Influence of Age, Farming Site, and Boiling on Pro-Vitamin A Content in Sweet Potato (*Ipomoea batatas* (L.) Lam.) Storage Roots

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Abstract

To maximize the availability of pro-vitamin A carotenoids in sweet potato and to recommend the appropriate start of piecemeal harvesting practice, the main carotenoids in storage roots of 17 different sweet potato cultivars were surveyed using HPLC and spectrophotometry methods, and their variation due to production site, storage root age, and boiling was assessed. There was significant variation in carotenoid content among cultivars. Six different carotenoids were consistenly detected in significant quantities. Orange-fleshed roots contained higher total carotenoid and β- -carotene content than white- and cream-fleshed lines, and all trans-β-carotene predominated. The effect of storage root age on carotenoid content was significant. Twelve weeks after planting, the yield and amount of pro-vitamin A present in roots of orange-fleshed cultivars evaluated were high enough to provide adequate dietary pro-vitamin A and suggest the start of piecemeal harvesting. The effects of farming site on total carotenoids was significant; however, the amount of β-carotene was not different over three testing sites. Boiling of roots for 30 min caused a reduction in total carotenoids which varied by cultivar; however, further boiling for up to 60 min did not exacerbate the reduction in total carotenoids.

Key Words: β-carotene; *Ipomoea batatas*; piecemeal harvesting; utilization; postharvest; vitamin A; Africa.

Introduction

Carotenoids represent the most widespread group of naturally occuring pigments in nature. They are primarily of plant origin and β-carotene, with few exceptions, predominates (Chandler and Schwartz, 1988). β-Carotene serves as an important nutritional component in foods, as a major precursor of vitamin A, and it provides pleasant yellow-orange colors to a decreased incidence of certain cancers in humans (Gester, 1993) and the possible role of carotenoids in immunity, fertility, and early prophylaxis of cardiovascular disease in livestock have generated interest in these compounds (Pfander, 1992).

Dietary vitamin A deficiency causes debilitating health problems such as xerophthalmia, corneal lesions, keratomalace, and, in many instances, death (Olson, 1989). Frequent report about these problems affecting young children from developing countries world-wide continue (WHO, 1995).

Since the early 1990s, the main strategy for combating vitamin A deficiency has been to distribute massive dose capsules (Kennedy and Oniango, 1993). However, the same effect could be achieved by consuming sufficient quantities of β-carotene- and vitamin A-rich foodstuffs. This is the safest approach to controlling vitamin A deficiency, and also the most sustainable in rural areas of developing countries where chronic deficiencies are common (Rahmathullah *et al.*, 1990).Food such as dairy and meat products containing preformed vitamin A are often too expensive for most people in developing countries. Therefore, it is important to make more potent and sustainable food sources of pro-vitamin A carotenoids available and improve their production, shelf-life, and consumer acceptance. This could make a tremendous contribution to improving human health.

Sweet potato has been receiving increasing attention from agriculturalists and ecologists interested in developing sustainable food production systems in the tropics, in part because it can grow on soils with limited fertility, is relatively drough tolerant, provides good ground cover, and is usually cultivated without fertilizer or pesticide (Ewell, 1990). Also, it has remarkable pro-vitamin A quantities (Woolfe, 1992). In parts of West, Central, and East Africa, sweet potato is an important staple food source of calories and is consumed by all age groups, but is particulary liked by children, who also are at most risk of vitamin A deficiency (Low *et al.*, 1997). Widely consumed varieties, however, are white or pale yellow in flesh color and contain very little β-carotene (Ameny and Wilson, 1997; Takahata *et al.*, 1993). Orange-fleshed sweet potato storage roots high in carotenoids and vitamin A-active β-Carotene (Purcell, 1962; Purcell and Walter, 1968; Simonne *et al.*, 1993; Takahata *et al*,. 1993) are less eaten. Consumption of orange-fleshed sweet potato roots and sweet potato-based processed foods would provide sustainable, cost-effective, and necessary vitamin A. Therefore, their use as a food source of carotenoids merits further attention.

Sweet potato production in Kenya is concetrated in the western region near Lake Victoria, although the crop is widely grown on a small scale throughout the country. Environmental conditions in these areas differ and this probably affects the nutritional quality of the sweet potato roots. Enviromental and biological stress from different farming sites has been reported to limit sweet potato production (Kays *et al.*, 1992). However, the influence of farming site on carotenoid content has not been conclusively established. While some researchers have noted a significant farming site influence, reports of insignificant variation in carotenoid content between farming sites have also been reported (Hammett, 1974; Woolfe, 1992). It is, therefore, important to investigate whether there is a significant variation in carotenoid content across farming sites.

Sweet potato roots are highly perishable and in East Africa are generally not harvested and stored for extended periods (Karuri and Ojijo, 1994). Instead, farmers piecemeal harvest the crop. The only kind of storage regularly practiced in the region is in-ground storage, by which farmers keep unharvested mature sweet potato in the field until they are needed for consumption or sale (Onwueme, 1982; Smit and Ocitti p'Obwoya, 1994). Some inconclusive reports say that carotenoid content changes during sweet potato storage root growth and development (Abubakar, 1981; Data *et al.*, 1987). Studying variation in carotenoid content, especially pro-vitamin A carotenoid content, during storage root development is relevant in the process of maximizing the availability of that nutrient. Proper recommendations could then be made to farmers to start practicing piecemeal harvesting.

Carotenoids are susceptibe to degradation upon exposure to heat, light, metal ions, acids, and even alkali, because of their highly conjugated structure (Goodwin, 1980; Wong, 1989). Processing and cooking usually expose foods to these degradation agents. Sweet potato roots are normally cooked before consumption; therefore, it is of interest to ascertain whether there is a significant change in their pro-vitamin A value during cooking.

