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Effects of Post-harvest Storage and Moist Heat Treatment on Carotenoids and Micronutrients in African Spinach (*Amaranthus hybridus*)

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Authors' contributions

This work was carried out in collaboration between both authors. Author PCO designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author FNO managed the analyses of the study, the literature searches and performed the statistical analyses. Both authors read and approved the final manuscript.

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ABSTRACT

The aim of this research was to investigate the nutrient composition of *Amaranthus hybridus* (raw,stored and as consumed) and evaluate its potentials for alleviating hidden hunger. Changes in carotenoids, selected vitamins and minerals of *Amaranthus hybridus* leaf during cooking (5 min,100°C) and room temperature storage conditions (29 ± 2°C, 85%RH) were determined. Carotenoids were separated by High performance liquid chromatography (HPLC) on a reversed-phase column. Lutein, β-cryptoxanthin, β-carotene and its *trans*-/cis-isomers were identified and quantified. Total-carotene was determined spectrophotometrically. The results indicated that the raw vegetable was rich in lutein (309.21 µg/gdwt) and β-carotene (174.86 µg/gdwt). β-cryptoxanthin was detected in relatively small amounts (11.02 µg/gdwt). Cooking significantly

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increased ($p<0.05$) the lutein (382.92 µg/gdwt) and β-carotene (208.94 µg/gdwt) contents of the leaf sample; however, it decreased the trans-β-carotene content. The contents of ascorbic acid, thiamin, niacin and riboflavin were as follows (in mg per 100 gfwt) 54.30, 0.14, 3.69 and 1.84 respectively, while phylloquinone (vitamin K_1) was 122.21 µg/100 gfwt. Cooking significantly ($p<0.05$) decreased the contents of the vitamins except phylloquinone. The mineral contents of raw sample were (in mg/100 g fwt), potassium, 5.50, calcium, 1.15, magnesium,1.20, zinc, 1.72 and iron, 10.40 respectively. Cooking of the leaf significantly ($p<0.05$) decreased the contents of potassium, calcium, zinc, magnesium, but increased the content of Iron. Storage caused a non-significant decrease in the content of niacin, potassium and magnesium. Consumption of 100-200 g/day of the leaf may be adequate to meet the recommended daily allowances (RDAs) of 900 µgRAE/day, 90 mg/day, 120 µg/day 1.3 mg/day and 9mg/day for vitamin A, vitamin C, vitamin K_1, riboflavin and Iron respectively. *Amaranthus hybridus* leaf will therefore improve the nutritional status of populations that consume adequate amount of the vegetable.

Keywords: Amaranthus hybridus; storage; processing; pro-vitamin A content; micronutrients.

1. INTRODUCTION

Amaranthus hybridus (African Spinach, Amaranth) belongs to the family *Amaranthaceae*. It is an annual herb, erect or less commonly ascending up to 2 m tall and often reddish tinted throughout [1]. The main uses of *A. hybridus*areas leaf vegetable prepared by cooking and consumed as a vegetable dish or as an ingredient in sauces [2].

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, minerals and fibre which are usually in short supply in daily diets [3]. Green leafy vegetables are very rich in carotenes. Besides the well-known provitamin-A activity of some carotenoids, they have also been associated with reduced risk of developing degenerative diseases, cataract and age related blurred or no vision in the eye [4]. 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 [5]. Several publications [6,7] have stressed the nutritional importance of traditional and indigenous leafy vegetables. However, the use of traditional and indigenous leafy vegetables by local is limited. Knowledge of indigenous plant use needs urgent scientific investigation and documentation before it is irretrievably lost to future generations [8].

The aim of this study is to document scientific information on the nutritional potentials of an underutilized crop, *Amaranthus hybridus* (raw, stored and as consumed) for combating hidden hunger. In rural populations of developing countries, vegetables are stored at ambient temperature especially when the market is saturated with fresh produce. Also in Africa, vegetables are often cooked before consumption. Therefore, another aim of this study is to evaluate quality and nutrient retention after storage and cooking. The results of this research work will determine whether *Amaranthus hybridus*could contribute effectively to the alleviation of micronutrient deficiencies among consumers. This will help to adequately establish its importance in human nutrition and provide basis for maximum utilization of the crop.

