

The Role of Rubber Seed Lipoxygenase in the Quality Restoration of Stored Lesser Yam

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Abstract

Partially purified rubber seed lipoxygenase was used to bleach lesser yam tubers (*Dioscorea esculenta*) that were browned by storing the cut yam tubers at room temperature. There was increase in the bleaching of polyphenols extracted from the browned yam tubers as the enzyme concentration and time of the incubation of the enzyme were increased. Moreover, there was increase in the bleaching of the browned yam tubers as the enzyme concentration was increased. The browned yam tubers were completely bleached to their original yellow colour at the enzyme concentration of 15 unit /ml.

Keywords: Rubber seed, lipoxygenase, lesser yam

Introduction

Lipoxygenase is an enzyme that initiates peroxidation in lipids (Tappel *et al.*, 1953). Free radicals formed at the enzyme's substrate (for examples, linoleic acid) during the formation of the enzyme's primary product (trans, cis-conjugated diene hydroperoxide), may abstract hydrogen atoms from easily oxidizable compounds, like carotenoids, in the medium thereby causing initial peroxidation on their structures. The peroxidation of the structures results in the breaking of bonds and destruction of the structures of such compounds (Braverman, 1963; Gurr and Harwood, 1991). According to Mitsuda *et al* (1967), there is a linear relationship between lipoxygenase concentration and the destruction of carotenoids.

Lipoxygenase had been used in food processing and preservation especially in baking industry (Boyer *et al.*, 1963). It was used extensively in bleaching carotenoids in wheat flour to produce white dough (Furia, 1975). The crude enzyme preparation had been used to enhance the rheological properties of dough in bread making and for flavour development in baked foods (Mitsuda *et al.*, 1967; Nanaki and Matsushita, 1980; Davis, 1988). The improvement of the rheological properties is due to the oxidation of gluten in wheat (Davis, 1988).

There is no improved way of storing processed yam. Yam flour, which is one of the two means of storing yam, turns brown when processed, and black, sticky and off-flavour when prepared as yam fufu (Onayemi, 1986); and such yam fufu is an unacceptable type of food by Nigerians. Yam flake, the other form of storing

processed yam, is not popular to West Africans. This is because the flake lacks the viscosity ('draw') of pounded yam (Onwueme, 1978). Moreover, yam flake becomes brown and rancid after 24 weeks of storage (Osagie, 1992).

When yam tubers are cut and exposed to air, they become brown at the cut surfaces. The browning is due to the oxidation of phenolic compounds such as catechol and caffeic acids to their corresponding o-quinones by the enzyme polyphenol oxidase in the presence of oxygen. The o-quinone and the polymerized forms are the brown pigments (polyphenols) seen on such cut plant tissues (Mondy and Mueller, 1977; Burda *et al.*, 1990).

Sulphur dioxide and its derivatives, such as metaspulphite, are used to prevent browning during the processing of food but once browning occurs, they cannot bleach the brownness (Braverman, 1963). Furthermore, they give bad flavour to food and are toxic when used in large quantity. National and international regulations either restricted their usage or banned them altogether when it was discovered that they could cause severe asthmatic disorder (Sharma and Ali, 1980).

Rubber seed lipoxygenase had been used to bleach yam tubers that had been allowed to brown for two days (Anokwulu, 2001). In this work, the use of rubber seed lipoxygenase in bleaching brown lesser yam tubers that were stored for three weeks, to their original yellow colour was investigated.

