AMINO ACID PROFILE AND PROXIMATE ANALYSIS OF FERMENTED AFRICAN LOCUST BEAN (*Parkia biglobosa*) SEEDS

ABSTRACT

Fermented *Parkia biglobosa* seeds nutritional content makes it suitable for use as a popular flavour intensifier in soups, stews and also as protein rich condiment in low protein content foods like vegetables. This study was aimed at determining the amino acid profile and proximate content of fermented African locust bean. The sample was prepared by boiling, dehulling and then fermenting the African locust bean. The analysis of theamino acid profile and proximate composition was done using standard procedures to evaluate the seeds nutritional potential. The results of amino acid profile revealed the following:3.58 ± 0.01 mg/100 g of lysine, 0.22 ± 0.01 mg/100 g of tryptophan, 2.69 ± 0.00 mg/100 g of leucine, 3.01 ± 0.01 mg/100 g of iso-leucine, 0.92 ± 0.02 mg/100 g of cysteine, 1.36 ± 0.02 mg/100 g of methionine, 1.11 ± 0.00 mg/100 g of valine, 1.34 ± 0.02 mg/100 g of tyrosine, 1.23 ± 0.01 mg/100 g of glycine, 3.00 ± 0.00 mg/100 g of arginine, 2.61 ± 0.02 mg/100 g of phenylalanine, 6.14 ± 0.01 mg/100 g of aspartic acid, 5.11 ± 0.00 mg/100 g of glutamic acid, 0.99 ± 0.00 mg/100 g of serine, 3.68 ± 0.02 mg/100 g of proline, 4.12 ± 0.01 mg/100 g of alanine, 1.15 ± 0.01 mg/100 g of histidine and 1.22 ± 0.00 mg/100 g of threonine. The results of proximate analysis showed the following: 4.78 ± 1.70 %of moisture content, 5.40 ± 0.07 %of ash content, 11.00 ± 0.29 % ofcrude fats, 6.00 ± 0.02 % ofcrude fibre, 20.56 ± 0.04 % ofcrude protein and 52.25 ± 0.01 % ofcarbohydrate content. The result showed that lysine and iso-leucine were the highest essential amino acid found while aspartic and glutamic acid where the highest for non-essential amino-acids.The crude protein content was high and could be due to the fermentation. The study on amino acid profile and proximate composition showed that fermented *Parkia biglobosa* seeds contained high protein content and can serve as a valuable dietary supplement, particularly in protein-deficient regions.

KEYWORDS: Fermented, African locust bean, Proximate, Amino acid profile and Nutritional content

1. INTRODUCTION

Most species of trees serve as sources of food and for medicinal purposes to different populations of people across the world.They also serve as source of income for many people dwelling in the rural areas [1]. Some of these trees and tree products are crucial as foods in West Africa due to their nutritional and sensory characteristicsand their availability in large quantities[2]. The seeds from these trees have protein content twice as much as that of cereals and usually contain more balanced profile of essential amino acids [3]. The protein content of legume grains ranges from 17 to 40 g/100 g which is much higher than that in cereals with a range of 7-11.0 g/100 g and approximately equal to the protein content of meat which ranges from 18-25 g/100 g [4].Seeds of legumes may account for up to 80 % of dietary protein for rural dwellers and may be the only source of protein for some groups [5]. One of the leguminous plants that produce seeds that can be consumed in meals is *Parkia biglobosa* (African Locust Bean) [1].The plant is commonly called the African locust bean tree and it is known to grow in different agro-ecological areas around the world [6]. African locust bean (*Parkia biglobosa*) falls within the category of plant proteins [7].

The African locust bean (Parkia biglobosa) is a tropical perennial and deciduous tree that grows mostly around Africa. It belongs to the subfamily Mimosoideae and family Leguminosae and grows mostly in the Sudan and Guinea savannah ecological zones [8]. The tree usually grows to height of 7 to 20 metres with broad leaves that bears broad crown and possesses brownish grey back and leaves. The leaves of the tree

are mostly used in treatment of various diseases such as diabetes Miletus, Malaria, inflammatory diseases, infections etc. The seeds, flowers, pulp, leaves, bark and roots can all be applied for different uses. However, the seed of the tree is the most used part and can be utilized for different applications such as in herbal medicine and as food condiments. The seeds can either be used directly or fermented under certain conditions before use. Nonetheless, it has been reported that the fermented locust bean is more utilized due to it better taste, higher nutritional content and flavour [8, 9].

