

Full Length Research Paper

# All trans-cis $\beta$ -carotene content of selected sweet potato (*Ipomoea batatas* (L) Lam) varieties as influenced by different levels of nitrogen fertilizer application

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Accepted 3 February, 2011

All trans-cis isomers content of sweet potatoes (*Ipomoea batatas* (L) Lam) as influenced by different levels of nitrogen fertilizer application were determined by high performance liquid chromatography (HPLC). The nitrogen fertilizer treatments were four nitrogen levels of 0 (control), 40, 80, 120 kg N/ha and four varieties of sweet potatoes: White fleshed TIS87/0087 and TIS8164, orange-fleshed, Ex-Igbariam and CIP Tanzania. The study area was Umudike Southeast Nigeria, located at latitude 05°29'N and longitude 07°33'E, and at elevation of 122 m above sea levels. Nitrogen fertilizer significantly ( $P < 0.05$ ) increased trans-cis isomers of  $\beta$ -carotene with incremental nitrogen fertilizer application up to 80 kg N/ha. TIS87/0087 and Ex-Igbariam varieties gave the highest trans-cis isomers of  $\beta$ -carotene at 40 to 80 kg N/ha when compared with the control (0 kg N/ha). Nitrogen fertilizer application above 80 kg N/ha did not increase  $\beta$ -carotene yield significantly ( $P > 0.05$ ) for the varieties studied except CIP Tanzania.  $\beta$ -carotene (trans-cis isomers) has the potential to improve vitamin A status among the vulnerable groups in Southeast Nigeria. Sweet potato being a stable crop in Southeast Nigeria can be effectively used as a vehicle for improving the Vitamin A intake in this ecological zone through biofortification with  $\beta$ -carotene. With nitrogen fertilizer increasing sweet potato production in this ecological zone, the evaluation of the trans-cis isomers of  $\beta$ -carotene content was a major objective.

**Key words:**  $\beta$ -carotene, trans-cis isomers, sweet potato varieties, nitrogen fertilizer application.

## INTRODUCTION

Sweet potato (*Ipomoea batatas* (L) Lam) belongs to the botanical family Convolvulaceae (morning glory family). It is a perennial crop that is usually grown annually. It grows from underground tuberous roots with trailing, twisting stems that can be as long as six meters. This tuber crop provides food to much of the world's population, occupying the position of the seventh most important food crop in the world by the beginning of the

21st century (Woolfe, 1992). This root crop has a long history of saving lives. It matures fast, rich in nutrients and is often the first crop to be planted after a natural disaster to provide abundant food supply to the population (Food and Culture Encyclopedia, 2003). The bulk of this staple crop is grown and consumed in the tropics (Kochlar, 1981). In Nigeria, sweet potato is an important part of the peoples diet, eaten boiled, fried for breakfast or as sweet potato chips (Aniedu and Oti, 2007). The National Root Crop Research Institute, Umudike, Southeast Nigeria, holds the sweet potato germplasm with over 50 local and CIP improved varieties in Nigeria.

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In the Southeastern population of Nigeria, like in other developing regions, vitamin A deficiency is prevalent affecting mostly children and women (Sight and Life, 2007). This problem has necessitated the recent emphasis by the Government of Nigeria on the fortification of essential food commodities (flour, vegetable oil and sugar) with vitamin A.

Some cultivars of sweet potato are high in pro-vitamin A,  $\beta$ -carotene (Almeida-Muradian and Penteadó, 1992). Concentration of  $\beta$ -carotene ranges from 0.2  $\mu$  g/g (white-fleshed sweet potato), Nepal (Vaida, 1995) to 218  $\mu$  g/g (orange fleshed sweet potato), Brazil (Almeida-Muradian and Penteadó, 1992).  $\beta$ -carotene is a potent vitamin A with 100% source of pro-vitamin A bioactivity and has been linked to immune function and decreased the risk of degenerative diseases (Oslo, 1999; Buri, 1997; Mayne, 1996). Vitamin A is also known to play crucial roles in vision, cellular differentiation and morphogenesis, haemopoiesis, skeletal growth and fertility in man (McLaren and Frigg, 2001).

