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PROXIMATE CONSTITUENT AND MINERAL CONTENT OF THE SEEDS OF AFRICA YAM BEAN (*SPHENOSTYLIS STENOCARPA*) PURCHASED IN EBONYI STATE, EASTERN NIGERIA

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ABSTRACT: *In sub-Saharan Africa there have been various calls for development of a crop which will be high yielding with increased protein content. One of such plants currently being speculated to be an important source of high protein, carbohydrates, as well as other nutritional substances is African yam bean (Sphenostylis stenocarpa Hochst ex A. Rich. Harms). This work is therefore designed to ascertain the proximate, mineral, vitamin and phytochemical composition of the seed of African yam bean. The seeds of African beans were analyzed for proximate mineral vectorment and phytochemical composition using standard methods. Data were subjected to statistical analysis using ANOVA. The results of the percentage proximate composition showed that the seeds contain 8.67% moisture, crude protein 22.22%, crude fiber 3.89%, ash 4.58%, fat 4.20%, and carbohydrate 56.44%. The mineral contents (mg/100g) were found to be 32.07 mg/100g (Sodium), 231.20 mg/100g (Potassium), 78.67 mg/100g (Magnesium), 159.66 mg/100g (Calcium), 133.53 mg/100g (Phosphorus), 2.32 mg/100g (Zinc), and 6.13 mg/100g (Iron). Africa yam bean is an important source of high-quality protein and carbohydrate, as well as other nutritious substances. The present constituent, especially their quantitative composition, may be an indication of the nutritional as well as medicinal importance of the seeds of Africa yam bean. Such interesting high content of certain bioactive compounds, such as phenol and flavonoid as well as elevated protein content, calls for improved cultivation of this crop for maximum yield and availability.*

KEYWORDS: Mineral content, Nutritional value, Proximate composition, *Sphenostylis stenocarpa*, Reagent.

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INTRODUCTION

Due to the increasing human population, the quest for food and more natural and herbal medicine has led to continuous efforts toward improvements of global and under explored foods and health systems. While the developed countries seem to have these systems stabilized, some parts of the world, especially Africa, still face enormous challenges. Protein is one of the essential food constituents required by man. In developed countries, sources of protein, especially animal proteins, are numerous. There are various techniques that help the developed world in animal production. Furthermore, many of their plants can accumulate and store high levels of protein as this is favored by their temperature. But in underdeveloped countries of Africa, especially the sub-saharan Africa, animal proteins are less available. The problem is increased by the inability of most of our plants to accumulate protein since our temperature is not friendly for such accumulation. It therefore becomes very necessary to preserve and adapt any plant that can increase food availability especially with increased protein constituents. One of such plants is the African yam bean. This is a legume indigenous to west and east Africa with nutritional content comparable to other common legumes.

African yam bean (AYB) (*Sphenostylis stenocarpa* Hochst. Ex. A. Rich. Harms) is an annual leguminous climber belonging to the class Magnoliopsida, family Leguminosae (Fabaceae), and subfamily Papilionoideae utilized for its edible seeds and underground tuber production. The seeds possess hard-to-cook characteristics which to a degree have contributed to its underutilization. It is widely cultivated in the Southeastern part of Nigeria (Idowu, 2014) and other parts of West and East Africa. AYB is mostly cultivated by local farmers for subsistence purposes. African yam beans perform better when intercropped than when grown as sole crops (Adeniyan, 2007) and it shows hypogeal type of germination. Cultivation of AYB for optimal yield starts from the end of May through July, which is the period of stable and maximum rainfall. Despite the nutritional value as well as health benefits of African bean, it is still largely under-utilized (Idowu, 2015).

The botany of this underutilized legume was first described (Okigbo, 1973). The vine produces linear pods, 20–30 cm long in which 20–30 seeds are borne. The seeds are usually brown, cream, orange-brown or mottled and ovoid in shape. Flowering occurs 80–130 days after planting and seed maturity after 150–300 days. Seed yield is very poor in Nigeria, about 300–500 kg/ha (Ezueh, 1984).

AYB is known with different dialectic synonyms that each local government in Ebonyi State has a separate local name for it. These synonyms within the state may include azuma (Afikpo South), azama (Afikpo North), eczema (Ohazara), azuaka (Ikwor) also known as azima in Ohafia, Abia State.

It is known that the nutritional quality health application of any plant part is in part associated with their major constituents, including proximate and minerals. Minerals are essential supplements for bones, teeth, soft tissues, hemoglobin, muscles and nerve cells. Mineral content of plant species may vary depending on the cultivar, ripening stage, agronomical practices and environmental conditions.

