Extraction of Carotenoides in Whiteflesh Sweet-Potatoes (*Ipomena batatas L*)

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Abstract

Whiteflesh sweetpotatoes (Ipomea batatas L) are an important source of b - carotene a precursor of vitamin A. A study was carried out to evaluate the carotenoid content of various white flesh sweet potatoes. The evaluation was done by spectrometric analyses, reverse phase HPLC and confirmation by mass spectrophotometry. b- carotene, a- carotene and canthaxanthin were identified by isocratic reverse phase HPLC. Total carotenoids ranged from 0 *ug*/100g to 260 *ugl* 100g (fwb). On processing into puree, there was reduction in total carotenoids, from 20 to 250 *ugl* 100g. The value of b - carotene ranged from 0 to 146ug / 100g (fwb). The presence of carotenoids were confirmed by mass spectroscopy and found to have m/z value of 537.8, 537.8 and 565.8.

Introduction

Carotenoids are natural pigments found in plants and some animals. The major component of this group is b-carotene (Simpson and Chichester, 1981). The main source of vitamin A in the diet of rural communities in Uganda is carotenoids obtained from vegetables, fruits, roots and tubers. Carotenoids in white fleshed sweetpotato cultivars were studied by Martin (1983), Almeida and penteado (1988). Chandler and Schwartz (1988), and Sweeny and Marsh (1971) found that carotene content was altered by processing of sweetpotatoes, the change being dependent on the treatment employed. The objective of this study was to determine the presence of carotenoids in whiteflesh African sweetpotatoes and extract the carotenoids and identify the carotenoids present by use of HPLC and Fast Atom Bombardment Mass Spectroscopy.

Materials and Methods

Four cultivars of sweetpotatos T3013, T1702, T3002 and T3006 were chosen for the study. The sweetpotatoes were grown at Hill farm, Lousiana State University, Baton Rouge, LA. U.S.A., following common cultural practices. After harvest the sweetpotatoes were cured at 30°C, 80 - 90% relative humidity and stored at 15°C and 80 - 90% relative humidity for 1-6 months until needed. The sweetpotato was prepared into puree as reported by Ameny *et al* (1994)

Carotenoids Extraction

Carotenoids content of raw material was extracted by the method of Ameny *et al* (1997). Samples were grated lengthwise on a cheese grater. 10 grams of each sample was weighed into a homogenizer with an ice jacket (Omni 17105), Omni international, Watersbury, CT. U.S.A). To this was added 20g of anhydrous sulfate, 1g of magnesium carbonate, and 100 ml of stabilized tetrahydrofuran (THF). For the processed purees (Ameny et al, 1997), 10g were weighed into a homogenizer with an ice jacket, into which was added an hydrous sodium sulfate, magnesium carbonate and stabilized THF. The samples were homogenized at a moderate speed for minutes, then vacuum filtered through a Buchner funnel fitted with Whatman #42 filter paper. The filtrate was then brought to a final volume of 500ml. The whole mi of the extract was evaporated at 40°C on a rotary evaporator (Buchi Laboratorimus - Technik AG, Flawil, Switzerland) with nitrogen and taken to a final volume of 10ml with stabilized THF. The extracts were stored with brown bottles with Teflon lined cap under nitrogen in a cold room at - 20°C until needed for spectrophotometic analyses and injection in the HPLC.

Spectrophotometry

Spectrophotometric analyses were done on the extracts using a scanning UV-vis spectrophotometer (Perkin Elmer, Norwalk, CT, U.S.A), to determine maximum absorption wavelength as described by Ameny *et al* (1997). Total carotenoids were determined at the maximum wavelength of absorption with b-carotene as a standard. Stock solutions of b-carotene were (Sigma Chemical Co, St Louis, MO, (USA) were prepared by weighing 25 mg into 100 ml brown low actinic volumetric flask bringing the volume to 100 ml with THF. Four working solutions were prepared by taking 1, 2, 3, or 4 ml from the stock and making upto 100 ml. Standards were stored at -20°C under nitrogen. A standard curve was prepared using the working solutions and the unknown b-carotene was determined against the standard, Ameny (1977).

