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Proximate analysis and anti-nutritional factors in three varieties of groundnut (*Arachis hypogaea*)

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Abstract
This study evaluated the nutritional qualities in seeds of three varieties of groundnut (SAMNUT-23, SAMNUT-24, and SAMNUT-26) as part of breeding lines undergoing genetic improvement at the Joseph Sarwuan Tarka University, Makurdi, Nigeria. From the result, the grand mean nutritional composition in groundnut seeds showed that lipid was the highest class of food (48.5%) followed by protein (22.7%). Others are carbohydrate (11.7%), moisture (8.6%) and ash (6.2%) while fibre content was the lowest (2.3%). Result indicated that SAMNUT-26 had the highest lipid (49.67%) and ash content (7.44%). SAMNUT-23 had the highest protein (24.45%) and SAMNUT-24 had the highest carbohydrate (15.94%) and moisture (8.73%), there was no significant difference among the varieties ($P>0.05$). Cyanide was not present in the three varieties studied while phytic acid was present as anti-nutrient but in small quantity with insignificant varietal differences ($P>0.05$). Oxalate content was the highest anti-nutrient obtained and it did not differ significantly among the varieties ($p<0.05$). Oxalate was 3.3 times higher than phytic acid. Oxalate was highest in SAMNUT-23 (15.0mg/100g) while the lowest amount was found in SAMNUT-26 (14.7mg/100g). Pearson's correlation showed that Lipid had a negative correlation with oxalate (-0.528*) and phytic acid (-0.706*), which indicates that lipid content suppresses oxalate and phytic acid contents drastically. From these findings, it could be observed that SAMNUT-26 variety appears to be the best variety because it had the highest lipid content and other nutrients but with a small amount of oxalate and phytic acid. There is need to improve the nutrition of groundnut and totally eradicate the anti-nutrients. This study is important to breeders in order to achieve food abundance in the country in terms of quality, quantity and safety.

Keywords: Groundnut, nutrition, anti-nutrition, breeding, food quantity

Introduction

The groundnut belongs to the family called Fabaceae (Leguminosae), they are also known as the legumes, bean, or pea family. Legumes host symbiotic nitrogen fixing bacteria in their root nodules. They require less fertilizer because of their ability to fix nitrogen thereby enriching the soil, making them available for crop rotation (McDonald *et al.*, 2016) [20]. Groundnut is grown with a component of a variety of crop mixtures including maize, millet, sorghum, and cowpea (Misari *et al.*, 2022) [21]. The main use of groundnut, is to produce oil (Cummings, 2012; Elegbede, 2021) [10]. Groundnut contain fatty acids, Palmitic acid which is the third most abundant fatty acid, and linolenic acid (omega-3) is present at a small amount (Onwuka, 2015) [27]. The by-products of ground nut contain enormous functional compounds like antioxidants, protein, minerals, vitamins, fibres and polyphenols that are mixed with processed foods as an active ingredient (Wu *et al.*, 2009; Zhao *et al.*, 2012) [34, 35].

Groundnut seed contains essential nutrient such as protein, oil magnesium, niacin, fibre, vitamin, phosphorus and manganese. Groundnut seed is a good source of vegetable protein, together with vitamins and high calories 100 per gram (Atasie *et al*, 2009) [7]. The importance of groundnut protein as food and feed sources has gone up, mostly in developing countries. According to Ogbe 2021, glutamic acid is said to be the most abundant amino acid in groundnut, which points to its potential to be isolated for use as a flavouring agent. The recent discoveries revealed that peanuts are good source of compounds such as phytostroels, phenolic acids, flavonoids and resveratrol which hinders cholesterol absorption from the diet (Limmongkon *et al.*, 2017; Sebei *et al.*, 2013) [19, 30].

