International Journal of Food Science and Nutrition
ISSN: 2455-4898
Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 3; Issue 1; January 2018; Page No. 117-122

Effect of various temperatures on the nutritional compositions of fermented African locust bean *(Parkia biglobosa)* seed

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Abstract

The effect of temperature on the nutritional values of fermented African locust bean (*Parkia biglobosa)* seed were studied. Temperatures ranging between 40 to 70^0 C were used for the fermentation. Biochemical and physiological analysis were evaluated. Highest nutritional values were obtained with samples fermented at lower temperatures (40 and 50^0 C) and they all had acceptable end products while all the samples fermented with higher temperature (60 and 70^0 C) gave poor nutritional values with unacceptable end products. *Bacillus subtilis* was used as starter culture and fermentation was carried out for 5 days (120 hours). An increase in moisture, protein, crude fat contents and decrease in total carbohydrate and crude fibre were noticed for all the fermentation temperature variation. The Organic functional groups were identified and characterized using Fourier Transform Infrared (FTIR) spectroscopy. The effect of temperature on the morphological structure of fermented samples.

Keywords: *Bacillus subtilis*, fermentation, Iru, *Parkia biglobosa*, starter culture

1. Introduction

Food fermentation is basically aimed at producing important nutrients or eliminating anti-nutrients [1]. This is necessary to improve the prevailing cases of malnutrition in Nigeria [2].

African locust bean seed was named *Parkia biglobosa* after a Scottish botanist and surgeon called Mungo Park [3]. *Iru* is a product of alkaline fermentation of *Parkia biglobosa* seeds which is popularly used as food seasoning and condiment in West African especially Nigeria. It is a good source of protein supplement for a protein deficient country and low income earners who could not afford animal protein.

Parkia biglobosa tree has application in both medicine and food additives to the indigenous people of Africa. The tree serves various medicinal purposes such as cure of diarrhea and hypertension since it contains a deposit of histamine which dilates the blood vessels and allows free flow of blood [4, 5].

[6] [7] reported fermented African locust bean seeds to be rich in protein with 39-47%, 31-40% of fat or lipid and 12-16% of carbohydrate. Published studies identified *Bacillus* spp. as the main microorganism involved in the fermentation of most legumes especially *Parkia biglobosa* with *Bacillus subtilis* as the predominant microorganism [7, 8, 8, 10, 11].

Fermentation of African locust bean seeds is a traditional art that is practiced in rural areas with rudimentary utensils which did not put hygiene into consideration. Since there is no standard method for the fermentation, every producer produces base on their culture and traditions which brings variation in the quality of *Iru* [12]. The length of production and also the methods of fermentation vary from one producer to another and with the final intended use, resulting to non uniformity in products [13]. Flavour is considered the quality index of fermented African locust bean and plays an important role in consumer acceptability [14]. Although fermented African oil bean seeds have typical flavour and appealing organoleptic quality, differences in flavour range and intensities exist. These vary perhaps as attributes of producer organisms which are due to the various volatile compounds produced by the fermenting population [15], this study used FTIR to study the variation in the volatile compounds as a result of change in temperature.

This study was carried out to know the effect of temperature variation on the nutritional composition and to bring out the optimum temperature that would yield the best nutrient composition for the fermentation of *Iru*.

2. Materials and Methods

Parkia biglobosa seeds used were purchased from local market.

All the chemicals used were of analytical grade from Sigma manufacturing industry. The starter culture used was prepared in Covenant University Microbiology laboratory, Sango-Ota, Nigeria.

2.1 Laboratory preparation of *Parkia biglobosa*

The *Parkia biglobosa* purchased from the market were processed using method of [4].

2.2 Preparation of *Bacillus subtilis*

Preparation of the inoculum used was carried out using method of [12].

2.3 Inoculation of Seeds
200 g of processed seed was inoculated with freshly prepared *Bacillus subtilis*. Five flasks labelled 24 hours to 120 hours were placed in a thermostatically controlled fermenter. At the end of every 24 hours samples were removed from the fermenter and packed into an air tight container which is stored in the freezer for further analysis.

