International Journal of Chemistry Studies
ISSN: 2581-348X
Impact Factor: RJIF 5.44
www.chemistryjournal.in
Volume 2; Issue 2; March 2018; Page No. 25-30

Biological and chemical changes during the aerobic and anaerobic fermentation of African locust bean

[*] Dr. Modupe Elizabeth Ojewumi, Abiodun James Omoleye, Atonye Stephanie Nyingifa
Department of Chemical Engineering, Covenant University, Canaan Land, Km 10 Idiiroko Rd., Ota, Ogun State, Nigeria

Abstract
The effect of aerobic and anaerobic fermentation on the nutritive value of African Locust beans were investigated. The seeds were soaked, boiled, dehulled and fermented for four days. The raw, unfermented and fermented seeds were subjected to physicochemical analysis. Fermentation caused a significant protein increase in the fermented seeds from 32 % to about 50 and 45% for both aerobic and anaerobic fermentation respectively. Decrease in some other proximate parameters were also observed, such as percentage ash content, carbohydrate, crude fibre with fermentation days for both aerobic and anaerobic fermentation. The product obtained from the aerobic fermentation was alkaline with a pH of 8.2, while the pH of the anaerobic seeds was slightly acidic 5.76.

Keywords: aerobic fermentation, anaerobic fermentation, proximate, sensory evaluation

Introduction

Fermentation is the biological conversion of complex **s**ubstrate such as starch or sugar into simple compounds by microorganisms [1]. It can also be defined as the production of energy from food without using oxygen. It is one of the oldest forms of food preservation known to man [2, 3].

Parkia biglobosa tree is known to be a native of Africa and is an important multipurpose tree of West African Savannah land, which is primarily grown for its pods that contain both a sweet pulp and valuable seeds [4]. The pods are flat and have irregular cluster of up to 30 seeds [5, 6].

African Locust bean with botanical name *Parkia biglobosa* is a leguminous plant found in the Savannah region of Nigeria. The botanical name *Parkia biglobosa* was given to it by Robert Brown, a Scottish botanist in 1826. He described the tree as genus of flowering plants in the legume group which belongs to the sub - family Mimosoideae and Leguminosae [3, 5, 7]. *Parkia biglobosa* (African locust bean seeds) is a perennial deciduous tree that grows from 7m to 20 meters high [8].

Parkia biglobosa seed is known as Iyere in Yoruba land while the fermented seed as Iru. Iru is one of the major sources of plant protein in African diet which is known as fermented vegetable protein 'Iru' [9]. Iru is consumed in many African countries, especially Nigeria. Iru known by different names in different countries - kinda in Sierra Leone. In Nigeria and Ghana it is called dawadawa or Iru, Benin Republic afintin and sonru; nététu in Senegal and Burkina Faso as soumbala; Japan as natto; and kinema in Nepal [10, 11, 12]. Apart from fermented *Parkia biglobosa* seeds (Iru) serving as a rich source of plant protein to man with low cost, it also serves as good source of protein for animal feeds, chick and fish (Livestock) [13, 14, 15].

There seems to be a general agreement on the spore-forming *bacillus* species as the main fermentation organisms [3, 16, 17, 18, 19, 20, 21, 22]. During the fermentation of African Locust bean seeds systematic investigation showed that *Bacillus subtillis* is the most dominant bacterium responsible for the fermentation [16, 23, 24, 25]. Literature revealed that some species of *bacillus* such as *Bacillus lichenioformis*, *Bacillus megaterium*, *L. mesenteriodes* and *Staphylococcus* are also found in the fermented condiment (Iru).

Fermentation can be carried out either in the absence or in the presence of air. When air introduced into the fermentation vessel, the type of fermentation which occurs is called aerobic fermentation. In aerobic fermentation, free oxygen acts as the hydrogen acceptor. Another type of fermentation pathway occurs when little or no air is introduced into the fermentation vessel which is known as anaerobic fermentation [25].

