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.

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, Amaranthus hybridus, Talinum triangulare, Pterocarpus mildbraedii and Gnetum africanum. Total–carotene was determined spectrophotometrically, while HPLC was used for detailed analysis of carotenoids. 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 – 11.0 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 (738.55 – 1756.51 µg/gdwt) contents of the samples, however, it decreased the % trans–β-carotene contents. Storage conditions in the study increased...
significantly ($P < 0.05$) the contents of total $\beta$-carotene and total-carotene except in the case of *Gnetum africanum* leaf. A regression model for the two methods of analysis of $\beta$-carotene with a coefficient of correlation $r = 0.925$ and coefficient of determination $r^2 = 0.856$, which allows for the calculation of total $\beta$-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 $\beta$-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 caroteneroid 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 throughput 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 carotenoides [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 $\beta$-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 carotenoides 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 activity of some carotenoides, 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 $\beta$-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*, *Talinum 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* (lnine), *Talinum triangulare* (mbolodi), *Pterocarpus mildbraedli* (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 mildbraedli* 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 polyethylene 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, C30 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)

\[ \frac{A_{fr1} \times volume (ml) \times 10^4 \times (DF)}{A_{1cm} \times sample \ weight} \]

Where,

\( A_{fr1} \) = Absorbance at 450 nm

Volume (ml) = volume of fraction 1

\( A_{1cm} \) = 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 oxy-carotenoids to lipophilic hydrocarbons, thus; Lutein, β-Cryptoxanthin, 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-Cryptoxanthin (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-Cryptoxanthin (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-Cryptoxanthin (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 constituent 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 et 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-carotene 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 than 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* after 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-caroten. 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/dwt). Also, raw *G. africanum* β-carotene concentration (246.93 µg/dwt) was significantly (P < 0.05) higher than other leaves, while *T. triangulare* raw leaf had the least concentration of β-carotene (45.42 µg/). Comparing the total β-carotene content of the leaves with previous reports, Schönfeldt and Pretorius [31], reported 79.6 µg/dwt in cooked *Cucurbita maxima* and 160 µg/dwt in raw *Amaranthus tricolor*. Ninomia and Godoy [32] recorded 85 µg/dwt in raw mint leaves. Also Žnidarčič et al. [33] reported 79 µg/dwt and 73 µg/dwt in garden rocket and chicory respectively. It seems therefore, that the leafy vegetables from the study are very rich dietary sources of β-carotene 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/dwt) and lowest in *P. mildbraedii* (5.05 µg/dwt). Also, the β-cryptoxanthin content in the cooked leaves was highest in *T. occidentalis*
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