3. Biochemical Analysis
3.1 Proximate Analysis of Fermented African Locust Bean Seeds: The parameters determined were % moisture, % crude protein, % fat, % ash, % crude fibre and % total carbohydrate compositions. These were determined using the [16] method.

3.2 Temperature Monitoring
This was done using the temperature probe fabricated with the fermenter used for the fermentation process.

3.3 Physiological Analysis
This is majorly the physical characteristics of the condiments such as taste, aroma, colour, texture and appearance.

4. Results & Discussion
The seed was fermented to a protein based vegetable condiment identified by its appearance, aroma, and taste. The smell was prominent after the third day (72 hours) of fermentation. The substrate became dark and soft with characteristics aroma similar to ammonia.

4.1 The pH

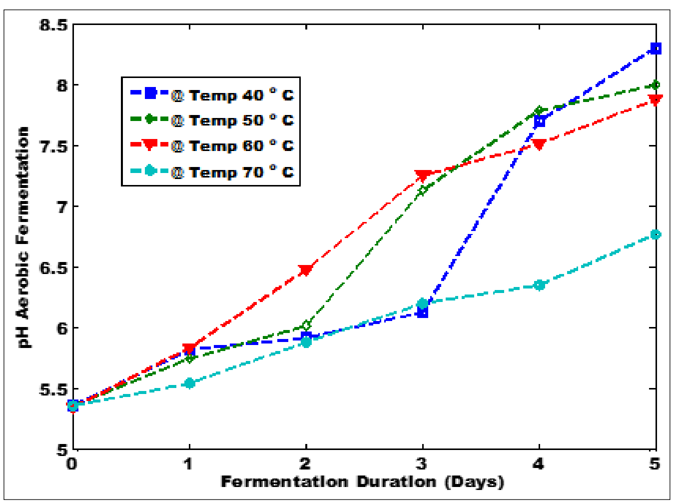


Fig 1: Effect various temperature on the pH of the fermented samples.

Prior literature works confirmed that the fermentation of African locust bean (*parkia biglobosa)* seed to Iru is an alkaline fermentation [12, 17, 18].

Figure 1 showed that lower temperature favoured increase in pH value towards alkalinity during fermentation. This is due to the ability of *Bacillus subtilis* to produce protease to hydrolyse protein and obtain amino acids and ammonia as source of carbon and energy for growth, respectively. Alkaline conditions provided a suitable environment for the hydrolysis of proteins by *Bacillus subtilis* which resulted in the production of amino acids and the release of ammonia which is alkaline in nature [14].

4.2 The Proximate Analysis of African Locust Bean Seeds
Figures 2a-5h shows the effect of temperation variation on the Proximate analysis of African locust bean seeds.

4.2.1 % Moisture content
The moisture content increased with fermentation duration in all the temperature [40-70ºC]. Analysis showed that the initial moisture content was 68 % which later increased to 76 % after the third day of fermentation. The raw unfermented seeds had 9.62 %, while raw cooked unfermented seeds had 56.33 % moisture content. During fermentation the higher the fermentation temperature, the higher the moisture content. The increase was probably due to the water used in subsequent processing such as soaking, dehulling and boiling of the raw seeds prior to fermentation processes and the increase in temperature which probably generated steam, since the fermenting vessels were tightly covered. [19, 20] reported that the metabolic activities of some microorganisms during fermentation gives out moisture as one of their end products. Increase in moisture content was noticed in all the fermentation temperature conditions.

4.2.2 % Ash content
Figures 2a-5h shows that ash content decreased with fermentation duration (days) and increased with fermentation temperature. Increase in ash content was noticed in fermentation temperature 40 and 50ºC while a decrease was also noticed at 60 and 70ºC. The ash content varied between 2.6-4.0 %. The decrease in percentage ash content was as a result of long hours of boiling, soaking in water and dehulling. A30-40% 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 [13, 19]. 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.

