Reduction or delay of post-harvest physiological deterioration in cassava roots with higher carotenoid content

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Running title: Post-harvest physiological deterioration and carotenoid in cassava

Abstract

Post-harvest physiological deterioration (PPD) is one of the most important constraints in cassava production and commercialization. It has been hypothesized that the antioxidant properties of carotenoids in yellow cassava roots may help reduce or delay PPD. The industrial sector prefers cassava with a high dry matter content. The latter has also been reported to have a positive correlation with PPD. The objective of this study was to determine the correlation between both the dry matter and total carotenoid contents and PPD in the roots of 101 cassava clones. PPD was positively but weakly associated with dry matter content (R^2 = 0.100, $P < 0.01$), and inversely associated with the total carotenoid content in roots (R^2 = 0.515, $P < 0.01$). In addition, total carotenoid content and color intensity were strongly and positively associated (R^2 = 0.769, $P < 0.01$), suggesting that the roots of cassava clones with a relatively high total carotenoid content can be selected through a simple visual inspection of the color intensity in the parenchyma.

Keywords: cassava root; postharvest physiological deterioration; carotenoid; dry matter; color

INTRODUCTION

Cassava (*Manihot esculenta* Cranzt) is a perennial crop native to tropical America.[1,2] About 70 million people in developing countries obtain more than 500 calories/day from cassava roots.[3,4] Compared with other staple foods, cassava offers the advantage of a flexible harvesting date, allowing farmers to keep the roots in the ground until needed. [5] The crop produces a reasonable yield under adverse climatic and soil conditions and is recognized as being important for food security because of its tolerance to drought, infertile soils, and an ability to recover from disease and pest attacks. Although the starchy root is the primary product, fresh leaves are also used for animal and/or human consumption.

A serious constraint to cassava production is the short shelf life of its roots due to post-harvest physiological deterioration (PPD). PPD begins within 24 hours,[6,7] and rapidly renders the roots unpalatable and unmarketable. Consequently, cassava roots need to be consumed soon after harvesting.[8] The short shelf-life severely limits the marketing options because it increases the likelihood of losses, marketing costs, and access to urban markets is limited to those close to the production sites.

PPD begins with vascular streaking, which is a blue-black discoloration of the xylem parenchyma, followed by general discoloration of the storage parenchyma. Occlusions and tyloses have also been observed in the xylem parenchyma.[9] Five to seven days later microbial activity causes further deterioration. Additionally, respiration is induced[10] resulting in starch hydrolysis.[11]

The processes involved in PPD resemble typical changes associated with the plant's response to wounding that triggers a cascade of biochemical reactions, which are frequently oxidative in nature.[6,10,11] Specific genes involved in PPD have been identified and characterized, and their expressions evaluated.[12-16]

PPD begins 24-48 hours after harvest (at 20-30 °C and 65-80% air relative humidity) but handling and storage conditions of the roots affect its speed and magnitude. Keeping roots at 10 °C and 80% air relative humidity delays the onset of PPD by two weeks. Maintaining roots in controlled atmosphere conditions (different treatments with less that 5% O_2) also delayed the onset of PPD.[17]

Recently, an international initiative that seeks to reduce micronutrient malnutrition using plant breeding to develop staple food crops rich in micronutrients, including provitamin A carotenoids, was initiated. The program, known as HarvestPlus, involves a global alliance of research institutions and implementing agencies in developed and developing countries. The first six focal crops that comprise the staple food for the majority of people in the world who have or are at high-risk of micronutrient deficiencies, are cassava, sweetpotato, maize, rice, wheat, and beans.