Groundnut seed can be processed and used in making soups, stew, peanut butter, oil and so many other products, the cake from groundnut can be used in feed and infant food formulations (Dhamsaniya *et al*., 2012; Timbabadiya *et al*., 2017) [11, 33]. Groundnut is also used in industries for making textiles materials, mixed nuts, make up, medicines, sauces pudding, solvents (Onyenuga, 2000) [28].
Groundnut is a valuable crop in terms of nutrition and wealth creation. There is need to know the nutritional contents of the three varieties of groundnut, this is necessary in order to solve the malnutrition and food insecurity in Nigeria. This work seeks to evaluate the proximate composition and determine the anti-nutritional factors (phytic acid, oxalate and cyanide) in the three varieties of groundnut. Specific objectives were to determine the amount of carbohydrate, protein, lipids, moisture, fiber, ash, cyanide, oxalate, and phytic acid content present in three varieties of groundnuts studied (SAMNUT 23, SAMNUT 24 and SAMNUT 26) and also to determine the relationship among the nutrients and anti-nutrient

Materials and Methods

The study area: This study was conducted in the Biochemistry Laboratory, College of Biological Sciences, Joseph Sarwuan Tarka University Makurdi, and Benue State.

Sample Collection, Identification and Preparation: Seeds of the three varieties were collected from the Department of Plant Breeding and Seed Science, College of Agronomy, Joseph Sarwuan Tarka University, Makurdi (JOSTUM). The varieties were part of the genetic resources of the ongoing molecular breeding work. They were approved and released by the Management of Molecular Biology Laboratory of the same department.

Proximate Analysis

Moisture determination: Moisture content was determined using the conventional method by (AOAC, 2021) [6]. Exactly 5g of each sample was put in each of the moisture cans, placed in the oven and dried at 105%C for 2 hours it was removed and placed in a desiccator to cool before weighing. The cycle of heating, cooling and weighing was repeated until constant weight was obtained. The moisture content was calculated using the formula;

$$\%\ \text{Moisture}\ \frac{w2-w3}{w2-w1}\ x\ \frac{100}{1}$$

$w1 = \text{weight of the empty moisture can}$
$w2 = \text{weight ofcan and sample before drying}$
$w3 = \text{weight of can and sample after drying}$

Crude protein determination: The micro-kjeldahl method was used as described by (AOAC, 2000) [6], in which samples were mixed with 10 ml of concentrated tetraoxosulphate (VI) acid in a kjeldahl digestion flask. Titration was done from the initial green color to a deep red or pink end point. The distillate obtained will be titrated again 0.02N tetraoxosulphate VI acid (H_2S0_4) solution. Titration will be done from the initial green color to a deep red or pink end point. The total nitrogen was calculated and multiplied with the factor, 6.25 to obtain the crude protein content.

$$\%\ \text{Crude protein} = \%\text{N6.25}$$

$$\%\ \text{N2} = \frac{(100x)\text{Nx14xVfxT}}{\text{w x 100 x VA}}$$

$W = \text{weight of the sample}$, N = Normality of filtrate ((H_2S0_4) = 0.02N, V_F = Total volume of the digest = 100mlV_A = Volume of the digest distilled

Fat determination: Fat content of the samples was determined by the solvent extraction method using a soxlet apparatus. Five grams (5g) of each samples will be wrapped in porous paper (Whatman number one filter paper), the wrapped sample will be put in a soxlet flask of the reflux connected to a condenser by heating the solvent in a flask through electro thermal heater, it will be vaporized and condensed into the reflux flask. The weight of the fat (oil) extracted as percentage of the sample weight (AOAC, 2000) [6].