4.2.3 % Crude protein
Several research works have been carried out on how fermentation enhances protein value of *Parkia biglobosa* seeds, this was established in figure 2a with increase in protein content at the third of fermentation. The protein composition decreases as temperatures increases. Figures below showed that at every fermentation temperature protein content increased from the first day up to the third day and declined from the fourth day. The % yield of protein from the fermentation of *Parkia biglobosa* at any given day of fermentation declines with increase in fermentation temperature from 40 to 70 ^{0} C. The protein yield at 40 ^{0} C on the third day of fermentation is 52.7 %, it reduced to 25.6 % on the third day at a temperature of 70 ^{0} C. This is shows that *bacillus subtilis* functions well at lower temperature (40-50 ^{0} C). Thus maximum yield of protein is achieved at the third day of fermentation with temperature 40 ^{0} C.

4.2.4 % Crude fibre
Fermentation reduced the percentage crude fibre of the

substrate (fermenting seed) with days of fermentation. An increase in fermentation temperature led to decrease in the percentage crude fibre. Temperature seems to have minimal or no impact in the Crude fibre of African locust bean seeds during fermentation. The patterns in figures 2a-5h reduced progressively, showing that there is a consistent decrease in the percentage crude fibre content.

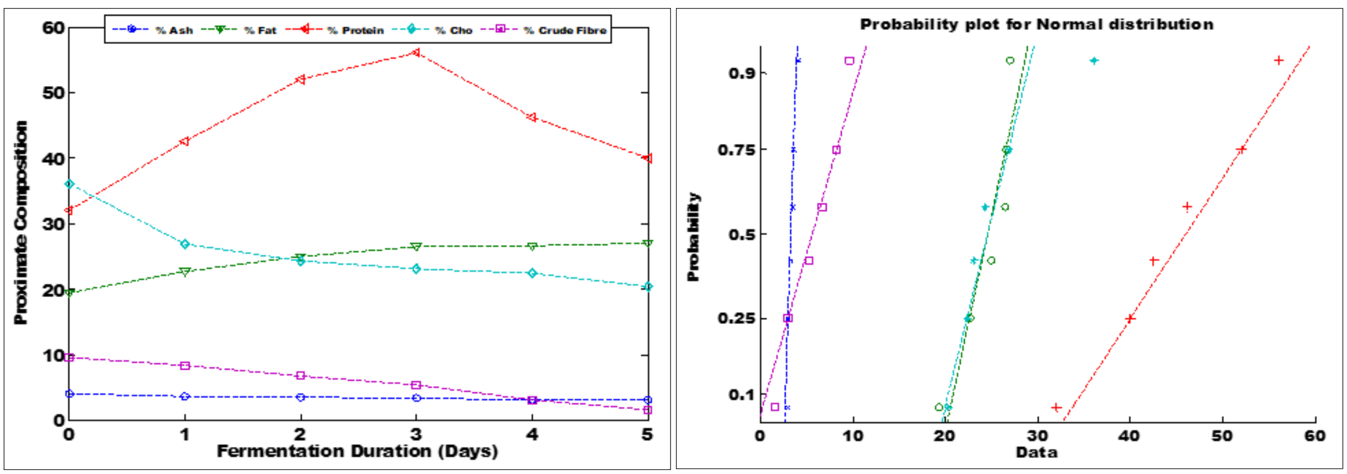
4.2.5 % Fat

Proximate analysis of both raw and cooked African locust bean seed gave about 17.33 % and 19.33 % of fat content respectively. Increase in fermentation temperature led to a decrease in % fat content. There was an increase in the % fat content with days of fermentation. The activity of lipolytic enzyme is faster with a slight increase in temperature during fermentation process but an elevated temperature above the optimum operating temperature of the fermenting microorganism- *bacillus subtilis* which is 40-50 ^{0} C will either make the organism go dormant and reactivated with a favourable conditions or die.

