

Nutrients Composition and Aflatoxin Contents of some locally cultivated Groundnuts in Sokoto State, Nigeria.

Muhibat Komolafe Bolanle^{1*}, Muhammad Nura Bello², Ekpa Emmanuel¹,
Abdulrahman Abdullateef¹, Jamila Saleh¹

¹ Department of Biology, Air Force Institute of Technology, Kaduna, Nigeria.

² Department of Biochemistry, Sokoto State University, Sokoto, Nigeria.

-----ABSTRACT-----

*This study evaluated the nutrients composition and aflatoxin contents of three varieties of groundnut (*Arachis hypogaea*;) locally cultivated in Sokoto state Nigeria using standard methods. The samples were collected from different markets within Sokoto state, dried at room temperature and homogenized using a blender. They were then stored in an air tight container until analysis. Nutrient composition determination was carried out on the samples according to Association of Analytical Chemists (AOAC, 2005) methods. Moisture content was observed to be low for all the three samples at 4.0-4.4%. Ash content was 1.50-2.03%, fat was 15.77-23.01%, carbohydrate was 50.72- 56.69%, energy was 470.90-499.32 kca and 2.92- 3.07% was the fibre content. Aflatoxin content using ELISA method was carried out. All the varieties analyzed showed an aflatoxin activities between 2.73 and 3.5ppb. The three different varieties show a significant difference in their nutrients and aflatoxin contents, except two pairs of the varieties having no significant difference based on their ash and aflatoxin contents at ($P < 0.05$). Thus, these groundnuts can be considered useful foodstuffs in minimizing protein-energy malnutrition (PEM) and serve as good sources of nutrients for food and feeds in Nigeria if properly harnessed*

Keywords: Groundnut, PEM, nutrient, Aflatoxin.

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I. INTRODUCTION

Groundnut (*Arachis hypogaea*), a rich source of protein is a widely consumed, but underutilized food in Sokoto state, Nigeria. Groundnut are among the major oilseeds in the world and are largely consumed in Europe and Africa [1]. Groundnut is an annual herbaceous plant growing 30 to 50cm (1.0 to 1.6ft) tall. As a legume, it belongs to the botanical family “fabaceae” and commonly known as the bean or pea family [2]. Groundnut is one of the leading agricultural crops of the world for the production of edible plant oil and protein. Groundnut contain a lot of important components for human nutrition [3, 4]. Foods like groundnuts can be utilized in a variety of ways to tackle malnutrition in children under the age of five. Malnutrition literally means “bad nutrition” and it technically includes both over and under nutrition. World Food Programme (WFP) defines malnutrition as “a state in which the physical function of an individual is impaired to the point where he or she can no longer maintain adequate bodily performance process such as growth, pregnancy, lactation, physical work and resisting and recovering from disease. Malnutrition can result from a lack of macronutrients (carbohydrate, protein and fat), micronutrients (vitamins and minerals), or both. Malnutrition has many causes, an extended shortage of food inevitably leads to potentially life- threatening malnutrition [5].

Malnutrition also refers to insufficient, excessive or imbalance consumption of nutrients. Although there are more people in the world that are malnourished due to excessive consumption, the greatest challenge in developing nations today is starvation, but insufficient nutrition that is the lack of nutrients necessary or the growth and maintenance of vital functions. The causes of malnutrition are directly linked to inadequate macronutrient consumption and disease, and are indirectly linked to household food security, maternal and child care, health services and the environment [6].

Malnutrition is often used to specifically refer to under nutrition where an individual is not getting enough calories, protein or micronutrients [7]. It increases the rate of infection and infectious disease, and moderate malnutrition weakens every part of the immune system [8]. Hence, there is need to consume food and agricultural produce with high nutrients, for the positive effectiveness of the body. Groundnut therefore is a good solution to all the challenges posed by malnutrition enumerated above.

Aflatoxins are known toxic substances found in groundnuts. Aflatoxins are a family of toxins produced by certain fungi that are found on agricultural crops such as maize, peanuts, cotton seeds and tree nuts. Aflatoxin-producing fungi can contaminate crops in the field, at harvest and during storage [9]. Aflatoxins are a

group of naturally occurring mycotoxins that are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, species of fungi that typically affect groundnuts which are abundant in warm and humid regions of the world [10].

