Kanem J Med Sci 2021; 15(2): 101-106
Open Access article distribution in the terms of the
Creative commons Attribution License (CC by 4.0)

ISSN 2006-4772
https://doi.org/10.36020/kjms.2021.1502.005

Original Article

DETERMINATION OF MINERALS, VITAMINS, ANTINUTRIENT AND AMINO ACID PROFILE OF PUMPKIN PIE PRODUCED PUMPKIN (CUCURBETA SPP) PUREE AND WHEAT (TRITIUM AESTIVUM) FLOUR SUPPLEMENTED WITH SPICES AND BUTTER

Falmata, A Sanda,[1] Bintu, BP,[1] Maryam, BK,[2] Zainab, B,[1] Goni, C,[1] Modu Sheriff.[1]

[1]Department of Biochemistry, Faculty of Science. University of Maiduguri Nigeria
[2]Department of Biological Science, Faculty of Science, University of Maiduguri Nigeria

Corresponding Author: Modu Sheriff. Department of Biochemistry, Faculty of Science. University of Maiduguri Nigeria
Email: shemodu@yahoo.com

ABSTRACT

Background: Pumpkin (*Cucurbita spp*.), is one of the most popular vegetables consumed in the world, has been recently recognized as a functional food. Traditional crops including the pumpkins, which are rich in micronutrients, are not consumed widely by smallholder farmers in Africa. However, the cultivation of high yielding, nutrient-rich, multipurpose crops-like pumpkin is important in solving the problems of malnutrition and contributing to food security in Africa including Nigeria. **Objectives:** Therefore, this work aimed at producing Pumpkin pie from Pumpkin puree and wheat supplemented with some indigenous spices alongside butter. **Methodology:** Formulations into various formed were made using Pearson's method. The formulations were assayed for mineral, vitamin, antinutrient and amino acid composition using standard laboratory methods. A significant ($P<0.05$) difference was observed in Zn and Ca content of all the samples, with sample D recording the highest value for Zn (1.30±0.01) mg/g and Ca (0.47±0.01) mg/g. However, samples A and D had the least values for Na and Fe. An increasing and decreasing pattern was observed in all the vitamin contents(B_1, B_2, B_6, B_{12} and C), moving from samples A through to D. Vitamin contents of samples B_1 and B_6 and higher than the RDA, while B_2, B_{12} and C were below RDA. The overall antinutrient contents observed were generally low, with sample A having the lowest values for Phytate, Oxalate and tannins. The B, C and D are more enhanced in terms of essential amino acids compared to the control group (sample A). **Conclusion**: Pumpkin pie can be produced from pumpkin and its supplementation of the spices can boost the pie with essential amino acids, minerals, and vitamin composition. This shows the potential of using pumpkin pie as snacks in-between meals for the prevention of Protein Energy Malnutrition (PEM).

Keywords: *Butter, Cinnamon, Ginger, Pumpkin*

Introduction

Background of the Study

The *Cucurbita* family includes pumpkins, squash, cucumbers, luffas, watermelons, and melons. Most of the plants in this family are vines. Pumpkins come in a multitude of colours, shapes, and sizes. On the farm, it grows green, yellow, red, white, blue and most of the pumpkins are multi-coloured striped. They can be huge, tiny, flat, short, tall, round, pear, necked, smooth, ribbed and even warty. Some pumpkins are fabulous for culinary uses.

Pumpkin is a member of the *Cucurbitaceous* family and has received considerable attention in recent years because of the nutritional and health-protective values such as anti-tumour, anti-bacterial, and anti-

Cite this article as: Falmata, A Sanda, Bintu, BP, Maryam, BK, Zainab, B, Goni, C, Modu Sheriff. Minerals, vitamins, antinutrient and amino acid profile of Pumpkin pie produced pumpkin (Cucurbeta spp) puree and wheat (Tritium aestivum) flour supplemented with spices and butter **Kanem J Med Sci 2021; 15(2): 107-118**

Submitted: 13th July 2021 **Accepted: 13th November 2021** **Published: 29th December 2021**

hypertensive.[1] In Bangladesh pumpkin is cultivated in about 14,000 ha of land with the production of 20-25 m tons/ha per year.[2] Protein-energy malnutrition is a major problem in many developing countries including Nigeria which affects many individuals, it occurs as a result of ignorance thereby making it out of reach of the common man. However, in Nigeria, the populace is unaware of the high nutritional and nutraceutical values of *Cucurbita*, rather it is regarded as traditional food mainly for the low-income earners, thus has not benefited from the same level of research attention given to other vegetable crops like cucumber, fluted pumpkin etc. Various products have been processed and preserved from pumpkin. However, no research has been carried out for the preparation and quality evaluation of pie from pumpkin.

Pumpkin (*Cucurbita spp*.), is one of the most popular vegetables consumed in the world, has been recently recognized as a functional food.[3] Traditional crops including the pumpkins, which are rich in micronutrients, are not consumed widely by smallholder farmers in Africa. However, the cultivation of high yielding, nutrient-rich, multipurpose crops-like pumpkin is important in solving the problems of malnutrition and contributing to food security in Africa.[4] Pumpkins are used as food and vegetables for consumption as both commercial and home garden crop. The multipurpose uses of pumpkins, their great diversity and adaptation to a wide range of environments indicate the potential of this crop. The most common product popular in most African countries is cooked matured fruit.

