GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 9, NO. 2, MARCH 2003: 165-169
COPYRIGHT(C) BACHUDO SCIENCE CO. LTD. PRINTED IN NIGERIA. ISSN 1118-0579


# CHANGES IN ACTIVITIES OF POLYPHENOL OXIDASE, ASCORBATE, PEROXIDASE, HYDROPEROXIDE AND LIPID LEVELS DURING DESICCATION OF *IRVINGIA GABONENSIS* (VARIETY EXCELSA) SEEDS.

**P. J. NYA, D. N. OMOKARO and A. E. NKANG**

(Received 3 September 2002; Revision accepted 6 January 2003).

## ABSTRACT

Activities of peroxidase (POD), Polyphenol oxidase (PPO), hydroperoxide and lipid contents were investigated during desiccation of cotyledonary tissues of *Irvingia gabonensis* at ambient temperature (26°C - 30°C), 35°C and 20°C. Activities of POD and PPO increased initially but declined in the latter desiccation period. However, loss of enzymic activity (POD and PPO) occurred generally at moisture content less than 20%. The hydroperoxide and lipid levels increased during desiccation irrespective of stage of seed development. Results suggest that seeds show peroxidation with drying, that is spontaneous oxidation of unsaturated fatty acids. Lipid peroxidation was very pronounced in *Irvingia gabonensis* seeds indicating the need for rapid drying at relatively high temperature in order to maintain seed quality.

Key Words: POD: Peroxidase; PPO: Polyphenol oxidase; RMC; Relative Moisture Content; *Irvingia gabonensis*

## INTRODUCTION

Bush mango (*Irvingia gabonensis)* is found growing in the forest and rarely cultivated. Cultivation is usually limited to areas where indigenous agricultural system embraces the cultivation of trees (Donna et. al, 1994 and Okafor, 1981). The uses of bush mango (or ogbono) are vast. Its seeds are used in soups as thickeners. Their cotyledons can be dried, ground and processed into a "cake" which is further smoked, stored and eaten readily with yam or plantain. In whatever form it is prepared "Ogbono" has a high nutritive and dietary value in terms of fats (72%) and protein (9%) (Okafor and Okolo, 1974; Ejiofor, 1994). The seedlings which could be used for farm planting are not easy to obtain because of the apparent dormancy of seed lots resulting in poor germination result (Omokaro et al, 1999).

There is therefore, the necessity to carry out procedures for the development of techniques of seed quality assessment for the selection of superior strains for commercial purposes.

There are great differences among seed in their content of food reserves. The composition of seeds influences their moisture content and consequently their storage behaviour (Street and Opik, 1977; and Vaughan and Duke, 1984). In some seeds including many legumes, high levels of proteins together with a higher amount of starch and a little lipid are found (Corners, 1978 and Kahn, 1983).

Increase in moisture content results in increased seed deterioration. Most seeds are better stored using conventional methods at low moisture contents. Seed with relatively high moisture contents up to 68.8% in fluted pumpkin, can germinate during storage (Ikediobi, 1985; Hailstones and Smiths, 1989).

Desiccation injury, loss of viability and membrane, enzyme or transport disruption may occur at low moisture content (Harrington, 1972; Smith and Adamson, 1989). For most seeds, drying below a certain moisture content induces loss of viability irrespective of the storage condition (Bass, 1979; and Barton, 1961). Seeds of many tropical trees are high in moisture content and cannot withstand intensive desiccation. These are recalcitrant seeds as opposed or orthodox seeds which can tolerate desiccation for low moisture contents (King and Robert, 1979).

Browning in most plant tissues is caused primarily by the activity of the enzyme polyphenol oxidase. The activity of polyphenol oxidase and the content of polyphenol are usually considered as the main factors contributing to the browning potential of tissues (Vaughan and Duke, 1984; Nkang and Chandler, 1986).

Knowledge of biochemical activities associated with moisture content, storage and desiccation of *Irvingia gabonensis* (Bush mango) is useful, as it will permit the assessment of seed

P. J. NYA, Department of Botany, University of Calabar, Calabar, Nigeria
D. N. OMOKARO, Department of Botany, University of Calabar, Calabar, Nigeria
A. E. NKANG, Department of Botany, University of Calabar, Calabar, Nigeria

quality. In this study investigations have been carried out on the PPO, POD, lipids and hydroperoxide contents of *Irvingia gabonensis* during desiccation of mature and immature seeds.

