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Effect of Prolonged Postharvest Physiological Deterioration on the Color Hues of Cassava Dried Chips and Flour

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Abstract

Off colors that accrue from oxidative postharvest physiological deterioration (PPD) of stored cassava tuberous roots pose a serious limitation to its large scale processing. Twelve white fleshed cassava genotypes were used to evaluate the effect of prolonged PPD on the color hues of cassava flour and dried chips produced from them at 1st, 7th and 9th day of storage. After relevant chemical/biochemical analyses (moisture, carotene) of the fresh and stored cassava roots, sensory analyses that used a color scale of 1(tan) to 10(white) was used to analyze the obtained color hues of the experimental dry feeding materials. The results showed that the freshly harvested roots had 0.89-1.75µg/g carotene and 31.68-42.37g/100g dry matter contents. Percentage retention of the carotene content at 7th and 9th day of root storage was 45.49-80.00% and 29.15-56.70% respectively. The color scores of cassava flour samples from these genotype were 5.4 to 7.6 on first day of storage, and 4.0 to 5.9 on 9th day of storage with the 'low cyanide' TMS 4(2)1425 cultivar maintaining appreciable good color stability. On the 9th day of storage, dried chips from TMS 4(2)1425 cultivar had a color score of 6.7 while others had color scores of 3.0 to 5.0. It could, however, be concluded that more work need to be done to deduce the reason why this low cyanide cassava cultivar gave the best product color hues more so, as cyanide is known to inhibit the mitochondrial oxidative electron transport chain that could have assisted in suppressing the oxidative PPD.

1. Introduction

Cassava (*Manihot esculenta* Crantz) belongs to the family *Euphorbiaceae*, and is extensively cultivated as an annual crop in tropical and subtropical regions of the world mainly for its edible starchy tuberous roots (Ugwu and Ukpabi, 2002; Burrell, 2003; Lokko *et al*, 2007; Ukpabi, 2008). Cassava ranks very high among crops that convert the greatest amount of solar energy into carbohydrate per unit geographic area, and is the cheapest source of calories for both human nutrition and animal feeding (Burrell, 2003). The tuberous starchy roots of cassava are presently being used for large scale production of dried cassava chips and flour in Nigeria (Taiwo, 2006; Ukpabi, 2009). Adequate cassava processing is known to detoxify the poisonous cyanogenic principles (linamarin, lotastraulin, cyanohydrin and free HCN) in cassava (Westby, 2002; Cardoso *et al*, 2005;

Hongbete *et al.*, 2009; Montagnae *et al.*, 2009). However, a serious limitation to large scale utilization of the crop is the poor shelf life of the tuberous roots. Freshly harvested roots from most cassava genotypes have only short storage life period of few days (Beeching *et al.*, 2002; Reilly *et al.*, 2003). The poor storability of these cassava roots is largely caused by a physiological disorder (vascular streaking or blue-black vascular discoloration) that is normally followed by microbial spoilage. This spoilage activity in newly harvested cassava is also known as Postharvest Physiological Deterioration (PPD) phenomenon (Wenham, 1995; Beeching *et al.*, 1997).

The oxidative vascular streaking and discoloration associated with postharvest physiological deterioration (PPD) have been observed to start as early as 48 hours after harvesting of the roots (Reilly *et al.*, 2003). However, Montaldo (1973) had earlier showed that the initial faint bluish streaks became brownish or blackish after 7 days of harvest; with a considerable variation of rate of severity amongst cassava varieties or genotypes as was also recorded by Sánchez *et al.* (2006) and Morante *et al.* (2010).

Beeching *et al.* (2002) and Reilly *et al.* (2003) observed that the abiotic stress response of cassava roots at harvest led to a flux of wound healing metabolic activities, with enhanced peroxidase, catalase, phenylalanine ammonia lyase (PAL) and polyphenol oxidase activities. These increased biochemical activities enhance the biosynthesis of secondary metabolites that give off colors (Beeching *et al.*, 2002; Reilly *et al.*, 2003). Though scopoletin was found as the major accumulating polyphenol in cassava PPD, the phenolic compounds in cassava roots include scopolin, esculin, proanthocyanidins, (+)-catechin, (+) gallicocatechin and rutin (Buschmann, *et al.*, 2000; Beeching *et al.*, 2002; Reilly *et al.*, 2003; Montagnae *et al.* 2009;)