In Nigeria *Cucurbita spp.* Also known as *Telfairia occidentalis* the most commonly cultivated pumpkin is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. Common names for the plant include fluted gourd, fluted pumpkin, (ugu) (in the Igbo language), and ikong-ubong (in the Efik and Ibibio languages). *T. occidentalis* is a member of the family *Cucurbitaceae* and is indigenous to southern Nigeria.[5] The fluted gourd grows in many nations of West Africa but is mainly cultivated in southeastern Nigeria and is used primarily in soups and herbal medicines. Although the fruit is inedible, the seeds produced by the gourd are high in protein and fat, and can, therefore, contribute to a well-balanced diet. The plant is a drought-tolerant; dioeciously perennial that is usually grown trellised.

Cucurbita seed which is considered an "oilseed", the fluted gourd is high in oil (30%).[5] Shoots of *T. occidentalis* contain high levels of potassium and iron, while seeds are composed of 27% crude proteins and 53% fats.[6] The leaves contain a high amount of antioxidants and hepatoprotective and antimicrobial properties.[7] The young shoots and leaves of the female plant are the main ingredients of a Nigerian soup,' ofe egusi. The large (up to 5cm), dark-red seed is rich in fat and protein and can be eaten whole, ground into powder for a kind of soup, or made into a fermented porridge.

In Zambia, the ripped fruit flesh is dried for longer preservation.[8] On the other hand, in southern Africa, the leaves are widely consumed as a leading leafy vegetable during the rainy season. In Zambia, 40 % of the households use pumpkin leaves as relish daily during the rainy season. In some parts of Zimbabwe, pumpkin leaves are the most popular leafy vegetable. In Cameroon and other parts of central and West Africa, *Cucurbita moschata sp.* is mainly grown for ripe seeds. The seeds are first roasted, the shells removed, and squashed into a paste and consumed with the main dish. Roasted seeds are also salted and eaten as a snack. The seed oil is edible and used as fuel.[8] However, the majority of farmers in the Lake Victoria basin of Kenya, Uganda and Tanzania do not consider pumpkins as a priority food and commercial crop and are largely utilizing it for home consumption.[9] They are mostly grown by low-income members of the community who mainly utilize the leaves as vegetables and occasionally the fruit when cooked.[9]

In human history and civilization, pumpkin is a sacred and purest food boldly mentioned in both divine sources of Abrahamic faith and scriptures; the Bible, and the Quran. From a spiritual and physical point of view, pumpkin has also been well versed in the Prophetic Tradition. Imam Ibn Al Qayyim wrote extensively on the benefits of pumpkin, which can be found in his book Tibb an Nabawi (Medicine of the Prophet).[10] There are a lot of health benefits of pumpkin that have come to light, including helping to reduce swellings, cooling down fever, helping with digestion, eliminating bile, etc. Its seeds also contain essential fatty acids that help maintain

healthy blood vessels, nerves, and tissues. It also includes anti-diabetic, antioxidant, anticarcinogenic and anti-inflammatory properties.[11] What is known, about "pumpkin" is a fruit that is defined as being the part of the plant which contains seeds. The average pumpkin contains about a cup of seeds, so they are most definitely a fruit. A pumpkin is a member of the *Cucurbita* family.

Methodology

Sample Collection

Freshly fully mature pumpkin, sugar, milk powder, wheat flour, sorghum, spices (cinnamon, cloves and ginger), vanilla extract and eggs were obtained from Maiduguri custom market and Monday market and were taken to the Department of Biological Science for identification.

Sample Preparation

Preparation of Pumpkin Puree

Firstly, fresh fully matured pumpkins were bought from the Maiduguri custom market and were identified, it was washed thoroughly. The unwanted portions including the seeds and skin were removed after slicing. Then it was put into the oven for 1 hour at 25^0c, the pulp was obtained from pumpkin. Finally, the pulp was blended to obtain the pumpkin puree and stored at refrigerator temperature. The puree was then used for the preparation of the pumpkin pie.

Preparation of sorghum and wheat flour

The sorghum grains was purchased from the Maiduguri custom market, the sorghum seed was dehulled using mortar and pestle, it was washed and sun-dried. The dried grains were ground into a fine powder and sieved using a 1mm pore sieve to obtain a fine powder. While the wheat flour was obtained from Maiduguri custom market, Borno State and immediately transported to the University of Maiduguri, it was sieved to remove dirt and was stored in a plastic container to prevent contamination.

Preparation of the spices (cinnamon, ginger and cloves).

These spices were bought from Maiduguri custom market, they were clean from dirt, then grounded with pestle and mortar to obtain a fine powder, and then it was stored in a plastic container to prevent contamination.

Preparation of pumpkin pies

Table 1: Formulation of samples for pie processing

Ingredients	A	B	C	D
Pumpkin puree	40	40	50	60
Wheat flour	40	10	8	8
Sorghum flour	10	10	10	10
Sugar	20	20	15	10
Milk powder	12	12	10	10
Eggs	10	10	10	8
Butter	8	8	6	6
Baking powder	0.5	0.5	0.5	0.5
Cloves	0.2	0.2	0.2	0.2
Ginger	0.2	0.2	0.2	0.2
Vanilla	3.0	3.0	3.0	3.0
Cinnamon	0.5	0.5	0.5	0.5
Total	100	100	100	100

Values are mean ± SEM n=3

Values with different superscript along the horizontal row are significant different ($p< 0.05$) Key:
A= pumpkin puree (40g) + sorghum flour (10g) + sugar + butter + egg + milk.
B= pumpkin puree (40g) + wheat flour (10g) + sugar + butter + egg + milk.
C= pumpkin puree (50g) + wheat flour (8g) + sugar + butter + egg + milk.
D= pumpkin puree (60g) + wheat flour (8g) + sugar + butter + egg + milk