Pro-vitamin A is required for the differentiation of epithelial cells, production of goblet cells and maintenance of an effective epithelial barrier against attack by pathogenic organisms. The all trans- isomer of  $\beta$ -carotene is the most useful form to the human body. The all trans  $\beta$ -carotene exhibits the highest pro-vitamin activity among carotenoids and is the usual configuration found in nature (Bovell- Benjamin, 2007). It can be isomerised to the cis-isomers on contact with acids, heat treatment and exposure to light. On isomerization, trans-carotenoids are converted to cis-carotenoids which are more readily oxidized to epoxy carotenoids, apo-carotenoids and hydroxycarotenoids. Compared to most staple root crops and vegetables, sweet potato has high protein content ranging from 4.41 to 16.11% (Ravindran et al., 1995; Grant et al., 1992 ) and vitamin C content ranging from 17.1 to 24.6 mg/100 g (US Dept of Agric., 2001).

Due to the importance of  $\beta$ -carotene to health and nutrition, four varieties of sweet potato, namely, CIP Tanzania and Ex-Igbariam (orange-fleshed), TIS 87/0087 and TIS 8164 (white-fleshed) are obtained from National Root Crop Research Institute, Umudike, Nigeria and four levels of nitrogen fertilizer application (0, 40, 80 and 120 kg N/ha) were evaluated within Umudike in southeast agro ecological zone. The purpose was to give focus to breeding efforts in sweet potato research and potentially improve the vitamin A status of the consumers. Analysis were performed according to HarvestPlus protocol using HPLC (Rodriguez-Amaya and Kimura, 2004) with the view to determining the all tran-cis  $\beta$ -carotene content of the sweet potato varieties.

## MATERIALS AND METHODS

### Experimental design

The experimental field was marked, ploughed and harrowed. A 4 x

4 factorial arrangement in a randomized complete block design (RCBD) was used. Four varieties of sweet potato (TIS87/0087, TIS8164, Ex-Igbariam and CIP Tanzania) and four levels of nitrogen fertilizer (0, 40, 80 and 120 kg N/ha) were the treatments. Sweet potato vine cutting of each variety (20 cm) were planted at a spacing of 1cm x 0.3 m. Nitrogen was applied as urea and each plot received a blanket application of 25 kg N/ha single super phosphate and 150 kg K/ha as muriate of potash. This fertilizer application was made four weeks after planting. Weeding was done once by hand pulling at 6 weeks after planting. The sweet potato storage root was harvested 16 weeks after planting. The study area was Umudike, southeast Nigeria, located at latitude 05°29'N and longitude 07°33'E, and at an elevation of 122 m above sea level. This soil is classified as sandy loam, acidic and characterized as an ultisol (Eke-Okoro, 2001).

## ANALYSIS OF $\beta$ -CAROTENE (HARVESTPLUS METHOD)

### Sample preparation

The method of Rodriguez-Amaya and Kimura (2004) was used. Freshly harvested sweet potato samples (from each variety) were washed with clean water, peeled, washed and quartered longitudinally. They were sliced to 1cm thickness and mixed manually. The samples were packaged in aluminum foil, labeled and stored at -80°C for subsequent trans- cis isomers of  $\beta$ -carotene analysis using HPLC.

## CAROTENOID EXTRACTION

Duplicates of the -80°C frozen and thawed samples were used for the analysis under a UV-filtered white fluorescent lighting to avoid carotenoid oxidation (Howe and Tanumihardjo, 2006). Carotenoids were extracted by grinding about 3 g of each sample in a mortar using pestle with about 50 ml of cold acetone. The residue was filtered in Butcher funnel equipped with filter paper (Whatman # 2 filter paper, Maidstone, England). The residue was returned to the mortar and the extraction was repeated using 20 ml of cold acetone until the residue was nearly colorless. The total extract was transferred to a separating funnel (250 ml) containing 20 ml of petroleum ether. One liter of distilled water was used to wash the organic phase which separated from the aqueous phase. The aqueous phase was discarded.

The organic phase was again washed with dilute brine solution to break-up any emulsion that may have formed. The organic phase was collected through anhydrous sodium sulphate (15 g) into 25 ml flat bottom flask. Ten (10 ml) of the sample extract was concentrated with a rotary evaporator (Buchi Waterbath B-481 Switzerland) and dried under vacuum for reverse-phase HPLC determination of the isomers of  $\beta$ -carotene at 450 nm.

