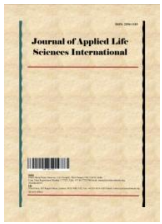

Journal of Applied Life Sciences International

23(12): 84-92, 2020; Article no.JALSI.64290
ISSN: 2394-1103

Effect of Aluminium Phosphide on Some Nutrients and Anti-Nutritional Factors in *Arachis hypogaea*

Chibuzo Carole Nweze[1*], Haruna Gambo Sunday[1], Aminu Dallah[1] and Tsalla Belove Danjuma[1]

[1]Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi P.M.B. 1022 Keffi, 93001, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author CCN designed the study and wrote the protocol. Author HGS performed the statistical analysis. Author AD wrote the first draft of the manuscript managed the literature searches. Author TBD managed the Laboratory analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2020/v23i1230208
Editor(s):
(1) Dr. Purnachandra Nagaraju Ganji, Emory University, USA.
Reviewers:
(1) Hugo Ruiz, The Nariño of University Pasto, Colombia.
(2) Uasley Caldas De Oliveira, University State of Feira de Santana, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64290

Original Research Article

Received 25 October 2020
Accepted 30 December 2020
Published 31 December 2020

ABSTRACT

Aims: To comparatively study the effect of aluminium phosphide preservative on some nutrientional and anti-nutritional factors in *Arachis hypogaea.*
Study Design: Randomized design
Place and Duration of Study: Department of Biochemistry and Molecular Biology Laboratory, Nasarawa State University, Keffi between May and August, 2019.
Methodology: Two portions of *A. hypogaea* weighing 500g each, were obtained, one portion preserved with Aluminium phosphide tablet while the second portion was without any preservative. The preservation lasted for 30 days after which the *A. hypogaea* was blended using a mechanical blender before analysis for nutritional and anti-nutritional compositions.
Result: The proximate compositions were significantly ($p<0.05$) higher in the NAPP than APP except the fiber (03.01±0.06 mg/100 g; 03.80±0.06 mg/100 g) and ash (01.00±0.06 mg/100 g; 02.23±0.06 mg/100 g). Carbohydrates (07.40±0.06 mg/100 g; 03.48±0.06 mg/100 g), protein (19.93±0.06 mg/100 g; 14.94±0.06 mg/100), fat (74.60±0.06 mg/100g; 68.08±2.75 mg/100 g), and moisture content (06.40±0.06 mg/100 g; 3.00±0.06 mg/100 g). Exception of phosphorus

**Corresponding author: E-mail: chibuzoihe@gmail.com;*

(65.00±0.06mg/100g; 63.00±0.06mg/100g), the minerals concentration was significantly($p<0.05$) higher in NAPP compared to APP. Iron (76.20±0.06 mg/100 g; 62.00±0.06 mg/100 g), potassium (38.01±0.06mg/100 g; 26.20±0.06mg/100g), manganese (26.02±0.06mg/100g; 15.10±0.06 mg/100 g), magnesium (11.00±0.06mg/100g; 06.00±0.06 mg/100 g), calcium (82.10±0.06 mg/100 g; 72.00±0.06 mg/100 g) and zinc (25.01±0.06 mg/100 g; 14.01±0.06 mg/100 g).The anti-nutritional factors showed significant difference ($p<0.05$) higher in tannin (9.33±0.69 mg/100 g; 1.90±0.02 mg/100 g), oxalate (32.50±0.60 mg/100 g; 42.50±.60 mg/100 g), phytate (16.72±0.60 mg/100 g; 5.48±0.60 mg/100 g), alkaloid (16.72±0.60 mg/100g; 5.48±0.60 mg/100 g) and saponins (40.74±0.60 mg/100 g; 38.20±0.60 mg/100 g) except cyanide (0.02±0.00 mg/100 g; 0.02±0.00 mg/100 g).

Conclusion: 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: Proximate compositions; food preservatives; anti-nutrients; phytochemicals; mineral compositions.

1. INTRODUCTION

Leguminous crops such as *Arachis hypogea* are good source of dietary proteins and could also serve as good source of slowly digestible carbohydrates. One of their prominent feature is the involvement in Nitrogen fixation in the soil where they are grown. 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. Leguminous plants are rich source of nutrient molecules such as protein, starch, minerals and vitamins. The presence of important health protective compounds such as phenolics, inositol phosphates and antioxidants may be found in them. 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 [1].

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 [2-4]. Food processing methods including soaking [5,6] 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 spoilage [7]. 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 [8-12]. Preservation may be of any kind but it should be long lasting for preservation of food and it should be value your money [13-17]. 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 acidulated water. Propyl and Methyl has been used as an anti-microbial preservative in foods, drugs and cosmetics for over 50 years [18-22]. There have been several previous safety assessments undertaken on this substance by several agencies, including FAO/WHO, FDA and FEMA [23-29].