Materials and Methods

Raw Materials

African locust bean seeds were bought from open market. The starter cultures used were freshly prepared in the Microbiology laboratory, Covenant University, Ota, Nigeria using method [3].

Preparation of seed for fermentation process

The seeds were processed according to [3] method.

pH determination

Method [26] was used for the determination of pH.

Aerobic fermentation

100 g of the seed sample was inoculated in a 200liter round bottomed flask using *Bacillus subtilis* and fermented for four days. Samples were removed daily (24 hours) and kept in a freezer for further analysis.

Anaerobic Fermentation

100 g of the seed sample was inoculated with *bacillus subtilis* using a 200litre Buckner flask. The flask was tightly corked with a rubber cork and the air inside was removed using 240V Monarch 1 phase induction motor vacuum pump for 15

minutes (de-vacuum). Fermentation was carried out for 4 days. Samples were collected daily and kept in the freezer for further analysis. Figure 1 shows the de-vacuuming process.

Fig 1: De-vacuuming of samples before anaerobic fermentation

Sensory Evaluation

The basic sensory evaluation of the fermenting stages of the bean was done by sight and smell tests of the seeds during various periods of fermentation.

Proximate analysis was carried out using method [25].

Results and Discussion

Raw Seed

The raw seed had a pungent but not offensive smell.

Table 1: Anaerobic Fermentation

	Odour/Aroma	Colour	Comments
Starting Sample	Beany Smell	Creamy Brown	Not Edible
Day 1	Lightly Pleasant	Brownish	Acceptable, no mucilage seen
Day 2	Ammonia smell	Browner	Mucilage noticed
Day 3	Stronger Ammonia smell	Browner	Not too acceptable with whitish mucilage
Day 4	Choking ammonia smell	Dark Brown	Presence of whitish mucilage had increased and the smell had become very strong

Table 2: Aerobic Fermentation

	Odour/Aroma	Colour	Comments
Starting Sample	Beany Smell	Creamy Brown	Not Edible
DAY 1	Lightly Pleasant	Brownish	Acceptable, no mucilage seen
DAY 2	Lightly pleasant	Browner	Acceptable with ammonia smell
DAY 3	Ammoniacal smell	Browner	Stronger ammonia smell
DAY 4	Choking	Dark Brown	Stronger ammonia smell

Inferences from the sensory evaluation

Tables 1 and 2 explained what was observed during the two fermentation processes. The mucilage formed during the fermentation was as a result of deterioration caused by other microorganisms growing on the substrate such as yeast or fungi. While the change in colour was due to the breaking down of amino acids and production of ammonia that occurs during fermentation. The appearance of the whitish mucilage and the stronger ammonia smell in the anaerobic fermentation before the aerobic fermentation may indicate that fermentation takes place faster during the anaerobic fermentation. Mist was also noticed on the walls of the flasks used, this might be as a result of heat released during the fermentation processes. The fermentation of African Locust bean seed is an exothermic process, where the temperature of the fermenting seed gradually increases from the ambient temperature to about 45^0 C [18]. The heat released during the fermentation condensed on the surface of the flask to appear as a mist.

Proximate Analysis

The proximate analysis of both the fermented and raw African locust bean seed for aerobic and anaerobic fermentation were shown in figures 2-7, while figure 8 shows the pH.

