

Comparison of Nutritional Composition, Antinutritional Factors and Antioxidant Potentials of Orange-Fleshed Sweet Potato Leaves

Henrietta Ayodele Oboh¹, Chiemela Enyinnaya Chinma^{2,3}, Fidelis Ehidiemen Olumese¹, Kingsley Oseren¹, Abraham Aluyor¹, Oluwakemi Toluwalope Savage¹, Obayagbona Joshua Oghosa¹

¹School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria

²Department of Food Science and Technology, Federal University of Technology, Minna, Nigeria

³Food Innovation Research Group, Department of Biotechnology and Food Technology, University of Johannesburg, Doornfontein Campus, Gauteng, South Africa

Email address:

henrietta.oboh@uniben.edu (Henrietta Ayodele Oboh), chinmachiemela@futminna.edu.ng (Chiemela Enyinnaya Chinma)

*Corresponding author

To cite this article:

Henrietta Ayodele Oboh, Chiemela Enyinnaya Chinma, Fidelis Ehidiemen Olumese, Kingsley Oseren, Abraham Aluyor et al. (2023). Comparison of Nutritional Composition, Antinutritional Factors and Antioxidant Potentials of Orange-Fleshed Sweet Potato Leaves. *International Journal of Nutrition and Food Sciences*, 12(6), 184-192. <https://doi.org/10.11648/j.ijnfs.20231206.14>

Received: August 19, 2023; **Accepted:** October 4, 2023; **Published:** December 22, 2023

Abstract: There is increasing consumer demand for functional and bioactive ingredients in foods to promote human health and ensure nutrition security in the developing regions of the world. Locally produced staples can be improved with specific micronutrients using conventional breeding methods. Orange fleshed sweet potatoes are a new variety of sweet potatoes; bred to produce nutrient dense products which can curb micronutrient deficiencies. In order to address vitamin A, macronutrient, and micronutrient deficits in sub-Saharan Africa and Asia, OFSP (Orange fleshed sweet potato) leaves can be used. In this study, the nutritional composition and antinutritional composition, and in vitro antioxidant potentials of orange-fleshed sweet potato (OFSP) leaves compared to fluted pumpkin (*Telfairia occidentalis*) leaves were investigated. Fresh OFSP and pumpkin leaves were washed, dried, pulverized into powder and used for subsequent analysis. The OFSP contained higher ($p \leq 0.05$) contents of protein, ash, dietary fiber, amino acids, minerals, β -carotene, vitamins C, D and E, lutein, total anthocyanin, phytochemicals, and antioxidant activities than fluted pumpkin leaves. The use of OFSP leaves could aid the reduction of micronutrient deficiencies and hidden hunger in poor urban and rural communities in developing countries including Nigeria. The high potassium content of OFSP leaves is advantageous to reduce hypertension and alleviate the scourge of cardiovascular diseases.

Keywords: Orange Fleshed Sweet Potatoes Leaves, Pumpkin Leaves, Phytochemicals, Nutrients, Antioxidants

1. Introduction

Large quantities of Green leafy vegetables (GLV) are available in Nigeria. They serve as cheap and important sources of nutrients because of the presence of minerals, vitamins, fiber, and proteins [1]. The nutrient dense characteristic of a diet can be improved by the inclusion of GLV. Health benefits associated with a diet rich in GLV, includes lower risks of cardiovascular diseases, stroke, and constipation [2]. The numerous phytochemicals, bioactive

compounds and robust antioxidants found in fresh green vegetables have anti-inflammatory activities which can combat illnesses [3].

Low consumption of vegetables contributes to increased morbidity and mortality [4, 5]. Despite the large quantities of GLV in Nigeria, the prevalent diet consists of a large intake of starchy root staples (cassava, sweet potatoes, rice), cereals (maize, millet, and sorghum) and poor intake of animal proteins, fruits, vegetables, and pulses. Biofortification of staple crops with significant amounts of macro and

micronutrients is a global agenda to end malnutrition [6]. In some communities in Nigeria, sweet potatoes were biofortified to enhance the intake of micronutrients and improve their nutritional status [7].

Orange fleshed sweet potato plant is a non-woody creeping plant with smooth, light, green-colored leaves. The vines have purple pigmentation which climbs by tendrils [8]. The roots are adventitious, mostly located within the top 25cm of the soil [9]. Production of green leafy vegetables alongside the tuber crops takes a secondary place; but they serve as a carrier of a variety of compounds with positive influence on human health [10].

OFSP leaves are underutilized in Nigeria. Their inclusion in diet can ensure food security during scarcity. The presence of macro and micronutrients could protect vulnerable groups in the community against hidden hunger, anemia, and oxidative stress.

In this study, the phytochemical, proximate, mineral, vitamin, antinutrient, amino acid, and in vitro antioxidant potentials of orange fleshed sweet potato (OFSP) leaves were assessed and compared to a commonly consumed GLV (*Telfaria occidentalis*, 'fluted pumpkin leaves')



Figure 1. Orange-fleshed sweet Potato leaves I (*Ipomoea batatas*).



Figure 2. 'Fluted pumpkin leaves' *Telfaria occidentalis*.

2. Materials and Methods

2.1. Plant Samples

The National Root Crop Research Institute (NRCI), Umudike, Abia State, Nigeria, provided the fresh leaves of orange-fleshed sweet potatoes and pumpkin in November 2022. They were verified at the University of Benin's Department of Plant Biology and Biotechnology in Benin City, Nigeria. At the department's Herbarium, a specimen

with the number UBH-1493 was stored.

The samples were cleaned with water to eliminate impurities before being air dried for 21 days at a temperature of about 25°C to maintain a constant weight. Using an electric mill, (Horus corn and wheat milling, China) the leaves were ground into a powder and filtered using a 0.55mm silk cloth to obtain dried powdered leaves.

2.1.1. Preparation of Aqueous Extracts

The pulverized leaves (60 g) were macerated in 2.5 Litres of water; stirred and soaked for 72 hours. The mixture was filtered through a cloth mesh, and the Chinese FD -10 bench top micro freeze drier was used to freeze-dry the crude extracts. The freeze-dried extracts were kept at around 4°C in sealed containers. 1g of this extract was utilised for phytochemical analyses.