Aflatoxins are found in various cereals, oilseeds, spices and nuts [11]. These aspergillus colonizes themselves and produces aflatoxins, which contaminate grains and cereals at various steps during harvesting or storage. Fungal contamination can occur in the field, or during harvest, transport and storage [12]. Aflatoxins affects humans following consumption of aflatoxins contaminated foods such as eggs, meat and meat products, milk and milk products, groundnut and so on [13]. Knowledge of its nutrient composition and safety, will be immensely useful in future development of food and nutrient composition database especially for the vulnerable population.



Fig 2: structure showing groundnut and groundnut seeds cut in half showing the embryos with cotyledons and primordial root [14].

II. MATERIALS AND METHOD

Reagents

Copper(II)tetraoxosulphate(VI), tetraoxosulphate(VI)acid (H_2SO_4), potassium hydroxide, Acetone, Trioxoborate(III)acid (H_3BO_3), Sodium hydroxide (NaOH), methyl red, Hydrochloric acid, Ethanol, Diethyl ether, Methanol and all other reagents were of analytical grade.

Experimental Design

Nutrient Analysis

The nutrient analysis of the samples were carried out in accordance with the procedure of (AOAC, 2005).

Determination of moisture content

A dried cooled platinum dish was weighed on an analytical balance as (w_1) and 2g of the sample was measured into the dish and weighed accurately (w_2). The dish and its content was transferred using a pair of tongs into a desiccator then placed into an oven at $105^\circ C$ to dry for about 3 hours. The dish was removed and re-weighed as (w_3).

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100\%$$

Where; W_1 = Weight of dish

W_2 = Weight of dish and sample

W_3 = Weight of dish and sample after drying in oven

Determination of ash content

A dried cooled platinum dish was accurately weighed on an analytical balance as (w_1) and 2g of the sample was measured into the dish and weighed as (w_2). Then it was transferred using a pair of tongs into a desiccator and placed into a muffle furnace at $550^\circ C$ until fully ash (colour changes to gray) then re- weighed as (w_3).

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100\%$$

Where; W_1 = Weight of dish

W_2 = Weight of dish and sample

W_3 = Weight of dish and sample after drying in oven

Determination of fat content

2g of the sample was weighed into a boiling tube. 10ml of conc. HCl and 10ml of distilled water was added and placed in a boiling water bath until solid particles dissolve and until mixture became brown. It was then taken off and cooled in a beaker containing water, then transferred into a separating funnel. 10ml of ethanol and 30ml of diethyl ether were added and shake to dissolve, it was then allowed to stand for some minutes so as to separate.

A clean dried conical flask was weighed as (w_1) and the ether layer was transferred into the flask. The extraction was repeated twice with 25ml of diethyl ether and the combined extract was evaporated in a water bath. The fat was dried at 105°C in an oven, cooled and re-weighed as (w_2).

$$\% \text{ Fat content} = \frac{W_2 - W_1}{W} \times 100\%$$

Where; W = Weight of sample

W_1 = Weight of conical flask

W_2 = Weight of sample and conical flask

Determination of crude protein

I. Digestion

1g of the sample was weighed on a top load balance and transferred into a digestion flask. A spatula-full of CuSO_4 salt was added, as well as 25ml of concentrated H_2SO_4 solution. A significant amount of antibump was added and the digestion flask was connected to a glass tube (with a condenser neck-off) whose joint was rubbed with Vaseline. The whole digestion set-up was connected to the lower chamber of the Kjeldahl apparatus and the heat knob was turned on. (Sample was heated until a clear solution was obtained).

II. Distillation

After complete digestion, 200ml of distilled water was added, as well as 85ml of 50% NaOH solution to the digest. The measuring cylinder used to measure the NaOH solution, was rinsed with 50ml distilled water and the content was transferred to the digestion flask. Antibump was added and the distillation set-up was connected to the upper chamber of the apparatus. 50ml of 2% H_3BO_3 was measured and transferred into a receiving flask. 3 drops of screened methyl red indicator were added giving a pinkish solution. The receiving flask was placed at the middle chamber of the apparatus, and the delivery tube was immersed into the pinkish solution in the receiving flask. The heat knob of the upper chamber was turned on for distillation to begin, and about 200ml of the resulting bluish solution was collected for titration.

