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Full Length Research Paper

Preliminary studies on polyphenol oxidase activity in plantain (*Musa paradisiaca)* cultivars

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Polyphenol oxidase (PPO) has been shown to be responsible for browning reactions and discolouration in many fruit and vegetable products. Crude polyphenol oxidase (PPO) of ripe and unripe plantain (Musa paradisiaca cultivars – Agbagba, Cardaba and plantain hybrid) was isolated and some of the characteristics of the PPO extracts investigated. The activity of the enzyme was evaluated using spectrophotomeric method. Plantain PPO (ripe and unripe) catalyzes oxidation of both various substrates with catechol being the most readily oxidized substrate. The optimum pH of the enzyme was between pH 6 and 7 for the three cultivars. Thermal inactivation data showed that activity of the enzyme was lost after heating for 15 and 4 min at 50 and 80°C, respectively. Browning reactions of plantain PPO were inhibited by sodium metabisulphite (0.1%), ascorbic acid (0.2%), malic acid (1.0%) and the least sodium chloride (>1.0%) which is similar to banana PPO. Plantain polyphenol oxidase was active towards catechol but not with cresol.

Key words: plantain (*Musa paradisiaca*) cultivars, polyphenol oxidase, catechol, enzyme activity.

INTRODUCTION

Plantain (*Musa paradisiaca*) is an important crop in Nigeria as in all humid tropical zones of Africa, Asia, Central and South America (Swennen, 1990). It is grown extensively in the southern states of Nigeria. Plantain is one of the most important sources of food energy throughout the African lowland humid forest zone. Plantain has a unique flavour and aroma and it contains high amount of potassium (K^+) and calcium (Ca^{2+}). Traditionally, plantain pulp is cooked when green and pounded to give ''*fufu*''-like dish which can be eaten with vegetable soup or sundried and milled into flour for *'amala'* while the ripe peeled plantain can be eaten directly or boiled or sliced and fried in vegetable oil. Industrially, plantain is an important raw material in the manufacture of plantain flour. Plantain flour is obtained by drying green fruit and can be used as diluents of bread flour. The flour from ripe fruit has high sugar content which makes it a useful component of infant diet and the carbohydrate have been found to be easily digested than that of cereal (Ladele et al., 1984). In some areas, the peel of plantain fruits are used in soap making and as animal feed when dried. In Nigeria, plantain is grown extensively in the southern states namely Anambra, Akwa-Ibom, Bendel, Cross-river, Imo, Lagos, Ogun, Oyo and Rivers. PPO is widely distributed in the plant kingdom and has been shown to be among the first enzymes to be studied according to Schoenbein (1856) as cited by Whitaker (1972). Being widely distributed in nature it has been studied in fruits such as banana (*Musa cavendishii*) (Fatemh et al., 2008), potato (*Colocasia antiquorum* var *esculenta*) (Lee and Park, 2007), grape (Munoz et al., 2004), lettuce (*Luctuca sativa* var*. capitata* L.) (Gawlik-Dziki et al., 2007) and pepper (*Capsicum annuum* L.) (Arnnork et al., 2010). Polyphenol oxidase has been shown to be responsible for browning reactions and discolouration in many fruits and vegetables (Marshall et al., 2000; Yoruk and Marshall, 2003). Enzymatic browning occurs in many fruits and vegetables such as when the tissue is bruised, cut or peeled. The injured tissue gradually darkens on exposure to air due to the oxidation of phenolic compounds to brown pigments. Therefore the objectives of this study are to isolate and determine some properties of enzymes of plantain (*M. paradisiaca*) cultivars and to evaluate methods of

controlling activity of the enzyme.

MATERIALS AND METHODS

Procurement of fruit samples

Plantain fruits (*M. paradisiaca*) cultivars (Agbagba, Cardaba and Hybrid) were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan. Unblemished fruit samples were obtained at their pre-climacteric stages and were transported to the laboratory in open top plastic containers within 1 h of harvest.

Sample preparation

The mature green fruits were cleaned with moist cotton wool to remove extraneous matter and fruit latex which may cause discolouration. For extraction of enzyme from unripe green stage (stage 1), the harvested fruits were used as such whilst the remaining fruits were kept in open plastic containers at ambient conditions (28 ± 1°C) and RH 68 ± 2%) until table ripe corresponding to stage 6 of the USA banana ripening chart (Adel, 2005).

Preparation of crude PPO extracts

Preparation of plantain pulps for PPO extraction was accomplished at two physiological stages namely: Mature green (colour index 1) and table ripe (colour index 6) (Adel, 2005). 2 g of fresh fruit pulp was homogenized with 18 ml of 1% polyethylene glycol buffered at pH 7 with 0.1M potassium phosphate (Palmer, 1963). The slurry was centrifuged in a Beckman centrifuge (model J-21B) at 4000rpm for 20 min at 8°C. The resultant supernatant was taken as the crude enzyme extract.

Assay of plantain PPO

The enzyme activity was determined spectrophotometrically by measuring the initial rate of the increase in absorbance at 470nm using CECIL UV spectrophotometer (model CE 202 series 2) as described by Palmer (1963). The sample cuvette contained 1 ml 0.033 M potassium phosphate (pH7), 1 ml 0.005 M substrate solution and quantity of enzyme to give a total reaction volume of 3 ml (Palmer, 1963). The reference cuvette contained the reaction mixture without the enzyme which was replaced with distilled water. Initial experiment was carried out using buffered homogenate (2 g of pulp in 20 ml 0.1 M potassium phosphate pH 7) (Palmer, 1963). One unit of PPO activity was defined as potency of enzyme that increased in absorbance of 0.01 to 0.05 per minute.