Formula for the Calculation

$$\%\ of\ fat = \frac{w2-w1}{w1}x\frac{100}{1}$$

Where,
W = Weight of the sample
W_1 Weight of empty extraction flask
W_2 = Weight of flask and oil extract

Ash determination

The furnace incineration gravimetric method recommendation (AOAC, 2000) [6], was used in the determination of the ash content. The crucibles were dried in an oven and cooled in the desiccators before weighing. Approximately 5 g of the sample was weighed and put into the crucible, covered and placed in a muffle furnace at a temperature of 70^{o}C. The crucibles containing the samples was weighed and the percentage ash content was determined Ash content was calculated using the formula:

$$\%\ Ash = \frac{w2-w3}{w2-w1}x\ \frac{100}{1}$$

W_1 = weight of the crucible
W_2 = weight of sample crucible
W_3 = weight of crucible + ash

Determination of crude fibre

This was determined by the Weende method as described by (Mubarak *et al.,* 2015). The defatted sample was treated with 200 ml of 1.2% H_2SO_4 and boiled under reflux for 30 minutes. The resultant mixture was filtered by washing with several portions of hot water using a two-fold muslin cloth to trap the particles.

The crude fibre content was calculated using the formula:

$$\%\ \text{Crude fibre} = \frac{\text{loss in weight incinertion}}{\text{weight of sample}}\text{x}\ \frac{100}{1}$$

$$= \frac{\text{w2} - \text{w3}}{\text{weight of sample}}$$

W_2 = weight of crucible sample after washing and drying in oven
W_3 = weight of crucible + sample ash

Carbohydrate determination

Carbohydrates determination was carried out by deference as Nitrogen free extraction (NFE).

% NFE = 100 - % (a + b + c + d + e)

Where,
A = Protein
B = Fat
C = Fibre
D = Ash
E = Moisture

Determination of anti-nutritional factors in mung bean: The oxalate content was calculated by taking 1ML of 0.05M $KMnO_4$ as equivalent to 2.2 mg oxalate. Alkaline picrate color method was used in Cyanide determination where the absorbance was read at 450 nm against blank, after color development (reddish brown color). Phytic phosphorus was determined by the method by (Ola and Oboh, 2000) [26] and the phytic acid content was calculated by multiplying the value of pp by 3.55. Each mg of iron equal 1.19 mg of pp.
Fe equivalent=1.15 x titer value
Pp= titer value x 1.19 x 1.95
Therefore, phytic acid =1.95 x 1.19 x 3.55 x titre value.

Data Analysis: Data was analyzed on the Minitab 16.0 software using the following statistical tools: Chi Square test of dependency, one way ANOVA ($p \leq 0.05$) and Pearson's correlation. Mean separation was done using Turkey's method at 95% confident limit (p-value=0.05 limit). Level of significant was determined at p =^0.5.

Results and Discussion

Table 1 shows the proximate composition of three varieties of groundnut: SAMNUT-23, SAMNUT-24, and SAMNUT-26. SAMNUT-26 had the highest lipid (49.67%) and ash content (7.44%). SAMNUT-23 had the highest protein (24.45%) and SAMNUT-24 had the highest carbohydrate (15.94%) and moisture (8.73%). The differences in nutrient did not vary significantly among the varieties (P>0.05). Grand mean nutritional composition in groundnut seeds showed that lipid was the highest class of food (48.5%) followed by protein (22.7%). Others are carbohydrate (11.7%), moisture (8.6%) and ash (6.2%) while fibre content was the lowest (2.3%) (Figure 1).

The lipid content (48.5%) in this work was a little higher than the value (47.00%) obtained by Atasie *et al*, (2009) [7] and Amom, (2009) [4]. However, Shashikant (2019) [31] reported a range of 39.45-41.48% while (Khalid *et al.*, 2017) [17] had a range of 31.50% to 45.75%. These constrasting reports show that there is an improvement in groundnut varieties. The high lipid content shows that oils from this plant can be extracted and imported into food or feed formulations that requires high levels of fat, this oil can be used in the processing of cosmetics and biofuel (Khalid *et al.*, 2017) [17]. The protein content in this research was high (22.7%) which agrees with the range obtained by Khalid *et al.*, (2017) [17] 20.71-25.34% but lower than the value (38.61), obtained by Atasie *et al*, (2009) [7], Musa *et al.*, (2010) [22] of (31.3%), and the range of 28.75 to 30.63 reported by Shashikant, (2019) [31]. This could be as a result of the improvement on their own varieties, suggesting an improvement in the seeds of groundnut to increase protein content.