Moisture Content

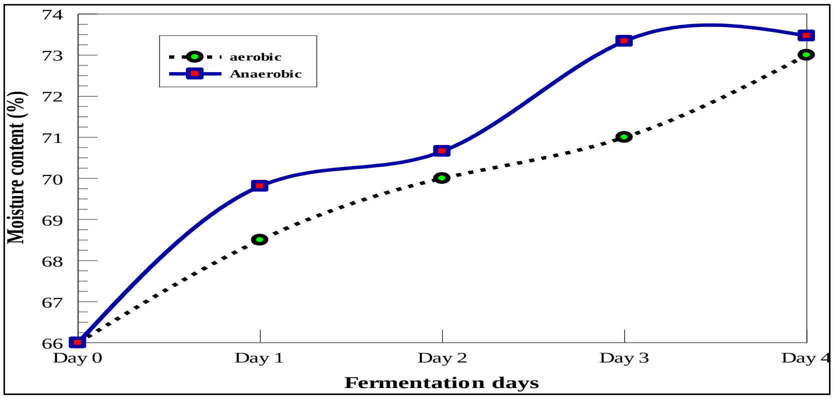

Fig 2: Percentage moisture content

In figure 2 increase in percentage moisture content was noticed for both aerobic and anaerobic fermentation. This could be as a result of various pre-fermentation processing activities carried out on the raw seed such as dehulling and boiling, since seeds were boiled for 4 hours and later left to soak overnight to soften the cotyledons before the de-hulling stage [28]. Reported that the metabolic activities of some microorganisms during fermentation time gives out moisture as one of their end products. Increase could also be due to the activities of the inoculum on the fermented sample as a result of extracellular enzymes production.

Ash Content

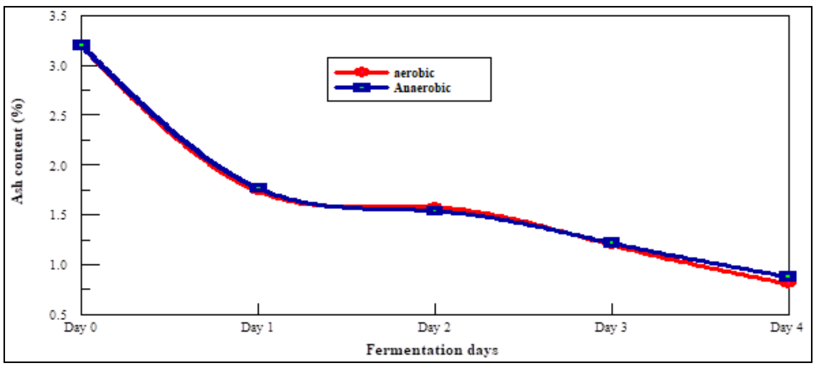

Fig 3: Percentage ash content

The ash content of the raw seed was seen to decrease after boiling and then gradually decrease during the fermentation for both fermentation processes. The decrease in percentage ash content was as a result of long hours of boiling, soaking in water and dehulling. A 40 - 45 % reduction was noticed, which implies that the total mineral content of the seeds resides in the hull of the seeds which are leached during processing [25,28]. The reduction in percentage ash content may also be due to the utilization of some essential salts during fermentation by microorganisms for their metabolic activities.

Crude Fibre

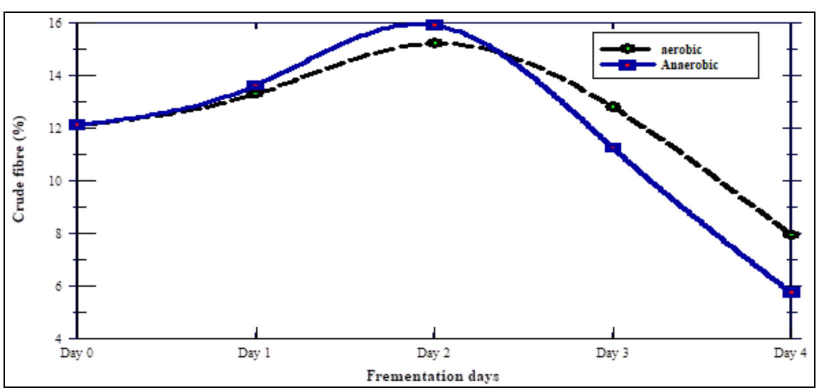

Fig 4: Percentage crude fibre

Figure 4 shows an increase in the percentage crude fibre during the first 48 hours for both fermentation processes. The pattern observed agrees with that reported by [19], who observed that fermentation increased the crude fibre by 30 % during the first 48 hours and then reduced it by about the same amount during the last 24 hours.

