Comparative study on effect of aluminium phosphide on some nutrients and anti-nutritional factors in *Arachis hypogaea*

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**Abstract**

The nutrients and anti-nutrients of freshly harvested *Arachis hypogaea* aluminium phosphide preserved (APP) non-aluminium phosphide preserved (NAPP) were investigated. The study aimed at determining the effect of aluminium phosphide on nutrients and anti-nutrients levels of legumes using *A. hypogaea* as a case study. The proximate composition, minerals profile and anti-nutrients were estimated using standard analytical methods. The proximate compositions showed that NAPP is significantly ($P<0.05$) higher than APP except the fiber (0.03±0.06mg/100g; 0.03±0.06mg/100g) and ash (0.01±0.06mg/100g; 0.02±0.06mg/100g) carbohydrate (0.07±0.06mg/100g; 0.03±0.06mg/100g), protein (0.93±0.06mg/100g; 1.49±0.06mg/100g), fat (0.76±0.06mg/100g; 0.68±0.02mg/100g), and moisture content (0.64±0.06mg/100g; 0.30±0.06mg/100g). Exception of phosphorus (0.65±0.06mg/100g; 0.63±0.06mg/100g), the minerals concentration was significantly ($P<0.05$) higher in NAPP compared to APP. Iron (0.76±0.06mg/100g; 0.62±0.06mg/100g), potassium (3.80±0.06mg/100g; 2.62±0.06mg/100g), manganese (2.60±0.06mg/100g; 1.50±0.06mg/100g), magnesium (11.00±0.06mg/100g; 0.06±0.06mg/100g), calcium (8.21±0.06mg/100g; 7.20±0.06mg/100g) and zinc (0.01±0.06mg/100g; 0.14±0.06mg/100g). The anti-nutritional factors showed significant difference ($P<0.05$) higher in NAPP compared to APP. Iron (0.76±0.06mg/100g; 0.62±0.06mg/100g), potassium (3.80±0.06mg/100g; 2.62±0.06mg/100g), manganese (2.60±0.06mg/100g; 1.50±0.06mg/100g), magnesium (11.00±0.06mg/100g; 0.06±0.06mg/100g), calcium (8.21±0.06mg/100g; 7.20±0.06mg/100g) and zinc (0.01±0.06mg/100g; 0.14±0.06mg/100g). The study showed that aluminium phosphide negatively affected the nutritional profile of *A. hypogaea*. Thus, the effect of aluminium phosphide should be further investigated in vivo.

**Keywords:** *Arachis hypogaea*, food preservatives, aluminium phosphide, anti-nutrients.

1. Introduction

Legumes occupy an important place in human nutrition and considered as poor man’s meat especially by those who are in developing countries. This is due to the reason that legumes are a good source of protein and slowly digestible carbohydrates. (Reyes-Moreno and Paredes-Lopez, 1993). They are very important in human and animal nutrition. Ideally the basic protein requirement is met by consuming proteins of plant and animal origin. Above all these facts, legumes contain more protein than any other plant proteins. They also have unique property of maintaining and restoring soil fertility. Legumes are a rich source of nutrients such as protein, starch, minerals and vitamins and also have important health protective compounds (phenolics, inositol phosphates and antioxidants) (Enujiugha, 2010). This advantageous composition of legume seeds, not only make them a meat replacer for vegetarians but also as a component of rational nourishment. They serve as a low-cost protein to meet the needs of the large section of the people. However, several anti-nutritional factors present in legume seeds are a major limiting factor for the increased consumption of legumes, whose presence degrades the nutritive value of legumes. This may even lead to health problems which could eventually become fatal to humans and animals if taken in larger amount. In spite of the increasing interest concerning cultivation of pulses, the growth in area and production of seeds, and their application is relatively small (Kozlowska et al., 1998) [19].

