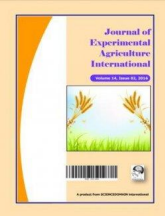

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Comparative Studies on Bioactive Components of Fluted Pumpkin, *Telfairia occidentalis* Hook F. Grown in Three Selected Solid Media

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KO and LAA designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors KO and SIM managed the analyses and literature searches of the study. All Authors read and approved the final manuscript.

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ABSTRACT

The amino acids, vitamins and proximate composition of fluted pumpkin, *Telfairia occidentalis* grown in three different selected solid media; humus soil, white-sand and wood sawdust were assessed and compared. Standard procedure and equipment were followed and used, respectively for the determination of the bioactive components of *T. occidentalis* leaf. Proximate composition of *T. occidentalis* in the three media were: carbohydrate (12.76%, 20.40% and 6.37%), crude fibre (4.76%, 5.70% and 5.59%), ash content (0.47%, 0.58% and 0.73%), crude lipid (0.30%, 1.00% and 0.10%) and moisture content (81.41%, 72.02% and 86.91%) for humus soil, white-sand and wood sawdust, respectively while the crude protein was constant at 0.30%. In that same order, the total amino acids were 9.75 g/100g, 7.53 g/100 g and 13.46%. The essential amino acids (5.85%, 4.49% and 7.94%) and non-essential amino acids (3.90 g/100 g, 3.04 g/100 g and 5.52 g/100 g) varied for

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humus soil, white-sand and sawdust, respectively. Among the amino acids, histidine (1.73 g/100 g, 1.34 g/100 g, and 2.45 g/100 g), cysteine (1.26 g/100 g, 0.98 g/100 g and 1.78 g/100 g) and threonine (1.23 g/100 g, 0.96 g/100 g and 1.75 g/100 g) were the most abundant while proline (0.01 g/100 g) was the least for humus soil, white-sand and sawdust as a medium of growth for pumpkin. The percentage water soluble vitamins (B_1, B_2, B_3, B_6, B_{12} and C): 26.16%, 71.42% and 135.53% and fat-soluble vitamins (A, E and K): 18.87%, 19.95% and 41.73% for humus soil, white-sand and sawdust, respectively. The water-soluble vitamins accounted for the high total vitamins obtained. The study has shown that *T. occidentalis* can be grown in white-sand and sawdust without losing the bioactive compounds rather improving its availability.

Keywords: Bioactive compounds; fluted pumpkin; solid media.

1. INTRODUCTION

A balanced diet of natural foods provides vitamins required for normal body functioning. In contrast to the inorganic mineral elements, they are essential for growth, health and reproduction. The moisture contents of fresh vegetables are comparatively high and affect the levels of water-soluble vitamins compositions. Ascorbic acid or vitamin C is acquired human nutrient. Vegetables are used as the main sources of nutrients and medicine in developing nations. Nigeria and most African countries consumed green leafy vegetable as diet or component of their meal in most cases. According to Hossain et al. [1], leafy vegetables are widely consumed in many countries because of their nutritional quality and anthocyanin, ascorbic acid, β-carotene, flavonoids, folic acid, polyphenol and alkaloid contents. Those components were earlier reported to have a wide range of biological functions such as antiallergic, anticancer, antidiabetic, antimicrobial, antioxidant, and anti-cardiovascular diseases [2]. Among the 20 amino acids required by humans for protein formation, only twelve can be produced within the body, whereas the other eight, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine must be obtained from the diet. In addition, adults are capable of synthesizing arginine and histidine essential for growth in children [3]. The amino acids (Asparagine, Glutamine, Serine, Glycine, Alanine, and Leucine) are naturally involved in osmolyte synthesis, cell metabolism, ammonia detoxification, antioxidant activity and alkaloid synthesis [4]. Food in the form of vegetable provides vital nutrients which are essential for life and other bioactive compounds which are necessary to promote health and prevent diseases [5].

Fluted pumpkin (*T. occidentalis*) is a perennial angiosperm plant with great economic importance in Nigeria. The plant originated in southern Nigeria, where it is used primarily in soups and herbal medicine [6]. The tender leaves and vines are consumed as vegetables by human or forage for livestock, while the young seeds are eaten as food and snacks [6,7,8]. The cooked leaves are stapled vegetable in soups and stews of various cultures throughout equatorial Africa [9,10]. In Nigeria, several works on *T. occidentalis* leaf as a leafy vegetable in the dietary food or as an infusion in medicinal preparation is being encouraged as a result of its medicinal properties which includes antidiabetic, anti-anaemic, hepatoprotective effect, and as a purgative [11,12,13,14].