Materials and Methods

Plant Material

Sweet potato cultivars from CIP's pathogen-tested collection (CIP, 1994) with storage root flesh colors ranging from white to orange were selected and used in this study. Apical sweet potato cutting 40 cm long were planted on flat at a spacing of 1.0 x 0.3 m and grown at the farm of the Faculty of Agriculture, University of Nairobi, Kabete. No irrigation, fungicides, or fertilizers were applied to the plots. Seventeen sweet potato cultivars were grown for 6 months and then tested to determine the main carotenoids.

Storage root sampling.

Samples of each cultivar were taken for carotenoid analysis in a two-storage random sampling. Three plants were randomly selected and three medium sized storage roots taken at random from each plant for carotenoid extraction and analysis. Roots were peeled, and about 2-cm-thick medial transverse slices were taken from each roots, and they were finely grated lengthwise using a cheese grater. These samples were thoroughly mixed, packed under nitrogen into plastic bags, and stored at - 20° C until used for carotenoid extraction. Carotenoid extraction was usually done within 10 days.

Root age. The effect of root age on carotenoid content was assessed by a sample of sweet potato roots grown at Kabete at 12, 16, 20 and 24 weeks after planting. At each stage, three plants were randomly selected, and the largest storage roots were piecemeal harvested from each randomly selected plant. Different plants were sampled at each harvest.

Farming site. The effect of farming site was studied using sweet potato storage roots from four cultivars grown for 5 months at three sites: Kabete (altitude 1800m, ambient temperature 20.3+3°C, and rainfall 1046 mm), Kiboko (975 m, 19.3+4° C, and rainfall 595 mm), and Kisii (1,765 m, 19.2 + 4.9 C, and rainfall 1,952 mm).

Cooking. The effect of cooking was evaluated using fresh roots grown for 6 months at Kabete. Unpeeled medium-sized roots from four cultivars were boiled in water for 30, 45, and 60 min. There boiled roots per cultivar were cooled, peeled, and sampled as above described.

Carotenoid Extraction

The extraction procedure from Khachik *et al.* (1992) was used. Two grams of grated root sample were mixed with 4 g anhydrous sodium sulfate, 0.2 g magnesium carbonate, and 10 ml of acetone: petroleum spirit (40-60° C) (PEP; 5:1). Carotenoid extraction was performed under ice to minimize possible thermal degradation and isomerization. Extract was then decanted and filtered through a funnel packed with glass wool into a flask. The residue was reextracted and filtered until the filtrate was devoid of yellow color. Combined filtrate was then partitioned between distilled water and PEP to remove acetone and other aqueous extract components. Organic PEP phase was recovered and dried through a column of anhydrous sodium sulfate and eluant made up to 50 ml with petroleum spirit. Aliquots were taken for spectrophotometric readings and HPLC carotenoid analysis.

Spectrophotometric Determination of Total Carotenoid and ß-Carotene Contents

Total carotenoids and β-carotene were determined spectrophotometrically as described by Imungi and Wabule (1990). A sample of 2-10g of grated storage roots was extracted with 20- ml aliquots of acetone by grinding in a mortar with pestle until the extract was colorless. The combined acetone fractions were transferred to a separatory funnel and mixed with 20-ml aliquots of petroleum spirit (40-60°C) until the petroleum spirit fraction was colorless. The petroleum spirit extract was brought to 100 ml and dewatered by the addition of 5 g anhydrous sodium sulfate. Samples were withdrawn for determination of total carotenoids using a spectrophotometer to measure the absorbance at 450nm. Concentrations were determined by comparison with a standard curve developed using pure β-carotene from Sigma (St. Louis, MO. USA). Then 25 ml of the petroleum spirit extract was concentrated using a rotary vacuum evaporator at 30° C, the residue was dissolved in 1 ml of petroleum spirit and eluted through a silica gel chromatographic column with β-carotene from Sigma as standard. Separation was run using petroleum spirit, and the first yellow fraction, which constituted the β-carotene, was collected and made to 25 ml for the reading of absorbance at 450 nm as above.

HPLC Carotenoid Analysis

One milliter of total carotenoid extract from 2 g of grated sweet potato sample was freeze-dried and reconstituted in the HPLC mobile phase of 90% methanol: 10% tetrahydrofuran. The rcconsituted samples were ultrafiltered through 0.5- µm microfilters before injection into the HPLC system. HPLC analyses were done as described by Ruddat and Will III (1985) at the laboratory of the International Livestock Reserch Institute (ILRI), Nairobi, Kenya. A 200-µl sweet potato extract sample was introduced into the Rheodyne 7010 sample injector by a 250-µl sweet potato extract sample was introduced into the Rheodyne nm. A Pharmacia HPLC with Pharmacia LKB VWM 2141 wavelengh monitor and Pharamacia LKB Gradient Pump ^249 was used. The HPLC column used was a silica-based octadecylsilyl Supelcosil LC-18, 5 um, 25 cm x 4.6 mm ID (pore size 100 A, surface area, 170 m2/g; pore volume, 0.6

ml/g; pH range, 2-7; carbon, 11%; frit pore size, 2μm). Isocratic nonaqueous reverse-phase (NARP) HPLC was used. The mobile phase consisted of 90% methanol: 10% tetrahydrofuran. The HPLC solvents were ultrafiltered using Ultipor Nylon-66 of 0.2-μm and 47-mm-diameter membrane filters. Degassing of the mobile phase by helium sparging for 15 min was done before daily runs to minimize baseline noise by reducing solvent out-gassing at the detector flow cell. The flow rate of the mobile phase was set at 1.5 ml/min. A back pressure of 7.5± 0.5 MPa was maintained during the HPLC runs. Chromatography was done at room temperature.

Standards used to identify different HPLC carotenoid chromatographic peaks were as follows. β-Carotene, α-carotene, lycopene from Sigma; ζ-carotene and β-cryptoxanthin were kindly donated by Drs. W. Schuep and J. Schierle from Hoffmann La Roche, Switzerland; and β-carotene-5,6 monoepoxide and β-carotene-5,6, 5^1, 6^1 - diepoxide were kindly donated by Dr. Peter Molnar from the University of Pecs, Hungary.