Khalid *et al.* (2017) [17] reported that high protein content is pertinent because some functional properties have long been associated with the activities of proteins (Khalid *et al.*, 2017) [17]. The high protein content obtained in this work indicates that groundnut is a good source of protein. The moisture content (8.6%) obtained in this report is lower than the safe level required for proper storage reported by Ofuga and Lale (2001), this report agrees with the findings of Musa *et al* (2010) [22], who reported the same value of (8.6%). Although it disagrees with the report of Atasie *et al*, (2009) [7], who had (5.80%). The differences in the various report could be attributed to the environment where the seeds were planted or the seeds itself. The higher the moisture content, the higher the attacks from microorganisms on the seeds (Ijarotimi and Keshinro 2020) [14]. However, seeds with low moisture content will last longer than those with high moisture content. The fibre content in this study was (2.3%), which was within the range (2.31 to 2.80 %) obtained by (Ameha and Leta 2020), but lower than what has been reported (3.8%) by (Atasie *et al.*, 2009) [7]. According to Shashikant, (2019) [31], the crude fiber shows the ability groundnut to maintain internal distention for a normal peristaltic movement of the intestinal tract, a physiological role that crude fiber plays. According to Amom, (2009) [4], diets that are low in crude fibre is undesirable because it can lead to constipation and such diets have link with diseases of colon like piles, appendicitis and cancer. The carbohydrate content in this study was (11.7%) which was higher than the range (4.22-7.21%) obtained by (Ameha and Leta 2020). The low content of carbohydrate indicates that groundnut is more of a body building food than energy food (Amom, 2009) [4].

Table 2 presents the anti-nutritional factors (cyanide, oxalate, and phytic acid) content in three varieties of groundnut seeds. Cyanide was completely absent in this report. Phytic acid content was low ranging from (3.67-5.33 mg/100 g) where SAMNUT-23 had the highest value with no significant difference among the varieties (P>0.05). Oxalate content was the highest anti-nutrient observed but did not differ significantly among the varieties (P>0.05). It was highest in SAMNUT-23 (15.0 mg/100 g) while the lowest amount was found in SAMNUT-26 (14.7mg/100g). Figure 2 shows that the overall mean Oxalate of 14.8 mg/100 g was 3.3 higher than phytic acid.

The anti-nutritional contents in these varieties are below the lethal level and permissible limit by WHO/FAO and are completely safe for consumption. Cyanide was completely absence in the groundnut seeds and phytic acid was relatively low in all the varieties measured. The lethal level for these anti-nutrients is 50-60rng/kg for cyanogenic glycoside and 2-5g/kg for oxalate and trypsin inhibitor (Onwuka, 2005) [27]. Phytic acid is generally a negatively-charged structure, which binds with positively-charged metal ions such as zinc, iron, magnesium and calcium to make complexes and reduce the presence of these ions by lowering their absorption rates. (Grases *et al.* 2017; Bora 2014) [13]. According to Kies *et al.* (2006) [18], phytic acid frustrates the activity of enzymes, which are needed for protein degradation in the small intestine and stomach.