Crude Protein

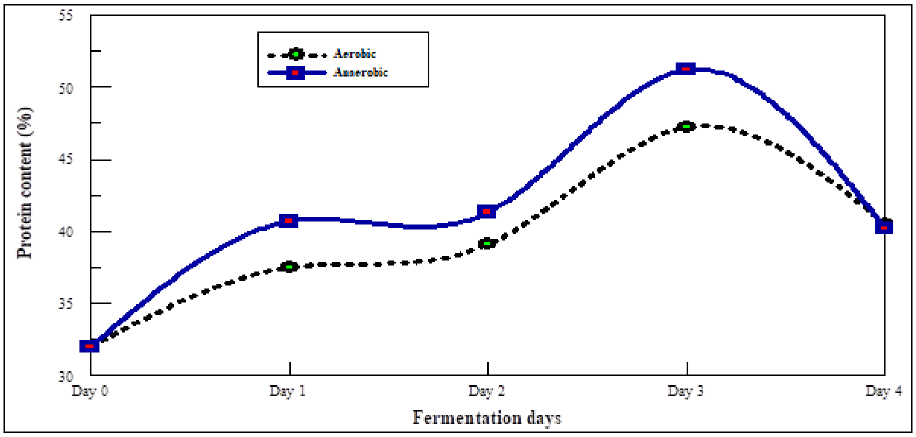

Fig 5: Percentage crude protein

Fermentation was seen to have a positive impact on the percentage crude protein of African locust bean seed in both processes. The percentage crude protein content of locust bean seed increases progressive throughout the processing until after the third day. The increase in the protein concentration was probably due to the ongoing reduction of the levels of the other components of the seed such as the crude fibre and the ash content. [26,27] reported that fermentation results in a lower proportion of dry matter in the food and the concentrations of vitamins, minerals and protein appear to increase when measured on a dry weight basis. Also some increase in the protein content may be attributed to the secretion of extracellular enzymes by *Bacillus subtilis* during the fermentation.

[27] also reported that apparent increase in the protein content was due to the microbial proliferation or multiplication in number of microorganisms in form of single cell proteins. Best products were observed to be the samples fermented for 72 hours (3 days) this supported what was recorded in literature. [19] reported that African locust bean does not come out well if fermentation is allowed to continue for more than 3 days, this encourages the fermented media to be suitable for the growth of yeast. Over-fermentation generates unacceptable levels of volatile fatty acids.