Soil plays a crucial role in the survival of plants [15]. Soil fertility decline and nutrient mining are perceived to be an important form of soil degradation in the agricultural land-use systems of the tropics [16]. Hence, any medium that possesses required nutrients has the capacity to sustain growth and development of plants. However, the levels of nutrients in different media differ depending on the media compositions and other environmental factors. The growth medium serves as anchorage and source of nutrition for plants.

Considering the fact that intake of green leafy vegetables in most regions of the world is on the increase either for food or medicine, the study seeks to identify and quantify some of the bioactive compounds in *T. occidentalis* leaf grown in three selected solid media.

2. MATERIALS AND METHODS

The seeds of *T. occidentalis* and sawdust (SD) used were sourced from Choba Market and Rumuosi Sawmill respectively while the white sand (WS) and humus soil (HS) were obtained from Choba River (4°54'0"N 6°54'0"E) and a garden in University of Port Harcourt (4°

53'54.30"N 6°54'56.90"E) in that order. Physicochemical analyses of humus soil, white sand and sawdust were carried out following standard procedures. The seeds were planted in three different media namely; white sand, sawdust and humus soil. After germination, the seedlings were allowed to stand for one month when it is matured and can be harvested to prepare food. The leafy vegetables were harvested and followed by the analyses of the proximate composition, amino acids and vitamins.

2.1 Proximate Analysis

Proximate analysis (moisture, ash, protein, carbohydrate, lipid content and crude fibre) was determined using the standard method of Association of Analytical Chemists (AOAC) [17].

2.1.1 Determination of crude protein (Kjeldahl method)

Plant samples weighing 0.1 g were put into different conical flasks, 3 g of digestion catalyst and 20 mL of concentrated sulphuric acid was added into the flask. The flasks were then heated gently to boil in a fume chamber until charred particles disappeared and a clear greenish grey solution was obtained. The resulting solution in the conical flask was heated for an additional 20 minutes and allowed to cool. The digest was diluted with water to 100 mL capacity and 20 mL was then measured into a distillation flask and this was connected to a condenser adapted to a receiver beaker containing 10 mL of 2% boric acid with 2 drops of double indicator. NaOH (40%) was added to the digest, the distillation flask was then heated to distil the nitrogen present as ammonia. The boric acid in the receiver was titrated with a standard 0.1N hydrochloric acid. The volume of HCl used was recorded as titre value. Total percentage nitrogen was calculated using the following formula:

%Nitrogen = (Titre value x 1.4 x 100) / (1000 x 20 x 0.1)

Titre value = Volume of Hydrochloric acid used.

1.4 = Nitrogen equivalent in relation to the normality of HCl used in titration.

100 = Percentage factor

1000 = Conversion factor from gram to milligram.

20 = Integral volume of digest analysed or distilled

0.1 = Weight of sample in gram digested

% Crude protein = %Nitrogen x 6.25

2.1.2 Determination of carbohydrate (Clegg Anthrone method)

Plant samples weighing 1 g were put into a 250 mL volumetric flask. Distilled water (10 mL) and 13 mL of 62% perchloric acid were added and the mixture was shaken to homogenize completely. The flask was made up to 250 mL of distilled water; the solution formed was filtered through a glass filter paper. Filtrate (10 mL) was collected and transferred into a 100 mL test tube; it was then diluted with distilled water. The hydrolysed solution was pipetted into a clean test tube and 5 mL of Anthrone reagent was added, and mixed together. The whole content was read at 630 nm wavelength using the 1 mL distilled water and the 5 mL anthrone prepared as blank. Glucose solution of 0.1 mL was also prepared and this was treated with the anthrone reagent. The absorbance of the standard glucose was calculated using the formula:

%CHO = (25 x Absorbance of sample) / (Absorbance of standard glucose x 1 g of sample used)

2.1.3 Determination of moisture using the air oven method

Samples weighing 1 g were placed in a clean dry porcelain evaporation dish. This was placed in an oven to maintain a temperature of 105°C for six hours. The evaporating dish was cooled to room temperature in a desiccator and re-weighed.

