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Carotenoids content of yellow-fleshed cassava genotypes grown in four agroecological zones in Nigeria and their Retinol Activity Equivalents (RAE)

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Abstract

Vitamin A deficiency (VAD) is a serious and widespread public health problem in developing countries. Therefore, it is essential to identify and improve provitamin A content of staple food crops that may be promoted for health improvements. Cassava (*Manihot esculenta*) is an important food crop in the tropics. Twenty-two yellow-fleshed and three white-fleshed cassava genotypes were grown in a randomized complete block design with two replications at five locations representing the major cassava-growing agro-ecological zones in Nigeria. The objective was to determine the quantity and quality of carotenoids in cassava storage roots. The varieties were harvested at 12 months after planting and analyzed for moisture content using a standard oven method and carotenoid content using HPLC. Results obtained indicate trans β-carotene was the major component of total carotenoids in cassava (53.5%), followed by α-carotene and 9-cis β-carotene (both at 19.3%). Other components were 15-cis β-carotene (14.9%), zeaxanthin (3.1%), α-cryptoxanthin (2.7%), 13-cis β-carotene (2.4%), and lutein (0.4%). The results revealed that 90% of the carotenoids in cassava is β-carotene. The mean Retinol Activity Equivalent for the genotypes across locations was 0.40, and the genotype TMS 01/1371 had Retinol Activity Equivalent of 0.63. The results from this study provide information that may be used in breeding programs to further enhance the carotenoid content of cassava storage roots.

Key words: Carotenoids, cassava, yellow-fleshed, β-carotene, trans-β-carotene, cis-β-carotene, Retinol Activity Equivalent, provitamin A.

Introduction

Vitamin A deficiency (VAD) is a threat to the health, sight, and life of millions of children in developing countries [1-3]. According to Ahmed and Darnton-Hill [4], vitamin A deficiency is, after protein-energy malnutrition and iron deficiency anaemia, the most widespread and serious nutritional disease among young children. In the early 1990s, the World Health Organization estimated that globally nearly 14 million children were affected annually by corneal blindness and 190 million were at risk of sub-clinical vitamin A deficiency [4]. Children deficient in this vitamin have poor appetite, are anaemic, and have increased susceptibility to infections, including measles, and are more likely to die in childhood [5, 6].

VAD can be caused by insufficient intakes of vitamin A, fat, protein, or zinc. Without fat, vitamin A cannot be absorbed; hence a diet low in fat can cause a deficiency by reducing vitamin A absorption. Protein deficiency can cause VAD because the retinol-binding protein needed to transport vitamin A cannot be made in sufficient quantities. The importance of zinc for vitamin A utilization is believed to be due to its role in protein synthesis [7].

Vitamin A in the diets of most populations comes from a wide variety of plant and animal sources. In industrialized countries, over two-thirds of dietary vitamin A is derived from animal sources as preformed vitamin A, whereas in developing countries, it is derived primarily from provitamin A carotenoids from plant sources [8, 9].

Carotenoids are fat-soluble compounds and can be divided into two main groups: carotenes or hydrocarbon carotenoids, composed only of carbon and hydrogen atoms, and xanthophylls, oxygenated hydrocarbon derivatives that contain at least one oxygen functional group, such as hydroxyl, keto, epoxy, methoxy, or carboxylic acid groups [10].

Provitamin A carotenoids can be found in many plant foods, such as yellow and orange fruits and vegetables [11] and in dark green leafy vegetables, such as amaranth and spinach. The colour of fruit and vegetables is not necessarily an indicator of the concentration of provitamin A [12, 13] since some carotenoids are not pre-cursors of vitamin A.

Some carotenoids, particularly α-carotene, β-carotene, γ-carotene, β-zeacarotene, and β-cryptoxanthin, can be converted to vitamin A in the intestinal mucosa [7, 13]. Furthermore, unconverted carotenoids also reach to the blood and tissues where they may function as antioxidants. Carotenoids have also been linked with enhancement of the immune system and decreased risk of degenerative diseases, such as cancer, cardiovascular disease, and cataract formation [11, 14-16]. β-Carotene is the most potent pro-vitamin A; it is also the most widespread. All *trans* carotenoids have more provitamin A activity than the *cis* isomers [17]. The conversion process is more efficient in vitamin A deficient individuals and less efficient in those who are vitamin A replete [4].

Because vitamin A deficiency remains a serious public health problem in developing countries, dietary sources and adequacy of provitamin A continue to be a major concern. The Consultative Group on International Agricultural Research (CGIAR) embarked on a project (HarvestPlus) that focuses on developing staple food crops with enhanced provitamin A content. Delivering such varieties to resource-poor farmers, consumers, and other end-users may contribute to improving the vitamin A status of women and children, especially, in developing countries.

Cassava (*Manihot esculenta*) is an important food crop in the tropics. Several hundred million people in developing countries depend on the crop as a major dietary source of energy [18]. Cassava is tolerant to low soil fertility and drought stress, is highly productive, is available throughout the year, and can be processed into different food products, depending on local customs and preferences [19]. The leaves are prepared and consumed as a vegetable in many countries and provide protein, minerals, and vitamins [20].