III. Titration

After complete distillation, the bluish receiving solution was then titrated with 0.05M H_2SO_4 solution until a permanent pink colour was observed, which indicated the end point.

$$\% \text{N} = \frac{\text{TV} \times 0.0014 \times 100}{W}$$

Where; %N= percentage Nitrogen

W= weight of sample

TV= Titre Value

%P (percentage protein) is calculated by multiplying the %N by the Jones factor, F, corresponding to the protein source, as shown below:

$$\% \text{ protein} = \% \text{N} \times F$$

Where; F = Jones factor which is 6.25 for groundnut

Determination of crude fibre content

1.0g of the sample was weighed into a glass crucible (W_s). The sample was hydrolyzed with 0.128M sulfuric acid, and boiled for 30mins. It was then hydrolyzed again using 0.223M potassium hydroxide solution, and boiled for 30mins. The residue was filtered and washed with distilled water (hot), it was cooled and washed with acetone. The residue and crucible were oven dried at 105°C , it was then cooled at room temperature in a desiccator and weighed as W_1 . The sample was then placed in a furnace to ash at 550°C , cooled to room temperature in a desiccator and re-weighed as W_2 .

$$\% \text{ Crude Fibre} = \frac{W_1 - W_2}{W_s} \times 100\%$$

Where; W_s = Weight of the sample

W_1 = Weight of the sample + Crucible before ashing

W_2 = Weight of the sample + Crucible after ashing

Determination of total carbohydrate content

By difference:

$$\% \text{ Total Carbohydrate} = 100 - (\% \text{moisture content} + \% \text{ash} + \% \text{protein} + \% \text{fat})$$

Determination of total energy content

By computation:

$$\text{Energy (Kcal)} = (\text{total carbohydrate} \times 4) + (\text{protein} \times 4) + (\text{fat} \times 9)$$

Aflatoxin Content Determination

Five grams of the grinded samples were weighed on a top loading balance into a weighing bottle. 25ml of 70% methanol was added and shaking vigorously at 250rpm for 3mins on the horizontal orbital shaker. The sample was then filtered using a whatman filter paper into a centrifuge tube. The filtrate was passed through mycosep multifunctional clean-ups columns to obtain clear extract. 200µl of conjugate solution was pipetted into the dilution wells and 100µl of the extracted sample was also pipetted into the dilution wells and mixed thoroughly. 100µl of the mixed solution from the dilution wells was pipetted into the antibody coated wells and incubated at room temperature for 15minutes. The content from the antibody coated wells was discarded and washed 5 times with distilled water, the antibody coated wells was taped to dry with absorbent paper towel until the remaining water has been removed. 100µl of the substrate solution was pipetted into the antibody coated wells and incubated at room temperature for 5 minutes which gives a blue colour. 100µl of stop solution was pipetted into the antibody coated wells and solution changes from blue to yellow. The content was mixed by sliding back and forth on the flat surface, the bottom of the micro wells was wiped with a dry cloth and the result was read within 20min after addition of stop solution. The strip was read with an ELISA reader using 450nm filter and 630nm differential filter.

Statistical Analysis

Statistical analysis was carried out using graphpad Instat version 3 and the results were expressed in mean ± SEM and also ANOVA test was carried out on the sample at (P< 0.05).

III. RESULTS

The results of Nutrients and Aflatoxin compositions of Groundnut (*Arachis hypogaea*) is presented in Table 1 and 2 respectively.

Table 1: Nutrient Composition (g/100g dry weight) of three varieties of Groundnut (*Arachis hypogaea*)

Variety	%Moisture	%Ash	%Protein	%Fat	%Carbohydrate	%Energy	%Crude Fibre
Kwankwaso	4.25±0.03 ^b	1.50±0.10 ^c	18.76±0.08 ^c	18.79±0.02 ^a	56.69±0.19 ^a	470.90±0.46 ^a	3.35±0.14 ^b
Erdakar	4.42±0.04 ^c	1.94±0.03 ^a	15.77±0.08 ^b	24.97±0.38 ^c	52.89±0.50 ^c	499.43±1.65 ^b	2.92 ±0.03 ^b
Kampala	4.01±0.01 ^a	2.03±0.00 ^a	20.22±0.15 ^a	23.01±0.09 ^b	50.72±0.25 ^b	490.89±0.42	3.07±0.08 ^b

Values are expressed as mean ± standard error of mean (n =3). Values with different superscript along the same column indicate significant difference but those with the same superscript along the same column indicates no significant difference (P<0.05).