### Analysis of trans-cis isomers of $\beta$ -carotene using reverse-phase HPLC

The analysis of all tran-cis  $\beta$ -carotene of the sweet potatoes using HPLC was adopted from published procedures (Howe and Tanumihardjo, 2006; Bushway, 1986). The concentrated and dried sample were reconstituted in methanol dichloroethane (1000  $\mu$ l, 50: 50 v/v) and injected (10  $\mu$ l) in to the HPLC. The HPLC consisted of a guard column, C30 YMC carotenoids column ( 4.6 x 2 50 mm, 3  $\mu$ m), 626 HPLC pump, 717 auto sampler, and a 2996 photodiode array detector ( Waters Corporation, Milford, M.A). Solvent A consisted of 100% methanol.

Solvent B consisted of 100% methyl tert-butyl ether. The isocratic elution was 50% of solvent A and 50% of solvent B at 1 ml/min for

**Table 1.** Effect of nitrogen concentration on trans-cis isomers of  $\beta$ -carotene ( $\mu$  g/g) in each sweet potato variety.

Isomer	Variety	0	40	80	120	LS D
9- cis	CIP Tanzania	0.875 <sup>b</sup> $\pm$ 0.021	0.59 <sup>d</sup> $\pm$ 0.028	0.76 <sup>c</sup> $\pm$ 0.042	1.71 <sup>a</sup> $\pm$ 0.042	0.0967
	Ex-Igbariam	1.84 <sup>b</sup> $\pm$ 0.184	3.945 <sup>a</sup> $\pm$ 0.035	3.865 <sup>a</sup> $\pm$ 0.714	0.76 <sup>c</sup> $\pm$ 0.042	2.22842
	TIS 87/0087	1.595 <sup>c</sup> $\pm$ 0.007	3.63 <sup>a</sup> $\pm$ 0.212	2.625 <sup>b</sup> $\pm$ 0.035	2.10 <sup>b</sup> $\pm$ 0.191	0.3993
	TIS 8164	1.87 <sup>b</sup> $\pm$ 0.283	2.26 <sup>b</sup> $\pm$ 0.127	2.91 <sup>a</sup> $\pm$ 0.304	0.595 <sup>c</sup> $\pm$ 0.035	0.6049
13-CIS	CIP Tanzania	1.03 <sup>b</sup> $\pm$ 0.183	1.00 <sup>b</sup> $\pm$ 0.014	1.18b $\pm$ 0.042	1.53a $\pm$ 0.057	0.2741
	Ex-Igbariam	1.445 <sup>b</sup> $\pm$ 0.050	2.66 <sup>a</sup> $\pm$ 0.042	2.51 <sup>ab</sup> $\pm$ 0.389	1.795 <sup>ab</sup> $\pm$ 0.686	1.0982
	TIS87/0087	1.46 <sup>d</sup> $\pm$ 0.042	2.595 <sup>b</sup> $\pm$ 0.007	3.22 <sup>a</sup> $\pm$ 0.156	1.77c $\pm$ 0.085	0.2531
	TIS8164	1.695 <sup>a</sup> $\pm$ 0.36	1.46 <sup>a</sup> $\pm$ 0.00	1.965 <sup>a</sup> $\pm$ 0.134	0.53 <sup>b</sup> $\pm$ 0.028	0.5356
15-CIS	CIP Tanzania	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.00
	Ex-Igbariam	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.00
	TIS87/0087	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.00
	TIS8164	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.29 <sup>a</sup> $\pm$ 0.00	0.00
Trans	CIP Tanzania	3.825 <sup>a</sup> $\pm$ 0.163	1.140 <sup>c</sup> $\pm$ 0.00	2.39 <sup>b</sup> $\pm$ 0.156	3.73 <sup>a+</sup> $\pm$ 0.354	0.5817
	Ex-Igbariam	3.505 <sup>a</sup> $\pm$ 0.191	6.125 <sup>a</sup> $\pm$ 0.106	6.025 <sup>a</sup> $\pm$ 1.633	4.32 <sup>a</sup> $\pm$ 1.612	3.1999
	TIS87/0087	3.325 <sup>c</sup> $\pm$ 0.064	6.81 <sup>b</sup> $\pm$ 0.707	11.33 <sup>a</sup> $\pm$ 1.089	4.275 <sup>c</sup> $\pm$ 0.205	1.8266
	TIS8164	3.195 <sup>b</sup> $\pm$ 0.516	4.27 <sup>a</sup> $\pm$ 0.283	4.61 <sup>a</sup> $\pm$ 0.325	0.86 <sup>c</sup> $\pm$ 0.071	0.9386
Total	CIP Tanzania	6.02 <sup>b</sup> $\pm$ 0.368	4.62 <sup>c</sup> $\pm$ 0.156	3.02 <sup>d</sup> $\pm$ 0.028	7.26 <sup>a+</sup> $\pm$ 0.339	0.7284
	Ex-Igbariam	7.075 <sup>a</sup> $\pm$ 0.417	13.02 <sup>a</sup> $\pm$ 0.184	12.685 <sup>a</sup> $\pm$ 2.737	8.50 <sup>a</sup> $\pm$ 3.78	6.5035
	TIS87/0087	6.67 <sup>d</sup> $\pm$ 0.099	13.33 <sup>b</sup> $\pm$ 0.905	18.105 <sup>a</sup> $\pm$ 1.124	8.96 <sup>c</sup> $\pm$ 0.32	2.0582
	TIS8164	7.05 <sup>b</sup> $\pm$ 1.150	8.275 <sup>ab</sup> $\pm$ 0.148	9.765 <sup>a</sup> $\pm$ 0.771	2.28 <sup>c</sup> $\pm$ 0.141	1.9535

