EFFECT OF FERMENTATION ON NUTRITIONAL COMPOSITION OF *PROSOPIS AFRICANA* SEEDS

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Astract

The study examined the effect of fermentation on the proximate, minerals, and amino acids composition of Prosopis Africana seeds. Proximate analysis conducted on the fermented and unfermented seed of Prosopis africana indicated that, fermentation results in increased protein (26.72 ± 0.50%) as against unfermented(16.57± 0.18%), ash content fermented (7.17 ± 0.58%);unfermented (4.50 ± 0.71%), moisture content fermented (13.33 ± 0.76); unfermented (2.67 ± 0.29%). On the other hand the carbohydrate contents of the fermented (22.07%); unfermented (36.59%), crude lipid fermented (8.83 ± 0.76%); unfermented (13.17 ± 0.76%), crude fiber fermented (21.83 ± 0.76%); unfermented (26.50 ± 1.00%) was observed to decreased as a result of the fermentation process. The mineral analysis revealed that the minerals content of the fermented seed such as Potassium, Sodium, Calcium, Magnesium and Phosphorus increased higher than the unfermented. Amino acid analysis shows that, the sample is rich in non-essential amino acid of which Aspartic acid (10.61g/100g) and Glutamic acid (14.54g/100g) are the predominant, while Leucine (7.21g/100g), lycine (4.32g/100g), and Valine (4.34g/100g) are the dominant essential amino acids present in the seeds. The amino acids score shows that isoleucine (126.8), leucine (109.2), threonine (102.9), and valine (124.0) are above the reference standard. The results generally indicate that fermented seed of Prosopis africana is a good source of mineral elements, crude protein, and some essential amino acids and as such if properly utilized it could supplement the body with some of the basic requirements.

Keywords: *Prosopis africana*, Mineral elements, Protein, Fermentation, Amino acids.

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1.INTRODUCTION

Fermentation has been used as a means of improving the quality of food for more than 6000 years. Probably no other process has had such an impact on the nutrition habits and food culture of mankind (Campbell-plat, 1994). Fermented food has been defined by Campbell-Platt (1987) as a food which has been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food. However, to the microbiologist, the term" fermentation" describes a form of energy-yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is incompletely oxidized, and an organic carbohydrate acts as the electron acceptor (Adams, 1990).

Fermentation process preserves and enriches food, improves digestibility and enhances the taste and flavor of the foods (Motarjemi, 2002). It is also an affordable process and therefore accessible to all population. Furthermore, fermentation has the potential of enhancing food safety by controlling the growth and multiplication of a number of pathogens in food. Its importance in modern-day life is underlined by a wide spectrum of fermented foods marketed both in developing and industrialized countries (Holzapfel, 2002).

Prosopis africana is a leguminous plant of the Fabaceae family. It is one of the about 45 species of the genus *prosopis*. It is a flowering plant that is locally called "Kiriya" in Hausa, "Gbaaye" in Tiv and "Ayan"in Yoruba languages of Nigeria. The trees of *Prosopis africana* are common in the middle belt, north and some parts of eastern and southern parts of Nigeria (Aremu *et al*., 2006). It grows to a height of 4-20m, with slightly rounded buttress roots and dark stem bark, which is scaly, slash and has white streaks. It flowers once a year. The fruits are smooth, hard pod-like capsules having banana fruit shape and are brown-black in color. The seeds are many, tiny and very hard, brownish-black in color with high resistance to water and insect attack. It is traditionally used for formulation of animal feeds and preparation of local condiments through boiling and fermentation processes (Aremu *et al*., 2006). The tree is of great economic value to man and animal, it fixes nitrogen to enrich the soil, generates hardy timbers, produces protein rich leaves and sugary pods used as feed stuffs for ruminants (Annongu *et al.*, 2004).

2.MATERIALS AND METHODS

2.1 Sample collection and Treatment

Prosopis africana fruits was purchased from Kara market Sokoto state, Nigeria. The pods were identified at the Botany unit of the Biological science Department, Usmanu Danfodiyo University, Sokoto. The seeds of *Prosopis africana* were extracted by beaten the pods on the concrete floor to break the pod.