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MATERIALS AND METHODS

Samples Collection

The seed samples of African yam Bean (*Sphenostylis stenocarpa*) were purchased from Nkwo market, Amangwu Edda, Afikpo south L.G.A., Ebonyi State, South East Nigeria. The plant sample was taken to the Laboratory where it was prepared and analyzed.

Preparation Of Samples For Analyses

The seed sample was cleaned and dried under room temperature for 5 days. The dried sample was first mashed slightly with mortar and pestle, and then they were ground to a fine powder using an electric blender. The dried powdered sample was however used for the various analyses.

Proximate Analysis

This was carried out mainly by using the method described by the Association of Official Analytical Chemists (A.O.A.C.) (2012). It involves the determination of crude protein, dry matter, ash, crude fiber, ether extract (fat), moisture content and carbohydrate content.

Crude Protein Analysis

The protein content of the samples was determined by the Kjeldahl method reported by James (1995).

The total nitrogen was determined and multiplied by the factor 6.25 to obtain the protein content. 0.5 g of the powdered sample was weighed into a Kjedahl digestion flask and a tablet of selenium catalyst was added to it. 10 ml of conc. H_2S0_4 was then added to the flask and digestion by heating under a fume cupboard until a clear solution was obtained in a separate flask. The acid and other reagents were digested but without sample to form the blank control.

All the digests were carefully transferred to a 100 ml volumetric flask and made up with distilled water to a mark in the flask. A 100 ml portion of each digest was mixed with equal volume of 45% NaOH solution in a Kjeldahl distilling unit. The mixture was distilled and the distillate collected into 10 ml of 4% boric acid solution containing three (3) drops of mixed indication [bromocresol green land methyl red]. A total of 50 ml distillate was obtained and titrated against 0.02 m of H_2SO_4 solution. Titration was done from the initial green color to a deep red end point.

The total nitrogen content was calculated as shown below:

$$\%\ N_2 = \frac{100}{W} \times \frac{N \times 14}{1000} \times \frac{VF}{VA}$$

where:

W = Weight of sample analyzed

N = Concentration of H_2SO_4 titrant

VF = Total volume of digest

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Va = Volume of digest distilled

T = Titer value-blank

Fat (Ether Extract) Analysis

Fat content of the samples were determined by the continuous solvent extraction method using a soxhlet apparatus. The method is described by James (1995) and Pearson (1996).

Five grammes (5.0 g) of each sample were wrapped in a porous paper (Whatman number one filter paper). The wrapped samples were put in a soxhlet influx flask containing 200 ml of petroleum ether. The upper end of the reflux flask was connected to a condenser. By heating the solvent in the flask through an electro-thermal heater, it vaporizes and condenses into the reflux flask. Soon, the wrapped samples were completely immersed in the solvent and remained in contact with it, the flask filled up and siphoned over, thus carrying oil extract from the sample down to the boiling flask.

This process was allowed on repeatedly for about 4 hours before the defatted sample was removed and reserved for crude fiber analysis. The solvent was recovered and the extracting flask with its oil content was dried in the oven at 600°C for 3 minutes [This is to remove any residual solvent]. After cooling in a desiccator, the flask was reweighed.

By difference, the weight of fat (oil) extract was determined and expressed as a percentage of sample weight. It was thereby calculated as:

$$\%fat = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

where:

W_1 = Weight of energy extraction flask

W_2 = Weight of flask and oil extract

Crude Fiber Analysis

This was determined by the Wende method (James, 1995). Exactly 5 g of each sample were defatted (during fat analysis). The defatted sample was boiled in 200 ml of 1.25% H2SO4 solution under reflux for 30 minutes. After that, the samples were washed with several proportions of hot (boiling) water using a two-fold muslin cloth to trap the particle. The washed samples were carefully transferred quantitatively back to the flask and 20 ml of 1.25% NaOH solution was added to it. Again, the samples were boiled for 30 minutes and washed as before with hot water. Then they were very carefully transferred to a weighed porcelain crucible and dried in the oven at 1050C for 3 hours.

After cooling in a desiccator, they were reweighed (W2) and then put in the muffle furnace and burned at 55ºC for 2 hours, until they became ash. Again, they were cooled in the desiccators and reweighed.