The qualitative and quantitative genetic variability of carotenoids in cassava storage roots has not been extensively investigated. Therefore, the objective of the present work is to: (a) characterize quantitatively and qualitatively the carotenoids of raw yellow-fleshed storage roots of improved cassava genotypes grown in different agroecological zones; (b) determine the relative proportion of the different carotenoids in relation to the total carotenoids and (c) estimate the Retinol Activity Equivalent (RAE) of yellow-flesh cassava genotypes.

Materials and Methods

Cassava storage roots: Twenty-five elite cassava genotypes consisting of twenty-two yellow-fleshed and three white-fleshed genotypes (TME1, TMS 30572, and TMS 91/02324) were grown under rain-fed conditions in the 2004/2005 growing season at five locations (Onne, Ubiaja, Ibadan, Mokwa, and Zaria) representing four major cassava growing agroecological zones in Nigeria namely humid forest, humid forest-savanna transition, southern Guinea savanna, and northern Guinea savanna (Table 1) [21]. The experimental design was a randomized complete block design with two replications at each location. Each plot consisted of 36 plants in six 6-plant rows. The ridges were 1 m apart, 30 cm high and 6 m long. Planting was done at the beginning of the rainy season. Spacing between plants was 1 m, giving a total plant population of 10,000 plants. No fertilizer or herbicides were applied during the course of the experiment, and hand weeding was done when necessary. The cassava storage roots were harvested at 12 months after planting.

Determination of moisture content: Moisture content was determined at the time of analysis using a standard oven procedure [22]. Samples were weighed into cans and placed in an oven (Fisher Scientific Co., USA, model 655F) maintained at 105±2°C. After 16-18 hours, the cans were transferred into a desiccator to cool, after which the final weight was taken and the percentage difference was determined.

Determination of carotenoids content: Five storage roots from three plants of each genotype were collected during harvesting and three storage roots of different sizes (large, medium, and small) were selected, washed, and air-dried. The roots were peeled, rinsed with de-ionized water, and cut longitudinally (from the stem end to the root end) into four equal parts. Two adjacent sections from each storage roots were taken, chopped into small pieces, combined, and mixed. The mixed sample was further divided into four equal parts. Adjacent sections were collected, mixed thoroughly, packed in a whirl pack, and wrapped with aluminium foil. All samples were prepared under red fluorescent lights to prevent photo-oxidation and isomerization.

Samples were stored at –80°C for 3 days and shipped in dry ice to the USA for analysis. Samples were analyzed at Iowa State University, Ames, Iowa State, USA by the method of Granado and colleagues [23] and modified by Li *et al.* [24] using HPLC. The modification involved introducing hot extraction with methanol at 50°C for 15 min. Interfering lipids were hydrolyzed in only 3 min by exposing small volumes of extract to excess potassium hydroxide while vortexing. The results are expressed in fresh weight (as is) basis. Duplicate analyses were conducted for a genotype in each replication and location.

Quality control during analysis: Calibration curves were validated using: 1) a non-commercial-available high-carotenoids cassava flour composite provided by the HarvestPlus program and stored at -70°C; and 2) Standard Reference Material (SRM) 2383 Baby Food Composite provided by the U.S. National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Inter-assay precision was evaluated using a composite of ground cassava flour prepared from conventional cassava and mixed with a commercially-available orange sweet potato flour to provide provitamin A carotenoids concentrations similar to those present in the cassava samples analyzed. The quality control composite was stored at -70°C. An aliquot of the quality control composite was analyzed with each assay. The inter-assay coefficient of variation (CV) for each carotenoid analyzed in the quality control composite was less than 7%.

Table 1. Agroecological characteristics of the locations [21].

Location	Agroecological zone	Soil type	Position	Altitude (m)	Rain (mm)	Wet season	Min/max temperature	Length of growing period (days)
Onne	Humid forest	Thionic Fluvisols	7°10' E; 4°46' N	30	2501.6	Feb-Dec	12-23/28-32°C	>270
Ubiaja	Humid forest	Dystric Nitosols	6°25' E; 6°40' N	210	1943.5	Mar-Dec	12-22/27-32°C	>270
Ibadan	Humid forest-savanna transition	Ferric Luvisols	3°54' E; 7°26' N	210	1252.8	Mar-Aug Aug-Nov	12-23/28-34°C	211-270
Mokwa	Southern Guinea savanna	Ferric Luvisols	5°4' E; 9°18' N	210	1235.2	Apr-Nov	13-24/28-36°C	181-201
Zaria	Northern Guinean savanna	Orthic Luvisols	7°38' E; 11°6' N	640	941.2	May -Jul	14-25/29-36°C	151-180

Assessment of carotenoid content and Retinol Activity Equivalents (RAE): Total carotenoid content was calculated from the HPLC analyses by adding all the different carotenoids; total *cis* β-carotene was calculated by adding 9- *cis, 13- cis,* and 15- *cis* β-carotene; and total β-carotene was calculated by adding total *cis* and *trans* β-carotene. Provitamin A activity was calculated by adding total β-carotene and half of α-carotene. Because carotenoids are less well absorbed and not completely converted to vitamin A, a correction factor, referred to as Retinol Activity Equivalents (RAE), must be applied to carotenoids to determine the amount of usable vitamin A they provide. The RAE was calculated by using 12 μg/g of β-carotene or 24 μg /g of α-carotene or β-cryptoxanthin [25].

