Correlation of High Performance Liquid Chromatography (HPLC) and Spectrophotometric Methods to Assess the Post Harvest Storage and Processing Changes in Total β-carotene Contents in Selected Nigeria Vegetables

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Original Research Article

ABSTRACT

The aim of this study was to correlate analytical methods (HPLC and spectrophotometric) in assessing the changes in total β-carotene contents in leafy vegetables during ambient temperature storage (29+2°C) and domestic processing (5 min,100°C.) The vegetables analyzed were: *Telfairia occidentalis*, *Amaranethus hybridus*, *Talinum triangulare*, *Pterocarpus mildbraedli* and *Gnetum africanum*. Total–carotene was determined spectrophometrically, while HPLC was used for detailed analysis of carotenoides. Lutein, β -cryptoxanthin and β-carotene isomers were identified and quantified. Results indicated that the raw vegetables were rich in lutein (124.03-655.95 μg/gdwt) and total β -carotene (45.42 – 246.93 μg/gdwt). *Beta*–cryptoxanthin was detected in small quantity (5.05-11 μg/gdwt). However, spectrophotometric result indicated a total–carotene content range (186.10 – 953.78 μg/gdwt). Cooking increased significantly (P< 0.05), the lutein (382. 92 – 1158.83 μg/gdwt), total β -carotene (738.53 – 1756.51 μg/gdwt) contents of the samples, however, it decreased the % *trans*–β-carotene contents. Storage conditions in the study increased

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significantly (P< 0.05) the contents of total β -carotene and total–carotene except in the case of *Gnetum africanum* leaf. A regression model for the two methods of analysis of β -carotene with a coefficient of correlation r = 0.925 and coefficient of determination r^2 = 0.856, which allows for the calculation of total β - carotene from total-carotene content was obtained.

Keywords: Correlation; leafy vegetables; beta-carotene; HPLC; regression.

1. INTRODUCTION

Vitamin A is an essential micronutrient required for vision and a variety of metabolic functions in the body. In developing countries more than 80% of the dietary vitamin A is supplied by carotenoides present in plant foods. The most predominant and active carotenoides in these foods is β-carotene [1]. Different carotenoides have been postulated to exhibit various beneficial effects on health. For example, carotenes are the sources of vitamin A [2]. Lutein and zeaxanthin are important factors for human vision [3]. The need for reliable data on the individual carroteroid content of those foods therefore become increasingly important.

Historically, much of the carotenoides data have been obtained by measuring total absorption at a specified wavelength, or more usually by open column chromatography followed by spectrophotometric quantification as in the Assonation of Official Analytical Chemists(AOAC) [4] method. This method is time consuming and does not quantify specific compound. Though interestingly it does not require expensive equipment [5]. Open Column Chromatography (OCC) has the advantage of using common laboratory equipment (recording UV – visible spectrophotometer). However, the sample through-put is low and reliability of results depends heavily on the expertise of the analyst [6]. More recently HPLC method have enabled more discrete analysis of carotenoids [7]. HPLC is expensive, especially in developing countries, and reliability of results directly depends on the accuracy of the standardization. Thus, the major difficulty in HPLC analysis of carotenoides is obtaining pure standards, especially for laboratories that have to import them [6]. Notwithstanding the difficulties in its execution, isomer separation using HPLC is necessary in the quantification of provitamin A content in foods.

The development of an HPLC method which can be used to routinely separate all the isomers of β-carotene present in food is imperative in assessing the fate of these compounds during processing and or storage because the *cis* isomeric forms of the provitamins are less potent, the need for their separate quantification in vitamin A assay has been increasingly accepted [8]. Sander et al. [9] took into account the hydrophobic characteristics of carotenoids and introduced a new reversed– phase, the triacontyl polymeric surface C_{30}. Also, they claimed that this phase is better for carotenoid separations and isomeric resolution than C_{18} columns. Appreciable levels of the isomers are formed during various food processing and the amount of each isomer coupled with their relative biological activities as vitamin A precursors should be used for accurate nutritional content measurements.

In Nigeria, as in most other tropical countries of Africa, where the daily diet is dominated by starchy staple foods, vegetables are the cheapest, accessible and available sources of nutrients, especially in rural areas where they contribute substantially to proteins, vitamins and fibre which are usually in short supply in daily diet [10]. Green leafy vegetables are very rich in carotenes. Besides the well-known pro-vitamin A activitiy of some carotenoids, they have also been associated with lowered risk of developing degenerative diseases, cataract and age related macular degeneration [11]. The compound possessing highest vitamin A activity and occurring most abundantly in fruits and vegetables is β-carotene.

Traditional vegetables grow wild and are readily available in the field as they do not require any form of cultivation. Communities in Africa have a long history of using traditional leafy vegetables to supplement their diets [12]. Several recent publications [13,14] have stressed the nutritional value of traditional and indigenous leafy vegetables. However, the use of traditional and indigenous leafy vegetables by local people is still a relatively under researched discipline in Nigeria. Knowledge of indigenous plant use needs urgent scientific investigation and documentation before it is irretrievably lost to future generations [15]. In rural populations of developing countries, vegetables are stored at

ambient temperature especially when the market is not enough for fresh produce. Also in Africa, vegetables are often cooked before consumptions. Five selected Nigerian leafy vegetable were analyzed. They are *Telfairia occidentalis*, *Amaranthus hybridus*, *Tailnum triangulare*, *Pterocarpus mildbraedli* and *Gnetum africanum*.

The aim of this study was to evaluate the changes in carotenoids incurred during the storage and processing of the leafy vegetables. Another aim was to establish a regression model that relates the total- carotene assessed by spectrophotometric method with the total β-carotene assessed by HPLC in the leaves.

2. MATERIALS AND METHODS

2.1 Selection of Vegetable Samples

Five traditional green leafy vegetables commonly consumed by both rural and urban communities in south-eastern Nigeria were identified and used for research work. They include *Telfairia occidentalis* (ugu) *Amaranthus hybridus* (Inine), *Tilanium triangulare* (mgbolodi), *Pterocarpus mildbreadli* (oha) and *Gnetum africanum* (okazi).

2.2 Collection of Samples

Three of the cultivated vegetables; *Telfaria occidentalis, Amaranthus hybridus* and *Talinum triangulare* were planted in October 2012, harvested and collected from two farms in Enugu state in December 2012. Natural organic manure was used as fertilizers for the three crops. The remaining two, *Gnetum africanum* and *Pterocarpus mildbreadli* are predominantly wild, and were collected from the wild. Leaves were selected at random from the plant area and picked by hand mid-morning during the harmattan season. A minimum of 1 kg per species was collected randomly from different plants within the field in each case. The leaves were placed in black polyethelene bags and transported to the Biochemistry Department of the University of Nigeria Nsukka for processing. Meanwhile carotenoid analysis on the dried and milled samples were carried out at IITA (International Institute of Tropical Agriculture) Ibadan.

