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Comparative studies on the amino acids, pesticide residue content, biogenic and toxic elements in an underutilized nut: Bambara groundnut (*Vigna subterranea*)

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ABSTRACT

Background: Bambara groundnut (*Vigna subterranea*) is an indigenous African leguminous crop commonly consumed in Ebonyi State in Nigeria.
Methods: This study determined the pesticide residue content, amino acids, toxic and biogenic elements of Bambara groundnut seeds obtained from three local government areas - Abakaliki, Ikwo, and Afikpo in Ebonyi State, Nigeria. The digested samples were analyzed using Inductively Coupled Plasma-Optical Emission (ICP-OES) for determination of minerals and toxic elements, while High Performance Liquid Chromatography (HPLC) system was used to determine the amino acids and pesticide residue content.
Results: Pesticide residue content varied from 0.001 ± 0.00 to 0.598 ± 0.03 μg kg^{-1} in Afikpo samples, 0.001 ± 0.05 to 0.50 ± 0.01 μg kg^{-1} in Abakaliki samples, 0.001 ± 0.00 to 0.47 ± 0.01 μg kg^{-1} in Ikwo samples. Pesticide residues detected in the samples were meaningless when compared to the maximum residue limit by World Health Organization (WHO). In the amino acid analysis, the composition of the essential amino acids was higher when compared to the WHO standard. Isoleucine was found to be the most abundant in the samples studied. The total percentage of amino acids in Afikpo sample (24.7 ± 1.4) were significantly higher ($p < 0.05$) than samples from Abakaliki (17.8 ± 1.0) and Ikwo (16.8 ± 0.1). The samples contained meaningless amounts of both macro and micro biogenic elements. Toxic elements such as lead, cadmium, mercury, arsenic, chromium and nickel were detected but lower than the WHO standard values.
Conclusion: This study indicates that this legume may be safe for consumption.

1. Introduction

Bambara groundnut (*Vigna subterranea* L.) Verd Court is a legume predominantly grown in Africa [1], especially in Nigeria. This nut is described as a hard-to-cook grain and therefore has some limitations [2] regarding its cooking. It is grown popularly by subsistence farmers and ranked the third amongst legumes, groundnut (*Arachis hypoga*ea L.) and cowpea (*Vigna unguiculata*) are ranked as the first and second respectively in Africa [3]. The legume has an affordable source of protein that improves the nutrition status of most rural households [4], while the nutritional composition of Bambara groundnut has been reported to provide a balanced diet when compared to most food legumes because of its high iron content and the protein profile [5–7]. It is also reported to be abundant in essential amino acids such as leucine, isoleucine, lysine, methionine, phenylalanine, threonine and valine when compared to groundnut [8,9]. The protein score of Bambara groundnut is reported to be 80% when compared to 65% for groundnut, 74% for soya bean and 64% for cowpea [10]. It contains about 63% carbohydrates, 24% protein, 6.5% lipids and 5.5% fiber [11]. The fatty acid profile consists of linoleic, palmitic and linolenic acids [12]. Tan et al. [13] reported that some macro minerals such as zinc, iron, calcium, sodium and potassium are found in this legume. This legume is also relevant in agriculture be-

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cause it improves soil fertility by fixing atmospheric nitrogen as nitrates [14], thereby improving soil yields [15].

This indigenous leguminous crop is commonly consumed as food and snack which plays an important role in the food and culture of individuals in the South East of Nigeria. Fresh and dry Bambara nuts are eaten in different ways after processing - the fresh pods are boiled with salt and consumed as a tasty snack while the dried nuts are roasted, grilled and chewed with dried palm kernel nuts as snack [16]. Despite all the nutritional potentials of Bambara groundnut, it still remains an underutilized plant [17]. It has also been reported to be a good source of starch for industrial purposes [1]. The ground seed is sieved to make fine flour, which can be used for different purposes such as in preparing cakes and puddings. An example is "Koki" which is a type of delicacy from Central Africa locally cooked to form a paste [18]. Also, "Okpa" is produced from Bambara groundnut flour which is a local delicacy cooked with other ingredients such as palm oil, "uziza" (*Piper guineense*) seeds and "scent leaf" (*Ocimum gratissimum*), mixed into a paste, wrapped and cooked in plantain leaves.