Table 2: Aflatoxin composition of three varieties of Groundnut (*Arachis hypogaea*)

Variety	Aflatoxin (ppb)
Kwankwaso	3.5±0.06 ^b
Erdakar	2.73±0.12 ^a
Kampala	3.07±0.03 ^a

Values are expressed as mean ± standard error of mean (n =3). Values with different superscript along the same column indicate significant difference but those with the same superscript along the same column indicates no significant difference (P<0.05)

IV. DISCUSSION

From the result of nutrient composition in [Table 1], the moisture content was lower than the value (4.12-9.26% DW) reported by [15] in “Nutrient composition of five varieties of commonly consumed Nigerian groundnut (*Arachis hypogaea*)” and also lower than (7.48%DW) recorded in the “chemical evaluation of food value of groundnut seeds” reported by [16]. The result obtained could be due to genetic variation as well as the climatic conditions in which the plant was grown. High moisture is associated with a rise in microbial activities during storage [17]. Hence the low moisture content makes the shelf-life to be long and contribute to the stability of *Arachis hypogaea* and prevent rancidity of the oil. The ash value obtained in previous research was relatively (3.0-7.4%DW) as reported by [18]. Therefore, the value of ash content from [table 1] shows an indication that it contains nutritionally important mineral elements and also a good source of nutrients for consumers. The fat content of groundnut have been reported as (33.6-54.95%DW) by [19] in “Chemical composition of groundnut, (*Arachis hypogaea*) Landraces” and (45.09-51.63%DW) by [20]. However, the value of fat content obtained from [table 1] are slightly lower. Hence, the variation existing from the reports seem to reflect the difference in locations where the groundnuts were cultivated as well as differences in the cultivars/ varieties evaluated in each study. The fat content in groundnut indicates high energy density that promotes fat soluble vitamin absorption without adding to the bulk of the diet [21]. The groundnut crude protein content as shown in [table 1] is similar to the ranges (19.02-27.16%DW) reported by [22] in “chemical composition of *Arachis hypogaea* Landraces”. Plant foods that provide more than 12% of its calorific value from protein are considered good source of potential source of protein. Hence, the protein in groundnut contributes to the growth and repair of worn-out tissues and also improve the nutrition of humans [23]. The main function of carbohydrate is for energy supply. The groundnut has a higher value of carbohydrate content from [table 1] compared to those reported within (19.02-27.16%) [24]. Hence, the carbohydrate content of groundnut shows that it could supplement the energy requirements for some of our daily activities which makes it a suitable source of nutrients.

The calorific value of the groundnut from the result in [table 1] is higher as compared to those reported at (289.40-352.59kcal/100g) [25]. This trend might also be due to the fact that improved varieties from research institutes were analyzed compared to the local varieties we analyzed. This calls for a need to circulate more improved varieties to our local farmers for cultivation and distribution to Nigerian groundnut consumers. The groundnut therefore if consumed in good quantity could be a good source of energy. The groundnut crude fibre content as shown in [table 1] is similar to the ranges (2.76-3.07%DW) reported [15]. Hence, the groundnut have low fibre content which is known to support bowel regularity, maintain normal cholesterol levels and blood sugar levels, reduce constipation and also prevention of heart diseases [26]. There is an evidence that crude fibre have a number of beneficial effects related to its indigestibility in the small intestine [27]. The result for aflatoxin content in groundnut [table 2] is within the range for consumption because high content of aflatoxin reduces the nutritional composition of groundnut making it not a good source of protein in which the difference may be due to different geographical origin, seasonal variation of the groundnut sample tested and weather conditions. From the result obtained it was observed that there is significant difference between the 3 varieties of groundnut based on their Ash, Moisture, fat, protein, carbohydrate, energy and aflatoxin content. This implies that the content between these 3 varieties of groundnut are different, that is; one has a higher content than the other. Also, from the result, it was observed that based on the Aflatoxin and Ash content, there is no significant difference between Erdakar and Kampala, and also no significant difference between the three pairs of groundnut sample based on their fibre content. Hence, this variation could reflect from the difference in the locations where the groundnuts are cultivated as well as differences in the cultivars/varieties evaluated in the study.

V. CONCLUSION

The findings obtained from the current study revealed that groundnuts are an excellent and affordable source of nutrition, supplementing vital nutrients to the human body such as carbohydrates, proteins, fats and so on. Groundnuts, when taken in adequate amounts in any form and free from contamination by aflatoxin through proper harvesting and storage, will supplement rich nutrients to the body that can promote growth and energy, and play a vital role in the prevention of diseases. A diet containing groundnut could provide all these vital nutrients and play a critical role in preventing disease and promoting good health.

Conflicts of interest

Authors have declared that no competing interests exist.

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