Materials and Methods

Lesser yam tubers (*Dioscorea esculenta* Lour) were purchased at the local market in Nsukka and identified as *D. esculenta* by the Taxonomical

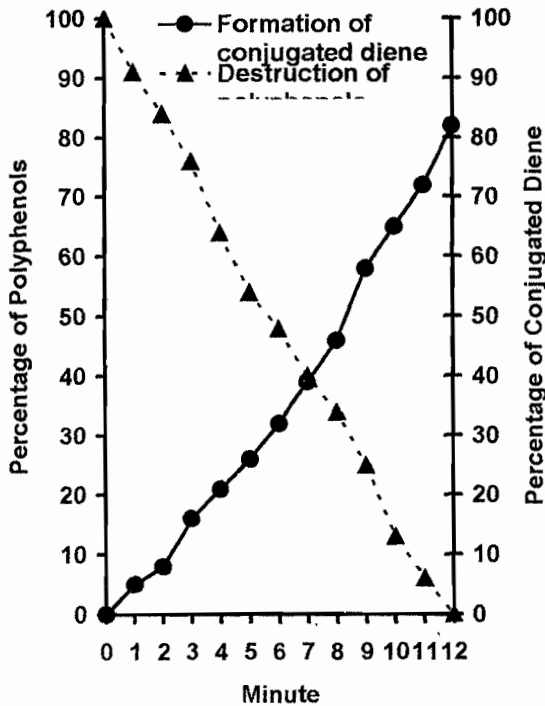


Fig. 1: Reaction of rubber seed lipoxygenase on polyphenols from lesser yam.

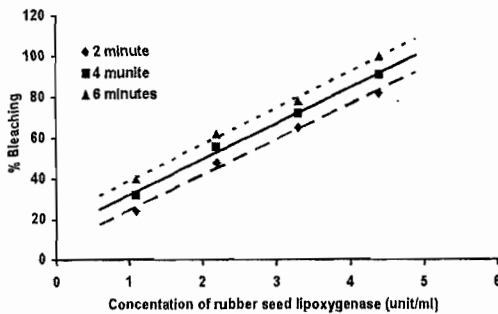


Fig. 2: Effect of rubber seed lipoxygenase concentration on the bleaching of polyphenols.

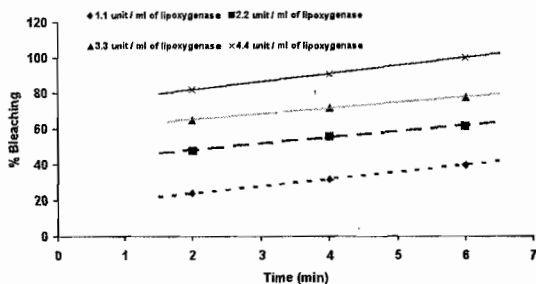


Fig. 3: Effect of time on the bleaching of polyphenols with rubber seed lipoxygenase

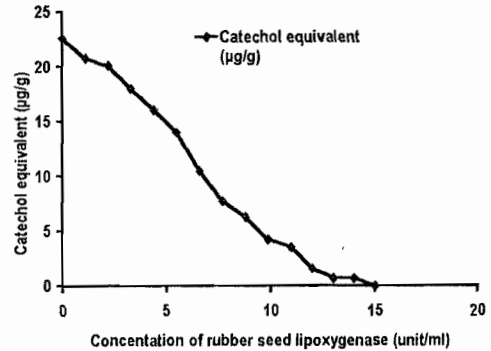


Fig. 4: Polyphenol content in the bleached yam cubes based on catechol equivalent.

polyphenols in browned lesser yam tubers was as described by Martin and Ruberte (1976).

Bleaching of polyphenol extract: Modified method of Tookey *et al* (1958) was used in the bleaching of the polyphenol extract. The substrate was prepared by dissolving 3 ml of the polyphenol extract and 2 ml of linoleic acid in 6 ml of ethyl ether. The mixture was gently warmed to prevent peroxidation. Then 15 ml of warmed ethanol were added to the mixture followed by the addition of 171 ml of 0.05 M borate buffer pH 7.0.

For the formation of conjugated diene from the product of linoleic acid, oxygen gas was passed over the surface of 5 ml of the substrate solution for 5 min. A volume of 1 ml of the enzyme solution was then added to the substrate solution. At 1 min intervals, 2 ml of the mixture of enzyme and substrate solutions were taken and applied to 4 ml of cold absolute ethanol to stop the reaction. A volume of 3 ml of the mixture was read in a spectrophotometer at 234 nm. The blank was the mixture without the enzyme solution.

