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Evaluation of Mineral Composition, Antinutritional Parameters and Microbial Count of Fermented *Prosopis africana* Seeds (*OKPEHE*)

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ABSTRACT
Boiled and fermented seeds of *Prosopis africana* were evaluated for mineral composition, anti-nutritional contents and microbial count were determined at 0 hr, 24 hrs, 48 hrs and 72 hrs of fermentation using standard procedures. The data obtained were subjected to descriptive statistics and one way ANOVA. The result of the quantitative phytochemical analysis showed general reduction in the entire anti- nutrients constituents as the fermentation progressed from 0 hr to 72 hrs. The Alkaloids content decreased significantly ($p < 0.05$) from 0.38mg/100g at zero hour to 0.13mg/100g at 72 hours. The Flavonoids content decreased significantly ($p < 0.05$) from 0.34mg/100g at zero hour to 0.17mg/100g at 72 hours. The mean Steroids content decreased significantly ($p < 0.05$) from 0.41mg/100g at zero hour to 0.21mg/100g at 72 hours. The saponins content decreased from 0.93mg/100g at zero hour to 0.55mg/100g at 72 hours. The resins content decreased from 0.87mg/100g at zero hour to 0.38mg/100g at 72 hours. The phenols content decreased significantly ($p < 0.05$) from 0.51mg/100g at zero hour to 0.22mg/100g at 72 hours. Tannis was not detected at any stage of fermentation and oxalate content decreased significantly ($p < 0.05$) from 0.13mg/100g at zero hour to 0.02mg/100g at 72 hours while results of minerals composition of *Okpehe* contained; Calcium (36.82ppm), Potassium (14.49ppm), Sodium (2.48ppm), iron (0.75ppm), copper (0.33ppm), Lead was not detected, cadmium (0.32ppm), nickel (0.03ppm) chromium were (0.01ppm). The bacterial load ranged between mean $\log_{10}$ (5.74- 5.92) cfu/g while Fungal count ranged between mean $\log_{10}$ (5.56-5.73) cfu/g after 72 hrs of spontaneous fermentation.
Keywords: *Prosopis africana* seeds, Fermentation, anti-nutrients, *Condiment*, legume, phytochemical

INTRODUCTION
Fermented *Prosopis africana* seed known as *Okpehe* by the Idoma people of North central of Nigeria is a very popular condiment traditionally prepared from *Prosopis africana* seeds. *Prosopis africana* is one of the lesser known leguminous seed crops used as food condiment in Nigeria (Asoiro and Ohagwu, 2017). *Prosopis* tree is a wild legume and many of these wild legumes are known for their

inexpensive proteins, essential amino acids, essential fatty acids, fiber, high calorific value and vitamins; but the presence of anti-nutrients in the seeds has limited their use (Bhat & Karim, 2009). Anti-nutritional factors are chemical compounds found in plants and food substances in general. They are poisonous to humans or in some ways limit nutrients availability to the body (Umar *e t al.,* 2013). Anti-nutritional factors are present in different plants substances in varying concentration depending on the kind of plants, mode of its propagation, chemicals used in growing the crop as well as those chemicals used in storage and preservation of the food substances. Anti-nutritional factors are known to interfere with metabolic processes such that growth and bioavailability of nutrients is negatively influenced (Umar *e t al.,* 2007). Anti-nutritional factors reduce the nutrient utilization intake of plants or plant products used as human foods or animal feeds and they play a vital role in determining the use of plants for humans and animals consumption (Ferguisin *et al.,* 1993) The effect of anti-nutritional factors, otherwise known as plants' secondary metabolites has not been properly addressed in most parts of the developing world. People have died out of ignorance, poverty and inadequate nutrition information and education, especially within sub-Saharan African regions. There are reports from time to time of deaths after consumption of some type of beans despite thorough cooking. Also, cases of renal and liver diseases are increasing and this call for a need to properly address the issue of thorough and inadequate processing of foods before consumption. The knowledge that these compounds elicit both toxic and advantageous biological responses has given rise to several investigations in recent times as to their possible physiological implications in various biological systems. These works thus aim at investigating the effect of fermentation on the mineral composition, anti-nutrients factors and microbial count of fermented *Prosopis africana* seeds (*Okpehe*).

