Evaluation of Nutritional and Anti-nutritional properties of raw and boiled seeds of African Yam Bean (*Sphenostylis stenocarpa*)

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Abstract The nutritional and anti-nutritional profiles of *Sphenostylis stenocarpa* were carried out using standard methods of food analysis. The results obtained showed that the raw and boiled seeds of *Sphenostyli*s *stenocarpa* contained 5.66% and 8.03% moisture, 3.79% and 2.21% ash, 3.32% and 2.13% crude lipid, 4.28.% and 2.38% crude protein, 24.90% and 15.65% crude fibre and 8.35% and 5.72% total carbohydrate, 45.10% and 54.09% respectively. The values are moderate compared to NAFDAC standard (12% protein, 0.5% lipid, 3.0% crude fibre, 13% moisture and 50% carbohydrate). The anti-nutritional analysis of the raw and boiled seeds revealed the results to be 0.28% and 0.14% phytate, 0.06% and 0.07% oxalate, 10.21.mg/kg and 8.50mg/kg HCN, 1.70% and 0.33% alkaloid and 1.06% and 0.28% Tannins. The results of the anti-nutritional factors in the raw and boiled samples showed that the values for phytate and tannins are below the permissible limit of WHO (250-500mg) 20mg/g of Tannins. The values of HCN for the raw and boiled seeds exceeds the permissible limits while the value of HCN for boiled seeds is within the permissible limits of WHO which is 1.0mg/100g, whereas the oxalate exceeds the permissible limit of 3-5mg/kg in both the raw and the boiled samples. The qualitative phytochemical anti-nutrients analysis revealed that phytate, oxalate, alkaloid and tannin were in trace amount. This composition shows that *Sphenostylis stenocarpa* could be a good source of Carbohydrate, dietary fibre and protein.

Keywords Anti-nutrients, *Sphenostylis stenocarpa*, Raw, Boiled, Phytochemicals, Proximate, nutritional

Introduction

Legumes are staple food for many people in different parts of the word. The seeds have an average of twice as much cereal by percentage and usually contain more balanced profile of essential amino acids. They range from the highly utilized legumes such as groundnut, cowpea, to the lesser-known ones like *Sphenostylis stenocarpa, Phaseolus vulgaris, Vigna radiate, Vigna subterranean, Mucuna pruriens* [1].

Sphenostylis stenocarpa is an underutilized food legume crop in the tropic. It is a typical African plant grown in most parts of the hot and humid tropical regions at middle or low altitudes and more specifically Nigeria [2]. It is

extensively cultivated in the western and southern parts of Nigeria. The protein content in African yam bean seeds ranges between 20 to 30%. Moreover, the amino acid content in African yam bean seeds is higher than those in cowpea, Bambara nut and pigeon pea [3]. *Sphenostylis stenocarpa* seed is rich in minerals such as phosphorus, potassium, magnesium, calcium, iron and zinc but low in sodium and copper {4]. *Sphenostylis stenocarpa* belongs to the family *fabaceae and class magnoliopsida,* sub family *fabiodeae* and *genus sphenostylis* and it is revealed in English as African yam bean. There are seven species in the genus *sphenostylis* and African yam bean is the most valuable [4].

African yam bean is a perennial climbing crop, 1-3m high, generally grown as an annual crop. It leaves are trifoliate with oval leaflet (2.7 to 13cm and 0.2 to 5.5 cm broad). It is cultivated for its edible seeds which contained in hard and tough 20-30 cm long pods. It is mainly used as human food but can be used to feed animals [5]. *Sphenostylis stenocarpa is a native to tropical west and central Africa.* In Nigeria, It is commercially grown in some parts of Benue where it is known as Ahuma in Tiv, Ulahi in Idoma and Ichiri in Igede. African yam bean is also cultivated in the western and southern part of Nigeria and is revealed as Otiili in Yoruba and Azima or Uzaaku in Igbo. It has been reported that the seeds of the plant has the potential to meet year-round carbohydrate and protein requirements of the most vulnerable population if domesticated [5, 6]. The seeds are known to contain high amount of carbohydrate, protein, minerals and fibre content. However, *Sphenostylis stenocarpa* is also known to contain anti-nutritional substances. Anti- nutritional factors are secondary metabolites found in plants and are known to be biologically active substances. These substances are found in fruits, seeds, and other parts. They occur in varying amounts depending on the kind of crop, and their mode of propagation [7, 8]. Research revealed that anti-nutrients are chemicals produced by plants for their defense and other biological functions. Most of these secondary metabolites have deleterious effect on human beings. However, they could be beneficial to humans and animals if consumed in appropriate amounts. It has been reported that the intake of phytate, tannins and Saponin at low levels reduces blood glucose and increases insulin responses to starchy foods, while phytate, tannins, Saponin, protease inhibitors and oxalate reduces cancer risks in both human and animals. Research has proved that oxalate and phytate form chelates with di and trivalent metallic ions such as Iron, Cadmium, Magnesium, to form poorly soluble compounds that are not readily absorbed by the gastrointrotestinal tract, thereby reducing their bioavailability in the body. These anti-nutritional factors can be reduced by some processing methods such as roasting, soaking, boiling, and fermentation, among others [9, 10]. Conventional food preparation techniques such as soaking, sprouting, boiling, and fermentation have been previously reported to improve flavor and palatability of legumes as well as to increase the bioavailability of nutrients, by deactivating anti-nutritional factors and also allowing the digestion and assimilation of starch and protein [10,11]. Hence, this shows why legumes should not be consumed raw.