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The crude fiber content was calculated mathematically as:

$$\%\text{crude fiber} = \frac{W_2 - W_3}{\text{Weight of sample}} \times \frac{100}{1}$$

where:

W_2 = Weight of crucible + sample after washing and drying in the oven.

W_3 = Weight of crucible + sample of ash.

Total Ash Content Analysis

This was done using the furnace incineration gravimetric method (AOAC, 1990).

A measured weight (5 g) of each powdered sample was in the previously weighed porcelain crucible. The sample in the crucible was put in the muffle furnace set at 550ºC and allowed to burn for 2–3 hours (until the sample becomes gray ash). The sample in the crucible was very carefully removed from the furnace (taking care not to allow air to blow away the ash) and cooled in a desiccator. It was reweighed by difference; the weight of ash was obtained and in percentage. It was, however, given by the formula:

$$\frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

where:

W_1 = Weight of crucible of Ash

W_2 = Weight of crucible + sample after drying to constant weight

Moisture Content Analysis

The moisture contents of the samples were analyzed by a simple method described by James (1995) and Pearson (1996).

A measured weight of each sample (5 g) was weighted into a weighted moisture can. The can and its sample content were dried in the oven at 1050C for 3 hours in the first instance. It was cooled in desiccators and reweighed. The weight was recorded while the sample was retained to the oven for further drying. The drying, cooling and weighing continued repeatedly until a constant was obtained. By the difference, the weight of moisture lost was determined and expressed as a percentage. It was calculated as shown below:

$$\%\text{moisture} = \frac{W_2 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

where:

W_1 = Weight of empty moisture Can

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W_2 = Weight of Can before drying

W_3 = Weight of Can + sample after drying to a constant weight.

Carbohydrate Determination

The carbohydrate content was calculated by arithmetic difference, as the Nitrogen Free Extractive (NFE), a method separately described by James (1995) and Pearson (1996). The NFE was calculated as:

% NFE = 100- % (A+ B+C+D+E)

where:

A = Crude protein

B = Fat (ether extract)

C = Crude fiber

D = Ash

F = Moisture

Determination of Mineral Elements

Samples were analyzed chemically, according to the official methods of analysis described by the AOAC (2005). All analysis was carried out in triplicate.

Calcium, Potassium and Sodium Content Determination

Apparatus: Heating mantle, crucible, glass rod, flame photometer, 100 ml volumetric flask, Whatman No. 1 filter paper, wash bottle, 10ml pipette, and funnel.

Reagents: 2 M HCl.

Determination: The ash of each sample obtained was digested by adding 5 ml of 2 M HCl to the ash in the crucible and heated to dryness on a heating mantle. Some (5 ml) of 2 M HCl was added again, heated to boil, and filtered through a Whatman No. 1 filter paper into a 100 ml volumetric flask. The filtrate was made up to mark with distilled water stoppered and made ready for reading of concentrations of calcium, potassium and sodium on the Jenway Digital Flame Photometer (PFP7Model) using the filter corresponding to each mineral element.

The concentration of each of the element was calculated using the formula: %Ca or %K or %Na

$$= \frac{\text{Meter Reading (MR) x Slope x Dilution factor}}{1000}$$

NB: MR x slope x dilution factor will give you the concentration in part per million (ppm or mg/kg). You get concentration in % when you divide by 1000.

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Phosphorus Determination: Phosphorus was determined routinely by the vanado-molybdate colorimetric or spectrophotometric method.

Apparatus: Spectrophotometer or colorimeter, 50 ml volumetric flask, 10 ml pipette, filter paper, funnel, wash bottle, glass rod, heating mantle, and crucibles.

Reagents: Vanadate-Molybdate yellow solution, 2 M HCl.

Determination: The ash of each sample obtained was treated with a 2 M HCl solution as described for calcium determination above. Then, 10 ml of the filtrate solution was pipette into 50 ml standard flask and 10 ml of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow development. The concentration of phosphorus was obtained by taking the optical density (OD) or absorbance of the solution on a Spectronic-20 spectrophotometer at a wavelength of 470 nm. The percentage phosphorus was calculated using the formula:

$$\%\text{Phosphorus} = \frac{\text{Absorbance} \times \text{Slope} \times \text{Dilution factor}}{10000}$$

Determination of Magnesium, Iron and Zinc Content

The digest of the ash of each sample above as obtained in calcium and potassium determination was washed into a 100 ml volumetric flask with deionised or distilled water and made up to mark. This diluent was aspirated into the Buck 210 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at their respective wavelengths with the irrespective hollow cathode lamps using appropriate fuel and oxidant combination.