2.1.2. Qualitative Analysis of Phytochemicals

The aqueous extracts were used for the evaluation of the presence of phytochemicals (Alkaloids, flavonoids, tannins, terpenoids and saponins) to were profiled following the methods of [11].

2.1.3. Proximate Analysis

The samples were analyzed using standard methods [12]. for moisture (method no. 925.09B), ash (method no. 923.03), fat (method no. 920.39C), protein (method no. 992.23), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) (method no. 991.43) and total dietary fiber (calculated as sum of IDF and SDF) [12].

2.1.4. Antinutritional Factors

Phytic acid and Tannins present in the green leafy vegetables were analyzed as described below.

2.1.5. Phytic Acid

One gram of the extract was mixed with 20 mL HCl to extract the Phytic acid in the sample. The filtrate was measured in a UV/Vis spectrophotometer (Genesys G10S, Thermo Fisher Scientific, Waltham, USA) at an absorbance of 500 nm, following the procedure described by [13]. The phytate value was measured from the phytic standard curve ($y = 0.513x + 0.0438$, $R^2 = 0.9978$) and the results were reported in mg /100 g.

2.1.6. Tannin

The sample extract (0.5 g) was mixed with with methanol (5 mL of 1%, v/v), to extract the Tannins. The filtrate was measured at an absorbance of 760 nm in a UV -Visible spectrophotometer (G10S, Thermo Fisher Scientific, Waltham, USA) [14]. Tannin content was measured from a standard curve of catechin ($y = 0.0093 x + 0.0541$, $R^2 = 0.99504$). The results were presented in CE /100 g.

2.2. Determination of Vitamins, Minerals, Amino Acids, and Antioxidants

2.2.1. Determination of β -carotene

The extraction and estimation of β -carotene were performed using a standard procedure [15]. The pulverized

sample (500 mg) was mixed with 10 ml of 80% acetone and were centrifuged for 3–4 minutes at 10,000 rpm. The supernatant was removed and brought up to 20ml in a volumetric flask, and the absorbance was measured at 620nm using a Hitachi U1800 spectrophotometer instrument (Hitachi, Tokyo, Japan). The β -carotene content was calculated using the following formula:

Amount of β -carotene = $7.6(\text{Abs. at } 480) - 1.49(\text{Abs. at } 510) \times \text{Final volume} / (1000 \times \text{weight of leaf taken})$

2.2.2. Determination of Vitamin C Content

The vitamin C content of the polar extracts was determined using the method of [16]. Briefly, 500 μL of a reaction mixture containing 100 μL of 13.3% (TCA, Trichloroacetic acid) and water received 75 μL of 2,4-dinitrophenylhydrazine, DNPH). At 37°C, the reaction mixtures were incubated for 3 hours. The medium was then added 0.5 ml of 65% H_2SO_4 (v/v), and their absorbance was measured at 520 nm. The vitamin C content of the samples was expressed as mg AA/100g.

2.2.3. Determination of Vitamin D and E

Vitamin D and E was profiled using a HPLC following a method described by [17]. while keeping the method of [18]. as the key reference with some modifications. Anhydrous sodium sulphate was used to dehydrate the vitamins after they had been extracted three times using partition chromatography and varying ether concentrations (50, 30, and 20 mL). Calibration curves were prepared for vitamins D and E. The vitamins were analysed with HPLC (Thermo Scientific™ Dionex™ UltiMate™ 3000 standard system, UK) while a C18 column (4.6 \times 250 mm, 5 μm) (Agilent ZORBAX Eclipse Plus™, USA, 5 μm , 4.6 \times 250 mm) was used with a linear gradient of methanol at a constant flow rate of 1 mL/min and UV detection recorded at 265 nm for vitamin D and 290nm for vitamin E. The results were reported as $\mu\text{g}/100\text{g}$.

2.2.4. Determination of Folic Acid

Folic acid content was determined according to a standard procedure (method 86–90.01), [18]. with a modification in sample extraction. Briefly, 5mL of 1N NaOH was used to hydrolyze the sample (0.5 g) for 1 hour at 50°C. 0.1N hydrochloric acid was used to bring the mixture's pH to 6.8. The extracts were filtered into vials for HPLC analysis (solvent flow rate was set at 0.8 ml/min with an increasing rate of solvent B (acetonitrile) over solvent A (30 mM phosphate buffer - pH 2.2) after being centrifuged at 2000 g for 15 min. The HPLC was equipped with a UV detector (Waters 2489 UV/ Visible Detector and Empower software, Milford, USA). The results were reported as μ / g .

2.2.5. Mineral Composition

Minerals were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) as previously reported by [19]. Briefly, 1g of each sample was mineralized by mixing with nitric acid and microwave digested (CEM One Touch TM Technology, CEM

Technologies, USA). The resulting solution was diluted with Milli-Q water (Millipore, Bedford, MA). Utilizing NIST traceable CRMs of the test minerals, stock and working standard solutions were prepared. The ICP-OES equipment (Spectro ARCOS, Spectro Analytical Instruments, Kleve, Germany) was used to analyse the extracts. The results were expressed as mg/100g.

2.2.6. Determination of Amino Acid

The amino acids of samples were determined using the Pico-tag method as reported by [20]. Briefly, 100 mg of sample was treated with 5 mL of 6 M HCl at 110°C for 24h to achieve complete hydrolysis, followed by precolumn derivatization and analysis by reversed-phase high-performance liquid chromatograph (Perkin Elmer, Glen Waverley, Australia) coupled with a photodiode array detector (MD-2010, JASCO, Tokyo, Japan) at 254 nm and expressed as mg/g protein.

2.2.7. Determination of Total Phenol, Flavonoids, and Antioxidant Activities

Total phenol

The total phenol content (TPC) was determined according to the method of [21]. A suitable dilution of the methanol extract of the sample was oxidized with 2.5 ml of 10% Folin-Ciocalteu's reagent (v/v), and 2.0 ml of 7.5% sodium carbonate was used to neutralize the reaction. The TPC was measured at 765 nm using a UV-visible spectrophotometer. as accordance with a calibration curve ($y = 1.1319 x + 0.0563$, $R^2 = 0.9955$), and expressed as mg/GAE/g. The reaction mixture was incubated for 40 minutes at 45 degrees Celsius, and the spectrophotometer was used to detect the absorbance at 765 nm. The gallic acid equivalent (GAE) of the total phenol concentration was then determined.

