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Proximate Composition and Amino Acids Contents in Selected Legumes and Hura crepitans seed in Wukari Taraba State

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***Abstract*:** The study evaluated the proximate composition and amino acid contents of selected legumes and Hura crepitans seed in Wukari,Taraba State, Nigeria. The Sand box seeds were collected from its tree in Wukari town, while black eyed pea and red kidney beans were purchased from Wukari market. The samples were processed into powder for proximate composition and amino profile. In all the samples, 18 amino acids were determined with higher values for aspartic acid, glutamic acid, leucine and phenylalanine while cysteine (0.43-0.79g/ 100g) had the lowest value in the samples analysed. Levels of amino acid ranged as: Sandbox seed had 0.79 ± 0.02 to 10.44 ± 0.02 g/100g, Black eyed pea seed had 0.51 ± 0.12 to 8.53 ± 0.03 g/100g, red kidney bean had 0.43 ± 0.02 to 7.93 ± 0.02 g/100g. The sand box seed had the highest protein content (33.71± 0.02 %), followed by red kidney bean (29.21 ± 0.02%). Nitrogen free extract had the highest value in black eyed pea (63.29±0.01%). The sand box seed had the highest crude fat (7.27 ± 0.02%). All the three crop samples had low safe moisture levels for storage. The study revealed the variation in the nutrients composition of the crop samples. However, sand box seeds had the highest protein content, coupled with high levels of amino acids detected, but the seed is yet to be consumed by humans. Hence, the need for further research on Hura crepitans with the view of reducing the deleterious effects of its antinutrients.

Keyword: Legumes, Proximate composition, Amino acids, High Performance Liquid Chromatography, Energy value

Introduction

Legumes refer to the seeds of *Leguminosae* including peas, beans and pulses. Legumes are considered as "poor man's meat" due to their high protein content and low cost compared to meat and meat products [1]. In most developing countries of the world, including Nigeria where diets are composed mainly of one plant staple food, the occurrence of protein-energy malnutrition especially among children is common[3]. As the cost of producing meat, milk, egg and fish which are foods of high biological values increase, plant proteins offer ready and affordable solution to the problem of a growing protein gap. Low cost, protein-rich and high energy food formulation based on cereal legume mixtures have been suggested [2, 3, 4]. The enrichment or fortification of traditional cereal based diets with other protein sources such as oilseeds and legumes has received considerable attention. This is because oilseeds and legumes proteins are rich in lysine, but deficient in sulphur containing amino acids [5]. Legumes generally contain relatively high amount of protein than other plant food stuffs. Cereals have low protein content and are in general deficient in lysine but are adequate in sulphur containing amino acids. Legume proteins are mainly used in food formulations to complement the protein in cereal grains because of their chemical and nutritional characteristics [6].The problem associated with meat consumption as source of protein has led to renewed interest in vegetation diet [7]. This phenomenon is reinforced by the fact that physicians have pointed out that consumers who eat too many animal products (rich saturated fat) and low amount of plant foods could lead to increase in the risk of cardiovascular diseases and some types of cancer [8]. To this end, intensive efforts were made to find alternative sources of protein from the underutilised leguminous plant in nutrition and in the formulation of new food products [8]. Kidney bean (*Phaseolus vulgaris*) , a grain legume, is one of the neglected tropical legumes that can be used to fortify cereal-based diets especially in developing countries, because of its high protein content [9]. It is also a rich source of vitamins, minerals and relatively high in crude fibre [10]. Kidney bean is one such protein source, which when used in the fortification or enrichment of cereal-based diets could go a long way in improving their nutritional status. This crop is among lesser–known beans grown in Nigeria especially by the people of Bokkos in Plateau State. Sandbox (*Hura crepitans*) seed is an evergreen tree of *Euphorbiaceae* family, that despite its abundance in Nigeria it is still underutilized. It is recognized by many dark pointed spines and smooth brown bark. These spines have caused it to be called "monkey no climb'' [11]. The bark of sandbox tree is used in herbal medicine for human and veterinary. Black eyed cowpea (*Vigna unguiculata*) is popularly known as Southern pea, China pea, black-eye bean or pea, cowgram in the United States or Nìébé in French speaking Africa [12]. Cowpea is well known for its good source of dietary protein for human consumption and of animal feed in the tropics, especially in Africa, Brazil and India. It thrives well in hotter more arid climates and more infertile soils than other food legume crops due to its symbiotic nitrogen fixing abilities which helps in maintaining soil fertility in peasant cropping systems. Over 65% of the cowpea crop is produced in Africa where Nigeria and Niger produce 50% of the world supply [3].