Antioxidants that include carotenes are considered capable of delaying PPD in cassava with resultant auto degradation or auto oxidation of the antioxidants (Tumuhimbise *et al.*, 2010; Ukpabi *et al.*, 2014). However, Zidenga *et al.* (2012) showed evidence of causal link between the biosynthetic cyanogenesis in cassava root and the onset of the oxidative burst that triggers PPD in cassava. They therefore, opined that cassava root shelf life can be extended via reduction of reactive oxygen species (ROS) through manipulating the cassava cyanogenesis pathway to reduce the cyanide content of the crop. This is based on the fact that cyanide as an inhibitor of the respiratory or oxidative electron transport chain in the mitochondrion (Lehninger, 2008) helps in the accumulation of ROS required for cassava PPD.

There is therefore, every need to investigate the changes in the carotene content (as an antioxidant marker) during prolonged post-harvest physiological deterioration (PPD) of improved cassava genotypes and the resultant effect on the color of their storable dry products that may be used as feeding materials. This is especially so as the color hues of products could affect their marketability.

2. Materials and Methods

2.1. Source of Materials

The experimental 12 cassava genotypes ((TMS 99/6012, TMS 92/0326, TMS 92B/0061, TMS 97/4763, TMS 4(2)1425, TMS 96/1632, TMS 97/0211, TMS M98/0040, TMS 91/02324, TMS 98/2226, TMS 98/2101, TMS 30572.) that are resistant to cassava mosaic disease and have minimal rotting (of cut roots) were originally developed at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and planted at the experimental plots of Cassava Program, National Root Crops Research Institute (NRCRI), Umudike, Nigeria (05° 29' N Latitude, 07° 33' E Longitude). The experimental tuberous roots were randomly harvested at 12 months after planting with the low cyanide TMS 4(2)1425 cultivar serving as a control. The analytical chemicals and reagents used during the study were manufactured by BDH, Poole, United Kingdom.

2.2. Postharvest Physiological Deterioration (PPD) Quantification

Unbroken Cassava roots were harvested for each of the experimental genotype and stored in heaps (of 10 kg each) on the floor of a room in the Biochemistry Laboratory Block of NRCRI, Umudike, Nigeria for a nine-day storage period. The ambient room conditions during the storage period were 26-32°C and 80-87% relative humidity (R.H.). Three roots were randomly selected from each genotype and cut transversely (seven 2 cm thick slices) to observe the level of discoloration of the root cortex. A scale of 1-10 was used to assess for potential to delay postharvest physiological deterioration at the seventh day of storage with 1=10% blue-black discoloration and 10=100% blue-black discoloration. In another experiment, the proximal and distal ends of the roots were cut off, with the distal end covered with cling film. At the seventh day, seven transversal slices, 2 cm thick were cut along the root starting from the proximal end. The same scale as above was used for assessment. Furthermore, the proximal and distal ends of the experimental roots were also cut off, with the distal end covered with Vaseline petroleum jelly. Seven transversal slices, 2 cm thick were also cut along the root starting from the proximal end and similar color assessment carried out as above.

2.3. Carotene Content of the Roots

The carotene content of the cassava flour samples was determined in triplicates using the HarvestPlus spectrophotometric method (Rodriguez-Amaya and Kimura, 2004). Acetone and petroleum ether were sequentially used as the extraction solvents (with light exclusion). The readings with the spectrophotometer (Jenway 6406, England) were done at the wavelength of 450 nm with 1 cm glass cuvette.

The carotene content was calculated as follows:

$$\text{Carotene content } (\mu\text{g/g}) = \frac{A \times V \times \text{DF} \times 10^4}{A_c \times \text{Sample Weight (g)}}$$

Where A = absorbance

V = Volume of extract

DF = Dilution factor

10⁴ = constant

A_c = Absorption coefficient of β-carotene in petroleum ether (2592)

2.4. Dry Matter Content of the Roots

Chopped root samples of about 1cm³ were used for the dry matter determinations as described by Bainbridge *et al* (1996). Replicated 5 g chopped fresh roots were dried in an oven (Gallenkamp, BS model Ov-160) at 105°C for 4 h. Dry matter content was expressed as the percentage of dry weight relative to fresh or wet weight.