Statistical analysis: Data from the different growing environments were subjected to combined analysis of variance and descriptive statistics using the Statistical Analysis System procedures software [26]. Data is presented as mean ± standard deviation on fresh weight basis.

Results and Discussion

Assessment of carotenoid content of cassava storage roots: Fig. 1 shows a representative chromatographic profile of carotenoids in fresh cassava storage roots. The mean percentage moisture content, carotenoid profile and concentrations in yellow-fleshed cassava storage roots grown at different locations are presented in Table 2. The presence of lutein, zeaxanthin, α-cryptoxanthin, α-carotene, 9-cis β-carotene, 13-cis β-carotene, 15-cis β-carotene, and *trans* β-carotene were identified in variable amounts in cassava storage roots. Among the different locations, *trans* β-carotene concentration was highest in cassava storage roots grown at Zaria (4.38 μg/g) compared to those grown at Ubiaja (2.52 μg/g); and total *cis* β-carotene content was highest in Mokwa and lowest at Onne. Total β-carotene content was higher in cassava storage roots grown in Zaria and Mokwa and similar among cassava storage roots grown in Ubiaja, Onne, and Ibadan. Cassava storage roots grown at Zaria had high total carotene than those grown at Onne. The mean percentage moisture content ranged from 65.3 (Ubiaja) to 76.3 (Onne) (Table 2).

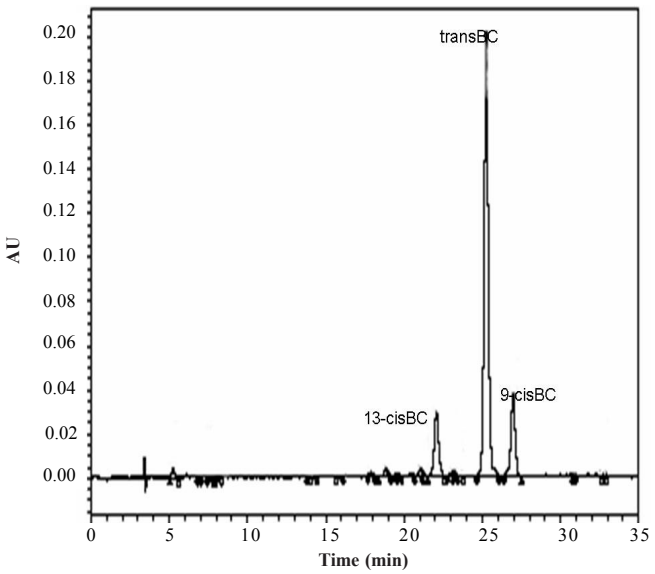

Figure 1. Representative chromatogram of carotenoids extracted from cassava storage roots. β–Cisomers were identified by comparison of elution profile and absorption spectra as described. Sudan I was added to cassava before extraction as recovery standard. See Materials and Methods for details.

Among the observed *cis* β-carotene isomers, 9-*cis* β-carotene was the major isomer with a mean of 0.92 μg/g followed by 15-*cis* β-carotene with a mean of 0.71 μg/g , and 13-*cis* β-carotene with a mean of 0.13 μg/g (Table 3).

Table 3. Location means of *cis* β-carotene content of elite cassava varieties grown at different locations in Nigeria.

Location	9-cis β-carotene (μg/g)	13-cis β-carotene (μg/g)	15-cis β-carotene (μg/g)
Ibadan	0.98	0.11	0.69
Mokwa	1.13	0.14	0.86
Onne	0.59	0.11	0.47
Ubiaja	1.07	0.12	0.67
Zaria	0.85	0.14	0.83
Mean	0.92	0.13	0.71
Min	0.59	0.11	0.47
Max	1.13	0.14	0.86
SE	0.096	0.007	0.069

[1] Means are for duplicate determination of each replications per genotype.

When the data for all the locations were pooled and analyzed, the mean moisture content across the locations was 69.1 ± 4.17% g. The β-carotene content of storage roots may be compared with values in the literature, using a moisture content of about 65 g/100 g [27]. The mean *trans* β-carotene concentration was 3. 07 μg/g; mean total β-carotene was 4. 81 μg/g; and mean total carotenoids was 5. 31 μg/g. Our results show a β-carotene content in yellow-fleshed cassava genotypes which is higher than the amount present in the white-fleshed cassava storage roots (TMS 30572, TMS 91/02324, and TME1). Among the genotypes, TMS 01/1335, TMS 01/1368, and TMS 01/1371 had the highest total β-carotene content compared to TMS 30572, TMS 91/02324, and TME. Most

Table 2. Location means of different carotenoids of elite cassava genotypes grown in four agroecological zone in Nigeria.