Identification of the various HPLC carotenoid peaks was based on consistent retention times and cochromatography. The former method entailed comparison of the HPLC mean retention times of main sweet potato carotenoids and those of pure carotenoid standards. Similarity and identity of sweet potato carotenoids and pure standards were statistically evaluated by *t* test. Cochromatography involved HPLC runs of mixtures of pure carotenoid standards and sweet potato extracts. Equal volumes (25 μl) and concentrations (0.025 μg/L) of carotenoid standards and sweet potato extract were injected into the HPLC and run under standard conditions. The HPLC cochromatographic traces were compared to traces generated by carotenoid standards and sweet potato extract. Elements considered during comparisons were coelution and the number and retention times of eluting carotenoid species. Absolute quantities were derived from peak area with concetration computed from HPLC standard curves of the respective carotenoids. Only provitamin A craotene, and β-carotene,-5,6 monoepoxide were quantified.

Statisical Analysis

One way and two-way ANOVA, Student's test correlation, and regression analyses were computed using Mstat computer statistical package (MSTAT-C, 1991).

Results

Identification of Sweet Potato Carotenoids

HPLC chromatograms (**Figs. 1 and 2**) from sweet potato extracts indicated that six main peaks of carotenoids were significantly present in most cultivars. The mean retention times of these carotenoids were 2.69, 3.75, 5.36, 8.61, 14.60, and 15.44 min (**Table 1**). Comparison of HPLC profiles of carotenoid standards, sweet potato extracts, and cochromatography indicated that three sweet potato carotenoid peaks profiled at 5.36, 8.61, and 14.60 min correlated to the cochromatographic peaks produced by β-carotene- 5,6,5,6- diepoxide, β-carotene-5,6-monoepoxide, and all-trans-β-carotene, respectively (**Table 1**). Carotenoids P1,P2, and P11 from sweet potato did not match with any of the standards used, while P5 was only present along with the standard β-cryptoxanthin.

Carotenoid Content of Sweet Potato Cultivars

Total carotenoid, β-carotene, and β-carotene-5,6-monoepoxide concentrations were significantly different among the 17 cultivars studied. Total carotenoid content ranged from traces of β-carotene equiv/100g fresh root in white-fleshed Naveto to 8.8 mg β-carotene equiv/100 g fresh root in orange-fleshed TIB 11 while β-carotene and β-carotene/ 5,6 monoepoxide contents varied, respectively, from traces to 8.0 mg β-carotene/100 g fresh root, and traces to 0.2 mg β-carotene-5,6 monepoxide/100 g fresh root (**Table 2**). Large intracultivar variation in carotenoid content was noted in carotenoid-rich cultivars like TIB 11, Japon Tresimesino Selecto, unknown, and W-220. The standard deviation was as high as 1.4 mg β-carotene/100 g fresh root in Japon Tresimesino Selecto.

Orange-and yellow-fleshed cultivars recorded higher total carotenoid, β-carotene, and β-carotene-5,6-monoepoxide contents than cream- and white-fleshed cultivars. Orange-fleshed cultivars had a higher percentage of β-carotene to total carotenoids than cream and white-fleshed cultivars (**Table 2**). Orange-fleshed TIB 11 recorded as high as 90% β-carotene as compared to 0.1 % in the white-fleshed Naveto.

HPLC chromatograms from the 17 cultivars studied showed that the unidentified carotenoid (P1) occurred in higher proportion in white-and cream-fleshed cultivars like Naveto, TIS 2534, KSP 11, LM 88.002, and Ex-Diani than in the orange- and yellow-fleshed cultivars. β-carotene- 5,6-monoepoxide, β-carotene-5,6,5,6-diepoxide, and unidentified carotnoid (P2)occured erratically in the tested cultivars.

Effect of Root Age on Carotenoid Content

The effect of root age on carotenoid content was studied for cultivars TIS 2534, KEMB 10, Kakamega 4, and Japon Tresmisino Selecto (**Fig.3**). HPLC chromatographic elutions run after 12, 16, 20, and 24 weeks for the extracts from four cultivars did not show any change in the identity of carotenoids present in the roots. The six main carotenoid peaks discerned in the general survey were present at all stages of root development. There was, however, a difference in peak area, indicating a carotenoid concentration change as roots aged.

Monthly analyses of carotenoid content revealed a significant effect of root age and a significant interaction between cultivar and root age (**Fig.3**). We noted that time taken by cultivars to attain maximum carotenoid content varied. Whereas cultivars like Japon Tresimesino Selecto and Kakamega 4 needed only 20 weeks to reach the highest content in total carotenoids; total carotenoid accumulation in KEMB 10 and TIS 2534 was still going on after 20 weeks. Changes in carotenoid content in relation to root age were not the same in all cultivars. The highest increase in total carotenoid content as a result of age occurred in early root growth between 12 and 16 weeks in cultivars Japon Tresimesino Selecto, KEMB 10, and Kakamega 4, whereas the highest increase in carotenoid content occurred between 16 and 20 weeks in TIS 2534. It was clear that 12-week-old roots contained less carotenoids than older roots in all four cultivars.

Effect of Farming Sites on Carotenoid Content

Total carotenoid content was significantly dependent on farming site and cultivar. Japon Tresimesino Selecto had a higher total carotenoid content in Kisii than elsewhere. The difference in β-carotene content for cultivars grown in Kiboko,

Fig 1. HPLC profiles showing main sweet potato carotenoids from cultivar Kakamega 4 at λ= 450 nm.