Recently, studies have shown that Bambara groundnut flour can be used in the production of food products such as milk, yogurt, bread, biscuits and dough nuts [19]. Apart from its nutritional uses, its medicinal uses have been reported. Its protein hydrolysate and peptide fractions have been reported to be used in the formulation of functional foods against high blood pressure and oxidative stress [20]. Despite the various nutritional and medicinal benefits of this legume, there are several constraints such as pest-infestation, hard-to-cook process and the presence of anti-nutrients such as phytic acid and trypsin inhibitor that could prevent the bioavailability of essential nutrients [21]. The incidence of toxic elements and pesticide residues present in various legumes and staple foods such as Bambara nuts consumed in Nigeria has been a source of concern. Therefore, the study aimed at investigating the pesticide residues, amino acid, biogenic and toxic element contents of Bambara groundnut samples obtained from three local governments in Ebonyi state, Nigeria with different soil types- Ikwo, Afikpo and Abakaliki.

2. Materials and methods

2.1. Sample collection

A total of nine samples were collected from different market locations in Ebonyi State- Afikpo, Abakaliki and Ikwo. They were identified and authenticated by a Botanist in the Department of Plant Science and Biotechnology, University of Nigeria. A voucher specimen (UNH 154a) was deposited at the herbarium. The samples were washed, dried in an open-air shade and manually ground to coarse powder. The samples were contained in air-tight sample bottles and stored till analysis.

2.2. Analysis of pesticide residues

Samples were prepared and analyzed following the method described by Tsochatzis et al. [22] as reported by David et al. [23]. Working standard solutions were prepared by appropriate dilution of the stock solutions with their respective dilution solvents. The samples were dissolved in 100 μL acetonitrile, vortexed for about 30 s and 20 μL were injected in the High-Performance Liquid Chromatography- Diode Array Detection (HPLC-DAD) system. The chromatographic system used was an Agilent Technologies (Waldbronn, Germany) 1200 series model which consisted of an on-line degasser system, a thermostated column compartment, a quaternary gradient pump, a Rheodyne (Cotati, CA, USA) injector valve with a 20 μL loop and a Diode Array Detection system. Chromatographic condition used was described by David et al. [23].

2.3. Analysis of amino acid profile

The amino acid profile of Bambara groundnut was determined according to the method of Thomas et al. [24] and reported by David et al. [23]. High Performance Liquid Chromatography (HPLC) was used to determine amino acid composition. 100 mg of the ground samples was mixed with 5 mL 6 M HCl and heated at 110 °C for 24 h. The hydrolysates obtained were mixed with 400 μL of 50 μmol mL^{-1} of L-α-amino-*n*-butyric acid (AABA) as internal standard.

2.4. Analyses of biogenic and toxic elements

Glasswares and plastic vials used were rinsed thoroughly with distilled water and then soaked in 14% HNO_3 (v/v) for 24 h, cleaned thoroughly with deionized water and dried. The employed chemicals and reagents were all of analytical grade. Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (A Perkin Elmer (Waltham, MA, USA), model Optima 8000) was used for the analysis of biogenic and toxic elements as described by Hafuka et al. [25]. Kjeldahl digester (VELPE Scientific, Japan) was used for digestion of coarse Bambara samples.

2 g of the Bambara samples was added to 80 mL of 0.1M HCl and the mixture was stirred at room temperature for 10 min. Following centrifugation for 10 min at 8000 g, the supernatant was filtered through a 1.0 μm pore- size cellulose ester membrane to obtain the extract. About 9 mL of extract solution and 1mL of concentrated HNO_3 were added into 10 mL test tubes. The digested samples were injected into the ICP-OES to determine the biogenic and toxic elements concentrations. All analyses were done in triplicate.

2.4.1. Estimated weekly intake of biogenic elements

Estimated weekly intake (EWI) was calculated based on the already determined concentrations of the biogenic elements using the following equation described by [26].

$$\mathrm{EWI} = \frac{\mathrm{ALC} \times \mathrm{c} \times 7}{\mathrm{BW}}$$

where: ALC is the assumed legume consumption which is set to be 20 g per day [27], c is the concentration of selected element (mg/kg), 7 is a factor for weekly duration and BW is an average body weight for a Nigerian adult which is 68 kg for adults [28] and 16 kg for children

2.5. Statistical analysis

Mean values were presented as Mean ± Standard error of mean (SEM). Analysis was carried out using one-way analysis of variance (ANOVA) to compare the experimental groups followed by Tukey's post hoc test. A test value of ($p < 0.05$) was considered to be statistically significant.