	Location				
Parameter	Ibadan	Mokwa	Onne	Ubiaja	Zaria
Lutein (ug/g)	0.03± 0.09	0.02± 0.09	0.01± 0.04	0.04± 0.10	0.03± 0.08
Zeaxanthin (ug/g)	0.12± 0.12	0.22± 0.17	0.05± 0.09	0.07± 0.11	0.25± 0.20
α-cryptoxanthin (ug/g)	0.14± 0.07	0.15± 0.08	0.11± 0.05	0.18± 0.09	0.14± 0.07
α-carotene (ug/g)	0.19± 0.10	0.22± 0.10	0.15± 0.09	0.22± 0.10	0.14± 0.10
Total cis-beta carotene (ug/g)	1.78± 0.78	2.13± 0.97	1.17± 0.54	1.87± 0.87	1.83± 0.87
Trans beta-carotene (ug/g)	2.64± 1.48	3.04± 1.90	2.83± 1.79	2.52± 1.19	4.38± 3.32
Total beta-carotene (ug/g)	4.42± 2.05	5.16± 2.48	4.00± 2.03	4.38± 1.88	6.20± 3.70
Total carotene (ug/g)	4.90± 2.20	5.78± 2.68	4.32± 2.09	4.89± 2.07	6.77± 3.68
Moisture content (%)	69.80± 7.35	67.36± 5.70	76.32± 5.88	65.29± 6.81	67.07± 4.57

[1] Means are for duplicate determination of each replications per genotype. [2] Data is expressed as mean ±SD (range) on fresh weight basis.

Table 4. Mean *trans* β-carotene, total β-carotene, total carotene, and moisture content of yellow-fleshed cassava genotypes.

Genotype	Trans β-carotene µg/g	Total β-carotene µg/g	Total carotene µg/g	% Moisture content
TMS 01/1115	5.71±3.37	6.91±3.52	7.35±3.42	72.70±4.91
TMS 01/1224	5.13±1.65	6.80±1.61	7.21±1.55	67.57±3.99
TMS 01/1235	3.30±1.19	5.45±1.33	5.94±1.33	70.84±6.94
TMS 01/1273	2.64±1.03	4.41±1.62	4.96±1.73	72.73±6.26
TMS 01/1277	3.48±1.49	5.84±1.82	6.44±1.83	69.44±5.85
TMS 01/1331	3.21±1.19	4.95±1.00	5.52±0.96	74.66±6.93
TMS 01/1335	6.08±3.21	7.39±3.49	7.82±3.42	70.23±6.25
TMS 01/1368	4.09±0.74	7.40±1.51	8.32±1.76	67.89±4.94
TMS 01/1371	4.83±1.30	7.53±1.39	8.16±1.47	74.15±7.52
TMS 01/1412	3.44±1.11	6.17±1.51	6.70±1.46	73.52±7.94
TMS 01/1413	3.27±1.17	5.37±1.78	5.87±1.89	71.78±5.21
TMS 01/1442	4.63±2.99	6.36±3.02	6.77±2.98	73.49±8.76
TMS 01/1610	3.25±1.14	4.92±1.23	5.77±1.27	70.79±7.04
TMS 01/1646	1.79±0.43	3.39±0.64	3.87±0.73	66.84±3.95
TMS 01/1649	3.50±0.98	5.61±0.83	6.28±0.89	68.26±8.40
TMS 01/1662	3.81±2.25	5.35±2.37	5.74±2.35	75.04±9.06
TMS 01/1663	4.50±1.93	6.33±2.09	6.74±2.11	72.30±4.23
TMS 30572	0.60±0.95	1.10±1.40	1.18±1.51	64.01±5.84
TMS 90/01554	1.28±0.69	2.45±1.12	2.70±1.22	62.98±5.37
TMS 91/02324	0.64±1.18	1.04±1.40	1.08±1.43	65.11±6.14
TMS 94/0006	1.11±0.52	2.31±1.05	2.79±1.33	62.95±4.52
TMS 94/0330	1.41±0.31	2.97±0.70	3.69±0.85	63.32±5.79
TMS 95/0379	1.98±0.49	4.31±1.14	5.05±1.37	68.35±7.24
TMS 98/2132	2.81±1.02	5.26±1.13	5.89±1.23	67.53±5.56
TME1	0.34±0.05	0.73±0.09	0.83±0.16	60.55±1.75
Mean	3.07	4.81	5.31	69.08
Std dev.	1.61	2.05	2.18	4.17
Min	0.34	0.73	0.83	60.55
Max	6.08	7.53	8.32	75.04

[1] Means are for duplicate determination of each genotype for each replication.

Table 5. Mean zeaxanthin, α-cryptoxanthin, α-carotene, and total cis β-carotene of yellow-fleshed cassava genotypes.