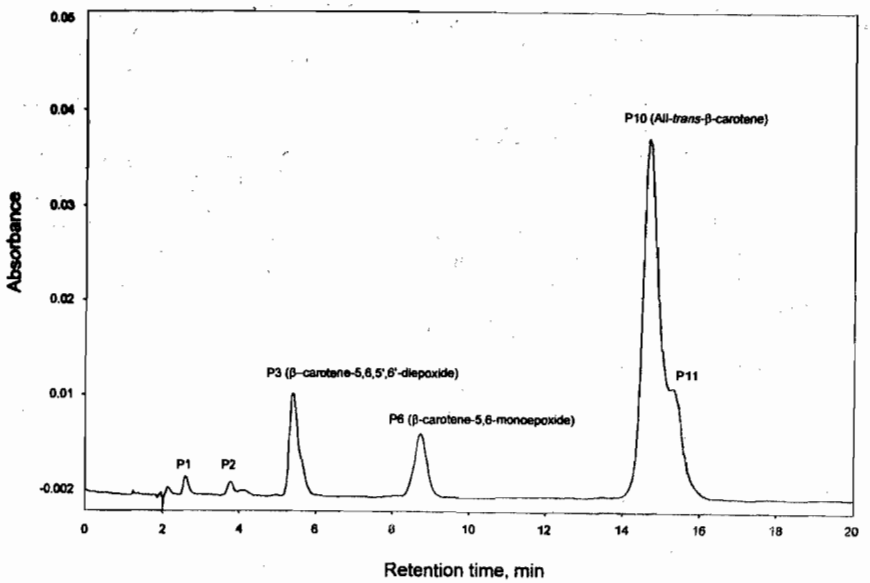


Fig 2. Cochromatography of carotenoid standards and sweet potato extract from Kakamega 4 at λ=450 nm (P1, unidentified; P2, unidentified; P2, unidentified; P3, β-carotene-5-6-5',6'-diepoxide; P4, β-cryptoxanthin; P5, unidentified; P6, β-carotene-5,6-monoepoxide, P7, ζ-carotene; P8, Lycopene; P9, α-carotene; P10, All-trans β-carotene; P11, inidentified).

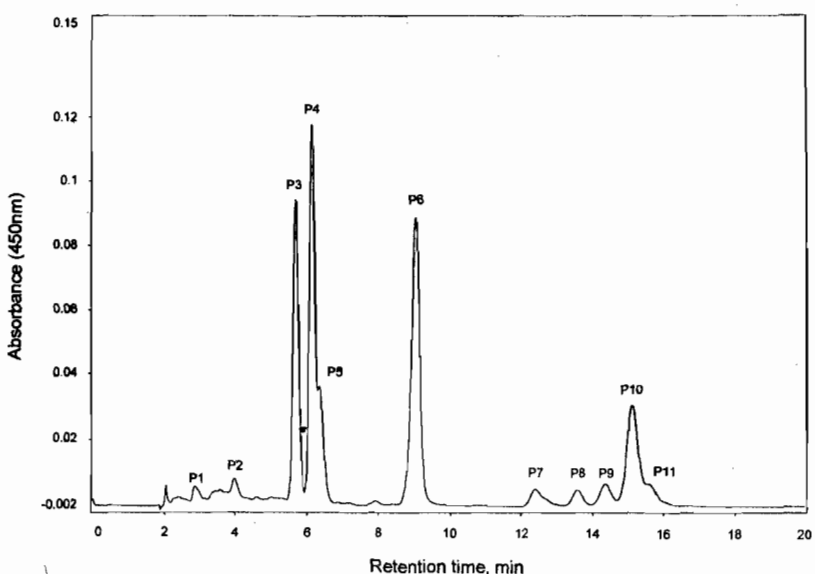
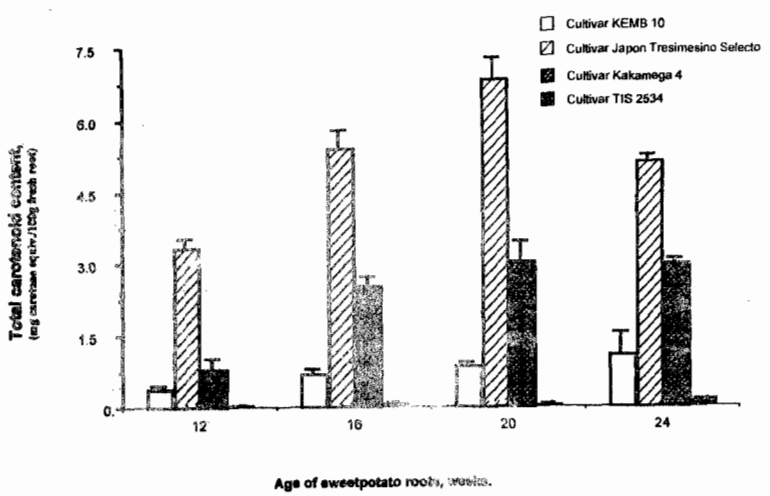


Fig 3. Total carotenoid contents of four sweetpotato cultivars at different root ages.



Kabete, and Kisii was not significant, except for the cultivar TIS 2534 which had a very low value at Kisii (**Table 3**). However, the HPLC profiles of each of the four cultivars used in this experiment were similar across farming sites in terms of number and had identical main carotenoid peaks (results not shown).

Effect of Boiling on Total Carotenoid Content

The effect of boiling on roots was assessed using three different boiling regimes, 30, 45, and 60 min. Boiling for 30 min resulted in a reduction of total carotenoid (**Table 4**). This reduction of total carotenoid content varied: 14% in TIS 2534, 26% in Japon Tresimesino Selecto, 34% in KEMB 10, and 59% in Kakamega 4. However, that reduction was not necessarily exacerbated by increasing the cooking time from 30 to 60 min, especially in Japon Tresimesino Selecto and Kakamega 4 which had the highest levels of initial total carotenoid content. After 1 h of boiling, KEMB 10 lost 57% of its initial total carotenoid content; TIS 2534 lost only 29%.

Discussion

Carotenoids in Sweet Potato Roots

HPLC results indicated that a good number of carotenoids occur in sweet potato root extracts. Six of these were present in significant amounts with the predominance for more than 80% of all-trans-β-carotene in most orange-fleshed sweet potato roots analyzed (**Fig.1**). All-trans-β-carotene, β-carotene-5,6 monoepoxide, β-carotene-5,6,5, 6-diepoxide, and unidentified carotenoid, denoted P1, were present in the 17 cultivars analyzed. The amount was, however, dependent on the cultivar. Comparisons of chromatographic profiles of the sweet potato extracts from different cultivars and the cochromatography (**Fig.2**) with carotenoid identified the presence of all-trans-β-carotene, β-carotene- 5,6-monoepoxide, and β-carotene-5,6,5,6-diepoxide (**Table 1**).