Legumes contain a wide variety of anti-nutritional factors such as raffinose family oligosaccharides (RFO’s), neurotoxin, proteinaceous compounds, lectins, goitrogenic factor,
amylase inhibitors, and phytic acid (Maia et al., 2000; Preet and Punia, 2000; Enneking, 2011) [21, 26, 10]. Food processing methods including soaking (Frias et al., 2000; Vidal-Valverde et al., 2002) [12, 34] germination, decortications, fermentations, cooking and addition of enzymes have been suggested to reduce the concentration of anti-nutritional factors in pulses which greatly influence their nutritive values. Cowpeas are highly susceptible to pest infestation, and this leads to huge post-harvest losses, lower food quality and poor food safety. To mitigate these losses, the majority of farmers and grain merchants employ various insect control measures, including the use of chemicals not minding the consequences of their actions. The use of chemicals for crop preservation has called attention of individuals, government agencies and organisations to food quality and safety in the country.

Food preservation is used from the ancient times. Food preservatives becomes an essential thing nowadays, this plays an important role during food transportation. Preservatives are the substances, which are used to prevent food spoilage from microorganism. This will preserve the food for a long duration from the spoilage (Norkulova, 2016) [23]. Food is an essential thing for human survival. Except our own garden plants, all the food used today has some preservatives. Recently, several microbial provoked teas got noticed in the Western place, probably not only because of trade expansions between west and china, but also because of several health beneficial claims associated with microbial fermented tea (Tarkhasi, 2016; Reena et al., 2016; Anisa et al., 2016; Amal et al., 2016; Nazni, and Karuna, 2016) [32, 28, 4, 3, 22].

Preservation may be of any kind but it should be long lasting for preservation of food and it should be value your money (Lourdes et al., 2016; Kumar, 2016; Obajulwu et al., 2016; Osakue et al., 2016; Rajani et al., 2016) [20, 19, 24, 25, 27]. An example of increasing a process would be to inspire fermentation of dairy products with microbes that convert lactose to lactic acid; an example of preventing a process would be stopping the browning on the surface of freshly cut Red Delicious apples using lemon juice or other acetalidated water. Propyl and Methyl has been used as an anti-microbial preservative in foods, drugs and cosmetics for over 50 years (Ahmed et al., 2016; Chugh et al., 2016; Trivedi et al., 2015; Bernardi et al., 2015; Khan et al., 2015) [1, 8, 33, 5, 17]. There have been several previous safety assessments undertaken on this substance by several agencies, including FAO/WHO, FDA and FEMA (Bhalla et al., 2015; Kataoka et al., 2015; Rufina et al., 2016; Darwish et al., 2017; Ahmed, 2017; Khan and Ahmed; Imlak et al., 2017) [6, 15, 30, 9, 1, 16].

Presence of anti-nutritional factors which are generated by normal metabolism of species in natural food stuffs and act to reduce nutrient intake, digestion, absorption and utilization and produces many other adverse effects. Studies are needed, that will provide ample solutions on the effect of preservative (aluminium phosphate) in A. hypogaea also known as peanut. The study aimed at determining the effect of aluminium phosphate on anti-nutritional factors, some mineral profile and proximate analysis of A. hypogaea. The results of this study would provide insight on to whether or not a preservative that is basically for storage is use to see it effect in anti-nutrient, whether the preservative reduces or increases the toxic anti-nutrient or altered the proximate composition or the mineral profile.

2. Materials and Methods
2.1 Sample Collection and Study Area
The study was carried out in the Biochemistry and Molecular Department of Nasarawa state University, Keffi.

2.2 Sample Collection and Preparation
The African peanut (Arachis hypogaea) seed samples were collected from farms in Keffi, Nigeria. The foreign particles in the sample were removed by hand picking. The peanut was then pounded, blended and pulverized into fine powder. The fine powder was used for the analysis.

2.3 Analysis of Samples
2.3.1 Determination of Proximate analysis

Moisture Content: Determination of moisture content was carried by the method of (AOAC 930.15, 2000 and ISO 6496. 1999). Dry matter was determined gravimetrically as the residue remaining after drying at 103°C in a ventilated oven.

Ash Content: Ash content was determined by gravimetric method according to (AOAC 942.05, 2000) using this equation:

\[
\text{% Ash} = \frac{W_3-W_1}{W_2-W_1} \times 100
\]

Where, \(W_1\) = weight of empty dish (g), \(W_2\) = weigh of the dish and sample (g), and \(W_3\) = weight of dish and residue after incineration (g).