Genotype	Zeaxanthin µg/g	α-cryptoxanthin µg/g	α-carotene µg/g	Total cis β-carotene µg/g
TMS 01/1115	0.12±0.14	0.16±0.04	0.16±0.09	1.20±0.27
TMS 01/1224	0.06±0.11	0.14±0.04	0.21±0.02	1.66±0.33
TMS 01/1235	0.10±0.13	0.15±0.03	0.24±0.02	2.15±0.65
TMS 01/1273	0.16±0.12	0.15±0.06	0.19±0.11	1.78±0.72
TMS 01/1277	0.22±0.13	0.14±0.03	0.24±0.03	2.36±0.48
TMS 01/1331	0.13±0.14	0.16±0.02	0.26±0.05	1.74±0.33
TMS 01/1335	0.10±0.16	0.15±0.04	0.17±0.07	1.31±0.63
TMS 01/1368	0.20±0.19	0.24±0.07	0.28±0.04	3.31±0.88
TMS 01/1371	0.09±0.14	0.22±0.07	0.27±0.05	2.70±0.87
TMS 01/1412	0.09±0.12	0.20±0.05	0.24±0.09	2.73±0.51
TMS 01/1413	0.10±0.14	0.20±0.07	0.21±0.12	2.10±0.75
TMS 01/1442	0.11±0.14	0.16±0.05	0.12±0.13	1.73±0.53
TMS 01/1610	0.29±0.18	0.16±0.03	0.17±0.09	1.67±0.51
TMS 01/1646	0.11±0.14	0.16±0.06	0.21±0.05	1.61±0.29
TMS 01/1649	0.23±0.18	0.19±0.05	0.25±0.03	2.11±0.59
TMS 01/1662	0.07±0.12	0.12±0.03	0.19±0.10	1.54±0.26
TMS 01/1663	0.07±0.14	0.14±0.07	0.21±0.09	1.83±0.58
TMS 30572	0.02±0.06	0.02±0.05	0.04±0.08	0.51±0.45
TMS 90/01554	0.04±0.08	0.06±0.07	0.16±0.07	1.16±0.44
TMS 91/02324	0.03±0.09	0.02±0.04	0.00±0.00	0.39±0.22
TMS 94/0006	0.23±0.18	0.12±0.07	0.13±0.11	1.20±0.53
TMS 94/0330	0.31±0.19	0.15±0.03	0.19±0.07	1.57±0.43
TMS 95/0379	0.31±0.16	0.19±0.09	0.25±0.05	2.32±0.65
TMS 98/2132	0.19±0.21	0.18±0.06	0.27±0.04	2.45±0.43
TME1	0.06±0.12	0.01±0.03	0.02±0.05	0.39±0.05
Mean	0.137	0.144	0.185	1.742
Std dev	0.087	0.059	0.076	0.719
Min	0.02	0.01	0.00	0.39
Max	0.31	0.24	0.28	3.32

[1] Means are for duplicate determination of each genotype for each replication.

of the studied genotypes (64%) had total β-carotene content and *trans* β-carotene (60%) above the overall mean (Table 4). TMS 01/1368 had higher total carotenoids compared to TME1. Although we added three check genotypes (white-fleshed) to serve as control, our results show that these genotypes were not devoid of carotenoids as their values for total carotenoids content ranged from 0. 83 µg/g for TME1 to 1. 18 µg/g for TMS 30572 (Table 4).

The mean zeaxanthin concentration was 0.137 µg/g; mean α-cryptoxanthin was 0.144 µg/g; mean α -carotene was 0.185 µg/g; mean total *cis* β-carotene was 1.742 µg/g (Table 5). The genotypes TMS 01/1368, TMS 98/2132, TMS 95/0379 had the highest total *cis* β-carotene compared to the other investigated genotypes.

We were also interested in determining what proportion of each type of carotenoids was present in the total carotenoids. *Trans* β-carotene was found to be the major component of total carotenoids in cassava (53.5%), followed by α-carotene and 9-*cis* β-carotene (both at 19.3%), 15-*cis* β-carotene (14.9%), zeaxanthin (3.1%), α-cryptoxanthin (2.7%), 13-*cis* β-carotene (2.4%), and lutein (0.4%). When we combined the *cis*-β-carotene to make total *cis*-β-carotene, total *cis*-β-carotene was second (36.7%) to *trans*-β-carotene. Furthermore, when we combined total *cis* and *trans*-β-carotene, the result revealed that over 90% of the carotenoids in cassava is β-carotene (Fig. 2). Our result is in agreement with that of Chávez *et al.* [28] who analyzed yellow-flesh cassava storage roots for total carotenoids spectrophotometrically, and β-carotene and α-carotene using HPLC. They reported that a large proportion of the carotenoids present in cassava storage roots is β-carotene. The authors did not indicate the proportion of *cis*- to *trans*-β-carotene, as these isomers differ in their provitamin A activity.

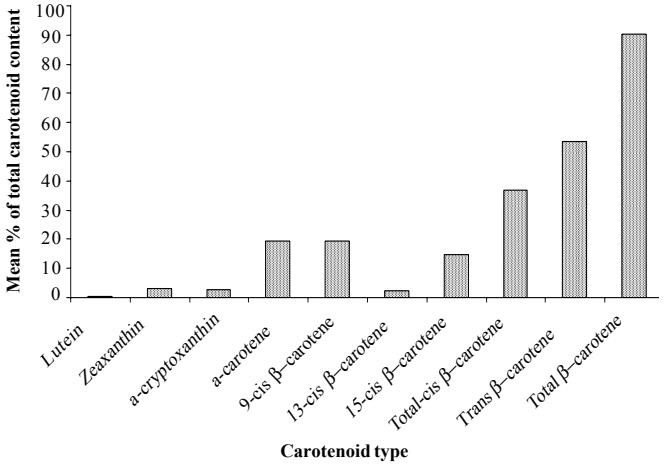

Figure 2. Mean carotenoids profiles (% of total carotene) of 22 yellow-flesh clones and 3 white-fleshed check clones grown in five locations in Nigeria.

Since the production and consumption of cassava worldwide exceed that of any other tropical root crop, it is surprising that there are few published studies on carotenoids. Emphasis on storage roots quality has previously been placed upon dry matter content, starch, cyanogenic potential, and pasting properties, therefore, there is limited information on the carotenoid composition and particularly on the individual components with provitamin A activity of yellow-fleshed cassava genotypes developed for tropical Africa. The present study has filled gaps in

the qualitative and quantitative information on cassava carotenoids. More recently, Chávez *et al.* [29] and Iglesias *et al.* [30] reported total carotenoids content of yellow-flesh cassava storage roots based on spectrophotometer.