Phytic acids affect the presence of minerals and also affect strongly the infants, pregnant and lactating women when they consume large portions of cereal-based foods (Chan *et al*. 2007; Al Hasan *et al.* 2016) [9, 2]. Sinha and Khare (2017) [32] reported that in diacotyledons like oilseed, nuts, and legumes, phytates are found in close interraction with proteins, which makes it difficult to be separated by a simple processing method like milling. Sarkiyayi and Kanu, (2019) [29] in their work on the Chemical Composition of Two Varieties of *Arachis hypogaea*, reported that both samples have high levels of phytate and oxalate then traces of Cyanogenic Glycoside. While Abdulrazaki *et al.*, (2014) [1], in their work on the proximate analysis and anti-nutritional factors of groundnut and melon husk revealed that groundnut shells have a higher oxalate and phytate content than the melon shell while the melon shells have a higher cyanogenic glycoside and trypsin inhibitor content than the groundnut shells. The phytic acid in this study was extremely low, indicating an improvement in phytic acid reduction in this new bred. The most important anti-nutrient studied was the Oxalate, being the highest examined (about 3.3 higher than phytic acid in groundnut seeds) although it is below the limit of risk. Anti-nutrients in nuts, legumes grains and vegetables, calls for a concern only when a person's diet is composed entirely of uncooked plant foods. Oxalate, for instance, prevents the absorption of calcium in the body by binding with it (Jiru and Urga, 1995) [16]. In sensitive people, even small amounts of oxalates can result in burning in the ears, throat, mouth and eyes; large amounts may lead to nausea, diarrhea, muscle weakness and abdominal pain, (Natesh and SK, 2018) [23]. The oxalate content obtained in this work was 15.0 mg/100 g which is higher than the value 8.0mg/100g obtained by (Aneta and Dasha 2019**)**, which suggests that groundnut seeds need to be improved upon in order to reduce the oxalate content so as to ensures that the right nutrients are absorbed.

Moisture had negative correlation with almost all the variables except carbohydrate (0.672*), oxalate (0.444*) and phytic acid (0.378*). Ash had a positive correlation with protein (0.893*). Lipid had a negative correlation with oxalate (-0.528*) and phytic acid (-0.706*), which indicates that lipid content suppresses oxalate and phytic acid contents drastically. SAMNUT-26 variety had the highest lipid but least in oxalate content and phytic acid content respectfully. The higher the lipid content the lower the oxalate and phytic acid contents. The lipid content should be increased in order to reduce the oxalate content in groundnut seeds.

Table 1: Proximate composition in three varieties of groundnut

Varieties	Moisture (%)	Ash (%)	Fibre (%)	Lipid (%)	Protein (%)	Carbohydrate (%)
SAMNUT-23	8.68±0.03	6.34±0.06	2.34±0.06	47.10±0.14	24.45±0.12	11.09±0.29
SAMNUT-24	8.73±0.12	4.68±0.16	2.23±0.06	48.81±0.28	19.61±0.17	15.94±0.52
SAMNUT-26	8.31±0.02	7.44±0.06	2.34±0.06	49.67±0.15	24.16±0.09	8.09±0.22
Grand mean	8.57±0.13	6.15±0.80	2.30±0.04	48.53±0.76	22.74±1.57	11.71±2.29

Moisture: χ^2 (Variety Vs Moisture content) = 0.012, P=0.994 (P>0.05)
Ash: χ^2 (Variety Vs Ash content) = 0.627, P=0.731 (P>0.05)
Fiber: χ^2 (Variety Vs Fiber content) = 0.004, P=0.998 (P>0.05)
Lipid: χ^2 (Variety Vs Lipid content) = 0.071, P=0.965 (P>0.05)
Protein: χ^2 (Variety Vs Protein content) = 0.648, P=0.723 (P>0.05)
Carbohydrate: χ^2 (Variety Vs Carbohydrate content) = 2.681, P=0.262 (P>0.05)