The people living in the rural areas in developing countries consume African locust bean in search for plant protein,due to the expensive nature of animal protein.This has made the useof the locust beanquite widespread[10]. The most popular form of consumption of the African locust bean seeds is in its traditional fermented form called dawadawa (Locust beans cake), a food condiment for seasoning of food[11]. It is a common food additive use as intensifier in soups, stews and also as protein source in low protein content foods like vegetables[12].This traditional fermented condiment is consumed by different ethnic groups across Africa, which is evident that the condiment plays a major role in the food habit of communities in the rural regions[13]. Locust beans cake is known as Nune by Tiv people, Iru by Yoruba people and Dawadawa in Hausa land. It has a black or brownish appearance with a strong pungent smell [12].Even though, the locust bean can be processed and used directly, the fermented form of it increases the protein content available, improves the flavour and reduces anti-nutrient content [14].Therefore, there is need to study the proximate content and amino acid profile of the fermented African locust bean because it has the potential to meet the protein need of the malnourish populace.

This study is therefore aimed at determining the proximate composition and amino acid profile of fermented *Parkia biglobosa* seeds. The study of the nutritional content of fermented *Parkia biglobosa* seeds will help to assess it potential as a source of plant protein, providing an important insight on dietary considerations and addressing the issue of nutritional deficiencies. The amino acid analysis will help to evaluate the protein quality, allowing for informed decisions regarding the use of fermented *Parkia biglobosa* seeds in diets especially in places where protein sources may be limited.

2. METHODOLOGY

2.1 Sample Collection and Pretreatment

Parkia biglobosa seeds were bought in Aliade market, Gwer East, Benue State. The seeds were packed in a nylon bag and transported to Chemistry Laboratory, Benue State University, Makurdi for analyses. Two(2)kilograms of the seeds were boiled for 12 hours and dehulled to separate the cotyledons from the hulls. The loosened hulls were removed by flotation. The dehulled cotyledons were boiled in excess water for 2 hours and drained through a raffia basket while still hot. It was then poured into a sack placed in a basket and covered with leaves and allowed to ferment for 48 hours. The resulting product was dried in an oven for 1 hourand crushed manually into powder using mortar and pestle and kept in an air tight container for use as sample [15].

2.2 Amino Acid Analysis

Exactly 10 g of defatted seed samples of *Parkia biglobosa* were analyzed for the amino acid profile using Amino Acid Analyzer (Technicon Instruments Corporation, and New York). The analysis period was 76 minutes with a column flow rate of 0.50 cm^3 per min at a temperature of 60 ^oC with reproducibility consistent within 5.0±3.0 %. The net height of each peak produced by the chart record of Amino Acid Analyzer was measured and calculated for the amino acid it represented [15].

2.3 Proximate Analysis

Proximate analysis (moisture, ash, fat, crude fibre, protein and carbohydrate contents) of the fermented *Parkia biglobosa* seedswheredetermined using the method of Association of Official Analytical Chemists (AOAC) [16].

2.3.1 Determination of moisture content

A crucible was thoroughly washed and dried in the oven at 100 °C for 30 minutes and allowed to cool inside desiccator. After cooling, it was weighed and recorded as (W_1). Exactly 1 g of the sample was transferred into a crucible and reweighed as (W_2). Then, the sample plus the crucible were placed in an oven at 100 °C for 2 hours and cooled in a desiccator. The process was repeated until a constant weight was obtained as (W_3) [1, 15]. The values obtained were used to calculate the percentage of moisture content through the equation:

$$\% \text{ Moisture content} = \frac{W2-W3}{W2-W1} \times 100 \qquad \text{Equ. 1.}$$

Where; W_1 = Weight of crucible, W_2 = Weight of crucible + sample, W_3 = Weight of sample after heating.