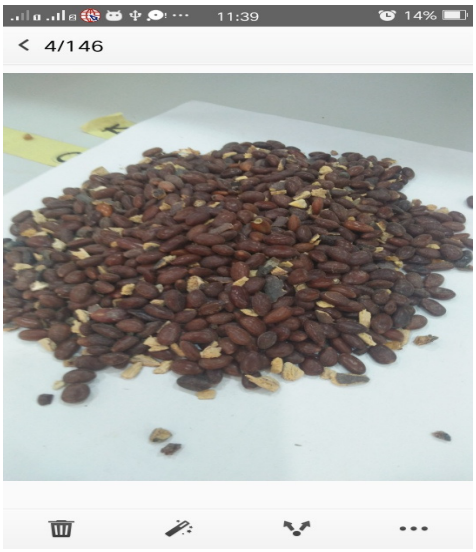
MATERIALS AND METHODS

Sources of Seeds and Authentication

One kilogram (1kg) of *Prosopis africana* seeds were purchased from the Brigade market in Kano State of Nigeria. The *Prosopis* seeds were

identified with a herbarium voucher number of BUKHAN 0193 at the Department of Plant Biology, Bayero University, Kano. *Prosopis africana* seeds were transported to the Department of Microbiology, Bayero University, in polythene bag for investigations.

Prosopis africana Tree



Prosopis africana seeds

Spontaneous Preparation of "*Okpehe*" from *Prosopis africana* Seeds

Three hundred grams (300g) of dried seeds of *Prosopis africana* was soaked overnight and later boiled in a large earthen-ware pot for about 1-2 days with fire wood, during which the seeds coats was soft and the seeds swollen. Seeds coats were removed by pressing between fingertips. The seeds coats were later decanted along with the washing water, leaving the clean seed cotyledons. The cotyledons were boiled for another 1-2 hours. The cotyledons were later drain through a sieve and wrapped with banana leaves. The wrapped cotyledons was stacked together and then covered with layers of sterile aluminium foil and kept in an incubating unit for about three days to produce the fermented *Prospis* mash – a strong-smelling mass of sticky brown cotyledons. (Ogunshe *e t al.*, 2007). The fermenting *Prosopis africana* seeds were removed for investigation at selected times of fermentation, 0, 24, 48 and 72 hours

Quantitative Determination of Mineral Contents and anti-nutrients screening of fermented *Prosopis africana* seeds

The mineral contents of samples were determined using X-Ray Fluorescence (XRF) spectroscopy method as described by Funtua (1999) and phytochemical analyses were performed using standard procedure ((Omolara and Ibrahim, 2014). fungal enumeration were carried out on Potato Dextrose Agar (PDA) incubated at 27±1°C for 72hrs and enumeration of Bacteria on nutrient agar using standard procedure.

RESULTS AND DISCUSSION

The result of the various analyses carried out on fermented seed s of *Prosopis africana* were presented below.

Mineral Composition of Fermented *Prosopis africana* seeds (*Okpehe*)

The mineral compositions of the *Okpehe* were shown in Figure 1. The results of minerals composition (Figure 1) shows that calcium was found to be the highest mineral observed. The presence of calcium in *P. africana* seed is good because Calcium in conjunction with other minerals is involved in bone formation. Potassium and sodium was observed in appreciable amounts (K=14.49ppm, Na=2.48ppm). The results were contrary to the report of Aremu *e t al.* (2006) who reported that potassium was the most predominant mineral in agricultural products, however is in agreement with the work of Umar *e t al.* (2008) they reported that calcium, is the most abundant mineral in *Okpehe*. The appreciable amount of potassium in this seed indicated that the seeds can be utilized beneficially in the diets of people who take diuretic medicines for the treatment of hypertension and peoples who suffered from excessive loss of potassium through the body fluid (Aremu *e t al.*, 2015).. From the study, concentration of iron was (0.75ppm) from fermented *Okpehe* seeds. Balogun (2012) detected iron in moderate amounts, 10.1mg/100g at zero time and 10.2mg/100g after 72 hours in *Okpehe*. Anhwange (2008) reported that, Iron carries oxygen to the cells and is necessary for the production of energy, synthesis of collagen and the proper functioning of the immune system. The amounts of cadmium (0.32ppm), nickel (0.03ppm) and chromium (0.01ppm) were

within the permissible limits (< 1) as reported by Adepoju-Bello *e t al.* (2012). It should be noted that these heavy metals is highly detrimental to humans when their concentrations exceeds tolerable limits by humans. Copper was detected (0.33ppm). Copper was among the minerals present in *P. africana* flour (Aremu *e t al.,* 2006). Copper is a trace element that serves as a co-factor and is required for enzyme function. Adejoh (2016) reported that copper is an important metal required by the human body for mental and physical development which is up taken through vegetarian foods such as nuts, seeds and grains.