2.2 Fermentation process

The traditional method of fermentation used in the production of a Nigerian local condiment *Daddawa* was used in this research work. The seeds were washed with water and spoiled seeds were removed. 300g of the seeds were boiled for 6 hours to soften the seed coat and facilitate extraction of the cotyledons. The seed coats were removed by pressing between fingertips. The cotyledons seeds were then placed into a container and covered with aluminum foil and allowed to ferment for three days. The fermented seeds were then spread on a tray to air dry. The dried products were ground to powder using mortar and pestle and was kept pending the time for the analysis.

2.3 Proximate Analysis

Standards methods of AOAC (1990) were used for the proximate analysis. The moisture content was determine by weighing two grammes (2g) of fresh seed kernel in a crucible and dried in an oven (Gallenkamp, UK) at 105^{o}C for 24 hrs. The dried sample was then cooled in a desiccator for 30 minutes and weighed.

The ash content was determined by the incineration of 2g dried sample in a muffle furnace at 55^{o}C for 2hrs. Crude lipid (CL) was Soxhlet extracted from 2g dried sample with n-hexane for 8hrs.The nitrogen (N) content was estimated by micro-Kjeldahl method and crude protein (CP) content calculated as N% x 6.25. Crude fiber (CF) content was determined by treating 2g dried sample with 1.25% (w/v) H_2SO_4 and 1.25% (w/v) NaOH. The available carbohydrate (CHO) was calculated by subtracting the percentages of ash, crude protein, crude lipid, and crude fiber from 100% moisture free sample.

2.4 Mineral Analysis

Mineral analysis was carried out after sample digestion of 2g of the dried sample with 24cm^3 mixture of nitric acid/perchloric/sulphuric acids in the ratio 9:2:1 respectively. Ca, Mg, Fe, Co, Mn, Cr, Ni, Cu and Zn were determined by atomic absorption spectrophotometry, Na and K by atomic emission spectrometry (AOAC, 1990), and P by the molybdenum blue colorimetric method (James, 1995).

2.5 Aminoacids Analysis

Amino acids composition of the seed kernel was determined using the method reported by Magdi (2004). Duplicate samples were hydrolyzed by transferring 50mg of the sample into a 15ml ampoule, adding 5ml of 6M HCl, sealing the vial under vacuum, flushed with nitrogen, and digesting at 110^{o}C for 24hrs. The sulphur-containing amino acids were determined using perfomic acid. Amino acids analyses were performed by high performance liquid chromatography (Shimadzu, G-C-14A, Kyoto, Japan).

2.6 Statistical Analysis

Data generated in triplicates were expressed as mean ± standard deviation using SPSS version 10 statistical packages.

3.0 RESULTS AND DISCUSSION

3.1 Results

The results of the proximate, mineral and amino acids composition of the fermented and unfermented seeds of *Prosopis africana* are presented in Table 1, Table 2 and Table 3 respectively. From the results, fermented seeds were found to contain high protein, low carbohydrate, high mineral values and higher essential amino acids when compared with the unfermented.

Table 1: Proximate composition of *Prosopis africana* seeds.

Parameters %	Fermented	Unfermented
Moisture content	13.33 ± 0.76	2.67 ± 0.29
Ash content	7.17 ± 0.58	4.50 ± 0.71
Crude protein	26.72 ± 0.50	16.57 ± 0.18
Crude lipid	8.83 ± 0.76	13.17 ± 0.76
Crude fiber	21.83 ± 0.76	26.50 ± 1.00
Carbohydrate	22.07	36.59

The values are mean ± standard deviation of three replicates

Table 2: Mineral composition of *Prosopis africana* seeds.

Mineral element (mg/kg)	Fermented	Unfermented
Calcium	0.42 ± 0.03	0.25 ± 0.05
Magnesium	1.82 ± 0.08	1.53 ± 0.05
Phosphorus	3.54 ± 0.02	3.16 ± 0.03
Sodium	103.33 ± 3.82	75.00 ± 2.50
Potassium	4033.33 ± 152.75	2200.00 ± 100.00

The values are mean ± standard deviation of three replicates

Table 3.a: Essential amino acids (g/100g Protein)

Amino acids	Fermented	Unfermented
Lycine	4.32	3.62
Tyrosine	3.17	2.70
Leucine	7.21	6.01
Isoleucine	3.55	3.23
Methionine	1.30	0.88
Cystine	1.72	1.46
Threonine	3.53	2.98
Valine	4.34	4.03
Phenylalanine	4.30	3.71