2.3 Experimental Design

The experiment has a randomized complete block design having vegetable types x 5 and processing (treatments) x 3 as some of the variations giving 5 x 3 = 15 observations. Each observation was repeated three times giving 15 x 3 = 45 observations for each parameter tested.

2.4 Processing of Samples

In the laboratory, the edible and inedible portions of each sample were separated. The inedible portions were discarded. The edible portions were washed with tap water. The edible portions of all the vegetables were divided in three equal parts. The first part was cooked for 5mins in boiling water with the lid on. The second sub-sample was wrapped in a newsprint and stored in the dark for 5 days, while the third sub-sample was kept raw.

2.5 Sample Preparation for Carotenoid Analysis

Both the raw, cooked and stored samples were oven dried in glass trays at 50°C for about 48 hours until there was no further moisture loss. The dried leaves were milled and sieved through a 1 mm stainless steel sieve to obtain a homogenized sample. Approximately 30 g of each of the sieved powdered samples were stored in sealed polyethylene bags and coded. The samples were stored at -20°C until they were analyzed at International Institute of Tropical Agriculture (IITA) Ibadan for carotenoid analysis.

2.6 HPLC Determination of Total β-carotene Content

The method of Howe and Tanumihardjo [16] was used. A Waters HPLC system (Water Corporation, Milford, MA) consisting of a Guard-column, C_{30} YMC carotenoid column (4.6 x 250 mm, 3 µl) water 626 binary HPLC pump, 717 auto sampler and a 2996 photodiode array detector was used for carotenoid quantification. Chromatograms were generated at 450 nm. Identification of lutein, β-cryptoxathin, and β-Carotene were carried out using standards and with verification of absorption spectrum. Standard curves for lutein. β-cryptoxathin, and β-Carotene standard already established in the crop utilization laboratory of IITA Ibadan, Nigeria were used. A solvent-gradient program was used to obtain adequate separation and detection of the carotenoids.

2.7 Spectrophotometric Determination of Total Carotene Content

Determination of total carotene content of the leaf samples was according to the method of Rodriguez-Amaya and Kimura [17]. The absorbance was read at 450 nm using Jenway Spectrophotometer (Model 752, England).

Total carotene content. (μg/g)

$$= \frac{A_{fr1} \times volume\ (ml) \times 10^4 \times (DF)}{A\ \frac{1\%}{1cm} \times sample\ weight}$$

Where,

A_{fr1} = Absorbance at 450 nm

Volume (ml) = volume of fraction 1

$A\ \frac{1\%}{1cm}$ = 2592 (absorption coefficient of β - carotene in petroleum ether (P.E)

3. RESULTS AND DISCUSSION

3.1 Chromatographic Profiles of Carotenoids

Figs. 1-2 show the typical HPLC chromatograms of raw, cooked and stored leaf samples. Two classes of carotenoids: xanthophylls and carotenes were identified and quantified. The components were eluted in order of decreasing polarity, from oxy-carotenoids to lipophilic hydrocarbons; Lutein, β-cryptoxanthin, 13– *cis*-β-carotene, 15–*cis*- β–carotene, *trans*- β-carotene and 9-*cis* β-carotene were detected under the employed running conditions at approximately 9, 16, 23, 24, 26 and 28 min respectively.

3.2 Chromatograms and Peak Areas

Fig. 1a – c show the HPLC Chromatograms of raw, cooked and stored *Telfairia occidentalis* leaf sample. A total of six different peaks were identified in the leaf sample. Identification of the peaks was based on comparison of unknown peaks to authentic standards in terms of elution time and absorption profile [18]. The absorption maxima of the standards were initially memorized in the HPLC database and were compared by overlay to each unknown peak. The components (Carotenoids) eluted in order of decreasing polarity from oxycarotenoids to lipophilic hydrocarbons, thus; Lutein, β-Crytoxanthin, 13-*cis*-, 15-*cis*-, *trans*- and 9-*cis*- β-carotene at their corresponding retention times

(RT); 9.573, 15.764, 23.229, 24.054, 26.575 and 28.517 min. respectively. Quantitative analysis was based on the peak area of the component in the chromatogram. Peak area was given in arbitrary unit while concentration was in µg/g. Peak areas of the chromatogram of cooked *T. occidentalis* sample (Appendix 2b) were β-Cryptoxanthin (45964) < 9-*cis*-(607938)< 12-*cis*-(1098753) < 15-*cis*-(1478983) < *trans*-(3295353) < Lutein (13863539) and corresponding concentrations, (Table 1) (µg/g): β-Crypoxanthin (7.13) < 9-*cis*-(50.78) < 13-*cis*- (90.72) < 15-*cis*-(121.67) < *trans*- (269.48) < Lutein (1158.83). Peak area of the chromatogram of cooked *T. occidentalis* sample was higher than the peak area of the raw and stored *T. occidentalis* samples. Peak areas of chromatogram of raw *T. occidentalis* sample (Appendix 2a) were β-Crytoxanthin (35970), 9-*cis*- (331726), 13-*cis*-(404828), 15-*cis*-(702118) *trans*-(1530355) and Lutein (8393924) and concentrations (µg/g) (Table 1): β-Cryptoxanthin (5.17), 9-*cis*-(26.47), 13-cis- (32.03), 15-cis- (54.65) trans-(117.68) and Lutein (655.70) respectively. However Peak area indicated in stored *T. occidentalis* sample (Appendix 2c)) showed a reduction in peak area of β-Cryptoxanthin (30338), 15-*cis*- (683556) and an increase in peak area of 9-*cis* (357738), 13-*cis* (45568) *trans*-(1657742) and lutein (11312365) with corresponding concentrations (µg/g): β-Cryptoxathin (4.99) 15-*cis*-(53.34), 9-*cis*-(28.50), 13-*cis*-(35.07) *trans*-(127.61) and Lutein (885.65) respectively. Similar trends relating peak area and concentrations were observed in other leaf samples (Fig. 2a-c) respectively. It is equally important to note that the two remaining parameters; % Area and Height followed similar trend as peak area and concentration. Peak area is proportional to sample size or sample concentration.

About 30 prominent and minor peaks were noted in the analysed leaf samples at 450nm. Unidentifies peaks include those of chlorophyll and its degradation products, some prominent and minor carotenoid constituuent of leaves like Neoxanthin, Violaxanthin, Zeaxanthin, α - carotene, α-cryptoxanthin etc.