The pumpkin pies were prepared as per the method described in the following flowchart;

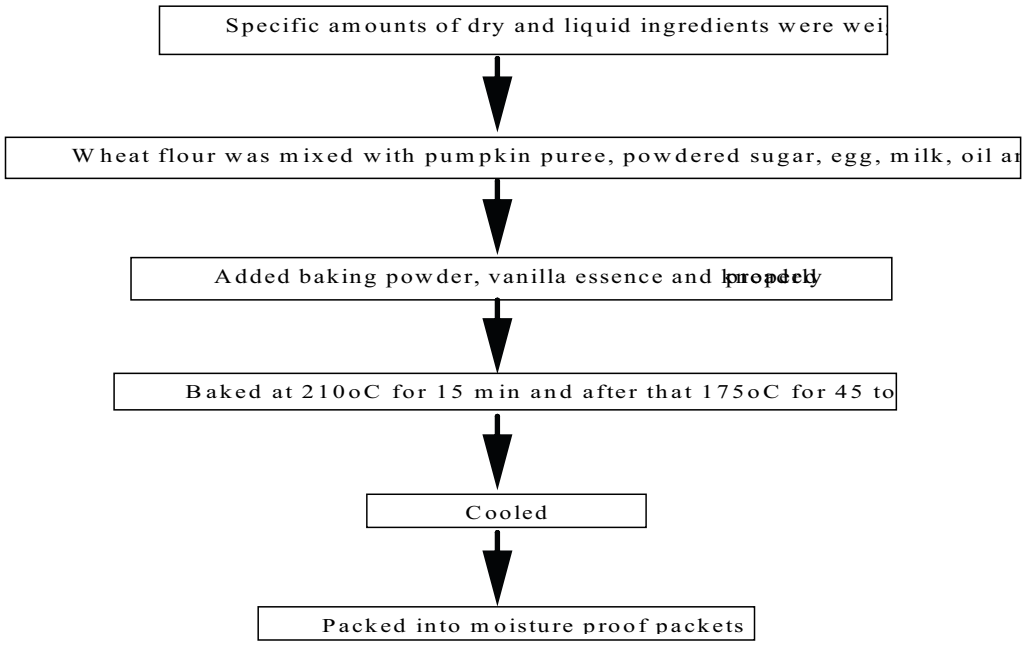

Figure 1: Flow chart for the preparation of pumpkin pies.

The first step is getting the edible part out of the pumpkin, the pumpkin was sliced into two halves and the seeds were removed. The two halves are heated until soft, in an oven. At this point, the pulp is scooped out and pureed. The pulp is mixed with eggs, evaporated milk, sugar, and a spice mixture called pumpkin pie spice, which includes (e.g., ginger, cinnamon, and cloves), then baked in a pie shell.

3.4 Methodology

Determination of mineral elements

Transfer 2.00g of sample into a Kjeldahl flask and add 25mls of digestion acid (Aqua regia HCl: HNO_3 3:1). Swirl and heat gently at first until frothing stops; then more strongly until a clear pale yellow solution results, allowed to cool and transfer digest into a 100ml volumetric flask, Made p to the mark with distilled water and Filtered using Whatman no. 1 filter paper. The filtrate was e taken to the Atomic Absorption Spectrophotometer (AAS) (Buck Scientific, VPG 230). A hallow cathode lamp of each element was installed into the instrument and the wavelength characteristics of that metal was set for the Determination of the mineral elements. Standards were run with corresponding lamps using air acetylene flame integrated mode and the concentration of each metal is obtained by extrapolation from the calibration curve of the standards.

Determination of Vitamins

To 5mL of sample hexane - extract added Potassium hydroxide (KOH) and saponify for 30 minutes and added an antioxidant. Transfer to a separator funnel extract by adding water and 1-1.5 volumes of hexane. The extract was washed several times with water and filtered through a filter paper containing 5g anhydrous sodium sulfate into a flask, rinsed with hexane and made up to volume. USP vitamin reference standards were used to prepare standard dilutions was prepared as blanking solution in a similar manner (5 mL water in place of sample extract). Read the absorbance of samples, standards after zeroing with a blank solution at 620nm with UV/Vis spectrophotometer. Vitamins concentrations were calculated using a five-point calibration curve.

Determination of Antinutrients by High-Performance Liquid Chromatography (HPLC)

The sample (5.00g) was weighed and extracted with hexane in a 50.0ml vial. The extract was filtered and the filtrate was then injected into a Buck scientific

(USA) BLC10/11 High-Performance Liquid Chromatography (HPLC) system fitted with a fluorescence detector (excitation at 295 nm and emission at 325 nm) and an analytical silica column (25 cm x 4.6mm ID, stainless steel, 5 μm). The mobile phase used was hexane: tetrahydrofuran: Isopropanol (1000:60:4 v/v/v) at a flow rate of 1.0ml/min. Standards of each antinutrient was prepared. The prepared standards were treated and analysed in the same manner as the samples using the same method. The area of each peak from both samples and standards chromatograph were calculated and recorded. The concentration of the antinutrient was calculated using the following formula:

[Conc. of Antinutrient] = [A SAMPLE x [STD] (ppm) x V HEX (ml)]/ [A STD x Wt SAMPLE (g)]

Where;
[Conc. of antinutrient] = concentration of Antinutrient in ppm
[STD] = concentration of standard
A SAMPLE = peak area of the sample
A STD = peak area of standard
V HEX = volume of hexane
Wt SAMPLE = weight of the sample

