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Comparative studies on some of the bioactive components of fluted pumpkin grown in three selected solid media

Abstract:

The amino acids, vitamins and proximate composition of pumpkin grown in three different selected solid media were assessed and compared. The solid media were humus soil, white-sand and wood sawdust. Standard procedure and equipment were followed and used, respectively for the determination of the bioactive components of pumpkin. Proximate composition of pumpkin 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 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/100g and 13.46%. The essential amino acids (5.85%, 4.49% and 7.94%) and non-essential amino acids (3.90 g/100g, 3.04 g/100g and 5.52 g/100g) varied for humus soil, white-sand and sawdust, respectively. Among the amino acids, histidine (1.73 g/100g, 1.34 g/100g, and 2.45 g/100g), cysteine (1.26 g/100g, 0.98 g/100g and 1.78 g/100g) and threonine (1.23 g/100g, 0.96 g/100g and 1.75 g/100g) were the most abundant while proline (0.01 g/100g) 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 accounts for the high total vitamins obtained. The study has shown that pumpkin can be grown in white-sand and sawdust without losing the bioactive compounds rather improving its availability.

Keywords: Bioactive compounds, fluted pumpkin, solid media

Introduction:

A well balanced diet of natural foods generally provide the vitamins requirements of man. In contrast to the inorganic mineral elements, they are essential dietary constituents ~~being~~ required for growth, health and reproduction. Vegetables are highly perishable and require careful processing in order to preserve the nutrients, especially the water soluble vitamins. Ascorbic acid or vitamin C is acquired human nutrient. Many nations of the world depend on vegetables as their main sources of several nutrients and medicine especially the less developed and developing nations. Nigeria and most African countries consume~~d~~ 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. These components were earlier reported to have a wide range of biological functions such as antiallergic, anticancer, antidiabetic, antimicrobial, antioxidant, anticardiovascular diseases [2]. Among the 20 amino acids required by humans for protein, only twelve can be produced within the body, whereas the other eight, isoleucine, leucine,

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lysine, methionine, phenylalanine, threonine, tryptophan and valine must be obtained from the diet. In addition, adults are capable of synthesizing arginine and histidine but these amino acids are believed to be essential to growing children, meaning that children cannot produce them on their own and must be obtained from other external supplies [3]. The amino acids (Asp, Glu, Ser, Gly, Ala, and Leu) are naturally involved in osmolyte synthesis, cell metabolism, ammonia detoxification, antioxidant activity and alkaloid synthesis [4]. It is interesting to ~~know~~ that many depend on vegetables intake in their diet instead of inorganic supplement for health concerns. In fact, food not only provides vital nutrients which are essential for life, but also other bioactive compounds which are necessary to promote health and prevent disease [5].

Fluted pumpkin (*Telfairia occidentalis* Hook F.) is a perennial angiosperm plant with great economic importance in Nigeria. This plant is indigenous to southern Nigeria, and used primarily in soups and herbal medicine [6]. The tender leaves and vines are consumed by humans as vegetables as well as livestock as forage, while the young seeds are eaten as food and snacks [6,7,8]. The cooked leaves are staple 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 antidiaebetic, antianeamic, hepatoprotective effect, and as a purgative leafy vegetable [11,12,13,14].

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 seek to identify and quantify some of the bioactive compounds in *Telfairia occidentalis* leaf grown in three selected solid media.

Materials and methods:

The seeds of *Telfairia 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 and a garden in University of Port Harcourt in that order. 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 the following analyses were carried out: proximate composition, amino acids and vitamins.

Proximate Analysis:

Proximate analysis (moisture, ash, protein, carbohydrate and lipid content) was determined using standard method of AOAC [15]. Fibre was determined by difference.

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 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

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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 standard 0.1N hydrochloric acid. The volume of HCl used was recorded as titre value. The formula for the calculation of total Nitrogen is:

%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 analyzed or distilled
0.1 = Weight of sample in gram digested
% Crude protein = %Nitrogen X 6.25

Determination of carbohydrate (CleggAnthrone 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 was added and the mixture was shaken in order for it to homogenize completely. The flask was made up to 250 ml with 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; this was also diluted to volume with distilled water. The hydrolyzed solution was pipetted into a clean test tube and 5 ml of Anthrone reagent was added, they were then mixed together. The whole mixture 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. Absorbance of the standard glucose was calculated using the formula below:

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

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

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 into 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.

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

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.

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 - per cent estimated proximate components represented the per cent fibre in the sample).

Determination of Amino acids using waters 616/626 LC (HPLC) Instrument:

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

***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 hydrolyzing 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 analyzer bottles for further analytical operations.