## MATERIALS AND METHODS

### Seed collection and desiccation

Fresh fruits of *Irvingia gabonensis* were harvested in Nsan village in Akamkpa Local Government Area of Cross River State of Nigeria between the months of June and July 1998. Seeds of approximately the same size and physiological maturity were used in carrying out the experiments.

The selected seeds were spread on the laboratory bench at ambient temperature (approx. 28°C – 30°C). Some seeds were dried in an incubator at 20°C and 35°C. Seeds were removed at daily intervals for the determination of moisture content, lipid and hydroperoxide levels and enzymes activity. The moisture content was determined on a fresh weight basis after oven-drying at 80°C for 72 hours.

### Extraction and Assay of polyphenol oxidase and ascorbate peroxidase

1. **Enzyme Extraction**: Enzyme extraction and assay were done in duplicate. One gram fresh weight of cotyledonary tissues from each desiccation treatment was ground into paste using a pestle and mortar in 20ml of extraction buffer (pH at 5°C). The homogenate or mixture was centrifuged at 10,000rpm for 3 minutes.

   The supernatant fraction was stored on ice and used as crude enzyme source.

2. **Enzyme Assay**

   (a) Polyphenol Oxidase

   To 2 mls of assay buffer (mixed 20Mm potassium phosphate buffer, potassium salts, pH 7.0) at 30°C was added to 500ml of enzyme preparation and 0.5mls of *dihydroxyphenylalanine* (10mM DOPA) for one minute. (50µl of 50% stock $H_2O_2$ and 5ml of distilled $H_2O$)

TABLE 1: CHANGES IN POD AND PPO ACTIVITIES IN MATURE FRUITS OF Irvingia gabonensis DESICCATION INTACT AT 28-300C (MEAN ±S.E)

| Desiccation | Peroxidase | | Polyphenol Oxidase | |
|---|---|---|---|---|
| (Days) | Absorbance | Activity (M/L) | Absorbance | Activity (M/L) |
| On collection | 1.11±0.00 | 2040.76±2.01 | 1.56±0.00 | 6.640±0.84 |
| 3 | 1.32±0.10 | 2426.86±4.00 | 1.99±0.01 | 8.471±0.63 |
| 7 | 1.33±0.00 | 2445.24±5.01 | 1.99±0.01 | 8.471±0.71 |
| 12 | 1.41±0.20 | 2592.32±4.09 | 1.64±0.00 | 6.981±0.16 |

The parameters are reported as mean ± Standard Error of five readings.

TABLE 2: CHANGES IN ACTIVITIES OF POD AND PPO IN IMMATURE FRIUTS DESICCATED AT 28-30$^0$C (MEAN ± S.E)

| Desiccation Time | Peroxidase | | Polyphenol Oxidase | |
|---|---|---|---|---|
| (Days) | Absorbance | Activity (M/C) | Absorbance | Activity (M/C) |
| On Collection | 0.38±0.00 | 269.64±2.20 | 0.96±0.01 | 4.087±0.60 |
| 3 | 1.37±0.01 | 2518.76±3.00 | 1.00±0.04 | 8.471±1.30 |

The parameters are reported as mean ± Standard Error of five readings

TABLE 3: CHANGES IN ACTIVITIES OF POD AND PPO IN MATURE FRUITS OF Irvinga gabonensis DURING DESICCATION AT 35°C (MEAN ±S.E)

| Desiccation Time | | RMC Peroxidase | | Polyphenol Oxidase | |
|---|---|---|---|---|---|
| (Days) | % | Absorbance | Activity N/L | Absorbance | Activity (N/L) |
| | | | | E | |
| On collection | 40.0±0.00 | 1.11±0.00 | 2040.76±0.80 | 1.56±0.00 | 6.641±0.16 |
| 3 | 30.0±0.01 | 1.30±0.01 | 2390.08±1.10 | 1.60±0.00 | 6.811±0.92 |
| 7 | 20.0±1.12 | 1.43±0.12 | 2629.09±1.12 | 1.97±0.00 | 8.343±0.70 |
| 12 | 10.0±1.20 | 1.00±0.60 | 2334.92±2.10 | 1.99±021 | 8.471±0.08 |
| 15 | 10.0±0.0 | 1.27±0.60 | 2334.92±2.10 | 1.99±0.21 | 8.471±0.10 |
| 18 | 10.0±0.10 | 1.98±0.16 | 1801.75±3.12 | 1.53±0.01 | 6.513±0.60 |