Determination of pH optima of PPO activity

The optima pH of plantain PPO was determined by measuring the activity as described in enzyme assay using (1) 0.1 M phosphate buffers pH 6 to 8, (2) 0.1 M citrate buffers pH 3.8 to 6, (3) Standard pH buffer pH 9.2. The cuvette contained the substrate (1 ml), buffer solutions (1 ml) at different pH and enzyme solution (1 ml) to give a reaction mixture of 3 ml (Palmer, 1963).

Determination of optima temperature of PPO activity

The thermal denaturation of the enzyme was determined using the method of Park et al. (1980). 5ml portion of enzyme solution were sealed in test tubes and heated in water bath at 50, 60, 70 and 80°C, respectively (Park et al., 1980). At various heating intervals 1ml of samples were withdrawn and immediately cooled by immersion in ice-water. The PPO activity was measured using spectrophotometer at 470 nm as described in the enzyme assay (Palmer, 1963).

Chemical inhibition of PPO activity

Investigation on the enzyme inhibition was determined by measuring activity using 1ml 0.005 M substrate solution, 1 ml of various concentrations of sodium metabisulphite, ascorbic acid, Malic acid, citric acid and sodium chloride from 0.05 to 5.0%, 1 ml 0.033 M phosphate buffer (pH 7) and 1 ml enzyme solution (Palmer, 1963).

RESULTS AND DISCUSSION

Substrate specificity

All of the three plantain cultivars responded actively towards o-diphenols but not for the monophenols (Table 1). The activity of PPO obtained from unripe plantains (stage 1) was higher than the activity of PPO obtained from ripe plantains. Cathecol was the most readily oxidized by the enzymes. This finding is in line with Sanjeev and Mishra (2011) work that banana PPO is active towards o-diphenolic compounds.

Effect of substrate concentration on plantain PPO

Preliminary study on the effect of substrate concentrations showed that the activity of unripe plantain PPO from different cultivars increased with increase in concentration. Polyphenol oxidase from Agbagba gave maximum activity between 5 and 40 mM after which the activity decreased. Cardaba and Hybrid plantains' PPO have maximum activities at 40 mM and decreased as substrate concentration increased. The activity of PPO extracted from ripe plantain of different cultivars also increased as substrate concentration increases. Ripe plantain PPO (Agbagba) has maximum activity at concentration of 50 mM while ripe Cardaba and ripe Hybrid PPO have maximum activity at 40 and 10 mM respectively. This corroborated the report of Yang et al. (2000) that banana PPO acts best at substrate concentration of 2.8 mM using dopamine as substrate. The difference in substrate concentration may be due to genetic difference between plantain and banana.

Effect of pH on plantain PPO activity

Maximum pH activity occurred at pH 6.4 and 7.0. The result showed decrease in enzyme activity at alkaline and acidic pH values for different cultivars. It is reported

Table 1. Substrate specificity of plantain PPO.

Substrate 40 Nm	Unripe plantain fruits			Ripe plantain fruits		
	Agbagba	Cardaba	Hybrid	Agbagba	Cardaba	Hybrid
Catechol	0.650	0.520	0.440	0.470	0.370	0.360
Pyrogallol	0.360	0.280	0.130	0.135	0.110	0.085
Quinic acid	0.460	0.250	0.120	0.125	0.090	0.085
M-cresol	0.000	0.000	0.000	0.000	0.000	0.000

Table 2. Effect of chemical concentration on plantain PPO.

Inhibitors	Concentrations (%)				
	0.05	0.1	0.2	0.5	1.0
Sodium metabisulphite	Brown colour after 5 min and becomes darker with time	No reaction for 24 h	No reaction for 24 h	No reaction for 24 h	No reaction for 24 h
Ascorbic acid	Colour developed after 2 min	Colour developed after 10 min	No reaction	No reaction	No reaction
Malic acid	Brown colour after 2 min	Brown colour after 5 min	Brown colour after 5 min	Brown colour after 5 min	No reaction
Sodium chloride	Brown colour in 2 min	Brown colour in 2 min	Brown colour in 5 min	Brown colour in 5 min	Brown colour after 20 min

that banana PPO is optimally active at pH = 7.0 (Sanjeev and Mishra, 2011) which is similar to results obtained for plantain PPO.

Effect of heat on plantain PPO activity

The activity of the PPO from all the cultivars decreased with increase in temperature. This is because the enzymes are proteinous and are denatured by heat. The enzyme lost its activity in 15 and 4 min when held at 50 and 80°C respectively.

Effect of chemical treatment on plantain PPO activity

Table 2 shows the effect of chemical inhibitors on the activity of plantain PPO using catechol as substrate. Polyphenol oxidase activity was inhibited at 0.1 and 0.2% solution of sodium metabisulphite and ascorbic acid respectively. Malic acid inhibited the PPO at 1% solution while sodium chloride inhibited the PPO at concentration greater than 1.0%.

Enzymes extracted with buffered polyethylene glycol from the pulp of unripe and ripe plantain cultivars are by substrate specificity a polyphenol oxidase which is similar to enzymes of banana (Palmer, 1963; Sanjeev and Mishra, 2011). The results reported here show that plantain PPO (ripe and unripe) catalyzed oxidation of various substrates with catechol being the most readily oxidized substrate. Browning reactions of plantain PPO were inhibited by sodium metabisulphite(0.1%), ascorbic acid(0.2%), Malic acid (1.0%), and the least sodium chloride (> 1.0%) which is similar to banana (Palmer,1963; Sanjeev and Mishra, 2011).

Conclusion

Polyphenol oxidase has been found to occur in the pulp of plantain fruits. The enzyme catalyzed the oxidation of various phenolic compounds and this study indicated that the cathecol is most readily oxidized. Plantain PPO extracted using buffered polyethylene glycol was relatively stable and had optimum value was pH 6.8 with cathecol as the substrate.

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