2. MATERIALS AND METHODS

2.1 Selection of Vegetable Sample

*Amaranthus hybridus***,** a green leafy vegetable that is commonly consumed by both rural and urban communities in South-Eastern Nigeria was identifiedbotanically and used for research work.

2.2 Collection of Sample

Details of the planting and harvesting of this crop has been described by Okpalanma et al. [9].The crop *Amaranthus hybridus*, was selected at random from the planting area (after 8 weeks of planting) and picked by hand mid-morning during the harmattan season. A minimum of 1 kg of the crop was collected randomly from different plants within the field. The leaves were placed in black polyethylene bags and transported to the Biochemistry Department of the University of

Nigeria Nsukka for processing. Meanwhile analyses of the dried (oven dried at 50°C for 48 h) and milled samples for carotenoids, vitamins and minerals were carried out at IITA (International Institute of Tropical Agriculture) Ibadan, Nigeria.

2.3 Processing of Samples

The methods used for sample processing were washing andshort time high temperature moist heat treatment.Storage was by wrapping dry fresh leaves in newspaper for 5 daysas described in Okpalanma et al. [9].

2.4 Sample Preparation for Carotenoid Analysis

The method used for sample preparation has been described in Okpalanma and Ojimelukwe [10]. Raw, cooked and stored samples were oven dried at 50°C for about 48 h. About 30 g of each sample was stored in sealed polyethylene bags. The samples were stored at -20^{0}C until they were used for carotenoid analysis because carotenoids are not stable at higher temperatures.

2.5 HPLC Determination of Total β-carotene Content

The method of Howe and Tanumihardjo [11] was used. 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 standards established in the crop utilization laboratory of IITA Ibadan, Nigeria were used.

2.6 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 [12]. The absorbance was read at 450 nm using Jenway Spectrophotometer (Model 752, England).

$$\text{Total carotene content. } (\mu g/g) = \frac{A_{fr1} \times volume\ (ml) \times 10^4 \times (DF)}{A_{1cm}^{1\%} \times sample\ weight}$$

Where,

A_{fr1} = Absorbance at 450nm

Volume (ml) = volume of fraction 1

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

2.7 Determination of Vitamins

Vitamin analyses were carried out on raw, cooked and stored leaves. Ascorbic acid, riboflavin, thiamin, vitamin k and niacin were analyzed. Raw, cooked and stored samples were refrigerated before analysis. The vitamin content of *Amaranthus hybridus* leaf was determined by standard methods described by AOAC [13].

2.7.1 Determination of ascorbic acid

Ascorbic acid was determined by weighing 3 g of each sample into an extraction tube. One hundred (100) ml of the extracting solution containing metaphosphoric acid and acetic acid was added to the tube in a 2:1 ratio. The resulting sample extract was centrifuged at 3000 rpm for 20 min. The obtained sample extract was transferred to 100 ml volumetric flask and made up to the mark with the extraction solution. Standard ascorbic acid solution was also prepared. Twenty (20) ml of both solutions were titrated against 2,6 dichloroindophenol to a pink end point. Ascorbic acid content was calculated using the formula:

$$\text{Ascorbic acid content} = \frac{C \times V \times DF}{WT}$$

Where,

V = Volume of dye used for diluting samples;
C = mg ascorbic acid/ml of dye
Df = Dilution factor; WT = Sample weight.

Loss of ascorbic acid (AA) was calculated as:

$$\%\ \text{Loss} = \frac{\text{AA content per 100 g of raw leaf-AA content per 100 g of processed leaf} \times 100}{\text{AA content per 100 g of raw leaf}}$$

2.7.2 Determination of niacin content

Three (3) grammes of each sample was weighed into a 50 ml conical flask containing 10 ml of 1N Sulphuric acid. It was heated to boiling point in a

water bath for 30 min and cooled. pH of the resulting extract was adjusted to 4.5. Sample extracts were put into volumetric flasks, made up to the mark with distilled water, purified b adding 17 g Ammonium sulphate and filtered. The purified filtered sample and standard niacin solution were mixed separately with 5 ml of 10% cyanogen bromide; 0.5 ml of 2% aqueous ammonium hydroxide; 2 ml of 10% Sulphuric acid and 0.5 ml of dilute hydrochloric acid. After leaving the sample to develop colour for 2 min, niacin concentration was estimated using a colorimeter (AE-11D HOSPIBRAND, USA) at 430 nm. Niacin content was calculated from the formula:

$$\text{Niacin content} = C \times \frac{DF}{WF}$$

Where C=Concentration of niacin; DF = Dilution factor; WT = Weight of sample.