Lead was not detected in fermented *Prosopis africana* seeds. Similar result was also obtained by Aremu *e t al.* (2006) for *P. africana* flour. This element is known to be toxic to the human body.

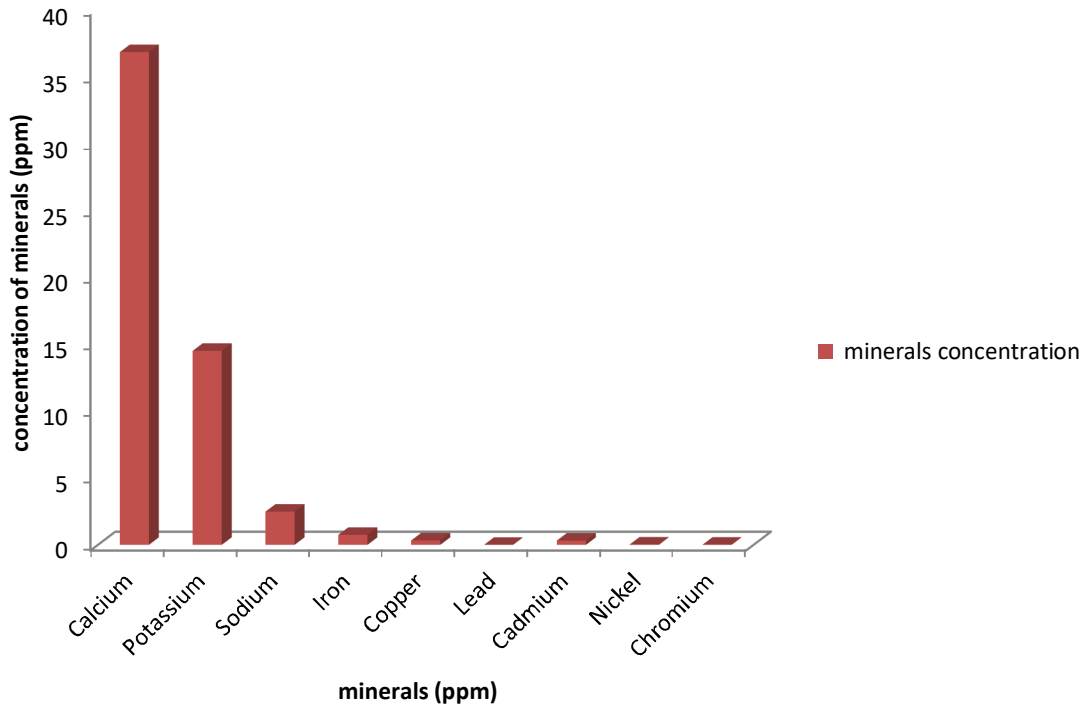


Figure 1: Minerals composition of fermented *Prosopis africana* seeds

The result of the quantitative anti-nutrients analysis (Table 1) shows general reduction in the entire anti- nutrients constituents as the

fermentation progressed from 0 hr to 72 hrs in the *Okpehe*, this could be attributed to the activities of the indigenous microbes as well as processing methods such as cooking and washing which could initiate the activities of some indigenous enzyme that degrade these anti-nutrients during fermentation (Mubarak, 2005). The reduction in concentration of anti-nutrients during fermentation was in line with the report of Aro *e t al.* (2008) on cassava starch residues and that of Oboh and Oladunmoye (2007). A significant decrease was observed in all the anti-nutrients content from 0 hr to 72 hrs in the *Okpehe* ($p<0.05$).

Knowledge of phytate levels in food is necessary because a high content can cause harmful effects on digestibility. Phytate has been recognized as an anti-nutrient due to its adverse effects. It reduced the bioavailability of minerals and caused growth inhibition. Phytate is capable of chelating divalent cationic minerals like calcium, iron, magnesium and zinc thereby inducing dietary deficiency (Airaodion *e t al.*, 2019a). Airaodion (2019a) also, suggested that the solubility of phytate and proportion of minerals bound to the complex depend on dietary calcium levels. Saponins are phyto-nutrients found in particular abundance in various plant species and are grouped by the soap-like foaming they produce when shaken in aqueous solution. Saponins when present in large amounts impart bitter taste to plant food and induce haemolysis and cholesterol binding (Bolarinwa *e t al.*, 2017).