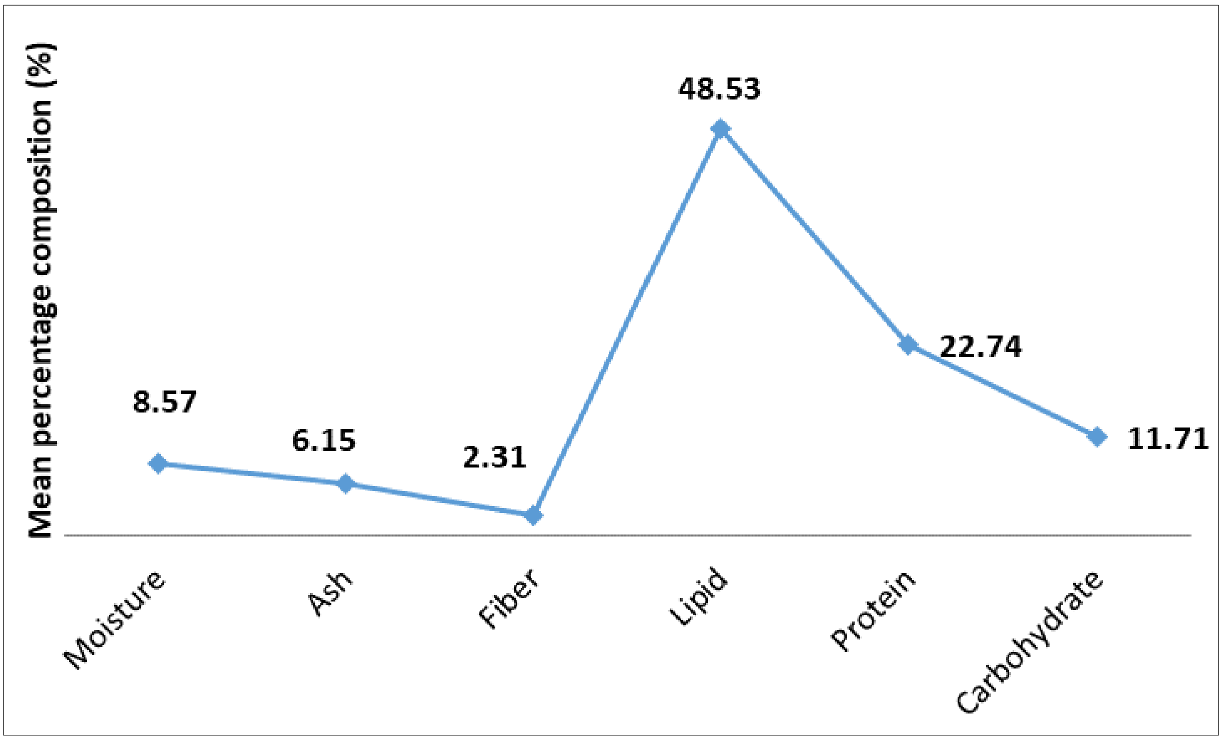

Fig 1: Grand mean of proximate composition in groundnut seeds

Table 2: Anti-nutritional factors in three varieties of groundnut

Varieties	Cyanide (mg/100g)	Oxalate (mg/100g)	Phytic acid (mg/100g)
SAMNUT-23	0.00±0.00	15.02±0.02[a]	5.33±0.67[a]
SAMNUT-24	0.00±0.00	14.77±0.09[a]	4.67±0.33[a]
SAMNUT-26	0.00±0.00	14.73±0.15[a]	3.67±0.33[a]
FAO/WHO limit			

F (Oxylate content) = 2.38, F=0.173 (P>0.05)
F (Phytic acid content) = 3.17, P=0.115 (P>0.05)
Means that do not share a letter are significantly different.