4.2.6 % Total Carbohydrate (Cho)

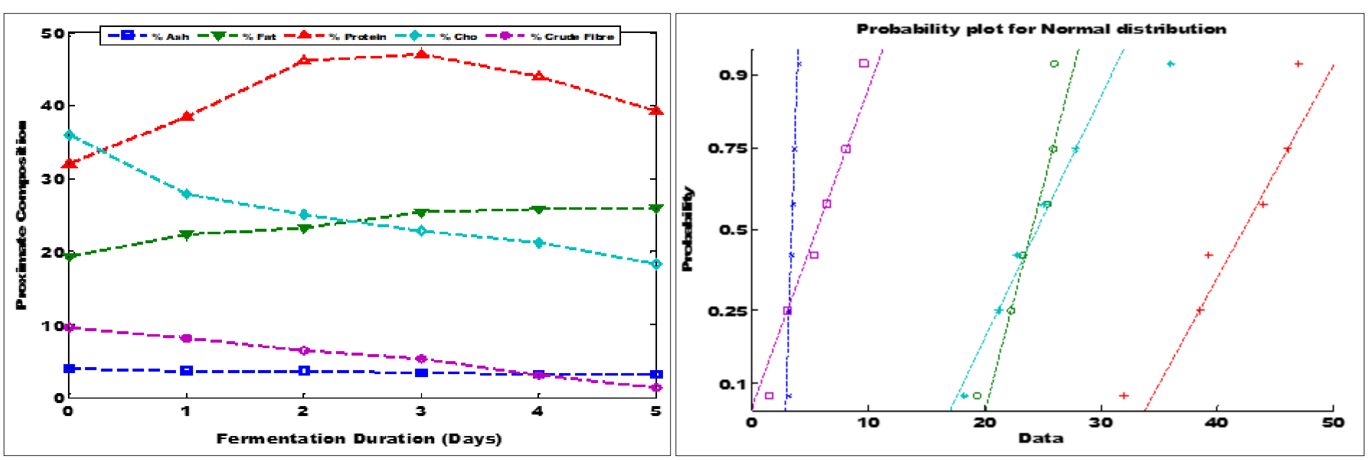
A decrease in total carbohydrate which is expected since it is the substrate that is been consumed by the fermenting microorganisms**.** The loss in carbohydrate during soaking and boiling is attributed to the leaching of soluble carbohydrates like sugars into the cooking water. Loss in carbohydrate during fermentation may also be as a result of the fermenting organisms that utilize some of the sugar for growth and metabolic activities [21]. The higher the temperature the lower the reaction since the fermenting microorganism cannot function well at high temperature, hence low conversion of carbohydrate to sugar.

In summary, the highest compositions were obtained on the third day of fermentation at temperature 40 °C, this gives the optimum operating temperature for the fermentation of African locust bean seeds. This is in support of the claims in literature that the fermenting microorgansim in African locust bean seeds has an optimum temperature with minimum value of 18 and a maximum value of 45 °C.



