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Short Communication

Antioxidant Enzyme Activity during Postharvest Deterioration of Cassava (*Manihot esculenta*) Root Tubers

ENEFE P. NDIDI[1] AND OYERINDE A. AKEEM†
Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Abuja, Nigeria
†*Department of Crop Science, Faculty of Agriculture, University of Abuja, Nigeria*
[1]Corresponding author's e-mail: nd1d1@yahoo.com; oyerindehyphae2002@yahoo.com

ABSTRACT

The antioxidant enzyme activities of superoxide dismutase and the subtypes copper/zinc superoxide dismutase (Cu/ZnSOD) and manganese superoxide dismutase (MnSOD) were studied during the postharvest physiological deterioration of cassava root tubers collected from the University of Benin Teaching and Research Farm. The enzyme activity was analyzed for four days. There was transient increase in the total superoxide dismutase enzyme activity with the peak value being obtained on the first day and thereafter, there was a consistent decline. Apart from the first day, the level of MnSOD was consistently higher than that of Cu/ZnSOD and the decreased level of MnSOD was compensated for by a corresponding increase in Cu/ZnSOD activity in that day, thereby conserving the total superoxide dismutase activity.

Key Words: Cassava; Superoxide dismutase; Antioxidant; Postharvest deterioration

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) also known as Manioc, tapioca or yucca, is a root tuber and an important staple food cultivated in the tropics (De Bruijn & Fresco, 1989). It provides a major source of calories for about 500 million people globally (Cock, 1985). The cassava root tuber is highly perishable and the postharvest shelf life is about 24–48 h (Asaoka *et al*., 1993). Rapid postharvest physiological deterioration of the root tuber, its bulkiness, low protein content and potential toxicity is major limitations of cassava. The potential toxicity of cassava is related to the ability of all parts of the plant to release hydrogen cyanogenic glucosides (linamarin & lotaustralin), a phenomenon known as cyanogenesis (Conn, 1994).

The dangers posed by the production of free radicals in living cells make their rapid elimination very necessary. Cassava root cells possess the ability to synthesize certain molecules known as antioxidants (superoxide dismutase, catalase, peroxidases). These molecules have the ability to trap, mop and inhibit the actions of highly reactive oxygen species (Halliwell, 1994; Foyer, 1994).

Superoxide Dismutase is a naturally occurring antioxidant enzyme, catalyses the disproportion of superoxide anions 0_2- to H_20_2 and 0_2. There are several types of the enzyme based on metal at the active site. They include MnSOD, Cu/ZnSOD and iron superoxide dismutase (FeSOD) (Fridovich, 1995). The Cu/ZnSOD is inhibited by the action of cyanide, possibly by formation of a cyanide metal enzyme complex, while the MnSOD is a cyanide insensitive enzyme (Ysebaert-Vanneste, 1980; Page, 2009).

Plumbey *et al.* (1981) carried out some studies on peroxidase (a known antioxidant) and vascular discoloration in cassava root tissue, while Osagie and Onigbinde (1998) established that cassava roots upon harvest and storage undergo chemical changes. However, there is paucity of data about the actual changes in total superoxide dismutase enzyme activity as well as the activities of the subtypes, Cu/ZnSOD and MnSOD during the early stages of postharvest deterioration of cassava root tuber and this forms the main focus of this work.

MATERIALS AND METHODS

Cassava (*Manihot esculenta* Crantz) root tubers were harvested from the Teaching and Research Farm, University of Benin, Nigeria and the analysis carried out in the Department of Biochemistry University of Benin Nigeria.

Preparation of cassava extracts/reagents: Two cassava tubers were peeled and washed with Ice-cold water then cut into small pieces. A 20 g of the cassava was homogenized with phosphate buffer (pH 7.0) and then the homogenates centrifuged for 15 min at 7000 rpm, while cool. The supernatant containing the crude enzyme extract was used for the enzyme assay. All the reagents used were stored stopper in a refrigerator at 4^{o}C except epinephrine, which was prepared fresh before use.

Enzyme assay: The Total Superoxide Dismutase enzyme activity was determined spectrophotometrically with the method of Fridovich (1995) as adopted by Isamah *et al.*

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(1995). Two ml of carbonate buffer (pH 10.2) was added to 0.2 mL of homogenate and epinephrine. The rate of epinephrine auto-oxidation was observed by monitoring the increase in absorbance at 480 nm every 30 sec for 150 sec. One unit of Superoxide Dismutase activity is the amount of the enzyme required for 50% inhibition of the oxidation of epinephrine to adrenochrome at 480 nm per min.

$$\text{Units/g fresh tissue} = \frac{\%\ \text{inhibition}}{Y} \times \frac{1}{50} \times \frac{1000}{1}$$

Where Y = mg of tissue per ml of reaction medium.

The MnSOD was assayed using the same method as above, except with the addition of NaCN to inhibit Cu/ZnSOD activity. The absorbance was read at 480 nm and the change monitored every 30 sec for 150 sec. The enzyme activity of Cu/ZnSOD was then determined as the difference between Total superoxide dismutase and cyanide–insensitive enzyme activity (Ysebaert–Vanneste, 1980).

Statistics: Data collected was subjected to parametric statistic tools of mean and standard deviation. This was further represented pictorially with a line graph for better comparison of deterioration of cassava root tubers.

