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Influence of tuber harvest time and storage period on polyphenoloxidase activity and rate of browning of white yam (*Dioscorea rotundata*)

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Abstract

In this study, the effects of stage of maturity and storage time of yam tubers on polyphenoloxidase (PPO) activity, and rate of browning of tuber parenchyma were investigated. Five Nigerian traditional varieties of *Dioscorea rotundata* were evaluated during the vegetative cycle. Final harvesting was done when foliage senescence was observed; analyses on the tubers continued during the storage of yam tubers in ambient conditions for three months. The rate of browning of the tuber parenchyma did not show any definite trend throughout the vegetative cycle and storage period and the varieties also behaved differently from one another. The three sections (proximal, middle, and tail) of the tuber also did not influence the rate at which browning occurred in the parenchyma. It could be deduced that the rate at which the color of the tuber parenchyma changes from white to brown upon exposure to atmospheric air is not dependent on the age and length of storage period of the tuber, but could be an inherent attribute of the variety. PPO activity reduced in most of the varieties with an increase in the age of the yam plant and exhibited irregular behavior during storage.

Key words: White yam, polyphenoloxidase, rate of browning, starch granule

Introduction

Browning of crops during storage and processing is a significant problem in the food industry and the home and is believed to be one of the main causes of quality loss during post-harvest handling. Browning can cause deleterious changes in the appearance and organoleptic properties of the food product, resulting in reduced consumer acceptance (McEvily et al. 1992).

Browning in yam occurs at all stages of the tuber's existence as a result of the oxidation of phenolic constituents, such as o-dihydroxy or vic-trihydroxyphenolics, by a phenol oxidase present in the tissue (Anosike and Ayaebene 1981). Ozo and Caygill (1986) identified cyanidin 3-glucoside, (+)-catechine and accompanying procyanidins as substances probably involved in the enzymatic browning reaction. Some yam cultivars are characterized by browning reactions caused by the action of polyphenol oxidases on the polyphenolic compounds present in the tubers. This occurs during processing when the tubers are exposed to the air (Adamson and Abigor, 1980).

The browning reaction causes formation of an ugly brown or gray color, which polymerizes to form melanin responsible for off-flavors and sometimes bitterness in yam (Osagie 1992). This discoloration affects the organoleptic appeal of fresh or processed yam and reduces its acceptability. Browning index (BI) represents the purity of brown color and is reported as an important parameter in the processing of crops in which both enzymatic and non-enzymatic browning have been observed (Buera et al. 1986; Guerrero et al. 1996; Palou et al. 1999).

Materials and methods

Five Nigerian traditional varieties of Dioscorea rotundata were obtained from IITA for the experiments. TDr 131 (abi), TDr 99-12 (ehuru), TDr 99-13 (omiefun), TDr 99-3 (akwuki), and TDr 335 (sebureke).

Planting: Whole small tubers and tuber setts of various sizes (100 – 300 g) for each of the varieties were planted at IITA, Ibadan, Nigeria, in a randomized complete block design with three replications. The varieties were randomly allocated to plots with 130 plants/plot and arranged as 10 rows of 13 plants/row at 1 m × 1 m spacing.

Selection and harvesting: Dates of emergence of vines were noted and only those that emerged within the first 5 weeks after planting were tagged for subsequent sampling. Harvesting was done on a monthly basis from 4 months after planting, which coincided with 3 months after vine emergence (MAVE). Harvesting for each month was spread over 3 weeks for the three replicates (i.e., one replicate/week). From 4 to 10 of the plants tagged for sampling were harvested at random. The tubers were washed and laid out in the shade to air-dry. Final harvesting was done at 7 MAVE when there was complete senescence of the vines. Selected tubers from the final harvest were stored for 3 months inside net bags under ambient conditions in a storage barn made of bamboo shelves nailed to live poles whose leaves and a thatched roof provided adequate shade (a modification of the traditional storage structures common in Nigeria). Sampling of tubers for analyses was done on a monthly basis.