Table 3.b: Non-Essential Amino Acids (g/100g Protein)

Amino acids	Fermented	Unfermented
Proline	3.86	3.46
Glycine	4.08	3.60
Arginine	5.52	4.75
Aspartic acid	10.61	9.86
Glutamic acid	14.54	13.63
Alanine	4.33	4.03
Histidine	2.27	2.02
Serine	4.01	3.53

Table 4: Amino Acid Scores of Fermented and Unfermented seeds of *Prosopis africana*

Amino acids	Fermented	Unfermented	Reference Value
Lycine	4.32(74.5)	3.62(62.4)	5.8
Tyrocine	3.17(50.3)	2.70(42.8)	6.3
Leucine	7.21(109.2)	6.01(91.1)	6.6
Isoleucine	3.55(126.8)	3.23(115.4)	2.8
Methionine	1.30(52.0)	0.88(35.2)	2.5
Cystine	1.72(68.8)	1.46(58.4)	2.5
Threonine	3.53(102.9)	2.98(87.6)	3.4
Valine	4.34(124.0)	4.03(115.1)	3.5
Phenylalanine	4.30(68.3)	3.71(58.9)	6.3
Scores	4/9	2/9	

Table 3.a and 3.b gives the Amino Acid Profile of Fermented and Unfermented seeds of *Prosopis africana* (g/100g protein) respectively. Table 4 gives the amino acid scores of Fermented and Unfermented seeds of *Prosopis africana* (g/100g protein).

3.2 Discussions

The results of proximate composition of the fermented and unfermented *Prosopis africana* seeds obtained in this research work indicated that the fermented seeds contain higher crude protein (26.72 ± 0.50%) when compared with the unfermented (16.57 ± 0.18%). The increase could be as a result of the activity of fermentative microbes and protein hydrolysis (Fadahunsi *et al*., 2010). The increase concurs with the finding of Balogun (2011) in the production of Okpehe (daddawa) from *Prosopis aricana* seeds.

The carbohydrate content of the fermented seed was observed to decrease (22.07%) when compared with the unfermented (36.59%).The decrease in the carbohydrate content of the fermented sample may be due to hydrolysis and subsequent utilization of sugar from the carbohydrate (Sani *et al*., 2013). The observed decrease is in agreement with the findings of Achinewu (1986), Fadahunsi and Sani (2010) and Sani *et al*., (2013) who observed a decrease in crude fiber and carbohydrate content. Therefore, fermentation is a good way of reducing carbohydrate content of the seed making it relatively safe for people requiring less sugar food.

Crude lipid of the fermented seed was observed to decreased (8.83 ±0.76%) when compared with the unfermented (13.17±0.76%). The reduction in the crude lipid indicates that the organism used for the fermentation has in addition to sugar, utilized fatty acid as its source of energy (Sani *et al*., 2013). Similar decrease was observed by Sani *et al*., (2013) on the fermentation of *Lagenaria Siceraria* Seeds.

The ash content of the fermented seed was observed to be higher (7.17±0.58%) when compared with the unfermented seed (4.50±0.71%), which is an indication that, fermented seed is rich in minerals. Similar increase in ash content was reported by Gernah *et al*., (2005) after 72 hours of fermentation of iru. This increase may be due to contribution by fermenting microorganisms (El Faki *et al*., 1991). The same trend was observed on the effect of fermentation on the nutrient status of locust bean where an increase of about 30% in ash content was recorded after fermentation (Eka, 1980).

The mineral composition of the fermented and unfermented *Prosopis africana* seed from Table 3.2 indicated that, the fermented seeds contain higher mineral values. Potassium was determined to be the highest mineral presence in the fermented seed (4033.33±152.75mg/kg) as against (2200.00±100.00mg/kg) in the unfermented. The significant amount of potassium presence in the fermented sample indicated that is a good source of potassium which helps in regulation of body fluids and maintenance of body pressure. It helps in controlling kidney failure and respiratory defect (Anhwange, 2008). The high content of potassium in the fermented seed also agrees with the findings of Aremu *et al*, (2006) that, potassium was the most abundant mineral in *Prosopis africana* seed.

Higher content of Calcium in the fermented seed (0.42 ± 0.03mg/kg) as against unfermented (0.25 ± 0.05mg/kg) is an indication that, fermenting a sample increases the amount of Calcium which in turn is needed for bone development and strong teeth (Aremu *et al*., 2006).The observed increase is in agreement with the findings of Makinde *et al*., (2013) on fermentation of *Sesame* seeds.