2.7.3 Determination of riboflavin content

Three (3) g of each sample was put into a 50 ml conical flask containing 20 ml of 2N HCl. The flask was heated to boiling in a water bath for 60 min and cooled. The pH of the sample extract was adjusted to pH 6 immediately using NaOH and readjusted to pH 4.5 using 1N HCl. The resulting solution was filtered and made up to 100 ml in a volumetric flask. Ten (10) ml of sample solution, 1 ml of water and 1 ml of standard riboflavin were put in separate test tubes, One (1) ml of glacial acetic acid and 0.5ml of 3% potassium permanganate were put into each test tube After 2 min 0.5ml of 3% hydrogen peroxide were added to the tubes The fluorescence of the sample containing distilled water was measured in a Fluorimeter (Shimadzu, RF 10A Kyoto, Japan) at an excitation wavelength of 470 nm and emission wavelength of 626 nm. Thereafter, 10 ml of Sodium hydrogensulphite solution was added to each sample and their fluorescence measured within ten seconds. Riboflavin content was calculated with the formula:

$$\text{Riboflavin} = \frac{X}{Y-X} \times \frac{1}{W}$$

Where,

A = Reading of sample-Reading of sample blank
Y = Reading of sample + standard – (Reading of sample + standard blank)
W = Weight of sample.

2.7.4 Determination of thiamin content

Three (3) g of each sample was mixed with 0.2N HCl in a 50 ml conical flaskand was heated to boiling in a water bath for 30 min. It was cooled and the pH was adjusted to pH 4.5 to 5.0 with HCl. Five (5) ml of 6% phosphatase was added to the solution which was incubated overnight at 37°C. The hydrolyzed sample extract was diluted to 100 ml in a volumetric flask. The mixture was filtered, passed through a column of silicate and eluted with 5ml hot acidic potassium chloride into a 25 ml volumetric flask. Ten (10) ml of both the sample and standard eluates were pipetted into four separate 50 ml conical flasks containing 1.5 g NaCl. Three (3) ml Potassium ferricyanide solution and 15 ml isobutanol were added to each sample. The flasks were shaken thoroughly for 2 min and the isobutyl fraction was decanted into fluorescent reading tubes. Fluorescence readings were taken at 365 nm excitation wavelength and emission wavelength of 435nm using a Fluorimeter (Shimadzu, RT-10A, Kyoto, Japan). The remaining two conical flasks containing standard and sample eluates were treated with 3 ml of 15% NaOH and their fluorescence measured and recorded as sample and standard blanks. Thiamin content was calculated from the formula:

$$\text{Thiamin content} = \frac{X \times 1 \times 25}{Y \times 5 \times V}$$

Where

X = Reading of sample-Reading of blank.
Y = Reading of standard-Reading of standard blank
V = Volume passed through the column.

2.7.5 Determination of vitamin K

Ten (10) g of each sample was refluxed for 3 h with 100 ml ethanol and filtered. The residue was extracted in the dark with 100 ml Petroleum ether (b.pt 40-60°C) for 48h using a Soxhlet apparatus. The ethanol/ether extracts were evaporated to dryness in a vacuum under nitrogen. It was re-dissolved in 100 ml of ether and 10 ml aliquot was mixed with equal volume of cold half saturated Barium hydroxide with 5 ml of water followed by 5 ml of 1:1 ethanol water solution. The washings were extracted with 10 ml of ether and all the combined ether extracts were dried over anhydrous Sodium sulphate and evaporated

in vacuo under nitrogen. The residue was re-extracted with 2 ml of 1:1 ethanol water solution. One (1) ml of 0.04% 2,4 –Dinitrophenylhydrazine in HCl (1:3 ratio) was added. The mixture was heated in a boiling water bath for 45 min and cooled. Two (2) ml of 8% NaOH and 1ml of amylalcohol were added and heated in a boiling water bath and cooled. The mixture was made up to 10 ml with ethanol and the absorbance was read at 620 nm in a spectrophotometer using water as blank. Vitamin K concentrations were calculated from the standard vitamin K curve.