Although we did not use diode array spectrometry to strengthen our results and unequivocally establish the identity of the carotenoid peaks of **Fig.2**, we ran each standarad one by one and the peaks were highly repetitive, suggestive of the homogeneity of the standards and consequently the repetitivity of our procedure.

Table 1. Retention Time of Carotenoid Standards and Sweet Potato Extract during HPLC Cochromatography

Peak Identity	Carotenoid Standards Retention time, min	Sweetpotato extract (Cultivar SPK 004), Retention time, min	Sweetpotato extract + carotenoid standard, Retention time, min
P1 (Unidentified)	-	2.69± 0.10	2.89 ±0.09
P2 (Unidentified)	-	3.75± 0.15	4.05 ±0.07
P3 (β-carotene-5,6,5',6'-diepoxide)	5.28±0.5	5.36± 0.22	5.83± 0.21
P4 (β-cryptoxanthin)	6.30±0.14	-	6.30±0.14
P5 (unidentified)	6.50±0.05	-	6.50±0.05
P6 (β-carotene-5,6-monoepoxide)	9.19±0.08	8.61±0.40	9.00±0.05
P7 (ζ-carotene)	12.42±0.18	-	12.57±0.20
P8 (Lycopene)	13.89±0.20	-	13.68±0.14
P9 (α-carotene)	14.36±0.06	-	14.43±0.07
P10 (All-trans-b-carotene)	15.72±0.49	14.60±0.87	15.21±0.08
P11 (Unidentified)	-	15.44±0.33	15.70±0.19

Table 2. Carotenoid and Vitamin A values of 17 Sweet Potato Cultivars Evaluated at Kabete, Kenya, in 1996.

Cultivar	Flesh Color	Total carotenoid content* (mg/100g fresh roots ±SD)	β-Carotene content* (mg/100g fresh root±SD)	β-Carotene-5,6-monoepoxine content (µg/100g fresh root ±SD)	β-Carotene to Total carotenoids, %	Vitamin A Value (RE/100g fresh root ±SD)
Naveto (CIP440131)	White	<0.1	<0.1	1.5 0.3	0.1	0.1 0.0
LM88.002 (CIP188001.1)	white	0.1 0.0	<0.1	0.1 0.0	4.5	0.9 0.6
KSP 11	White	0.2 0.0	<0.1	<0.1	12.5	3.3 0.3
TIS 2534 (CIP440062)	White	0.1±0.0	<0.1	0.1±0.0	12.1	2.8±0.3
Ex-Diani	White	0.2±0.0	<0.1	0.1±0.1	10.1	3.2±0.6
Phillippine (CIP440160)	Dark cream	0.2±0.0	<0.1	0.3±0.2	3.2	0.9±0.3
TIS 70357 (CIP440078)	Cream	0.2±0.0	<0.1	0.2±0.0	15.8	6.6±1.2
NG 7570 (CIP420053)	White	0.2±0.0	<0.1	0.1±0.0	9.9	3.4±0.8
Capadito (CIP440377)	Pigmented	0.2±00	<0.1	ND	15.0	6.0±1.0
KEMB 10	Cream	0.4±0.0	0.1±0.0	2.3±0.2	39.6	21.1±1.8
Maria Angola (CIP420008)	Pale Orange	0.4±0.0	0.1±0.0	0.5±0.1	28.4	18.5±1.6
Kakamega 4 (SPK 004)	Orange	2.6±0.2	1.5±0.1	68.0±0.0.0	59.0	258.2±23.3
Zapallo (CIP420027)	Pale Orange	4.3±0.0	2.9±0.5	111±19.3	67.7	493.8±80.2
Japon Tresmensino Seleco (CIP420009)	Intermediate orange	5.5±0.3	4.6±1.4	90.2±2.7	82.7	768.4±228.8
Unknown	Pale Orange	7.5±0.7	6.2±0.0	98.5±5.8	83.1	1047.3±15.8
W-220 (CIP440015)	Intermediate orange	8.4±0.4	6.0±0.0	208.9±56.9	71.7	1021.3±82.1
TIB 11 (CIP440057)	Orange	8.8±0.7	8.0±0.3	91.0±4.7	90.8	1338.2±56.9

* Values less than 0.05 mg/100 g fresh root are indicated as 0.0

Table 3. Total Carotenoid and β-Carotene Contents of Four Sweet Potato Cultivars across three testing sites in Kenya.

	Kabete (altitude =1,800 m, temperature = 20.3±3° C, and rainfall = 1,046 mm)		**Kiboko** (altitude =975 m, temperature = 19.3±4° C, and rainfall = 595 mm)		**Kisii** (altitude =1,765 m, temperature = 19.2±4.9° C, and rainfall = 1,952 mm)	
Cultivars	Total carotenoid contents* mg/100 g fresh root ± SD	β-carotene contents* mg/100 g fresh root ± SD	Total carotenoid contents* mg/100 g fresh root ± SD	β-carotene contents* mg/100 g fresh root ± SD	Total carotenoid contents*. mg/100 g fresh root ± SD	β-carotene contents* mg/100 g fresh root ± SD
Japon Tresimesino Selecto (CIP420009)	5.5 ± 0.3	4.6 ± 1.4	5.5± 0.4	5.5± 0.4	7.7 ± 0.3	5.9 ± 0.4
KEMB 10	0.4 ± 0.0	0.1 ± 0.0	0.2± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.1± 0.0
TIS 2534 (CIP440062)	0.1 ± 0.0	<0.1	0.1 ± 0.0	<0.1	0.1 ± 0.1	<0.1
Kakamega 4 (SPK 004)	2.3± 0.1	1.5± 0.1	2.6 ± 0.2	1.5 ± 0.1	2.7± 0.1	1.9 ± 0.2

* Values less than 0.05 mg/100 g fresh root are indicated as 0.0

Table 4. Total carotenoid content of boiled sweet potao storage roots from four cultivars.