HPLC Determination of b-carotene

A rapid non-aqueous reversephase HPLC was used, modified from Bushway (1985). Conditions were as follows: column packing, C-18 5* Vydac 201TP54; column, 150 mm X 4.6 mm; isocratic, solvent, methanol/chloroform (90:10); flow rate, 1 ml/min. at 500 psi; ambient temperature, 20 C. b-carotene was used as a standard to determine the retention time (Ameny *et al* 1997)

Fast Atom Bombardment (FAB) MASS Spectrometry (MS)

Positive-ion FAB mass spectra were obtained using TSQ70 (Finigan Mat, San Joe, Ca)double focusing mass

Table 1. Absorption wavelength and total carotenoids in sweetpotatoes

Total carotenoid content (fwb) Ug/100g				
Samples	Treatment	Range	Mean	Distinct peak n nm
T1702	Raw Puree	220-260 90-190	249.14±17.04a 157.14±68.18b	428, 454 428
T3006	Raw Puree	90-240 70, 120	144.87±48.15b 93.33±15.98c	428, 453 428
T3002	Raw Puree	0-20 40-250	6.42±10.25f 91.57±80.82c*	- -
T3013	Raw Puree	30-40 20-50	43.14±10.30d 24.57±10.09f	- -

Numbers with the same letter are not significantly different at P<0.05
*Means for readings, the rest are means for seven readings. Duncan's Multiple Range Test.

Table 2. Quantitative value of β–carotenes in sweetpotatoes

Samples	Treatment	β–carotenes µg/mg (fwb)		RE
Sweetpotato		Range	Mean	µg
T1702	Raw Puree	78.25-146.0 4.40-17.13	104.99±22.15 11.44±4.09	19.1 1.90
T3006	Raw Puree	5.83-34.03 2.95-7.17	21.62±8.96 5.01±1.43	3.72 0.83