Vitamin K = Y/0.946

Where Y = Absorbance reading of samples

2.8 Determination of Minerals

Potassium, calcium, magnesium, zinc and iron were analyzed. Raw, cooked and stored samples for minerals analysis were wet-digested before analysis. Mineral content was determined after wet digestion of samples using standard AOAC methods [13]. One-half gramme of each sample was weighed into a kjeldhal flask. Twenty five (25) ml of a mixture of conc. HCl and HNO_3 (3:1 ratio) was added to the flask. The flask and its content was heated until the content became colourless. It was transferred to a 250 ml volumetric flask and was made up to the mark with distilled water. The solution obtained after wet digestion was evaluated for its content of potassium, calcium, magnesium, zinc and iron in an Atomic Absorption Spectrophotometer (AAS) (Buck Scientific 210VGP). The sample was analyzed for different elements after calibrating the AAS for each selected element using prepared standards with known concentrations. The absorbance values were measured for each element at the corresponding wavelength: K (703 nm); Ca (422.7 nm); Mg (285.2 nm); Zn (307.6 nm) and Fe (372 nm).

2.9 Statistical Analysis

The means, standard deviations and analysis of variance (ANOVA) of all the data obtained from the study were computed using the Statistical Package for Social Science (SPSS) version 17. Analysis of Variance was used to detect significant differences ($p \leq 0.05$) among the sample means followed by the application of Least Significant Difference test (LSD) for the separation of significant means.

2.10 Experimental Design

The experimental design was randomized complete block having one vegetable type and three processing (treatments) giving 1 x 3 = 3 observations. Each observation was repeated three times giving 3 x 3 = 9 observations for each parameter tested.

3. RESULTS AND DISCUSSION

3.1 Chromatographic Profiles of Carotenoids

Fig. 1 (a-c) show the HPLC chromatogram of raw, cooked and stored *Amaranthus hybridus* leaf samples. The xanthophylls and carotene components identified and quantified, were eluted in order of decreasing polarity: Lutein>β-cryptoxanthin>13-*cis*-β-carotene>15-*cis*-β-carotene>*trans*-β-carotene and 9-*cis*-β-carotene.

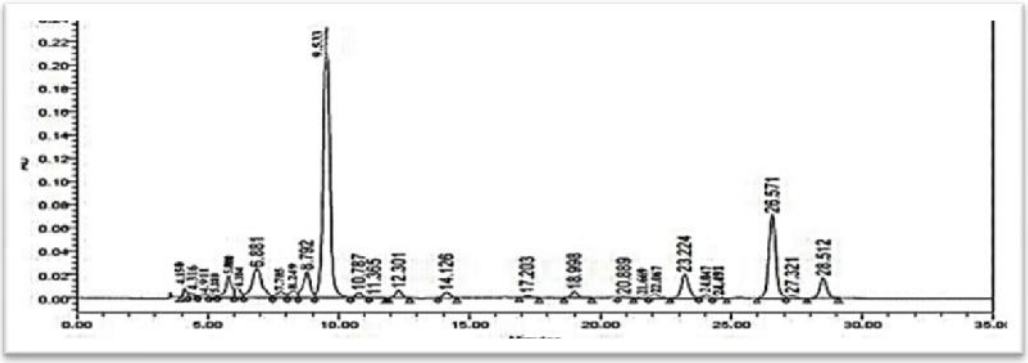
3.2 Total β-carotene Content

Table 1 shows the carotenoid concentrations of *Amaranthus hybridus* leaf as influenced by ambient temperature storage and cooking treatments. The total β-carotene (tβC) level was significantly ($p<0.05$) higher in cooked leaf (208.94 µg/gdwt) than in raw leaf (174.86 µg/gdwt). This correlates with the results from Faber et al. [14]. Processed samples have greater extractability of carotenoids which could explain the higher tβC levels in the cooked samples [15]. Cooking also resulted in 119.48% apparent retention when compared with the raw leaf (Table 2). Dietz and Erdman [16] reported that cooking resulted in greater than 100% retention of β-carotene in vegetables, because denaturation of carotene binding proteins release the carotenoids so they can be extracted more easily.