% Moisture = [(weight of fresh sample – weight of dried sample)/ Weight of sample used] x 100

2.1.4 Determination of lipid by Soxhlet extraction method

Samples weighing 2 g was inserted into a filter paper and placed into the Soxhlet extractor. The extractor was fitted into a pre-weighed round bottomed dry distillation flask and the solvent acetone was added to it through the condenser. The extractor and the flask were held in place with a retort stand clamp. Cold water was passed into the condenser via the rubber tubing and the

flask was heated such that the solvent refluxed continuously within the enclosure. The lipid in the solvent chamber was extracted through this process of continuous refluxing. After the lipid had been extracted completely from the sample, the condenser and the extractor were disconnected, the acetone solvent was distilled off and the lipid concentrate was cooled in the oven and re-weighed.

% Lipid = [(Weight of flask and extract − Weight of empty flask) / Weight of sample extracted] x 100

2.1.5 Determination of ash by furnace method

The dried sample weighing 1 g was placed into a porcelain crucible which had been preheated and weighed. The crucible was inserted into a muffle furnace and regulated to a temperature 630°C. After three hours it was removed from the furnace and allowed to cool to room temperature, and then it was re-weighed.

2.1.6 Determination of fibre

The fibre content was determined by difference. The other five proximate components were summed and the value gotten was subtracted from 100% giving the fibre content (100 - percent estimated proximate components represented the percent fibre in the sample).

2.2 Determination of Amino Acids Using Waters 616/626 LC (HPLC) Instrument

The sample preparation and determination were carried out in the following four stages:

2.2.1 Hydrolysis

The samples (0.5 g) were weighed into a sterile furnaces hydrolysis tube 5 nmols Norleucine was added to the samples and then dried under a vacuum. The tube was again placed in a vial containing 10.05 N HCl with a small quantity of phenol, thereby hydrolysing the protein by the HCl vapours under vacuum. This stage of hydrolysis of the sample lasted for between 20 – 23 hours at 108°C. After the hydrolysis, the samples were dissolved in ultra-pure water grade, containing ethylene diamine tetraacetic acid (EDTA). The EDTA chelates the metal was present in the samples. The hydrolysed samples now are stored in HPLC amino acid analyser bottles for further analytical operations.

2.2.2 Derivatisation

The hydrolysed samples were derivalised automatically on the water 616/626 HPLC by reacting the five amino acid, under basic situations with phenylisothiocyanate (PITC) to get phenylthiocarbamyl (PTC) amino acid derivatives. The duration for this is 45 minutes per sample, as calibrated on the instrument. A set of standard solutions of the amino acids were prepared from Pierce Reference Standards H (1000 µmol) into auto-sampler crops and they were also derivatised. These standards (0.0, 0.5, 1.0, 1.5, 2.0 µmol) were used to generate a calibration file that was used to determine the amino acids contents of the samples. After the derivatisation, a methanol solution (1.5*N*) containing the PTC-amino acids was transferred to a narrow bore waters 616/626 HPLC system for separation.

2.2.3 HPLC separation and quantization

The separation and quantization of the PTC-amino acids were done on a reverse phase (18 silica column) and the PTC chromophone were automatically and digitally detected at the wavelength of 254 nm. The elution of the whole amino acids in the samples took 30 minutes. The buffer system used for separation was 140 mm sodium acetate pH 5.50 as buffer A and 80% acetonitrile as buffer B. The program was ran using a gradient of buffer A and buffer B concentration and ending with a 55% buffer B concentration at the end of the gradient.

2.2.4 Data processing/interpretation and calculation

The intensity of the chromatographic peaks areas were automatically and digitally identified and quantified using a Dionex chromeleon data analysis system attached to waters 616/626 HPLC System. The calibration curve or file prepared from the average values of the retention times (in minutes) and areas (in Au) at the amino acids in 5 standards runs was used.

Since a known amount of each amino acid in the standard loaded into the HPLC, a response factor (Au/pmol) was calculated by the software interphase with the HPLC. The response factor was then used to calculate the amount of each amino acid (in pmols) in the sample and displayed on the system digitally. The amount of each amino acid in the sample is finally calculated by the software by dividing the intensity of the peak area of each (corrected for

the differing molar absorptivity's of the various amino acids) by the internal standard (Pierce) in the chromatogram and multiplying this by the total amount of internal standard added to the original sample.