| Parameters | Species | Raw  | Cooked | Stored |
|------------|---------|------|--------|--------|
| Lutein     | Telfairia occidentalis | 655.70±4.95 | 1158.83±0.05 | 885.65±2.36 |
|            | Amaranthus hybridus    | 309.21±2.33  | 382.92±1.61   | 312.84±2.61  |
|            | Talinum triangulare    | 124.03±0.58  | 593.24±3.55   | 235.68±1.83  |
|            | Pterocarpus mildbraedii| 261.96±9.20  | 507.97±2.27   | 343.33±0.32  |
|            | Gnetum africanum       | 528.87±1.24  | 504.92±1.85   | 394.31±1.27  |
| β-Cryptoxanthin | Telfairia occidentalis | 5.17±0.07   | 7.13±0.15     | 4.99±0.14    |
|            | Amaranthus hybridus    | 11.02±0.25   | 5.76±0.38     | 10.47±0.53   |
|            | Talinum triangulare    | 5.11±0.18    | 4.86±0.12     | 5.15±0.02    |
|            | Pterocarpus mildbraedii| 5.05±0.05    | 5.59±0.02     | 4.88±0.45    |
|            | Gnetum africanum       | 5.77±0.25    | 6.22±0.25     | 4.79±0.29    |
| 13-cis-β-Carotene | Telfairia occidentalis | 32.03±1.38   | 90.72±0.09    | 35.97±0.11   |
|            | Amaranthus hybridus    | 34.30±0.57   | 45.56±0.77    | 32.78±0.89   |
|            | Talinum triangulare    | 7.79±1.00    | 71.31±1.23    | 20.25±1.37   |
|            | Pterocarpus mildbraedii| 16.26±1.21   | 40.26±1.17    | 17.89±0.94   |
|            | Gnetum africanum       | 34.35±0.52   | 36.90±0.45    | 8.10±0.19    |
| 15-cis-β-Carotene | Telfairia occidentalis | 54.65±0.62   | 121.67±2.19   | 53.34±0.49   |
|            | Amaranthus hybridus    | 5.69±0.42    | 6.11±0.21     | 5.28±0.07    |
|            | Talinum triangulare    | 3.39±0.76    | 29.71±0.18    | 9.07±0.67    |
|            | Pterocarpus mildbraedii| 4.84±0.16    | 12.14±1.95    | 4.43±0.09    |
|            | Gnetum africanum       | 91.84±1.95   | 89.14±2.94    | 27.56±2.34   |
| Trans-β-Carotene | Telfairia occidentalis | 117.68±2.17  | 269.48±0.69   | 127.61±2.23  |
|            | Amaranthus hybridus    | 107.97±3.45  | 125.54±3.70   | 102.48±5.27  |
|            | Talinum triangulare    | 25.71±0.48   | 209.29±2.41   | 70.09±2.84   |
|            | Pterocarpus mildbraedii| 50.13±1.30   | 132.79±0.46   | 65.78±1.48   |
|            | Gnetum africanum       | 96.53±1.45   | 95.56±0.86    | 65.41±2.02   |
| 9-cis-β-Carotene | Telfairia occidentalis | 26.47±2.29   | 50.78±1.04    | 28.50±0.72   |
|            | Amaranthus hybridus    | 26.89±0.22   | 31.72±2.53    | 26.45±0.56   |
|            | Talinum triangulare    | 8.53±0.76    | 44.29±1.30    | 20.91±0.19   |
|            | Pterocarpus mildbraedii| 12.30±1.28   | 27.25±0.36    | 12.56±0.36   |
|            | Gnetum africanum       | 24.21±1.68   | 26.51±0.76    | 21.56±1.35   |
| Total β-carotene | Telfairia occidentalis | 230.82±4.96  | 532.66±13.94  | 245.42±7.96  |
|            | Amaranthus hybridus    | 174.86±2.81  | 208.94±2.98   | 166.99±1.73  |
|            | Talinum triangulare    | 45.42±3.53   | 354.60±2.95   | 120.31±1.13  |
|            | Pterocarpus mildbraedii| 83.53±0.89   | 212.44±1.40   | 100.65±3.39  |
|            | Gnetum africanum       | 246.93±6.68  | 248.10±3.05   | 122.63±5.50  |

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

![Fitted Line Plot](image)

**Fig. 3a. Linear Relationship between Total-carotene and β-carotene**

**Quadratic Model:**

![Fitted Line Plot](image)

**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

| Model | 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\(^a\)        |

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. |
|-------------|----------------|----|---------------|-------|------|
| 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

d. 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 |     |     |
| TC      | .292           | .033       | .925  | 8.801 | .000 |
| (Constant) | -7.031      | 27.151     | -.259 | .800  |     |

