Vitamin C and pro-vitamin A contents of wholesome cassava flour as affected by short period pre-processing storage of fresh cassava roots

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Abstract

The rapid oxidative postharvest physiological deterioration of cassava storage root is a serious limitation to its large scale processing into cassava flour. Freshly harvested roots from TME 419 (white fleshed), TMS 30572 (creamy white fleshed) and UMUCASS 38 (yellow fleshed) cassava varieties were used to determine the vitamin C (ascorbic acid) and pro-vitamin A (carotene) contents of produced wholesome cassava flour as affected by short period (0-2 days) pre-processing storage at ambient room conditions (26-30°C, 82-94% relative humidity). The storage effect on the bulk density and colour of the flour samples were also determined. Results showed that the wholesome (<10mg/kg cyanide) cassava flour samples from the storage roots stored for two days had significant (P<0.05) reduction (10.08-33.96%) in the antioxidative carotene content of the flour samples from non stored roots (0.53-14.35µg/g). The reduction rate (25.00-37.50%) for the ascorbic acid content of the flour samples from non stored roots (0.08-0.10mg/g) was not significantly (P<0.05) different from the whitish flour samples from TME 419 variety (unlike those of TMS 30572 and UMUCASS 38 varieties) even after two days of storage. Though the short period cassava root storage did not significantly (P<0.05) affect the packed bulk density (0.63-0.67 g/cm³) of the whitish flour samples, noticeable colour changes were observed amongst some of the flour samples. It could be deduced that wholesome cassava flour production from yellow cassava (for appreciable pro-vitamin A content) should, as much as possible, be prepared from freshly harvested roots.

Keywords : Vitamin C, pro-vitamin A, cassava root, pre-processing storage and Cassava flour

Introduction

The tuberous starchy roots of cassava (*Manihot esculenta*) are presently being used for large scale production of cassava flour in Nigeria (Taiwo, 2006 and Ukpabi, 2009). The tuberous cassava roots, like potato (*Solanum tuberosum*) and yam (*Dioscorea* species) tubers have an appreciable quantity of the nutritionally important ascorbic acid or vitamin C (Chávez et al., 2000; Okaka and Okaka 2001; Bradbury and Singh, 2006). However, unlike the yam and potato tubers that have an appreciable postharvest storage life that runs into weeks or few months (Okaka and Okaka, 2001), freshly harvested cassava roots have only short storage life period of a few days (Ejiofor and Ohambele, 1997; 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 (Noon and Booth, 1977; Rickard et al., 1979; Wenham, 1995; Ejiofor and
Ohambele, 1997; Reilly et al., 2003). This spoilage activity in newly harvested cassava is also known as Post-harvest Physiological Deterioration (PPD) phenolmenon (Wenham, 1995; Beeching et al., 1997). Observed PPD of cassava roots within 48-72 hours of harvest (Wenham, 1995; Ejiofor and Ohambele, 1997) has been identified as a serious limitation to large scale processing of cassava (Reilly et al., 2003) into wholesome secondary food products such as cassava flour (Westby, 2002). Local producers of high grade cassava flour (largely from white fleshed cassava roots) known as the High Quality Cassava Flour (HQCF) in Nigeria are currently advised to process their cassava roots strictly within one day of harvesting them.

Recently, scientists in National Root Crops Research Institute (NRCRI), Umudike, Nigeria and International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria have developed and released (to local farmers in Nigeria) yellow cassava roots that have considerable levels (on dry matter basis) of vitamin C ($\approx 1.00$mg/g) and pro-vitamin A ($\approx 25$µg/g) substances in their fresh roots (Ukpabi et al., 2014). Earlier experimentation with freshly harvested cassava roots showed that these micronutrients with vitamin activity can be retained to a limited extent in wholesome (with innocuous cyanide level) cassava flour processed with intermediate technology (Ukpabi et al., 2014). Adequate processing of the crop, that involves the hydrolytic activity of the endogenous glucosidase or linamarase enzyme helps to drastically reduce the anti-nutritional and toxic cyanogenic glucosides (linamarin and lotaustralin) in cassava roots to innocuous levels (Nambisan and Sundaresan, 1991; Bokanga, 1995; Ukpabi, 2008).