RE: Retinal Equivalent, µg
Means are from 10 readings

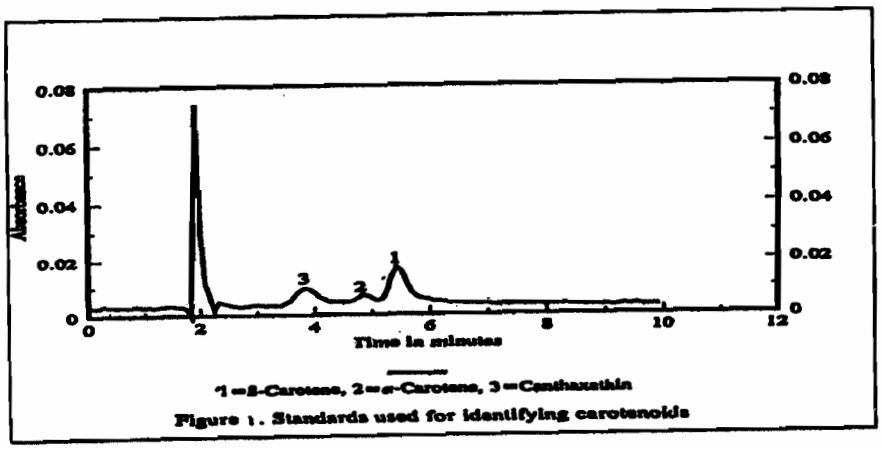

Figure 1. Standards used for identifying carotenoids

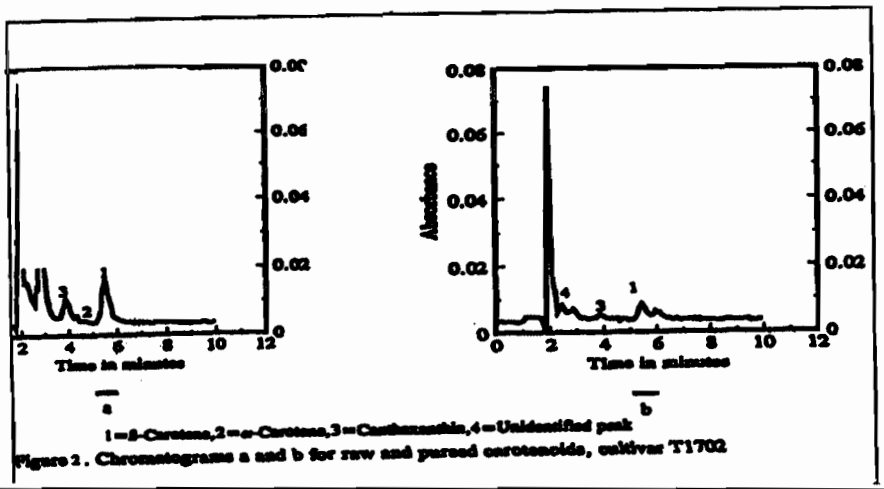

Figure 2. Chromatograms a and b for raw and pureed carotenoids, cultivar T1702

spectrometer attached to an ICIS Data System as described Schmitz *et al.*, 1992; Ameny *et al* 1997). Static FAB-MS analysis was carried out by applying 1 ml of extract containing at least 0.1 mg of individual carotenoid to the probe tip.

Statistical Analyses

Analyses of variance (ANOVA) of the results of carotenoid contents were computed by General Linear Model (GLM) procedure using the Statistical Analysis System (SAS International, Inc. Cary NC, 1994). The difference between the means were determined by Duncan's Multiple Range Test.

Results and Discussion

The maximum absorption was at 454nm and 428nm for cultivar T1702 and 453nm and 428nm for cultivar T3006 extract, and the standard b-carotene was 458nm.

The other two sweetpotato cultivars did not produce distinguishable maximum wavelength when scanned. The cultivarT1702, however, had the most distinctive peak. On processing there was a shift in the wavelength from 454nm to 428nm for cultivar T1702 and from 453nm to 428nm for cultivar T3006. The other sweetpotato cultivars, T3013 and T3002 did not show any change in the absorption maxima. The total carotenoids in the sweetpotatoes were then analysed for UV/VIS characteristics in comparison to a b-carotene standard at 458nm to determine the approximate total carotenoid content in the extracts shown in **Table 1**. The total carotenoids content varied from 0 to 260ug/ 100g in the raw extracts and from 20 to 190 ug/100g in the extract of the processed samples (Table 1). There was a change in total crude carotenoid on processing into puree, a decrease of 62% for cultivar T1702, 75% for T3006, and an increase of 800% for T3013 (Table 1)

HPLC Determination of carotenoids in sweetpotatoes.

The reversed-phase HPLC separation of the carotenoids was possible using retention times with the eluent being

monitored at 450nm. Pure standards were used for comparison (**Fig 1**). b-carotene (5.7 mins) was detected in the two (T3002 and T3013) other sweetpotato cultivars. The other carotenoids were alpha-carotene 6.21ug/100g (4.4mins), canthaxanthin 15.14ug/100g (3.8mins) and an unidentified peak (2.5mins). They were present in cultivars T1702 and T3006 only (Fig.2). The other cultivars, T3013 and T3002, did not reveal the presence of carotenoids on the reverse-phase HPLC run even though some carotenoids were detected by total carotenoid extraction method. The carotenoids were able to elute under ten minutes making this a quick method. Individual carotenoid contents are shown in **Table 2**. b-Carotene content ranged from 5.83ug/100g to 146ug/100g (fwb) in the raw sweetpotatoes. Cultivar T1702 had the highest value with a mean of 104ug/100g on fresh weight basis, and T3006 had a mean of 21 ug/100g. On processing into puree, there was a decrease in alpha-carotene to between 2 to 34ug/100g. T1702 had 11.4ug/100g and T3006 had a mean of 5ug/100g and T3006 had a mean of 5ug/100g. These variations may be due to the variety of sweetpotatos studied as sweetpotatoes are known to vary considerably in colour. Unlike previous investigators, the present research found some cultivars (T1702 and T3006) of whiteflesh African sweetpotatoes to contain b-carotene at rather low levels, ranging from 2.95ug/100g to 146ug/100g of sample (fwb), alpha-carotene and canthaxanthin were also found to be present.

Fast atom bombardment determination of carotenoids

It was possible to identify three carotenoids based on their m/z ration. A specific ion of interest formed during FAB ionization was selected by using a double-focusing mass spectrometer, fragmented by collision activation, and then analyzed via B/E - linked scanning to yield fragmentation data characteristic of the selected ion. b-carotene had m/z of 537.8, alpha carotene had 537.8 and canthxanthin had 565.8. Previous report by Martin (1980) and Almeida and Penteado (1988) had shown various carotenoids in white flesh sweetpotatoes with B-carotene, alpha-crotene and canthaxanthin, and b-carotene was the most abundant.

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