Quantification of the carotenoid concentrations were made by reading off the peak area of sample on internal standard calibration curve (peak area vs concentration) already established for IITA Ibadan HPLC. The HPLC employed in the analysis was equipped with a computerized data handling systems that generated automatically the peak areas and concentrations of the samples'carotenoids.

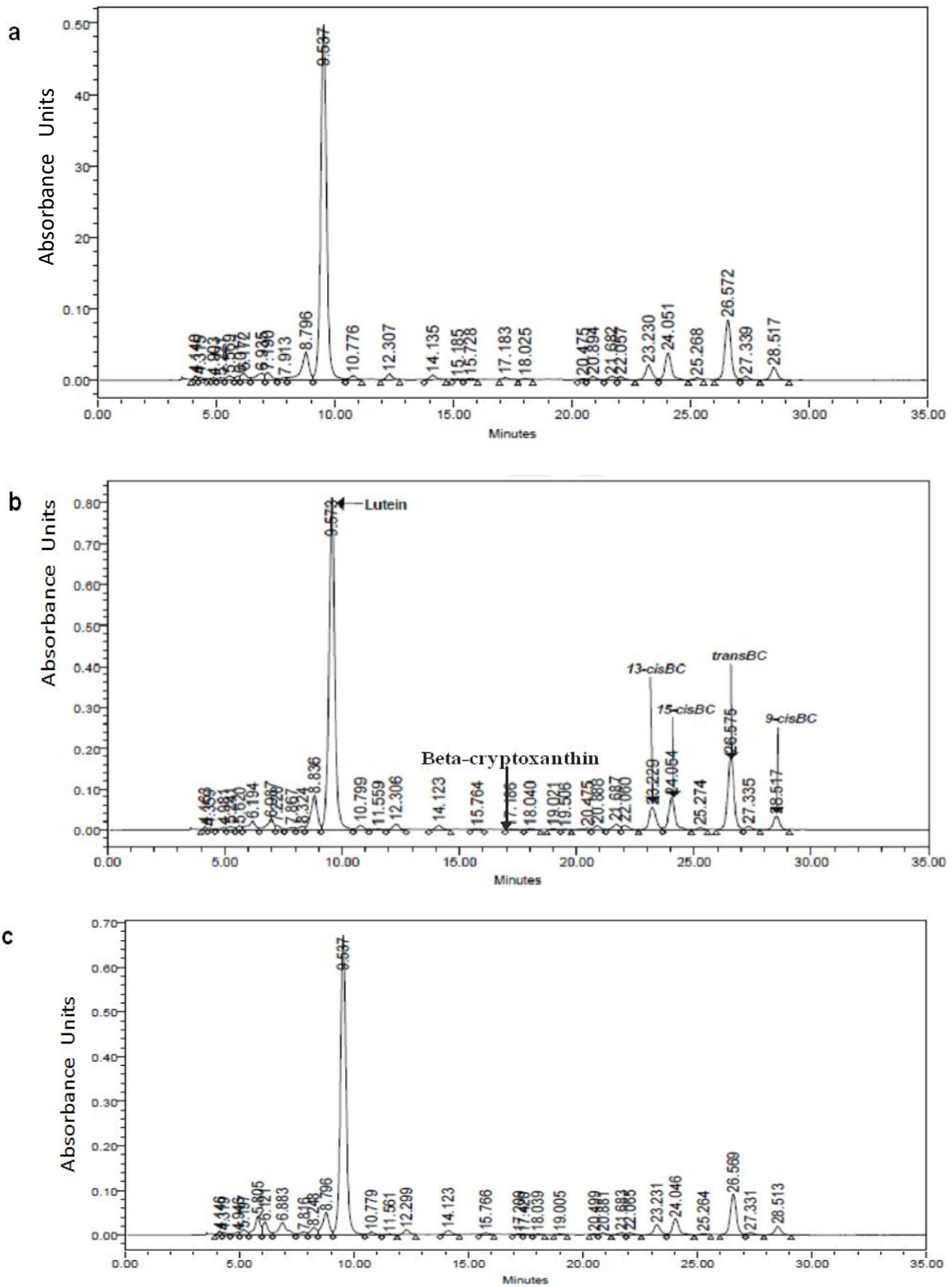**Fig. 1a-c. Carotenoid profile of *Telfairia occidentalis* (Ugu) leaf samples by HPLC (a) Raw (b) Cooked (c) Stored**
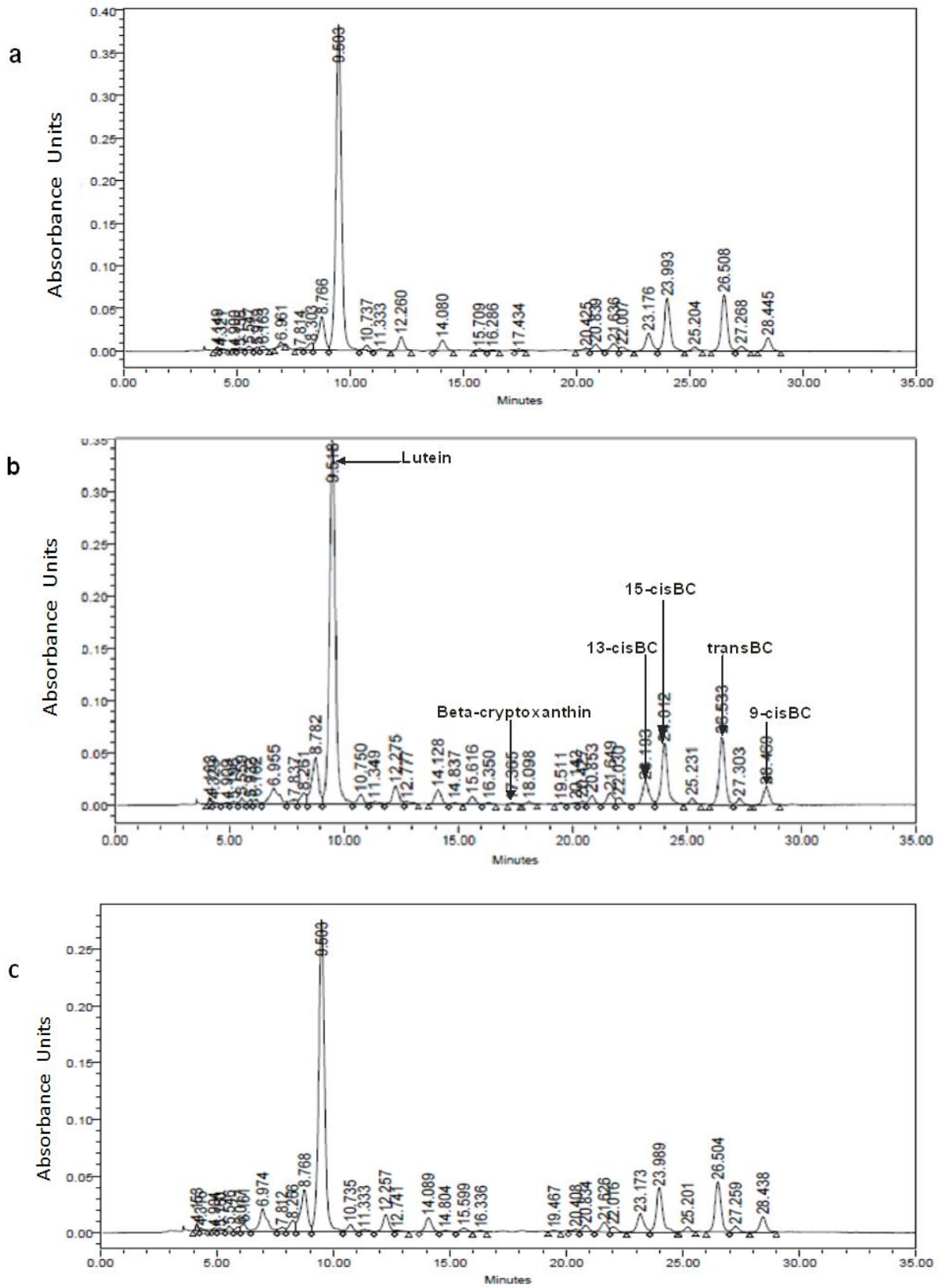
Fig. 2a-c. Carotenoid profile of *Gnetum africanum* (Okazi) leaf samples by HPLC (a) Raw (b) Cooked (c) Stored