Presently, there is a dearth of information on the vitamin C and pro-vitamin A contents of freshly processed wholesome cassava flour made from cassava roots that had a short-term pre-processing storage period of few days. Therefore, this study was aimed at determining the effect of few days’ post harvest storage of matured cassava roots on the ascorbic acid (vitamin C) and carotene (pro-vitamin A) contents of wholesome cassava flour with nutritionally safe level of cyanide. It is believed that the result of this study would be of immense benefit to industrial food processors and research food chemists/biochemists in cassava producing regions of the world.

**Materials and methods**

**Sources of materials**

Fresh storage roots from white fleshed TME 419, creamy white fleshed TMS 30572 and yellow fleshed UMUCASS 38 cassava varieties used in this study were randomly harvested at 12 months after planting from the experimental plots of Cassava Programme, NRCRI, Umudike, Nigeria ($05^\circ 29'$ N Latitude, $07^\circ 33'$ E Longitude). The experimental cassava flour samples were produced from fresh and stored (1-2 days) roots of the experimental cassava varieties. These experimental roots were stored in a room at the Biochemistry Division of NRCRI, Umudike, Nigeria under ambient room conditions ($26-30^\circ$C, $82-94%$ relative humidity.). The analytical chemicals and reagents used for the experimental chemical analyses were manufactured by BDH (British Drug Houses), Poole, England.

**Processing of cassava flour samples**

The experimental cassava flour samples were prepared with the fresh and stored tuberous roots in a dark food laboratory (with windows covered with dark curtains) using the modified method of Ukpabi (2008) as depicted by the unit and sub-unit operations shown in Fig. – 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 grating was, however, done mechanically with a grater (a field marshal model with 7.5Horse power diesel engine). Dewatering of the grated cassava pulp (bagged in sacks) was done with a screw press. The pulverized
Dewatered cassava mash was effectively dried at 60-65°C 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 mash was done with a single disc attrition mill (A446A model) while the 250μm mesh sieve was used to get the fine flour for each experimental cassava variety (stored and non-stored). Each experimental flour sample was packaged in sealed black polyethylene bags prior to chemical (carotene, ascorbic acid and cyanide) and physical (colour and bulk density) analyses.

**Carotene determination**

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), while the readings with the spectrophotometer (Jenway 6406, England) was done at ƛ450 nm with 1 cm glass cuvette.

The carotene content was calculated as follows:

\[
\text{Carotene content (µg/g)} = \frac{Ax V x DF x 10^4}{A_c x \text{Sample Weight (g)}}
\]

Where:
- \(A\) = absorbance
- \(V\) = Volume of extract
- \(DF\) = Dilution factor
- \(10^4\) = constant
- \(A_c\) = Absorption coefficient of β-carotene in petroleum ether (2592)

**Ascorbic acid determination**

The ascorbic acid (vitamin C) content of the experimental flour samples was determined in triplicates using the titration method as described by James (1995) using 2,6-dichlorophenolindophenol (DCIP) as an indicator to get the titre values (at 15 seconds persistent pinkish end point). Freshly prepared standard ascorbic acid solution was used to calculate equivalent to 1ml of the DCIP dye solution.

**Cyanide determination**

The total cyanide content of the flour samples was also determined in triplicates by the colorimetric Alkaline Picrate method of Ikediobi et al. (1980) as described by Onwuka (2005). The yellowish alkaline picrate was prepared by dissolving 1g picric acid and 5g sodium carbonate in distilled water. The liquid filtrate (1.0ml) from the cyanide extraction process was added to 4.0ml alkaline picrate solution in a test tube and corked. The mixture was incubated at 50°C for 5 minutes to allow for colour development. After colour development (from yellowish colour to reddish colour) and cooling, the absorbance was read at 490nm wavelength with UV/Visible spectrophotometer (Jenway 6405, England). Diluted potassium cyanide (KCN) was used to prepare the standard curve that was employed in calculating the cyanide content of the experimental samples.