Objectives of the Research
The work is aimed at evaluating the nutritional and anti-nutritional components of raw and boiled seeds of *Sphenostylis stenocarpa*

Materials and Methods
Study Area
The research was conducted in Makurdi, town the Benue State capital. The town is located at latitude 7° 38'N - 7° 50'N and longitude 8° 24'E - 8° 38'N. It is situated in the Benue valley in the North Central Nigeria.

Sample collection and Preparation
Dried seeds of African yam bean were purchased from Ajio market, Kwande Local Government Area of Benue State and were taken to Department of Biology Science of Benue State University Makurdi for Identification and authentification. The dried seeds were cleansed and sorted to remove dirt's and other extraneous materials and damaged seeds. Exactly 100g of the seeds were divided into two different portions. The first portion was boiled for 6 hours, this was then sun dried and pounded. The other portion was properly sun dried and pounded using mortar and pestle. These samples were labeled and properly stored in an air tight container to be used for further analysis.

Determination of Proximate Composition

Moisture content

The method of Association of Official Analytical Chemists (AOAC) (1995) was employed using hot air-drying oven. Empty clean crucible dish was dried in the oven at a temperature of 105ºC for one hour and cooled in a desiccator. 5g of the sample was sample weighed and put in the dish and heated for 3 hours at a temperature of 105ºC [11]. The dish was then removed from the oven, cooled in a desiccator and weighed. The moisture content was calculated as:

$$\text{Moisture (\%)} = \frac{\text{weight loss}}{\text{ample weight}} \times 100 \tag{1}$$

Ash content

The method of AOAC (1995) was used to determine the percentage of ash content. Exactly 5 g of the sample was weighed in to a pre-heated and cooled crucible and incinerated in a muffle furnace at 200ºC for 4 hours. The ash was then cooled in a desiccator and weighed. The ash content was calculated using;

$$\text{Ash (\%)} = \frac{\text{weight of Ash}}{\text{weight of sample}} \times 100 \tag{2}$$

Crude fibre

Exactly 5g of the powdered was weighed and placed in 500mL conical flask containing 200 cm^3 of 1.25% H_2SO_4 and were boiled gently for 30 minutes. The content was filtered and the residue was scrapped back in to the flask with a spatula. 200 cm^3 of 1.25% NaOH was added and were allowed to boil gently for 30 minutes. The content was filtered and washed thoroughly with hot distilled water. The precipitate was rinsed once with 10% HCl and twice with ethanol. The content was allowed to dry and the residue was scraped into a weighed crucible and was dried overnight at 105ºC in hot oven. It was cooled in desiccator. The sample was then heated at 600ºC for 90 minutes in a furnace. It was finally cooled in a desiccator and weighed again [11].

The percentage crude fibre was calculated using the equation below:

$$\text{Crude fibre (\%)} = \frac{\text{weight loss on ignition}}{\text{weight of sample}} \times 100 \tag{3}$$

Crude Lipid

Exactly 2g of the dried sample was weighed into a porous thimble, and its mouth covered with cotton. The thimble was then placed in an extraction chamber, and then suspended above a receiving flask containing petroleum ether (BP. 40 – 60ºC). The flask was then heated on hot mantle and the oil was extracted. The extraction continued for eight hours after which the thimble were removed from the Soxhlet and heated over water bath, the flask containing the oil was disconnected, cleaned up and placed in an oven at 100ºC for 30 minutes. The flask was then cooled in desiccator and weighed. The percentage crude lipid content was calculated using [10, 11].