The parameter are reported as mean ± Standard Error of five readings

TABLE 4: CHANGES IN ACTIVITIES OF POD AND PPO IN MATURE FRUITS OF Irvingia gabonensis DURING DESICCATION AT 20°C (MEAN ±S.E)

| Desiccation Time | Peroxidase | | | Polyphenoloxidase | |
|---|---|---|---|---|---|
| Days | RMC% | Absorbance | Activity (N/C) | Absorbance | Activity (N/L) |
| On collection | 46.0±1.30 | 1.11±0.00 | 2040.76±0.71 | 1.56±0.00 | 6.641±0.60 |
| 3 | 38.4±0.97 | 1.40±0.00 | 2573.93 ± 1.33 | 1.55±0.00 | 6.598 ± 0.60 |
| 7 | 30.0±2.04 | 141±0.00 | 2592.32±3.01 | 1.99±0.00 | 8.471±0.84 |
| 12 | 30.0±2.04 | 1.52±0.00 | 2794.56±4.10 | 1.99±0.00 | 8.471±0.16 |
| 13 | 20.0±2.01 | 1.40±0.00 | 2573.93±3.10 | 1.99±0.00 | 8.471±0.07 |

The parameters are reported as mean ± Standard Error of five readings.

The mixture was incubated for one minute at 30°C and the absorbance measured colorimetically at 470mM against a blank of water. Activities of PPO are expressed as Mmole quinone product $Sec^{-1}$ $L^{-1}$ and calculated using an extinction coefficient of 1433m – $cm^{-1}$ (Jimenez and Garcia-Carmora, 1995).

**Ascorbate Peroxidae**

To 3ml of assay buffer was added 200µl of enzyme preparation. To this was added 100µl of substrate (100mm ascorbate). The reaction was started with the addition of 50µl of $H_2O_2$ preparation (50µl of 50% stock $H_2O_2$ in 5mls of $H_2O_2$. The absorbance of the mixture after 60 seconds were measured colorimetrically at 430nM with water as blank. POD activity expressed as Mmol ascorbate product oxidized $Sec^{-1}$ was calculated using an extinction coefficient of 6.391 $mol^{-1}$ $cm^{-1}$ for ascorbate (Putter, 1974).

***Extraction of Lipids***

Lipids were extracted as described by Hailstone and Smith, (1988). Lipids were extracted from 0.3g dry weight of ground cotyldonary tissue in 20mls of a solvent system of petroleum ether and methanol (2: 1, V/V) containing 0.006% of the antioxidant butylated hydroxytoluene. Lipids were determined graimetrically after extraction at 65°C.

**Determination of Hydroperoxide**

Hydroperoxides were determined in the

pid extracts. To 50ml of 0.14m ferrous chloride vas added 2mls of benzene and 1ml methanol ınd shaken. 50µl OF 3 M potassium thiocyanate. The absorbance of the resulting solution was read :olorimtrically at 505nm against a blank of the eagents (Smith and Adamson, 1989).

## RESULT

Activities of peroxidase (POD) increased gradually during desiccation of mature *Irvingia gabonensis* seeds at ambient temperature. Polyphenol oxidase (PPO) activity increase similary but with activity decreasing at the end of the desiccation period (Table 1).

The relative moisture content in the seeds of Irvingia gabonensis decreased with time irrespective of the desiccation temperature and stage of maturity (Table 3 and 4). The seeds dried at 35°C lost moisture more rapidly than the others (Table 3).

In general , activities of POD and PPO increased initially but declined with prolonged desiccation in seeds kept at ambient temperature and 35°C.

In seeds dried at 20°C PPO activity increased throughout the desiccation period. On the other hand, PPO activity increased initially but thereafter decreased slightly at the end of the desiccation treatment (Table 4).

Seeds of *Irvingia gabonensis* demonstrated a relatively high moisture content at harvest (Table 5 and 6). The seeds dried at 35°C lost moisture more rapidly to those dried at 20°C, moisture content declined with desiccation in both cases. In general, lipid and hydroperoxide level appeared to increased gradually with time during desiccation at both temperatures (Table 5 and 6).