Crude Protein: Crude protein was determined using Macro Kjeldahl Method (AOAC 990.03, 2000) using this formula: Percentage nitrogen

\[
\%N = \frac{V_s-V_b}{W_1} \times M (\text{HCl}) \times 1 \times 14.007 / (W \times 10)
\]

Where, \(V_s =\) ml HCl needed to titrate sample, \(V_b =\) ml HCl needed for the blank test M (HCl), I = the acid factor, 14.007 = molecular weight of N, 10 = conversion from mg/g to %, \(W =\) weight of the sample (g) and \(F = N \times F\) where F is a factor equal to 6.25.

Crude Lipid: The crude lipid was determined using petroleum ether extract (AOAC 920.39, 2000) with the relation % crude fat = \(W_3 - W_2 \times 100/W_1\)

Where \(W_1\) = initial sample weight in grams, \(W_2\) = tare weight of flask in grams, \(W_3\) = weight of flask and fat residue in grams.

Crude Fiber: Crude fiber was determined by filtration method (ISO 6865, 2000) using Percent crude fibre (%CF) = \((W_2-W_3) \times 100 / W_1\)

Where \(W_1\) = weight of the sample (g), \(W_2\) = weight of crucible and residue after drying (g), and \(W_3\) = weight of crucible and residue after incineration (g)

Carbohydrate Content: The carbohydrate content was calculated by subtracting the summed up percentage
compositions of protein, lipid, fiber, moisture and ash contents from 100 (A.O.A.C., 1990). \% C = 100 - (\%P + \%F + \%A + \%W + \%Fi)

Where; C = carbohydrates, P = protein, F = fat, A = Ash, W = water, Fi = fiber

2.3.2 Determination of Mineral composition
The mineral analysis was determined in accordance to the method described by (ISO, 1998; A.O.A.C., 2000). The absorbance of calcium, phosphorus, zinc, potassium, manganese, and magnesium was measured in the solution at 578 nm and 430 nm respectively, using a spectrophotometer against the blank.

2.3.4 Determination of Anti-nutritional factors
Phytate Content: Phytate content was determined using the method described by Haugh and Lantzsch (1993). Absorbance was read at 519nm against a blank (distilled water) in a spectrophotometer (Atomic Absorption spectrophotometer – AAS Model SP9) using

\[
\% \text{ phytate} = \frac{a_u}{a_s} \times \frac{C}{W} \times \frac{V_f}{V_a} \times \frac{100}{1}
\]

Where: \(a_u\) = Absorbance of test sample, \(a_s\) = absorbance of standard solution, \(C\) = concentration of standard solution, \(W\) = weight of sample used, \(V_f\) = total volume of extract, \(V_a\) = volume of extract used

Cyanide Content: The cyanide contents of the sample were determined using the method described by Bradbury et al. (1985) using

\[
\text{HCN (mg/kg)} = 1000 \times 0.05 \times W \times \frac{a_u}{a_s}
\]

Where: \(W\) = Weight of sample, \(a_u\) = absorbance of the test sample, \(a_s\) = absorbance of standard solution

Oxalate Content: Determination of Oxalate was carried out according to AOAC (2005) using

\[
\% \text{ oxalate} = \frac{V_t}{W_s} \times V_{me} \times \text{Titre} \times 100
\]

Where: \(V_t\) = total volume of titrate = 100, \(W_s\) = weight of sample = 2g EQU, \(V_{me}\) = volume – mass equivalent.

Tannins Content: Tannins content was determined by Folin Denis colometric method. The tannin content was calculated as

\[
\% \text{Tannins} = \frac{100}{W} \times \frac{a_u}{a_s} \times \frac{C}{V_t} \times \frac{V_t}{V_a}
\]

Where: \(W\) = weight of sample, \(a_u\) = absorbance of test sample, \(a_s\) = absorbance of standard tannin solution, \(C\) = concentration of standard tannin solution, \(V_t\) = total volume of extract, \(V_a\) = volume of extract analysed.