3.3 Total β-carotene Content

Table 1 shows the effect of storage and processing on the carotenoid concentrations of the vegetables. The total β-carotene levels were significantly (P < 0.05) higher in cooked leaves than in raw leaves except in the case of *Gnetum africanum.* These results correlate with the results of Faber at al. [19]. Cooking (5min, 100°C) did not soften the *G. africanum* leaves. This could be explained by the inherent hard and tough attributes of freshly harvested *eru* leaves. However more than 100% Apparent retention was observed in all the cooked samples when compared with raw samples (Appendix 1). The high Apparent retention (780%) of β-caretene in *Talinum triangulare* could be attributed to its relatively high moisture and soluble solids content. Cooking and subsequent drying of the leaf sample resulted in much loss of its water and soluble solids thereby concentrating the carotenoids per unit weight of the leaf. Dietz and Erdman [20], reported that cooking resulted in greater than 100% retention of β-carotene in vegetables, because denaturation of carotene binding protein releases the carotenoids so that they can be extracted more easily. Rodriguez-Amaya [21] reported carotenoid retentions of over 100% in cooked foods calculated on a dry weight basis. In all cases, cooking resulted in higher retention than raw and stored leaves. This apparent increase could simply be due to the greater ease with which carotenoids were extracted from cooked or processed samples compared with carotenoids in fresh foods where they were physically protected or combined with other food compounds [22].

As expected, the % *trans* –total β-carotene was lower in the cooked than in the raw leaves (Appendix 2). During the cooking process, some of the *trans*- β -carotene could have been converted to *cis* –isomers or other oxidation products [8]. The levels of *cis* –isomers of β – carotene were therefore higher in cooked leaves then in raw leaves [23]. Thermal processing of foods result in *trans* - to *cis* –isomerisation. The consequences of *trans-/ cis* –isomerization are changes in bioavailability and physiological activity [24]. However, only *trans-β*-carotene can be preferentially converted to retinol (vitamin A) in the enterocyte [25]. The cooked leaves'*β*-carotene are three times more bioavailable than the raw leaves' *β*-carotene [26]. Also, the percentage trans -*β*-carotenes was higher in the stored than in raw leaves (Appendix 2). The levels of *cis*-isomers in stored leaves are

therefore lower than in raw leaves. The most abundant *cis* – isomer of β –carotene in the raw, cooked and stored leaf samples was 13 – *cis* – isomer. Several different geometric isomers of 13-*cis*- β–carotene, 15 – *cis* -β–carotene,*trans* – β–carotene, 9-*cis* β–carotene, etc exist in foods and human tissues. The major β-carotene isomer in the circulation of humans are *trans*- β-carotene, with small amounts of 13-*cis*- β-carotene and 9-*cis*- β-carotene [27].

A significant (P < 0.05) increase in total β-carotene content in stored leaves was observed when compared with raw leaves (Appendix 1). This could result from continuation of physiochemical activities [28]. However, there was a significant (P < 0.05) decrease in the total β-carotene content of *G. africanum a*fter storage. This could be attributed to the degree of lignifications of the tissues resulting in obvious reduction in carotenoids extractability. Immature tissues with high respiratory rates often exhibit hardening and lignifications during storage [29]. Consequently, the storage conditions employed in our study may have preserved the *trans*- β-carotenes. However, carotenoid losses during post-harvest storage were reported in leaves [30], especially under exposure to light and conditions that favour wilting.

Among the vegetables in the study, cooked *T. occidentalis* leaf had the highest β-carotene concentration (532.66 µg/gdwt). Also, raw *G.africanum* β-carotene concentration (246.93 µg/gdwt) was significantly (P < 0.05) higher than other leaves, while *T. triaigulare* raw leaf had the least concentration of β-carotene (45.42 µg/g). Comparing the total β-carotene content of the leaves with previous reports, Schönfeldt and Pretorius [31], reported 79.6 µg/gdwt in cooked *Cucurbita maxima* and 160 µg/gdwt in raw *Amarauthus tricolor.* Ninomia and Godoy [32] recorded 85 µg/gfwt in raw mint leaves. Also,Žnidarčič et al. [33] reported 79 µg/gfwt and 73 µg/gfwt in garden rocket and chicory respectively. It seems therefore, that the leafy vegetables from the study are very rich dietary sources of β-caretene when compared with the commercially available vegetables.

3.4 Xanthophylls Content

The β-cryptoxanthin contents (Table 1) in the raw leaves was highest in *A. hybridus* (11.02 µg/gdwt) and lowest in *P. mildbraedli* (5.05 µg/gdwt). Also, the β-cryptoxanthin content in the cooked leaves was highest in *T. occidentalis*

(7.13 µg/gdwt) and lowest in *T. triangulure* (4.86. µg/gdwt). The variation could be explained by differences in species. These results were in agreement with previous reports by Daly et al. [34] and Khorkhar et al. [35] for Coriander and Sage herbs respectively. β-cryptoxanthin is a minor provitamin A constituent of leaves [36].