2.3.2Determination of ash content

A crucible was washed with clean water and oven-dried for 30 minutes at 100 ^{o}C. The crucible was removed from the oven and cooled in a desiccator after which it was weighed. Exactly 5 g of the powdered sample was weighed and transferred into the pre-weighed crucible and put into muffle furnace at 600 °C for 3 hours until light gray ash was obtained. The crucible was removed from the furnace, put in a desiccator to cool and was reweighed to obtain the weight of ash [17]. The percentage ash was calculated using the equation;

$$\% \text{ Ash content} = \frac{(\text{Weight of crucible} + \text{ash}) - (\text{Weight of crucible})}{\text{Weight of sample}} \times 100 \qquad \text{Equ. 2}$$

2.3.3 Determination of fats content

Fat content of the *Parkia biglobosa* seeds was determined using the Soxhlet extraction method. The extraction flask was washed, dried, cooled and weighed prior to extraction. Exactly 5 g of the sample was weighed into a beaker and introduced into a thimble. About 250 mL of N-Hexane was added to the flask for extraction in the Soxhlet apparatus. After this, the extract was dried in an oven for 15 minutes at 100 ^{o}C to remove any remaining solvent, cooled in the desiccator and reweighed [17]. The percentage fat content was calculated using the equation:

$$\% \text{ Fat} = \frac{(\text{Weight of flask} + \text{oil}) - \text{Weight of flask}}{\text{Total weight of sample}} \times 100 \qquad \text{Equ. 3}$$

2.3.4 Determination of crude fibre

Exactly 5 g of the sample was weighed into a 500 mL beaker and 200 mL hydrochloric acid (1 %) was added to the content and boiled for 30 minutes. The suspension was filtered and the residue was washed to neutral pH. The sample residue was then boiled again in 200 mL sodium hydroxide solution for 30 minutes, it was then filtered through Whatman no.1 filter paper and the residue obtained was transferred into a crucible and heated in hot air oven at 80 ^{o}C for 30 minutes. The dried residue was then cooled in a desiccator and weighed. The weighed sample residue was ashed in a muffle furnace at 550 ^{o}C for 30 minutes. The sample was removed from the furnace when its temperature was 200 ^{o}C. It was then cooled in a desiccator and weighed [18]. The loss in weight of the incinerated residue before and after incineration was taken as the crude fibre content and calculated using the equation:

$$\% \text{ Crude ibre} = \frac{\text{Total weight of fibre}}{\text{Weight of sample}} \times 100 \qquad \text{Equ. 4}$$

2.3.5 Crude protein

Exactly 2 g of the sample was weighed and transferred into a 100 mL Kjeldahl digestion flask. Exactly 2 g of anhydrous sodium sulphate (Na_2SO_4), 0.5 copper sulphate ($CuSO_4$) (catalyst) and 5 mL of concentrated sulphuric acid (H_2SO_4) were added. The flask was heated gently in a fume cupboard until the solution turned black. After this, heat was increased to obtain a clear solution, cooled, washed and transferred into 25 mL volumetric flask and rinsed with distilled water to mark. Exactly 5 mL of 2 % boric acid was measured into a 250 mL beaker and 2 drops of methyl red indicator was added. A quantity of 5 mL of the digest was poured into the distillation flask and the apparatus was set up. The heating system was switched on for 25 minutes and the receiver beaker was then removed [17, 19]. The collected distillate was cooled and titrated against 0.1 M HCl acid to an end point (indicated by change in colour from gray to purple). The titre value (Final burette reading - Initial burette reading) was recorded [19]. Crude protein was determined using the equation:

$$\% \text{ Crude protein} = \frac{14.01 \times 0.1 \times 6.25 \times \text{T}}{\text{W}} \times 100 \qquad \text{Equ. 5}$$

Where; 14.01 = Atomic weight of nitrogen, 0.1 = Molarity of acid, 6.25 = Protein conversion factor, T = Titre value and W = Weight of sample.

2.4.6 Carbohydrate content

Total carbohydrate content of the sample was determined by difference (subtracting crude protein, moisture, fat, fibre and ash content from 100%) [17]. The total carbohydrate content of the sample was obtained using the equation:

$$\text{Carbohydrate} = 100 - (\% \text{ protein} + \% \text{ fat} + \% \text{ ash} + \% \text{ crude fibre} + \% \text{ moisture}) \qquad \text{Equ. 6}$$

3. RESULTS

The proximate composition and amino acid profile of fermented *Parkia biglobosa* seeds were analyzed and presented in Table 1 and 2 respectively.