Crude Fat

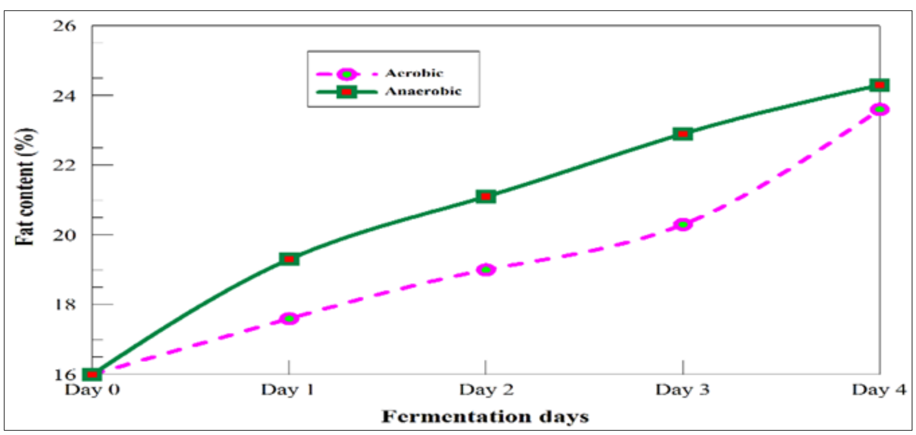

Fig 6: Percentage crude fat

Increase in fat content was also noticed in both aerobic and anaerobic fermentation. Oil was noticed at the walls of the vessels used for the cooking of raw seeds and during processing, which show that the seeds contain oil. We can conclude from this that long hours of cooking brings out the oil in seeds through the fractionating intact part of the oil bodies and rupturing of cellular structure [9,28]. The increase in fat content of the fermented African locust bean seeds can also be attributed to the increase in the activities of lipolytic enzymes, which hydrolyses fat to glycerol and fatty acid.

Carbohydrate Content

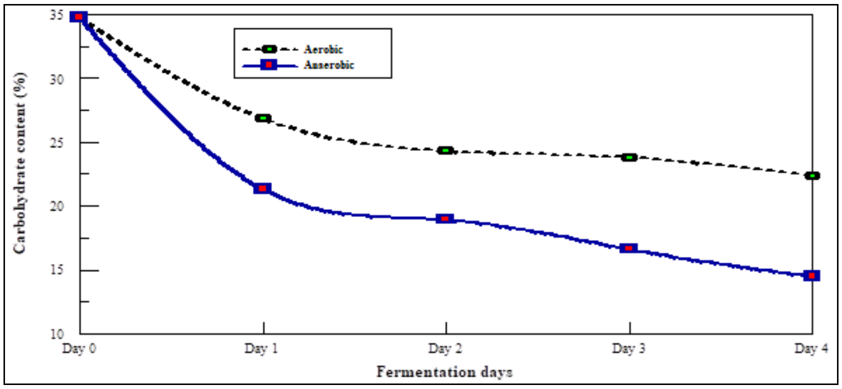

Fig 7: Percentage carbohydrate content

The carbohydrate content of the locust bean seed reduced during both the aerobic and anaerobic fermentation. This was due to the leaching of soluble carbohydrates like sugars into the water used for boiling and soaking. The loss on fermentation may be due to the utilization of some of the sugars by the fermenting organisms for growth and metabolism. When *Bacillus* is acting as an anaerobe (i.e. in an anaerobic environment), the substrate itself becomes the primary source of the energy needed for growth, survival and metabolism. *Bacillus* species have been found to be producers of certain enzymes such as amylase, galactanase, glucosidase, fructofuranosidase and galactosidase [19, 29]. These enzymes are involved in degrading carbohydrates. Both microbial amylase and galactanases hydrolyzes carbohydrates to sugars, which are the more digestible form. This explains the successive decrease in the carbohydrate content during the fermentation period.

pH

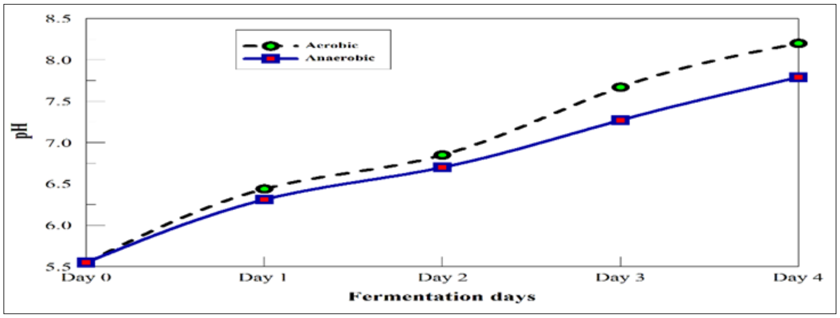

Fig 8: pH

The pH for the aerobic fermentation increased from 5.9 in the raw seed to about 8.2 in the fermented seed. The pH obtained during the aerobic fermentation fits perfectly with the results obtained by Odunfa [11, 25]. Odunfa reported a gradual increase in pH during the first 30 hours of fermentation and later a sharp increase to 8.1 subsequently. From the results obtained, the aerobic fermentation of the African Locust bean seed is an alkaline fermentation. The increase in the pH during the fermentation may be because of the formation of ammonia which is basic.