## DISCUSSION

Activities of peroxidases have been implicated and associated with various physiological processes including germination, ripening, abscission and fruit development (Gasper *et al*, 1972; Asins *et al* 1984). In this study, activities of peroxidase (POD) increased gradually during desiccation of mature *Irvinga gabonensis* seeds at ambient temperature. The

TABLE 5: EFFECT OF DESICCATION IN Irvingia gabonensis SEEDS AT 20°C (MEAN ± S.E)

| Desiccation Period Days | Relative Moisture Content (%) | Lipid Content (Mg Lipid g-IDW) | Hydroperxide (Abs. 505 mm) |
|---|---|---|---|
| 0 | 39.1±0.4 | 38.1±1.3 | 0.35±0.5 |
| 3 | 38.4±0.3 | 40.1±1.0 | 0.47±0.1 |
| 6 | 30.0±1.1 | 42.4±1.4 | 0.45±0.0 |
| 9 | 30.0±1.2 | 56.1±1.7 | 0.50±0.6 |
| 12 | 20.0±0.3 | 48.3±1.2 | 0.50±0.1 |
| 15 | 20.0±0.6 | 50.5±1.3 | 0.50±0.0 |

The parameters are reported as mean ± Standard Error of five readins.

TABLE 6: EFFECT OF DESICCATION IN Irvingia gabonensis SEED AT 35°C (MEAN ±S.E)

| Desiccation Period Days | Relative Moisture Content (%) | Lipid Content (Mg Lipid g-IDW) | Hydroperoxide (Abs. 505 nm) |
|---|---|---|---|
| 0 | 40.0±0.06 | 38.1±0.42 | 0.35±0.02 |
| 3 | 30.0±0.94 | 40.8±0.71 | 0.35±0.01 |
| 6 | 20.0±0.03 | 42.4±0.54 | 0.43±0.07 |
| 9 | 10.0±0.00 | 41.0±0.61 | 0.45±0.01 |
| 12 | 10.0±0.20 | 56.1±0.10 | 0.53±0.03 |
| 15 | 10.0±0.30 | 56.1±0.21 | 0.53±0.00 |

The parameters are reported as mean ± Standard Error of five readings.

pattern of polyphenol oxidase (PPO) activity was similar to that of POD, increasing initially and thereafter declining with severe desiccation. This agrees with a suggestion (Harrington, 1972) that desiccation injury, loss of viability and membrane, enzyme or transport disruption may occur at low moisture.

The subsequent declines in the peroxidation activity occurred generally at moisture content less than 20% and may be associated with some level of deterioration (stress damage). This went to confirm what (King and Robert, 1979) reported, that seeds of many tropical trees are high in moisture and cannot withstand intensive desiccation. However, *Irvingia gabonensis* seeds exhibits recalcitrant viability characteristics.

The loss of enzymic activity with prolonged desiccation was apparent in all the treatments. This may be as result from the depletion of substrates of reduced transport or accessibility of substrates as a results of desiccation. This agrees with a suggestion (Bass, 1979) that increase endogenous hydrolytic activity might lead to distribution in metabolism which could result in enzyme inactivation. Increase activity of ascorbate peroxidase may be part of an endogenous mechanism that protect membrane and enzymes during desiccation.

Seeds of *Irvingia gabonensis* showed greatly increased hydroperoxide levels with desiccation. This suggests a change in the level of unsaturated fatty acids with desiccation. Lipid levels also increased with desiccation in *Irvingia ganbonensis* seeds. This agrees with what (Hailstone and Smiths, 1989; Smith and Adamson, 1989) suggested that spontaneous oxidation of unsaturated fatty acids in lipids produce highly reactive intermediates, hydroperoxides and secondary products. Also peroxidation of seed lipids during storage could lead to changes in the relative percentages of constituent fatty acids due to the preferential breakdown of unsaturated fatty acids. However, seeds of *Irvingia gabonensis* seem to show peroxidation with drying. The quality of the final product may therefore guaranteed if the seeds are dried at a higher temperature (about 60°C).

## ACKNOWLEDGEMENT

Dr. Omokaro and Mr. Paul Nya acknowledge with thanks the support of the University of Calabar Senate Research Grant for the on-going studies in the physiology and biochemistry of *Irvingia gabonensis* seeds.

## REFERNCES

Asins, M. j., Benito, C. and Perezde la Vaga, 1983. A Comparative study of the changes of peroxidase patterns during wheat, rice, triticale germination Canadian Journal of Botary. 61: 3393 - 3398.