The cultivation and consumption of different varieties of leguminous seeds in Wukari, Taraba State and Nigeria as a whole have increased over time. Different fertililizers, soil types, texture and other environmental factors can affect yields as well as bioavailability of nutrients in legumes and other crops. There is dearth of information on the nutritional profile of these three crops produced in Wukari, Taraba state. Hence this research was embarked on to furnish this information.

Materials and Methods

Location of study

The study was conducted at Federal University Wukari, Taraba State, which is located in the North eastern region of Nigeria.

Collection of samples

Black eyed pea and red kidney beans were purchased from Wukari market, in Wukari Local Government Area of Taraba State, Nigeria. The sandbox seed were picked from the sandbox tree at Anguwan Marmara in Wukari, Taraba state.

Preparation of samples

All foreign particles from the black eyed pea and kidney beans were removed by hand picking. The sandbox seeds enclosed in hard shells were then soaked in water for about 24h for easy decortication. The seeds were then sun-dried and ground into fine powder prior to analysis.

Determination of proximate composition of samples

The proximate composition of the three crop samples **(**black eyed pea, kidney beans and sand box) was determined using the standard methods [13]. Nitrogen free extract (soluble carbohydrates) was determined by difference.

100- sum of the content of crude protein, crude fibre, ether extract and ash

Determination of gross energy content of samples

The energy content of the sample was calculated by multiplying the protein content by four, carbohydrate content by four and fat by nine [14] as:

Energy Value = (% crude protein X 4) + (% crude fat X 9) + (%carbohydrate X 4). Values used in this formula are based on dry matter basis which was determined by subtracting moisture content from 100.

Determination of amino acid profile

Sample preparation

Two (2g) of the powdered sample was weighed and transferred into a beaker. The beaker was heated in a water bath at 40^{o}C to melt the sample. To the sample was added 6M HCl and heated at 110 ^{o}C for hydrolysis of protein. Then, the digested sample was transferred into 50ml standard volumetric flask and the volume was made up by adding distilled water to the 50ml mark. The extract was centrifuged, filtered and 30ml of water was added to the residue, which was then sonicated for 30minutes. The solution was then passed through a 0.22µm Millipore membrane and the filtrate was transferred to a 100mL volumetric flask and diluted with water to make up to volume. This solution was passed through a 0.22µm Millipore membrane and the filtrate was used as follows:

Derivatization of Samples

The mixed amino acid solution or filtered sample (10µL) was transferred to a full recovery sampler to which 70µL sodium hexane sulphonic acid buffers was added. The solution was vortexed briefly and then 20µL of reconstituted buffer, and the solution was mixed by vortexing for several seconds. It was then heated on a heating block at 55ºC for 10 minutes. Derivatives were stable at room temperature for up to one week.

Preparation of standard amino acid solution 6

Standard solutions of the amino acids (all essential and the entire non-essential) were prepared. The solution were prepared by adding 0.1M HCl. The concentrations of the standard solutions were serially diluted to give 25, 20, 15, and 10nM each. They were stored in a thermocool freezer at 4ºC till used. The mixed standard solution contained 25pmol of each amino acid derivative.

Chromatographic conditions and Procedure

Chromatographic separation of prepared samples was carried out on a Buck scientific BLC10/11-model of HPLC equipped with UV 338nm detector, AC18, 2.5 X 200nm, 5um column and a mobile phase of 1:2:2 (100mM sodium phosphate, pH 7.2: Acetonitrile: Methanol v/v/v) at a flow rate of 0.45 mL / minute and an operating temperature of 40ºC. Standard solutions were analysed in a similar manner. In terms of retention time, the composition of each peak was confirmed and using peak area of each amino acid, the concentrations were determined in accordance with both the external standard (by extrapolating from the calibration curve, prepared by plotting a graph of peak area versus concentration of each amino acid and standard solution.

Results

Table 1: Proximate Composition of black eyed pea, red kidney beans and sand box

Parameters (%)	Crude protein	Ether extract	Crude fibre	Ash	Moisture	Nitrogen free extract
Sandbox *(Hura crepitans*) seed	33.71±0.02^{a}	7.27±0.02^{a}	0.95±0.01^{a}	8.22±0.00^{a}	12.40±0.00^{a}	49.85±0.02^{c}
Black eyed pea *(Vigna unguiculata)*	27.21±0.02^{c}	3.27±0.02^{b}	2.04±0.01^{b}	4.19±0.01^{b}	9.25±0.00^{b}	63.29±0.01^{a}
Red kidney bean *(Phaseolus lunatus)*	29.21±0.03^{b}	2.68±0.02^{c}	2.13±0.01^{a}	3.98±0.06^{c}	9.10±0.00^{c}	62.00±0.03^{b}

Data are presented as mean ± standard deviation of three replicates. Means within a column with the same superscript were not significantly different (p>0.05).