3.4. Determination of Amino acid Profile of the samples

The defatted samples were utilized to estimate amino acids. The sample (30mg) was hydrolyzed with 6N HCI at 110°C for 24h. Amino acid analysis was performed on reverse phase-high Performance liquid chromatography (HPLC) (Buck scientific BLC 10/11 USA) equipped with UV 338nm detector. A C18, 2.5 x 200mm, 5um column and a mobile phase of 1:2:2 (100mM sodium phosphate, pH 7.2: Acetonitrile: methanol) was used at a flow rate of 0.45mL/minute and an operating temperature of 40°C. Mixed standards were analyzed similarly for identification. Peak identification was conducted by comparing the retention times of authentic standards and those obtained from the samples, these data were integrated using peak Simple chromatography data system processor; (Buck SCi.chromatopac data processor). (AOAC 2000).

The lipids were extracted from samples by a Soxhlet extractor using hexane as a solvent. Fatty acids were transformed into methyl ester according to the ISO procedure (ISO, Method 5509, 2008). The fatty acid methyl esters (FAMEs) were extracted with petroleum ether and were analyzed by High-Performance liquid chromatography (HPLC) (Buck scientific BLC 10/11 USA) equipped with a flame ionization detector and integrator. The mobile phase is (59:41) Acetonitrile: 2-propanol and the column (Prevail C-18,5u, 150 x 4.6mm) flow rate was 1 mL/min. The oven temperature was maintained at 210ºC for 45 min. The fatty acids were identified by comparing their retention times with those of standards (AOAC, 2020).

3.5 Statistical Analysis:

All determination was carried out in triplicate. All data collected were subjected to analysis of variance (ANOVA) using SPSS statistical package version 21.0 and Duncan's multiple ranges was used to compare the means (Steel and Tone, 1986).

Results

Mineral Composition

The result of the mineral composition of the pies sample was presented in table 4.1. The Zn contents were 0.48± 0.01mg/g, 0.70±0.01mg/g, 0.57±0.002mg/g and 1.30±0.02mg/g for the sample A, B, C and D respectively. The difference was significant ($p<0.05$). The values for the calcium ranges between 0.10± 0.01 to 0.47± 0.01mg/g. sample D recorded higher value (0.47±0.1 mg/g) for calcium while the control had 0.43± 0.01mg/g, while sample D recorded a least value of 0.10±0.00mg/g.

There were no significant ($p>0.05$) difference observed between the manganese level observe B (0.02± 0.00mg/g) and sample D (0.01 ± 0.01mg/g) and sample D (0.01±00 d/dg) and also with the control sample A (0.01 ± 0.01 dg/g). However, a significant ($p < 0.05$) was observed when compared with the Mn composition of sample C (0.23 ± 0.01dg/g).

A significant ($p < 0.05$) difference was observed in the Na level of the samples B (0.28 ± 0.02 dg/g), C (0.1±0.02mg/g) and D (0.55± 0.01 mg/g).
The Na levels of Sample D and B were higher than in the control group A (0.21 ± 0.01 mg/g), while The Na level was lower in group C compared to control. A

significant ($p < 0.05$) difference was observed in the Fe composition of sample B (0.77 ± 0.02 mg/g) when compared with sample C (1.21 ± 0.01 mg/g) while no significance ($p > 0.05$) difference was observed between the control sample A (1.18 ± 0.01 mg/g) and sample C (1.21 ± 0.01 mg/g).

Table 1: Mineral Element Composition (mg/g)

Sample	Zn	Ca	Mn	Na	Fe
A	0.48± 0.01^{d}	0.043±0.02^{b}	0.01±0.01^{b}	0.21± 0.01^{c}	1.18±.0.01^{a}
B	0.70±0.01^{b}	0.10±0.01^{d}	0.02±0.00^{b}	0.28± 0.02^{b}	0.77±.0.2^{b}
C	0.57±0.02^{c}	0.3±0.01^{c}	0.23±0.01^{a}	0.19±.0.02^{c}	1.21±.0.01^{a}
D	1.30±0.02^{a}	0.47±0.01^{a}	0.01±0.01^{a}	0.55±.0.01^{a}	0.43±.0.01^{d}

Values are mean ± SEM n=3

Values with different superscript along the horizontal row are significant different ($p< 0.05$) Key:
A= pumpkin puree (40g) + sorghum flour (10g) + sugar + butter + egg + milk.
B= pumpkin puree (40g) + wheat flour (10g) + sugar + butter + egg + milk.
C= pumpkin puree (50g) + wheat flour (8g) + sugar + butter + egg + milk.
D= pumpkin puree (60g) + wheat flour (8g) + sugar + butter + egg + milk

Vitamin Composition

Table 4.2 presents the vitamin composition of the pie sample. The vitamin B_1 Composition of sample A (1.72 ± 0.26 mg/g), B (1.84 ± 0.01 mg/g) and C (1.94 ± 0.01 mg/g) showed gradual increase in the level of Vit B_1, but not significant ($p > 0.05$) difference. Also, no significant ($p > 0.05$) difference was observed in the B_1 composition of sample D (1.43 ± 0.01 mg/g) and samples B and the control sample A, also when sample D and control compared

The Vitamin B_2 composition of the pie samples ranged between 0.23 ± 0.01 mg/g to 0.26 ± 0.01 mg/g with the control sample A having the highest value (0.26 ± 0.01 mg/g), while the least value was recorded by pie sample C (0.023 ± 0.01 mg/g). The B_2 composition of sample B (0.24 ± 0.01 mg/g) show no difference ($p > 0.05$).