Cultivar	Total Carotenoid content* (mg/100g boiled root ± SD)			
	Raw sweetpotato	Roots boiled for 30 min.	Roots boiled for 45 min.	Roots boiled for 60 min.
KEMB 10	0.9± 0.0	0.6± 0.1	0.6 ± 0.0	0.4± 0.1
Kakamega 4 (SPK 004)	3.1± 0.1	1.2± 0.1	1.7± 0.0	1.7± 0.3
Japon Tresimesino Selecto (CIP420009)	6.7± 0.0	5.0± 0.1	6.6± 0.1	6.6± 0.1
TIS 2534 (CIP440062)	0.1± 0.0	0.1± 0.0	0.1± 0.0	0.1± 0.0

* Values less than 0.05 mg/100 g fresh root are indicated as 0.0

P1 predominated in white- or cream-fleshed cultivars like KSP 20, Naveto, and KEMB 10 where it formed a significant proportion of total carotenoids. The possible identity of this carotenoid was able to be postulated on the basis of its elution pattern. Early elution of P1, as well as that of P2, strongly suggest that it is a xanthophII, possibly lutein, and well as that of P2, zeaxanthin. Craft (1992) reported that during nonaqueous reversed-phase HPLC, xanthophyII, which are more polar, partition more preferentially in the polar mobile phase and therefore elute earlier than the less polar carotenes. P5 and P11 were thought to be, respectively, the cis forms of β-chryptoxanthin and β-carotene.

Large variation was observed in carotenoid content among the 17 cultivars studied. This was a reflection of the wide spectrum of the root flesh color of sweet potato. White-flesh roots like those from cultivars NG 7370, TIS 2534, and KSP 20 had the lowest total carotenoid, while orange-fleshed cultivars like TIB 11, Japon Tresimesino Selecto,Kakamega 4, and W-220 had the highest. Our results agree with the conclusion that carotenoids,especially β-carotene, are largely responsible for the orange-flesh color in sweet potato storage roots (De Almeida-Muradian *et al.*, 1992; Gracia *et al.*,1970; Picha, 1995; Takahata *et al.*, 1993). The depth of orange-flesh color was mainly a function of the concentration of all trans β-carotene, as was similarly reported by Simonne *et al.* (1993).

Large variations in carotenoid content were noted in carotenoid-rich cultivars like Japon Tresimesino Selecto and W-220. These variations, also noted by Woolfe (1992), suggest a within-site difference in carotenoid content for cultivars, and their causes are still to be investigated. Low *et al.* 1997) suggested that cultivars having more than 100 μg retinol equivalent per 100g fresh roots were good sources of vitamin A. **Table 2** shows the vitamin A values of the 17 cultivars. Cultivars TIB 11, W-220, Unknown, Japon Tresimesino Selecto, Zapallo, and Kakamega 4 have sufficient levels of retinol equivalents to meet this criteria. Their cultivation, consumption, and utilization in different dishes should be encouraged in combating nutritional vitamin A deficiency.

Our results suggest that the commerical use of carotenoid-rich sweet potato cultivars as sources of β-carotene concentrate can be considered. In fact, 1 kg of sweet potato roots from cultivar TIB 11 can yield about 0.008 g of β-carotene. To produce 1 kg of β-carotene, therefore, about 12,500 kg of roots would be needed. Given that the market price for stabilized, dispersible poweders containing 5-10% of β-carotene is US$ 600, 1 kg of 100% β-carotene can fetch about $12,000. At this market rate, the value of 1 kg of sweet potato roots from cultivar TIB 11 which is currently US$ 0.10-0.25 in Kenya should be economically comopetitive.

Effect of Roots Age

Sixteen- to twenty-week-old roots contained higher carotenoid concentrations than younger roots (Fig. 3.) These differences in total carotenoid content between young and older roots depended on the cultivar. Sixteen-week-old roots from Kakamega 4 were twofold higher in total carotenoid content than 12-week-old roots. Orange-fleshed cultivar Japon Tresimesino Selecto had two-thirds of its total carotenoid content available after just 12 weeks. The concentration of total carotenoid continued to increase up to the 24th week in low-carotenoid-content cultivars TIS 2534 and KEMB 10 (**Fig.3.**) Therefore, to receive the maximum pro-vitamin A benefits from the sweet potato, piecemeal harvesting and consumption of roots from Japon Tresimesino Selecto could begin after 12 weeks, after 16 weeks from Kakamega 4, and after 20 to 24 weeks from the lower carotenoid content cultivars.

There have been conflicting reports on the effects of root age on carotenoid content in sweet potato. Abdel-Kader (1991) and Kimbrough *et al.* (1946) indicated that time of planting and harvesting had no effect on the carotene content. Simpson (1990), Abubakar (1981), and Ezell *et al.* (1952) contended that carotenoid concetration changed with root age. A significant variation noted in this study (**Fig. 3**) suggests that there is a general increment in carotenoid content with sweet potato roots age.

Effect of Farming Site

There was a significant effect from farming site on the concentration of total carotenoids in the four sweet potato cultivars studied. The interaction of cultivars and farming sites also influenced the total carotenoid content. Farming sites, however, did not affect the β-carotene content. Lower total carotenoid content was observed in roots grown in Kiboko. Cultivar TIS 2534 showed large differences in total carotenoid content; Japon Tresimesino Selecto exhibited relatively lower differences. Overall, the difference in total carotenoid content across farming sites was inconsequential in terms of vitamin A value because β-carotene was not affected by farming site (**Table 3**).