The sodium content of the fermented seed (103.33 ± 3.82mg/kg) appears to be higher compared to the unfermented (75.00 ± 2.50mg/kg). Sodium is very important for osmoregulation and fluid maintenance within the body.

Phosphorus content was observed to be higher in the fermented seed (3.54 ± 0.02mg/kg) when compared with the unfermented (3.16 ± 0.03mg/kg). Phosphorus is needed in the body for teeth and bone development (Makinde *et al*., 2013). The fermented samples could therefore be referred to as good sources of and Phosphorus. The observed increase is in agreement with the finding of Makinde *et al*. (2013) on fermentation of *Sesame* seeds. Magnesium content of the fermented seed was determined to be higher than the unfermented.

The result of the amino acid analysis of the seeds (g/100g protein) is expressed in Table 3.3a and 3.3b. Amino acid score for the individual essential amino acid is shown in Table 3.4. As seen in Table 3.3a, nine essential amino acids, namely lycine, tyrocine, leucine, isoleucine, methionine, cystine, threonine, valine, phenylalanine and eight non-essential amino acids were identified in both the fermented and unfermented sample. The non-essential amino acids identified are proline, glycine, arginine, aspartic acid, glutamic acid, alanine, histidine and serine.

Glutamic acid having (14.54g/100g) was the most concentrated amino acid in the two sample. This observation agrees with the Chidi *et al*. (2012) that glutamic acid is the most

concentrated amino acid in the raw and fermented *Prosopis africana* seeds.
Methionine was the least concentrated amino acid having (0.88g/100g), the observed low concentration of methionine in this sample is in agreement with the reported least concentration of methionine in the raw and fermented sample of *Prosopis africana* seeds (Chidi *et al*., 2012).
The amino acids score in Table 3.4 shows that, Leucine, Isoleucine, Threonine and Valine are the essential amino acids with percentage amino acid above reference standard indicating that, the fermented sample could be a good source of these essential amino acids.
Fermentation significantly increased both essential and non-essential amino acids compositions of *Prosopis africana* which is in agreement with the similar observed effect of raw and locally fermented *Prosopis africana* and *Ricinus Communis* seeds (Chidi *et al*., 2012).

4. CONCLUSIONS

Fermentation of *Prosopis africana* seed serves as a good way of enhancing its protein and minerals composition while a decrease in the carbohydrate content of the seed was observed. The process results in increased, nutritional values of the fermented foods, adds a variety of flavour and extends the shelf life over the unfermented. The amino acid content of the seed shows that, it could be a good source of Leucine, Isoleucine, Threonine and Valine essential amino acids. Therefore, fermentation is one of the important process that converts the less to the more nutritious forms of the seed by the use of oxidizing agent such as bacteria.

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REFERENCES

[1] Achinewu,S.C. (1986). The Effect of Fermentation on Carbohydrate and AcidComposition of Oil African Seed (Pentaclethra macrophylla), *Food Chem*emistry, **19:** 105-16. Cited in: Sani N.A., Hassan, L.G., Dangoggo, S.M., Ladan, M.J., Umar,KJ (2013): Effect of Fermentation on the nutritional and Antinutritional Composition of *Lagenaria Siceraria* Seeds.

[2] Adams MR. (1990): Topical aspects of fermented foods. *Trends in Food Science andTechnology*, **1:** 141-144

[3] Amoo. Isiaka Adekunle and Jakotagba Olorutobi Adenuke. (2012). Effect of Fermentation on the Nutritive Value of Aspergillus Niger Fumigatus Fermented Hura Crepitance Seed Flour.*Greener Journal of Physical Scinces.* **2(3):** 85-88.

[4] Anhwange, B.A., (2008). Chemical Composition of Musa Sapientum (Banana) Peels. *Journal of Food Technology*, **6:** 263-266.

[5] Annongu, A.A., J.K. Joseph and F. Liebert. (2004). Effect of anaerobic fermentation and Lyle treated Prosopis africana seed meal on the nutritional and hematological responses of Harco chicks. J*ournal of Raw Material Research*, **1:** 33-41.