A significant ($p < 0.05$) difference was observed in the B_6 composition of the pie samples B, C and D when a sample with the control pie A. a higher value of B_{12} was recorded by control sample B (0.05 ± 0.01 mg/g) and sample C (0.03 ± 0.01 mg/g).

The ascorbic acid composition of samples A, B and C did not show any significance ($p > 0.05$) difference but when compared to sample D, a significant ($p < 0.05$) difference was observed.

Table 2: Composition of Vitamins (mg/100g)

Sample	B_1	B_2	B_6	B_{12}	C
A	1.72± 0.26^{ab}	0.26±0.01^{a}	1.68±0.01^{b}	0.18± 0.00^{a}	0.32±.0.2^{b}
B	1.84±0.01^{ab}	0.24±0.01^{b}	1.80±0.0.62^{a}	0.05± 0.01^{b}	0.28±.0.01^{b}
C	1.94±0.01^{a}	0.23±0.01^{c}	1.52±0.01^{c}	0.03±.0.01^{b}	0.30±.0.01^{b}
D	1.43±0.01^{b}	0.24±0.01^{b}	1.50±0.01^{d}	0.44±.0.01^{c}	0.57±.0.02^{a}
RDA	0.6	0.6	0.6	1.2	25

Values are mean ± SEM n=3

Values with different superscript along the horizontal row are significant different ($p< 0.05$) Key:
A= pumpkin puree (40g) + sorghum flour (10g) + sugar + butter + egg + milk.
B= pumpkin puree (40g) + wheat flour (10g) + sugar + butter + egg + milk.
C= pumpkin puree (50g) + wheat flour (8g) + sugar + butter + egg + milk.
D= pumpkin puree (60g) + wheat flour (8g) + sugar + butter + egg + milk
RDA =Recommended Daily Alowance

Antinutritional Factors of Sample

The antinutritional factors of the pie samples including the control were presented in table 3. the value for the phytate composition samples A, B C and D were 1.41 ± 0.02 mg/g, 1.67 ± 0.01 mg/g, 1.71 ± 0.01 mg/g and 1.77 ± 0.01 mg/g respectively. Significance ($p < 0.05$) difference was observed in the oxalate content of D (1.84 ± 0.01 mg/g) compared with samples B and C, a significant ($p < 0.05$) was also observed. However, the oxalate content of samples B (1.73 ± 0.01 mg/g) and C (1.76 ± 0.01 mg/g) did not show any significance ($p > 0.05$) difference.

The results of the tannin content of the control sample A (2.35 ± 0.01 MG/G) showed no significance ($P > 0.05$) difference, also that of sample C (2.45 ± 0.03 mg/g) and sample D (2.49 ± 0.03 mg/g) did not show any significant ($p > 0.05$) difference.

Table 3: Composition of Antinutrient (mg/100g)

Sample	Phytate	Oxalate	Tannins
A	1.41 ± 0.02^{d}	1.68 ± 0.01^{c}	2.31 ± 0.01^{b}
B	1.67 ± 0.01^{c}	1.73 ± 0.01^{b}	2.35 ± 0.01^{b}
C	1.71 ± 0.01^{b}	1.76 ± 0.01^{b}	$2.45 \pm .0.03^{a}$
D	1.77 ± 0.01^{a}	1.84 ± 0.01^{a}	$2.49 \pm .0.02^{a}$

Values are mean ± SEM n=3

Values with different superscript along the horizontal row are significant different ($p < 0.05$) Key:
A= pumpkin puree (40g) + sorghum flour (10g) + sugar + butter + egg + milk.
B= pumpkin puree (40g) + wheat flour (10g) + sugar + butter + egg + milk.
C= pumpkin puree (50g) + wheat flour (8g) + sugar + butter + egg + milk.
D= pumpkin puree (60g) + wheat flour (8g) + sugar + butter + egg + milk

Essential Amino Acids

The result of this research for amino acid composition of pumpkin pie sample produced from pumpkin pulp is shown in table 4 and 5 respectively.

The lysine content ranged from 1.84 ± 0.02 to 3.17 ± 0.01, with the highest value observed in sample D and sample A as the lowest value. The lysine content showed that there was no significant ($p > 0.05$) differences between sample B and C, while there was significant ($p < 0.05$) differences between sample A and D.

The methionine content ranged from 1.64 ± 0.01 to 3.13 ± 0.01, with the highest value observed in sample D and the lowest value as sample A. The methionine content showed that there was a significant ($p < 0.05$) difference between the whole sample formulations.

The threonine content ranged from 2.12 ± 0.01 to 3.65 ± 0.01 with the highest value observed in sample D and sample A as the lowest value. The threonine content showed that there was no significant ($p > 0.05$) difference between sample B and sample C while there was a significant ($p < 0.05$) difference between sample A and sample D formulations.

The isoleucine content ranged from 3.14 ± 0.0.1 to 4.03 ± 0.01 with the highest value observed in sample D and sample A as the lowest value. The isoleucine content showed that there was a significant ($p > 0.05$) difference between sample A and sample B while there was significant ($p < 0.05$) differences between sample C and sample D formulation.

The leucine content ranged from 6.31 ± 0.01 to 7.49 ± 0.01 with the highest value observed in sample D and sample A as the lowest value. The leucine content showed that there were significant ($p < 0.05$) differences between the whole sample formulations.