Environmental and biological stresses often limit sweet potato production (Keys, 1992). Different farming sites presented different sets of environmental factors that might have exerted a significant effect on carotenogenesis. The effect of soil moisture levels were increased, there was a significant decrease in percentage of dry matter, in total carotenoid pigments of fresh roots, and in root protein content. The observation of Constantin *et al.* (1974), however, was not consistent with

our results; lower total carotenoid content was recorded in Kiboko, a drier site than Kabete and Kisii. Further studies on the relationship between moisture availability and carotenoid content are still needed. Constantin *et al.* (1977) showed that soil nutrients had a variable effect on carotenoid content. Applications of phosphorus (0-74 kg/ha) had no effect on dry matter or carotenoid content. However, potassium has been observed to improve the nutritional value of sweet potato by inducing greater carotene accumulation in the root (Greig and Smith, 1961). Differences in carotenoid content across the farming sites of Kabete, Kiboko, and Kisii could have been attributed to varitations in such nutrients which may have affected carotenogenesis.

Effect of Boiling

Boiling sweet potato roots reduced the carotenoid contents for all the cultivars studied (**Table 4**); the magnitude of reduction was important only for the first 30 min and varied with cultivar. Carotenoid content from cultivar KEMB 10 was highly reduced after boiling for 30 min, whereas orange-fleshed Japon Tresimesino Selecto and Kakamega 4 were less affected by boiling for 60 min. Chandler and Schwartz (1988) noted that carotenoids in cultivars less rich in carotenoids are more susceptible to degradation than richer ones.

Reduction in carotenoid content could be explained by the fact that carotenoids are heat-labile compounds and undergo oxidation and degradation upon exposure to heat, light, acids, peroxides, metals, and enzymes. Carotenoids are easily oxidized because of the large number of conjugated double bonds found in the compounds (Krinsky *et al.*, 1990). β-Carotene formed a significant proportion of total carotenoids in most of the cultivars studied, especially orange- and yellow-fleshed ones. Carotenoid degradation produced as a result of the boiling conditions used in this study could have been cis isomers, epoxide, and lower molecular weight fragments associated with further oxidation. Thermally mediated cis isomerization may not necessarily lead to the complete loss of vitamin A activity of carotenoids. In vivo studies with the ferret model have shown that 9-cis-β-carotene has good bioavailability and is a precursor of 9-cis-retinoic acid, which can be converted to vitamin A (Kays *et al.*, 1992). If such metabolism occurs in human then boiling-associated cis isomerization may not necessarily lead to the comp!ete loss of vitamin A values.

The carotenoid content of roots boiled for 45 min was higher than in roots boiled for 30 min, and the apparent increase in carotenoid content was higher in orange-fleshed Kakamega 4 than in KEMB 10. This apparent increase in carotenoid content upon heating has been attributed to leaching of soils and enhanced extraction (Bradbury and Holloway, 1988; Reddy and Sistrunk, 1980). Heating extricates the hydrophilic matrix of roots and therefore enhance extraction by improving the access of solvents.

Conclusion

A number of carotenoids are present in sweet potato cultivars grown in Kenya. Upto 10 carotenoids occurred in the cultivars studied. Six main carotenoids were present in significant amounts; the rest were present in trace amounts. Of these, three were positively identified: β-carotene-5,6-monoepoxide, β-carotene-5,6,5,6-diepoxide, and all-trans-β-carotene. Significant variation in carotenoid content exists among sweet potato cultivars in Kenya. This variation can be appreciated from the range of flesh colors in different cultivars. Orange-fleshed cultivars are richer in carotenoids and vitamin A value than yellow, cream, and white cultivars. Most of the cultivars preferred by Kenyan farmers and consumers are white- or cream-flesh colored and have a low pro-vitamin A value. Root age exerted a significant effect on carotenoid content and significant interaction between cultivar and root age. The highest increase in carotenoids occurred between 12 and 16 weeks of growth in most cultivars studied.

Farming site had noeffect on β-carotene content and is of no significant nutritional importance.

Boiling for 30 min generally reduced the carotenoid content of sweet potato. Loss of carotenoids was higher in cultivars with a low carotenoid content. Boiling should therefore be minimized for maintaining pro-vitamin A. Prolonged cooking of sweet potato and other pro-vitamin A-rich products reduces the vitasmin A value.

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References

Abdel-Kader, Z. M. (1991). Determination of carotenoids in food by HPLC. Die Nahrung. 35, 689-695.

Abubakar, M. (1981). Effect of harvest dates and intra-row spacing on yield and quality of three sweetpotato cultivars. Dissertation Abstracts of Internation Bulletin, Vol. 41, P. 4335.

Ameny, M. A., and Wilson, P. W. (1997). Relationship between Hunter color and b-carotene content in white-fleshed African sweetpotato. J. Sci. Food Agric. 73, 301-360.

Bradbury, H. J., and Holloway, D. W. (1988). Effect of Cooking and Storage in Sweetpotato. Australian Center for International Agriculture.

Chandler, L.A.,and Schwartz, (1988). Isomerization and losses of trans-β-carotene in sweetpotatoes as affected by processing treatments. J.Agri. Food Chem. 36, 129-133.

CIP (International Potato Center) (1994). Pathogen-Tested Sweetpotato Cultivars for Distribution, 3rd ed. Lima, Peru.

Constantin, R.J., Hernandez, T.P., and Jones, L.G.(1974). Effects of irrigation and nitrogen fertilization on quality of sweetpotato. J.Am. Soc. Hort. Sci. 99, 308-310.

Constatin, R.J.,Hernandez, T.P., and Jones, L.G.(1974). Effects of irrigation and nitrogen fertilization on quality of sweetpotato. J. Am. Soc. Hort. Sci. 99, 308-310.

Craft, N.E. (1992). Carotenoid reversed-phase HPLC methods: Reference compendium. Methods Enzymol.213, 185-205

Data, E.S., Villamayor, F.G., Jr., Reoma, and Anzano. D.R. (1987). Yield and chemical composition of cassava and sweetpotato at different ages of harvest. Philippine J. Crop Sci. 12,44.