Total flavonoid content

The total flavonoid content of the methanol extracts of the samples was determined using a slightly modified method reported by [22]. Briefly, 1.4 ml water, 50 μL 1M potassium acetate, 50 μL 10% AlCl_3 , and 0.5 ml of diluted sample extract were combined and left at room temperature for 30 minutes, to obtain the flavonoid in the reaction. The reaction mixture's absorbance was measured at 415 nm after being tested in a UV-Visible spectrophotometer, and the results were expressed as mg QE/100g.

Determination of total anthocyanin

Total anthocyanins content (TAC) was determined following the method described by [23]. The sample was diluted and stored in the dark for 2h. Thereafter, total anthocyanins content was calculated using the formula: $\text{TAC (mg/g)} = \text{OD}_{535} \times \text{V} \times \text{N} \div 98.2 \div \text{m} \times 100$. The result was expressed as mg/g.

Determination of Lutein

Lutein content was determined by the method of [24]. Samples were extracted in a 25 mL solution of acetone while being continuously shaken at room temperature ($28 \pm 1^\circ\text{C}$) in the dark. Butylated-hydroxy-toluene (0.3g) was added to stop carotenoids from oxidizing. The residues were extracted three times with 25 mL acetone for 4 hours respectively. All

extracts were combined and dried under vacuum and used for HPLC analysis. The results are presented as mg/g.

Antioxidant activities

Determination of DPPH

The free radical scavenging ability of extracts of the samples against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical were evaluated as described by [25]. To the sample extract, methanolic solution (0.4 mM) containing DPPH radicals was added and was kept in the dark for 30 minutes. Trolox was used as a standard. The absorbance was measured at 516 nm. The ability of the DPPH to scavenge free radicals was expressed as a percentage of the control.

Determination of ABTS

The ABTS radical cation decolorization (ABTS⁺) method as described by [26] with a slight modification. Briefly, 0.1ml of diluted sample extracts was added to ABTS⁺ radical (7.0 mM ABTS with 2.45 mM potassium persulfate at 2:1 (v/v), thoroughly mixed, and then left at room temperature for 6 minutes. The absorbance was determined at 734 nm. The positive control was ascorbic acid. The following equation was used to determine the scavenging activity: ABTS radical scavenging activity (%) = $(1 - A_1/A_0) \times 100$. The ability of the ABTS to scavenge radicals was expressed as a percentage of the control.

2.2.8. Data Analysis

The results were compiled using a Microsoft Excel spreadsheet (version 10) before being subjected to descriptive analysis. The results were expressed as mean \pm standard deviation. Statistical Package for Social Sciences, SPSS®, Version 21.0 was used.

3. Results and Discussions

Phytochemical screening

Phytochemical screening of the aqueous extracts of leaves is represented in Table 1. Saponins, alkaloids, tannins, flavonoids, terpenoids and phenols were present. Alkaloids were not present in the leaves.

Table 1. Qualitative Phytochemical Screening of OFSP aqueous leaves extract.

Phytochemical	OFSP leaves	Pumpkin leaves
Alkaloids	-ve	-ve
Flavonoids	+ve	+ve
Phenols	+ve	+ve
Saponins	+ve	+ve
Tannins	+ve	+ve
Terpenoids	+ve	+ve

*Interpretation: + Trace, +ve Indicates the presence of phytochemicals.

Phytochemicals, proximate and antinutrient analyses

The phytochemicals found in OFSP leaves may exhibit beneficial pharmacological activities. Phytochemicals have a vast range of activities. Saponins have been shown to have anticholesterolemic, antidiabetic and anticancer activity; Tannins are bioactive substances, their presence in the extracts can be anti-carcinogenic [27]. Flavonoids, phenols,

tannins and terpenoids are a large group of polyphenolic and aromatic compounds that could exert positive benefits through their antioxidant activity and protection against oxidative stress [22].

Table 2 represents the proximate composition of OFSP and pumpkin leaves. The moisture content of OFSP and pumpkin leaves were 11.25 ± 0.34 g/100g and 12.13 ± 0.55 g/100g, respectively. The crude protein results showed that OFSP and pumpkin had 5.84 ± 0.07 g/100g, and 3.29 ± 0.40 g/100g respectively. The fat content in OFSP and pumpkin leaves were 0.63 ± 0.03 g/100g and 0.56 ± 0.03 g/100g, respectively. The OFSP and pumpkin leaves contained ash content of 3.39 ± 0.01 g/100g and 2.89 ± 0.01 g/100g, respectively. The OFSP leaves contained higher concentration of insoluble, soluble, and total dietary Fiber than pumpkin leaves. The proximate composition of OFSP and Pumpkin leaves varied significantly ($p \leq 0.5$). However, there were no significant differences ($p \leq 0.5$) in the Antinutritional factors.

Table 2. Proximate composition and antinutritional factors of orange fleshed sweet potato and fluted pumpkin leaves.

Parameters	Pumpkin leaves	OFSP leaves
Moisture (g/100 g)	12.13 ± 0.55^a	11.25 ± 0.34^a
Protein (g/100 g)	3.29 ± 0.40^b	5.84 ± 0.07^a
Fat (g/100 g)	0.56 ± 0.03^b	0.63 ± 0.03^a
Ash (g/100 g)	2.89 ± 0.01^b	3.39 ± 0.01^a
Crude fiber (g/100g)	2.36 ± 0.11^b	2.51 ± 0.13^a
Insoluble dietary fiber (g/100 g)	2.41 ± 0.01^a	2.17 ± 0.01^b
Soluble dietary fiber (g/100 g)	1.97 ± 0.01^a	1.88 ± 0.01^b
Total dietary fiber (g/100 g)	4.38 ± 0.03^a	4.05 ± 0.01^b
Antinutritional factors		
Phytic acid (mg/100g)	0.03 ± 0.00^a	0.01 ± 0.01^a
Tannins (mg/100g)	0.18 ± 0.04^a	0.11 ± 0.01^a

Mean \pm standard deviation of triplicates.

Means with no common letters within a row significantly differ ($p \leq 0.05$). All values are on dry weight basis.