Saponin Content: Saponin level was done by the double solvent extraction gravimetric method (Harborne, 1973) updated 2018 in Toxicology Laboratory N.V.R.I. Vom, Jos Plateau state, Nigeria using

\[
\% \text{Saponin} = \left(\frac{W_2-W_1}{W}\right) \times 100
\]

Where: \(W\) = weight of sample used, \(W_1\) = weight of empty evaporating dish, \(W_2\) = weight of dish + saponin extract

Alkaloids Level: Alkaloids level was determined by the alkaline precipitation gravimetric method (Harborne, 1973) was used. The weight of alkaloid was determined and expressed as a percentage of the sample

\[
\% \text{Alkaloid} = \left(\frac{W_2-W_1}{W}\right) \times 100
\]

Where: \(W_1\) = weight of empty filter paper \(W_2\) = weight of filter paper + alkaloid precipitate

2.4 Statistical Analysis
Descriptive statistics was used to analyse data obtained from test procedures and the mean values were compared using Microsoft Excel 2013 with significant difference at 5% level of confidence (\(P<0.05\)).

3. Results
Proximate analysis of the studied sample is shown in the figure below. Results obtained showed significant difference (\(P<0.005\)) in all the nutrients between the unpreserved and preserved samples except for crude fiber (03.01±0.06, 03.80±0.06) and ash (01.23±0.06, 02.00±0.06) respectively. Meanwhile, the levels of crude fat are much higher than other nutrients.

Fig 1: Effect of aluminium phosphide (APP) on proximate composition of Arachis hypogaea
Analysis of mineral profile in the sample is revealed in the chart below. Results obtained showed significant difference \((P<0.005)\) in all the minerals between the unpreserved and preserved samples except for phosphorus \((65.00 \pm 0.06, 63.00 \pm 0.06)\) respectively. The unpreserved samples maintained higher levels of minerals than the preserved ones.

![Chart showing mineral profile](image)

Figure 3 below shows the results of the analysis of anti-nutrients in the analysed sample of \(A.\ hypogaea\). The levels of cyanide for both the preserved and unpreserved samples were infinitesimal. Oxalate, phytate and alkaloid levels showed significant difference \((P<0.005)\) with increase in the preserved samples. On the contrary, tannin and saponins levels indicates decrease in the preserved samples.

![Chart showing anti-nutrient factors](image)

4. Discussion

The proximate analysis of \(A.\ hypogaea\) seeds has been studied which showed significant decrease \((P>0.05)\) in fat which makes it a suitable source of nutrient that can improve the energy density of man and animals. This is due to the fact that aluminium phosphide has effect on fat. The protein in groundnut seeds contributes to the growth and repair of worn-out tissues, will also improve the nutrition of humans and animals. Therefore, aluminium decreases the nutritional content of protein. there was no significant \((P>0.05)\) difference in the ash content which is relatively low, since the ash contains the minerals which can be estimated from it by atomic absorption spectrophotometry, it can be a good source of nutrients for consumers this shows that aluminium phosphide does not have any effect on both samples. The crude fibre is not high enough but can aid digestibility in humans, aluminium phosphide shows no effect on crude fiber. The carbohydrate content decreases due to the effect of aluminium phosphide, makes it not suitable for nutrient. Aluminium phosphide decreases the moisture content there by making it low, this makes the shelf-life to be long and contribute to the stability of \(A.\ hypogaea\) and prevent rancidity of the oil.