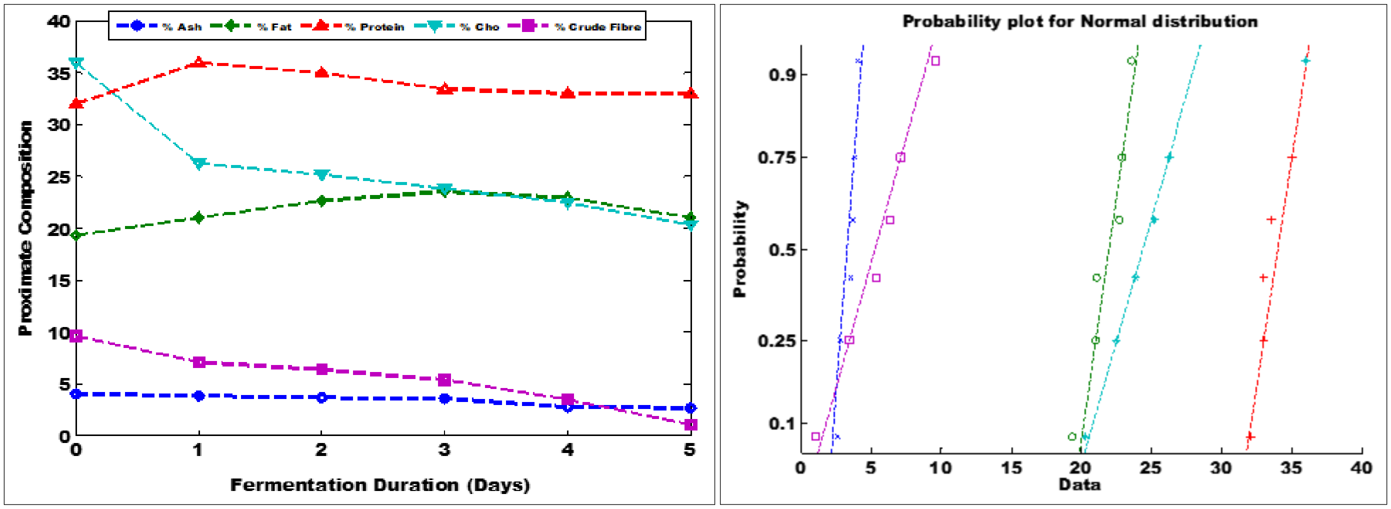
A B

Fig 2a&b: proximate analysis at 40o C



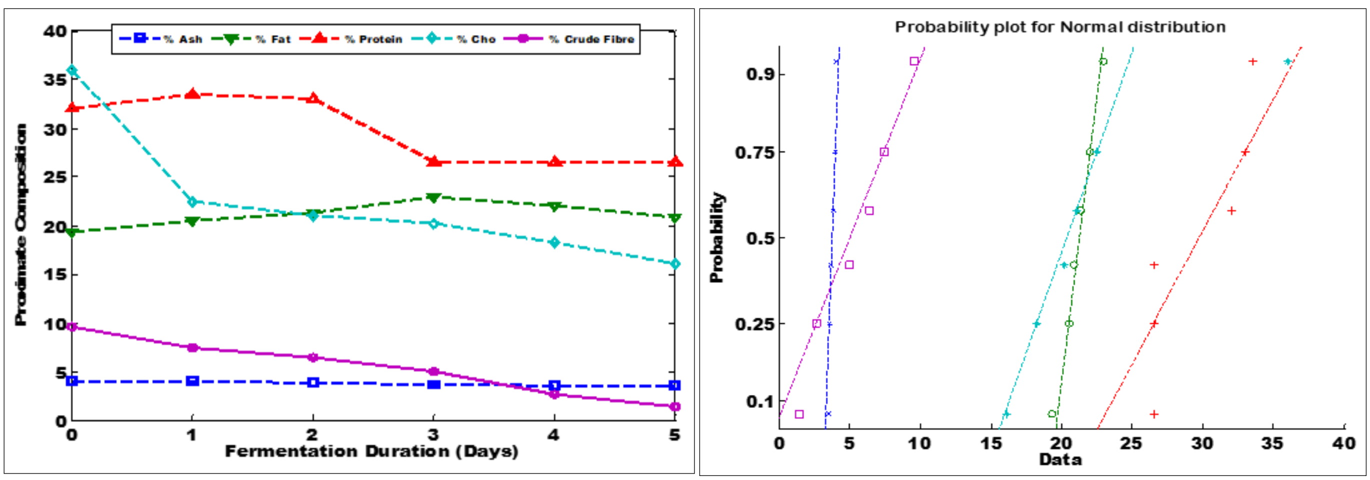
C D

Fig 3c&d: proximate analysis at 50 ° C



E F

Fig 4e&f: proximate analysis at 60 º C



G H

Fig 5g&h: proximate analysis at 70 o C

The normal distributions of each components show that at 40 ºC, the probability of obtaining crude fibre, ash, cho, fat and protein is 0.25, 0.25, 0.6, 0.6 and >0.9 (Figure 2b). Hence, the probability of distribution is proportional to the proximate composition (Figure 2a).

The normal distributions of each components show that at 50 ºC, the probability of obtaining crude fibre, ash, cho, fat and protein is 0.25, 0.25, 0.5, 0.5 and >0.8 (Figure 3d). Hence, at an increased temperature, the probability of distribution for cho, fat and protein decreased while crude fibre and ash remained constant (Figure 2a). Hence, at higher temperature, the probability of distribution in composite-crude fibre and ash is not proportional to the proximate composition (Figure 3c).