***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.

***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.

***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 which is attached to the 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.

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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 that was interphased with the HPLC. This response factor was used to calculate the amount of each of the 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

$$\text{mg/ml (in sample)} = \frac{\mu\text{g/ml in extract x sample volume}}{\text{Wt. of sample}}$$

Vitamins:

Vitamin C was determined using titrimetric method [16].

Vitamin A extraction and determination using waters 616/626 HPLC

Plant sample (0.5 g) was weighed into a ~~cornical~~ 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 added to lower the pH to 4.5. The solution was made up to 50 ml, ~~shake~~ 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 added and mixed well. Finally, 20 mg of sodium hydrogen sulphate added and shake properly. The extract was ~~ran~~ 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 wavelength of 328 nm.

2.7.2 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 at the temperature of ~~40%~~ for 2 hours, allowed to cool to room temperature and transferred to a set of plastic centrifuged tubes. The later was shake 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

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working standard (0, 0.2, 0.4, 0.6, 0.8 ppm) and determination was carried out wavelength range of 240 - 465 nm.

2.7.3 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 shake 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 compound. 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 wavelength of 356 nm.

Results and Discussion:

Proximate composition of the leaf of *T. occidentalis* grown in humus soil, white-sand and sawdust are presented in Table I. 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 [17], high moisture content is linked with increased microbial activities during storage. Interestingly, in white-sand growth medium, *T. occidentalis* 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 is surprisingly low, ranging from 0.10 – 1.00%. This values is low compared to values (1.83 - 27%) reported by other researchers in some leafy vegetables consumed in Nigeria and Niger Republic [18,19]. These low crude lipids is of great importance, considering that excess intake of lipid leads to health challenges such as obesity and heart related problems.

Table I: Proximate composition of *T. 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

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Amino acids content of *T. occidentalis* nurtured in three different solid medium are presented in Table II. The presence of essential (9) and non-essential (11) amino acids were observed. These amino acids were below the limit documented by FAO [20], indicating that *T. occidentalis* can possibly 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/100g) and cysteine (1.78 g/100g) for each respectively. Also, histidine (1.73 g/100g; 1.34 g/100g) and cysteine (1.26 g/100g; 0.98 g/100g) were the 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/100g, the least concentrated amino was tryptophan with values of 0.05, 0.04 and 0.08 g/100g in that order which is comparably lower than the value (0.43 g/100g) reported for *Cucurbita maxima* leaves [21].

Table II: Amino acids present in *T. occidentalis* grown in different media

Types of amino acids	Amino Acids (g/100g)	Growth medium			*FAO (g/100gcp)
		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/100g)		9.75	7.53	13.46	

*Source: FAO [20]

The proportion of vitamins in *T. occidentalis* leaf under different growth media are shown in Table III. There is variation in vitamins content of same plant grown in different medium. Surprisingly, *T. occidentalis* leaf grown in sawdust had the highest water-soluble and fat-soluble vitamins compared to others. The study suggest that soil physicochemical

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composition affects the mineral nutrition of plants grown in it. B-Vitamins obtained is within the recommended daily allowance for adults (19-64 years) in the United Kingdom. However, *T. occidentalis* contains high B-vitamins in relation to the work of Ball [22], who reported that *Geranium robertianum* is a good source of vitamin complex (B_1 – 288.17±0.12 mg/100g; 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/100g) by Wasagu *et al.* [19] for *Pistia stratiotes* leaf. Lawal *et al.* [23] reported that fresh pumpkin contains 64.33±4.48 mg/100g vitamin C. The mitochondrial functions are affected by low and dietary intake of B-vitamins and vitamin C [24,25,26,27,28]. Okwu and Josiah [29] reported that ascorbic acid is crucial for body performance. Vitamin C is also antioxidant which performs as an electron donor for eight human enzymes [30]. The vitamin E content of *T. occidentalis* varied in different solid media. Kornteiner *et al.* [31] earlier reported that vitamin E always increases with temperature during seed maturation and also during drought. This observation is in line with result for 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 an important oil especially in cosmetic applications, human diet, nutrition and health [32]. The vitamin E content of *T. occidentalis* leaf in different solid media were higher compared to pumpkin seed oil as reported by Karanja *et al.* [32]. Presence of vitamin K in *T. occidentalis* as a fat soluble vitamin is important to the overall human health. Douthit *et al.* [33] reported the importance of vitamin K intake on cardiovascular health of an adult. Previous works on the intake of vitamin K have been inconsistent [34,35,36,37,38,39,40,41,42,43]. Also, Jie *et al.* [34] 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 in those without aortic calcifications.

Table III: Percentage vitamins present in *T. 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 [44]

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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 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 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.

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