin browning in bananas', *Phytochemistry*, Vol. 10, No. 4, pp.771–777.

Umit Unal, M. (2007) 'Properties of polyphenol oxidase from Anamur banana (*Musa cavendishii*)', *Food Chemistry*, Vol. 100, No. 3, pp.909–913.

Vamos-Vigyazo, L. (1981) 'Polyphenol oxidase and peroxidase in fruits and vegetables', CRC *Critical Review of Food Nutrition*, Vol. 15, No. 1, pp.49–127.

Von Loesecke, H.W. (1950) *Banana*, p.189, Interscience publication, New York.

Vuylsteke, D. and Swenne, R. (1990) 'Somaclonal variation in African plantains', *IITA Research*, Vol. 1, No. 1, pp.4–10.

Whitaker, J.R. (1972) 'Polyphenol oxidase', *Principle of Enzymology for the Food Sciences*, pp.571–582, Marcel Dekker, New York.

Wissemann, K.W. and Lee, C.Y. (1980) 'Purification of grape polyphenoloxidase with hydrophobic chromatography', *Journal of Chromatography A*, Vol. 192, No. 1, pp.232–235.

Wuyts, N., De Waele, D. and Swennen, R. (2006) 'Extraction and partial characterization of polyphenol oxidase from banana (*Musa acuminata* Grande naine) roots', *Plant Physiology and Biochemistry*, Vol. 44, No. 5, pp.308–314.

Yang, C.P., Fujita, S., Ashrafuzzaman, M., Nakamura, N. and Hayashi, N. (2000) 'Purification and characterization of polyphenol oxidase from banana (*Musa sapientum* L.) pulp', *Journal of Agricultural and Food Chemistry*, Vol. 48, No. 7, pp.2732–2735.

Yang, C.P., Fujita, S., Kohno, K., Kusubayashi, A., Ashrafuzzaman, M. and Hayashi, N. (2001) 'Partial purification and characterization of polyphenol oxidase from banana (*Musa sapientum* L.) peel', *Journal of Agricultural and Food Chemistry*, Vol. 49, No. 3, pp.1446–1449.

Yemenicioglu, A., Ozkan, M. and Cemeroglu, B. (1997) 'Heat inactivation kinetics of apple polyphenoloxidase and activation of its latent form', *Journal of Food Science*, Vol. 62, No. 3, pp.508–510.

Yoruk, R. and Marshall, M.R. (2003) 'Physicochemical properties and function of plant polyphenoloxidase', *Journal of Food Biochemistry*, Vol. 27, No. 5, pp.361–422.

Zhou, P., Smith, N.L. and Lee, C.Y. (1993) 'Potential purification and some properties of Monroe apple peel polyphenol oxidase', *Journal of Agricultural and Food Chemistry*, Vol. 41, No. 4, pp.532–536.