The normal distributions of each components show that at 60 ºC, the probability of obtaining crude fibre, ash, Cho, fat and protein is 0.15, 0.15, >0.1, >0.1 and >0.9 (Figure 4e). Hence, the probability of distribution is proportional to the proximate composition (Figure 4f).

The normal distributions of each components show that at 70 ºC, the probability of obtaining crude fibre, ash, Cho, fat and protein is 0.40, 0.40, 0.7, 0.7 and >0.9 (Figure 2b). Hence, at a specific temperature, the probability of distribution is inversely proportional to the proximate composition (Figure 2a).

5. Physiological/Organoleptic Analysis

Panel of 10 assessors were used with the following results;

Table 1: Physiological/Organoleptic Analysis of the Final Product

	Colour	**Taste**	**Texture**	**Appearance**
Dislike Extremely	None	None	None	None
Dislike Slightly	None	10%	None	None
Neither Like Nor Dislike	20%	None	10%	None
Like Moderately	80%	90%	70%	70%
Like Extremely	90%	90%	90%	90%

Purchase Interest 100% while Overall Acceptability of 90% were obtained.

Only the sample fermented for 72 hours with fermentation temperature 40^0 C was acceptable with respect to taste, colour, appearance and aroma, this shows the optimum fermentation conditions for the fermentation of African locust bean seed.

6. Identification of Organic Functional Groups Present in African Locust Bean (*Parkia biglobosa*) Seeds.

The Organic functional groups were identified and characterized using Fourier Transform Infrared (FTIR) spectroscopy (Bruker VideoMVP™ Single Reflection ATR Microsampler Spectrometer model). This is an accurate and effective way of determining the absence or presence of different functional groups in a molecule.

The spectrum in raw unfermented African locust bean has a characteristics strong stretching broad bond of O-H, hydrogen-bonded Alcohol, Phenols and carboxylic acids compound, with absorption frequency range of 3271.68 cm^{-1}. A stretching vibration of C-H, Alkynes with strong and sharp intensity is observed at the same absorption frequency. Similarly, a stretching broad N-H medium but secondary band amine was also noticed. Bands such as stretching frequency of alkane C-H and alkene =C-H with strong and medium intensity was noticed at absorption frequency of 2924.52 cm^{-1} respectively. All sample analysed shows the same properties as above but at different characteristics absorption of frequency range. This explains the similarity in aroma (odour) during fermentation at various temperature. The intensity of each compounds found at different stages of fermentation temperature differentiates them from one another, this accounts for the increase in aroma (odour) during and after fermentation.

C-Cl and C-Br (Alkyl halides and aromatics compounds) are the compound which was observed only on the 5th day of fermentation at temperature 50 °C. This probably might be responsible for the strong undesirable odour perceived after the fourth day of fermentation at higher temperatures (60-70ºC).

7. Morphological Structure

The Morphological structures of African Locust Bean (*Parkia biglobosa*) Seeds viewed using SEM (Scanning Electron Microscope) revealed the following;