<sup>A b c</sup> means  $\pm$  SD in the same row with similar superscript are not significantly different ( $P > 0.05$ ).

15 min. Isomers of  $\beta$ -carotene were eluted between 6 to 8 min. Chromatograms were generated at 450 nm. Identification of trans-cis isomer of  $\beta$ -carotene were done using standards and with the verification of their absorption spectrum.

#### Standard reagents used

The following standard reagents were used for the analysis:

1. Acetone reagent (ACS) 99.5%, GFS Chemicals, Mekinley Ave. Columbus
2. Petroluem ether, 40 to 60°C, Sigma-Aldrich, St Louis, USA.
3. Sigma carotene, from carrots, minimum 95% HPLC, Stein Hein, Germany.

These reagents were supplied from the biochemistry laboratory of IITA, Ibadan, Nigeria.

#### Statistical analysis and calculations

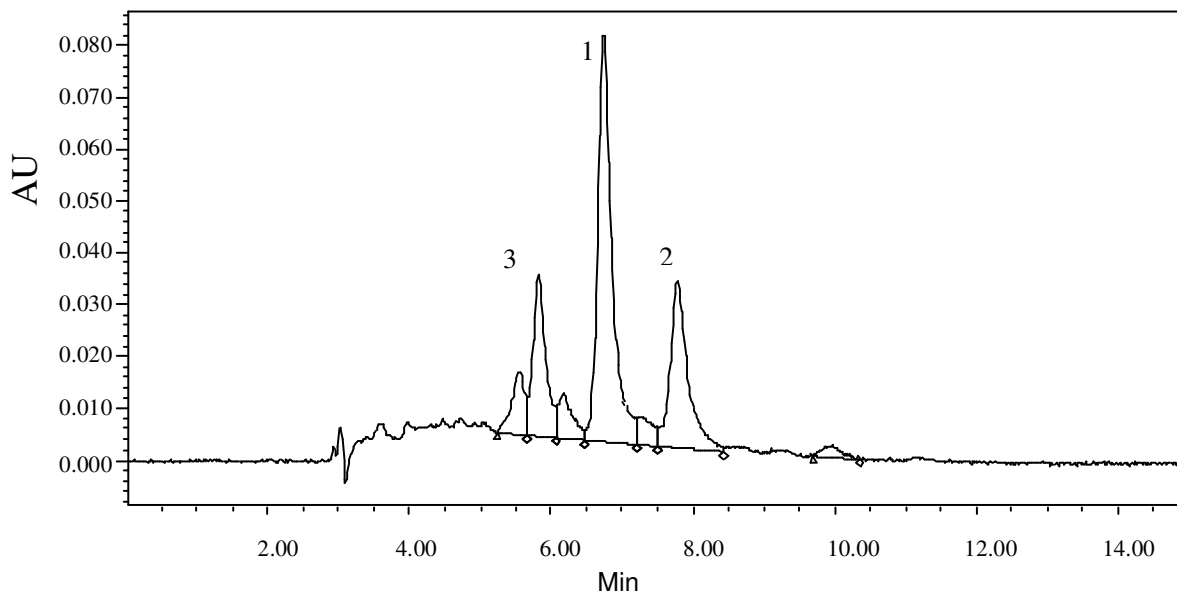
The mean, standard deviation and analysis of variance (ANOVA) of the data obtained from the study were computed using Statistical Package for Social Science (SPSS) version B. Means were separated using least significant difference test (LSD) at  $P < 0.05$ . Analysis of variance (ANOVA) was specifically performed to check for significant difference ( $P < 0.05$ ) between means.