After the picomole by the intensity of the height of each amino acid has been ascertained by the software, the data, the digital chromatographic software extrapolate back to 5 nmoles of the internal standard (Norleucine), and displays for the total amount that was pipetted into the hydrolysis tube at the beginning of the analysis as below:

Calculation:

mg/mL (in Extract) = Dilution factor x Peakheight intensity
mg/mL (in sample) = (mg/mL in extract x sample volume) / Wt. of sample

2.3 Vitamins

Vitamin C was determined using titrimetric method [18].

2.3.1 Vitamin A extraction and determination using waters 616/626 HPLC

Plant sample (0.5 g) was weighed into a flask, 20 mL of 0.2*N* HCl dispensed and allowed to stand for 1.5 hours. The solution was cooled and the pH adjusted to pH 6, using NaOH. Also, 1*N* HCl was added to lower the pH to 4.5. The solution was made up to 50 mL and centrifuged for 10 minutes at 3000 rpm. The supernatant was separated, 1 mL of acetic acid (CH_3COOH) added and mixed properly. Also, 0.5 mL of 3% H_2O_2 was added and mixed well. Finally, 20 mg of sodium hydrogen sulphate was added and shake properly. The extract was run on HPLC (Waters 616/626). Water 616/626 accessories used had Merck Lichrosphere WOCH-18/2 (5 µm) at 40°C column (stationary phase) and mobile phase (Solvent 'A' was 30 mM sodium acetate, pH 6.5 containing 5% dimethyl flormamide and solvent 'B' was acetonitrile) with fluorescence detector, range of working standard (0, 2, 4, 6, 8 ppm) and determination was carried out at the wavelength of 328 nm.

2.3.2 Vitamin B_2, B_6, B_{12} combined extraction and determination using waters 616/626 HPLC

Plant sample (2.5 g) was weighed into a set of digestion tubes, and an extraction solution (Ultra-pure water: HCl: 0.1*N* H_2SO_4, in the ratio 5:2:3) dispensed. The tube was warmed to the temperature of 40°C for 2 hours, allowed to cool to room temperature and transferred to a set of plastic centrifuged tubes. The later was shaken for 10 minutes and centrifuged at 3000 rpm. The supernatant was set in autoanalyser tubes and ran on HPLC. Water 616/626 accessories used had Merck Lichrosphere WOCH-18/2 (5 µm) at 40°C column (stationary phase) and mobile phase (Solvent 'A' was 30 mM sodium acetate, pH 6.5 containing 5% dimethyl flormamide and solvent 'B' was acetonitrile) with fluorescence detector, range of working standard (0, 0.2, 0.4, 0.6, 0.8 ppm) and determination was carried out at wavelength range of 240 - 465 nm.

2.3.3 Vitamin E extraction and determination using waters 616/626 HPLC

Plant sample (0.5 g) each was weighed into a set of digestion tubes, 20 mL of diluted hydrochloric acid (HCl) added and shook vigorously for 2 hours. The extract was further treated with phosphatase to liberate free vitamin E into the solution. The extract was purified by passing through base exchange silicate alkaline column to remove interfering compounds. Thereafter, the extract was stored in a set of vials for analysis using HPLC. Water 616/626 accessories used had Merck Lichrosphere WOCH-18/2 (5 µm) at 40°C column (stationary phase) and mobile phase (Solvent 'A' was 30 mM sodium acetate, pH 6.5 containing 5% dimethyl flormamide and solvent 'B' was acetonitrile) with fluorescence detector, range of working standard (0, 0.2, 0.4, 0.6, 0.8 ppm) and determination was carried out at the wavelength of 356 nm.

3. RESULTS AND DISCUSSION

3.1 Growth Media

The physicochemical composition of the three growth media used for growing *T. occidentalis* is presented in Table 1. The composition varied from one growth medium to another.

3.2 Proximate Composition

Proximate composition of the leaf of *T. occidentalis* grown in humus soil, white-sand and sawdust are presented in Table 2. The results varied in the three growth media. *Telfairia occidentalis* leaf showed appreciable levels of crude protein (0.30%, 0.30% and 0.30%), carbohydrate (12.76%, 20.40% and 6.37%), crude lipid (0.30%, 1.00% and 0.10%), crude

fibre (4.76%, 5.70% and 5.59%), ash (0.47%, 0.58% and 0.73%), and moisture content (81.41%, 72.02% and 86.91%) respectively. The moisture contents of *T. occidentalis* were high in the three media, which directly affects the shelf life. This has serious economic implications. According to Abdullahi [19], high moisture content is linked with increased microbial activities during storage. Interestingly in white-sand growth medium, *T. occidentalis* leaf with the least moisture content had the highest carbohydrate, crude fibre and crude lipid. The particle sizes of the growth media probably affected the availability of water to the plant. Crude lipid was surprisingly low, ranging from 0.10 – 1.00%. These values were low compared to values (1.83 - 27%) reported by other researchers in some leafy vegetables consumed in Nigeria and Niger Republic [20,21]. These low crude lipids are of great importance, considering that excess intake of lipid leads to health challenges such as obesity and heart-related problems.

