EVALUATION OF CHEMICAL COMPOSITION OF *Acacia niloticaSEEDS*

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Abstract: *Acacia nilotica* seeds purchased from local markets in Minna, Nigeria were analyzed for chemical composition, anti-nutrient contents and amino acid profile. The seed flour contained 6.67% moisture content, 2.80% ash, 23.33% crude fat, 6.53% crude fibre, 30.95% crude protein and 29.72% carbohydrate. The calorific value of the flour was 452.65 Kcal/100g. The saponins, tannins, flavonoids, alkaloids, oxalates and cyanogenic glycosides contents were 2.40, 0.11, 6.20, 8.70 and 0.22% as well as 0.23 mg/100g. The bulk density, pH, emulsification capacity, gelation capacity, absorption capacity, oil absorption capacity, foam stability, wettability and viscosity were 0.62 g/cm^3, 5.30, 32.22 cm^3, 2.15 %, 2.30 g, 1.06 g, 1.08 sec, 21.25 sec and 6.00 mPa.sec, respectively. This seeds flour was good sources of essential minerals such as K, Mg, Ca, P and Na with the most abundant of these being potassium (1168±62.36 mg/100g). The amino acids profile of the seed showed that essential amino acids such as arginine, leucine and phenylalanine were 11.22, 6.23 and 4.7 g/100g, respectively. The results of this study, suggest that the seeds of *Acacia nilotica* seed has the potential of being exploited as a source of oil and protein for food formulations.

Keywords: *Acacia nilotica* seed, amino acid, anti-nutritional factor, essential minerals

Introduction

Wetland Food is mainly eaten in order to get adequate nutrients, for the normal functioning of the body which includes healthy growth and living (Syazzie*et al.,* 1994). In local foodstuffs, the supply of nutrients such as protein, carbohydrate, fats, water, minerals and vitamins is in different amounts. In general, wild seeds and fruits are consumed by both rural and urban dwellers, chiefly among low income earners to fill the gaps of malnutrition (Fagbemi*et al.,* 1991). Some of these wild seeds have greater nutritional values in comparison with the levels found in some cultivated foods. Although, some of them have some anti-nutritional factors that affect the availability of other nutrients needed necessary for the body.

In the past, man has used some 3,000 plant species for foods, but only few of them have been commercially cultivated to some extent. This has led to the underutilization of a large percentage of this vast natural resource (NAS, 1984). In advanced countries, wild plants are in use as sources of food and other life supporting commodities which offer sufficient levels of nutrition to human beings and their animals (AberoumandandDeokule, 2010). These wild plants offer essential constituents to human diet by providing the body with minerals, vitamins and contain hormone precursors as well as protein and energy (Akubugwo*et al.,* 2007).

Acacia nilotica L. wild (Mimosacae) is an essential plant (Kaur*et al.,* 2005). *Acacia nilotica* (English name: Gum arabic, Hausa: Bagaruwa and Nupe: Gabaruwa). This plant is a member of the family Fabaceae-mimosaceae high with a thick spherical crown. Its branches are usually sinister and black coloured, with grey pinkish slash and fissured bark which exude a reddish low quality gum (Mann *et al.,* 2003). It is an ever green tree, growing up to 10 meters in height. It is thus a small tree with dark brown or black longitudinally fissured bark or crown with slender and pubescent when young (KritikarandBasu, 2003). It is known as babul in the dried parts of India. In this region, it is the most important tree where almost all its parts are used in medication including root, bark, flower, gum and pods (Said, 1997). *Acacia* species contains secondary metabolites which include alkaloids, cyanogenic glycosides, diterpenes, phytosterol and triterpenegenins and saponins, flavonoids and tannins (Seigler, 2003). The plant contains amino acid such as cystine, methionine, threonine, lysine and tryptophan and also serves as sources of macro elements like potassium, phosphorus, magnesium, iron and manganese (Singh *et al.,* 2008). *A. nilotica* is an essential multipurpose plant that has been used broadly for the treatment of various diseases such as in the treatment of an earache, dysentery, diabetes and diarrhea, and as a tonic. The pods are used for treating impotency, primogenital disorder and dry cough. The seeds and leaves extract are used for general body vigor for ethno-medicinal applications of *Acacia nilotica*. This study investigated chemical composition of the *A. nilotica* seeds for possible utilization of the seeds by human and animals.