3. Results and discussion

3.1. Pesticide residues

Table 1 shows the levels of various pesticide residue content in Bambara groundnut samples. Some pesticide residues found were cyclodienges, cyclohexane, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD), dicofol, perthane, methoxychlor, aldrin, dieldrin, heptachlor, chlordane, endosulfan, hexachlorobenzene, pentachloro benzene, mirex, toxaphene, alpha-hexachlorocyclohexane (HCH), beta-HCH and gamma-HCH. The levels ranged from 0.001 to 0.598 μg/kg. Pesticide residue content varied from (0.001 ± 0.00 to 0.598 ± 0.03 μg kg^{-1}) in Afikpo, (0.001 ± 0.00 to 0.50 ± 0.01 μg kg^{-1}) in Abakaliki and Ikwo had (0.001 ± 0.00 to 0.470 ± 0.01 μg kg^{-1}). However, there was a significant ($p < 0.05$) higher concentration of dieldrin in Afikpo sample relative to the

Table 1
Pesticide residues (µg kg^{-1}) in Bambara groundnut in comparison with MRL ORIGIN.

Pesticides	Afikpo	Abakaliki	Ikwo	MRL(µg kg^{-1})
Cyclodienges	0.11 ± 0.0^{a}	0.50 ± 0.0^{b}	0.47 ± 0.0^{b}	NA
Cyclohexane	0.01 ± 0.0^{a}	0.03 ± 0.0^{b}	0.04 ± 0.0^{c}	NA
DDT	0.004 ± 0.0^{a}	0.007 ± 0.0^{b}	0.009 ± 0.0^{c}	50*
DDD	0.002 ± 0.0^{a}	0.013 ± 0.0^{b}	0.076 ± 0.0^{c}	50*
Dicofol	0.004 ± 0.0^{a}	0.004 ± 0.0^{b}	0.003 ± 0.0^{a}	50***
Perthane	0.11 ± 0.0^{a}	0.07 ± 0.0^{b}	0.06 ± 0.0^{c}	10*
Methoxychlor	0.29 ± 0.0^{a}	0.07 ± 0.0^{b}	0.03 ± 0.0^{c}	10**
Aldrin	0.02 ± 0.0^{a}	0.02 ± 0.0^{a}	0.04 ± 0.0^{c}	20**
Dieldrin	0.6 ± 0.03^{a}	0.4 ± 0.02^{b}	0.3 ± 0.0^{c}	50$^{\#\#}$
Heptachlor	0.06 ± 0.0^{a}	0.06 ± 0.0^{a}	0.06 ± 0.0^{a}	50$^{\#\#}$
Chlordane	0.003 ± 0.0^{a}	0.004 ± 0.0^{a}	0.005 ± 0.0^{b}	20$^{\#\#}$
Endosulfan	0.001 ± 0.0^{a}	0.001 ± 0.0^{a}	0.001 ± 0.0^{a}	50*
Hexachlorobenzene	0.001 ± 0.0^{a}	0.002 ± 0.0^{b}	0.002 ± 0.0^{b}	NA
Pentachlorobenzene	0.002 ± 0.0^{a}	0.003 ± 0.0^{b}	0.006 ± 0.0^{c}	NA
Mirex	0.01 ± 0.0^{a}	0.01 ± 0.0^{a}	0.01 ± 0.0^{a}	10*
Toxaphene	0.005 ± 0.0^{a}	0.007 ± 0.0^{b}	0.008 ± 0.0^{b}	100$^{\#}$
alpha-HCH	0.02 ± 0.0^{a}	0.01 ± 0.1^{c}	0.02 ± 0.0^{a}	200**
beta-HCH	0.065 ± 0.0^{a}	0.027 ± 0.0^{b}	0.024 ± 0.0^{b}	200**
gamma-HCH	0.003 ± 0.0^{a}	0.002 ± 0.0^{b}	0.003 ± 0.0^{a}	10*

Values are reported as mean ± SEM of three determinations. (n = 3). $^{a–c}$different superscripts along the rows are significantly different (p < 0.05). MRL: Maximum residue level; * European Union MRL [29]; **FAO/WHO MRL [30]; ***Thailand MRL [31], # Leonards et al. [32], ## China MRL [33]; DDT: Dichlorodiphenyltrichloroethane; DDD: Dichlorodiphenyldichloroethane; HCH: hexachlorocyclohexane (HCH); NA: not available at the time of this report.