Flavonoids have been reported to be an important phyto-chemical due to its antioxidant properties (Airaodion *e t al.*, 2019b). Some plants have been reported to prevent ulcer due to their flavonoid content (Airaodion *e t al.*, 2019b). Tannins have been reported to form complexes with proteins and reduce their digestibility and palatability (Eka, 2005). They also bind iron, making it unavailable (Airaodion *e t al.*, 2019). However, tannins are water soluble compounds and as such can be eliminated by soaking followed by cooking (Singh, 2008). The tannins content in *Okpehe* was below the detrimental dose of 0.7–0.9% (Airaodion *e t al.*, 2019c). These anti-nutrients form complexes with metals. The lower concentration of the anti-nutrients in *Okpehe* may

enhance absorption when they form complexes with metals. The values of alkaloids observed (Table 1) were relatively lower when compared with literature values for similar plants. Alkaloids have been reported to cause gastrointestinal upset and neurological disorders especially when taken in excess. This implies that these anti-nutritional factor needs to be eliminated during processing before consumption. Phenols have been shown to demonstrate antimicrobial properties (Mace *e t al.*, 2017) while resins and steroids may either be beneficial or detrimental (De and Pal, 2014). These factors may however not perform significant functions since they occur in inconsiderable amounts in *Okpehe* analyzed. Despite their toxicity level.

The results of mean viable cell counts during spontaneous fermentation of *Prosopis africana* seeds to produce *Okpehe* were presented in figure 2. An increase in aerobic mesophilic bacterial and fungal counts was observed during the spontaneous fermentation of *Prosopis africana* seeds to produce *Okpehe* (figure 2). Bacteria count ranging from 5.5 x 10^5 at ohr to 8.3 $x10^5$(cfu/g) at 72hrs while Fungi counts ranging from 3.6 x 10^5 at ohr to 5.4 $x10^5$(cfu/g) at 72hrs. Yabaya (2006) reported an increase in the microbial count from 2.8 $\times$ 10^7 at 24hr to 1.8 $\times$ 10^{10} at 72hr of fermentation and attributed it to the breakdown of protein, lipid, starch and other nutrients to their simpler forms which the organisms use as their source of carbon and nitrogen. Bukar and Mohammad (2017) also observed an increase of microbial load from 4.49 *log* to 4.70 *log* counts, but was slightly lower than the result that has been reported in this study which observed an increase from 1.25 - 1.22 during the course of the fermentation. This shows that even though precautions have been taken quite seriously, it is difficult to eliminate contamination completely under the spontaneous fermentation process.

	Anti-nutrients parameters (mg/100g)							
Time of fermentation	Alkaloids Content	Flavonoids Content	Steroids content	Saponins Content	Resins content	Phenols Content	Tannins Content	Oxalates content
0hr	$0.38^{a}\pm0.010$	$0.34^{a}\pm0.012$	$0.41^{a}\pm0.007$	$0.93^{a}\pm0.009$	$0.87^{a}\pm0.003$	$0.51^{a}\pm0.007$	0.00 ± 0.00	$0.13^{a}\pm0.00$
24hrs	$0.27^{b}\pm0.007$	$0.27^{b}\pm0.006$	$0.33^{b}\pm0.009$	$0.86^{b}\pm0.012$	$0.73^{b}\pm0.009$	$0.37^{b}\pm0.007$	0.00 ± 0.00	$0.08^{b}\pm0.003$
48hrs	$0.22^{c}\pm0.003$	$0.21^{c}\pm0.007$	$0.27^{c}\pm0.007$	$0.71^{c}\pm0.007$	$0.47^{c}\pm0.007$	$0.28^{c}\pm0.003$	0.00 ± 0.00	$0.04^{c}\pm0.003$
72hrs	$0.13^{d}\pm0.007$	$0.17^{c}\pm0.007$	$0.21^{d}\pm0.007$	$0.55^{d}\pm0.007$	$0.38^{d}\pm0.003$	$0.22^{d}\pm0.003$	0.00 ± 0.00	$0.02^{d}\pm0.003$
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 1: **Changes in anti-nutrients parameters during fermentation of *Prosopis africana* seeds**

$p \leq 0.05$

Values are means ±SE of three replicate fermentations. Means in the same column with the different superscript are statistically significant.