The lutein contents in the raw leaves ranged from 124.03 to 655.7 µg/gdwt for *T. occidentalis* and *A. hybridus* respectively (Table 1). The results of this study are in agreement with previous reports. Kopsell et al. [37] reported lutein concentration range from 48 to 134 µg/gfwt and 65 to 130 µg/gfwt for kale and Spinach. Dias et al. [38] recorded values from 52 to 64 µg/gfwt and 36 to 56 µg/gfwt for Kale and Beet leaf respectively. Lutein though not vitamin A active, is of some health benefits. According to Wisniewska and Subczynski [39], the presence of lutein and/or zeaxanthin in the diet may be beneficial for reducing the incidence of two common eye diseases of age - related macular degeneration and cataract formation.

Table 2 shows the total-carotene content in selected and processed green leafy vegetables. The total-carotene content in cooked leaves was significantly (P > 0.05) higher than in raw and stored leaves. This could be explained by higher extractability of carotenoids in cooked leaves. Cooking softens or breaks the cell wall and denatures proteins complexed with carotenoids, thus facilitating the release of carotenoids from the food matrix [40]. There were no statistical differences between raw and stored leaves. This implies that the conditions under which the leaves were stored did not degrade the carotenoids. However, there were numerical increases in total-carotene in stored *T. occidentalis* (1194.64 µg/g), *T. triangulare* (393.08 µg/g), and *P. mildbraedi* (528.75 µg/g) when compared to their corresponding raw leaves (953.78, 186.10 and 429.70 µg/g respectively). The opposite was found in leaves of *A. hybridus* (492.01 µg/g) and *G. africanum* (608µg/g) with lower levels of total carotene when compared with their corresponding raw leaves content; (533.92 and 694.30 µg/g respectively). Cooked *T. occidentalis* (1756.5 µg/g) had the highest total carotene concentration while cooked *A. hybridus* (660.46 µg/g) had the lowest. Raw *T. occidentalis* (953.78 µg/g) had the highest total –carotene, while raw *T. triangulare* (186.10 µg/g) had the lowest. Also stored *T. occidentalis* (1194.64 µg/g) contained the highest level of total carotene, while stored *T. triangulare* (393.08

µg/g) contained the least. The differences could be explained by differences in species and method of processing. The low value of raw *T. triangulare* (186.10 µg/g) could be as a result of its high water/solid contents that diluted the carotenoids and increased unit weight both of which decreases the carotenoid concentrations. Total carotene values were higher than their corresponding total β- carotene values. Total carotene consists of α - carotene, β- carotene and its isomers. Total-carotenoids differ from total carotene in that the former contains the carotenes (total carotene) and oxygenated carotenes or xanthophylls.

3.5 Regression and Correlation Analysis

Table 3 shows the data obtained by both the spectrophotometric and the HPLC methods for five leaf samples. In our study we proceeded to demonstrate that total carotene obtained by spectrophotometric method and total β-carotene measured by HPLC showed a linear relation. In this research work both total carotene and β-carotene were considered as the variables to regress or model. The regression was shown below using SPSS (statistical package for social sciences) (Figs. 3a-c). Fig. 3a showed scatter plot of variables considered with linear equation as well as coefficient of correlation, r = 0.925 and coefficient of determination r^2 = 0.856 of the model formulated. From the figure, the model is Y= -7.03+0.292x and r^2=0.856 which implies total carotene could explain 85.6% of variability in β-carotene. The constant term of − 7.031 shows the least value β-carotene can assume in such experiment and 0.292 was the rate of change of β-carotene with respect to total carotene which could also be interpreted as a unit increase in total carotene will lead to 0.292 unit increase in total β-carotene. The rate of change (b=0.292) was significant at 5% since the P value of t-test was 0.000. Also, the P-value of the model was 0.000 which implied the model was adequate in prediction of total β-carotene when the value of total carotene was known. r^2 values from 0.81 is interpreted as strong linear trend and r values from 0.91 as strong positive correlation [41].

Regression output of linear model is presented in Tables 4 a-c. There was a strong correlation between the variables (0.925). The positive correlation value implies that increase in one of the variables corresponds to increase in other variable and r^2 of the linear model was shown in column 3 as 0.856 (85.6%) and adjusted r^2 as 0.845 (84.5%). This implies adequacy of the

model with significance at 1%. The residual of 2245.558 was considerably low when compared to 173931.3 considered variation. Regression output showed the coefficients of the dependent variable, β-carotene and also showed the model formulated and significance of parameters involved using T-test. The model was Y=-7.031+0.292x.

Table 1. Effects of storage and moist heat treatment on the carotenoid content of green leafy vegetables

Parameter	Species	Carotenoid Content ($\mu g/g$ edible portion, dry weight basis)		
		Treatment		
		Raw	Cooked	Stored
Lutein	*Telfairia occidentalis*	655.70[b]±4.95	1158.83[a]±0.05	885.65[b]±2.36
	Amaranthus hybridus	309.21[b]±2.33	382.92[a]±1.61	312.84[b]±2.61
	Talinum triangulare	124.03[b]±0.58	593.24[a]±3.55	235.68[b]±1.83
	Pterocarpus mildbraedli	261.96[b]±9.20	507.97[a]±2.27	343.33[b]±0.32
	Gnetum africanum	528.87[a]±1.24	504.92[a]±1.85	394.31[b]±1.27
β-Cryptoxanthin	*Telfairia occidentalis*	5.17[b]±0.07	7.13[a]±0.15	4.99[b]±0.14
	Amaranthus hybridus	11.02[b]±0.25	5.76[a]±0.38	10.47[b]±0.53
	Talinum triangulare	5.11[b]±0.18	4.86[a]±0.12	5.15[b]±0.02
	Pterocarpus mildbraedli	5.05[a]±0.05	5.59[a]±0.02	4.88[b]±0.45
	Gnetum africanum	5.77[a]±0.25	6.22[a]±0.25	4.79[b]±0.29
13-cis-β-Carotene	*Telfairia occidentalis*	32.03[b]±1.38	90.72[a]±0.09	35.97[b]±0.11
	Amaranthus hybridus	34.30[b]±0.57	45.56[a]±0.77	32.78[b]±0.89
	Talinum triangulare	7.79[a]±1.00	71.31[a]±1.23	20.25[b]±1.37
	Pterocarpus mildbraedli	16.26[b]±1.21	40.26[a]±1.17	17.89[b]±0.94
	Gnetum africanum	34.35[a]±0.52	36.90[a]±0.45	8.10[b]±0.19
15-cis-β-Carotene	*Telfairia occidentalis*	54.65[b]±0.62	121.67[a]±2.19	53.34[b]±0.49
	Amaranthus hybridus	5.69[b]±0.42	6.11[a]±0.21	5.28[b]±0.07
	Talinum triangulare	3.39[b]±0.76	29.71[a]±0.18	9.07[b]±0.67
	Pterocarpus mildbraedli	4.84[b]±0.16	12.14[a]±1.95	4.43[b]±0.09
	Gnetum africanum	91.84[a]±1.95	89.14[a]±2.94	27.56[b]±2.34
Trans-β-Carotene	*Telfairia occidentalis*	117.68[b]±2.17	269.48[a]±0.69	127.61[b]±2.23
	Amaranthus hybridus	107.97[b]±3.45	125.54[a]±3.70	102.48[b]±5.27
	Talinum triangulare	25.71[b]±0.48	209.29[a]±2.41	70.09[b]±2.84
	Pterocarpus mildbraedli	50.13[b]±1.30	132.79[a]±0.46	65.78[b]±1.48
	Gnetum africanum	96.53[a]±1.45	95.56[a]±0.86	65.41[b]±2.02
9-cis-β-Carotene	*Telfairia occidentalis*	26.47[b]±2.29	50.78[a]±1.04	28.50[b]±0.72
	Amaranthus hybridus	26.89[b]±0.22	31.72[a]±2.53	26.45[b]±0.56
	Talinum triangulare	8.53[b]±0.76	44.29[a]±1.30	20.91[b]±0.19
	Pterocarpus mildbraedli	12.30[b]±1.28	27.25[a]±0.36	12.56[b]±0.36
	Gnetum africanum	24.21[a]±1.68	26.51[a]±0.76	21.56[b]±1.35
Total β-carotene	*Telfairia occidentalis*	230.82[b]±4.96	532.66[a]±13.94	245.42[b]±7.96 LSD= 2.03
	Amaranthus hybridus	174.86[b]±2.81	208.94[a]±2.98	166.99[b]±1.73 LSD = 3.41
	Talinum triangulare	45.42[b]±3.53	354.60[a]±2.95	120.31[a]±1.13 LSD= 5.07
	Pterocarpus mildbraedli	83.53[b]±0.89	212.44[a]±1.40	100.65[a]±3.39 LSD= 3.55
	Gnetum africanum	246.93[a]±6.68	248.10[a]±0.35	122.63[b]±5.50 LSD= 4.32