The amount of moisture present in the OFSP leaves were lower than the values reported by [28]. The amount of moisture determines the texture of the leaves and the maintenance of the shelf life. The reduced moisture content obtained in OFSP leaves could be that the sampling period was characterized by intense sunlight and dryness. The protein values of OFSP leaves were significantly higher than pumpkin leaves. Different amino acids combine to form Proteins, which are utilized for diverse purposes in the body. It is needed to form blood cells, muscles, skin, hair connective tissues, bone marrow and vital organs [29]. Protein deficiency can cause growth retardation and reduced intelligence. The fat content obtained for OFSP is generally low. Their intake will reduce weight increase and risk of obesity and other cardiovascular diseases. The quantity of ash content is directly related to the mineral composition. The values obtained for OFSP were higher than that reported in fluted pumpkin leaves (Akinwunmi *et al.* 2016). This suggests that the leaves of OFSP are rich in organic matter.

The soluble, insoluble, and total dietary fiber of OFSP leaves were significantly ($p \leq 0.5$) lower than those obtained for the pumpkin leaves. Adequate fiber in the diet has been

shown to lower serum cholesterol levels and prevent constipation by increasing bulk and promoting motility and peristalsis in the GIT. High fiber content in the diet can also reduce the risk of diabetes, hypertension, and breast cancer.[30]. The values obtained for phytic acid and tannins were low. The phytic acid content of OFSP and pumpkin leaves were 0.01 ± 0.01 mg/100g and 0.03 ± 0.00 mg/100g, respectively. The tannin of OFSP and pumpkin leaves were 0.11 ± 0.01 mg/100g and 0.18 ± 0.04 mg/100g, respectively.

Phytates taken in by diet should be 25mg/100g or less to prevent mineral deficiencies [31]. This is useful because phytic acid functions as an anti-nutrient by forming insoluble complexes with minerals, which prevents them from being absorbed through the gut walls; thus, reduces the bioavailability of minerals. Antinutrients can negatively interfere with the digestibility of proteins and starches.

Micronutrients Carotenoids and vitamins

Beta-carotene, lutein values were 44.18 ± 0.13 mg/100g, 27.05 ± 0.01 mg/100g in OFSP leaves. However, Vitamin C, D and E levels were 21.84 ± 0.26 mg AA /100g, 1.68 ± 0.03 µg/100g and 3.27 ± 0.05 µg/100g. The total anthocyanins content and folic acid present in OFSP leaves was 0.35 ± 0.01 mg/g and 0.02 ± 0.01 µg/g, respectively. The values obtained were significantly higher than those obtained for pumpkin leaves.

Table 3. Vitamin composition of samples.

Parameters	Pumpkin leaves	OFSP leaves
β-carotenoids (mg/100g)	38.47 ± 0.16^b	44.18 ± 0.13^a
Lutein (mg/100g)	22.30 ± 0.10^b	27.05 ± 0.08^a
Vitamin C (mg AA/100 g)	19.52 ± 0.23^b	21.84 ± 0.26^a
Vitamin D (µg /100g)	1.17 ± 0.03^b	1.68 ± 0.03^a
Vitamin E (µg /100 g)	2.54 ± 0.01^c	3.27 ± 0.05^a
Total anthocyanin (mg/g)	0.25 ± 0.01^b	0.35 ± 0.01^a
Folic acid (µ/g)	0.03 ± 0.01^a	0.02 ± 0.01^a

Mean± standard deviation of triplicates.

Means with different superscripts within a row are significantly different ($p \leq 0.05$).

Beta-carotene and lutein, perform a protective role from age-related degeneration in the eyes. Lutein, zeaxanthin, and carotenoids are present in the eye lens and macular region of the retina. [32]. Millions of children around the world have an increased risk of blindness, and other illnesses because of inadequate dietary intake of vitamin A [33]. In addition, Beta-carotene (a provitamin of vitamin A), carotenoids and xanthophylls are powerful antioxidants that can mop off free radicals and reduce oxidative stress. The Recommended daily allowance (RDA) for beta-carotene is 15-180 mg a day. The result from this study, shows that OFSP has a significant amount of lutein and beta carotene that can meet the daily requirements to maintain good health. Increased consumption of carotenoid rich vegetables produces better outcomes than the use of dietary supplements. [34]. The skin, skeleton tissues and respiratory organs all depend on carotenoid, which is a precursor of vitamin A [35].

Antioxidant vitamins (Vitamin C and E) can mop off free radicals generated in the cells and protect the cells from

oxidative damage. The OFSP leaves are good sources of the antioxidant vitamins; while they are poor sources of vitamin D, E and Folic acid. The values obtained from this present study are low. Our results and those reported in recent times also showed that pumpkin leaves are good sources of antioxidants and antioxidant activity.

Folic acid is a B vitamin, water-soluble compound, important for the prevention of anemia and normal neural tube development; low maternal folate status could result in neural tubes defects (NTDs) in newborns [36]. Some Nigerian green leafy vegetables were reported to be good sources of folate in the raw form; ranging from 21.5 µg/100 g in *Solanum macrocarpon* leaves to 183.4 µg/100 g in *Corchorus olitorius*[37].

Amino acid composition

Amino acid profile is an indication of the quality of protein contained in a food sample. Amino acids profile of OFSP and pumpkin leaves are represented in Table 3. The results revealed that the total essential amino acids (TEAAs) of pumpkin and OFSP leaves were 63.50 and 70.60 mg/g, respectively. Leucine was the predominant essential amino acids recorded in both samples. The total essential amino acids (TEAA) were higher in the OFSP leaves than the values of fluted pumpkin leaves. OFSP leaves had a higher total amino acid (TAA) with 115.66mg/g sample. The sulphur containing amino acids (methionine and cysteine) were limiting essential amino acids; with 0.88 ± 0.01 mg/g and 0.65 ± 0.00 for OFSP and pumpkin leaves, respectively and cysteine with values of 0.59 ± 0.01 mg/g and 0.44 ± 0.00 mg/g respectively. The highest concentration of essential amino acids were leucine, lysine, valine, and phenylalanine. This result agrees with those reported by [38] in leaves of *Melanthera scandens*.