The phenylalanine content ranged from 3.13 ± 0.13 to 7.94 ± 0.01 with the highest value observed in sample D and sample A as the lowest value. The phenylalanine content showed that there were no significant ($p > 0.05$) differences between the whole sample formulations.

The valine content ranged from 2.51 ± 0.01 to 3.65 ±

0.01 with the highest value observed in sample D and sample A as the lowest value. The valine content showed that there were significant ($p< 0.05$) differences between the whole sample formulations.

The tryptophan content ranged from 3.63 ± 0.01 to 5.25 ± 0.02 with the highest value observed in sample D and sample A as the lowest value. The tryptophan content showed that there were significant ($p< 0.05$) differences between the whole sample formulations.

The histidine content ranged from 2.05 ± 0.01 to 3.85 ± 0.01 with the highest value observed in sample D and sample A as the lowest value. The histidine content showed that there were significant ($p <0.05$) differences between the whole sample formulations.

The arginine content ranged from 6.23 ± 0.01 to 8.33 ± 0.01 with the highest value observed in sample D and sample A as the lowest value. The arginine content showed that there were significant ($p< 0.05$) differences between the whole sample formulations.

Table 4 : Amino acid (Essential) Profile of Pumpkin Pie

Amino acid	A	B	C	D
Lysine	1.84 ± 0.02^{c}	2.10 ± 0.01^{b}	2.13 ± 0.01^{b}	3.17 ± 0.01^{a}
Methionine	1.64 ± 0.01^{d}	2.75 ± 0.04^{c}	2.85 ± 0.01^{b}	3.13 ± 0.02^{a}
Threonine	2.12 ± 0.01^{c}	2.94 ± 0.01^{b}	3.10 ± 0.01^{b}	3.65 ± 0.01^{a}
Isoleucine	3.14 ± 0.01^{c}	3.16 ± 0.01^{c}	3.53 ± 0.02^{b}	4.03 ± 0.01^{a}
Leucine	6.31 ± 0.01^{d}	6.53 ± 0.01^{c}	6.85 ± 0.02^{b}	7.94 ± 0.01^{a}
Phenylalanine	3.13 ± 0.13^{a}	3.16 ± 0.01^{a}	3.76 ± 0.02^{a}	3.87 ± 0.01^{a}
Valine	2.51 ± 0.01^{d}	3.15 ± 0.01^{c}	3.22 ± 0.01^{b}	3.65 ± 0.01^{a}
Tryptophan	3.63 ± 0.01^{d}	4.34 ± 0.02^{c}	4.89 ± 0.01^{b}	5.25 ± 0.02^{a}
Histidine	2.05 ± 0.01^{d}	2.13 ± 0.01^{c}	2.83 ± 0.02^{b}	3.85 ± 0.01^{a}
Arginine	6.23 ± 0.01^{d}	7.11 ± 0.01^{c}	8.22 ± 0.02^{b}	8.33 ± 0.01^{a}

Values are mean ± SEM n=3

Values with different superscript along the horizontal row are significant different ($p< 0.05$) Key:
A= pumpkin puree (40g) + sorghum flour (10g) + sugar + butter + egg + milk.
B= pumpkin puree (40g) + wheat flour (10g) + sugar + butter + egg + milk.
C= pumpkin puree (50g) + wheat flour (8g) + sugar + butter + egg + milk.
D= pumpkin puree (60g) + wheat flour (8g) + sugar + butter + egg + milk.

Non-Essential Amino Acid

The serine content ranged from 3.45 ± 0.01 to 4.97 ± 0.01 with the highest value observed in sample A and sample D as the lowest value. The serine content showed that there were significant ($p< 0.05$) differences between the whole sample in table 5.

The cystine content ranged from 3.97 ± 0.01 to 4.76 ± 0.01 with the highest value observed in sample A and sample D as the lowest value. The cystine content showed that there were significant ($p <0.05$) differences between the whole sample formulations.

The tyrosine content ranged from 5.06 ± 0.01 to 5.81 ± 0.01 with the highest value observed in sample D and sample A as the lowest value. The tyrosine content showed that there were significant ($p< 0.05$) differences between the whole sample formulations.

The alanine content ranged from 6.55 ± 0.01 to 8.83 ± 0.01 with the highest value observed in sample D and sample A as the lowest value. The alanine content showed that there were significant ($p< 0.05$) differences between the whole sample formulations.

The aspartic acid content ranged from 6.83 ± 0.01 to 8.23 ± 0.01 with the highest value observed in sample A and sample D as the lowest value. The aspartic acid content showed that there were significant ($p< 0.05$) differences between the whole sample formulations.

The glutamic acid content ranged from 3.13 ± 0.01 to 4.34 ± 0.01 with the highest value observed in sample A and sample C as the lowest value. The glutamic acid content showed that there were no significant ($p> 0.05$) differences between sample C and sample D while there were significant ($p<0.05$) differences between sample A and sample B formulations.

The glycine content ranged from 5.69 ± 0.01 to 6.82 ± 0.02 with the highest value observed in sample B and sample A as the lowest value. The glycine content

showed that there were no significant ($p > 0.05$) differences between the whole sample formulations.

The proline content ranged from 2.36 ± 0.01 to 3.87 ± 0.02 with the highest value observed in sample D and sample A as the lowest value. The proline content showed that there were significant ($p < 0.05$) differences between the whole sample formulations.