3.3 Amino Acids

Amino acids content of *T. occidentalis* nurtured in three different solid media are presented in Table 3. The presence of essential (9) and non-essential (11) amino acids were observed. These amino acids were below the limit reported by FAO [22], indicating that *T. occidentalis* can possibly serve as one of the natural sources of amino acids for human consumption. Amongst the solid media, *T. occidentalis* grown in sawdust had the highest essential and non-essential amino acids with histidine (2.45 g/100 g) and cysteine (1.78 g/100 g) for each respectively. Also, histidine (1.73 g/100 g; 1.34 g/100 g) and cysteine (1.26 g/100 g; 0.98 g/100 g) were the

Table 1. Physicochemical composition of the growth media

Parameters	Growth medium		
	Humus soil	White sand	Sawdust
pH (1:1) H_2O	4.63	5.32	ND
pH (1:1) KCl	3.59	4.87	ND
% Organic Carbon	1.129	0.075	ND
% Organic matter	1.952	0.130	ND
% Nitrogen	0.117	0.008	0.008
P (mg/g)	13.690	2.128	0.086%
Ca (Cmol/kg)	0.316	0.729	0.180%
Mg (Cmol/kg)	1.017	0.586	0.038%
K (Cmol/kg)	0.169	0.051	0.007%
Na (Cmol/kg)	0.652	0.661	100.47mg/g
Al (Cmol/kg)	0.000	0.000	ND
Acidity (Cmol/kg)	0.410	0.400	ND
ECEC (Cmol/kg)	2.564	2.427	ND
Mn (mg/g)	64.250	1.010	136.970
Fe (mg/g)	106.780	12.960	118.970
Cu (mg/g)	10.750	1.340	0.000
Zn (mg/g)	6.020	5.080	13.990
% Sand	73.8	95.8	ND
% Silt	11.4	1.4	ND
% Clay	14.8	2.8	ND

ND - Not Determine

Table 2. Proximate composition of *Telfairia occidentalis* grown in different media

Proximate composition (%)	Growth medium		
	Humus soil	White sand	Sawdust
Crude protein	0.30	0.30	0.30
Carbohydrate	12.76	20.40	6.37
Crude lipid	0.30	1.00	0.10
Crude fibre	4.76	5.70	5.59
Ash	0.47	0.58	0.73
Moisture content	81.41	72.02	86.91

Table 3. Amino acids present in *Telfairia occidentalis* grown in different media

Type of amino acids	Amino Acids (g/100 g)	Growth medium			*FAO (g/100 gcp)
		Humus soil	White sand	Sawdust	
Essential	Threonine	1.23	0.96	1.75	4.00
	Leucine	0.22	0.17	0.32	7.00
	Isoleucine	0.62	0.48	0.87	4.00
	Lysine	0.92	0.67	1.00	5.50
	Methionine	0.52	0.40	0.67	3.50
	Phenylalamine	0.49	0.38	0.70	6.00
	Tryptophan	0.05	0.04	0.08	1.00
	Valine	0.07	0.05	0.10	5.00
	Histidine	1.73	1.34	2.45	
Non-essential	Alanine	0.51	0.40	0.73	
	Aspartic acid	0.19	0.15	0.27	
	Asparagine	0.27	0.21	0.38	
	Glutamic acid	0.14	0.11	0.20	
	Glutamine	0.70	0.55	1.00	
	Glycine	0.18	0.14	0.26	
	Proline	0.01	0.01	0.01	
	Serine	0.05	0.04	0.06	
	Arginine	0.43	0.33	0.61	
	Cysteine	1.26	0.98	1.78	2.00
	Tyrosine	0.16	0.12	0.22	2.80
Total Amino acid (g/100 g)		9.75	7.53	13.46	

**Source: FAO [22]; gcp represents gram crude protein*

most concentrated essential and non-essential amino acids in humus soil and white sand respectively. The essential amino acids content of *T. occidentalis* in three solid media with their values ranged from 0.04 – 2.45 g/100 g, the least concentrated amino was tryptophan with values of 0.05, 0.04 and 0.08 g/100 g in that order which is comparably lower than the value (0.43 g/100 g) reported for *Cucurbita maxima* leaves [23].