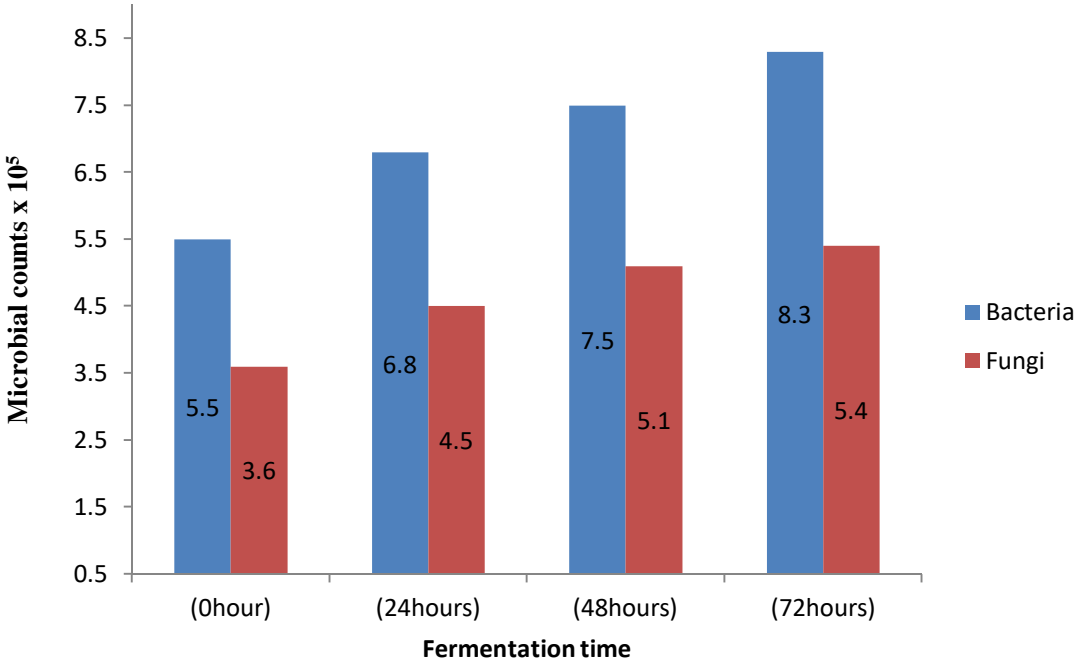


Figure 2: Mean aerobic bacteria and Fungi counts (cfu/g)

CONCLUSION AND RECOMMENDATION

This work has shown that fermentation leads to appreciable reduction in the amount of anti nutritional factors present in the fermented seeds, while improving the minerals availability of the fermented products. Fermented *Prosopis africana* seeds can thus be seen as functional foods, having high medicinal values in addition to the well-known and well established nutritional values.

REFERENCES

Adejoh, I.P. (2016). Assessment of heavy metal contamination of soil and cassava plants within the vicinity of a cement factory in north central, Nigeria. *Advances in Applied Science Research*, **7**(3):20-27.

Adepoju-Bello, A.A.; Oguntibeju, O.O., Onuegbu, M.T., Ayoola, G.A.A. and Coker, H.A.B. (2012). Analysis of selected metallic

impurities in soft drinks marketed in Lagos, Nigeria. *African Journal Biotechnology,* **11**, 4676–4680.

Airaodion, A.I., Ibrahim, A.H., Ogbuagu, U., Ogbuagu, E.O., Awosanya, O.O., Akinmolayan, J.D., Njoku, O.C., Obajimi, O.O., Adeniji, A.R. and Adekale, O.A. (2019b). Evaluation of phytochemical content and antioxidant potential of *Ocimum gratissimum* and *Telfairia occidentalis* leaves. *Asian Journal of Research in Medical and Pharmaceutical Sciences,* **7**(1):1-11.

Airaodion, A.I., Ogbuagu, U., Ogbuagu, E.O., Airaodion, E.O., Agunbiade, A.P., Oloruntoba, A.P., Mokelu, I.P., Ekeh, S.C. (2019c). Investigation of aqueous extract of *Zingiber officinale* root potential in the prevention of peptic ulcer in albino rats. *International Journal of Research and Innovation in Applied Science,* **4**(2):64-67.

Airaodion, A.I., Olatoyinbo, P.O., Ogbuagu, U., Ogbuagu, E.O., Akinmolayan, J.D., Adekale, O.A., Awosanya, O.O., Agunbiade, A.P., Oloruntoba, A.P., Obajimi, O.O., Adeniji, A.R. and Airaodion, E.O.(2019a). Comparative assessment of phytochemical content and antioxidant potential of *Azadirachta indica* and *Parquetina nigrescens* leaves. *Asian Plant Research Journal;* **2**(3):1-14.