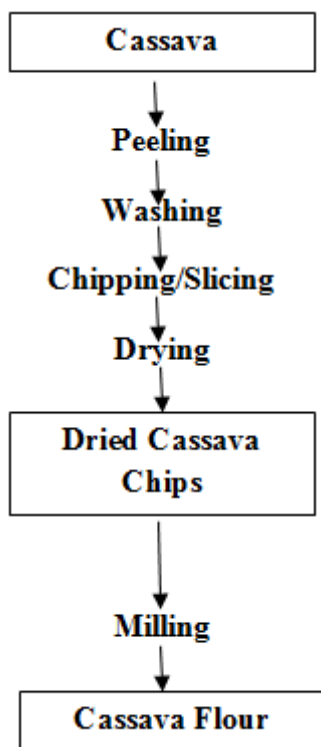


Figure 1. Flowchart for the production of the experimental cassava dried chips and flour.

2.5. Production of Cassava Dried Chips and Flour Samples

The experimental cassava roots stored at different periods (1st, 7th and 9th day) were used to produce dried cassava chips and flour samples. The unit and sub-unit operations employed included peeling, washing, dicing and oven-drying (70^oC) as shown in Figure 1. The peeling of the fresh cassava roots was done manually with a sharp kitchen knife while washing was also done manually with clean water. The chipping of the peeled roots (to about 2mm thickness) was done with a chipping machine (Crypto Peerless, Birmingham, England).

The wet chips were effectively dried to brittleness (with moisture content of <10%) in an electric hot air, thermo-regulated oven (Gallenkamp, BS model Ov-160). Milling of the dry cassava chips was done with a single disc attrition mill (A446A model) while a 250µm mesh sieve was used to get the fine flour for each experimental cassava variety.

2.6. Sensory Evaluation for Products Color Hues

Seven trained sensory assessors were used to evaluate the color of the cassava dried chips and cassava flour. The assessors noted the colors of the dried cassava chips and cassava flour using a sliding scale of 0-10 to score the products. In the sliding scale, 10 represented white while 0 represented brown (tan).

2.7. Statistical Analysis

Statistics Analysis System (SAS)/PC software (Lincence site 0022206002) of International Institute of Tropical Agriculture, Ibadan, Nigeria was used for mean separations and standard deviations of data. The copyright of the system belongs to SAS Institute Incorporated, Cary, North Carolina, U.S.A.

3. Results and Discussion

Table 1. The experimental cassava genotypes with early and delayed vascular streaking or discoloration*.

Early vascular discoloration (Score =6 to 10)*	Delayed vascular discoloration (Score= 1 to 5)*
TMS 99/6012	TMS 4(2)1425
TMS 92/0326	TMS 96/1632
TMS 92B/0061	TMS 97/0211
TMS 97/4763	TMS M98/0040
	TMS 91/02324
	TMS 98/2226,
	TMS 98/2101

Using a color sliding scale of 0-10, where 1=10% blue-black discoloration and 10=100% blue-black discoloration.

The observed cassava genotypes that had early and delayed vascular streaking for uncut or whole harvested roots are shown in Table 1. Though cutting the roots enhances PPD, whole root approach for PPD quantification in Table 1 was used, as Morante *et al.* (2010) had suggested using the farmers’ and food processors’ storage method in order to get better advisory results for cassava end users. However, the employed subjective visual scores need to be improved upon by the use of relevant laboratory equipment, especially as the locally popular TMS 30572 (scoring between 5 and 6) could not be justifiably classified as a genotype with either early or delayed vascular streaking in Table 1. Visual observation during the study also showed that amongst the cut roots (covered with plastic film), TMS M98/0040 genotype was least susceptible (at 9th day) to discoloration due to vascular streaking while TMS 99/6012 genotype was the most

susceptible (at the 7th day). However, cut roots of TMS 4(2)1425 covered with the petroleum jelly showed minimal vascular streaking even at the 9th day of storage. Vaseline (petroleum jelly) and cling film used in covering the cut experimental roots were meant to limit systematic oxidation. Presently, all the experimental genotypes have been officially released to Nigerian farmers for cultivation (IITA, 2010) and it is important to note that all the genotypes with delayed vascular streaking also had delayed rotting during this study.

Table 2. Carotene content of the cassava roots during the days of storage.