Barton, L. V., 1961. Seed preservation and longevity. Interscience publishers Inc. New York, pp320.

Bass, L.N., 1979. Physiological and other aspects of seed preservation In: The plant seed development, Preservation and Germination. (Irwin Rubenstein, Ronald C. Phillips, Charles E. Green and Gengenbach, B.G. eds,). Academic Press, London, pp 145-165.

Corners, E. J. H., 1976. The seeds of dicotyledons Vol. 1. Cambridge University Press, London, pp. 113-124.

Donna, M. A., Ogar, A. F. and Otu, I. I., 1994. A user survey on *Irvingia gabonensis* in Cross River State-Nigeria. Local Storage methods of Bush Mango. A paper presented at the pregermplasm collection meeting on *Irvingia gabonensis* organized by ICRAFT, Nairobi, Kenya held at I.T.T.A., Ibadan, Nigeria, 10-14 May, 1994, pp. 1-8.

Ejiofor, M. A. N., 1994. Nutritional values (and utilities) of "Ogbono", Irvingia gabonesis var. excelsa. A paper presented at the Germplasm collection meeting at IITA, sponsored by International Centre for Research in Agroforestry (ICRAF), 10- 11 May, 1994, IITA, Ibadan, Nigeria, Pp 1-7.

Gasper, T. H., Penel, G., Thorpe, T. and Gripin, H., 1982. Peroxidase: 1970 1980. A survey of their biochemical and physiological role in higher plants University Of Geneva.

Hailstones, M. A. and Smith, M. T., 1988. Lipid peroxidation in relation to declining Vigor of seeds of Soya (Glycine max) and Cabbage (Brassica oleracea). Journal of Plant physiology, 133: 452-453.

Harrington, J. F. and Roberts, E. H. (1972). Seed storage and longevity. In: *Seed Biology* Vol. 3. (T. T. Kozlowiski). New York, pp. 145-245.

Ikediobi, C. O., 1985. Biochemistry and Physiology of yam storage. In Advances in yam Research. Osuji, G. ed. Biochemistry Society of Nigeria, Anambra State University of Science and Technology, Enugu.

Jimenaz, M. and Garcia-Carmona, F., 1995. pH – induced Hysteris of latent Broad Bean polyphenol oxidase: Phytochemistry 40: pp 373-376.

Khan, A. A., 1993. The Physiological and Biochemistry of

seed development, Dormancy and Germination. Elsevier Bio-Medical Press, Amsterdam, New York, pp. 185 –371.

King, M. N. and Robert, E. H., 1979. The storage of recalcitrant seeds: Achievement and possible approaches. IBPGER. Executive Secretariat Rome.

Nkang, A. and Chandler, C., 1986. Changes during embryogenesis in rain forest seeds with orthodox and recalcitrant viability characteristics. Journal of Plant Physiology. 126: 243-256.

Okafor, J. C. and Okolo, H. C., 1974. Potentialities of some indigenous fruits trees of Nigeria. Proc. Fifth Annual Conference Forestry. Association of Nigeria, p. 15.

Okafor, J. C., 1981. Woody Plants of Nutritional Importance in Traditional farming System of the Nigerian Humid Tropics, Ph. D. Thesis, University of Ibadan (unpublished). Pp. 12-20.

Omokaro, D. N., Nkang, A and Nya, P. J., 1999. Effects of Desiccation and Subsequent Dehydration on the germination of Irvingia gabonensis Var. excelsa seeds. Seed Science and Technology. 27: 877-884.

Putter, J., 1974. Peroxidase: In: Methods of Enzymatic Analysis (ed. H. U. Bergmey). Verlay Chemic Meinheim, pp. 685-690.

Smith, M. T. and Adamson, J. H. A., 1989. Volatile lipid peroxidation breakdown products and viability in seeds of lettuce (*Lactrua Sative* L.) south Africa Journal of Science. 85: 63-64.

Street, H. E. and Opik, H., 1977. The physiology flowering plants. 2nd Edition, English Language Book Society (ELBS) and Edward Arnold (Publishers) Ltd., London, pp. 5-28.

Vaughan, K. C. and Duke, S. O., 1984. Functions of Polyphenol oxidase in higher plants. Plant Physiology. 60: 106-112.