$$\text{Crude fat (\%)} = \frac{\text{weight of oil extractd}}{\text{weight of sample}} \times 100 \tag{4}$$

Crude protein

5g of the sample was weighed in to a Kjeldahl digestion flask and catalyst Na_2SO_4, $CuSO_4$ and selenium Oxide in (10:5:1) were added to the sample which were followed by 10 cm^3 of concentrated H_2SO_4. The content in the flask was then heated in the Kjeldahl digestion flask for one and half hour, ensuring that digestion was completed. The flask was cooled and the content diluted with 10mL distilled water. The diluted content was filtered in to 100mL volumetric flask and was made up to the mark with distilled water. 10 cm^3 of the aliquot was taken into digestion flask and 20cm^3 of 45% NaOH solution were added to it. The content was diluted to about 200 cm^3 with distilled water and distilled using micro Kjeldahl distilled apparatus. The distillate was received into a flask containing 10 cm^3 boric acid solution indicators after the distillation.

The distillate was then titrated against 0.01M HCl to the end point [11].

Crude protein (%) $= \frac{TV \times C \times F \times V1}{W \times V2} \times 100$ (5)

Where: TV = Titre Value of the Acid; C = Concentration of Acid used; V1 = Volume of the distilled water used for diluting the digest; V2 = Volume of aliquot used for titration;

W = weight of Sample used; F = protein Multiplication Factor 6.25

Carbohydrate

The total amount of carbohydrate in the sample was obtained by using the weight difference percentage. This was done by subtracting the percentage sum of the food nutrients (% crude protein, % crude fat, % crude fibre, % moisture content and % ash) from 100% dry weight. Percentage carbohydrate was calculated, using the formula below [11].

Carbohydrate (%) = 100 – (Protein + Fat + Fibre + Ash + Moisture).   (6)

Determination of Anti-nutritional Factors
Determination of Tannins

Exactly 1g of the sample was weighed and transferred into a bottle. 10mL of distilled water was and added and stirred at 5 minute interval for 30 minutes then filtered. A total volume of 2.5mL of the filtrate, (sample) standard tannic acid solution and distilled water was added into test tubes, labelled sample standard and blank respectively. 1.0 of Folin-Denis reagent was added to all the test tubes followed by 2.5mL of saturated sodium bicarbonate solution was then added and allowed to incubate at room temperature for 90 minutes. The absorbance of the sample and the standard was read against the blank at 490nm [11].

The percentage of tannin will be calculated thus:

Tannin (%) $= \frac{AT \times 100 \times Vf}{AS \times W \times Va} \times C$ (7)

Where: AT = Absorbance of the test sample

AS = Absorbance of the standard solution

C = Concentration of standard solution

W = Weight of the sample used

Vf = Total volume of the extract

Va = Volume of the extract analyzed

Determination of Alkaloids

About 5 g of the sample was weighed and added into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered with aluminum foil and allowed to stand for 4 hours. After which the solution will be filtered with whatman filter paper. 12 mL of ammonium hydroxide solution will be added to the filtrate and allowed to cool. The precipitate will be dried in the oven at 60ºC and reweighed to determine the weight of the alkaloid.

Alkaloid (%) $= \frac{(\text{Weight of filter paper} + \text{alkaloid}) - (\text{weight of filter paper})}{\text{Weight of sample used}} \times 100$

(8)

Determination of Cyanogenic Glycosides

The sample (1g) was soaked in 10 mL of 70% alcohol for 2 hours and then filtered. The extract obtained will then be purified using lead acetate and Na_2HPO_4 solution. Freshly prepared Buljet's reagent (containing 95 mL Aqueous picric acid and 5 mL 10% aqueous NaOH). The difference between the intensity of colours of the experimental and blank samples (distilled water and Buljet's reagent) gives the absorbance and is proportional to the concentration of the glycosides [12]

Determination of Phytate

The method used was that of [13] with slight modification. 0.2 g of the samples was weighed into 250 mL conical flask. This was then soaked in 100 mL of 20 % HCl for 3 hours, the samples were then filtered and 50 mL of the

filtrates was placed in a 250 mL beaker and 100 mL distilled added to the samples. 10 mL of 0.3 % ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195 g iron per 1 mL.