On the other hand, samples from the anaerobic fermentation experienced a decrease in pH to become slightly acidic fermented products.

5. Conclusions

The African Locust bean contains an appreciable amount of essential macro-nutrients, especially protein and carbohydrate which are important parts of the daily diet of every household. Therefore, increase intake of the locust beans will reduce the risk of nutrient deficiencies in consumers.

Conflict of interest

The authors declare no conflict of interest.

6. References

1. Ojewumi ME, Omoleye JA, Ayoola AA, Ajayi AA, Adekeye BT, Adeyemi AO, *et al*. Effects of Salting and Drying on the Deterioration Rate of Fermented *Parkia biglobosa* Seed. Journal of Nutritional Health & Food Engineering. 2018; 8(1):1-5.
2. Omafuvbe BO, Olumuyiwa S, Falade BA, Steve RA, Adewusi. Chemical and Biochemical changes in African Locust Beans (*Parkia biglobosa*) and Melon (*Citrullus vulgaris*) seeds during fermentation to condiments. Pakistan Journal of Nutrition. 2004; 3(3):140-145.
3. Ojewumi ME, Omoleye JA, Ajayi AA. Optimum

Fermentation Temperature for the Protein Yield of *Parkia biglobosa* Seeds (Iyere). Proceeding of the 3rd International Conference on African Development Issues (CUICAD), 2016a; 584-587, Ota, Ogun-state, Nigeria. ISSN 2449-075X.
4. Ojewumi ME, Omoleye JA, Ajayi AA. Optimization of Fermentation Conditions for the Production of Protein Composition in Parkia biglobosa Seeds using Response Surface Methodology. International Journal of Applied Engineering Research. 2017a; 12(22):12852-12859.
5. Modupe EO, Eluagwule O, Ayoola AA, Ogunbiyi AT, Adeoye JB, Emetere ME, *et al*. Termiticidal effects of African locust bean seed oil extract. International Journal of Current Research. 2017b; 9(07):53929-53934.
6. Odunfa SA. Biochemical changes during production of ogiri, a fermented melon (*Citrullus vulgaris* orchard) product. Qualities Planetering Plant Food for Human Nutrition. 1983; 32:11-18.
7. Abdoulaye O. Influence of process condition on the digestibility of African Locust Bean (Parkia biglobosa) Starch. American Journal of Food Technology. 2012; 7:552-561.
8. Teklehaimanot Z. Exploiting the potential of indigenous agroforestry trees: *Parkia biglobosa* and *Vitellaria paradoxa* in sub-Saharan Africa. Agroforestry Systems. 2004; 1(3):207-220.
9. Ojewumi ME, Omoleye JA, Emetere ME, Ayoola AA, Obanla O, Babatunde E, *et al*. Effect of various temperatures on the nutritional compos-itions of fermented African locust bean (*Parkia biglobosa*) seed. International Journal of Food Science and Nutrition. 2018; (3)1:117-122.
10. Azokpota P, Hounhouigan DJ, Nago MC. Microbiological and chemical Changes during the fermentation of African locust bean (*Parkia biglobosa*) to produce afintin, iru and sonru, three traditional condiments produced in Benin, International Journal Food Microbiology. 2005; 107:304-309.
11. Odunfa SA. A note on the microorganisms associated with the fermentation of African locust bean (*Parkia filicoidea*) during iru production, Journal Plant Foods. 1981a; 3:245-250.
12. Azokpota P. Esterase and protease activities of *Bacillus* spp. from afitin, iru and sonru; three African locust bean (*Parkia biglobosa*) condiments from Benin. African Journal Biotechnology. 2006; 5(3):265-272.