**Bulk Density (BD) determination**

The packed BD and loose BD determinations were carried out in triplicates by the method of Okezie and Bello (1988) as modified by Ukpabi and Umeh (2001) using the knowledge that density is equal to mass per volume. Using 50g flour sample and a previously weighed or tared 100ml measuring cylinder, the volume for loose BD was got by gentle pouring of the floury sample into the measuring cylinder, while the volume for packed BD was got by gentle tapping of the laboratory bench in which the measuring cylinder was kept, until a final volume was got. The original volume \((V_1)\) and the final volume \((V_2)\) were then used for the calculation of loose BD and packed BD as follows:

\[
\text{Loose Bulk density (g/cm}^3\text{)} = \frac{\text{Weight of sample (g)}}{\text{Initial volume (}V_1\text{) of the sample (cm}^3\text{)}}
\]

\[
\text{Packed Bulk density (g/cm}^3\text{)} = \frac{\text{Weight of sample (g)}}{\text{Final volume (}V_2\text{) of the sample (cm}^3\text{)}}
\]
Weight of sample (g)

\[ \text{Packed bulk density (g/cm}^3) = \frac{\text{Final volume (V}_2\text{) of the sample (cm}^3)}{\text{Weight of sample (g)}} \]

**Flour colour determination**

The colour hues of the produced flour samples were determined visually with the assistance of a numbered colour chat (colour chat for carotene analysis) where 1 represented white, 2 represented light cream, 3 represented cream, 4 represented light yellow, 5 represented yellow, 6 represented yellow deep, 7 represented orange and 8 represented pink.

**Statistical analysis**

Statistical Analysis System (SAS) PC software (License site 0822206002) belonging to International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria was used for the statistical analysis.

**Results and Discussion**

Table 1 shows the effect of the few days' post-harvest storage of the experimental cassava roots on the pro-vitamin A carotene content of the produced cassava flour samples. Though only the cassava flour samples produced from the yellow fleshed UMUCASS variety had an appreciable quantity of carotene (12.76 - 14.35 µg/g), all the experimental flour samples, including those from white and cream fleshed cassava roots (with 0.35-1.13 µg/g carotene content), had significant (P<0.05) reduction (10.08-33.96%) in their pro-vitamin A content with two days pre-processing root storage at ambient room conditions (26-30ºC, 82-94% R.H.).

Plant carotene pigments impart yellow-orange colour on some tissues or organs of carotene-rich crops (Onimawo and Akubor, 2005). Fíkselová et al. (2010) reported post-harvest storage carotene losses (27.3% in three months) in orange coloured carrot roots stored at above chilling temperature of 8ºC, while Ukpabi et al. (2014) gave remarkable (about 40%) processing loss of carotene during conversion of yellow cassava roots to cassava flour under a warm condition (26-32ºC). In the study of Ukpabi et al. (2014) at NRCRI, Umudike, Nigeria, the freshly harvested cassava roots of TME 419, TMS 30572 and UMUCASS 38 cassava varieties respectively had carotene content (on dry matter basis) of 3.66 µg/g, 5.49 µg/g and 24.85 µg/g. On fresh wet basis, the yellow fleshed roots of UMUCASS 38 had 5.78 µg/g carotene content with low dry matter content of <25% at NRCRI, Nigeria (Ukpabi et al., 2014).

Ukpabi et al. (2005) in their biochemical characterization study of cassava PPD at NRCRI, Umudike, Nigeria also found that the carotene content of cassava storage roots decreased with time of storage. Though the plastid localized carotene biosynthesis that goes through the isoprenoid pathway (Cazzonelli, 2011), has been found to occur in freshly harvested carrots (Lee,1986), long-term storage of carrots above freezing temperatures had been found to lead to significant losses in their carotenoids content (Matějokavá and Petriková, 2010; Ilić et al., 2013). However, the effect of postharvest storage on the carotene content of carrots seemed to vary with the crop variety as we found in the cassava flour samples produced with the stored cassava roots. Freshly harvested cassava roots are known to undergo oxygen stress that affect certain oxidative metabolic reactions prior and during cassava PPD (Beeching et al.,1998; Reilly et al., 2003), and endogenous plant antioxidants such as tocopherols, carotenoids and ascorbic acid have been identified as oxygen scavengers in the system (Reilly et al., 2003). Enzyme catalysed biochemical reactions identified with cassava PPD include those of catalase and peroxidases (Reilly et al., 2003). Therefore, the reduction of the antioxidants might lead to their degradation if there are no adequate reversible reactions.