Table 1. Results of proximate composition of fermented *Parkia biglobosa* seeds

S/N	Parameter	Composition (%)
1	Moisture content	4.78 ± 1.70
2	Ash content	5.40 ± 0.07
3	Fat content	11.00 ± 0.29
4	Crude fibre	6.00 ± 0.02
5	Crude protein	20.56 ± 0.04
6	Carbohydrate content	52.25 ± 0.01

NB: Data are presented as mean ± SD value (n = 3).

Table 2. Amino acid profile of fermented *Parkia biglobosa* seeds

S/N	Amino acid	Composition (mg/100 g)
1	Lysine	3.58 ± 0.01
2	Tryptophan	0.22 ± 0.01
3	Leucine	2.69 ± 0.00
4	Iso-leucine	3.01 ± 0.01
5	Cysteine	0.92 ± 0.02
6	Methionine	1.36 ± 0.02
7	Valine	1.11 ± 0.00
8	Tyrosine	1.34 ± 0.02
9	Glycine	1.23 ± 0.01
10	Arginine	3.00 ± 0.00
11	Phenylalanine	2.61 ± 0.02
12	Aspartic acid	6.14 ± 0.01
13	Glutamic acid	5.11 ± 0.00
14	Serine	0.99 ± 0.00
15	Proline	3.68 ± 0.02
16	Alanine	4.12 ± 0.01
17	Histidine	1.15 ± 0.01
18	Threonine	1.22 ± 0.00

NB: All values are presented as mean ± standard deviation in duplicates

4. DISCUSSION

Moisture content of a material is defined as the amount of water present in the material. The value is often represented as a percentage of the material mass [20]. High moisture content encourages microbial growth whereas low moisture content indicates a lesser propensity for microbial contamination [21]. From the results shown in Table 1, the fermented *Parkia biglobosa* seeds contained 4.78 ± 1.70 % moisture. This value is slightly greater than the value of 4.47 % reported by Tor *et al*. [15] but lesser compared to the value of 9.70 % reported by Adeyeye, [1]. The moisture content value obtained in this study is an indication of low amount of water in fermented *Parkia biglobosa* seeds hence giving it a longer shelf life.Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. The ash content is a measure of the total amount of minerals present in a food [22]. Ash is a crude indicator for minerals, and its content will not be affected by drying [23]. The result of ash content in this study was 5.40 ± 0.07 % (Table 1). Olakunle and Adebola, [24] and Ezegwu *et al*. [25] reported results (3.55 %) and (4.19 %) respectively which are less than the value presented in this work. Also, Ajegena *et al*. [26] reported an ash content value of 2.5 % which is far less than the value presented in this study. The value of ash content obtained in this work indicates that the fermented *Parkia biglobosa* seeds contains few mineral components and is safe for consumption.

This refers to the amount of fat present in a particular food or substance, usually measured as a percentage of the total weight [27]. Fat in food determine the amount of energy available. A diet providing 1-2 % of its caloric energy as fat is said to be sufficient to human beings [28]. The crude fat value of fermented *Parkia biglobosa* seeds was found to be 11.00 ± 0.29 % (Table 1). The value is lower than 24.21 % reported by Olakunle and Adebola [24]. Also, the value falls outside the range (10.50-8.50 %) and (18.78-19.62 %)

reported by Ajegena *et al*. [26] and Ibrahim *et al*. [29] respectively. The value of fat content obtained in this work indicates appropriate concentration of dietary fats.Fiber is necessary for the lowering cholesterol level as well as the smooth intestinal functioning [27]. Fiber is characterized by low or no nutritional value however, its effect on digestive system may help to fight diabetes and lower high blood cholesterol level [30]. Low level crude fiber is considered appropriate [31] because high level can cause intestinal irritation, lower digestibility and decreased nutrient usage [32]. The result of crude fibre obtained in this study was 6.00 ± 0.02 % (Table 1). The value is slightly greater than 5.53 % reported by Ibrahim *et al*. [29], but appreciably higher than 3.55 % reported by Adeyeye, [1] and 3.84 % reported by Ezegwu *et al*. [25]. The value of crude fibre reported in this study is relatively high and shows that the fermented *Parkia biglobosa* seeds are rich in crude fibre which is beneficial to health.