Vitamin A is an essential micronutrient for the normal functioning of the visual and immune systems, growth and development, maintenance of epithelial cellular integrity and for reproduction.[18] Improving the vitamin A status of children can reduce mortality rates by 23% to 32%.[18,20] Between 100 and 140 million children are vitamin A deficient.[21] In addition to the direct effect of vitamin A

deficiency, there is growing evidence that vitamin A metabolism interacts with that of iron and zinc thus, improving vitamin A status has benefits beyond vitamin A status alone.[22] Provitamin A carotenoids from vegetables and fruits contribute to two-thirds of dietary vitamin A intake worldwide, and to more than 80% of intake in the developing world.[23]

The genetic variability in the type and amount of carotenoids in cassava has not been extensively studied. Iglesias *et al.*[5] evaluated the total carotenoid content in the roots of 632 clones. A more extensive evaluation has been done by Chávez *et al.*[24] who found the total carotenoid content in roots ranged from 1.02 to 10.40 μg/g fresh tissue. Based on preliminary findings, Chávez and colleagues proposed that the total amount of carotenoids is inversely associated with PPD, implying that carotenoids might reduce or delay the onset of PPD. The objective of this study was, therefore, to further determine the effect of the total carotenoid content in cassava roots on the reduction or delay of PPD. The correlation between the dry matter content and PPD and the association between total carotenoid content and color intensity were also investigated

MATERIALS AND METHODS

The roots of 101 cassava clones from 15 sources were used in this study (**Table 1**). The clones came from one of two categories: those produced in breeding programs at the International Center for Tropical Agriculture (CIAT, Colombia) and those from landraces in the germplasm collection held at CIAT.

Because of the limitations in the number of roots that could be analyzed each day, harvest took place from April 12 to April 28, 2004. A sample of roots was collected each day for carotene quantification and PPD determination, about 11 months after planting.

Sampling procedure and handling

One to three plants per genotype were harvested and their roots selected based on their health status and commercial size. The distal and proximal extremes of each root were cut. The central portion of the root was used for PPD quantification, which was taken individually for each root. The peeled distal and proximal sections of all the roots from a given genotype were pooled together for dry matter content and carotene quantifications, which were made on the pooled root samples. These samples were cut into small pieces and grated.

Post-harvest physiological deterioration (PPD)

The central sections of the roots described above were used for PPD quantification. Measurements were made individually for each root. PPD was determined using the method of Wheatley *et al*.[29] with one modification: prepared roots were stored for 7 days instead of 3 days. Roots were kept in a controlled environment chamber at 25°C and 60-80% relative humidity before PPD quantification. The proximal and distal root ends were removed and covered with clingfilm. After one week, seven 2-cm thick transversal slices were cut along the

root, starting at the proximal end. A score between 1 and 10 was assigned to each slice, corresponding to the percentage of the cut surface showing discoloration (1=10%, 2=20%, etc). The mean PPD score for each root was calculated by averaging the scores of the seven slices.

Total carotenoid concentration

Total carotenoid assays were carried out immediately after harvest at CIAT on the pooled samples from all the roots of each genotype. The grated root samples were blended using a household food processor for extraction.

Carotenoids were extracted following the method of Safo-Katanga *et al.*[25] except that acetone was used together with petroleum ether (35-60^{o}C fraction). The separation between the solid and liquid phases, however was through centrifugation and not by filtration as suggested by Katanga et al. [25] Approximately 5 g of tissue was homogenized for 1 minute with 10 ml acetone:petroleum ether (1:1) using a Polytron homogenizer, followed by centrifugation at 3000 rpm, for 10 minutes, at 10^{o}C to separate the liquid extract from the solid residue. The former was collected and extraction repeated until the residue was colorless (usually three times). The extracts were then combined, water added, and the petroleum ether phase containing the carotenoids separated from the lower aqueous-acetone phase. Quantification was done by visible absorption spectrophotometry using a Beckman DU 640 recording spectrophotometer. Total carotenoid content was

calculated using absorbance at 450 nm and the absorption coefficient of β-carotene in petroleum ether (2592). [26]

Standard deviations for measurements of roots from different plants of the same clone, of different roots from the same plant and of different samples from the same root represented 7.7, 7.0 and 2.8% of the mean carotene concentrations, respectively.[27] In a different study, carotene concentrations in different sections of the roots were found to vary but this variation was small enough to fail reaching statistical significance. [28]

Dry matter content

From the same pooled-sample of roots used for carotene measurement another sub-sample was taken for the quantification of dry matter content. To estimate it, 20-30 g of the chopped and grated fresh roots were dried in an oven at 60°C for 24 h. Dry matter was expressed as the percentage of dry weight relative to fresh weight.