Table 4. Table Amino acid composition (mg/g) of OFSP and pumpkin leaves.

Parameter	Pumpkin leaves	OFSP leaves
Essential amino acid		
Histidine	4.55 ± 0.01^b	5.90 ± 0.01^a
Isoleucine	7.86 ± 0.01^b	8.77 ± 0.02^a
Leucine	11.40 ± 0.01^b	12.20 ± 0.01^a
Lysine	10.05 ± 0.01^b	11.97 ± 0.01^a
Methionine	0.65 ± 0.00^b	0.88 ± 0.01^a
Phenylalanine	10.73 ± 0.01^b	11.14 ± 0.01^a
Threonine	7.21 ± 0.02^b	8.11 ± 0.01^a
Valine	11.05 ± 0.01^b	11.63 ± 0.01^a
TEAA Non-essential	63.50	70.60
Alanine	9.41 ± 0.01^b	10.31 ± 0.01^a
Arginine	11.05 ± 0.00^b	12.11 ± 0.01^a
Aspartic acid	28.51 ± 0.07^b	29.63 ± 0.05^a
Cysteine	0.44 ± 0.00^b	0.59 ± 0.01^a
Glutamic acid	30.68 ± 0.06^b	31.53 ± 0.04^a
Glycine	0.34 ± 0.01^b	10.84 ± 0.02^a
Proline	9.20 ± 0.01^a	8.36 ± 0.02^b
Serine	6.53 ± 0.02^b	7.10 ± 0.01^a
Tyrosine	4.20 ± 0.01^b	5.19 ± 0.01^a
TNEAA	100.36	115.66
TAA	163.86	186.26

Mean ± standard deviation of triplicates.

Values with different superscripts in a row are significantly different ($p \leq 0.05$).

The quality of dietary proteins is dependent on the presence and amount of the essential amino acid. High quality dietary proteins will promote growth in children and repair tissues. The body can incorporate essential amino acids into new proteins as the cells need them. Non-essential amino acids are produced from other precursors in the cell. Total non-essential amino acids (TNEAAs) recorded in pumpkin and OFSP leaves were 100.36 mg/g and 115.66 mg/g, respectively. Glutamic acid was the predominant essential amino acids recorded in both samples. The amount of non-essential amino acids: glutamic and aspartic acids were high for OFSP leaves [39] made similar observations in the amino acid profile of three GLV consumed in Nigeria. Alanine is an important source of energy for muscle tissue while glycine aids the release of energy and facilitates the synthesis of hormones needed for a strong immune system. Arginine strengthens immune responses to bacteria and viruses, promotes wound healing and growth hormones needed for growth optimization and tissue repair.

Our findings shows that OFSP leaves were significantly higher ($P \leq 0.5$) in proteins, ash, vitamins, and amino acids than pumpkin leaves. Fluted pumpkin leaves were however reported to be superior in nutritional values (amino acids, proteins and energy values) to other GLV (spinach Leaf (*Amaranthus hybridus*), bitter leaf (*Vernonia amygdalina*) and water leaf (*T. triangulare*) analysed [40]. Therefore, OFSP leaves are good sources of essential amino acids and could serve as an alternative source of cheap, quality protein.

Mineral composition

The mineral content of OFSP and pumpkin leaves are represented in Table 5. This is supported by the higher ash content represented in Table 2. The leafy vegetables contain varying amounts of minerals. OFSP leaves had significantly higher ($p \leq 0.5$) values than those obtained for pumpkin leaves. However, sodium was significantly lower in the OFSP leaves.

Table 5. Mineral composition of orange fleshed sweet potato and fluted pumpkin leaves.

Parameters	Pumpkin leaves	OFSP leaves
Calcium (mg/100g)	1316.43±12.18 ^b	1448.70±19.43 ^a
Manganese (mg/100g)	15.30±0.75 ^b	17.81±0.93 ^a
Magnesium (mg/100g)	496.82±13.29 ^b	583.52±12.36 ^a
Sodium (mg/100g)	12.10±0.22 ^a	7.05±0.09 ^b
Iron (mg/100g)	18.04±0.20 ^b	20.89±0.10 ^a
Potassium (mg/100g)	2120.40±24.73 ^b	2516.77±18.34 ^a
Phosphorus (mg/100g)	130.63±1.15 ^b	157.90±1.13 ^a
Selenium ((g/100 g))	6.13±0.08 ^b	9.72±0.06 ^a
Zinc (mg/100g)	2.86±0.01 ^b	3.85±0.11 ^a

Mean± standard deviation of triplicates.

Means with no common letters within a row significantly differ ($p \leq 0.05$). All values are on dry weight basis.

The values in the OFSP leaves, indicate they are nutrient minerals dense. The presence of micronutrients minerals in diet are essential to sustain numerous physiological roles that leads to optimal healthy functions. They function as cofactors in enzymatic reactions. Minerals have been reported to have very good stability during food processing [41]. They

provide the alkaline minerals that will counteract the excess acid foods.

Potassium, magnesium, and calcium (macro elements) are present in adequate amounts, the Dietary Reference Intake (DRI) of 4,700 mg of potassium [42] cannot be met by any of the vegetables at 100g per day serving. Studies have shown that diets high in potassium can reduce the risk of hypertension and possibly stroke by a mechanism independent of blood pressure [43,44]. The leafy vegetables contain chlorophyll which make them rich sources of magnesium. The vegetables studied have enough to meet the recommended daily values of 300 mg [45] at a serving of 100g per day. Magnesium interacts with phosphate in stabilizing nucleic acids. Also, more than 300 enzymes require magnesium ions as cofactors for their catalytic activities [46]. The vegetables, as presented in Table 5, are also rich in calcium. 100g per day serving with any of the vegetables will meet the calcium recommended adequate intake of 1200 mg/day [47]. The high Calcium levels observed in this study agrees with those reported by [48]. The values can meet the RDA of 800 to 1200 mg/day for calcium in adults. Calcium is needed for the development of bones and sustainability of strong bones, muscle contraction and formation of blood clots.

OFSP leaves contained high amounts of zinc, which is known to play a significant role in the normal functioning of the immune system, protein metabolism and enhance the ability to promote healthy skin and hair. [48]. It also improves reproductive function. Zinc deficiency led to retarded growth and delayed sexual maturity due to its function in the metabolism of nucleic acids and protein production.