Abakaliki and Ikwo samples. Interestingly, the levels of all residues in these samples were lower when compared to the maximum residue limits (MRL) established by national and international bodies as listed below Table 1.

In Nigeria, pesticides are widely used in agriculture by farmers and traders to protect their growing plants on the farm from pests and stored products from insect-borne diseases. Though the pesticide residue content in the samples of Bambara groundnut were found in meaningless amounts when compared to the Maximum Residue Limit (MRL) set by various MRL standards as listed in Table 1. Dieldrin was higher in all three samples studied though meaningless when compared to the Maximum Residue Limit (MRL) in China. These meaningless amounts of pesticides detected in these samples could have accumulated during growth or storage to preserve the grains from pest attacks. This is in agreement with reports by Puri [34] who reported that pesticide residues detected in crops could be as a result of application of pesticide during cultivation and to some extent from residues in the soil. Other ways by which pesticides can be found in these grains could be during pesticide production, distribution of agricultural produce, surface run off of pesticides, accidental discharge or spillage near groundwater or potable water [35]. Reports have shown that the presence of pesticide residues and antinutrients such as trypsin inhibitors in legumes could limit the bioavailability of the essential nutrients [36] required for human health. Adequate processing methods such as boiling, fermentation and roasting of the Bambara groundnut samples could reduce the level of pesticide residues. The meaningless amount of these residues suggest that these Bambara groundnut samples are safe for consumption.

Table 2
Biogenic elements in Bambara groundnut (mg/100g).

	ORIGIN		
Element	Afikpo	Abakaliki	Ikwo
Calcium	58.2 ± 0.5^{a}	24.2 ± 0.1^{b}	39.4 ± 0.2^{c}
Manganese	1.8 ± 0.4^{a}	0.7 ± 0.1^{b}	0.9 ± 0.1^{c}
Potassium	36.5 ± 0.9^{a}	18.2 ± 0.1^{b}	24.3 ± 0.7^{c}
Sodium	12.0 ± 5.3^{a}	21.2 ± 10.3^{b}	36.0 ± 9.4^{c}
Phosphorus	122.4 ± 0.2^{a}	124.5 ± 0.1^{a}	111.4 ± 0.0^{b}
Sulphur	0.5 ± 0.1^{a}	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}
Magnesium	157.0 ± 2.1^{a}	73.0 ± 0.0^{b}	88.2 ± 1.7^{b}
Iron	94.0 ± 1.4^{a}	58.0 ± 1.1^{b}	65.0 ± 0.7^{b}
Zinc	12.6 ± 1.0^{a}	7.4 ± 1.5^{b}	8.1 ± 0.5^{b}
Copper	43.1 ± 1.1^{a}	17.1 ± 0.10^{b}	22.1 ± 0.1^{b}
Cobalt	0.007 ± 0.0^{a}	0.008 ± 0.0^{a}	0.009 ± 0.0^{b}
Aluminium	0.003 ± 0.0^{a}	0.003 ± 0.0^{a}	0.003 ± 0.0^{a}
Silver	0.003 ± 0.0^{a}	0.002 ± 0.0^{b}	0.003 ± 0.0^{a}
Molybdenum	0.9 ± 0.0^{a}	0.4 ± 0.0^{b}	0.5 ± 0.0^{c}
Selenium	0.002 ± 0.0^{a}	0.002 ± 0.0^{a}	0.002 ± 0.0^{a}
Boron	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
Vanadium	1.0 ± 0.0^{a}	0.6 ± 0.0^{b}	0.3 ± 0.0^{c}
Barium	0.001 ± 0.0^{a}	0.001 ± 0.0^{a}	0.003 ± 0.0^{b}
Silicon	0.002 ± 0.0^{a}	0.002 ± 0.0^{a}	0.002 ± 0.0^{a}
Titanium	0.002 ± 0.0^{a}	0.003 ± 0.0^{b}	0.002 ± 0.0^{a}
Gold	0.04 ± 0.0^{a}	0.02 ± 0.0^{b}	0.02 ± 0.0^{b}

$^{a–d}$Different superscripts along the rows are significantly different (p < 0.05). Values are presented as mean ± SEM of three determinations (n = 3).