This refers to the total protein content present in a food or substance. It contains essential amino acids responsible for growth and repair of worn-out tissues in humans [30]. The value of crude protein was found to be 20.56 ± 0.04 % (Table 1). The value is considerably less than 35.69 % reported by Tor *et al*. [15] and 36.5 % reported by Ajegena *et al*. [26], but slightly less than 21.24 % reported by Ezegwu *et al*. [25]. The value presented in this study indicates that fermented *Parkia biglobosa* seeds has a high crude protein which is a good source of amino acid requirement necessary for growth, maintenance and repair.This refers to the amount of carbohydrates present in a particular food or substance, usually measured as a percentage of the total weight [33]. Carbohydrate is the primary source of energy in the body [26]. Carbohydrates and lipid are the principal sources of energy [27]. The percent carbohydrate content was found to be 52.25 ± 0.01 % as shown in Table 1. The value is significantly greater than 37.14 % reported by Tor *et al*. [15] and 45.80 % reported by Ezegwu *et al*. [25]. The carbohydrate value in this study shows that the fermented condiment has high amount of carbohydrates.

Amino acids are needed for the synthesis of most body tissues, enzymes, hormones and other metabolic molecules [34]. The results of amino acid analysis shown in Table 2 revealed that *Parkia biglobosa* contained varying concentrations of essential amino acids such as lysine (3.58 ± 0.01 mg/100 g), methionine (1.36 ± 0.02 mg/100 g), phenylalanine (2.61 ± 0.02 mg/100 g), leucine (2.69 ± 0.00 mg/100 g), threonine (1.22 ± 0.00 mg/100 g), tryptophan (0.22 ± 0.01 mg/100 g), histidine (1.15 ± 0.01 mg/100 g), iso-leucine (3.01 ± 0.01 mg/100 g), valine (1.11 ± 0.00 mg/100 g) and some non-essential amino acids such as arginine (3.00 ± 0.00 mg/100 g), aspartic acid (6.14 ± 0.01 mg/100 g), glutamic acid (5.11 ± 0.00 mg/100 g), serine (0.99 ± 0.00 mg/100 g), proline (3.68 ± 0.02 mg/100 g), alanine (4.12 ± 0.01 mg/100 g), cysteine (0.92 ± 0.02 mg/100 g), tyrosine (1.34 ± 0.02 mg/100 g) and glycine (1.23 ± 0.01 mg/100 g), which could carry out important roles in the body. For the essential amino acids, lysine was found to be highest, followed by iso-leucine and tryptophan the least. Similar research on amino acid profile of fermented African locust bean reported highest values for lysine, leucine and phenylalanine [35]. The variation in amount of the essential amino acids could be due to difference in location, soil type and weather.The presence of essential amino acids suggested that the fermented seeds of *Parkia biglobosa* could serve as a good source of amino acids.

The non-essential amino acids were also observed to be in varying concentrations with highest amount been aspartic acid followed by glutamic acid while cysteine was found to be the lowest. Same non-essential amino acids were also report by another [35].These amino acids help in regulating the blood sugar levels, promoting the growth and recovery of muscles and bones, as well as the production of the growth hormones. Isoleucine takes part in the formation of haemoglobin, used for energy by muscular tissues. Methionine helps remove toxic waste from the liver and assist in regeneration. Glycine enhances sleep and supports whole-body health, maintains the strength and support of muscles and bones. Cysteine helps prevent damages from alcohol and tobacco use, stimulates white blood cell activity, while glutamic acids are a chemical that helps nerve cells in the brain, send and receive information from other cells [36]. In summary, the results of amino acids indicated that the seeds of *Parkia biglobosa* are good sources of both essential and non-essential amino acids which can supplement diets.

5. CONCLUSION

This study has shown that fermented *Parkia biglobosa* seeds contain appreciable nutrients required for the body. The result of the proximate composition showed high levels of protein and carbohydrates but moderate moisture, fat, fibre and ash contents. The results of amino acid analysis revealed that *Parkia biglobosa* contained varying concentrations of essential and non-essential amino acids. In summary, the study on amino acid profile and proximate composition showed that fermented *Parkia biglobosa* seeds contained high protein content and can serve as a valuable dietary supplement, particularly in protein-deficient regions.

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