Materials and Methods

Sample collection and preparation

The matured dried pods of *Acacia nilotica* fruits were purchased from the local markets in Minna, Nigeria. The seeds were separated from the pods and the seeds were soaked in water for 24 h to remove the epicarp. The hydrated seeds were then sun dried for one week. The dried seeds were milled in attrition mill, sieved through 200 µm wire mesh, packed in a plastic container which was sealed with aluminum foil and stored at ambient temperature prior to analysis.

Proximate analyses

The moisture, ash, fat and protein contents of the *Acacia nilotica* seeds flour were carried out as described by the Association of Official Analytical Chemists methods (AOAC, 2006). Total carbohydrate content was determined by subtracting percentage moisture, ash crude fiber, protein, and fat from 100%. The energy value (kcal/100g) was estimated by multiplying the percentage of crude protein, crude lipid and carbohydrate by 4, 9 and 4 respectively as conversion factors.

Mineral analyses

The sample was digested by weighing 1.0 g into a beaker and 10 cm^3 of the acid mixture ($HC10_4$:H_2SO_4:$HN0_3$) in the ratio of 1:4:3 was added to the sample in the beaker. The mixture was swirled and left in a fume cupboard overnight. The sample was then digested on a Kjedhal digestion block until the solution became quite clear. The digest was allowed to cool, diluted with 20 cm^3 of water, filtered using Whatman filter papers, made up to mark with deionized water in 100 cm^3 volumetric flasks and then transferred into sample bottles. The sample was analyzed for their mineral contents of interest using atomic absorption spectrophotometer (AAS) Buck model 210 VGP. A flame photometer (Jenway model) was used for the determination of potassium and sodium, while

phosphorus was determined colorimetrically using the vanudo-molybodate colorimetric method.

Functional properties

The functional properties investigated in this study were pH, water absorption capacity, bulk density, foam stability, oil absorption capacity, wettability, gelation capacity, gelatinization temperature and viscosity. They were all determined using the standard methods described by AOAC (2006).

Determination of amino acid profile

The sample was defatted using chloroform/methanol mixture at 2:1 proportion. About 4.0 g of the sample was kept in extraction thimble and extracted for 15 h in Soxhlet extractor (Niemane*t al.*, 1992).

Nitrogen determination

In each case, 200 mg of the powdered sample was weighed, wrapped in Whatman filter paper (No.1) and kept in the Kjeldhal flask. 0.5 g of sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) at 10:5:1 ratio was added into the digestion flask. 10 cm^3 of concentrated sulphuric acid and anti-bumping agents were also added to the mixture. The distillate was then titrated with standardizing 0.01 $moldm^{-3}$ hydrochloric acid.

$$\text{Percentage Nitrogen} = \frac{(a-b)\times 0.01\times 14\times V\times 100}{W\times C} \quad (1)$$

Where: a = titre value of the digested sample; b = titre value of the blank sample; V = volume after dilution (100 cm^3); W = weight of dried sample (mg); C = aliquot of the sample used (10 cm^3)

2.0 g of the dried sample was weighed into extraction thimble and defatted with 2:1 chloroform and methanol mixture using Soxhlet extractor. From the defatted sample, 1.0 g was weighed into glass ampoule and 7 $cm^3$of 6.0 $moldm^{-3}$HCl was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was sealed and placed in an oven preset at 105±5^{0}C for 22 h. The ampoule was allowed to cool before breaking open at the tip and the content filtered. The filtrate was then evaporated to dryness at 40^{o}C and the residue was dissolved in 5 cm^3 acetate buffer (pH 2.0) and stored in plastic specimen bottles. 5-10 µl was dispensed into the cartridge of the sequential multi-sample amino acid analyzer (TSM). The net height of each peak produced by the chart recorder of TSM was measured. The half-height of the peak on the chart was found and the width of the peak on the half height was accurately measured and recorded. Approximately area of each peak was then obtained by multiplying the height by the width at half-height.

The norcleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$\text{NE} = \frac{\text{Area of Norceucine Peak}}{\text{Area of each amino acid}} \quad (2)$$

A constant S was calculated for each amino acid in the standard mixture:

Where S_{std}= NE_{std} x Molecular weight x µMAA_{std}

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the following formula:

Concentration (g/100g protein) = NH x W@NH/2 x S_{std} x C (3)

$$\textbf{where } C = \frac{\text{Dilution factor}\times 16}{\text{Sample weight (g)}\times\%N\times 10\times\text{vol loaded}} \div N_h \times W_{(\text{norleucine})} \quad (4)$$

Where N_h = Net height, W = Width at half height and $W_{(\text{norleucine})}$ = Width of norleucine.

Determination of the predicted protein efficiency ratio

The predicted protein efficiency ratio (P-PER) was determined using the following equation:

$$P - PER = 0.468 + 0.454\ (Leu) - 0.105\ (Tyr) \quad (5)$$

Evaluation of anti-nutritional factors

The alkaloid, tannins, flavonoids, saponins, cyanide and oxalate contents were determined using the methods of Harbone (1998), Makkar and Goodchild (1996), Bohan and Kocipai-Abyazan (1974), Obadani and Ochuko (2001), Onwuka (2005) and Day and Underwood (1986), respectively.

Statistical analysis

All determinations were performed in triplicate. The statistical analyses were conducted using analysis of variance (ANOVA). The mean and standard deviation of each set of values were also calculated.

Results and Discussions

Proximate composition

Table 1 shows the proximate composition of *Acacia nilotica* seeds which showed that the seeds are good sources of protein, lipid, fibre and carbohydrate. The crude fat and crude protein contents were higher than the results reported in *Acacia nilotica* fruit as reported by Bwai*et al.* (2015). The moisture content of the seeds was 6.67±0.12% which was low in comparison with that of *Casiatora*seeds (11.50%) reported by Adamu*et al*. (2013). The low moisture content will give long shelf life to *Acacia nilotica* seed flour. The crude fat was considerably high (23.33±0.58%), a value higher than the 15.48% reported for *Parkiabiglobosa*seeds (Elemo*et al.*, 2011) and 16.01% reported for *Casiatora* seeds by Adamu*et al*. (2013). The high fat content suggests that *A. nilotica*seeds can be classified as an oil-rich seed. The crude protein content of the seed flour was 30.95±0.85%. The was higher than 27.9% crude protein reported for *Parkiabiglobosa seeds* by Elemo*et al.* (2011) and the 21.0 % obtained for *Pigeon pea* (Abdelrahman*et al.*, 2010). The carbohydrate content of *Acacia nilotica* seed flour was 29.72±0.10% which was considerably higher than the 8.2±0.32% reported for *Acacia sieberina* seeds by Abubakar*et al*. (2014). These values make the seeds of *Acacia nilotica* a better source of energy than the most of the report seeds.

Table 1: Proximate composition of *Acacia nilotica* seeds (% dry matter basis) and their calorific value

Parameters	Contents
Moisture	6.67±0.12
Ash	2.80±0.10
Crude fat	23.33±0.58
Crude fibre	6.53±0.15
Crude protein	30.95±0.85
Carbohydrate	29.72±0.10
Calorific value (Kcal/100g)	452.65

Table 2: Some mineral contents of *Acacia nilotica* seeds in mg/100g

Mineral	Concentration
Sodium	958.00±8.60
Potassium	1168.00±62.36
Calcium	809.00±49.27
Magnesium	305.80±81.16
Iron	213.00±9.50
Manganese	50.00±1.00
Copper	30.33±1.53
Zinc	148.00±10.00
Phosphorous	674.00±6.93
Na/K	0.83
Ca/P	1.2

Na/K = Sodium to potassium ratio. Ca/P = Calcium to phosphorus ratio

Mineral composition

Table 2 shows the mineral composition of *Acacia nilotica* seeds. The most abundant minerals in the sample were potassium, sodium, calcium and phosphorus with values of