Aremu, M. O., Awala, E.Y., Opaluwa, O.D., Odoh, R. and Bamidele, T. O. (2015). Effect of Processing on Nutritional Composition of African Locust Bean (*Parkia biglobosa*) and Mesquite Bean (*Prosopis africana*) Seeds. Communications in Applied Sciences, **3**(1):22-41

Aremu, M. O., Olonisakin, A., Atolaye, B. O. and Ogbu, C. F. (2006). Some nutritional and functional studies of *Prosopis africana. Electronic Journal of Environment, Agriculture and Food Chemistry* 5(6): 1640-1648.

Asoiro, F.U. and C. J. Ohagwu, C. J. (2017). Some moisture dependent thermal properties and bulkdensity of Prsopis African a seeds. Nigerian ***Journal of Technology,*** *Vol.ume 36, No. 3, pp.* 936 – 943.

Balogun, M.A. (2012). A Fermentation study and Physicochemical Analy sis of *Prosopis africana* seeds to produce okpehe, a Local Condiment. University of Ilorin Institutional Repository 24-60.

Bhat, R and Karim, A. A., 2009. Exploring the Nutritional Potential of Wild and Underutilized Legumes. *Comp. Rev. Food Sci. & Food Safety.* 8, (4): 305 – 331.

Bolarinwa, I.F., Ogunleye, K.Y. and Rasheed, G.A. (2017).Effect of processing on beta-carotene content and other quality attributes of cassava flakes (Gari) produced from yellow cassava varieties. *Nigeria Food Journal,* **82:25** 34.

De, P.K. and Pal, A. (2014). Effects of aqueous young leaves extract of *Mangifera indica* on gram-negative microorganisms causing gastro-intestinal disorders. *Asian Journal of Plant Science and Research* **4:** 23-27.

Eka, O.U.(2005). The chemical composition of yam tubers. In: Advances in yam research.The biochemistry and technology of yam tuber. Biochemical Society of Nigeria Enugu, Nigeria, **1:**51–75.

Fergusin, E. L., Gibson, R.A., Opara-obisaw, O., Stephen, A.M. and Thomson, L. U. (1993). The Zinc, calcium, copper,magnesium, non-starchpolysaccharide and phytate content of seventy eight locally grown and prepared African foods. *Journal of Food Analysis.* **6**: 337-342.

Funtua, I. I. (1999). Application of the transmission-emission method in EDXRF for the determination of trace elements in geological and biological materials. Trace Microproble Technology, **17:**293-297.

Macé, S., Hansen, L.T. and Rupasinghe, H.P.V. (2017) Anti-Bacterial Activity of Phenolic Compounds against Streptococcus pyogenes. Medicines **4:** 25.

Mubarak, A.E.(2005). Nutritional composition and anti nutritional factor of mung beans (*Phaseolus aureus*) as affected by some home traditional processes. Food Chemistry.**89:**489-495.

Oboh, G. and Oladunmoye, M.K. (2007). Biochemical changes in micro fungi fermented cassava flour produced from low and medium cyanide variety of cassava tubers. Nutrition and Health.**18**:355-367.

Ogunshe, A. A. O., Omotosho, M. O. and Ayanshina, A. D. V. (2007). Microbial studies and biochemical characteristics of controlled fermented afiyo- a Nigerian fermented food condiment from Prosopisafricana (Guill and Perr.) Taub. Pakistan *Journal of Nutrition* **6** (6):620-627.

Omolara, o.o. and Ibrahim O. B. (2014).Ant- nutritional and phytochemical evaluation of raw and fermented African locust beans(*Parkia biglobosa*) seeds .*Global journal of pure and applied sciences,* **20**: 105-109.

Singh, U. (2008). Anti-nutritional factors of chickpea and pigeon pea and their removal by processing. Plant Foods for Human Nutrition, **38**:251-261.

Umar, K.J., Hassan, L.G., Usman, H. and Wasagu, R.S.U. (2013). Nutritional Composition of the Seeds of Wild Melon (Citrullus ecirrhosus). *Parkistan Journal of Biological Sciences,* **16**: 536 -540.

Umaru, H.A., Adamu, R., Dahiru, D. and Nadro. M.S. (2007). Level of Antinutritional Factors in Some Wild Edible Fruits of Northern Nigeria. *African Journal of Biotechnology* **6**(6): 1935 – 1938.

Wasagu, R.S.U., Lawal, M., Shehu, S., Alfa, H.H. and Muhammad, C. (2013). Nutritive values and Antioxidant properties of Pistiastratiotes (Water lettuce). *Nigerian Journal Basic and Applied Sciences,* **21**(4): 253.