The assay for the bleaching of polyphenol extract was carried out by also taking 2 ml of the mixture of the enzyme and substrate solutions and discharging the aliquots into a mixture of 5 ml of ethanol and 8 ml of hexane. A volume of 3 ml of the ethanol layer, containing the polyphenols, was read in the spectrophotometer at 420 nm. Controls without the enzyme were also prepared.

Determination of percentage bleaching: The method for determining percentage bleaching was according to Holden (1965). The formula for percentage bleaching was:

$$\text{Percentage bleaching} = \frac{A_0 - A}{A_0} \times 100$$

where A_0 is the untreated polyphenol extract and A is the treated polyphenol extract.

Section of Botany Department, University of Nigeria, Nsukka. Lipoxygenase was extracted from rubber seeds and purified as describe by Anokwulu (2003).

Extraction of polyphenols from browned yams: The method for the extraction of

Bleaching of browned yam: Lesser yam tubers were cut into pieces of about 2 x 2 x 2 cm and stored for three weeks at room temperature. The substrate was prepared by dissolving 2 ml of linoleic acid in 6 ml of ethyl ether and 15 ml of ethanol. Then 171 ml of 0.05 M borate buffer pH 7.0 were added to the mixture. The enzyme solution (0 –14 ml) was added to 45 ml of the substrate solution after the substrate solution was saturated with oxygen gas. The browned yam cubes were then immersed in the mixture of enzyme and substrate solutions. This was followed by shaking the conical flask containing the mixture in a mechanical flask shaker for 1h at room temperature. The yam cubes were then rinsed with distilled water and dried in the Gallenkamp oven at 60 °C.

Standard curve: Catechol was used in constructing the standard curve for the estimation of the concentration of polyphenols in the bleached yam cubes. A volume of 4 ml of a 1:1 volume of 0.2 % solutions of ferric chloride and potassium ferricyanide was added to 1 ml of each of 6 series of dilutions of catechol solution (1.0 to 0.0 ml) prepared. Each of the 6 test tubes was shaken vigorously. The colour was allowed to develop by placing the test tubes in a water bath at 37°C for 10 min. The absorbance of each of the solutions was read at 420 nm and the concentrations of catechol ($\mu\text{g/ml}$) were plotted against the corresponding absorbance at 420 nm.

Results and Discussion

There was increase in the formation of conjugated diene as time increased (Fig. 1). This is similar to the result obtained by Tookey *et al.* (1958) in the destruction of carotene. It could be the free radicals formed during the formation of the primary product of the substrate, linoleic acid, that destroyed the polyphenols. The plot of percentage bleaching against the concentration of rubber seed lipoxygenase is presented in fig. 2. The bleaching of polyphenol extract increase as the enzyme concentration was increased. Moreover, there was increase in the bleaching of polyphenol extract as time increased (Fig 3.). The increase in the bleaching of polyphenols as the enzyme concentration was increased could be due to the increase in the formation of free radicals at the increased enzyme concentration. This increase in the formation of free radicals resulted to increase in the destruction of polyphenols.

The increase in the bleaching of the browned yam cubes as the enzyme concentration

was increased is presented in fig 4. It shows therefore, it was the destruction of the polyphenols that led the bleaching of the browned yam cubes. There was a complete bleaching of the browned yam cubes to yellow ones at the enzyme concentration of 15 unit/ ml. The results show that lipoxygenase could bleach polyphenols. The bleaching of polyphenols in yam cubes resulted in the removal of brown colour that could make browned yam tubers unacceptable as food product and the bleaching of polyphenols could also help in preserving yam flour.

The use of rubber seed lipoxygenase is of economic importance. This is because in Nigeria, only latex from rubber trees is used commercially. According to Ononogbu (1994), there are over 300000 hectares of rubber tree plantations in Nigeria and these rubber trees produce over 120000 tons (12×10^7 kg) of seeds yearly. The rubber seeds, apart from being used for seedlings, are allowed to waste. The use of rubber seed in processing yam is a way of using the rubber seeds, that could have been wasted, for a commercial purpose.

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