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 phosphide) in *A. hypogaea* also known as peanut. The study aimed at determining the effect of aluminium phosphide on nutrients and anti-nutrients levels of legumes of *A. hypogaea* as a case study. To determine the effect of aluminium phosphide on anti-nutritional factors, some mineral profile and proximate analysis of *A. hypogaea*.

2. MATERIALS AND METHODS

2.1 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 *A. hypogaea* was then pounded, blended and pulverized into fine powder. The fine powder was used for the analysis.

2.2 Sample Preservation

The sample of *A. hypogaea* was divided in to two portions and labelled "preserved with AlP" (Aluminium phosphide) and the second labelled "preserved without AlP". One tablet of AlP; a synthetic preservative was inserted into the sample labelled preserved with AlP while the second sample was devoid of AlP preservative. Both were preserved for one month (30 days) after which they were blended using a mechanical blender and analysed for nutritional and anti-nutritional indices.

2.3 Determination of Proximate analysis

2.3.1 Determination of moisture content

Determination of moisture content was carried by the method of [30]. Dry matter was determined gravimetrically as the residue remaining after drying at 103^0C in a ventilated oven.

2.3.2 Determination of ash content

Ash content was determined by gravimetric method according to [30] using this equation:

$$\% \text{ Ashe} = \frac{W3-W1}{W2-W1} \text{ X } 100$$

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).

2.3.3 Determination of crude protein

Crude protein was determined using Macro Kjeldahl Method [30] using this formula:

Percentage nitrogen (%N) = (Vs-V_b) x M (HCl) x 1 x 14.007/ (W x 10)

Where, Vs = ml HCl needed to titrate sample, V_b = ml HCl needed for the blank test M (HCl) = molarity of HCl, 1 = the acid factor, 14.0076 = molecular weight of N, 10 = conversion from mg/g to % , W = weight of the sample (g) and % protein = N×F where F is a factor equal to 6.25.

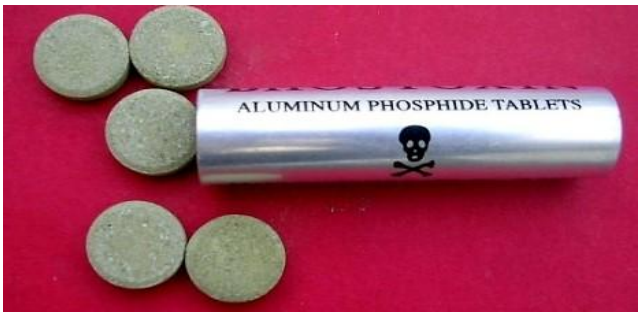

Fig. 1. Aluminium phosphide tablets

2.3.4 Determination of crude lipid

The crude lipid was determined using petroleum ether extract [30] with the relation

$$\% \text{ crude fat} = W_3 - W_2 \times 100/W_1$$

Where W_1 = initial sample weight in grams, W_2 = tare weight of flask in grams, W3 = weight of flask and fat residue in grams.

2.3.5 Determination of crude fiber

Crude fiber was determined by filtration method (ISO 6865, 2000) using

$$\text{Percent crude fibre (\%CF)} = (W_2 - W_3) \times 100 / W_1$$

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)

2.3.6 Determination of Carbohydrate Content

The carbohydrate content was calculated by subtracting the summed up percentage compositions of protein, lipid, fiber, moisture and ash contents from 100 [30]. % C = 100 - (%P + %F + %A + %W + %Fi)

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

2.3.7 Determination of Mineral composition

The mineral analysis was determined in accordance to the method described by [30]. 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.

2.4 Determination of Anti-nutritional Factors

2.4.1 Determination of phytate content

1ml of the sample was added in a clean dry test tube and in another test tube for blank 1ml of D/H_2O was added. 3mls of copper acetate buffer was added in the test tube and blank. 0.56mls of 5% ammonium molybdate was added and mixed thoroughly. About 0.5mls of ethanol was added (freshly prepared before used) and allowed to stand for 5minutes. Absorbance was read at 519nm against a blank (distilled water) in a spectrophotometer (Atomic Absorption spectrophotometer –AAS Model SP9) using

$$\% \text{ phytate} = \frac{au}{as} \times \frac{C}{W} \times \frac{Vf}{Va} \times \frac{100}{1}$$

Where: au = Absorbance of test sample, as = 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

