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Lipid peroxidation, o-diphenolase, superoxide dismutase and catalase profile along the three physiological regions of *Dioscorea rotundata* Poir cv Omi

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Abstract

The head region of *Dioscorea rotundata* Poir CV Omi is bitter in taste when eaten but is the most productive region for propagation when compared to the other two physiological regions; middle and tail. In this study some biochemical parameters are highlighted to explain these two differences. o-Diphenolase activity was highest in the head region, followed by the tail region and lastly the middle region while the level of lipid peroxidation was highest in the middle region, followed by the tail and lastly the head region. The activities of superoxide dismutase and catalase exhibited a similar pattern of distribution with the highest activities recorded in the tail region and followed by the head region. The middle region had the least antioxidant enzyme activities.

1. Introduction

Polarity has been reported on the activity of o-diphenolase in potato tubers (Tanaka & Uritani, 1977). Similarly, the three physiological regions head, middle and tail in yam tubers have been demarcated using the level of o-diphenolase activity (Isamah, 1984); glycolytic enzymes and pH (Oluoha & Ugochukwu, 1985). Mlingi (1995) used the level of cyanogenic glucosides to delimit cassava tubers.

The superoxide radical O_2^- is formed in living cells during several biochemical reactions (Fridovich, 1974). The free radicals initiate lipid peroxidation probably resulting in the deterioration of cell membranes (Dhindsa, Plumb-Dhindsa & Thorpe 1981).

Lipid peroxidation is under the control of efficient endogenous cellular defence systems to ensure the maintenance of cell integrity as well as optimum metabolic and functional performance. When the function of an endogenous protective system is depressed, oxygen radicals have been considered as a basic factor in the acceleration of cell ageing and other specific degeneration diseases (Rauchova, Ledvinkova, Kalous & Drahota, 1995). Dhindsa et al., (1981) have shown that the deterioration of membranes during leaf senescence is accompanied by a decrease in superoxide dismutase (SOD) activity.

Polyphenoloxidases are believed to be ubiquitous in the plant kingdom and they are primarily associated with enzymatic browning and off-flavour generation. Both phenomena are of vital importance to manufacturers, as they impair, not the sensory properties, but also nutritive value (Vamos-Vigyazo, 1981). The head region of *D. rotundata* Poir cv Omi is bitter in taste and the most productive region for propagation. The aim of this paper was to investigate some of the biochemical parameters responsible for the difference in the sensory properties and the higher productive potential of the head region of *D. rotundata* Poir cv Omi.

2. Material and methods

2.1. Materials

The tubers of *D. rotundata* Poir cv Omi were obtained from a local farm in Obinomba in Delta State of

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Nigeria. The tubers selected for this study were in storage for two months and were neither damaged during harvesting nor affected by disease. All the reagents used were the best available and were of analytical grade.

2.2. Methods

2.2.1. Extraction and assay of o-diphenolase

The head, middle and tail regions of the selected yam tubers were peeled and washed with ice-cold water and cut into small pieces. The yam tissues (50g) from each of the physiological regions were added to 100 cm^3 of ice-cold 0.05 M phosphate buffer, pH 7.0. Polyethylene glycol (1g) and 1% (w/v) Triton x-100 were also added to the mixture and blended in a MSE blender immersed in ice. The extract was squeezed quickly through a double layer of cheesecloth and the filtrate clarified by centrifugation at 8000 g for 15 min, at 4°C. The resulting supernatant was used as the crude enzyme without further purification. For the assay of o-diphenolase activity, the method of Adamson and Abigor (1980) was used with catechol as substrate while the protein content of each region was estimated by the method of Lowry, Rosebrough, Farr and Randal (1951). One unit of o-diphenolase activity is the amount of enzyme that caused a 0.001 change in absorbance per min at 420 nm.

2.2.2. Preparation of extracts for the determination of lipid peroxidation

The yam tuber pieces (50 g) from each physiological region were separated, washed with ice-cold water and homogenized with 100 cm^3 of ice-cold 0.05M phosphate buffer pH 7.O.

The extract so obtained was clarified by centrifugation for 20 min, at 7000 g at 4°C. The supernatant (S_1) so obtained was used for the determination of the level of lipid peroxidation by the method of Hunter, Gebicki, Hoffstein, Weinstein and Scott (1963) as modified by Gutteridge and Wilkins (1982).

2.2.3. Extraction and assay of catalase

Catalase was measured with a similar (S_1) fraction after addition of 1% (v/v) of ethanol and incubation in the cold (0–4°C) for 15 min. This treatment is reported to reverse the inactivation of catalase which takes place by the formation of compound 11 (Cohen, Dembiec & Marcus, 1970). Catalase activity was determined essentially as described by Kaplan and Groves (1972) in which the disappearance of hydrogen peroxide was described by the equation for first-order reaction kinetics:

$$\mathrm{Log}_{10}A = \mathrm{Log}_{10}A_0 - \frac{kt}{2.3}$$

where A_0 is the absorbance due to hydrogen peroxide at 0 time, A is the absorbance due to hydrogen peroxide at time t, k is the first-order rate constant and t is the time interval over which the reaction is measured (70 s). Thus each catalase unit specifies the relative logarithmic disappearance of hydrogen peroxide per min at 240 nm and is expressed as k min^{-1}.

2.2.4. Extraction and assay of superoxide dismutase (SOD)

An aliquot of the (S_1) supernatant was precipitated on ice with 0.30 volume of chloroform/methanol (3:5 v/v) according to the method of Aksnes and Njaa (1981).