Values are means ± standard deviations of triplicate determinations
Means with different superscripts within the same specie are significantly different (p< 0.05)

Table 2. Effect of storage and processing methods on total -carotene content of selected indigenous green leafy vegetables

Leaf space	Treatment	Total carotent (µg/g edible portion, dry weight basis) Mean ± S.D
Telfairia occidentalis	Raw	953.78±4.05
	Cooked	1756.51[a]±36.22
	Stored	1194.64[b]±5.04 (LSD= 7.34)
Amaranthus hybridus	Raw	533.92[b]±0.42
	Cooked	660.46[a]±0.41
	Stored	492.01[b]±0.81 (LSD= 6.31)
Talinum triangulare	Raw	186.10[b]±0.43
	Cooked	976.01[a]±1.21
	Stored	393.08[b]±1.63 (LSD= 7.21)
Pterocarpus mildbraedli	Raw	429.70[b]±14.04
	Cooked	738.53[a]±0.40
	Stored	528.75[b]±0.86 (LSD= 6.25)
Gnetum africanum	Raw	694.30[b]±1.26
	Cooked	768.35[a]±0.85
	Stored	608.56[b]±0.43 (LSD= 6.03)

Values are means ± standard deviations of duplicate determinations on dry weight basis.
Means with different superscripts within the same (species) column are significantly different (P ≤ 0.05)

Table 3. Total- Carotene (Spectrophotometric method) and Total *β-carotene* (HPLC method) values determined for five leafy vegetables and used for regression analysis

Leaf species	Treatment	Total carotene (µg/g)	Total β-carotene (µg/g)
Telfairia occidentalis	Raw	953.78	230.82
	Cooked	1756.51	532.66
	Stored	1194.64	245.42
Amaranthus hybridus	Raw	533.92	174.86
	Cooked	660.46	208.94
	Stored	492.01	166.99
Talinum triangulare	Raw	186.10	45.42
	Cooked	976.01	354.60
	Stored	393.08	120.31
Pterocarpus mildbraedii	Raw	429.70	83.53
	Cooked	738.53	212.44
	Stored	528.75	100.65
Gnetum africanum	Raw	694.30	246.93
	Cooked	768.35	248.10
	Stored	608.56	122.63

In the study, quadratic model, (Fig. 3b) was also considered and the regression equation was $Y=3.725+0.264 x +0.0000146x^2$. The P-value of the model was 0.000 with r = 0.926 and r^2=0.857. Although the r-square value of quadratic model was slightly higher than that of linear model, none of the parameters was significant at 5%. This showed the superiority of linear model over quadratic model. Thus, in measuring the relationship between the variables, linear model was better. Regression output of quadratic model was presented in Tables 5 a-c. The model had a correlation value of 0. 926 between the variables which could be interpreted as strong positive relationship between the variables. The positive correlation value implies that increase in one of the variables corresponds to increase in the other variable and R-square of the quadratic model was shown in column 2 as 0.857 (85.7%) and adjusted R-square as 0.83.3%). The model is adequate and has a significance level at 1%. The residual of 2422.762 was considerably low when compared to 87025.186 considered variation. The coefficients also showed the model formulated and significance of parameters involved using t-tests. The regression equation

was $Y=3.725 + 0.26439x + 0.0000146x^2$. The scatter plot (Fig. 3c) of β-carotene versus total carotene is presented, and both the least squares line and the fitted quadratic model are super imposed. Even though the coefficients of the models are different, the two curves are almost identical. There is no reason to include the quadratic term in the model. It makes the model more complicated, without improving the fit.