3.2. Biogenic elements

Table 2 presents biogenic elemental composition in Bambara groundnut samples collected at three local governments -Afikpo, Abakaliki and Ikwo. The result shows that the three samples were rich in biogenic elements and therefore could be beneficial for both humans and animal health. Macro biogenic elements determined in the samples include Na, K, Mg, Ca and P. The levels of Ca, Mg and K in Afikpo sample were significantly (p < 0.05) higher than that of Abakaliki and Ikwo; whereas Na level in Ikwo sample was significantly (p < 0.05) higher than the other two samples. The micro biogenic elements reveal that sulphur, manganese, iron, zinc, copper and boron levels were significantly (p < 0.05) higher in Afikpo than in Abakaliki and Ikwo samples. Cobalt level in Ikwo sample was significantly (p < 0.05) higher when compared to the other samples.

Biogenic elements perform important physiological functions in the human body [37]. The most abundant elements in the three samples of Bambara groundnut are Ca, K, Mg, P, Cu and Na. This confirms what other authors have reported about the most abundant elements which are Mg and P in Bambara groundnut [38,39]. These macro biogenic minerals are required in large amounts in the human body. The average daily dietary intake for each micronutrient that is required to sus-

Table 3
Recommended Dietary Allowances and Adequate Intakes for selected biogenic elements.

Biogenic elements										
Life Stage	Group	Ca (mg/d)	Cu (mg/d)	Fe (mg/d)	Mg (mg/d)	Mn (mg/d)	P (mg/d)	Zn (mg/d)	K(g/d)	Na(g/d)
Children	1-3yrs	700	340	7	80	*1.2*	400	*3*	*3*	*1*
Children	4-8yrs	1000	440	10	130	*1.5*	500	*5*	*3.8*	*1.2*
Male	19-50yrs	1000	900	8	420	*2.3*	700	*11*	*4.7*	*1.5*
Female	19-50yrs	1000	900	18	320	*1.8*	700	*8*	*4.7*	*1.5*
Pregnancy	19-50yrs	1000	1000	27	360	*2*	700	*11*	*4.7*	*1.5*
Lactation	19-50yrs	1000	1300	9	320	*2.6*	700	*12*	*5.1*	*1.5*

This table presents Recommended Daily Allowance (RDA) in ordinary type and *Adequate Intake (AI) in italics type.* mg/d- milligrams per day. The table was adapted from Dietary Reference intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc [49]; and Dietary Reference intakes for Water, Potassium, Sodium, Chloride, and Sulphates [50]. These reports may be accessed via www.nap.edu. The values of daily intake, Recommended Dietary Allowances (RDA) or Adequate Intakes (AI), as well as the Upper Intake Levels (UL) recommended by the Food and Nutrition Board/Institute of Medicine (US) for the minerals analyzed were used as a reference for adults

Table 4
Estimated Weekly intake (EWI) of some selected minerals.

EWI (mg/kg)		
Element	Adults	Children
Ca	83.59	355.25
Cu	56.47	240.01
Fe	148.91	632.89
Mg	218.38	928.11
Mn	2.33	9.89
P	245.88	1045.01
Zn	19.29	81.99
K	54.21	230.39
Na	47.50	201.86

EWI- Estimated weekly intake.

tain normal physiologic functions is measured in milligrams or smaller quantities. These macro elements are known for their specific functions in the metabolic regulation of biological systems. Calcium is needed for growth, maintenance of bone, teeth and muscles in children and adults [40]. Zinc and calcium participate in glucose metabolism by enhancing insulin production [41]. The intake of essential elements such as calcium, potassium and iron promote growth, health and cognitive development both in children and adults [42] (Table 3).