Sorting and preparation of tubers: Freshly harvested tubers were sorted into three classes: big (the biggest of the lot), medium (tubers of sizes between the biggest and the smallest) and small (the smallest of the lot). Three sections (proximal, middle and tail) of three tubers, which represented the three size categories, were cut open to expose the parenchyma. Single point measurements on the three tubers were made.

Determination of rate of browning: A Color-meter was used to measure the L*a*b* values of the flesh of freshly peeled yam tuber at 0 minute. Measurement was taken again after 10 mins. The BI was calculated to represent the purity of the brown color. Palou et al. (1999) explained in their report that it is as an important parameter in processes where enzymatic and non-enzymatic browning takes place. The BI of the yam flesh at 0 min and after 10 min was obtained from the calculation below:

$BI = [100 (x – 0.31)] / 0.172$
where $x = (*a + 1.75*L) / (5.645L + *a – 3.012*b)$

Δ BI was calculated as difference between the BI values after 10 min and the initial BI value (0 min)
Rate of browning $^{-min}$ (Δ BI/min) = Δ BI / 10 (min)

Determination of PPO activity: Crude polyphenol oxidase (PPO) was obtained by homogenizing 9 g of freshly peeled yam tissue in 45 ml of cold buffer (0.05 M sodium phosphate, pH 7.0) in Ace homogenizer at 0.42 g for 4 min. The homogenate was centrifuged at 75.75 g (4 oC, 10 min) and the supernatant was used as the source of crude enzyme (modified method of Omidiji and Okpuzor [1996].

The PPO activity of yam tissue was determined using 12.5 mM catechol as substrate, according to the modified method of Adamson and Abigor (1980). The assay medium contained 5.4 ml of 0.2 M sodium phosphate buffer, pH 7.0, containing 12.5 mM catechol, and 0.1 ml of crude enzyme extract, while the reference cuvette contained 5.4 ml of the buffer-catechol mixture and 0.1 ml of the crude enzyme extract which had been previously boiled and cooled. The change in absorbance at 440 nm was read after 90 s in a Spectronic 401 spectrophotometer (Milton Roy). One unit of PPO activity is defined as a change of 0.01 absorbance unit at 440 nm at room temperature (29±2 ^{o}C).

Results

Rate of browning: The rate of browning of the tuber parenchyma did not show any definite trend throughout the vegetative cycle and storage period (Table 1) while the varieties also behaved differently from one another. Difference in the portions of tuber examined also did not influence the rate at which browning occurred in the parenchyma (Table 2). The negative values obtained could be attributed to the sensitivity of the color meter. It could be deduced that the rate at which the tuber parenchyma changes from white color to brown upon exposure to atmospheric air is not dependent on the age and length of storage of the tuber, but could be an inherent attribute of the variety. Abass et al. (2003) also reported that there were no significant changes in the color indices as well as the BI measured on yam tubers' parenchyma (D. rotundata varieties) during storage. The mean values of the effect of the age of the plant and length of storage period on the rate of browning of the tuber parenchyma of five varieties are shown in Table 3.

Polyphenol oxidase activity: PPO activity of the yam varieties with age and length of storage of tubers is as shown in Figure 1. No result was indicated at 7

Table 1: Effect of age of plant and storage period on rate of browning (min^{-1}) of yam tuber parenchyma

Months after vine emergence (MAVE)	Months of storage	Variety				
		TDr 131	TDr 99-12	TDr 99-13	TDr 99-3	TDr 335
3		0.38abc	0.27b	0.45abc	0.18a	0.30ab
4		0.70a	0.37b	0.19bc	0.24a	0.03ab
5		0.64ab	0.07b	0.65a	0.34a	0.27ab
6		0.28abc	0.14b	0.24abc	0.32a	0.28ab
7		0.53abc	0.38b	0.04c	0.79a	1.20a
	1	0.18bc	0.13b	0.61ab	0.48a	0.31ab
	2	0.08c	2.52a	0.32abc	ND	-0.22b
	3	0.41abc	0.78b	0.24abc	ND	0.23ab