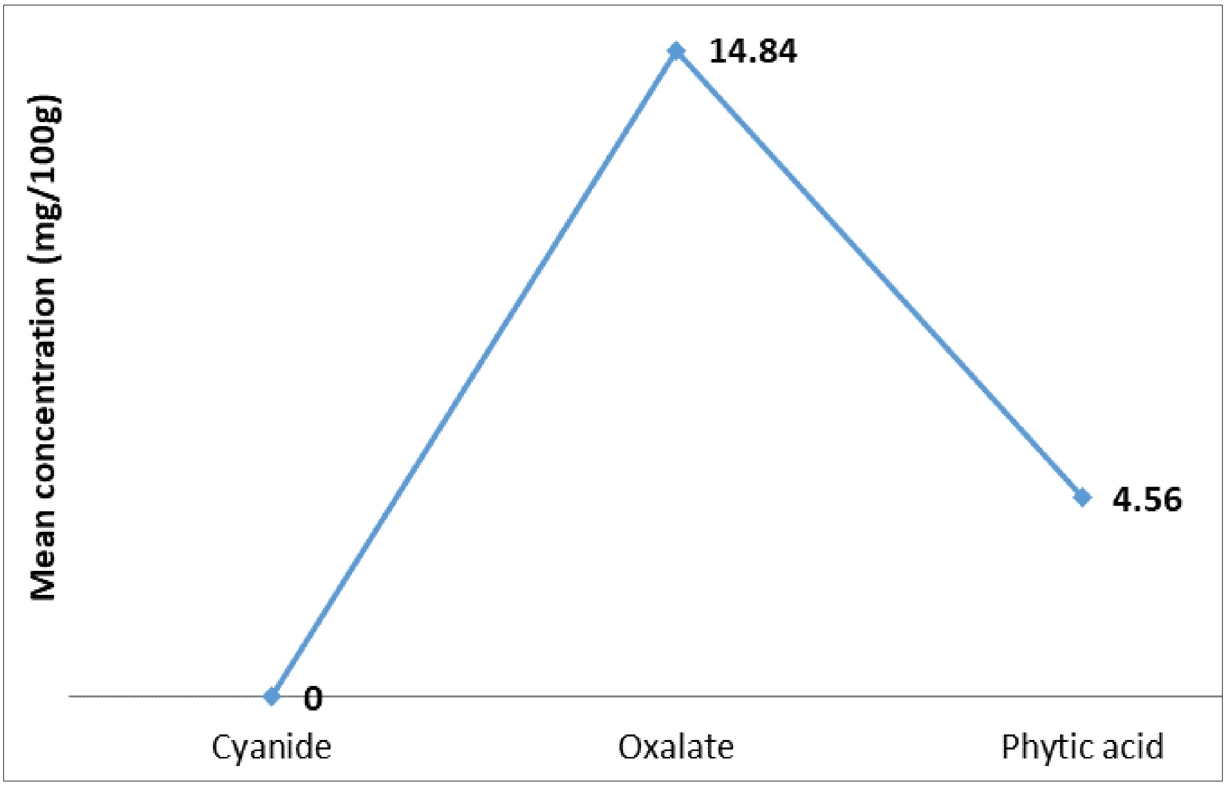

Fig 2: Comparative analysis of anti-nutritional factors in groundnut

Table 3: Pearson's Correlation Matrix

	Moisture	Ash	Fiber	Lipid	Protein	Carbohydrate	Oxalate	Phytic acid
Moisture	1							
Ash	-0.712	1						
Fiber	-0.305	0.418	1					
Lipid	-0.526	0.202	-0.109	1				
Protein	-0.445	0.893	0.474	-0.224	1			
Carbohydrate	0.672	-0.990	-0.442	-0.218	-0.901	1		
Oxalate	0.444	-0.006	0.045	-0.528	0.313	-0.063	1	
Phytic acid	0.378	-0.387	0.213	-0.706	-0.059	0.382	0.553	1

Strength of correlation
0.00-0.39 = Weak correlation
0.40-0.69 = Moderate correlation
0.7-0.9 = High correlation
>0.90 = Very high correlation

Conclusion

Groundnut seeds are rich in oil and other nutrients and are of high economic importance. The three varieties have good nutritional values, SAMNUT-26 variety appears to be the best variety because it had the highest lipid content and other nutrients but had a minimal amount of oxalate and phytic acid. It is pertinent to improve the oil contents of the other varieties in order to reduce oxalate and phytic acid drastically. This information is important to farmers and consumers of groundnut.

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