The 25.00 - 37.50% reductive effect of the short period pre-processing root storage on the ascorbic acid...
Table - 1. Effect of pre-processing root storage on carotene content (µg/g) of the cassava flour samples

<table>
<thead>
<tr>
<th>Storage period</th>
<th>Cassava Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TME419</td>
</tr>
<tr>
<td>0 day</td>
<td>0.53a</td>
</tr>
<tr>
<td>1 day</td>
<td>0.41a,b</td>
</tr>
<tr>
<td>2 days</td>
<td>0.35b</td>
</tr>
</tbody>
</table>

Values in the same column with different letters are significantly different (P<0.05)

Table - 2. Effect of pre-processing root storage on ascorbic acid content (mg/g) of the cassava flour samples

<table>
<thead>
<tr>
<th>Storage period</th>
<th>Cassava Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TME419</td>
</tr>
<tr>
<td>0 day</td>
<td>0.08a</td>
</tr>
<tr>
<td>1 day</td>
<td>0.07a</td>
</tr>
<tr>
<td>2 days</td>
<td>0.06a</td>
</tr>
</tbody>
</table>

Values in the same column with different letters are significantly different (P<0.05)

Table - 3. Effect of pre-processing root storage on cyanide content (mg/kg) of the cassava flour samples

<table>
<thead>
<tr>
<th>Storage period</th>
<th>Cassava Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TME419</td>
</tr>
<tr>
<td>0 day</td>
<td>4.74a</td>
</tr>
<tr>
<td>1 day</td>
<td>4.69a</td>
</tr>
<tr>
<td>2 days</td>
<td>4.00b</td>
</tr>
</tbody>
</table>

Values in the same column with different letters are significantly different (P<0.05)

Table - 4. Effect of pre-processing root storage on the colour of the cassava flour samples

<table>
<thead>
<tr>
<th>Storage period</th>
<th>TME419 Flour</th>
<th>TMS 30572 Flour</th>
<th>UMUCASS38 Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>White (1)</td>
<td>Light cream (2)</td>
<td>Light yellow (4)</td>
</tr>
<tr>
<td>1 day</td>
<td>white (1)</td>
<td>Light cream (2)</td>
<td>Light yellow (4)</td>
</tr>
<tr>
<td>2 days</td>
<td>Creamy white (1-2)</td>
<td>Light cream (2)</td>
<td>Very light yellow(3-4)</td>
</tr>
</tbody>
</table>

where 1 represented white, 2 represented light cream, 3 represented cream, 4 represented light yellow, 5 represented yellow, 6 represented yellow deep, 7 represented orange and 8 represented pink.

Table - 5. Pre-processing root storage effect on loose and packed bulk densities of the cassava flour samples

<table>
<thead>
<tr>
<th>Storage period</th>
<th>TME 419 Flour</th>
<th>TMS 30572 Flour</th>
<th>UMUCASS 38 Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bulk density (g/cm³)</td>
<td>Bulk density (g/cm³)</td>
<td>Bulk density (g/cm³)</td>
</tr>
<tr>
<td>Loose</td>
<td>Packed</td>
<td>Loose</td>
<td>Packed</td>
</tr>
<tr>
<td>0 day</td>
<td>0.42a</td>
<td>0.65a</td>
<td>0.42a</td>
</tr>
<tr>
<td>1 day</td>
<td>0.41a</td>
<td>0.64a</td>
<td>0.42a</td>
</tr>
<tr>
<td>2 days</td>
<td>0.41a</td>
<td>0.63a</td>
<td>0.42a</td>
</tr>
</tbody>
</table>

Values in the same column with different letters are significantly different (P<0.05)
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The content of the freshly prepared cassava flour samples (0.05-0.10 mg/g) as shown in Table 2 seem to suggest that significant (P < 0.05) ascorbic acid anti-oxidative effect during the initial PPD biochemical reactions in this study was more specific to TMS 30572 and UMUCASS 38 varieties over those of the TME 419 variety. Earlier work by Ukpabi et al. (2014) showed that even in post-processing storage, cassava flour samples from TME 419 in sealed black polythene bags, unlike those from relatively carotene-rich UMUCASS 38 variety do not significantly (P < 0.05) loose their ascorbic acid content even after four weeks of storage at ambient temperature of 26 - 32°C. Degradation of ascorbic acid primarily involves oxidation to dehydroascorbic acid followed by hydrolysis to 2, 3 diketogulonic acid, further oxidation, dehydration and polymerization to form coloured nutritionally inactive products.