2.4.2 Determination of cyanide content

10 mls of distilled H_2O and 10mls of stock solution of potassium was added in beaker No.1, mixed thoroughly and transfer 10mls to beaker No.2 by serial dilution up to the last beaker No.6 where 10ml was discarded from beaker No.6 then warmed on a water bath including the blank for 10-15mins. The absorbance was taking at 490nm wave length for three (3) times then the average was recorded for each beaker, hence compared to the values of the samples tested for the concentration of cyanide. Cyanide was expressed as mg per kilogram of the sample.

$$\text{HCN (mg/kg)} = 1000 \times 0.05 \times W \times \frac{au}{as}$$

Where: W = Weight of sample, au = absorbance of the test sample, as = absorbance of standard solution

2.4.3 Determination of oxalate content

Determination of Oxalate was carried out according to [30] using

$$\% \text{ oxalate} = \frac{Vt}{Ws} \times Vme \times Titre \times 100$$

Where: V_t = total volume of titrate = 100, W_s = weight of sample = 2g EQU, Vme = volume – mass equivalent.

2.4.4 Determination of tannins content

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

$$\% \text{ Tannins} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{vt}{va}$$

Where: W = weight of sample, au = absorbance of test sample, as = absorbance of standard tannin solution, C = concentration of standard tannin solution, Vt = total volume of extract, Va = volume of extract analysed.

2.4.5 Determination of saponin content

Saponin level was done by the double solvent extraction gravimetric method [31] updated 2018 in Toxicology Laboratory N.V.R.I. Vom, Jos Plateau state, Nigeria using

$$\% \text{ Saponin} = \frac{W2-W1}{W} \times 100$$

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

2.4.6 Determination of alkaloids level

Alkaloids level was determined by the alkaline precipitation gravimetric method [31] was used. The weight of alkaloid was determined and expressed as a percentage of the sample

$$\% \text{ Alkaloid} = \frac{W2-W1}{Weight\ of\ sample} \times 100$$

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

2.5 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 AND DISCUSSION

3.1 Results

3.1.1 Proximate composition of Aluminium phosphide and Non-aluminium phosphide preserved *A. hypogaea*

Results of the proximate analysis of the samples is shown in Fig. 1. Results obtained showed significant difference (P<0.05) in all the nutrient compositions between the non-aluminium phosphide preserved (unpreserved) and the Aluminium phosphide (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 when compared to the other proximate compositions.

3.1.2 Mineral content of Aluminium phosphide and Non-aluminium phosphide preserved *A. hypogaea*

Results of the analysis of mineral content in the sample is shown in Fig. 2. Results obtained showed significant difference (P<0.05) in all the minerals between the non-aluminium phosphide preserved (unpreserved) and the Aluminium phosphide (preserved) samples except for phosphorus (65.00±0.06, 63.00±0.06) respectively where the difference in concentration was not statistically significant (P>0.05). The unpreserved samples maintained higher levels of minerals than the preserved ones.

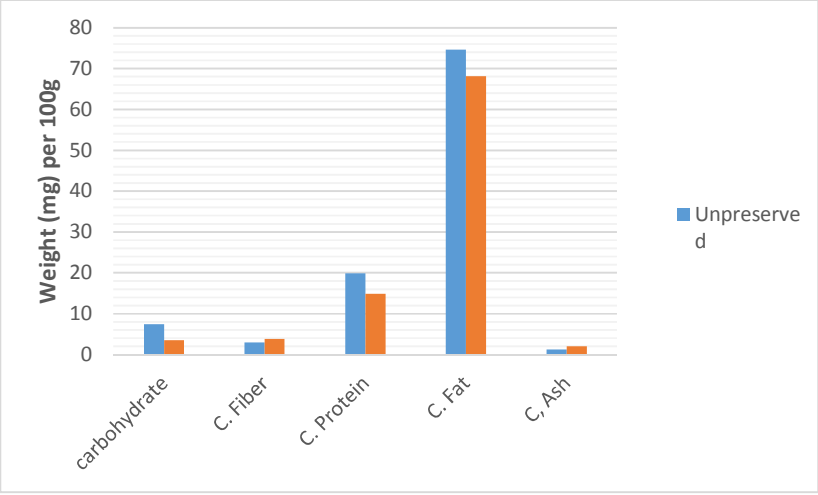

Fig. 1. Effect of aluminium phosphide (APP) on proximate composition of *Arachis hypogaea*