De Almeida-Muradian, L.B., De Penteado, M.V.C., and De Ferreira, V.L.P. (1992). Relationship between carotenoid content and hunter colour parameters of Brazilian sweetpotato. Rev. Espanol. Ciencia Technol. Alimen. 36,611-619.

Ewell, P.T. (1990). Sweetpotato in Eastern and Southern Africa Paper presented at the workshop on sweetpotatoes in the food systems of Eastern and Southern Africa, Nairobi, Kenya.

Ezell, B.D., Wilcox, M.S., and Crowder, J.N. (1952). Pre-and post-harvest changes in carotene, total carotenoids and ascorbic acid content of sweetpotatoes. Plant Physiol. 27, 355-369.

Garcia, E.H., Querido, I.B., and Cahanop, A.C. (1970). The relationship of carotene and starch content of some twenty-six varieties of sweetpotatoes (Ipomoea carotenoids. Int. J. Vitam. Nutr. Res 63, 93-121

Goodwin. T.W. (1980). Chemistry and Biochemistry of Plant Pigments. Academic Press London, UK.

Greig, J.K., and **Smith, F.W. (1961).** Sweetpotato growth, accumulation and carotenoid content affected by cation level in the growth medium. Proc. Am. Soc Hort. Sci. 77, 463-472.

Hammett, H.L (1974) Total carbohydrate and carotenoid content of sweetpotato as affected by cultivar and area of production. HorScience 9,467-468.

Imungi, J.K., and Wabule, M.N. (1990). Some chemical characteristics and availability of vitamin A and vitamin C from Kenya varieties of papaya (Carica papaya L.). Ecol. Food Nutr. 24,115-120.

Karuris, E.G., and Ojijo, N.K.O. (1994). Storage studies on sweetpotato roots: Experiences with KSP20 cultivar. Acta Hort. 368, 441-452.

Kennedy, E.T., and Oniango, R. (1993). Household and preschooler Vitamin A consumption in southwestern Kenya. J.Nutr. 123, 841-846.

Kays, S.J. **(1992).** The chemical composition of sweetpotato. In Sweetpotato Technology for the 21st Century (W.A.Hill, C.K. Bonsi,and P.A.Loretan, Eds). Tuskegee Univ., AL.

Khachik, F., Beecher, G.R., Goli, M. M., and Lusby, W.R. (1992). Separation and quantitation of carotenoids in foods. Methods Enzymol. 213, 347-359.

Kimbrough, W.D., Fieger, E.A., and Lewis, H. (1946). Effects of date of planting and time of harvesting on the carotene of sweetpotato of the Puerto Rico variety. Proc. Am. Soc. Hort. Sci. 47, 400-402.

Krinsky, N.I., Mathews-Roth , M.M., and Taylor, R.F. (1990). Carotenoids: Chemistry and Biology. Penum Press, New York.

Low, J., Kinyae, P., Gichuki, S., Oyunga, M.A., Hagenimana, V., and Kabira, J. (1997). Combating Vitamin A Deficiency throught the Use of Sweetpotato. Results form Phase I of an action research project in South Nyanza, Kenya. International Potato Center, Lima, Peru.

MSTAT-C Program (1990). A software program for the design, management, and analysis of agronomic research experiments. Michigan State University, MI.

Olson, J.A. (1989). Biological action of carotenoids introduction. J. Nutr. 119, 94-95.

Onwueme, I.C. (1982). The Tropical Tuber Crops: Yams, Cassava, Sweetpotato, and Cocoyams. English Language Book Society and John Wiley and Sons, Chichester, U.K.

Pfander, H (1992). Carotenoids: An overview. Methods Enzymol. 213, 3-13.

Picha, D. H. (1985). Crude protein, minerals and total carotenoids in sweetpotatoes. J.Food Sci. 50, 1768-1769.

Purcell, A.E., and Walter, W.M.,Jr. (1968). Carotenoid of Centennial variety of sweetpotato.J. Agric. Food Chem. 16, 769-770.

Rahmathullah, L., Underwood, B.A., Thulasiraj, R.D., Milton, R.C., Ramaswamy, K., Rahmathulla, R., and Babu, G. (1990). Reduced mortality among children in southern India receiving a small weekly dose of vitamin A. N. Engl. J.Med. 323, 929- 935.

Reddy, N.W., and Sistrunk, W.A. (1980). Effect of cultivar, size, storage and cooking method on carbohydrates and some nutrients of sweetpotatoes. J. Food Sci, 45, 682-684.

Ruddat, M.,and Will III, O. H. (1985). High perfomance liquid chromatography of carotenoids. Methods Enzymol. 111, 189-200. Simon, P. W. (1997). Plant pigments for color and nutrition. HortScience 32, 12-13.

Simonne, A. H., Kays, S.J., Koehler, P.E., and Eitenmiller, R.R. (1993). Assessment ofβ-carotene content in sweetpotato breeding lines in relation to dietary requirements. J.Food Compos. Anal. 6, 336-345.

Simpson, L.K. (1990). Use of food composition tables for retional and provitamin A carotenoid content. Methods Enzymol. 190, 237-241.

Smit, N.E., and Ocitti P'Obwoya, C.N. (1994). Piecemeal harvesting of sweetpotato: Its effect on yield loss due to sweetpotato weevils. Paper presented at the Third Triennial Conference of the African Potato Association, 9-13 May 1994, Sousse, Tunisia.

Takahata, Y., Noda, T., and Nagata, T. (1993). HPLC determination of β-carotene content in sweetpotato cultivars and its relationship with color value. Japan. J. Breed. 43, 421-427.

WHO (1995), World Health Organisation Micronutrient Deficiency Information System. Global Prevalence of Vitamin A Deficiency, MDIS working paper 2. WHO/NUT/ 95.3, Geneva.

Wong, D.W.S. (1989). Mechanism and Theory in Food Chemistry. Van Nostrand Reinhold, New York. Woolfe, J.A. (1992). Sweetpotato: An Untapped Food Resource. Cambridge University Press, Cambridge, UK.