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 |
|---------------|----|----------|-------------------|---------------------------|
| R            | .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 carotenoids 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 (Spectrophotometric 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-Saharan 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<sup>a</sup>             | 106.32<sup>a</sup> |               |
| Amaranthus hybridus      | 119.48<sup>a</sup>             | 66.90<sup>a</sup>  |               |
| Talinum triangular       | 780.71<sup>b</sup>             | 264.88<sup>b</sup> |               |
| Pterocarpus mildbraedii  | 254.32<sup>a</sup>             | 120.49<sup>a</sup> |               |
| Gnecatum Africanum       | 100.47<sup>a</sup>             | 49.72<sup>a</sup>  |               |
### 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 |
|----|-------|--------|--------|
| 1  | 4.140 | 45004  | 0.32   | 4929  |
| 2  | 4.315 | 43218  | 0.31   | 3495  |
| 3  | 4.903 | 25660  | 0.18   | 2151  |
| 4  | 5.221 | 42663  | 0.31   | 2392  |
| 5  | 5.669 | 87619  | 0.63   | 5368  |
| 6  | 0.017 | 63007  | 0.40   | 5073  |
| 7  | 6.172 | 144278 | 1.03   | 8374  |
| 8  | 6.935 | 21615  | 1.52   | 8730  |
| 9  | 7.190 | 163214 | 1.17   | 9643  |

| RT | Area  | % Area | Height |
|----|-------|--------|--------|
| 10 | 7.913 | 50821  | 0.36   | 2542  |
| 11 | 8.796 | 832516 | 5.96   | 37484 |
| 12 | 9.537 | 8363924| 60.14  | 495319|
| 13 | 10.776| 99228  | 0.71   | 5749  |
| 14 | 12.307| 129719 | 0.93   | 7683  |
| 15 | 14.135| 117449 | 0.84   | 0130  |
| 16 | 15.185| 20610  | 0.15   | 1000  |
| 17 | 15.728| 35970  | 0.26   | 1615  |
| 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 | 20.984| 83958  | 0.60   | 4179  |
| 22 | 21.662| 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 *Telfaria 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   | 4046   |
| 4    | 5.241  | 132901 | 0.52   | 7496   |
| 5    | 5.620  | 126019 | 0.49   | 9122   |
| 6    | 6.104  | 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   |

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

| RT   | Area   | % Area | Height |
|------|--------|--------|--------|
| 10   | 9.324  | 149094 | 0.58   | 8584   |
| 11   | 9.836  | 159593 | 0.63   | 62636  |
| 12   | 9.573  | 1386359| 54.15  | 612052 |
| 13   | 10.799 | 205407 | 0.80   | 11219  |
| 14   | 11.559 | 43623  | 0.17   | 1610   |
| 15   | 12.306 | 258758 | 1.01   | 14068  |
| 16   | 14.123 | 189601 | 0.74   | 10156  |
| 17   | 15.764 | 45664  | 0.18   | 2708   |
| 18   | 17.186 | 34290  | 0.13   | 1200   |
| 19   | 18.046 | 49629  | 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 | 189058 | 0.74   | 10094  |
| 24   | 21.687 | 259845 | 1.01   | 13763  |
| 25   | 22.090 | 221762 | 0.67   | 11273  |
| 26   | 23.229 | 1098753| 4.29   | 55511  |
| 27   | 24.054 | 1478083| 5.78   | 78030  |
| 28   | 25.274 | 75439  | 0.29   | 4651   |
| 29   | 26.575 | 3295353| 12.87  | 179905 |
| 30   | 27.335 | 157238 | 0.61   | 8514   |
| 31   | 28.517 | 607938 | 2.37   | 32293  |

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| RT | Area   | % Area | Height |
|----|--------|--------|--------|
| 10 | 8.796  | 969714 | 5.12   | 48736  |
| 11 | 9.537  | 11312365| 59.77  | 671038 |
| 12 | 10.779 | 98172  | 0.52   | 5876   |
| 13 | 11.561 | 21953  | 0.12   | 934    |
| 14 | 12.299 | 17777  | 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 | 51826  | 0.27   | 3146   |
| 23 | 21.683 | 87024  | 0.46   | 4731   |
| 24 | 22.065 | 79849  | 0.42   | 4161   |
| 25 | 23.231 | 455768 | 2.41   | 22061  |
| 26 | 24.045 | 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   | 18802  |

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