It was then calculated as;

$$\textbf{phytic acid} = \frac{\textbf{titre value} \times \textbf{0.001} \times \textbf{1.19} \times \textbf{1}}{\textbf{2}} \tag{9}$$

Determination of oxalate

2g of the sample was weighed and extracted twice at 50 °C, stirred for 1hr with 20mL of 0.3M HCl. The combined extract was diluted to 100mL with distilled water and used for total oxalate estimation. The oxalate was estimated by pipetting about 5mL of the extract which was made alkaline with 1mL of 5M ammonium hydroxide. About 2 drops of phenolphthalein were added to the extract and acetic acid was added in drops. 1 mL of 5% aqueous Calcium chloride was then added to the mixture and allow to stand for 2hrs after and was centrifuged at 3000rpm for 15 min. The supernatants were discarded and the precipitate was washed three times with hot water, thoroughly mixed. About 2mL of 3M sulphuric acid was added and the precipitate was dissolved by warming in bath at 75° c. The content of the test tube was then titrated with freshly prepared 0.01M potassium per manganese at room temperature until the first pink color appeared. This was then warned at 75 °C and titrated until pink color persisted [13]

% Oxalate = Vt/Ws × Vme × Time

Where Vt = Total Volume of titrate

Ws = Wight of the sample

Vme = Vol – Mass equivalent (1 cm^3 of 0.005M $KMnO_4$ is equivalent to 0.00225g anhydrous oxalic acid)

Results and Discussion

Table 1: Nutritional content of raw and boiled seeds of *S. stenocarpa*

Parameters (%)	Concentration Raw Seeds	Concentration Boiled Seeds
Moisture	5.66±0.12	8.03±0.10
Ash	3.32±0.20	2.13±0.14
Crude lipid	4.28±0.09	2.38±0.03
Crude protein	24.90±0.05	15.65±0.03
Crude fibre	8.35±0.32	5.72±0.44
Total carbohydrate	45.10±0.45	54.09±0.47

Values are mean duplicate of ± S.D determination

Table 2: Anti-nutritional content of raw and boiled seeds of *S. stenocarpa*

Parameters (%)	Concentration Raw Seeds	Concentration Boiled Seeds
Phytate	0.28±0.01	8.03±0.10
Oxalate	0.06±0.00	2.13±0.14
Cyanogenic glycoside	10.21±0.30	8.50±0.36
Alkaloid	1.70±0.02	0.33±0.04
Tannin	1.06±0.09	0.28±0.05

Values are mean duplicate of ± S.D determination

Table 3: Qualitative anti-nutrient screening of raw and boiled seeds of *S. stenocarpa*

Parameters	Amount
Phytate	+
Oxalate	+
Cyanogenic glycoside	++
Alkaloid	+
Tannin	+

(+): Trace Amount Present, (+) (+) : Abundant Amount Present, (-) : NO Amount Present

Discussion

Moisture content

The mean value of the moisture content obtained from this study were 5.66 % for raw and 8.03 % for boiled as reported in Table 1. These values are higher than those obtained [14, 15] which were 2.98 % for raw and 4.16 % for boiled. These show a significant difference between the samples under study with respect to moisture. It has been reported that high moisture content leads to difficulty in storage and hence spoilage. The significant difference between these two samples could be accustomed to the drying time of the samples before usage for analysis [15].

Ash Content

The mean values of ash for the present work ranges from 3.32 % and 2.13 % for raw and boiled samples, the results of this study are similar to those obtained by [16,17] with values 3.20 % for raw and 2.13 % for boiled samples. These results are different from those obtained by [18, 19] which are 1.17 % and 1.18 % respectively. The difference could be attributed to the method of processing the seeds and the drying time.

Crude Protein

The values of protein obtained from this study are 24.90 % and 15.65 % as shown in table 1 for the raw and boiled samples. This shows a high protein content in the raw sample. The protein content obtained from this study was higher than those reported by [20] with values 11.06 % for the raw and 5.71% for the boiled. The result obtained was in consonant with [21] with a value of 23.80 % and 13.52% for raw and boiled samples. Studies have shown that boiling denatures proteins. From literature it has been reviewed those plants with a protein content of greater than 12 % are considered a good protein source [21]. Hence, the seeds of *S. stenocarpa* studied are then a good source of protein if consumed in the right proportions [22].

Crude Lipid

The ranges of values obtained for Lipid in this study are 3.28 % and 2.38 % for raw and boiled samples. This trend shows a variation in the results obtained by [23] in his study of the Proximate Analysis, Phytochemical and Mineral Composition of Boiled and raw seeds of *Cajanus cajan* which shows the results at 1.23 % for raw and 0.86 % for boiled respectively [24]. The result obtained from this study shows that the lipid is higher than those obtained by[25,26] which are 0.01 % and 1.49% This result is lower than that obtained by [25] with 10.73 %. The lower lipid content of African yam bean allows it better storage stability while avoiding rancidity and it's also an ideal food for weight control, reducing the risk of overweight.