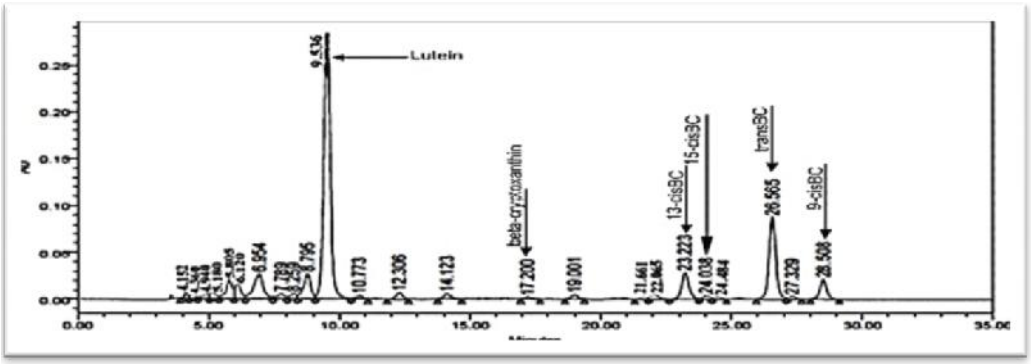
As expected, the *trans*-β-carotene (Table 2) was lower in the cooked than in raw leaves. During cooking, some of the *trans*-β-carotene could have been converted to cis-isomers or other oxidation products [17]. Literature suggests that the consequences of *trans*-cis-isomerization are changes in bioavailability and physiological activity [18].

Table 1 also shows a non-statistical decrease in stored leaf tβC contents (166.99 µg/gdwt) when compared with the raw leaf (174.86 µg/gdwt). Total β-carotene decrease during the shelf-life period (5 days) could result from sustained

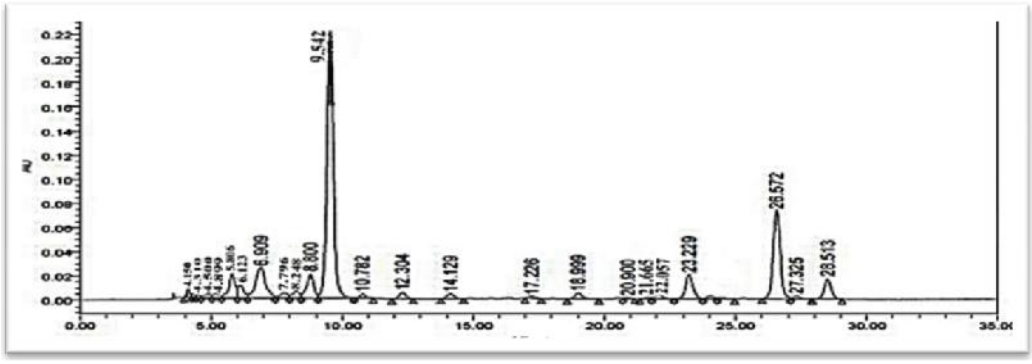
enzyme activity, physiological degradation and further respiration of plant tissue. Beta-carotene near the outer cell wall would be more susceptible to heat and oxidative damage [19]. However, the slight decrease during storage resulted in an apparent retention of 95.49% of tβC (Table 2). The importance of this observation, was that the storage conditions in our study did not degrade much of the carotenoids. Comparing the tβC of *A. hybridus* with previous reports [20] recorded similar trend. They reported that tβC content of cooked leaves ranged from 23.43 µg/gdwt (*Amaranthus tricolor)* to 61.53 µg/gdwt (*Corchorustridens*) and of raw leaves from 16.01 µg/gdwt (*Amaranthus tricolor)* to 36.63 µg/gdwt (*Corchorus tridens).*



(a) Raw *Amaranthus hybridus*



(b) Cooked *Amaranthus hybridus*



(c) Stored *Amaranthus hybridus*

Fig. 1. (a-c). Carotenoid Profile of *Amaranthus hybridus* (spinach) Leaf Samples by HPLC (a) Raw (b) Cooked (c) Stored