Root color and other measurements

A 1 (white) to 9 (pinkish) scale chart for root color was developed to standardize the visual measurement of the root parenchyma, which can vary from white to cream to yellow to orange and to even pinkish roots. Although alternative and more precise methods for scoring color intensity could be used, this chart was

preferred because it can easily be used in the field when cassava is harvested in isolated areas where no access to laboratories is available.

Statistical analysis

Many PPD values, which are expressed as percentages, fell below 10%; thus, the data were transformed using the Arcsin √ percentage.[30] The associations between the PPD and dry matter content, PPD and total carotenoid content, and total carotenoid content and color intensity were evaluated using linear regression analysis. Different models were considered for each case and the best model selected using the Statistix 8 analytical software.[31] One root with a color score of 8 and a total carotenoid content 4.81 µg/g fresh root was clearly an outlier and excluded from the analysis of color intensity and total carotenoid content.

RESULTS

The mean and standard deviation (SD) dry matter content of the 101 fresh roots was 34.5±5% (range 16.9-45.1%). After one week of storage, the variation in PPD was large and ranged from 0–73% with a mean and SD of 20.1 ± 20.4. The mean and SD total carotenoid content was 2.06±1.79 µg/g fresh root (range 0.20–7.74 µg/g fresh root). Color varied from off white to nearly pink (range 1–8) with a mean and SD intensity of 3.1±1.4 (**Table 2)**. Many roots had a low total carotenoid content reflected in a white parenchyma, resulting in an asymmetrical distribution for total carotenoid concentration in the roots (skewness value = 1.34).

The association between PPD and the dry matter content of roots was significant and positive, although weak (R^2 = 0.100, P < 0.01) as shown in **Figure 1**. PPD values varied considerably at total carotenoid concentrations below 1.0 µg/g fresh root, but tended to peak to a maximum of about 25 % at higher total carotenoid concentrations (**Figure 2**). Regression analysis showed PPD to be negatively and significantly associated with total carotenoid content (R^2 = 0.51, P < 0.01). A significant proportion of the variability in PPD, therefore, could be explained by the total carotenoid content.

Root parenchyma color intensity was correlated with total carotenoid content. The association was exponential with increasing color intensity (R^2 = 0.769, P < 0.01) (**Figure 3**).

DISCUSSION

The average values for PPD and dry matter content are in accord with those observed in other studies.[16,24] The variation in the total carotenoid content of cassava roots observed in this study are also consistent with those reported by Chavez et al.[24] Like van Oirschot *et al*.,[8] we found that PPD was positively and significantly correlated with the dry matter content in the roots, although our association was weak (R^2=0.0995). An important objective in cassava breeding programs is to increase root dry matter content because the buyer pays for starch rather than water and a higher dry matter facilitates drying of roots and the extraction of starch. While our finding related to PPD and dry matter content was

somewhat discouraging, an important finding was the good correlation between the total carotenoid content of the roots and the reduced or delayed PPD after 7 days (R^2 = 0.515). This may be due to the antioxidant property of the carotenoids. Deterioration of cassava roots requires oxyen[7,32,33] and oxidative stress has been shown to be involved in PPD.[15]

The inverse association between total carotenoid concentration and PPD is encouraging because it suggests that cassava roots with higher total carotenoid levels are not only more nutritious, but may also be more marketable because of their reduced or delayed PPD. This increased shelf life may only be one or two additional days and would not overcome the serious problem of marketing cassava roots. Nevertheless, it may still encourage farmers to grow yellow rooted pro-vitamin A cassava clones and should be pursued, particularly where cassava is used for human consumption

The implications of these findings for the genetic improvement of cassava are important. In addition to the nutrition benefit for human populations dependent on this crop, yellow pro-vitamin A cassava roots are of higher value to the animal feed industry. Carotenoid content and dry matter content are independently inherited. A higher dry matter is particularly desirable for the animal feed and starch industries. Among people who eat boiled roots, preference is for varieties that have intermediate levels of dry matter. [34] It is specifically the market for fresh cassava destined for human consumption that faces the problems of PPD and

marketability; consequently any reduction or delay in PPD will be of most benefit to this market.