Determination of Mineral Elements

Samples were analyzed chemically according to the official methods of analysis described by the AOAC (2005). All analysis was carried out in triplicate.

Calcium, Potassium and Sodium Content Determination

Apparatus: Heating mantle, Crucible, Glassrod, Flame photometer, 100 ml Volumetric flask, Whatman No. 1 Filter paper, Wash bottle, 10 ml pipette, funnel.

Reagents: 2 M HCl.

Determination: The ash of each sample obtained was digested by adding 5 ml of 2 M HCl to the ash in the crucible and heated to dryness on a heating mantle. Some (5 ml) of 2 M HCl was added again, heated to boil, and filtered through a Whatman No. 1 filter paper into a 100 ml volumetric flask. The filtrate was made up to mark with distilled water stoppered and made ready for reading of concentrations of calcium, potassium and sodium on the Jenway Digital Flame Photometer (PFP7Model) using the filter corresponding to each mineral element.

The concentration of each of the element was calculated using the formula: %Ca or %K or %Na

$$= \frac{\text{Meter Reading (MR)} \times \text{Slope} \times \text{Dilution factor}}{1000}$$

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NB: MR x slope x dilution factor will give you the concentration in part per million (ppm or mg/kg). You get concentration in % when you divide by 1000.

Phosphorus Determination: Phosphorus was determined routinely by the vanado-molybdate colorimetric or spectrophotometric method.

Apparatus: Spectrophotometer or colorimeter, 50 ml volumetric flask, 10 ml pipette, filter paper, funnel, wash bottle, glass rod, heating mantle, crucibles.

Reagents: Vanadate-Molybdate yellow solution, 2 M HCl.

Determination: The ash of each sample obtained was treated with a 2 M HCl solution as described for calcium determination above. Then, 10 ml of the filtrate solution was pipette into 50 ml standard flask, 10 ml of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow development. The concentration of phosphorus was obtained by taking the optical density (OD) or absorbance of the solution on a Spectronic-20 spectrophotometer at a wavelength of 470 nm. The percentage phosphorus was calculated using the formula:

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Determination of Magnesium, Iron and Zinc Content

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Statistical Analysis

The experimental results were presented in mean ± SD of the mean of three replicates. The sample means were compared using Analysis of Variance (ANOVA) to determine the level of significance. Differences in mean values were considered significant at $P<0.05$.

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RESULTS

The sample of the AYB seed was analyzed for different proximate composition including moisture content, ash content, crude lipid content, crude protein content, crude fiber content, and carbohydrate content using different standard techniques, and the results are presented in Table 1. The results for the analysis of some mineral elements including calcium, magnesium, potassium, sodium, phosphorus, iron and zinc are presented in Table 2.

Table 1: Proximate compositions of the seeds of AYB (%)

Constituents	Quantity (%)
Moisture	8.67 ± 0.042
Protein	22.22 ± 0.025
Fat	4.20 ± 0.038
Fiber	3.89 ± 0.229
Ash	4.58 ± 0.040
Carbohydrate	56.44 ± 0.020

Values are means of triplicate analysis ± standard deviation.

MINERAL ASSAY (mg/100 g)

Table 2: Mineral composition of the seeds of AYB on dry weight basis expressed as mg/100 g

Mineral	Amount (mg/100 g)
Calcium	159.66 ± 1.46
Magnesium	78.67 ± 0.31
Potassium	231.20 ± 1.65
Sodium	32.07 ± 0.83
Phosphorus	133.53 ± 1.65
Iron	6.13 ± 0.03
Zinc	2.32 ± 0.04

Values are means of triplicate analysis ± standard deviation.

DISCUSSION

From Table 1, which shows the proximate screening of AYB seeds, the moisture content was found to be 8.67%. This low moisture content of AYB is of great advantage because it enables the seed to be stored for a very long period of time. An average crude protein content of 22.22% slightly lower than that of Glycine max was obtained in this research. This agrees with the reported crude protein ranges by some authors: 22.33–25.78% (Adeyeye et al., 1994), 22.72–26.68% (Ajibola & Olapade, 2016), 21.84–23.41% (Baiyeri et al., 2018). The observed differences in these ranges of crude protein values could be attributed to environmental and genetic variations. This high amount of crude protein in AYB seeds increases its value as feedstuff for both human and animals and it is also one reason for the economic advantage that AYB seeds have over other leguminous seeds. However, the incorporation of AYB food into a diet could be an important means of managing protein deficiency cases such as kwashiorkor

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and marasmus. Therefore, they can be used to fortify other foods low in protein to address protein malnutrition in the society.