[6] Anon. (1996). *Food Processing for Food Security and Income Generation*, Intermediate Technology, UK

[7] AOAC (Association of Official Analytical Chemicals). (2006). Official Method of Analysis of the AOAC (W. Horwitz Editor Eighteen Edition, Washington; D. C., AOAC.

[8] Aremu, M.O., A. Olonisakin, B.O. Atolaye and C.F. Ogbu. (2006). Some Nutritional and Functional Studies of Prosopis Africana.*Electronic Journal of Environment, Agriculture and Food Chemistry***,** **5:** 1640-1648.

[9] Assean manual of food analysis. (2011). Regional Centre of ASSEAN Network of Food data system Institute of Nutrition, Mahidol University, Thailand.

[10] Balogun M.A, Oyeyiola, G.P (2011): "Microbiological and Chemical Changes during the Production of Okpehe from *Prosopi safricana* Seeds".*Journal of Asian Scientific Research*, **1(8):** 390-398.

[11] Benitez, L. V. (1989). Amino Acid and fatty acid profiles in aquaculture nutrition studies, p.

23- 35. in S.S. De Silva (ed.) Fish Nutrition Research in Asia. Proceedings of the Third Asian Fish Nutrition Network Meeting. Asian fish. Society Special Publication. 4, 166 p. Asian Fisheries Society, Manila Philippines.
[12] Campbell-platt. G. (1994). Fermented foods – a world perspective. *Food Research International*, **2:** 253-257
[13] Campbell-Platt. G. (1987). *Fermented Foods of the World – A Dictionary and Guide*. London, Butterworths. ISBN: 0-407-00313-4.
[14] Chidi. U. Igwe., Okey. A. Ojiako., Ken C. Anugweje., Linus. A. Nwangu and Cosmas O. Ujumundu. .(2012). Amino acid profile of raw and locally processed seeds of Prosopis Africana and Ricinnus Communis: Potential antinodes to protein malnutrition. *Functional Health and Diseases*, **2(4):**107-119.
[15] El Faki AE, Dirar AA, Collins MA and DB Harper. (1991). *Biochemical and microbiological investigation of Sigda:* A Sudanese fermented food derived from sesame seed oil cake. *Journal of Science and Food Agriculture*, **57:** 351-361.
[16] Eka OU (1980).*Effect of fermentation on the nutrient status of locust beans*. *Food Chememistry*, **5:** 305-308. 23.
[17] Fadahunsi IF, Sani AI (2010). *Chemical and Biochemical Changes in Bambra Nut during Fermentation to "Tempeh"*. Electronic J Environ Agric D. *Food Chemistry*, **9(2):** 275-283 Cited in: Sani N.A., Hassan, L.G., Dangoggo, S.M., Ladan. M.J., Umar, KJ. (2013): Effect of Fermentation on the Nutritional and Antinutritional Composition of *Lagenaria siceraria* Seeds.
[18] Fetuga, B.L; Babatunde, G.M and Oyenuga, V.A. (1974.) *Protein quality of some unusual protein foodstuffs: Studies on the African Locust Bean* (Parkiafiliciodea Welv) Seed. *British Journal of Nutri*tion, **32:** 27-36.
[19] Fermented Frutis and Vegitables. A global Perspective .(1998). online, Available at: http://www.fao.org/docrep/x0560e04.htm. Accessed on: 21/4/2013.
[20] Gernah, D. I., Inyang, C.U. and Ezeora, N.I. (2005) "*Effect of Incubation Materials on Fermentation of African Locust Beans (Parkia biglobosa) in the Production of 'dawadawa'*". *Nigerian Food Journal*, **23:** 166-173.
[21] Holzapfel, W.H., (2002). Appropriate Starter Culture Technologies for Small-scale Fermentation in Developing Countries. *.International Journal Food-microbial*., **75:** 197-212.
[22] James, C. A. (1995). *Analytical Chemistry of Food*. Chapman and Hill, London.
[23] Khetarpaul N & Chauhan BM. (1990). *Fermentation of pearl millet flour with yeasts and lactobacilli: in vitro digestibility and utilisation of fermented flour for weaningmixtures.* Plant Foods for Human Nutrition 40, 167-173. Cited in:*Fermentation as a Method of Food Processing production of organic acids, pH-development and microbial growth infermenting cereals*,Licentiate thesis May 1999,Peter SahlinDivision of Applied Nutrition and Food Chemistry, Center for Chemistry and Chemical Engineering, Lund Institute of Technology,Lund University.
[24] Lockwood, J.A., Burney,J.C., Debrey, R.A. and Pradt, R.E. (1994). A Reversed Fauna Grasshopper Glacier Unique Insight to Acridid Biology. *Bolletin Samdad Vegetal*, **20:** 223-236.
[25] Magdi, A. Usman. (2004). Chemical and Nutrient Analysis of Baobab (*Adansonia digitata*) Fruit and Seed Protein Solubility. *Plant Food for Human Nutrition* **52:** 29-33.
[26] Makindele, F.M., Akinoso, R. and Adepoju, A.O. (2013). Effect of Fermentation Containers on the Chemical Composition of Fermented Sesame (Sesamun indicatum L) seeds, *African Journal of Food Agriculture and Development*, **13(1):** 35-45.
[27] Mortarjemi, Y. (2002). Impact of Small-Scale Fermentation Technology on Food Safety in Developing Countries. *International Journal Food-microbial*., **75:** 213-229.
[28] Muhammad, N.O. and O.B. Oloyede, (2009). Protein Fractions and amino acid profile of Aspergillus niger- fermented Terminalia Catappa Seed Meal. *African Journal of Microbiology*, **3:** 101-104.
[29] Nason NJ, Field ML .(1992). Influence of temperature on the nutritive value of lactic acid fermented cornmeal, *Journal of food science*, vol. 49, pp 958 – 959. Cited in: *Fermentation as a Method of Food Processing production of organic acids, pH-development and microbial growth in fermenting cereals*, Licentiate thesis May 1999 ,Peter Sahlin ,Division of Applied Nutrition and Food Chemistry, Center for Chemistry and Chemical Engineering, Lund Institute of Technology,Lund University.
[30] Obizoba, IC., Atii, JV. (1991): Effect of soaking, sprouting, fermentation and cooking