Figs. 3a – c. Regression Plots of Total - carotene and β-carotene
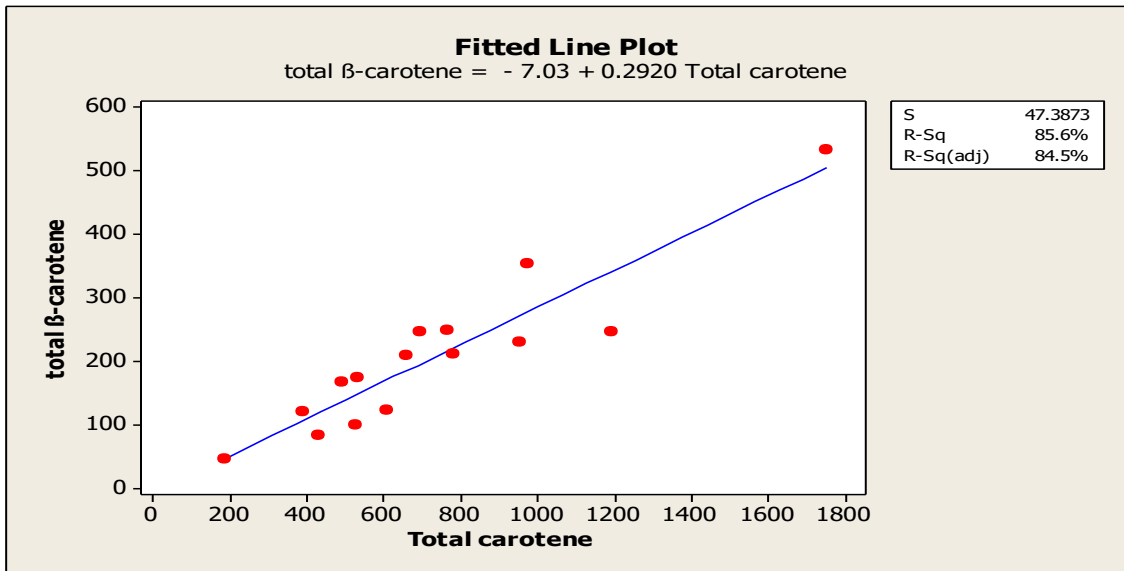
Linear Model:

Fig. 3a. Linear Relationship between Total-carotene and β-carotene
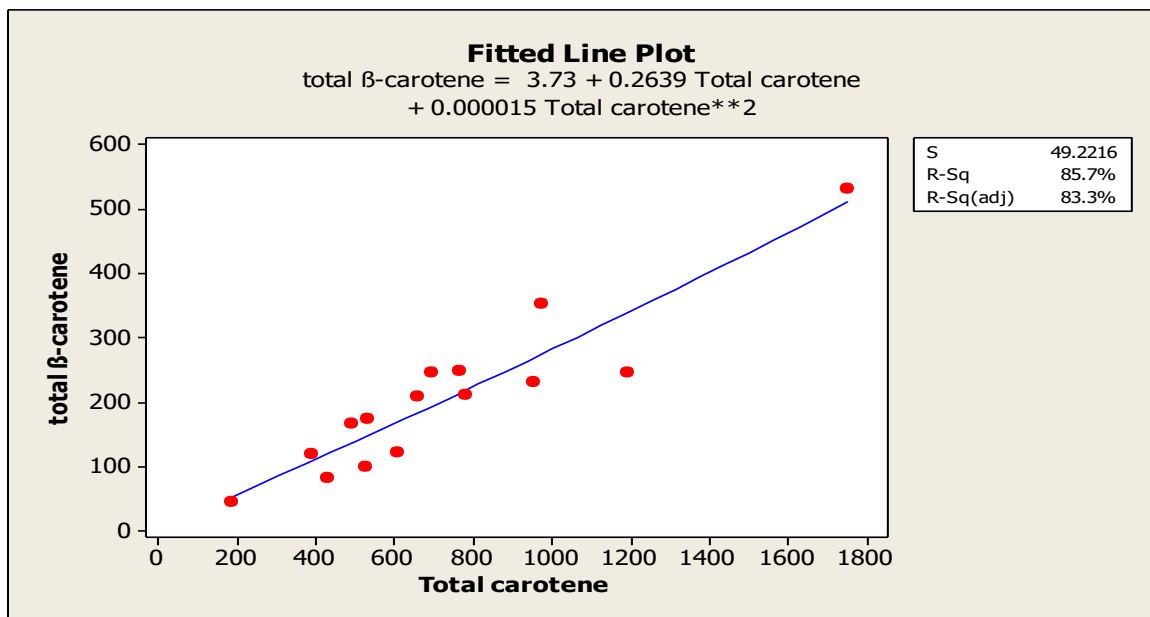
Quadratic Model:

Fig. 3b. Quadratic relationship between Total-Carotene and β-carotene
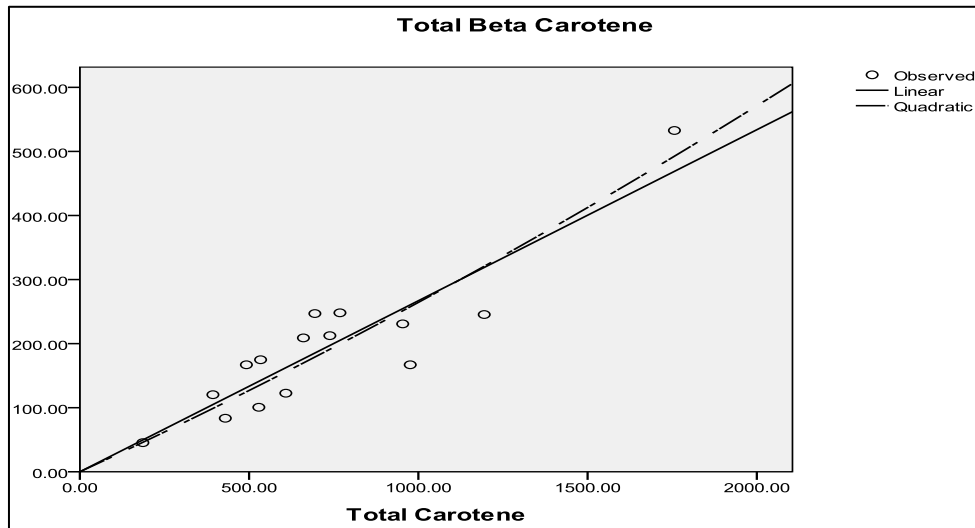
Fig. 3c. Spss Graph for linear and quadratic relationships between total-carotene and beta-carotene of the green leafy vegetables

Tables 4a- c. Regression Output of linear model

Table 4a. Shows summary of the model with correlation between the variables as 0.925

A. **Linear Model**

Model	R	R square	Adjusted R square	Std. error of the estimate	Change Statistics				
					R square change	F change	df1	df2	Sig. F change
1	.925[a]	.856	.845	47.38732	.856	77.456	1	13	.000

a. Predictors: (Constant), Total Carotene

Table 4b. Shows adequacy of the model with significance level as 0.000 which implies the model is significant at 1%

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	173931.261	1	173931.261	77.456	.000[a]
	Residual	29192.256	13	2245.558		
	Total	203123.516	14			

a. Predictors: (Constant), Total Carotene
b. Dependent Variable: Total β-carotene

Table 4c. This shows the model formulated and significance of parameters involve using t-test

Model		Unstandardized coefficients		Standardized coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-7.031	27.151		-.259	.800
	TC	.292	.033	.925	8.801	.000

a. Dependent Variable: Total β-carotene

The modes is:

Y= - 7.03+0.292x

Table 5a-c. Regression output for Quadratic Model

Table 5a. Shows summary of the model with correlation between the variables as 0.926

Model summary			
R	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
.926	.857	.833	49.222