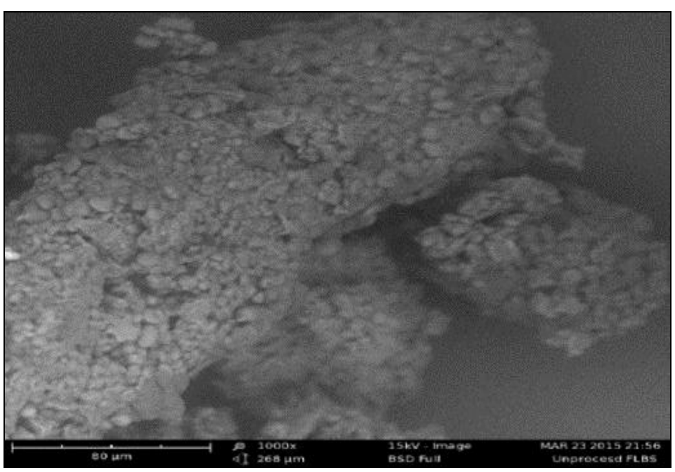


Fig 6: The morphological structure of unprocessed African locust bean seeds

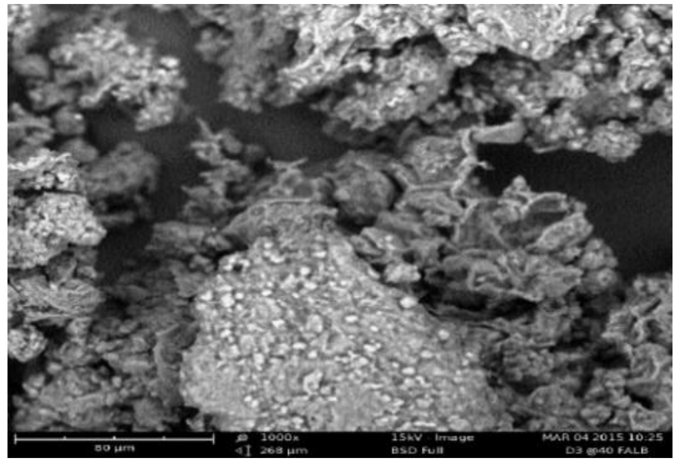


Fig 7: The morphological structure of fermented sample on the third day at 40°C

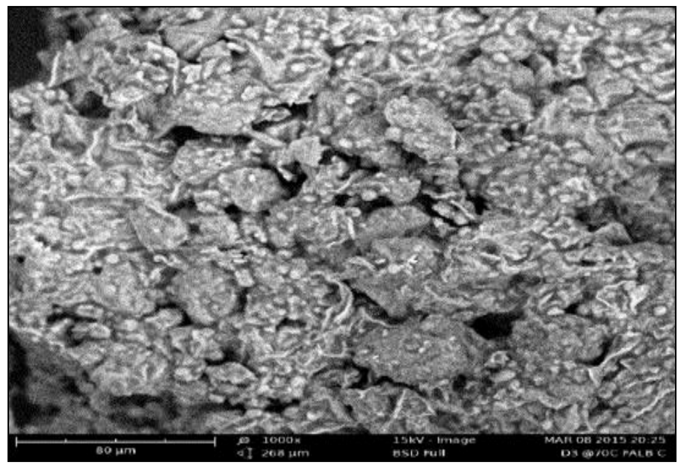


Fig 8: The morphological structure of fermented sample on the third day at 70°C

The result obtained from SEM showed the effects of temperature and fermentation on the morphological structure of both raw and fermented sample. This revealed the differences in processing and fermentation stages. Figure 6 revealed the morphological structure of the unprocessed seed with an agglomerated cohering image with a coarse and wrinkled corrugated surface (No reaction or fermentation observed). Figure 7 revealed the morphological structure of sample fermented for three days at temperature 40ºC. A wider agglomerated and non-cohering structure with a wider pores compared to figure 6, this was attributed to the introduction of heat. This confirmed that compounds were been broken down into smaller units such as carbohydrates. Granules were also still visible, probably protein but adhere more to the surface of the structure (Reaction observed).

Figure 8 revealed the morphological structure of the third day fermented sample at fermentation temperature 70°C**.**

Agglomerated with a cohering structure was seen. Since the structure were compact and dense, disintegration is probably to be low or absent, hence little or no reaction.

This is probably the reason why all the products at this fermentation temperature were not acceptable. Narrower pores with more fissures were also noticed all over the surface. [22, 23] reported that the morphology of fermented and unfermented African locust bean seeds depends on the botanical sources of starch.

8. Conclusion

The highest nutritional composition of fermented *Parkia Biglobosa* was obtained at fermentation temperature 40^0 C on the third day (72 hours). Elevation in the fermentation temperature denatures nutrient and the nutritional values. The higher the temperature, the lower the nutritional values in the fermented seed.

This work discovered that fermented *Parkia biglobosa* was discovered to be very rich in protein (about 52%), increase in its consumption will reduce the risk of nutrient deficiencies in consumers.

9. Conflict of Interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

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