Table 5: Amino acid (Non-essential) Profile of Pumpkin Pie

Amino Acids	A	B	C	D
Serine	4.97 ± 0.01^{a}	4.78 ± 0.01^{b}	3.87 ± 0.01^{c}	3.45 ± 0.01^{d}
Cystine	4.76 ± 0.01^{a}	4.58 ± 0.04^{b}	4.34 ± 0.01^{c}	3.97 ± 0.01^{d}
Tyrosine	5.06 ± 0.01^{d}	5.16 ± 0.02^{c}	5.53 ± 0.01^{b}	5.81 ± 0.01^{a}
Alanine	6.55 ± 0.01^{d}	8.25 ± 0.03^{c}	8.44 ± 0.02^{b}	8.83 ± 0.02^{a}
Aspartic Acid	8.23 ± 0.01^{a}	8.03 ± 0.02^{b}	$7.94 \pm o.o1^{c}$	6.83 ± 0.02^{d}
Glutamic Acid	4.34 ± 0.02^{a}	3.28 ± 0.01^{b}	3.13 ± 0.01^{c}	3.14 ± 0.02^{c}
Glycine	5.6 ± 0.01^{a}	6.82 ± 1.00^{a}	5.90 ± 0.01^{a}	6.07 ± 0.02^{a}
Proline	2.36 ± 0.01^{d}	3.25 ± 0.02^{c}	3.66 ± 0.01^{b}	3.87 ± 0.02^{a}

Values are mean ± SEM n=3

Values with different superscript along the horizontal row are significant different ($p < 0.05$) Key:
A= pumpkin puree (40g) + sorghum flour (10g) + sugar + butter + egg + milk.
B= pumpkin puree (40g) + wheat flour (10g) + sugar + butter + egg + milk.
C= pumpkin puree (50g) + wheat flour (8g) + sugar + butter + egg + milk.
D= pumpkin puree (60g) + wheat flour (8g) + sugar + butter + egg + milk.

Discussion

Mineral

The increase in the mineral elements levels might be the decrease in the antinutritional factors during processing. However, the decrease in the level of Mn, Na and observed in the control sample A might be due to the loss of a total ash content during the processing of Sorghum.

Similar work reported by Akingbala *et al.*[12] and Oyerekua[13] reported that more than 50% of the ash in sorghum was leech out of steep water and washed away. Laminu *et al.*[14] also reported a decrease in the levels Na, K, Mg, Ca, P, Zn and Fei processes sample.

Vitamin

The increases in the vitamin levels of B groups observed in samples B and C compared to A and D might be a result of wheat used in the sample. USDA[12] reported that what have high vitamin composition compared to sorghum. The increase in C vitamin observed in D might be due to the high proportion of puree added. However, the concentration of B vitamin were below be RDA 4-8 year. B_{12} and C levels are below RDA for 4-8 years.

Antinutritional Factor

Phytate is regarded as an anti-nutrient because of its high affinity to minerals such as calcium, magnesium, iron, copper and zinc[16] the differences between level of phytate formed in the control sample (A) might be as a result of the processing reduces the level of antinutrient. Similar work was reported by Falmata *et al*.[17] A high level of phytate acid in the samples may result in the formation of insoluble complexes with minerals like calcium and magnesium, sequestering them and making them inaccessible for metabolic processes.

Oxalate was also regarded as an antinutrient because it has a strong affinity to minerals e.g calcium [18] Generally, the low levels of oxalate observed in the samples might be due to the baking temperature during pits production.

Tannins are water-soluble phenolic compounds that form insoluble complexes with proteins, precipitating them from aqueous solutions, thereby interfering with their bioavailability The reduction observed in the tannin might be due to processing and baking as reported by Ijarotimi and Esho.[19]

Essential Amino Acid:
There was an increase in methionine and lycine content observed in sample B – D compared to sample A might be probably due to the supplementation of more pumpkin puree which has more protein content than sample A with a low amount of pumpkin puree as reported by Zhang *et al*[20]

There was an increase in threonine, leucine and isoleucine content observed in sample B – D compared to sample A might be probably due to supplementation of wheat flour which have more protein content than sorghum in sample A as reported by Adams *et al.*[21]

Equal content of phenylalanine in all samples A – D might be probably due to supplementation of equal content of milk in the formulation which is highly rich in protein profile as reported by Playne *et al*[22].

Increased valine and tryptophan content observed in sample B – C compared to sample A, might be probably due to supplementation of more pumpkin puree which has more protein content than sample A with a low amount of the pumpkin puree as reported by Zhang *et al*.[20]

Increased histidine and arginine content observed in sample B – C compared to sample A, might be probably due to supplementation of wheat flour which have more protein content than sorghum in sample A as reported by Adams *et al.*[21]

Non-essential Amino Acid:
Increased in serine and cysteine content in sample A compared to sample B - D might be probably due to supplementation of sorghum flour which is rich in cystine than wheat flour in sample B – D as reported by USDA.[15]

Increased in glutamic acid and aspartic acid content in sample A compared to sample B - D might also be probably due to supplementation of milk and egg which is richer in protein than in sample B – D as reported by Playne *et al.*[22]

Equal content of glycine in all samples A – D might be probably due to supplementation of equal content of milk in the formulation which is highly rich in protein profile as reported by Playne *et al.*[22]

Increased in alanine, tyrosine and proline content in sample B – D compared to sample A, might be probably due to supplementation of wheat flour which have more protein content than sorghum in sample A as reported by Adams *et al*[21]

In conclusion
Therefore, this work revealed that pumpkin pie can be produced from pumpkin. The supplementation of the spices has further boosted the pie with essential amino acids, minerals, and vitamin composition. This further reveals the potential of using pumpkin pie as snacks in-between meals for the prevention of Protein Energy Malnutrition (PEM).