Studies on a Nigerian population shows that Zinc deficiency affects 20% of children less than five years; 28.1% of mothers and 43.9% of pregnant women [49]. Important intracellular and extracellular cations are sodium and potassium, respectively. Sodium is involved in the regulation of acid-base balance, action potential for signal transduction and maintaining plasma volume.[49]. The magnesium content of OFSP leaves (583.52mg/100g) obtained in this study meets the recommended daily allowance (RDA) of 400mg/day for men 19-30 years old and 310mg/day for women 19-39 years old [49].

Red blood cell and hemoglobin synthesis require iron. Deficiency of Iron in diet leads to anaemia. The RDI for iron is 18 mg; Children (7-10 years) and adult males require 10 mg of iron; adult females require 15 mg and pregnant and lactating mothers require 13 mg [50]. This present study shows that OFSP leaves have higher amounts of iron to meet the RDI and prevent deficiency diseases.

Nutritionally essential selenium is a constituent of more than two dozen selenoproteins that play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, and protection of cells and tissues from oxidative damage, diseases and infection. [51]. Selenium is a cofactor in glutathione peroxidase, which functions to mop off hydrogen peroxide thereby acting as antioxidants and anti-

inflammatory agents. The RDA of children and adults for selenium ranges from 15-70 µg. This study shows that OFSP leaves are excellent sources of selenium, with values of 9.72g/100g. Iron, zinc and β-carotene (vitamin A) act synergistically to ensure haematopoiesis and prevent anaemia.

Total phenolic and invitro antioxidant activities

The total phenolic (TPC) and flavonoid content, and antioxidant capacities (ABTS and DPPH) of the GLV (OFSP and fluted pumpkin leaves) are shown in Table 6. In the leafy vegetables studied, the total phenolic and flavonoids content ranged from 53.19 to 67.51 mg/GAE/g and 34.54 to 62.34 CE /100g QE/g respectively. Antioxidant activities of the vegetables ranged for DPPH activity from 53.80 % to 62.34% for DPPH activity, ABTS radical scavenging ability % 66.35 to 71.60. The values obtained with the OFSP leaves were significantly higher ($p \leq 0.05$) than those obtained in pumpkin leaves.

Table 6. Total phenolic content and antioxidant activities of orange fleshed sweet potato and fluted pumpkin leaves.

Parameters	Pumpkin	OFSP
TPC (mg/ GAE/g)	53.19 ±0.03 ^b	67.51 ±0.20 ^a
Total flavonoid (mg CE/100 g)	34.54 ±1.10 ^b	49.26 ±1.94 ^a
DPPH (%)	53.80 ±0.71 ^b	62.34 ±0.59 ^a
ABTS radical scavenging ability (%)	66.35 ±0.51 ^b	71.60 ±0.44 ^a

Mean± standard deviation of triplicates.

Means with no common letters within a row significantly differ ($p \leq 0.05$).

All values are on dry weight basis

Polyphenols (catechin and quercetins) and flavonoids are secondary plant metabolites in green leafy vegetables. The polyphenolic extracts from plants were evaluated and shown to exhibit health promoting effects which includes high radical-scavenging activity, antimutagenicity, potential chemo preventive properties, and antidiabetic effects [52]. Polyphenols can be converted by the gut microbiome to bioactive compounds with therapeutic effects [52]. The radical scavenging abilities of the OFSP and pumpkin leaves measured by DPPH and ABTS confirms that the orange sweet potato leaves are a source of antioxidants. Similar findings were reported by [40] in pumpkin leaves.

Flavonoids are compounds present in plants that are anti-inflammatory, antioxidant, and anti-carcinogenic activities [53]. Large intake of flavonoids has been associated with health benefits which includes protection against cardiovascular diseases, stroke, and cancer. The leafy vegetables had higher ABTS values relative to the DPPH scavenging activity. There could be other compounds in the vegetables responsible for the difference obtained.

4. Conclusion

The OFSP crop are produced by genetic modification and biofortification. This could be the reason for the superior plant products that are nutrient-dense and may be consumed as food products. The investigated leafy greens are good

sources of vitamins, minerals (macro and micronutrients), proteins, amino acids, and crude Fiber. The presence of significant amounts of polyphenols and flavonoids in the vegetables studied is beneficial to the maintenance of good health. The green leafy vegetables have strong antioxidant properties and, when ingested often and in large amounts, would aid in the prevention of degenerative illnesses, hypertension, and oxidative stress in adults and the elderly population. It will help lessen malnutrition among growing children and adolescents in underprivileged communities. They should therefore be used as part of a strategy to reduce malnutrition, hidden hunger; and to curtail food insecurity. However, future studies are still required to identify and quantify specific phenolic compounds and profile the health promoting constituents present in the samples as well as their bioavailability.

Funding Sources

We thank Tertiary Education Trust Fund (TETFund) for supporting the project with the Institutional based research grant (IBR, 2022).

ORCID

Henrietta Ayodele Oboh: No. 0000-0003-3515-4555
Chiemela Enyinnaya Chinma: 0000-0001-5874-5695

Acknowledgments

We thank Emetole, JM of National Root crop research Institute (NRCRI), Umudike, Abia State, Nigeria for providing the OFSP leaves used in this study.

References

- [1] Dixon, B. M Harris, E. M (2004) Nigeria food consumption and nutrition survey, 2001-2003.
- [2] Nyanchoke M. A van Stuijvenberg, M. E Tambe A. B Zuma M. K Mbhenyane, X. G (2022). Fruit and Vegetable Consumption Patterns and Risk of Chronic Diseases of Lifestyle among University Students in Kenya. *Int. J. Environ. Res. Public Health*. 19: 6965. <https://doi.org/10.3390/ijerph19126965>.
- [3] Miller Jones J. (2004). Dietary fibre intake, disease prevention, and health promotion: An overview with emphasis on evidence from epidemiology. In: *Bioactive Carbohydrates for Food and Feed*. JW van der Kamp ed. Academic Publishers, Wageningen, Netherlands.
- [4] Ukoma A. N. and Obi J. A (2018). Comparative Evaluation of the Nutrient Composition and Phytochemical Content of Selected Vegetables Consumed in Nigeria. *International Letters of Natural Sciences* 71: 43-50.
- [5] Uusiku N. P. Oelofse A. Duodu K. G. Bester M. J Faber, M. (2010). Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. *Journal of food composition and analysis*, 23(6): 499-509.