The independent variable is Total Carotene

Table 5b. Shows adequacy of the model with significance level as 0.000

ANOVA					
	Sum of squares	**Df**	**Mean square**	**F**	**Sig.**
Regression	174050.372	2	87025.186	35.920	.000
Residual	29073.145	12	2422.762		
Total	203123.516	14			

The independent variable is Total Carotene

Table 5c. This shows the model formulated and significance of parameters involve using t-test

Coefficients				
	Unstandardized coefficients		**Standardized coefficients**	**t**
	B	**Std. Error**	**Beta**	
TC	.264	.131	.836	2.009
TC ** 2	1.460E-5	.000	.092	.222
(Constant)	3.725	56.113		.066

The model is;
$Y = 3.725 + 0.264x + 0.0000146x^2$

5. CONCLUSION

The vitamin A potentials of five leafy Nigerian vegetables were evaluated in this research work. Storage and cooking of the leaves after harvest resulted in changes in carotenoides in the leaves. The levels of nutrient retention after domestic processing support the inclusion of these leaves in a daily diet to overcome vitamin A deficiency and age-related macular degeneration and cataract. Total-carotene (Spectrophotomeric method) showed a good correlation with total β-carotene (HPLC method), then it was possible to determine total - β-carotene content by using the spectrophotometric method. This is important especially to the developing countries in sub-Sahara Africa.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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APPENDIX

Appendix 1. Calculation of percentage Apparent Retention of Total β-carotene in selected green leafy vegetables

Species	% Retention (Total *β*-carotene)	
	After cooking	After storage
Telfaria occidentalis	230.76[a]	106.32[a]
Amaranthus hybridus	119.48[a]	66.90[a]
Talinum triangular	780.71[b]	264.88[b]
Pterocarpus mildbraedii	254.32[a]	120.49[a]
Gnectum Africanum	100.47[a]	49.72[a]

Appendix 2. Calculation of percentage *trans* of total β-carotene in selected leafy vegetables

Leaf species	Treatment	% *Trans* of total β-Carotene
Telfaria occidentalis	Raw	51.0
	Cooked	50.6
	Stored	52.0
Amaranthus hybridus	Raw	62.0
	Cooked	60.1
	Stored	61.3
Talinum triangulare	Raw	56.6
	Cooked	59.0
	Stored	58.3
Pterocarpus mildbraedii	Raw	62.5
	Cooked	60.0
	Stored	65.4
Gnetum Africanum	Raw	39.1
	Cooked	38.3
	Stored	53.3

Appendix 2a. HPLC peak area of carotenoids of raw *Telfairia occidentalis* (ugu) leaf

	RT	Area	% Area	Height		RT	Area	% Area	Height
1	4.140	45004	0.32	4929	10	7.913	50821	0.36	2542
2	4.315	43218	0.31	3495	11	8.796	832516	5.96	37484
3	4.903	25660	0.18	2151	12	9.537	8393924	60.14	496319
4	5.221	42663	0.31	2392	13	10.776	99228	0.71	5749
5	5.569	87619	0.63	5368	14	12.307	129719	0.93	7683
6	6.017	63607	0.46	5073	15	14.135	117449	0.84	6130
7	6.172	144278	1.03	8374	16	15.185	20610	0.15	1000
8	6.935	211615	1.52	8730	17	15.728	35970	0.26	1815
9	7.190	163214	1.17	9643	18	17.183	44474	0.32	2555

	RT	Area	% Area	Height
19	18.025	26174	0.19	1544
20	20.475	24058	0.17	1506
21	20.894	83958	0.60	4179
22	21.682	93932	0.67	4929
23	22.057	76063	0.54	4038
24	23.230	404828	2.90	20413
25	24.051	702118	5.03	37172
26	25.268	43314	0.31	2531
27	26.572	1530355	10.96	83528
28	27.339	89112	0.64	4693
29	28.517	331726	2.38	17513

Appendix 2b. HPLC peak area of carotenoids of cooked *Telfairia occidentalis* (ugu) leaf

	RT	Area	% Area	Height
1	4.163	52160	0.20	6742
2	4.359	48928	0.19	3502
3	4.981	90663	0.35	4946
4	5.241	132901	0.52	7496
5	5.620	126019	0.49	9122
6	6.194	447131	1.75	18866
7	6.987	488491	1.91	22695
8	7.228	148747	0.58	11088
9	7.867	113251	0.44	5131

	RT	Area	% Area	Height
10	8.324	148094	0.58	8564
11	8.836	1595093	6.23	82636
12	9.573	13863539	54.15	812052
13	10.799	205407	0.80	11219
14	11.559	43823	0.17	1610
15	12.306	258578	1.01	14068
16	14.123	189601	0.74	10156
17	15.764	45964	0.18	2708
18	17.186	34290	0.13	1200
19	18.040	49029	0.19	2795
20	19.021	36238	0.14	2069
21	19.506	18511	0.07	1076
22	20.475	84502	0.33	4071
23	20.888	189056	0.74	10094
24	21.687	258946	1.01	13763
25	22.060	221762	0.87	11273
26	23.229	1098753	4.29	55511
27	24.054	1478983	5.78	78030
28	25.274	75439	0.29	4651
29	26.575	3295353	12.87	179965
30	27.335	157236	0.61	8514
31	28.517	607938	2.37	32293

Appendix 2c. HPLC peak area of carotenoids of stored *Telfairia occidentalis* (ugu) leaf

	RT	Area	% Area	Height
1	4.146	61873	0.33	7026
2	4.319	57190	0.30	4210
3	4.946	77044	0.41	4675
4	5.197	171525	0.91	10490
5	5.805	649902	3.43	40641
6	6.121	425634	2.25	26927
7	6.883	659381	3.48	26279
8	7.816	112119	0.59	5217
9	8.248	278462	1.47	15505

	RT	Area	% Area	Height
10	8.796	969714	5.12	48736
11	9.537	11312365	59.77	671038
12	10.779	98172	0.52	5676
13	11.561	21953	0.12	934
14	12.299	177777	0.94	10549
15	14.123	165061	0.87	8620
16	15.766	56260	0.30	3326
17	17.209	30338	0.16	1635
18	17.420	21039	0.11	1266
19	18.039	23253	0.12	1375
20	19.005	28047	0.15	1792
21	20.499	20161	0.11	1338
22	20.881	51828	0.27	3146
23	21.683	87024	0.46	4731
24	22.065	79849	0.42	4161
25	23.231	455768	2.41	22661
26	24.046	683556	3.61	35759
27	25.264	40073	0.21	2355
28	26.569	1657742	8.76	90342
29	27.331	97077	0.51	5115
30	28.513	357738	1.89	18602

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The peer review history for this paper can be accessed here:
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