The fiber content of the sample was found to be 3.89%. Though that may be slightly low when compared with staple grain legumes such as soybean meal with 6.5% crude fiber (Akinmutimi, 2007), the presence of fiber in foods is known to be beneficial. The presence of fiber in AYB makes them a potential source of important functional foods to consumers because it acts as a diluent. Its absence in diets leads to the incidence of a wide range of diseases which includes colon biventricular, diabetes mellitus, obesity and coronary artery diseases (Oke et al., 2007). Fibre also has some physiological effects in the gastrointestinal tract. These effects include variation in fecal water, fecal bulk and transit time and elimination of bile acids and neutral steroids which lower the body cholesterol pool and can also cut the risk of colon cancer (Ogbemudia et al., 2017).

The crude fat content of 4.20% in this study is an indication that AYB seeds possess essential oils and it is higher compared to other alternative protein sources such as pigeon pea (2.33%), velvet bean, *Mucuna cochinchinensis*, sword bean, etc.

According to this result, AYB has ash content of 4.58%, a value that compares favorably with other alternative vegetable protein sources. This is an indication that the bean seeds have high mineral content. African yam beans contain a large amount of carbohydrate (CHO) (56.44%), comparable to some other legumes. According to Maphosa and Jideani (2017), the bulk of CHO in the seed is starch and this starch has slowly digestible properties, which are fit for the consumption of diabetic patients since they do not spike the glycemic index and blood glucose of individuals suffering from diabetes. Besides the starch content, the bean is also a good source of non-starch polysaccharides (NPS) such as cellulose, hemicellulose and arabinose. NPS lowers the risk posed by cardiovascular disorder, colorectal cancer, breast cancer, coronary heart diseases (CHD), laxative disorder, type 2 diabetes and other lifestyle disorders (Kumar et al., 2012).

Generally, the proximate results obtained in this study suggest that AYB can be a good source of protein, carbohydrate, minerals, and crude fiber and therefore can reduce the problem of nutrient deficiency in the society. Hence, it should be exploited as a commercial source to supplement both for animal and human consumption and also be used as staple food.

Mineral Assay

The mineral composition of *Sphenostylis stenocarpa* seeds in mg/100 g is presented in Table 2. The major minerals in AYB are potassium, phosphorus, magnesium, calcium, sodium, iron and zinc. In particular, this study revealed potassium, 231.20 mg/100 g, to be the most abundant mineral element which according to literature was widely distributed in plants and was rarely deficient in diet (Geleijnse, 2004). Calcium (159.66 mg/100 g) was found to be the second abundant mineral, an important macro mineral essential for strong bones and teeth. Phosphorus 133.53mg/100 g and magnesium (78.67mg/100 g) are essential minerals important for a variety of cellular metabolic activities and sometimes have the fortification and enrichment of less nutritious staples. Sodium (32.07 mg/100 g) was found to be the fifth abundant mineral. Sodium and potassium work hand in hand throughout the body. Potassium naturally balances the metabolic action of sodium such that diets low in potassium and high in sodium increase the risk of high blood pressure and cardiovascular diseases (WHO, 2012). The ratio of sodium

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to potassium (Na+/k+) in the diet is an important predictor of hypertension than the amount of either one alone (WHO, 2012). From the results obtained, the ratio of sodium to potassium (Na+/k+) was 0.139. Iron (6.13 mg/100 g) is important for its role in oxygen and electron transport. Zinc (2.32 mg/100 g) is an important micronutrient for the production of hormones. These elements play an important role in human metabolism. On the other hand, they can be risk elements when taken in abnormal quantities. The order of the levels of the elements in the samples was determined to be: K > Ca > P > mg > Na > Fe > Zn.

CONCLUSION

Just like many other leguminous seeds, African yam bean is an important food. It is rich in protein, carbohydrate, fiber, minerals, and vitamins and very low in fat. Generally, the results obtained in this study suggest that AYB can be a good source of protein, carbohydrate, minerals and crude fiber, and therefore should be exploited as a commercial source to supplement both animal and human consumption. Inclusion into food would not only enrich or fortify foods but would also serve as a natural way of staying healthy for consumers and, as such, the consumption should be encouraged. The nutritious properties of AYB suggest that it has potential uses in ameliorating malnutrition in our society and this could be achieved either by consuming it directly or using it to fortify foods.

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