- [6] Nelson D. L. M. E., Cox (2005). *Lehninger principles of Biochemistry* 4th ed. Palpigraph Macmillan Company, New York/Basinstoke pp1-1119.
- [7] Bouis H. E. Saltzman A (2017). Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Global food security* 12: 49-58.
- [8] Morris M. C, Wang Y, Barnes L. L, Bennett D. A, Dawson-Hughes B, Booth S. L. (2018). Nutrients and bioactives in green leafy vegetables and cognitive decline: Prospective study. *Neurology*. 16; 90(3): e214-e222.
- [9] Heuzé V. Tran G. Hassoun P. Renaudeau D. and Bastianelli D.(2015). Sweet potato (*Ipomoea batatas*) tubers. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO.
- [10] Khush G. S. Lee S., Cho J.-I., Jeon J.-S. (2012). Biofortification of crops for reducing malnutrition. *Plant. Biotechnol. Rep.* 6: 195–202.
- [11] AOAC (1990). *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington DC.
- [12] AOAC. *Official Methods of Analysis*. (2005). In *The association of official analytical chemists (18th ed.)* North Fredrick Avenue Gaithersburg, Maryland. Latta M. Eskin M. (1980). A simple method for phytate determination. *Journal of Agricultural and Food chemistry*, 28: 1313-1315.
- [13] Latta M. Eskin M. (1980). A simple method for phytate determination. *Journal of Agricultural and Food chemistry*, 28: 1313-1315.
- [14] Udomkun P. Tirawattanawanich C. Ilukor J. Sridonpai P. Njukwe E. Nimbona P Vanlauwe B. (2019). Promoting the use of locally produced crops in making cereal-legume-based composite flours: An assessment of nutrient, antinutrient, mineral molar ratios, and aflatoxin content. *Food chemistry*, 286: 651-658.
- [15] Jensen A. (1978). Chlorophylls and carotenoids. In J. A. Hellebust, & J. S. Craigie (Eds.), *Handbook of physiological methods and biochemical methods*. pp 5-70. Cambridge: Cambridge University Press.
- [16] Benderitter M. Maupoil V. Vergely C. Dalloz F. Briot F. Rochette L. (1998). Studies by electron paramagnetic resonance of the importance of iron in the hydroxyl scavenger properties of ascorbic acid in plasma: Effects of iron chelators. *Fundamentals Clinical Pharmacology*. 12: 510–516.
- [17] Sami R. Li Y. Qi B. Wang S. Zhang Q. Han, F, Ma Y, Jing J, Jiang L, (2014). HPLC Analysis of Water-soluble Vitamins (B2, B3, B6, B12, and C) and Fat-soluble Vitamins (E, K, D, a, and β -carotene) of *Okra (Abelmoschus Esculentus)*.
- [18] AACC (1999). Method 56-30-water hydration capacity of protein materials, Reappr. *Approved Methods of the American Association of Cereal Chemists*. The Association, St Paul, MN.
- [19] Chinma C. E. Abu JO. Asikwe B. N. Sunday T. & Adebo, O. A. (2021). Effect of germination on the physicochemical, nutritional, functional, thermal properties and in vitro digestibility of Bambara groundnut flours. *LWT-Food Science and Technology* 140: 110-749.
- [20] Chinma C. E. Ilowefah, M. Shammugasamy B. Ramakrishnan Y. Muhammad, K. (2014). Chemical, antioxidant, functional and thermal properties of rice bran proteins after yeast and natural fermentations. *International Journal of Food Science & Technology*, 49: 2204-2213.
- [21] Singeleton, V. L., Orthofer, R. Lamuel-raventos, R. M. (1999). Analysis of total phenols and other oxidation substrate and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymology*, 299: 152–178.
- [22] Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline content in Burkina Faso honey, as well as their radical scavenging activity. *Food Chemistry*, 91, 571–577.
- [23] Fuleki T Francis F. J. (1968). *Quantitative Methods for Anthocyanins*; Blackwell Publishing Ltd.: Hoboken, NJ, USA, 33: 266–274.
- [24] Liu Y. Perera CO. Suresh V (2007). Comparison of three chosen vegetables with others from Southeast Asia for their lutein and zeaxanthin content. *Food Chemistry*, 101(4): 1533-1539.
- [25] Gyamfi M. A. Yonamine M. Aniya Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana *Thonningia sanguinea* on experimentally induced liver injuries. *General pharmacology*, 32: 6661-6667.
- [26] Cai Y. Luo Q. Sun, M. Corke H. (2004). Antioxidant activity and phenolic compounds of traditional Chinese medicinal plants associated with anticancer. *Life Science*, 74: 2157-218. Cambridge University Press.
- [27] Manikyam H. K. Joshi S. K. Vakadi S. Patil S. B. (2021). Anticancer activity of terpenoid saponin extract of *Psidium guajava* on MCF-7 cancer cell line using DAPI and MTT assays. *African Journal of Pharmacy and Pharmacology*, 15(12): 206-211.
- [28] Kumar D, Satendra K Shekhar C. (2020). Nutritional components in green leafy vegetables: A review *Journal of Pharmacognosy and Phytochemistry* 9(5): 2498-2502.
- [29] Beaulah A. C, Rajamanickam Swaminathan, V. (2020). Nutritive Values and Importance of Tropical Green Leafy Vegetables in Human Diet – A Review. *Int. J. Curr. Microbiol. App. Sci.* 9(09): 656-669.
- [30] Da Silva D. J. C. Imai S. (2017). Vegetables consumption and its benefits on diabetes. *Journal of Nutritional Therapeutics*. 6(1): 1-10.
- [31] Onomi S, Okazaki Y, Katayama T (2004). Effect of dietary level of phytic acid on hepatic and serum lipid status in rats fed a high sucrose diet. *Bioscience. Biotechnol Biochem.* 68(6): 1379-1381.
- [32] Carazo A. Macáková K. Matoušová K. Krěmová LK. Protti M. Mladěnka P. (2021). Vitamin A Update: Forms, Sources, Kinetics, Detection, Function, Deficiency, Therapeutic Use and Toxicity. *Nutrients*. 13(5): 1703. doi: 10.3390/nu13051703. PMID: 34069881; chemists (18th ed.) North Fredrick Avenue Gaithersburg, Maryland.
- [33] Cocate P. G. Natali A. J. Alfenas RG. de Oliveira A. (2015). Carotenoid consumption is related to lower lipid oxidation and DNA damage in middle-aged men. *Br. J. Nutr.* 114: 257–264. doi: 10.1017/S0007114515001622.
- [34] Maseko, I.; Mabhaudhi, T.; Tesfay, S.; Araya, H. T.; Fezzehazion, M.; Plooy, C. P. D. (2018). African leafy vegetables: A review of status, production, and utilization in South Africa. *Sustainability* 10: 16.