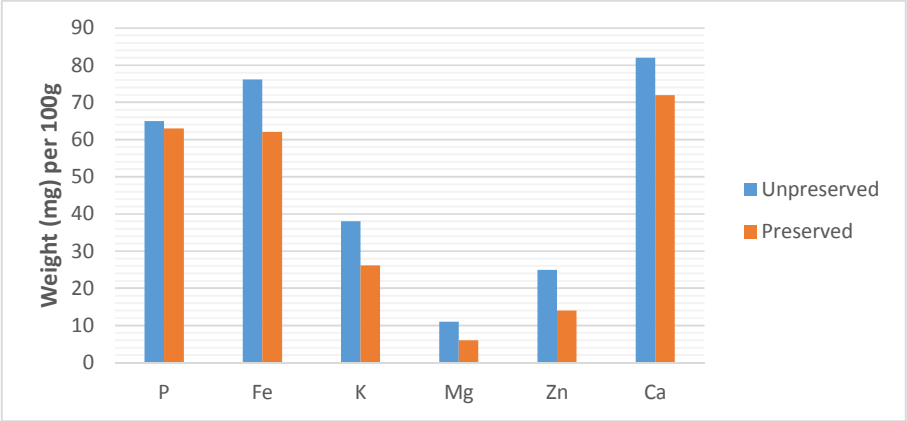

Fig. 2. Effect of Aluminium phosphide (APP) on mineral profile of *Arachis hypogaea*

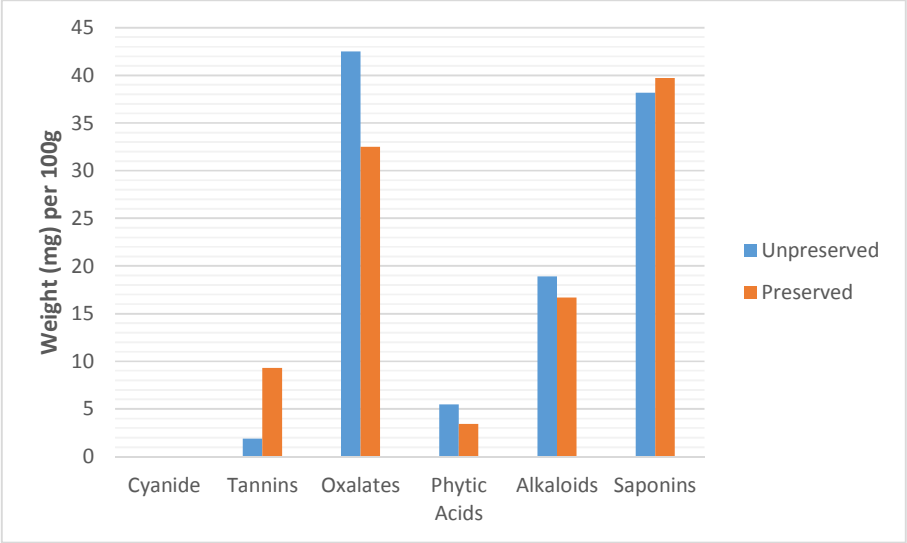

Fig. 3. Effect of APP on anti-nutrient factors of *A. hypogaea*

3.1.3 Anti-nutritional composition of Aluminium phosphide and Non-aluminium phosphide preserved *A. hypogaea*

Fig. 3 shows the results of the analysis of anti-nutrients in the analysed sample of *A. hypogaea*. The levels of cyanide for both the non-aluminium phosphide preserved (unpreserved) and the Aluminium phosphide (preserved) samples were infinitesimal. Oxalates, phytates and alkaloids levels showed significant difference ($P<0.05$), been higher in the non-aluminium phosphide (NAP) preserved samples. On the contrary, tannin and saponins levels were higher in the Aluminium phosphide (APP) preserved samples.

3.2 Discussion

In this study, we used Aluminium phosphide to preserve a certain portion of *A. hypogaea* samples and preserved another portion of the *A. hypogaea* without adding any preservative, this was to enable us compare the effect of the preservative on the nutritional composition of the legume by comparing the results of analysis of the two categories of samples.

The proximate analysis of the APP *A. hypogaea* seeds has been studied which showed significant decrease ($p<0.05$) in fat thereby making 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 200 mg/kg [32]. 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 oxidative phosphorylation and ATP production is stopped, intracellular oxygen utilization ceases, cell is then forced to use anaerobic metabolism which could lead to lactic acid 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 [33-35]. High level of alkaloids exerts toxicity and adverse effects to human, especially in physiological and neurological activities.

4. CONCLUSION

The study demonstrated that application of the artificial preservative; Aluminium phosphide (AlP) to preserve *A. hypogaea* resulted in a significant($p<0.05$) increase in anti-nutrient factors such as phytic acid, oxalates, tannins, saponins and alkaloids but no significant increase or decrease in cyanide activity was observed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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