on nutrient composition and some anti-nutritional factors of sorghum (Guinesia) seeds. *Plant Foods for Human Nutrition*, (41): 203-212. Cited in: Fermentation as a Method of Food Processing production of organic acids, pH-development and microbial growth in fermenting cereals,Licentiate thesis May 1999 ,Peter Sahlin ,Division of Applied Nutrition and Food Chemistry, Center for Chemistry and Chemical Engineering, Lund Institute of Technology,Lund University.

[31] Odebunmi, E.O., Oluwaniyi, O.O. and Bashiru, M.O. (2010): Comparative Proximate Analysis of Some Food Condiments.*Journal of Applied Science Research*, **6(3):** 272-274.

[32] Oyenuga, V.A., 1968. *Nigeria Foods and Feeding Stuffs; their Chemistry and Nutritive Value.* 3rd Edition., Ibadan University Press, Ibadan, Nigeria, pp: 99.

[33] Parades-Lopez,O., (1992), Nutrition and Safety Considerations in Applications of Biotechnology to Traditional Fermented Foods, report of an Ad Hoc Panel of the Board on Science and Technology for International Development, National Academy Press, Washington D.C., USA.

[34] Paredes-López O & Harry GI .(1988). Food biotechnology review: traditional solid-state fermentations of plant raw materials - application, nutritional significance and future prospects. CRC Critical Reviews in Food Science and Nutrition 27, 159-187. Cited in: Fermentation as a Method of Food Processing production of organic acids, pH-development and microbial growth in fermenting cereals, Licentiate thesis May 1999,Peter Sahlin ,Division of Applied Nutrition and Food Chemistry, Center for Chemistry and Chemical Engineering, Lund Institute of Technology,Lund University.

[35] Passmore, Eastwood, J .(1986). *Human Nutrition and Dieties*, *8th Edition Nutrition Foundation*, Church Chill, Living Stone pp43-107.

[36] Vogel, A.I. (1978). *Text Book of Organic Chemistry*, 4th Edition Longman Group Ltd, London. Pp 220-224.

[37] Sani N.A. Hassan, L.G., Dangoggo, S.M., Ladan, M.J., Umar, KJ. (2013): Effect of Fermentation on the nutritional and Antinutritional Composition of Lagenaria Siceraria Seeds. *Journal of Applied Chemistry,* **5(2):** 01-06.

[38] Walker, P.M.B. (1988). *Chambers Science and Technology Dictionary*, Chambers, Cambridge University Press, UK.