- [35] Rana A, Santiya M, Dhewa T, Mishra V, Aluko, RE(2022). Health benefits of polyphenols: A concise review. *J. FoodBiochem* 46(10): 3569-4805.
- [36] Delchier N, Herbig A. L, Rychlik M, Renard C. M(2016). Folates in fruits and vegetables: Contents, processing, and stability. *Comprehensive Reviews in Food Science and Food Safety* 15(3): 506-528.
- [37] Ejoh S. I, Wireko-Manu F. D, Page D. and Renard CMG (2019). Estimation of folate content of cultivated and uncultivated traditional green leafy vegetables in Nigeria *African Journal of food science*. 13(9) 191-195.
- [38] Aja P. M, Ale B. A, Ekpono E. U, Nwite I, Aja L, Asouzu N. C, Njoku A. (2021). Amino acid profiles of *Solanum aethiopicum*, *Amaranthus hybridus*, and *Telfairia occidentalis*, common leafy vegetables in Nigeria. *Sci Prog*. 104(3): 368504211032079. doi: 10.1177/00368504211032079.PMID 34261343.
- [39] Omoyeni O. A, Olaofe O, Akinyeye, R. O (2015). Amino Acid Composition of Ten Commonly Eaten Indigenous Leafy Vegetables of South-West Nigeria. *World Journal of Nutrition*.
- [40] Usunobun, U. and Egharebva, E (2014). Phytochemical Analysis, Proximate and Mineral Composition And In Vitro Antioxidant Activities In *Telfairia Occidentalis* Aqueous Leaf Extract. *Journal of Basic and Applied Sciences* 1(1): 74–87.
- [41] Asaolu S. S, Adefemi O. S, Oyakilome I. G, Ajibulu K. E Asaolu M. F.(2012). Proximate and mineral composition of Nigerian leafy vegetables. *Journal of Food Research*. 1: 214-218.
- [42] Fagbohun E. D, Lawal O. U, Ore M. E. (2012). “The proximate, mineral and phytochemical analysis of the leaves of *Ocimum g.*, *Lasianthera m. Scandens a.* and *L. guinesis* and their medical value”. *International of Applied Biological Pharmaceutical Technology*, 3: 15-22.
- [43] Olowoyo P, Okekunle AP Ojagbemi A Akpa O. M, Akinyemi R, Lackland, D, Bruce B, Ovbiagele, Owolabi, M. (2021). Green leafy vegetable intakes are inversely related to the incidence of stroke, *European Journal of Preventive Cardiology*, 28: (18), e21–e23.
- [44] Whelton PK, He J, Cutler JA, Brancati FL, Appel LJ, Follmann D, Klag MJ. (1997). Effects of oral potassium on blood pressure. Meta analysis of randomized controlled clinical trials. *JAMA*. 277(20): 1624–32. DOI: 10.1001/jama.1997.03540440058033. PMID 9168293. 32.
- [45] Romani AMP. (2013). Chapter 3. Magnesium in Health and Disease. In Astrid Sigel; Helmut Sigel; Roland K. O. Sigel. Interrelations between Essential Metal Ions and Human Diseases. *Metal Ions in Life Sciences* 13. Springer. 49–79. DOI: 10.1007/978-94-007-7500-8_3. 34.
- [46] FNB. (1997). Food and Nutrition Board, Institute of medicine: Dietary reference intakes for calcium, phosphorus, magnesium. Washington DC, National Academy Press.
- [47] Ross CA, Jones G, Abrams SA, Kovacs CS, Aloia JF, Manson JE, Brannon PM, Mayne ST, Clinton SK, Rosen CJ, DurazoArvizu RA, Shapses SA, Gallagher JC, Gallo RL. Dietary reference intakes for calcium and vitamin D. Institute of Medicine, National Academy of Science; 2010.
- [48] Onwordi C. T, Anuoluwa I, Ogungbade M Adedjoja I Wusu D. (2009). The proximate and mineral composition of three leafy vegetables commonly consumed in Lagos, Nigeria. *African Journal of pure and Applied chemistry*3(6) 102-107.
- [49] Grim CE, Luft FC, Miller JZ, Meneely GR. (1980). Racial differences in blood pressure in Evans County, Georgia: relationship to sodium and potassium intake and plasma renin activity. *Journal of Chronicle Diseases*. 1980; 33(2): 87–94. DOI: 10.1016/0021-9681(80)90032-6. PMID 6986391 31.
- [50] Johnson-Wimbley T. D Graham D. Y. (2011). Diagnosis and management of iron deficiency anemia in the 21st century. *Ther. Adv. Gastroenterol*. 4: 177- 184. 5.
- [51] Nestel, P (1993). Food Fortification in Developing Countries. US Agency for International Development.
- [52] Krochmal-Marczak B, Cebulak T, Kapusta I, Oszmiański J, Kaszuba J, Żurek N. (2020). The Content of Phenolic Acids and Flavonols in the Leaves of Nine Varieties of Sweet Potatoes (*Ipomoea batatas* L.) Depending on Their Development, Grown in Central Europe. *Molecules*. 25(15) 3473.
- [53] World Health Organization (WHO) (2018). Increasing Fruit and Vegetable Consumption to Reduce the Risk of Non-Communicable Diseases. In World Health Organization, E-Library of Evidence for Nutrition Actions (eLENA); World Health Organization: Geneva, Switzerland, 2018.