1168, 958, 809 and 305.8 mg/100g respectively. The major minerals, potassium and sodium among others play key roles in controlling the osmotic and acid base balance of the body fluid (Hamad*et al.*, 2011). The levels of potassium, phosphorus, magnesium, sodium and calcium were different from values reported by Bello and Abdu (2011) for the seeds of the same plant. These differences could be to differences in environmental conditions in which they were grown. The ratio of sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) are shown in Table 2. The Na/K in the body is important for prevention of high blood pressure. Na/K ratio of *Acacia nilotica* was less than one which signifies that the sample would probably be a good source for electrolyte balance and may be used in the treatment of high blood pressure. Similar report was made by Aremu*et al.* (2006). Calcium and phosphorus are of great concern in the formation of strong bones and teeth, growth, normal nerve and muscle action, blood clotting, heart function and cell metabolism (Rolfes*et al.*, 2009). Food is considered good if the Ca/P ratio is above one and poor if the ratio is less than 0.5. The Ca/P ratio in the present study was 1.20, indicating that these seeds could serve as a good source of minerals for bone formation.

Functional properties

Table 3 shows the functional properties of *Acacia nilotica* seed flour. The bulk density (0.62±0.01 g/cm^3), pH (5.30±0.10), water absorption capacity (23.00±0.20 %), oil absorption capacity (10.60±0.08%), foam stability (1.08±0.00 s), emulsification capacity (33.22±0.84%), wettability (21.26±1.03 s), viscosity (6.00±0.01 mPa.s) and foam stability (2.15±0.01%). The bulk density of flour is of importance in food packaging (Snow, 1974). Low bulk density implies that less quantity of the food samples would be packaged in a constant volume, thereby ensuring an economical packaging. However, the packed bulk density of the *A. nilotica*seeds was high which would ensure that more quantities of the food samples can be packed in a packaging material, but less economical to the producer of the food products. The pH of the *A. nilotica*seed flour was slightly acidic which could alter the pH of the system into which it is introduced and when used in food formulation (Appiah*et al.*, 2011). Water absorption capacity is an index of the maximum amount of water that a food product can absorb and retain (Ijarotimi*et al.*, 2012). With respect to water absorption capacity, Giami and Bekeham (1992) reported that the microbial activities of food products with low WAC would be reduced. Hence the shelf-life of such a product would be extended. The foam stability of the seeds is a submission that the seeds would be most useful as foam enhancers in food systems. The water absorption capacity, foam capacity and foam stability of *A. nilotica* seeds obtained in this study were lower than those reported by Adenike and Adekunle (2012) for *Tamarindusindica* pulp and *Ziziphusspina Christi* fruit and seed and Mustapha *et al.* (2015) for undecorticated groundnut seeds. However, the general functional properties of these seeds positioned them as excellent constituents in food formulations.

Table 3: The functional properties of *Acacia nilotica*seed flour

Parameters	Values
Bulk Density (g/cm^3)	0.62±0.01
pH	5.30±0.10
Emulsification Capacity (%)	33.22±0.84
Gelation Capacity (%)	2.15±0.01
Gelatinization Temperature (^{0}C)	60.00±1.00
Water absorption Capacity (%)	23.00±0.20
Oil absorption Capacity (%)	10.60±0.08
Foam Stability (s)	1.08±0.00
Wettability (s)	21.25±1.03
Viscosity (mPa.s)	6.00±0.01

Values are means ± SD of 3 replications

Table 4: The anti-nutrient contents of *Acacia nilotica* seeds

Parameters	Contents
Saponin (%)	2.40±0.20
Tannin (%)	0.11±0.01
Flavonoid (%)	6.20±0.10
Alkaloid (%)	8.70±0.12
Oxalate (%)	0.22±0.01
Cyanide (mg/100g)	0.23±0.01