Micro minerals such as Se, Cu, Zn, Mn and Fe were obtained in reasonable amounts. These micro elements act as cofactors for some protective antioxidant enzymes. For example, superoxide dismutase contains Mn, Cu, Zn, which protect cells from pro-oxidant molecules especially free radicals. Iron concentration ranged from (58.0 ± 1.1 to 94.0 ± 1.4) mg/100g. These were lower in values when compared to iron values obtained other reports from Bambara groundnut (150.34 ± 0.51 to 159.99 ± 0.82) obtained from different market locations in Nigeria [43].

Studies have also shown that concentration and bioavailability of these biogenic elements can be influenced by duration of storage, adequate processing method, location of elements in the seeds and mineral chelation [44]. The variability in the composition of these elements observed in all samples could be attributed to soil, climatic conditions, seasonal variations and even legume maturity as reported by [45]. Both macro and micro biogenic elements are known for their specific functions which include regulation of blood glucose homeostasis, osmo-regulation, hormonal and metabolic functions, regulation of enzyme activity and heme synthesis.

Children had higher values for the various selected minerals when compared to adults as shown in estimated weekly intake in Table 4. This could be attributed to their rapid growth rate, development and their need for a balanced diet [46]. In the estimated weekly intake table, P, Mg and Fe had higher values for children when compared to the Recommended Daily Allowance and Adequate intake values. These minerals have been involved in growth and development in children especially during early childhood [47]. Therefore, dietary element intake must be sustained in children and adults to maintain physical health as acute imbalances of these nutrients may be fatal [48].

3.3. Toxic elements

Toxic elements in Bambara groundnut samples which includes lead, cadmium, mercury, arsenic, nickel and chromium are presented in Table 5. However, the levels of Pb, Cd and Hg in Afikpo sample were significantly ($p < 0.05$) lower than the corresponding toxic elements measured in Abakaliki and Ikwo samples. The toxic elements levels in all samples were lower than the FAO/WHO standard.

The level of toxic elements (Pb, Cd, Hg, As, Ni and Cr) in Bambara groundnut samples were relatively lower when compared to the FAO/WHO standard. Bioaccumulation of these toxic elements could result to various abnormalities which includes organ toxicity and oxidative stress in the human body which could be deleterious to body tissues and organs [52]. Most farmers and traders use pesticides to prevent pests and fungi so therefore this could lead to accumulation of such toxic elements during growth process and storage. Food stuffs or farm produce stuffs can also be contaminated by toxic elements during harvest, transportation, storage and processing stages [23]. For example, mercury is used as fungicides to prevent fungi attack on plants and also known as an environmental pollutant, some of its compounds have been applied to mining in the extraction of gold and some industrial processes [52].

Pollution of soil, air and water by toxic elements are detrimental to human health. The local governments- Afikpo, Abakaliki and Ikwo have mining sites where lead and some heavy metals are mined. Lead is an environmental pollutant and can find its way into food samples through contaminated water and through lead mining sites in found in these local governments in Ebonyi state. Lead exposure can lead to disturbances in physiological functions of the body and could predispose one to so many diseases [52]. The accumulation of these toxic metals such as Pb could have arisen to the mining sites located in these areas. High levels of Cd in water, air, and soil can occur due to industrial activities which can be a substantial exposure to humans [52]. Arsenic (As) is present in our natural environment and its concentration increases when human activities such as pesticide application, mining and smelting operations [53,54]

Some of these elements become toxic when their permissible ranges are exceeded [55]. Adequate processing methods are required in order to eliminate the presence of these toxic elements. Scientific reports on Bambara groundnut have shown that traditional processing methods such as fermentation, germination and roasting could increase the nutrient content of Bambara groundnut seeds [56] and therefore decrease the levels of these toxic elements.

Table 5
Comparison of toxic elements (mg kg^{-1}) in Bambara groundnut with FAO/WHO standards.