Mineral content for the peanut showed no significant \((P>0.05)\) difference in phosphorus content which is due to the fact that aluminium phosphide does not have any effect on both the preserved and preserved. There was a significant \((P>0.05)\) decrease on the preserved sample on iron, potassium, magnesium, zinc, calcium and manganese. This is due to the fact that aluminium phosphide forms a complex with these ions and there by reduces the bioavailability. Sun-dried groundnut is a good source of magnesium and iron while the roasted groundnut is a good source of potassium, calcium, zinc and phosphorus. The availability of calcium, magnesium, phosphorus is a good indication that the groundnut is so rich in the minerals for bone formation. Calcium is very essential in blood clothing, muscles contraction and in certain enzymes in metabolic processes. Results of the anti-nutrient contents showed that there was no significant \((P>0.05)\) difference in the cyanide...
concentration of the aluminium phosphide on preserved and unpreserved peanuts. The present result suggests that the cyanide content in *A. hypogaea* is within the permissible level of 200mg/kg (Richard & Thompson, 1997) [29]. This is probably due to no remarkable difference in the degradation of cyanide (HCN) in the two samples, indicating that preservative might have no effect on cyanide accumulation in the sample. This point to the fact that aluminium phosphide does not affect cyanide concentration in peanuts. Cyanide when ingested at low concentration is converted to thiocyanide in the body which is less harmful and can be detoxified by the body while accumulation binds to ions in the cytochrome and stops electron transport as a result of production and metabolism acidosis. There was significant (*P > 0.05) increase in tannins concentration of the preserved but decrease in unpreserved. This has clearly shown that the release of phosphine gas from aluminium phosphide (ALP) decreases the tannins content. The analyzed result suggests that oxalate content in all the samples is lower than the permissible level of 250mg/100g which cannot induce toxicity in man but high level of it above the permissible level can because oxalate is consumed in high amount so it binds to minerals, vitamins and other nutrient there by reducing the bioavailability of these nutrient in the body resulting into nutritional problems for example oxalate binds to calcium forming crystals which result to kidney stones. Similarly, the concentration of phytate in both samples is also below tolerant level of 600-800mg/100g but high amount may result in nutritional problems, such as rickets, goiter, which is a result of calcium and iodine deficiency respectively. Excessive intake of saponins result to high toxicity due to its haemolytic property, in which it ruptures erythrocytes and release haemoglobin. It reduces nutrient utilization and conversion efficiency as in ruminant (Cheeke, 1989; Sen et al., 1998) [3]. High level of alkaloids exerts toxicity and adverse effects to human, especially in physiological and neurological activities.

### Table 1: Effect of APP on proximate composition of *A. hypogaea* [Weight (mg) per 100g]

| Compositions  | Unpreserved      | Preserved        |
|--------------|------------------|------------------|
| Carbohydrate | 07.40±.06*       | 03.48±.06        |
| Crude Fiber  | 03.01±.06        | 03.80±.06        |
| Crude Protein| 19.93±.06*       | 14.94±.06        |
| Crude Fat    | 74.60±.06*       | 68.08±.06        |
| Crude Ash    | 01.23±.06        | 02.00±.06        |
| Moisture     | 06.40±.06*       | 03.00±.06        |

KEY: Mean ± Standard Deviation. *P <0.05 are considered statistically significant.

### Table 2: Effect of aluminium phosphide on minerals profile of *Arachis hypogaea* [Weight (mg) per 100grams of sample]

| Composition       | Unpreserved        | Preserved         |
|-------------------|--------------------|-------------------|
| Phosphorus (P)    | 65.00±.06          | 63.00±.06         |
| Iron (Fe)         | 76.20±.06*         | 62.00±.06         |
| Potassium (K)     | 38.01±.06*         | 26.20±.06         |
| Magnesium (Mg)    | 11.00±.06*         | 06.00±.06         |
| Zinc (Zn)         | 25.01±.06*         | 14.01±.06         |
| Calcium (Ca)      | 82.10±.06*         | 72.00±.06         |
| Manganese (Mn)    | 26.02±.06*         | 15.10±.06         |

KEY: Mean ± Standard Deviation. *P <0.05 are considered statistically significant.

### Table 3: Effect of aluminium phosphide on anti-nutrients factors of *A. hypogaea* [Weight (mg) per 100grams of sample]

| Anti-nutrients | Unpreserved      | Preserved         |
|----------------|------------------|-------------------|
| Cyanide        | 0.0173±.002      | 0.0163±.002       |
| Tannins        | 1.903±.015       | 9.330±.690        |
| Oxalates       | 42.500±.600      | 32.500±.600       |
| Phytic Acids   | 5.48±.600        | 3.420±.600        |
| Alkaloids      | 18.880±.600      | 16.720±.600       |
| Phosphine      | 38.200±.600      | 39.740±.600       |

KEY: Mean ± Standard Deviation. *P <0.05 are considered statistically significant.

### 4. Conclusion

The study has demonstrated that artificial preservative also known as aluminium phosphide (APP) has resulted in a significant(*P<0.05) increase in anti-nutrient such as phytic acid, oxalate, tannin, saponins and alkaldoids in leguminous grain (peanut) but no significant increase or decrease in cyanide activity. These results clearly indicate that artificial preservative which is used for storage purpose against insect, microbes and so on is harmful to living organism as excess accumulation can lead to metabolic acidosis, respiratory distress syndrome and shock, and there is no specific or effective antidote.

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