The variation in the total carotenoids content reported in the present study is lower than that found in the literature [31] with a mean total carotenoid concentration of 2.06 mg/100 g fresh root with a range of 0.20 to 7.74 mg/100 g. Chávez *et al.* [29] evaluated 2457 cassava genotypes from 1998 through 2001 for total carotenoids and reported that total carotenoid content ranged from 0.01 to 1.04 mg/100 g fresh tissue. The authors did not indicate the moisture content of the storage roots at the time of analysis, as moisture content may affect the concentration of total carotenoids through concentration or dilution. The highest observed value in the current analysis was 8.32 µg/g for the genotype TMS 01/1368 whereas as much as 2.4 mg/100 g fresh root have been reported by Iglesias and colleagues [29].

The result is in agreement with that of McDowell and Oduro [32] that the amount of β-carotene in yellow cassava storage roots was as much as 1 mg/100 g on a dry weight basis, about 100 times the amount present in white storage roots. Penteado and Almeida[33] conducted an analysis of five cassava genotypes produced in Sao Paulo; the β-carotene content of the varieties ranged from 0.1 to 0.6 mg/100 g. When Nassar and colleagues [34] screened 15 indigenous genotypes in Brazil for carotenoids content, the genotype UnB400 had 236 mg/kg lutein and 1.24 mg/kg trans β-carotene. The β-carotene content of 21 cassava genotypes from exotic and indigenous collections was evaluated by Moorthy *et al.* [35], the authors reported a variation from 0.04 to 0.79 mg/100 g edible portion and concluded that there was considerable variation in β-carotene content in cassava storage roots. Chavez *et al.* [36] screened 601 genotypes for carotenoids content based on spectrophotometer and showed that genotypic variability was high with a range of 0.6 to 2.4 mg/100 g of fresh root even for deep yellow and orange roots. All the cassava genotypes presented the same major carotenoids in different concentrations. In general, it has been observed that α-carotene sometimes accompanies β-carotene at a much lower concentration [37].

Assessment of provitamin A activity and Retinol Activity Equivalent of cassava storage roots: Provitamin A value was calculated by adding the total β-carotene and half of the α-carotene assuming 100% activity for β-carotene and 50% α-carotene [38]. In the present investigation provitamin A activity of the cassava genotypes ranged from 0.074 to 7.66 µg/g with a mean of 4.90 µg/g. The genotypes TMS 01/1335, TMS 01/1368, and TMS 01/1371 had the highest observed provitamin A activity value of 7.47, 7.54 and 7.66 µg/g, respectively (Table 6). The units for vitamin A used on food and in nutrient databases include Retinol Equivalents (RE) and International Units (IU). One RE equal µg or 6 µg β-carotene, while using IU, 1 RE equl 3.33 IU retinol or 10 IU β-carotene [39]. Recently, new units for vitamin A (Retinol Activity Equivalents, RAE) were proposed. These units reflect recent knowledge that conversion to retinol of food carotenoids is only about half as efficient as was previously believed. Thus, the new conversion factor for foods is 12 µg β-carotene per µg retinol instead of 6 µg and 20 IU β-carotene per µg retinol instead of 10 IU [40, 41]. When we converted provitamin A activity to RAE, the RAEs were 0.62 for TMS 01/1335; 0.62 for TMS 01/1368; and 0.63 for TMS 01/1371 across locations. Across locations, the mean RAE for the genotypes was 0.40 (Table 6).

Table 6. Provitamin A activity and Retinol Activity Equivalent (RAE) of cassava storage roots grown at different agroecological zones in Nigeria.

Genotype	Provitamin A Activity (µg/g)	Retinol Activity Equivalent (RAE)
TMS 01/1115	6.99	0.576
0TMS 01/1224	6.91	0.567
TMS 01/1235	5.57	0.454
TMS 01/1273	4.50	0.367
TMS 01/1277	5.96	0.487
TMS 01/1331	5.08	0.412
TMS 01/1335	7.47	0.616
TMS 01/1368	7.54	0.617
TMS 01/1371	7.66	0.627
TMS 01/1412	6.29	0.514
TMS 01/1413	5.48	0.447
TMS 01/1442	6.42	0.530
TMS 01/1610	5.00	0.410
TMS 01/1646	3.49	0.282
TMS 01/1649	5.73	0.467
TMS 01/1662	5.44	0.446
TMS 01/1663	6.43	0.527
TMS 30572	1.12	0.092
TMS 90/01554	2.53	0.204
TMS 91/02324	1.04	0.087
TMS 94/0006	2.37	0.192
TMS 94/0330	2.88	0.232
TMS 95/0379	4.43	0.359
TMS 98/2132	5.39	0.438
Std Dev	2.085	0.171
TME1	0.74	0.061
Mean	4.898	0.401
Min	0.74	0.06
Max	7.66	0.62

The vitamin A value of a food has been reported in International Units (IU). However, since the dietary provitamin A carotenoids are utilized poorly compared with retinol, the expression of the total vitamin A activity of a diet in IU has had to be qualified by indicating the percentage of the activity contributed by the provitamin A carotenoids. As indicated above, it has been recommended that a correction factor, referred to as Retinol Activity Equivalent (RAE), must be applied to determine the amount of usable vitamin A [40, 41]. Bradbury and Holloway [41] used a factor of 6 µg/g and reported RAE of 0.11-6.9 for sweet potato and 0.18 for yam. In the present study, we used 12 µg/g; therefore, it becomes apparent that yellow-fleshed cassava storage roots are intermediate between orange flesh sweet potato and yam.