The starch industry is unlikely to favor yellow cassava because many end-users require a white product that could not be obtained from yellow roots. The animal feed industry, in contrast, may benefit from yellow cassava roots because the reduction in PPD may help neutralize the faster deterioration expected from the high dry matter roots that this industry require.

The association between the total carotenoid content in the roots and color intensity is of practical relevance. Adequate laboratory facilities for quantifying carotenoids are unavailable in many developing countries, particularly where cassava is an important human food crop. The positive correlation between color intensity and total carotenoid content indicates that simple screening based on visual scoring of color is adequate to initially select clones with a high total carotenoid content in their roots. Because of the exponential nature of this association, efforts should be directed at improving the color chart on which the color intensity score was based, particularly for scores ranging from 4 to 8.

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Table 1. Summary of the origin of the 101 cassava clones included in the present study

Colombia	31	Argentina	4	Guatemala	1
Brazil	27	Costa Rica	2	Mexico	1
CIAT - FS[a]	15	Indonesia	2	Panama	1
CIAT-HS[a]	6	Malaysia	2	Thailand	1
Peru	6	Ecuador	1	Venezuela	1

[a] CIAT-FS and CIAT-HS refer to improved clones derived from full-sib and half-sib families, respectively.

Table 2. Characteristics of roots from 101 cassava clones.

	Color intensity (Scale 1-9)	Carotene content (µg/g fresh root)	Post-harvest physiological deterioration (%)	Dry matter content (%)
Mean (SD)	3.1 (1.4)	2.1 (1.8)	20.1 (20.4)	34.5 (5.0)
Range	1–8	0.2–7.7	0–73.1	16.9–45.1

SD = standard deviation

Figure 1. Association between PPD and dry matter content based on roots from 101 cassava clones. The regression analysis was based on PPD data transformed by Arcsin √ percentage function (standard error for regression coefficient = 0.298)