Genotype	Carotene content (µg/g) during storage*		
	DAY 1	DAY 7	DAY 9
TMS M98/0040	1.00 ^a	0.65 ^b	0.44 ^c
TMS 92/0326	1.13 ^a	0.51 ^b	0.48 ^c
TMS 99/6012	1.27 ^a	0.83 ^b	0.37 ^c
TMS 91/02324	0.93 ^a	0.69 ^b	0.53 ^c
TMS 98/2101	1.64 ^a	0.82 ^b	0.59 ^c
TMS 30572	1.73 ^a	1.09 ^b	0.84 ^c
TMS 97/0211	1.75 ^a	1.12 ^b	0.89 ^c
TMS 92B/0061	1.48 ^a	0.87 ^b	0.57 ^c
TMS 96/1632	1.72 ^a	0.93 ^b	0.71 ^c
TMS 98/2226	0.95 ^a	0.76 ^b	0.43 ^c
TMS 97/4763	0.89 ^a	0.61 ^b	0.35 ^c
TMS 4(2)1425	1.31 ^a	0.84 ^b	0.51 ^c

*Values in a row with different letters are significantly different (P=0.05)

Table 3. Postharvest carotene retention in the experimental cassava roots.

Genotype	Carotene content (%) during storage*		
	DAY 1	DAY 7	DAY 9
TMS M98/0040	100.00 ^a	65.00 ^b	44.40 ^c
TMS 92/0326	100.00 ^a	45.49 ^b	42.30 ^c
TMS 99/6012	100.00 ^a	65.40 ^b	29.15 ^c
TMS 91/02324	100.00 ^a	74.51 ^b	56.70 ^c
TMS 98/2101	100.00 ^a	50.15 ^b	36.18 ^c
TMS 30572	100.00 ^a	63.19 ^b	48.81 ^c
TMS 97/0211	100.00 ^a	64.11 ^b	50.86 ^c
TMS 92B/0061	100.00 ^a	59.17 ^b	38.59 ^c
TMS 96/1632	100.00 ^a	53.74 ^b	41.14 ^c
TMS 98/2226	100.00 ^a	80.00 ^b	44.74 ^c
TMS 97/4763	100.00 ^a	68.61 ^b	39.24 ^c
TMS 4(2)1425	100.00 ^a	64.14 ^b	39.22 ^c

*Values in a row with different letters are significantly different (P=0.05)

The effect of PPD on carotene levels in the stored cassava roots is shown in Tables 2 and 3. The rapid decline of carotene content of the experimental cassava roots was remarkably different from those observed in stored white Guinea yam (*Dioscorea rotundata*) by Ikediobi and Oti (1983). Ikediobi and Oti (1983) found that the carotenoid content of stored white yam remained fairly stable after one month of barn-storage at ambient temperature in Nigeria. It might be necessary to suggest the possibility of fully identifying how the genes responsible for yam wound healing mechanism differ from that of harvested cassava roots. Though extensive work had been initiated on the genetic mapping of the genes responsible for cassava PPD (Iglesias

et al.,1997), there is also a need to consider the role of cassava acyanogenesis in delaying PPD (Zidenga *et al.*, 2012). This is more so as the storable white Guinea yam has no cyanogenic toxicity and Siritunga and Sayre (2003) have demonstrated the feasibility of generating cyanogen free transgenic cassava. The effective use of this biotechnology tool could lead to better storage life for cassava roots at ambient conditions in Nigeria.

In Nigeria, cassava roots for industrial use are recommended to have dry matter content of at least 30%. Table 4 shows that all the roots of the experimental genotypes (at harvest in Umudike, Nigeria) exceeded this figure. The experimental roots as respiring living plant tissues had varying dry matter content during the storage period possibly due to respiratory moisture loss (Lehninger, 2008) and the effect of the warm and humid ambient storage conditions (26-32^oC, 80-87% R.H).

Table 4. Dry matter content of the cassava roots during the days of storage.