FAO/WHO [42] recommended a daily intake of 400 µg RAE for infants and children and 600-800 µg RAE for adults. Given that the recommended daily intake is 400 µg retinol equivalents for infants and children; we estimate the consumption of 200 g of cassava storage roots per day from yellow-flesh genotypes, this might supply between 88.8 and 127.8 RAE, provided that there is minimum loss during processing and that adequate fat is consumed to enhance bioavailability. It should be noted that the genotypes used in the present study were not developed specifically for enhanced provitamin A carotenoids, therefore, yellow-flesh cassava has the potential to contribute in alleviating vitamin A deficiency.

Conclusions

The results from this investigation have provided information on the quantitative and qualitative information on carotenoids of yellow-fleshed cassava storage roots. *Trans* β-carotene was found to be the major component of total carotenoids in cassava, followed by α-carotene and 9-*cis* β-carotene, 15-*cis* β-carotene, zeaxanthin, α-cryptoxanthin, 13-*cis* β-carotene, and lutein. Total *cis*- β-carotene was second to *trans* β-carotene; and 90% of the carotenoids in cassava is β-carotene. The RAE was lower than that of orange flesh sweet potato.

With the characterization of the carotenoids in cassava genotypes, it is now possible to estimate, with more accuracy, after taking to account losses during processing and bio-availability, the amount that is needed to have a nutritional impact. Furthermore, it may be possible to determine the contribution of yellow-flesh cassava genotypes to vitamin A intakes of populations where cassava is a major staple food crop and makes up a large part of the diet. The result from this study has provided information that may be used in breeding programs to further enhance the carotenoids content of cassava storage roots. Further investigation is needed to determine carotenoids retention during processing and storage, and their bioavailability.

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References

[1]Ross, D. A. 1998. Vitamin A and applied health. Proc. Nutr. Soc. **57**:159-165.

[2]West, Jr. K. P. 2002. Extent of vitamin A deficiency among preschool children and women of reproductive age. J. Nut. **132**:2857-2866.

[3]Tee, E. S. 1992. Carotenoids and retinoids in human nutrition. Crit. Rev. Food Sci. and Nutr. **31**:103-163.

[4]Ahmed, F. and Darnton-Hill, I. 2004. Vitamin A deficiency. In Gibney, M. J., Margetts, B. M., Kearney, J. M. and Arab, L. (eds). Public Health Nutrition. Blackwell Science Ltd. Pub., Oxford, UK, pp. 192 – 215.

[5]Underwood, B. A. 1998. Micronutrient malnutrition: Is it being eliminated?. Nutr. Today **33**:121-129.

[6]McLaren, D. S. and Frigg, M. 2001. Sight and Life Manual on Vitamin A Disorders (VADD). 2nd edn. Task Force Sight and Life, Basel, Switzerland.

[7]Smolin, L. A. and Grosvenor, M. B. 2003. Nutrition Science and Applications. 4th edn. John Wiley and Sons, Inc, Hoboken, New Jersey, USA.

[8]West, K. P. and Jr. Darnton-Hill, I. 2000. Vitamin A deficiency. In Semba, R. D. and Bloem, M. W. (eds). Preventive Nutrition. Vol. 3. Nutrition and Health in Developing Countries. Humana Press, Totowa, New Jersey, USA.

[9]Ruel, M. E. 2001. Can food-based strategies help reduce vitamin A and iron deficiencies? A review of recent evidence. International Food Policy Research Institute, Washington, DC, USA.

[10]Rodriguez-Bernaldo de Quiros, A. and Costa, H.S. 2006. Analysis of carotenoids in vegetable and plasma samples: A review. J. Food Comp. and Anal. **19**:97-111.

[11]Rodriguez-Amaya, D. B. 1997. Carotenoids and food preparation: The retention of provitamin A carotenoids in prepared, processed, and stored foods. Universidade Estadual de Campinas, Campinas, SP, Brazil.

[12]Speek, P. F., Speek-Saichua, S. and Schreurs, W. H. P. 1988. Total carotenoids and β-carotene contents of Thai vegetables and the effect of processing. Food Chem. **27**:245-257.

[13]Gross, J. 1991. Pigments in Vegetables: Chlorophylls and Carotenoids. Van Nostrand Reinhold Pub., New York, New York, USA.

[14]Byers, T. and Perry, G. 1992. Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. Ann. Rev. Nutr. **12**:139-159.

[15]Krinsky, N. I. 1990. Carotenoids in medicine. In Krinsky, N. I., Mathews-Roth, M. M. and Taylor, R. F. (eds). Carotenoids: Chemistry and Biology. Plenum Press, NY, pp. 279-291.

[16]Krinsky, N. I. 1994. The biological properties of carotenoids. Pure and Appl. Chem. **66**:1003-1010.

[17]Rodriguez-Amaya, D. B. 1993. Nature and distribution of carotenoids in foods. In Charalambous, G. (ed.). Shelf-Life Studies of Foods and Beverages. Chemical, Biological, Physical, and Nutritional Aspects. Elsevier Science Publishers, Amsterdam, The Netherlands, pp. 547-589.