Recommendations
1. Based on the result of this research we recommend that government should educate both the urban and rural community based on the functions and nutritional value of pumpkin which is been neglected by many people.
2. The In vivo study of pumpkin pie should be carried out to study the nutritional value of the pie within the system.
3. The shelf-life study or duration of the sample should be observed for it limited usage.

References

1. Caili, FU., Huan Shi, Ouanlong LI. A Review on Pharmacological Activities and Utilization Technologies of Pumpkin. Pubilcation Food Human Nutrition 2006; **61**:73-80
2. Sharma, S, Ramana, Rao. T.V. Nutritional Quality Characteristics of Pumpkin Fruit as Revealed by its Biochemical Analysis. *Int. Food Res. Journal* 2013; **20**, 2309–2316.
3. Nwanna, Esther Emem, *et al.* Antioxidant and Hepatoprotective Properties of *Telfairia occidentalis* Leaf (Fluted Pumpkin). Thesis and Dissertations (Biochemistry) (2008): n. pag. Archived Copy. Archived from the Original on 2013-12-06. Retrieved 2013-12-06.
4. Tolonen M (Ed). Vitamins and minerals in health and nutrition. First Edition. Elsevier 1990.
5. Akoroda, M.O. Ethnobotany of Telfairia Occidentalis (*Curcurbitacae*) Among Igbos of Nigeria. Economic Botany 1990; 29-39.

JSTOR 4255209
6. Aiyelaagbe, I.O.O, Kintomo, A A. Nitrogen Response of Fluted Pumpkin (*Telfairia occidentalis* Hook. F) Grown Sole or Intercropped with Banana. *Journal of Nutrient Cycling in Agro Ecosystems* 2002; **64**: 231-35
7. Nwanna, M., Kolahsa, R, Eisenreinch K. Pretreatment of Dietary plant foodstuffs with phytate and its effect on growth and mineral concentration in common cap. Journal of animal science.2008; 4(11); 285-297
8. Grubben, G.J.H. and Chigumira Ngwerume, F. Cucurbitamoschata Duchesne In: Grubben, G.J.H. and Denton, O.A. (Editors). PROTA 2 Vegetables/Légumes. 2004. [CDRom]. PROTA, Wageningen, Netherlands
9. Ondigi, AN., Toili, WW., Ijani, ASM, Omuterema, SO. Comparative Analysis of Production Practices and Utilization of Pumpkins (Cucurbitapepo and Cucurbita maxima) by Smallholder Farmers in the Lake Victoria Basin, East Africa. *African Journal of Environmental Science and Technology 2008;* **2**(10):296-304.
10. Ibn al-Qayyim. Al-Tibb al-Nabawi. Beirut: Mu'assasah al-Risalah. IbnMajah and Al-Busayri. (n.d.). 'Ithaaf', V.**4**, 1985. page:310.
11. Zhou, Y., Wang, T., Meng, Q. and Zhai, S. Association of Carotenoids with Risk of Gastric Cancer: A Meta-analysis. Clinical Nutrition 2016: **35**(1):109-16.
12. Akingbala, J.O. A laboratory procedure for the preparation of Ogi, A Nigerian fermented food". *Journal of Food Science 1981;* **46** (5): 1523-1526.
13. Oyarekua, M.A, Eleyinmi A.F. Comparative evaluation of the nutritional quality of corn, sorghum and millet 'ogi' prepared by modified traditional technique. *Food Agricultural Environment 2004;* **2** (2), 94-99.
14. Laminu, H. H., Modu, S, Muhammad, A. A. Evaluation of the chemical composition, anti-nutrients and mineral element level of a composite meal from pearl millet, wheat, cowpea and groundnut. *Sky Journal of Food Science* 2014; **3** (6): 061 – 070
15. USDA. National nutrient database for seeds and legumes 2021. Accessed 12th December 2020.
16. Steel, R. G. D, Torie, J. H. Principle and Procedures of Statistics. Abiometrical Approach (2nd edn). M.C. Graw Hill. International Aucklanal, 1981; 125p
17. Falmata, A.S., Modu, S., Badau, H. D., Babagana, M, Bintu, B.P. Formulation and evaluation of complementary weaning food prepared from single and combined sprouted/fermented local red sorghum (*S. bicolor*) variety blended with cowpea. (*Vigina unguiculata*) and groundnut (*Arachid hypogea*). *International Journal of Biotechnology and Food Science*. 2014; 8: 149-155
18. Ravani A, Joshi, D. C. Processing of valu added of underutilized fruit crops. Trends in Post-Harvest Technology 2014; 2(2); 15-21
19. AOAC. *Association of Official Analytical Chemists*. Official Methods of Analytical Chemists. 18th edition. Washington D.C. 2000.
20. Zhang, C., Postma, J. A., York, L. M, Lynch, J. P. Root Foraging Elicits Niche Complementarity- Dependent Yield Advantage in the Ancient 'Three Sisters' (Maize/Bean/Squash) Polyculture." *Annals of Botany*. 2014; DOI: 10.1093.
21. Adams, R. M., Hurd, B. H., Lenhart, S, Leary. N. Effects of global climate change on agriculture: an interpretative Review. Climate Research 2002; 11:19-30
22. Playne M.J., Bennett L.E., Smithers G.V. Functional dairy foods and ingredients. Austral. J. Dairy Technol 2003; 58: 242–264.