Homogenization was performed for 20 min at 7000 g at 4°C. The supernatant (S_2) was used for the assay of SOD activity which is based on its ability to inhibit the oxidation of epinephrine by superoxide anion (Misra & Fridovich, 1972). One unit of SOD activity is the amount of the enzyme required for 50% inhibition of the oxidation of epinephrine to adrenochrome at 480 nm per min.

2.2.5. Statistical analysis

The mean and standard error of mean (SEM) were analysed according to the procedure outlined by Bailey (1981).

3. Results and discussion

The activity of o-diphenolase in *D. rotundata* Poir cv Omi was highest in the head region, followed by the tail region and lastly the middle region (Table 1). Onayemi and ldowu (1988) have established that the levels of polyphenolic and glycoalkaloid substances in stored *D. rotundata* Poir and *D. cayenensis* Lam increased and became concentrated at the head region. Thus, this high polyphenolic substance in the head region will enhance the activity of o-diphenolase, leading to the accumulation of elevated levels of tannins, polymerized products of o-diphenolase activity. The tannin content in *D. rotundata* is 20.00 ± 0.05 mg/100 g dry matter

Table 1
o-Diphenolase activity in the three physiological regions of *D. rotundata* Poir cv Omi[a]

Physiological regions	o-Diphenolase activity (units/mg protein)
Head	300 ± 327 a $n = 5$[b]
Middle	2400 ± 502 $n = 5$
Tail	2760 ± 467 $n = 5$

[a] Results are expressed as mean ± standard error of mean (S.E.M.).
[b] n = Number of yam tubers.

Table 2
The level of lipid peroxidation in the three physiological regions of *D. rotundata* Poir cv Omi[a]

Physiological regions	Level of lipid peroxidation MDA (nmol ml^{-1})
Head	10.8 ± 1.57 $n = 5$[b]
Middle	14.5 ± 2.40 $n = 5$
Tail	12.4 ± 1.49 $n = 5$

[a] Results are expressed as mean and standard error of mean (S.E.M.).
[b] n = Number of yam tubers.

Table 3
Superoxide dismutase (SOD) activity in the three physiological regions of *D. rotundata* Poir cv Omi[a]

Physiological regions	SOD activity (units/g wet tissue)
Head	26.4 ± 0.03 $n = 5$[b]
Middle	26.0 ± 0.03 $n = 5$
Tail	26.6 ± 0.01 $n = 5$

[a] Results are expressed as mean and standard error of mean (S.E.M.).
[b] n = Number of yam tubers.

Table 4
Catalase activity in the three physiological regions of *D. rotundata* Poir cv Omi[a]

Physiological regions	Catalase activity K (min^{-1})
Head	3.60 ± 0.02 $n = 5$[b]
Middle	3.31 ± 0.20 $n = 5$
Tail	3.67 ± 0.01 $n = 5$

[a] Results are expressed as mean and standard error of mean (S.E.M.).
[b] n = Number of yam tubers.

(Udoessien & Ifon, 1992). Tannins may decrease protein quality by decreasing digestibility and palatability (Osagie 1998). The head region of *Dioscorea rotundata* Poir cv Omi is bitter in taste and this explains the cultural practice of removing the head region of *D. rotundata* Poir cv Omi before cooking. Other nutritional effects which have been attributed to tannins include damage to the intestinal tract, interference with the absorption of iron and a possible carcinogenic effect (Butler, 1989; Osagie, 1998).

The peak value of lipid peroxidation was obtained in the middle physiological region, whilst the head region had the lowest level (Table 2). This increase in lipid peroxidation in the middle region coincided with the relatively low levels of antioxidant enzyme components (SOD and catalase) in this region (Tables 3 and 4). Endogenous catalytic oxygen scavengers, namely SOD and catalase, play key roles in cellular defence against reactive oxygen species under physiological conditions (Coudray, Boucher, Pucheu, Leiris & Favier, 1995). Ketiku and Oyenuga (1973) have reported a higher level of glucose in the bottom (tail region) compared to the head region of *D. rotundata* Poir. Bryszewska, Zavodnik, Niekurzak & Szosland (1995) had established that auto-oxidation of glucose is an additional source of free radicals. This may explain the higher level of lipid peroxidation in the tail region compared to the head region.

When lipid peroxidation proceeds in any biomembrane, several fatty acids and lysophospholipids are released leading to changes in biomembrane microviscosity and kinetic properties (Halliwell & Gutteridge, 1989; Ytrehus & Hegstad, 1991). Massive attack of membranous structures by reactive oxygen molecules may account for the characteristic tissue disintegration association with rotting of stored healthy yam tubers (Ikediobi, 1985). Desmarchelier, Novoa Bermudez, Coussio, Ciccia and Boveris (1997) have reported that polyphenols and flavonoids have antioxidant activity and, as would be expected, this justifies the low level of lipid peroxidation in the head region of *D. rotundata* Poir which accumulates polyphenolic substances in the head region with storage (Onayemi & ldowu, 1988).

That the head region of the yam tuber is the most productive part for propagation (Coursey, 1967) hinges on two factors. Firstly, the high o-diphenolase activity in the head region will enhance propagation, since it has been reported that polyphenoloxidases may play a role in plant growth regulation and also in auxin biosynthesis (Vamos Vigyazo, 1981). Secondly, the compromised level of lipid peroxidation in the head region will reduce the amount of reactive oxygen radicals and their accompanying innocuous membranous disintegration, thereby extending the shelf-lives of the head region for a more promising propagation.

From the results obtained in this study, it is pertinent to state that the activities of o-diphenolase, antioxidant enzymes and the level of lipid peroxidation have significant roles to play in the palatability and shelf-lives of *D. rotundata* Poir cv Omi.

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