## RESULTS AND DISCUSSION

Table 1 shows the effect of nitrogen concentration on the trans-cis isomers of  $\beta$ -carotene in the sweet potato varieties. The main carotenoid identified in the sweet potato varieties was pro-vitamin A carotenoid, ( $\beta$ -carotene) in its trans-cis isomers, namely:

all-trans, 9-cis, 13-cis and 15-cis  $\beta$ -carotene isomers.

Trans- $\beta$ -carotene had the highest concentration in all four varieties followed by 9-cis- $\beta$ -carotene and 13-cis- $\beta$ -carotene respectively. The isomerization of the trans-carotenoid, the natural configuration, to the cis-isomers may have been promoted by heat generated during carotenoid extraction. The effect of different levels of nitrogen fertilizer application from 0 to 80 kg N/ha produced a significant ( $P < 0.05$ ) difference with increasing values of trans-cis isomers of  $\beta$ -carotene with the exception of CIP Tanzania (Table 1). At zero (0 kg N/ha) level of nitrogen fertilizer concentration, the total  $\beta$ -carotene and the trans-cis isomers of  $\beta$ -carotene maintained closely the same value for the different varieties of sweet potato (Table 1). The value of the geometrical all trans isomer of  $\beta$ -carotene increased



**Figure 1.** Chromatogram of Ex-Igbariam cultivar at 40 Kg N/ha.

progressively for Ex-Igbariam from 3.5 (0 kg N/ha) to 6.025  $\mu$  g/g (80 kg N/ha), TIS87/0087 increased from 3.3 (0 kg N/ha) to 11.3  $\mu$  g/g (80 kg N/ha) while TIS8164 increased from 3.2 (0 kg N/ha) to 4.6  $\mu$  g/g (80 kg N/ha) respectively. For the cis geometrical isomers, 9 and 13 cis  $\beta$ -carotene, similar increases from 0 to 80 kg N/ha was maintained. This resulted to the higher  $\beta$ -carotene yield for Ex-Igbariam (13.02  $\mu$  g/g) at 40 kg N/ha, TIS87/0087 (18.10  $\mu$  g/g) at 80 kg N/ha and TIS8164 (9.76  $\mu$  g/g) at 80 kg N/ha (Figure 1).