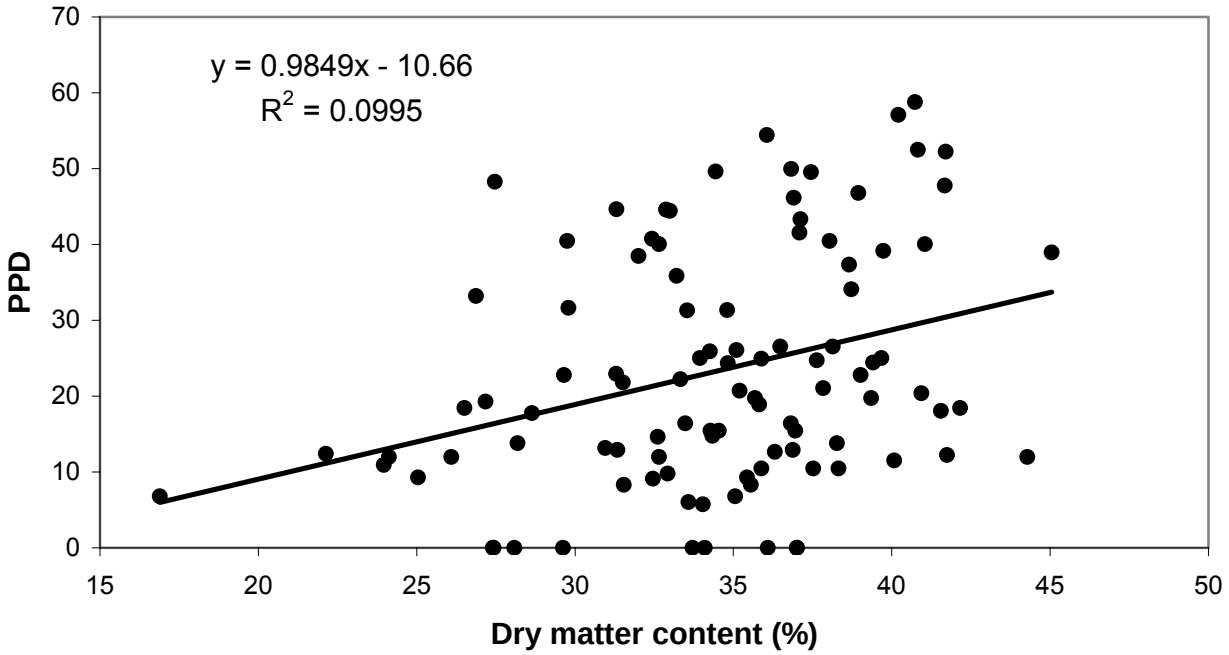

Figure 2. Association between PPD and total carotenoid content based on roots from 101 cassava clones. The regression analysis was based on PPD data transformed by Arcsin √ percentage function (standard error for regression coefficient = 1.136)

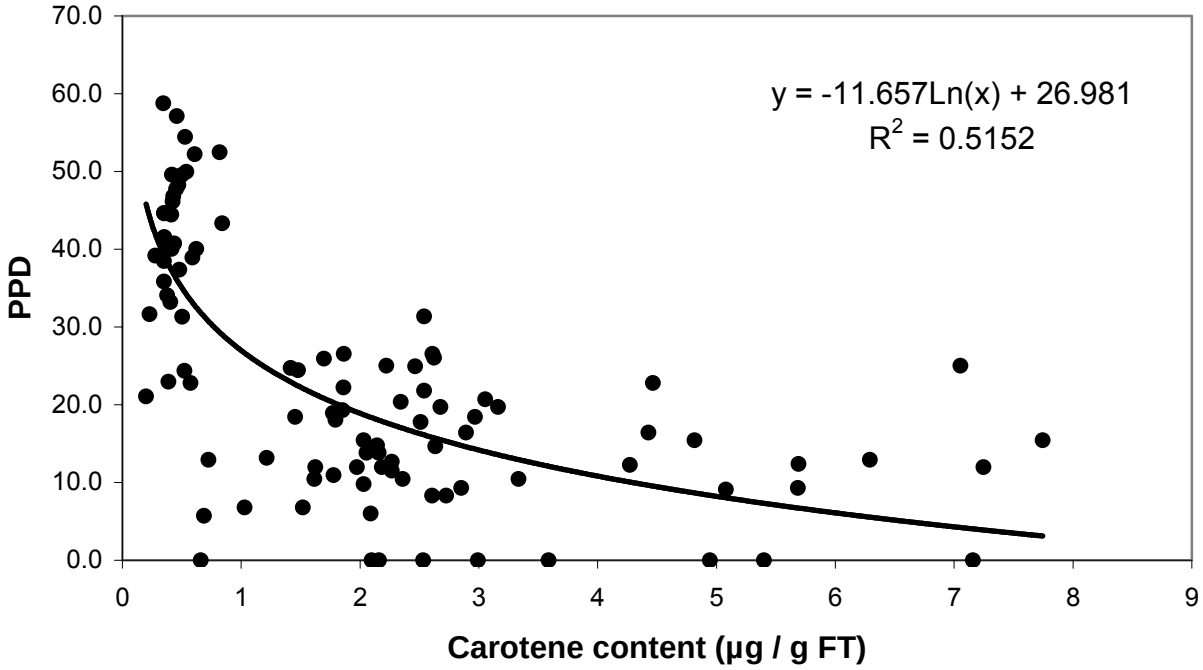

Figure 3. Association between total carotenoid content and color intensity in roots from 101 cassava clones (standard error of regression coefficient = 0.03).