ORIGIN				
Toxic element	Afikpo	Abakaliki	Ikwo	FAO/WHO reference[#]
Lead	0.001 ± 0.0[a]	0.004 ± 0.0[b]	0.005 ± 0.0[b]	0.10
Cadmium	0.01 ± 0.0[a]	0.02 ± 0.0[b]	0.01 ± 0.0[a]	0.05
Mercury	0.02 ± 0.0[a]	0.02 ± 0.0[a]	0.02 ± 0.0[a]	NA
Arsenic	0.002 ± 0.0[a]	0.002 ± 0.0[a]	0.007 ± 0.0[b]	0.10
Nickel	0.015 ± 0.0[a]	0.006 ± 0.0[b]	0.004 ± 0.0[b]	0.20
Chromium	0.018 ± 0.0[a]	0.014 ± 0.0[b]	0.020 ± 0.0[a]	NA

[a,b]Different superscripts along the rows are significantly different ($p < 0.05$). Values are presented as Mean ± SEM of three determinations ($n = 3$). NA: Not available [#]Food and Agriculture Organization/ World Health Organization [51].

Table 6
Percentage (%) composition of amino acids.

ORIGIN			
Amino acid	Afikpo	Abakaliki	Ikwo
Alanine	0.005 ± 0.0[a]	0.003 ± 0.0[b]	0.004 ± 0.0[c]
Arginine	0.002 ± 0.0[a]	0.003 ± 0.0[b]	0.002 ± 0.0[a]
Asparagine	0.12 ± 0.0[a]	0.08 ± 0.0[b]	0.001 ± 0.0[c]
Aspartic acid	0.001 ± 0.0[a]	0.001 ± 0.0[a]	0.001 ± 0.0[a]
Cystine	0.04 ± 0.0[a]	0.04 ± 0.0[a]	0.05 ± 0.0[a]
Glutamic acid	0.037 ± 0.0[a]	0.007 ± 0.0[b]	0.005 ± 0.0[c]
Glutamine	0.012 ± 0.0[a]	0.007 ± 0.0[b]	0.007 ± 0.0[b]
Glycine	0.009 ± 0.0[a]	0.006 ± 0.0[b]	0.006 ± 0.0[b]
Histidine	0.02 ± 0.0[a]	0.02 ± 0.01[a]	0.01 ± 0.00[b]
Isoleucine	8.6 ± 0.8[a]	10.4 ± 0.5[a]	9.3 ± 0.8[a]
Leucine	2.2 ± 0.1[a]	0.6 ± 0.0[b]	0.3 ± 0.0[c]
Lysine	1.3 ± 0.0[a]	1.6 ± 0.0[a]	1.4 ± 0.0[a]
Methionine	3.0 ± 0.0[a]	1.7 ± 0.0[b]	1.0 ± 0.0[c]
Phenylalanine	0.02 ± 0.0[a]	0.02 ± 0.0[a]	0.02 ± 0.0[a]
Proline	0.01 ± 0.0[a]	0.02 ± 0.0[b]	0.02 ± 0.0[b]
Serine	0.02 ± 0.0[a]	0.02 ± 0.0[a]	0.02 ± 0.0[a]
Threonine	9.3 ± 0.7[a]	3.3 ± 0.4[b]	4.6 ± 0.1[b]
Tryptophan	0.002 ± 0.0[a]	0.003 ± 0.0[b]	0.004 ± 0.0[b]
Tyrosine	0.013 ± 0.0[a]	0.009 ± 0.0[b]	0.014 ± 0.0[a]
Valine	0.02 ± 0.0[a]	0.01 ± 0.0[b]	0.01 ± 0.0[b]
Total amino acids (%)	24.7 ± 1.4[a]	17.8 ± 1.0[b]	16.8 ± 1.00[b]

[a–c]Means with different superscripts along the rows are significantly different ($p < 0.05$). Values are presented as Mean ± SEM of three determinations ($n = 3$).

Table 7
Percentage of essential amino acids of Bambara groundnut in comparison with the WHO reference standard for dietary proteins.