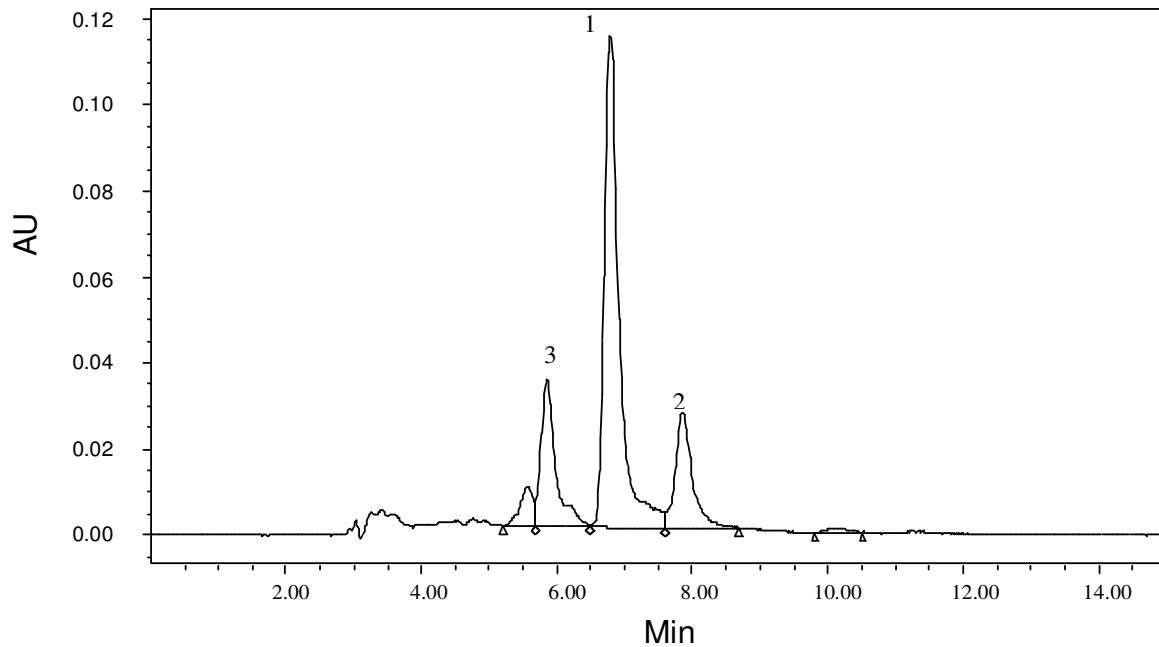
Above 80 kg N/ha, there was a decline in the all-trans-cis- $\beta$ -carotene content of the sweet potato varieties with the exception of CIP Tanzania. This trend established that the total  $\beta$ -carotene and all-trans-cis isomers of  $\beta$ -carotene yield are better at 40 to 80 kg N/ha (Table 1). The  $\beta$ -carotene yield from these varieties of sweet potatoes confirms the  $\beta$ -carotene values from sweet potato varieties common to Africa. Low (2007), of the International Sweet potato Center, noted that the  $\beta$ -carotene content of sweet potato common to Africa ranged from 100 to 1,600  $\mu$  gRAE/100g, thus agreeing with the  $\beta$ -carotene values obtained in this work for most of the varieties. The implication of this result is that nitrogen fertilizer application for optimum  $\beta$ -carotene yield will depend on each variety and environmental variations (Villagaria, 1999).

This may be the reason TIS87/0087, (a white-fleshed, and improved elite variety) and Ex-Igbariam, (a local orange-fleshed variety) with high nitrogen response ability and high photosynthetic activity (Okon, 2006) yielded higher  $\beta$ -carotene and trans-cis  $\beta$ -carotene values than CIP Tanzania and TIS8164 varieties. CIP Tanzania was observed to have the least  $\beta$ -carotene concentration at 40-80 kg N/ha due probably to low nitrogen response

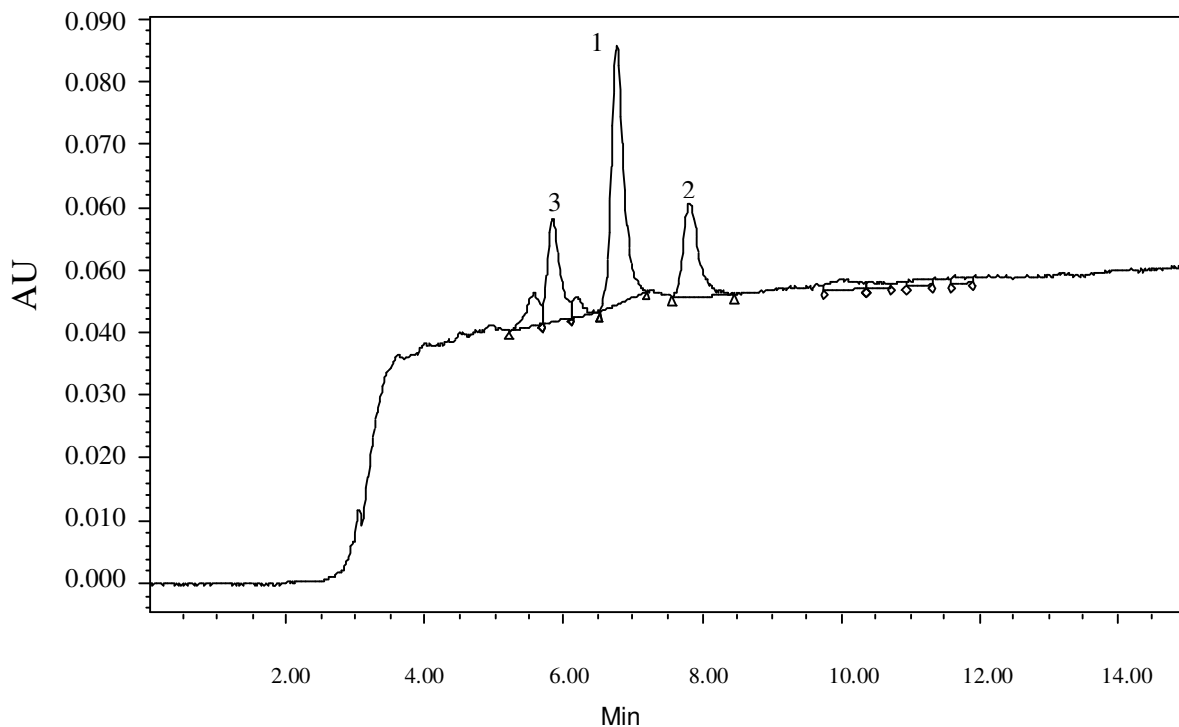
and poor environmental adaptation.