Genotype	Dry matter content (%)*		
	DAY 1	DAY 7	DAY 9
TMS M98/0040	39.88 ^b	40.64 ^{a,b}	41.17 ^a
TMS 92/0326	35.85 ^b	41.20 ^a	42.13 ^a
TMS 99/6012	35.88 ^b	42.40 ^a	43.03 ^a
TMS 91/02324	37.96 ^b	40.63 ^a	35.91 ^a
TMS 98/2101	42.37 ^b	37.28 ^c	45.56 ^a
TMS 30572	34.87 ^b	37.90 ^a	33.29 ^b
TMS 97/0211	39.65 ^a	35.01 ^b	36.65 ^b
TMS 92B/0061	36.42 ^b	40.87 ^a	38.20 ^a
TMS 96/1632	31.68 ^c	33.64 ^b	41.31 ^a
TMS 98/2226	37.96 ^a	35.62 ^b	37.39 ^a
TMS 97/4763	31.68 ^b	35.82 ^a	36.90 ^a
TMS 4(2)1425	36.34 ^b	42.26 ^a	40.02 ^a

*Values in a column with the same letter are not significantly different (P=0.05)

The color characteristics of the experimental cassava dried chips and flour are shown in Table 5. The color ratings of the produced dried chips and flour of TMS 4(2)1425 were similar at 1st, 7th and 9th days of storage (Table 5). As remarked earlier, more research work needs to be done to deduce the reason why this low cyanide cassava cultivar (<5 mg HCN/100 g root) that generally has relatively low cyanide content in Nigeria (Okpara *et al.*, 2014; FAO, 2015) gave the best product color hues especially as cyanide is known to inhibit the mitochondrial oxidative electron transport chain that could have assisted in suppressing the oxidative PPD in cassava. However, all these seem to support the idea that systematic acyanogenic condition suppresses the oxidative burst at the onset of PPD in cassava (Zidenga *et al.*, 2012).

Generally, there were varietal effect on color of the dried chips and flour produced from the roots that underwent PPD in this study (Table 5). It should also be noted that enzymatic and non-enzymatic 'browning' with varying intensities amongst varieties, also contribute to discoloration of processed tuberous roots such as cassava (Hongbete *et al.*,

2009; Okaka and Okaka, 2001). Therefore, future research work with these experimental cassava genotypes would determine the levels of cyanogens and phenolics in their roots in order to properly elucidate research results.

Table 5. Color characteristics of the experimental fresh cassava roots, dried chips and flour samples*.

Genotype	Day of storage	Fresh root color score	Dried chips color score	Flour color score
TMS 30572	1	Creamy white	5.3	5.6
TMS 30572	7		4.1	4.7
TMS 30572	9		4.1	4.7
TMS 4(2)1425	1	White	7.1	7.1
TMS 4(2)1425	7		7.0	6.3
TMS 4(2)1425	9		6.7	5.9
TMS 91/02324	1	White	7.4	7.6
TMS 91/02324	7		4.9	7.3
TMS 91/02324	9		4.7	5.1
TMS 92B/0061	1	White	5.7	5.9
TMS 92B/0061	7		5.7	5.0
TMS 92B/0061	9		4.6	5.0
TMS 92/0326	1	White	6.0	7.4
TMS 92/0326	7		4.0	4.0
TMS 92/0326	9		3.0	4.0
TMS 96/1632	1	White	5.1	7.1
TMS 96/1632	7		3.4	5.3
TMS 96/1632	9		3.3	4.4
TMS 97/0211	1	Creamy white	6.1	5.4
TMS 97/0211	7		4.7	4.4
TMS 97/0211	9		4.7	4.1
TMS 97/4763	1	White	5.6	7.0
TMS 97/4763	7		4.0	4.7
TMS 97/4763	9		3.7	4.4
TMS 98/2101	1	Creamy white	6.9	6.7
TMS 98/2101	7		5.4	5.7
TMS 98/2101	9		5.0	4.7
TMS 98/2226	1	White	5.6	7.4
TMS 98/2226	7		4.1	5.3
TMS 98/2226	9		4.3	5.1
TMS 99/6012	1	White	7.1	7.4
TMS 99/6012	7		5.1	4.9
TMS 99/6012	9		4.1	4.1
TMS M98/0040	1	Cream	6.2	7.4
TMS M98/0040	7		5.1	5.3
TMS M98/0040	9		4.0	5.3
		SE	0.20	0.31

*Using a color sliding scale of 0-10, where 0 = tan/brown and 10 = white.

4. Conclusion

This study showed a pronounced varietal effect on the effect of prolonged PPD on color hues of the experimental dry cassava products. Therefore, to make for prolonged storage of cassava roots for large scale processing of cassava dried chips and cassava flour, especially for livestock feed production, there may be need to develop transgenic cassava varieties that will have minimal PPD reactions. In the interim, the potential of low cyanide TMS 4(2)1425 roots in maintaining dry product color stability could be exploited by

scientists, cassava agro industrialists and other end users.

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