[18]Cock, J. H. 1982.Cassava: a basic energy source in the tropics. Science **218(**4574):755-762.

[19]Nweke, F. I., Spencer, D. C. and Lynam, J. K. 2002. The Cassava Transformation. Michigan State University Press, East Lansing, Michigan State, USA.

[20]Achidi, A. U., Ajayi, A. O. and Bokanga, M. 2003. Cassava use utilization as a vegetable source for humans in Africa. In Proceedings of Eighth Triennial Symposium of The International Society of Tropical Root Crops, Africa Branch (ISTRC-AB). Ibadan, Nigeria. International Institute of Tropical Agriculture, Ibadan, Nigeria.

[21]Jagtap, S. S. 1993. Climate, soils and agro-ecological characteristics at breeding sites. Draft for Crop Improvement Division (CID) internal review, Nov, 1993. International Institute of Tropical Agriculture, Ibadan, Nigeria.

[22]AOAC 1990. Official Methods of Analytical Chemists. 15th edn. AOAC Arlington, Virginia, USA.

[23]Granado, F., Olmedilla, B., Gil-Martinez, E. and Blanco, I. 2001. A fast, reliable, and low-cost saponification protocol for analysis of carotenoids in vegetables. J. Food Comp. and Anal. **14**:479-489.

[24]Li, S., Tayie, F., Zheng, Y. and White, W. S. 2005. A rapid extraction method for HPLC quantification of carotenoids in maize. FASEB J. **19**:A474.

[25]Food and Nutrition Board, Institute of Medicine 2001. Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academy Press, Washington, DC, USA.

[26]SAS 2001. Statistical Analysis Software (SAS) systems. SAS Institute Inc. Cary, NC, USA.

[27]Bradbury, J. H. and Holloway, W. D. 1988. Chemistry of Tropical Root Crops. Significance for nutrition and agriculture in the Pacific. ACIAR, Canberra, Australia, pp. 53-77.

[28]Chávez, A. L., Sánchez, T., Ceballos, H. and Tohme, J. 2004. Nutritional and agronomic advantages of yellow rooted cassava. Available at: http://www.ciat.org/yuca/pdf/poster_nutritional_agronomic.pdf.

[29]Chávez, A. L., Sánchez, T., Jaramillo, G., Bedoya, J. M., Echeverry, J., Bolanõs, E. A., Ceballos, H. and Iglesias, C. A. 2005. Variation of quality traits in cassava roots evaluated in landraces and improved clones. Euph. **143**:125-133.

[30]Iglesias, C., Mayer, J., Chavez, L. and Calle, F. 1997. Genetic potential and stability of carotene content in cassava storage roots. Euph. **94**:367–373.

[31]Sánchez, T., Chávez, A. L., Ceballos, H., Rodriguez-Amaya, D. B., Nestel, P. and Ishitani, M. 2006. Reduction or delay in postharvest physiological deterioration in cassava roots with higher carotenoids content. J. Sci. Food and Agric. **86**:634-639.

[32]McDowell, I. and Oduro, K. A. 1983. Investigation of the β-carotene content of yellow varieties of cassava (*Manihot esculenta*). J.Plant Foods **5**:169-171.

[33]Penteado, M. V. C. and Almeida, L. B. 1988. Ocorrencia de carotenoidses em raizes de cinco cultivares de mandioca (*Manihot exculenta* Crantz)

do Estado de Sao Paulo. Review Farm Bioquim Univ. Sao Paulo **24**:39-49.

[34]Nassar, N. M. A., Vizzotto, C. S., Silva, H. L., Schvartz, C. A. and Pires Junior, O. R. 2005. Potentiality of cassava cultivars as a source of carotenoids. Journal of Food, Agriculture & Environment **3**(3&4):33-35.

[35]Moorthy, S. N., Jos, J. S., Nair, R. B. and Sreekumari, M. T. 1990. Variability of β-carotene content in cassava germplasm. Food Chem. **36**:233–236.

[36]Chávez, A. L., Bedoya, J. M., Sánchez, T., Iglesias, C. A., Ceballos, H. and Roca, W. 2000. Iron, carotene, and ascorbic acid in cassava roots and leaves. Food and Nutr. Bull. **21**(4):410-413.

[37]Rodriguez-Amaya, D. B. 2002. Brazil: A bounty of carotenoids sources. Sight and Life Newsletter **4**:3-9.

[38]Simpson, K. L. and Chichester, C. O. 1981. Metabolism and nutritional significance of carotenoids. Ann. Rev. in Nutr. **1**:351-374.

[39]Barr, S. I. 2003. Dietary Reference Intakes: Vitamins. The Whitehall-Robins Report **12**(2):1-2.

[40]Institute of Medicine 2000. Dietary Reference Intakes for vitamin C, Vitamin E, selenium, and carotenoids. National Academy Press, Washington, DC.

[41]Institute of Medicine 2001. Dietary Reference Intakes for vitamin A, Vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, zinc and carotenoids. National Academy Press, Washington, DC.

[42]FAO/WHO 1988. Requirements of vitamin A, iron, folate, and vitamin B_{12}. Report of a Joint FAO/WHO Expert Consultation, FAO Food and Nutrition Series No. 23; Food and Agriculture Organization, Rome, Italy.