Sweet potato has an adequate to excellent bioavailability of  $\beta$ -carotene, a pro-vitamin A, with a 100% vitamin A activity (Rodriguez-Amaya and Kimura, 2004). The requirement for vitamin A activity is an un-substituted  $\beta$ -ring with an 11-carbon polyene chain. The rapidly growing world population may rely on dietary pro-vitamin A carotenoids such as  $\beta$ -carotene derived from staple food sources like sweet potato to maintain Vitamin A functions in the body (Von-Lintig, 2007). This was supported by Low (2007) who showed that orange-fleshed sweet potato eaten by young children in Zambazia (Mozambique) had a positive impact on vitamin A status of the children during her assessment period.  $\beta$ -carotene is also important because it is an effective natural antioxidant.  $\beta$ -carotene has the potential to act as a lipid-soluble chain breaking antioxidant to quench singlet oxygen and interact with free radicals to prevent oxidative stress (Palozza and Krinsky, 1992). This action has the effect of lowering the risk of chronic diseases such as cardiovascular diseases (Mayne, 1996). McLaren and Frigg (2001) also contributed that vitamin A plays a crucial role in vision, cellular differentiation and morphogenesis, haemopoiesis, skeletal growth and fertility in man.

Besides, Pro-vitamin A is important for epithelial cells differentiation, goblet cells production and the maintenance of an effective epithelial barrier against attack by pathogenic organisms. This research finding (especially for Ex-Igbariam and TIS87/0087) has identified locally developed sweet potato varieties with immense potentials for the supply of  $\beta$ -carotene and energy, thus enhancing vitamin A status and food security in the Southeast Zone of Nigeria.