Table 1. Effects of storage and moist heat treatments on the carotenoid content of *Amaranthus hybridus* leaf

Carotenoid concentration (µg/g edible portion dry weight basis)	Treatment		
Carotenoid	**Raw**	**Cooked**	**Stored**
Lutein	309.21^{b} ±2.3	382.92^{a} ±1.61	312.84^{b} ± 2.61
β -cryptoxathin	11.02^{b} ± 0.25	5.76^{a} ± 0.38	10.47^{b} ± 0.53
13-*cis*-β-carotene	34.30^{b} ± 0.57	45.56^{a} ± 0.77	32.78^{b} ± 0.89
15-*cis*-β-carotene	5.69^{b} ± 0.42	6.11^{a} ± 0.21	5.28^{b} ± 0.07
Trans-β-carotene	107.97^{b} ± 3.45	125.34^{a} ±3.70	102.48^{b} ± 5.27
9-*cis*-β-carotene	26.89^{b} ± 0.22	31.72^{a} ± 2.53	26.45^{b} ± 0.56
Totalβ-carotene	174.80^{b} ± 2.81	208.94^{a} ± 2.96	166.96^{b}±1.73

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

Table 2. Provitamin A content, % trans of total β-carotene and % retention of total β-Carotene

Treatment	Provitamin A (µg/g)	Vitamin A Activity (µg RAE)/100g)	%Trans Total β-carotene	% Retention (Total β-carotene)
Raw	180.37	1190	61.74	-
Cooked	211.82	1390	60.08	119.84 (after cooking)
Stored	172.22	1144	61.36	95.49 (after drying)

The data shown on Table 1 indicated that the most abundant *cis*-isomer of β-carotene in the raw, cooked and stored samples was 13-*cis* β-carotene. Several different geometric isomers of β-carotene; *trans*-, 9-*cis*-, 13-*cis*, 15-*cis* isomeric forms exist in food and human tissues [21]. The major β -carotene isomers in the circulation of humans is *trans*- β -carotene with small amounts of 13-*cis*, 9-*cis*- β -carotene. The levels of *cis*-isomers of β-carotene are much higher in leafy vegetables [22]. Therefore, isomer separation is needed for the accurate determination of the vitamin A activity of leaf meals.

3.3 Xanthophyll Content

The β-cryptoxanthin content of the leaf was relatively in small quantity (Table 1) β-cryptoxanthin is a minor pro-vitamin A constituent of leaves [23]. The β-cryptoxanthin content of the cooked leaves (5.76 µg/gdwt) was significantly ($p<0.05$) lower than in both the raw (11.02 µg/gdwt) and stored (10.47 µg/gdwt) leaf sample.

Lutein content of the cooked leaf (382.95 µg/gdwt) was significantly ($p<0.05$) higher than in both the raw (309.21 µg/gdwt) and stored (312.847 µg/gdwt) leaf samples. Several previous reports on lutein contents of leafy vegetables had been reported [24] and showed that lutein concentration ranges from 4.8 to 13.4 mg/ 100 g fwt) for Kale and from 6.5 to 13.0 mg /100 g fwt for spinach. Similar trend was reported by Dias et al. [25] who observed from 0.52 to 4.0 mg/100 g fwt for leaf beat and turnip greens. According to Wisniewka and Subczynski [26], the presence of lutein and /or zeaxanthin in the diet may be beneficial for reducing the incidence of the two common eye diseases of aging; age-related macular degeneration and cataracts formation.

3.4 Pro-vitamin A Content

The pro-vitamin A concentration in the leaf sample was calculated (Table 2) by adding the value of total β-carotene to one-half the value of the corresponding β-cryptoxanthin [12]. The principal pro-vitamin A carotenoids is β-carotene, while the β-cryptoxanthin is a minor constituent [12]. The pro-vitamin A content of the raw, cooked and stored samples were 180.37, 211.82 and 172.22 µg/g respectively. Vitamin A is expressed as β-carotene, retinol, retinol equivalent or retinol activity equivalent. Hence 1 µg RAE = 1 µg retinol = 12 µg *trans*- β-carotene= 24 µg β-cryptoxanthin [27]. Using the above conversion factors, the vitamin A activity (in µg RAE/100 g) (Table 2) of the raw, cooked and stored leaf samples were 1190, 1390 and 114 respectively. It follows therefore that the consumption of about 100 – 200 g /day of the

cooked leaves would meet the vitamin A recommended Daily Allowance (RDA) of 900 µg RAE/day for men and 700 µg RAE/day for women 19-30 years old [27]. Though the amount which is bioavailable cannot be assumed to be the same as that calculated from analyzed levels in foods, reliable information on the types and concentrations of the various carotenoids found in fruits and vegetables in these regions is essential as a result of the increased attention paid to the problem of vitamin A deficiency and xerophthalmia.