Though fresh vitamin C rich edible storage roots generally contain more vitamin C than the stored ones (Lee and Kader, 2000), there has been observed cultivar difference in the vitamin C content of carrots (Matějková and Petriková, 2010), orange fleshed sweet potato (Tumuhimbise et al., 2010) and other horticultural crops (Lee and Kader, 2000). Actually, Ukpabi et al. (2014) also found varying content of ascorbic acid in the fresh cassava roots used for this study as follows: 0.20 mg/g for TME 419 variety, 0.21 mg/g for TMS 30572 variety and 0.23 mg/g for UMUCASS 38 variety.

Table 3 shows that the studied cassava flour samples had 3.09-5.75 mg/kg cyanide content or potential. These values are below the recommended safe level of ≤10 mg/kg cyanide for human food materials (FAO/WHO, 1991). Therefore, based on cyanide content, the newly produced flour samples from the experimental cassava varieties could be considered wholesome for human consumption. Although the fresh cassava roots used for the production of the flour samples had 51.58-74.99 mg/kg cyanide on fresh weight basis (Ukpabi et al., 2014), the dewatering and drying operations used during the flour processing (Fig.- 1) and the activity of the endogenous membrane bound linamarase enzyme (Mpong et al., 1990) probably contributed in getting these low cyanide contents. Hydrocyanic acid or hydrogen cyanide (HCN) is known to be highly water soluble and volatile at ≥25.7°C (Westby, 2002) and cell disruptive activity during the rasping of the roots with a grater (Fig.-1) allowed the hydrolytic glucosidase enzyme to assist in the effective breakdown of the water soluble toxic cyanogenic glucosides.

![Flow chart for the production of the experimental cassava flour](image-url)
(Nambisa and Sundaresan, 1991) to innocuous glucose, and the volatile toxic HCN and/or the equally water soluble toxic (largely unstable) ketone cyanohydrin that normally breaks down to HCN and non toxic ketone in aqueous solutions at neutral or near neutral pHs.

The visually observed white-yellow colour hues of the cassava flour samples from the three investigated cassava varieties are shown in Table 4. Minimal colour change was, however, observed in the flour samples produced from the stored roots in the course of this study. It needs to be mentioned that vascular discolorations associated with cassava PPD had been reported in the roots of some other cassava varieties even after 24 hours of harvesting (Ejiofor, and Ohambele, 1997; Beeching, et al., 2002). Based on the fact that cassava can be processed into many other wholesome non floury food products that do not require colour consistency and micronutrients retention (Balagopalan, 2002; Westby, 2002), we suggest that cassava flour industries should convert some of their harvested starchy roots after one day of storage to some of these products. We also suggest that biochemists in advanced laboratories should endeavour to unambiguously pinpoint the catabolic pathway of ascorbic acid and carotenes in fresh cassava roots during the crop’s PPD. Table 5 shows the loose packed density (0.41-0.46g/cm³) and packed bulk density (0.63-0.67g/cm³) of the flour samples. The short period pre-processing root storage only had significant (P=0.05) effect on the bulk density (BD) of UMUCASS 38 flour samples. Information on food BD is not only important to food industrialists in packaging and handling of floury foods, but it is also required by food technologists in preparing some food products (Kulkarni et al., 1996 and Malomo et al., 2012).

**Conclusion**  
Reactions associated with the oxidative cassava PPD seemed to have contributed, to a limited extent, to the obtained varying negative effect on the carotene and ascorbic acid contents of wholesome cassava flour samples produced from the experimental white fleshed, cream fleshed and yellow fleshed cassava varieties stored for 1-2 days. Only the whitish flour samples did not have significant (P=0.05) reduction in their pro-vitamin A (carotene) and vitamin C (ascorbic acid) contents after one day of pre-processing root storage. It could be deduced from this study that wholesome cassava flour production from yellow cassava (for appreciable pro-vitamin A content) should, as much as possible, be prepared from fresh roots <24 hours of harvest.

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Beeching, J.R., Reilly, K., Gómez-Vásquez, R., Li, H., Han, Y., Rodriguez, M. X., Buschmann, H.,


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