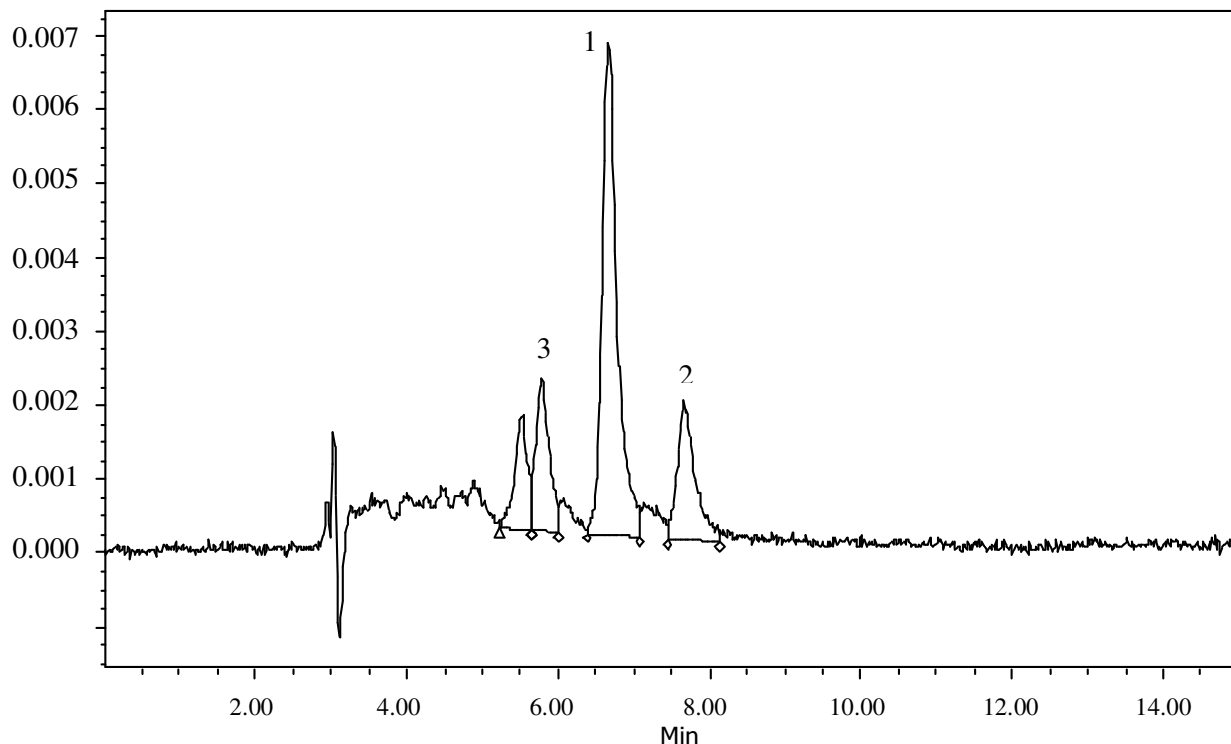
**Figure 2.** Chromatogram of TIS87/0087 cultivar at 80 KgN/ha.



**Figure 3.** Chromatogram of CIP Tanzania cultivar at 40 Kg N/ha.

The chromatograms for the carotenoids of the four sweet potato varieties are shown in Figures 1, 2, 3 and 4 respectively. CIP Tanzania (in particular) had many minor peaks whose carotenoids other than  $\beta$ -carotene could not

be identified due to lack of internal standards. This collaborates with other research findings which attributed about 36% of total carotenoid to other minor carotenoids in some sweet potato varieties (Rodriguez-Amaya and



**Figure 4.** Chromatogram of TIS 8164 cultivar at 40KgN/ha. Peaks are (1) Trans- $\beta$ -carotene, (2)9-cis  $\beta$ -carotene, (3)13-cis  $\beta$ -carotene.

Kimura, 2004; Almeida and Penteado, 1992). The peak areas for Ex-Igbariam and TIS870087 are larger than the peak areas for CIP Tanzania and TIS8164. This may directly accounted for higher carotenoid concentrations in Ex-Igbariam and TIS87/0087 than in CIP Tanzania and TIS 8164. This trend has thus established that Ex-Igbariam and TIS87/0087 are the best quantitative  $\beta$ -carotene cultivars. At a fertilizer application level of 40 kg N/ha, the all trans- $\beta$ -carotene level of TIS87/0087 was highest. In addition to the identified peaks, Ex-Igbariam had two other broad but minor peaks that were unidentified (Figure 1). TIS87/0087 and CIP Tanzania had at least one minor but broad peak in each case (Figures 2 and 3) while TIS 8164 potato variety had at least two unidentified peaks (one broad peak and another sharp peak).

## Conclusion

Nitrogen fertilizer application to sweet potato production significantly affected the all tran-cis isomers and total  $\beta$  carotene yield particularly at 40 to 80 kg N/ha. The high  $\beta$ -carotene yielding varieties are Ex-Igbariam and TIS87/0087. At 13.33 to 18.10  $\mu$  g/g (~1333 to 1810  $\mu$  g/100g) of  $\beta$ -carotene bioavailability from Ex-Igbariam and TIS87/0087 varieties of sweet potato,  $\beta$ -carotene can potentially improve the vitamin A status of the vulnerable

groups. This result can direct breeding and production needs of sweet potato cultivars in Southeast Nigeria. The precision of the analysis at 90% and above using coefficient of variation limited the errors in carotenoids extraction and  $\beta$ -carotene concentration.

## ACKNOWLEDGEMENT

The authors are grateful to Sir E. A Nwauzor and Mr J. I Chukwu of the sweet potato Programme, National Root Crop Research Institute, Umudike, Nigeria for supplying the sweet potato cultivars.

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