3.5 Total Carotene Content

Fig. 2 shows the total-carotene content of the leaf samples. The total carotene content in cooked leaf (660.46 µg/gwt) was significantly ($p<0.05$) higher than in raw (533.92 µg/gwt) and stored (492.01 µg/gwt) leaf. This could be explained by higher extractability of carotenoids in cooked leaves. There was no statistical difference in total-carotene content in raw leaf sample when compared with the stored sample. Total-carotene values were higher than corresponding total-β-carotene values. The differences were attributed to the assay method. The spectrophotomeric method gives higher values for samples that contain a complex mixture of carotenoids, because it measures total carotenoids/total-carotene [28].

3.6 Vitamin Content

The vitamin content of *A. hybridus* is shown in Table 3. Vitamin concentrations (in mg/100 g fwt) were 154.30 1.84, 0.14 and 3.69 for ascorbic acid, riboflavin, thiamin and niacin respectively. Vitamin K_1 concentration was 122 µg/100 g fwt. Cooking significantly decreased ($p<0.05$) the contents of the water soluble vitamins. Water soluble vitamins leach into cooking water and some portion of it may actually be destroyed by heating [29]. However, cooking significantly increased ($p<0.05$) the content of vitamin K_1 in the leaf. Vitamin K_1 is located in the chloroplasts in plants. Cooking by boiling may disrupt the cell wall, thereby releasing vitamin K_1thereby making it available for measurements [30]. The loss of ascorbic acid observed in the study was 43%. The reported cases of ascorbic acid loss during blanching or cooking are enormous and may vary between 40 and 70% in some cooked vegetables when processed at 100°C for 10 min [31]. The loss in ascorbic acid after storage in this study was 37%. Other researchers have also reported postharvest losses in ascorbic acid. A 29-50 and 34-38% losses were recorded by P [32] in *Cassia tora* and *Corchorus tridens* leaves stored at 20°C for 8 days. Initial contents of some of the vitamins were in agreement with previous reports [33] reported 155.1 mg/100 g vitamin C in A hybridus. Lower values of these vitamins were reported by Agtel et al. [34] and Uusiku et al. [35] It is therefore evident that concentrations of Ascorbic acid, Riboflavin and vitamin K_1 in *A. hybridus* are high and adequate to meet the RDAs of 90mg/day ascorbic acid, 1.3 mg/day riboflavin and 90 µg/day vitamin K_1 in children and adults respectively [27].

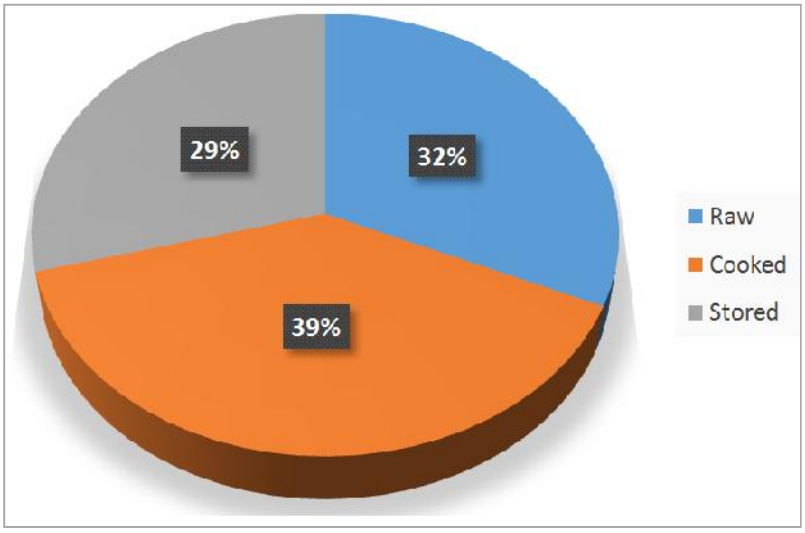


Fig. 2. Total Carotenoid content of raw, cooked and stored *Amaranthus hybridus*

Table 3. Effects of storage and processing on selected vitamins of *Amaranthus hybridus* leaf