Table 2: Amino Acids Profile of sandbox seed, black eyed pea and red kidney bean

Amino acid(g/100g protein)	**Sandbox *(Hura crepitans*) seed**	**Black eyed pea*(Vigna unguiculata)***	**Red kidney bean*(Phaseolus lunatus)***
Lysine	3.63±0.03[a]	3.41±0.01[b]	3.25±0.03[c]
Methionine	0.87±0.01[b]	0.91±0.02[a]	0.81±0.01[c]
Threonine	2.10±0.02[a]	1.55±0.15[b]	1.95±0.02[a]
Isoleucine	2.75±0.02[c]	3.11±0.01[a]	2.86±0.02[b]
Leucine	8.35±0.03[a]	7.62±0.01[b]	6.77±0.02[c]
Phenylalanine	9.33±0.03[a]	4.02±0.02[b]	3.83±0.02[c]
Valine	5.34±0.02[a]	4.11±0.12[b]	3.97±0.02[b]
Tryptophan	4.01±0.02[a]	3.00±0.02[b]	2.96±0.02[c]
Histidine	2.22±0.02[a]	1.92±0.02[b]	1.84±0.02[c]
Arginine	5.31±0.02[a]	4.00±0.01[b]	3.82±0.02[c]
Serine	3.62±0.02[a]	2.93±0.04[b]	2.73±0.02[c]
Cysteine	0.79±0.02[a]	0.51±0.12[b]	0.43±0.02[c]
Tyrosine	3.56±0.01[a]	3.21±0.12[b]	3.31±0.02[b]
Alanine	3.83±0.02[a]	3.54±0.01[b]	3.34±0.02[c]
Aspartic acid	10.44±0.02[a]	8.06±0.03[b]	7.53±0.02[c]
Glutamic acid	9.04±0.01[a]	8.53±0.03[b]	7.93±0.02[c]
Glycine	4.00±0.00[a]	3.87±0.02[b]	3.44±0.03[c]
Proline	3.04±0.02[a]	2.11±0.03[b]	2.03±0.02[c]

Data are presented as mean ± standard deviation of three replicates. Means within the same row carrying different superscripts are significantly different at ($p<0.05$).

Table 3: The energy content of black eyed pea, red kidney beans and sand box.

	Gross energy	
Samples	**Kcal/100g**	**KJ/100g**
Sandbox *(Hura crepitans*) seed	399.73±0.02[a]	1672.47±0.02[a]
Black eyed pea *(Vigna unguiculata)*	392.21±0.02[b]	1641.01±0.02[b]
Red Kidney bean*(Phaseolus lunatus)*	389.96±0.03[c]	1631.59±0.03[c]

Data are presented as the mean ± standard deviation of three replicates. Means within the same column carrying different superscripts are significantly different ($p<0.05$).

Table 1 shows the mean proximate composition of the crops analysed. The crude protein values of the crop samples were significantly different ($p<0.05$) among samples with the highest value observed in sandbox seed with 33.71 ± 0.02%, while the least was gotten for red kidney bean as 29.21 ± 0.03%. Also, it was revealed that there were significant differences ($p<0.05$) in the values of ether extract determined. Other proximate parameters such as crude fibre, ash content, moisture content and nitrogen free extract (NFE)

estimated show significant differences among samples with sand box seeds having the highest values of ash and moisture content. However, the highest content of crude fibre was observed in red kidney bean (2.13 ± 0.01%), while the highest value of NFE of 63.29 ± 0.01was revealed in black eyed pea.

Table 2 shows the amino acid profile of three crop samples analysed in this study. The following essential amino acids were detected in these samples: lysine, methionine, threonine, isoleucine, leucine, phenylalanine, valine, tryptophan, histidine and arginine. The remaining eight amino acids in Table 2 not mentioned here were the non-essential amino acids. Variation was observed in the amino acid profile of these samples with sand box seeds having the highest values in the following amino acids: lysine, threonine, leucine, phenylalanine, valine, tryptophan, histidine, arginine, serine, cysteine, tyrosine, alanine, aspartic acid, glutamic acid, glycine and proline.

Table 3 shows the energy content of the crop samples analysed. The variation observed showed significant differences ($p< 0.05$) among samples studied with the highest values of energy content observed in sand box seeds with 399.73 ± 0.02 kcal/ 100g followed by black eyed pea which had value 392.21 ± 0.02 kcal/100g, while the least energy content was found in red kidney bean as 389.96 ± 0.03 kcal/ 100g.