Crude Fibre

The value of crude fibre obtained from this study range from 8.35 % for the raw and 5.72 % for the boiled. The results obtained from this study work were lower than those obtained by [26, 27] reported higher values at 15.35 % and 18.25 % respectively. The presence of crude fibre in food or plant is an indication of the level of non-digestible carbohydrate and lignin [27].

 Total Carbohydrate

The carbohydrate values of the raw and boiled sample of *S. stenocarpa* obtained from this study were 45.10% and 54.09% as reported in table 1. These results show that our samples are rich in carbohydrate. The results obtained from this study are lower than those of [28, 29] which reported values of 55.25% for raw and 72.38% for boiled. From the study it can be drawn that carbohydrates are the major macronutrients of *S. stenocarpa* seeds. The protein content of African yam bean is comparable to most leguminous foods such as rice bean, chick pea, cowpea, pigeon pea, and velvet bean [30].

Phytic and Oxalic acids

Legumes have been reported to possess anti-nutritional factors which affect their nutritional quality. ANFs are capable of changing the taste, protein digestibility and bioavailability of nutrients [31]. They can be toxic and may

negatively affect the nutrient value of seeds by impairing protein digestibility and mineral availability. However, they are heat-labile and hence may be inactivated by processing methods involving heat generation. On the other hand, a number of the compounds, such as phytic acid, phenols, and tannins, usually considered anti-nutritional compounds, are currently considered potential antioxidants containing health-promoting effects. For example, phytic and oxalic acids has now been revealed to have rich antioxidant, hypoglycaemic activities and also to possess anti-carcinogenic properties. Therefore, elimination of these compounds depends upon the consumer's preferences [32]. The results for ANFs in processed samples observed in the current study were similar to [32] where there was a significant reduction in phytate, contents following cooking. The values for the present study for phytic acid were 0.28% and 0.14% for the raw and boiled samples and 0.06% and 0.07% for oxalic acids. Phytates are responsible for the inhibition of absorption of minerals such as iron, zinc and calcium to the body compounds which are not voluntarily absorbed from the intestine, thus interfering with the bioavailability of these essential minerals as well as inhibiting enzymatic digestion of both starch and proteins [32]

Cyanogenic Derivatives

Hydrogen cyanide is of high detriment for human consumption. Small amount of it in the human body can cause harm to one's health. Table 2 of this study showed the value of the cyanogenic derivative at 10.21% and 8.50%.This result is in agreement reported on previous work on the nutritional and anti-nutritional composition of *Vigna unguiculata* [33]. It can be deduced from this study that the cyanide concentration reduces on boiling, this is because during boiling, disruption of tissue and parenchyma occur and this facilitates the release of the free cyanide into the boiling water and also the enzyme, beta glucosidase is destroyed. Also, evaporation of free cyanide is volatile to heat. It is also worthy of note that the disruption of tissue and parenchyma during boiling leads to softening of the shoots which makes it palatable for consumption [33, 34].

Alkaloids

Alkaloids are the most efficient therapeutically significant plant substances. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and anti-bacterial properties [35]. The value obtained from this study show the mean value of the raw and boiled seeds at 1.70% and 0.33% respectively. These values were closely related to that of [35]) in his study of the phytochemical constituents and nutrient evaluation of *Phaseolus vulgaris.* These values are however lower than those reported from previous work [35].

Tannin

The range of values for tannins obtained from this study for raw and boiled samples are 1.06% and 0.28%. The values obtained show a minor difference between the raw and boiled sample of the African yam bean seeds. The values obtained from this research are lower than those of [35]. The presence of tannin in the plant indicates the astringent properties of African yam bean seeds and in addition, aid in the healing of wounds and burns [35].

Conclusion

The results of the present study showed that the African yam bean seeds have considerable good nutritional profiles with high levels of protein, carbohydrate, and other nutrients comparable with those of other common legumes. Seed processing by drying and boiling reduced the level of anti-nutrients with low effect on nutritional quality. *Sphenostylis stenocarpa* has great potential for combating protein energy malnutrition. Renewed efforts should be made towards increased cultivation by farmers, with a briefing on utilization such as a food nutrient for humans as well as livestock.

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