3.4 Vitamins

The proportion of vitamins in *T. occidentalis* leaf under different growth media are shown in Table 4. There is variation in vitamins content of the same plant grown in a different medium. Surprisingly, *T. occidentalis* leaf grown in sawdust had the highest water-soluble and fat-soluble vitamins as compared to others. The study suggests that soil physicochemical composition affects the mineral nutrition of plants grown in it. B-Vitamins obtained are within the recommended daily allowance for adults (19-64 years) in the United Kingdom [24]. However, *T. occidentalis* contained high B-vitamins in relation to the work of Ball [25], who reported that *Geranium robertianum* was a good source of vitamin complex (B_1 – 288.17±0.12 mg/100 g; B_2 – 818.21±0.07 mg/100g; B_3 – 319.13±0.12 mg/100g). The vitamin C value present in *T. occidentalis* leaf grown in sawdust and white-sand (17.27%, 9.75% respectively) were higher compared to that reported (5.6 g/100 g) by Wasagu et al. [21] for *Pistia stratiotes* leaf. Lawal et al. [26] reported that fresh fluted pumpkin leaf contains 64.33±4.48 mg/100g vitamin C. The mitochondrial functions are affected by low dietary intake of B-vitamins and vitamin C [27,28,29,30,31]. Okwu and Josiah [32] reported that ascorbic acid is crucial for the body performance. Vitamin C is also antioxidant which performs as an electron donor for eight human enzymes [33]. The vitamin E content of *T. occidentalis* varied in the different solid media. Kornteiner et al. [34] earlier reported that vitamin E always increases with temperature during seed maturation and also during drought. This observation is in line with the result of vitamin E, which showed *T. occidentalis* grown in sawdust had the highest vitamin E content, a reflection of the temperature difference in the media. Vitamin E in pumpkin seed oil makes it important oil especially in cosmetic applications, human diet, nutrition and health [35]. The vitamin E content of *T. occidentalis* leaf in the different solid media were higher compared to pumpkin seed oil as reported by Karanja et al. [35]. Presence of vitamin K in *T. occidentalis* as a fat-soluble

Table 4. Percentage vitamins present in *Telfairia occidentalis* grown in different medium

Types of vitamins	Vitamins (%)	Growth medium			*FSA (%)
		Humus soil	White sand	Sawdust	
Water-soluble	Vitamin B_1	6.13	9.22	12.54	13
	Vitamin B_2	0.80	10.34	21.04	28
	Vitamin B_3	1.25	3.02	7.66	
	Vitamin B_6	2.12	5.01	11.66	10
	Vitamin B_{12}	12.70	34.08	65.36	
	Vitamin C	3.16	9.75	17.27	
Fat-soluble	Vitamin A	17.27	16.63	29.65	
	Vitamin E	0.97	2.15	8.07	
	Vitamin K	0.63	1.17	4.01	
Total Vitamins (%)		45.03	91.37	177.26	

**FSA - Food Standards Agency [24]*

vitamin is important to the overall human health. Douthit et al. [36] reported the importance of vitamin K intake on cardiovascular health of an adult. Previous works on the intake of vitamin K have been inconsistent [37,38,39,40,41, 42,43,44,45,46]. Also, Jie et al. [37] suggested that vitamin K intake might be linked to the development of atherosclerosis because they found that phylloquinone intakes were significantly lower in postmenopausal women with aortic calcifications than without aortic calcifications.

4. CONCLUSION

The use of humus soil for the cultivation of plants has been a long-standing practice for most farmers. It has been reported severally that seeds need water for germination to commence. On the basis of this prerequisite for seeds, pumpkin grown in other solid media has shown the capacity to sustain growth and development of several bioactive compounds such as vitamins, amino acids and proximate composition, which serve as the building blocks of living system. Pumpkin grown in sawdust gave the highest histidine, threonine and cysteine content of the amino acids when compared to other media. Similar results were obtained for water-soluble vitamins and fat-soluble vitamins with variation in proximate composition among pumpkin grown in humus soil and white-sand. However, the amino acids and vitamins obtained by using the three media were inferior to the standard set by FAO and FSA. The study recommends that further studies be carried out on other solid media as a medium for growing plants and subsequently harness improved bioactive compounds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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