Anti-nutritional properties

Table 4 shows the anti-nutritional factors present in the seeds of *Acacia nilotica*. The saponins, tannins, flavonoids, alkaloids, oxalates and cyanogenic glycosides were 2.40±0.20 (%), 0.11±0.01 (%), 6.20±0.10 (%), 8.70±0.12 (%), 0.22±0.001 (%) and 0.23±0.01 (mg/100g), respectively. Flavonoid has anti-oxidant effects on human (Eleazu*et al.*, 2011). The availability of flavonoids helps in biological functions such as protection against allergies, inflammation ulcers, hepatoxin and also against carcinogenic diseases. Oxalate, tannins and cyanogenic glycoside also bind to minerals thereby reducing their bioavailability (Adamu*et al.*, 2013). This tends to render calcium unavailable by binding to the calcium to form complexes. The oxalate prevents the absorption of calcium and also precipitates this element around the renal tubules (Nkafamiya and Manji, 2006). The level of oxalate obtained in this study was lower than the value obtained in *Brideliaferruginea* by Jonathan and Funmilola (2014). Tannins have the ability to precipitate certain protein and thereby making them indigestible. The 0.11±0.01 % tannins analyzed in the samples in the study was higher than the 21.0 mg/100 g for *D. oliveri* by Mann and Otori (2014). Saponins in seeds or fruits impose astringent taste that affects palatability, reduce feed intake and affects the utilization of protein and consequently body growth. The saponin content obtained in this study was higher than the 0.96 % reported for *Lablab purpureus*seeds by Shaahu*et al.* (2015). The anti-nutritional features of *Acacia nilotica* seed could serve as therapeutic used as anti-cancer, anti tumours, antiscorbutic, astringent, anti-oxidant and antispasmodial (Saini, 2008). In general, the levels of anti-nutrients in *Acacianilotica* seed are low to significantly interfere with nutrients utilization.

Amino acid composition

The amino acid profiles of *Acacia nilotica* seed flour are presented in Tables 5 and 6. The sample contains significant quantities of glutamic acid, arginin and aspartic acid whose values were 18.18, 11.22 and 11.50 g/100g, respectively. These amounts were higher than the values found in *Solanumaethiopicum* as reported by Adeyeye and Adanlawo (2001). The total essential and non-essential amino acids for *Acacia nilotica* were 43% and 57.02%, respectively. The *Acacia nilotica* contained all the essential amino acids, although leucine and the sulphur containing amino acid methionine and cystine were in low quantities. The total aromatic amino acid in the seeds of this plant (9.83%) was an indication that its combination with other diets could increase the net protein. On the other hand, the neutral amino acid in the sample accounted for 12.00%. The amino acid profile of the studied sample suggests that its protein is of high nutritive value which can be exploited by the feeds industries for feeds formulations.

Table 5: The amino acids profile of *Acacia nilotica* seeds

Amino acids	Concentration (g/100g)
*Lysine	3.46
*Histidine	2.30
*Arginine	11.22
Aspartic acid	11.20
*Threonine	2.80
Serine	4.61
Glutamic acid	18.18
Proline	3.87
Glycine	5.36
Alanine	3.72
Cystine	1.39
*Valine	4.29
*Methionine	1.35
*Isoleucine	3.42
*Leucine	6.23
*Tyrosine	3.49
*Phenylalanine	4.70
Tryptophan	0.90

*Essential amino acid

Table 6: Concentration of essential, non-essential, acidic, neutral, sulphur containing aromatic and basic amino acids of *Acacia nilotica* seeds

Parameter	Level
Total amino acid (TAA %)	92.49
Total non-essential amino acid (TNEA)	52.74
TNEAA (%)	57.02
Total essential amino acid with histidine	39.77
Total essential amino acid without histidine	37.47
% TEAAwith histidine	43.00
% TEAAwithout histidine	40.51
Total aromatic amino acid (TArAA)	9.09
% TArAA	9.83
Total neutral amino acid (TNAA)	11.10
% TNAA	12.00
Total acidic amino acid (TAAA)	29.38
% TAAA	31.77
Total basic amino acid (TBAA)	16.98
% TBAA	18.36
Total sulphur containing amino acid (TSAA)	2.74
% TSAA	2.96
Cystine in TSAA (%)	50.73
[a](P-PER)	2.93
Leu/Ile (ratio)	1.82
Leu-Ile (difference)	2.81

[a](P-PER): Calculated predicted protein efficiency ratio

Conclusion

The results of this study showed that the proximate and mineral compositions make *Acacia nilotica* seed justify possible valuable for human and animal nutrition. The *Acacia nolitica* seeds contained some bioactive secondary compounds which have essential in pharmacological effects and can be used as medication such as skin regeneration, anti-inflammatory and diuretic properties.Particularly the seeds are rich in potassium, phosphorus and magnesium which make it an excellent source of these major minerals needed in larger amounts by the body. The Na/K of the seed flour was less than one making the flour potentially useful in preventing high blood pressure.

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