13. Uaboi-Egbenni PO, Okolie PN, Sobande AO, Alao O, Teniola O, Bessong PO, *et al*. Identification of subdominant lactic acid bacteria in dawadawa (a soup condiment) and their evolution during laboratory-scale fermentation of *Parkia biglobosa* (African locust beans), African Journal Biotechnology. 2009; 8(25):7241-7248.
14. Ademola IT, Baiyewu RA, Adekunle EA, Omidiran MB, Adebawo FG. An Assessment into Physical and Proximate Analysis of Processed Locust Bean (*Parkia biglobosa*) preserved with common salt, Pakistan Journal Nutrition. 2011; 10(5):405-408.
15. Campbell-Platt G. African locust bean (*Parkia* species) and its West African fermented food products, Dawadawa. Economics Food Nutrition Journal. 1980; 9:123-132.
16. Sanni AL. Biochemical changes during production of Okpehe, a Nigeria Fermented food condiment. Chemistry Microbiology Technology Lebanism. 1993; 15:97-100.
17. Odunfa SA. Microbiology and amino acid composition of Ogiri - A food condiment from fermented melon seeds. Die Nahrung. 1981b; 25:811-816.
18. Odunfa SA, Oyewole OB. African fermented foods in Microbiology of Fermented Foods. Wood, B.J.B. 1998; 713-746.
19. Omafuvbe BO, Abiose SH, Shonukan OO. Fermentation of soybean (*Glycine max*) for soy-daddawa production by starter cultures of *Bacillus*. Journal of Food Microbiology. 2002; 19:561-566.
20. Ouoba LII, Cantor M, Diawara B, Traore A, Jakobsen M. Degradation of African locust bean oil by *Bacillus subtilis* and *Bacillus pumillus* isolated from soumbala, a fermented African locust bean condiment. Journal of Applied Microbiology. 2002; 95:868-873.
21. Ouoba LII, Rechinger KB, Barkholt V, Diawara B, Traore AS, Jakobsen M, *et al*. Degradation of proteins during the fermentation of African locust bean (*Parkia biglobosa*) by strains of *Bacillus subtilis* and *Bacillus pumilus* for Soumbala. Journal of Applied Microbiology. 2003; 94:396-402.
22. Ouoba, LII, Diawara B, Amoa-Awua, Traore AS, Moller PL. Genotyping of starter cultures of *Bacillus subtillis* and *Bacillus pumilus* for fermentation of African locust bean (*Parkia biglobosa*) to produce Soumbala. International Journal Food of Microbiology. 2004; 90:197-205.
23. Odunfa SA. African fermented foods. Microbiology of Fermented foods, 2ed. B.J.B. wood. Elsevier Applied Science. 1985; 155-191.
24. Antai SP, Ibrahim MH. Microorganisms associated with African locust Bean (*Parkia filicoidea Welw*) fermentation for dawadawa production. Journal of Applied Bacteriology. 1986; 61:145-148.
25. Ojewumi ME. Optimizing the conditions and processes for the production of nutrient from *Parkia biglobosa*. PhD. dissertation submitted to the Department of Chemical Engineering, Covenant University, Nigeria, 2016c.
26. Eka OU. Effect of fermentation on nutrient status of locust beans. Journal of Food Chemistry. 1979; 5:305-308.
27. Oboh G. Nutrient and antinutrient composition of condiments produced from some fermented underutilized legumes. Journal of Food Biochemistry. 2006; 30(5):579-588.
28. Omafuvbe BO, Olumuyiwa S, Falade BA, Osuntogun S, Adewusi RA. Chemical and Biolochemical changes in African Locust Beans (*Parkia biglobosa*) and Melon (*Citrullus vulgaris*) seeds during fermentation to condiments. Pakistan Journal of Nutrition. 2004; 3(3):140-145
29. Aderibigbe EY, Odunfa SA. Growth and extra cellular enzyme production by strains of *Bacillus* species isolated from fermenting African locust bean, Iru. Journal of Applied Bacteriology. 1990; 69:662-671.