ORIGIN				
Amino acids	Afikpo	Abakaliki	Ikwo	WHO[a]
Phenylalanine	0.08	0.1	0.1	6.0
Histidine	0.08	0.1	0.07	NA
Isoleucine	34.8	58.3	55.3	4.0
Leucine	8.9	3.2	1.9	7.0
Lysine	5.4	9.0	8.5	5.5
Methionine	12.1	9.5	6.1	3.5
Threonine	37.4	18.6	27.2	4.0
Tryptophan	0.01	0.02	0.02	3.6
Valine	0.09	0.07	0.06	5.0

[a] World Health Organization [57]. Calculation of each essential amino acid is expressed as a percentage of the total amino acids obtained as shown in Table 5.Values are based on three determinations ($n = 3$).). NA: Not available

3.4. Percentage composition of amino acids

Table 6 presents the percentage composition of amino acids in Bambara groundnut samples. The percentage of amino acids ranged from 0.001 to 10.39%. The percentage composition of isoleucine, threonine, methionine, leucine and lysine were relatively high in all three samples. However, the highest percentage of amino acid (10.39% isoleucine) was obtained in Abakaliki sample. The total percentage of amino acids in Afikpo sample (24.7 ± 1.4)% were found to be significantly ($p < 0.05$) higher than samples from Abakaliki (17.8 ± 1.01)% and Ikwo (16.8 ± 0.99)%.

3.5. Essential amino acids

Table 7 shows the comparison of essential amino acid composition of Bambara groundnut samples with the WHO standards. All three samples contain essential amino acids and the percentage content of isoleucine, lysine and threonine are comparable and higher than the WHO standard. However, leucine and lysine values obtained from the sample from Afikpo are comparable to the WHO standard.

Amino acids are the building blocks of peptides and proteins. They are starting materials to a variety of biomolecules such as hormones, coenzymes, nucleotides, alkaloids, pigments and neurotransmitters [58]. Both essential and non-essential amino acids are present in the Bambara groundnut samples. This legume contains essential amino acids which are required to protect against protein deficiencies and therefore could combat malnutrition because of its rich protein content. Scientific evidence has revealed that Bambara groundnut is used in improving the protein quality of a staple diet- maize gruel also called "*ogi*" due to its high protein content [19].

Leucine and lysine were comparable to the WHO standard and this confirms reports by authors who reported that leucine and lysine were high in the dehulled and whole seeds of Bambara groundnut [59]. Isoleucine, threonine, methionine, leucine and lysine are relatively high in percentage compared to the WHO standard. The values of these amino acids could be attributed to the physiological status and seasonal variations of this plant where the Bambara groundnut samples were sourced. Isoleucine is a branched chain amino acid which plays a key role in maintaining the body immune function and necessary in various functions of the human body, such as growth and immune function, metabolism of protein and transport of glucose [60].

In summary, essential amino acids play variety of roles in the body which includes, physiological functions, growth, immune functions, protein metabolism, glucose transportation, protein folding functions. Bambara groundnut has been widely used in enriching traditional foods because of its relatively high nutritional value and fairly balanced amino acid profile. This confirms reports by various authors who reported that Bambara groundnut is a good source of amino acids therefore can serve as a food supplement [2,59].

4. Conclusion

The importance of Bambara groundnut as an underutilized crop has vast prospects for food production and has the capability to combat malnutrition both in adults and children. Bambara groundnut samples obtained from various locations -Ikwo, Abakaliki and Afikpo contain some reasonable levels of biogenic elements and essential amino acids. Chemical pesticides both in farming and storage of food products should be appropriately monitored to avoid long term side effects on human health. There is need to control and monitor pesticides usage in these areas where the samples were sourced because of their potential toxicity. Although our study reports that the toxic element concentrations were within the permitted limits. However, adequate processing methods of the Bambara groundnut seeds could result to zero levels of both pesticide residue contents and toxic elements. Therefore, Bambara groundnut makes a complete and safe edible diet for human consumption in Ebonyi State.

Ethical approval

The conducted research is not related to either human or animals.

Data availability statement

All data generated or analysed during this study are included in this published article and its supplementary information files.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

CRediT authorship contribution statement

Chima A. Egedigwe-Ekeleme: Conceptualization, Methodology. **Ademola C. Famurewa:** Conceptualization, Methodology. **Uchenna O. Egedigwe:** Writing – review & editing. **Chimaraoke Onyeabo:** Writing – review & editing. **Shedrach C. Kanu:** Investigation, Resources. **Shedrack C. Ogunwa:** Investigation, Resources. **Chinwendu A. Onuora:** Writing – original draft. **Anthonia O. Agbo:** Writing – original draft.

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