Means followed by the same letters along the column are not significantly different (P<0.05).
ND – Not determined

Table 2: Variation in rate of browning (min^{-1}) of different portions of yam tuber parenchyma

Tuber portion	Variety				
	TDr 131	TDr 99-12	TDr 99-13	TDr 99-3	TDr 335
Head	0.57a	0.65a	0.36a	0.33a	0.19a
Middle	0.38ab	0.15a	0.37a	0.43a	0.49a
Tail	0.28b	0.37a	0.24a	0.42a	0.30a

Means followed by the same letters along the column are not significantly different (P<0.05).

Table 3: Mean effect of age of plant and storage period on rate of browning (min^{-1}) of tuber parenchyma of five varieties

Months after vine emergence (MAVE)	Months of storage	Rate of browning (min^{-1})
3		0.30a
4		0.28a
5		0.18a
6		0.05a
7		0.53a
	1	0.29a
	2	0.36a
	3	0.35a

Means followed by the same letters along the column are not significantly different (P<0.05).

(MAE) due to a logistics problem. Results obtained showed a decreasing trend in values during growth to the month of October (6 MAE), while the varieties showed irregular behavior at storage time. A similar decrease in activity was reported during the development of medlar fruit (Aydin and Kadioglu, 2003) and in Cajanus cajan leaves (Mukherjee and Rao, 1993). Ikediobi and Oti (1983) also reported a steady decrease of PPO activity during the storage of D. rotundata tubers. However, the activities of phosphorylase, hexokinase, glucose-6-phosphate, and alcohol dehydrogenase in D. rotundata increased with the age of tubers (Ugochukwu et al. 1977).

Adamson and Abigor (1980) and Anosike and Ayaebene (1981) associated yam tissue browning with PPO activity. Omidiji and Okpuzor (1996) reported that only 40% of browning in D. rotundata is due to PPO activity while the remainder is non-enzyme mediated. There was no observed correlation between the rate of browning and PPO activity. This might

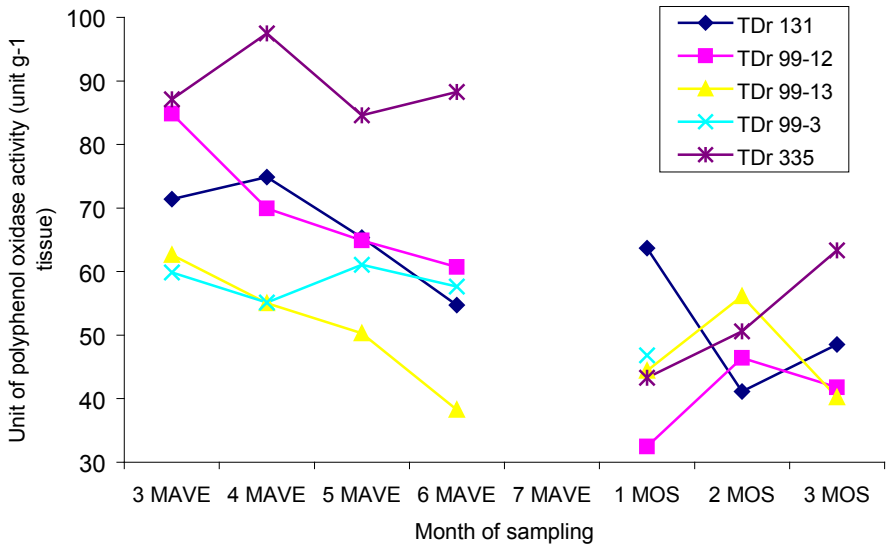

Fig. 1: Polyphenol oxidase activity of tubers with age and length of the storage period

be due to the short time (10 min) used for the study of rate of browning of the tuber parenchyma in this work.

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