Vitamin content per 100g edible portion (fresh weight basis)					
Treatment	**Ascorbic acid (mg)**	**Riboflavin (mg)**	**Thiamin (mg)**	**Niacin (mg)**	**Vitamin K (µg)**
Raw	$154.30^{b}\pm0.14$	$1.84^{b}\pm0.02$	$0.14^{b}\pm0.01$	$3.69^{b}\pm0.01$	$122^{a}.21$
Cooked	$67.59^{a}\pm0.01$	$1.41^{a}\pm0.71$	$0.03^{a}\pm0.01$	$0.47^{a}\pm0.01$	$122^{a}.56$
Stored	$87.16^{a}\pm0.01$	$1.26^{a}\pm0.06$	$0.09^{a}\pm0.01$	$2.05^{b}\pm0.04$	$117^{b}.09$

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

Table 4. Effects of storage and processing methods on selected mineral content of *Amaranthus hybridus* leaf

Mineral content of *Amaranthus hybridus* (mg/100g edible portion, fresh weight basis)					
Treatment	**Iron (Fe)**	**Potassium (k)**	**Calcium (ca)**	**Magnesium (Mg)**	**Zinc (Zn)**
Raw Amaranthus hybridus	$10.40^{a}\pm0.28$	$5.50^{b}\pm0.14$	$1.15^{b}\pm0.07$	$1.20^{b}\pm0.28$	$1.72^{b}\pm0.14$
Cooked *A. hybridus*	$12.70^{b}\pm0.14$	$4.35^{a}\pm0.49$	$0.70^{a}\pm0.14$	$0.85^{a}\pm0.07$	$1.05^{b}\pm0.07$
Stored *A. Hybridus*	$11.60^{b}\pm0.56$	$5.1^{b}\pm0.14$	$1.05^{a}\pm0.07$	$1.3^{b}\pm0.14$	$1.7^{b}\pm0.07$

**values are mean±standard deviation of triplicate determination means with different superscripts within the same column are significantly different (p=0.05)*

3.7 Effects of Storage and Processing Methods on Selected Vitamins of Indigenous Green Leafy Vegetables

Table 3. shows the effects of storage and processing methods on selected vitamins of *Amaranthus hybridus.*

3.8 Mineral Content

The effects of storage and cooking on the selected mineral contents of *Amaranthus hybridus* was presented on Table 4. The mineral content of the raw leaves were (in mg/100 g) potassium 5.50, calcium 1.15, magnesium 1.20, zinc, 1.72 and Fe, 10.40 respectively. Cooking, significantly ($p<0.05$) decreased the levels of the elements. Oladumaye et al. [36] observed significant ($p<0.05$) reductions in K, Na, Ca and Fe contents of cooked tender and matured cassava leaves. During the cooking process, minerals leach into cooking water [37].The reductions could be due to effects of oxidizing agents, exposure to heat, light and extremes of pH and other factors that affect organic nutrients [38]. The results of mineral analysis of Amaranth suggest the consumption of large quantities to meet the recommended daily allowance (RDAs) for minerals. Recommendations on mineral intake are usually related to age groups [39,40]. Green leafy vegetables constitute the cheapest dietary source of minerals for combating hidden hunger [41].

4. CONCLUSION

Storage and cooking of the leaves resulted to increases in carotenoids, vitamins and minerals and ascorbic acid. Results observed in this study indicated that consumption of about 100-200 g/day of spinach (*A. hybridus*) may be adequate to meet the RDAs of vitamin A (900 µg RAE/day), vitamin K_1 (120/day), vitamin C (90 mg/day) riboflavin (1.3 mg/day) and iron (9 mg/day) in adults and children respectively. Spinach is therefore an important vegetable for combating micronutrient malnutrition.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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