Discussion

Proximate Composition

The proximate composition of the three crops are shown in Table1. The sandbox seed contained the highest protein value (33.71%) while the black eyed pea had the least protein content. The value obtained in this study is higher than 25.16±0.22% for sandbox seed reported by NRC [15]. The amount of protein in the samples make them nutritionally rich and can contribute to the daily protein need of 33.6g for human adults [16]. The high crude protein content of *Hura crepitans (sand box)* seed suggests that it is a good source of protein which can be useful in building up and repair of worn out tissues.

The crude fibre contents of the three legumes were 0.95%, 2.04% and 2.13% for the sandbox seed, black eyed pea and red kidney bean respectively. These fibre contents were lower than 5.30% reported by [17] for donkey-eye bean or ox-eye beans (*Mucuna Sloanei).* The low crude fibre contents of the seeds, in addition to their high protein contents would make them suitable for inclusion in the diets of non-ruminants such fish and poultry where low fibre content is desirable.

The crude fat (ether extract) contents of the samples were within the range of 2.68 -7.27%. The crude fat value of *Hura crepitans* was lower than the value reported by [18,19] and also lower than the values reported for oil seeds which generally have crude fat content that ranged from 18% in soya bean to 43% in groundnut oil.

The low moisture contents of the samples show that the legumes were properly dried to safe level for storage. For the black eyed pea (9.25%), and red kidney bean (9.10%), their low moisture contents would confer longer shelf-life compared to sandbox seeds that contained 12.40% moisture. The low

moisture content of the sand box sample in this study is similar to what was reported for sandbox seed [20].

The ash contents of the crop samples ranged between 3.98 and 8.22%. The ash contents of the seeds were higher than that of water melon (*Citrullus lanatus)* (Elegbede, 1998), The ash contents of the black eyed pea and kidney bean were within the range of 3.0-4.8% in the present study, which is higher than the ash contents of grain cereals which ranged from 0.56 % for rice to 1.67 % for millet [21].

The NFE content of the crop samples ranged from 49.85 to 63.29%. The NFE contents of the black eyed pea and red kidney bean were higher than those of three varieties of beans reported by [22]. However, the sandbox seed had the least NFE content of 49.85 ± 0.02 among the crops analysed in this study.

Energy Value

Among the three crop samples evaluated in the present study, the sandbox seed (399.73KCal/100g) had the highest energy value, followed by the black eyed pea (392.21Kcal/100g) and then the red kidney bean (389.96Kcal/100g). The high energy content may be attributed to carbohydrate and ether extract content in the crops. However, the values were lower than the values reported for some leguminous oilseeds [23].

Amino Acid Profile

The amino acid profiles of the three crops are presented in Table 2. In all the amino acids analyzed, higher values were recorded for phenylalanine, glutamic acid and leucine while aspartic acid had the highest value as observed for sand box seeds, cysteine had the lowest value in all the three crops analysed. The following ranges were obtained for the levels of amino acids of the samples: sandbox seed had 0.79 ± 0.02 to 10.44 ± 0.02 g/100g, black eyed pea seed had 0.51 ± 0.12 to 8.53 ± 0.03 g/100g, red kidney bean had 0.43 ± 0.02 to 7.93 ± 0.02 g/100g. The amounts of cysteine and methionine were low in all the seeds ranging from 0.81-0.91 g/100g for methionine and 0.43-0.79 g/100g for cysteine g/100g. [24] showed that the proteins of alfalfa seed contained more arginine, cysteine, glutamic acid, and histidine, and less alanine, aspartic acid, and tryptophan whereas higher values were obtained in the present study for aspartic acid, phenylalanine and leucine. This was similar to earlier research work carried out by [25] who showed that leucaena seed contained high amount of crude protein (30.8%) and was rich in aspartic acid and glutamic acid but low in methionine.

Conclusion

This research revealed that black eyed pea (Vigna unguiculata), red kidney beans (Phaseolus lunatus) and sand box (Hura crepitans) seed contained high protein contents with appreciable amount of energy and other nutrients. All the crop samples contained varying amounts of all essential amino acids. Based on the results of this study, the legumes (black eyed pea and red kidney beans) could be used in the formulation of infant diets for reducing Protein Energy Malnutrition (PEM) in Taraba State and Nigeria. However, more work still needs to be carried out on sandbox seeds in the areas of toxicological study

and antinutritional factors, before it can justify its consumption by humans.

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