RESULTS AND DISCUSSION

The result showed a transient increase in the total SOD activity with the peak value was obtained on the first day and thereafter a continued decline (Fig. 1). The level of MnSOD was consistently higher than that of Cu/ZnSOD (Fig. 2 & 3). However, on first day the decreased level of MnSOD was compensated for by a corresponding increase in Cu/ZnSOD activity (Fig. 2 & 3). Consequently, the total level of SOD activity was preserved.

Considering that Superoxide Dismutase is directly involved in detoxification of 0_2-, measurement of activities of superoxide dismutase and related antioxidative enzymes provide information about the extent to which tissue is exposed to reactive oxygen species. The available evidence clearly suggests that the production of ROS may be a general alarm signal that serves to alert metabolism and gene expression about possible modification (Foyer *et al*., 1994). Free oxygen radicals are important factors involved in the phenomenon of biological aging; a consequence of oxidative stress (Nohl, 1993).

Yu and Rengel (1999), in an earlier work reported that the activities of antioxidative enzymes usually increase in the initial stage of the stress, providing a certain degree of protection from oxidation damage and then decline with the duration of stress due to either reduced synthesis, enhanced degradation or inactivation of the enzymes or all these factors, as observed in the present study. In fact, an increase in Total SOD activity followed by a decrease in the activity in response to drought or high light intensity stress was reported at the initial stage of the stress in wheat species (Mishra *et al*., 1995; Page, 2009).

Fig. 1: Change in the level of Total SOD during post-harvest deterioration of cassava

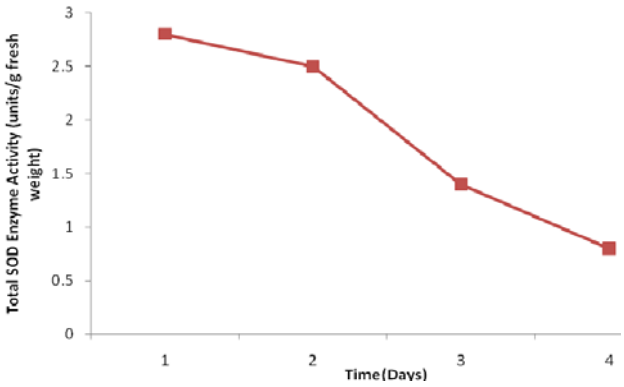


Fig. 2: Change in the level of MnSOD during post-harvest deterioration of cassava

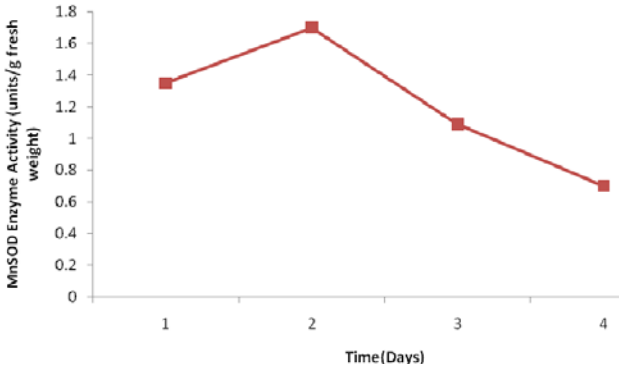
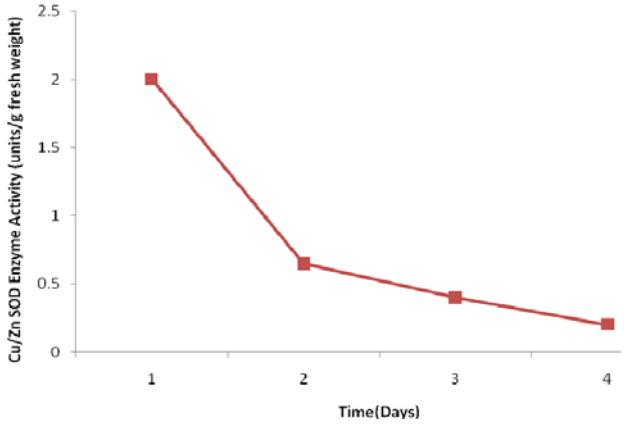


Fig. 3: Change in the level of Cu/ZnSOD during postharvest deterioration of cassava. Results are expressed as mean ± standard error of mean



Apart from the first day, MnSOD was consistently higher than Cu/ZnSOD activity (Fig. 2) despite the fact that MnSOD represents only 5-20% of the Total SOD activity (Ysebaert–Vanneste, 1980). There is no report of FeSOD enzymes in higher plants. Also reciprocal responses were obtained in the activities of CuZn. SOD and MnSOD on the first day (Figs. 2 & 3) such that decrease in one type of

enzymes resulted in compensatory increase in the other. This argues directly for the importance of the shared enzymatic activity of these two proteins and also presents evidence that organisms always tend to maintain homeostasis (Nohl, 1993).

CONCLUSION

This study showed that some enzymes such as lipoxygeneses, ATPases and lipases have possible roles in the postharvest deterioration of cassava root tubers. However, the present study showed the actual changes in the level of the antioxidant enzymes superoxide dismutase and the subtypes; hence their possible role in the postharvest deterioration of cassava roots tubers.

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