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Molecular Compositions and Morphological Structures of Fermented African Locust Bean Seed (*Parkia biglobosa*)

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Abstract: The effect of production processes on molecular compositions and structure of fermented *Parkia biglobosa* were investigated in this study. The protein-based condiment was obtained from fermented *P. biglobosa* seed. Fermentation took place for five days with *Bacillus subtilis* used as a starter culture. The raw seeds were processed to bring out the edible seed for fermentation. There were examined the effect of fermentation with respect to time and temperature on identifying organic functional groups using FTIR (Fourier Transform Infrared Spectroscopy) and morphological structure of the seed using SEM (Scanning Electron Microscopy. Different magnifications were used for the SEM analysis, and the ones with the best images were reported in this work. Images were described based on the surface pattern morphology.

Keywords: fermentation; SEM; FTIR; *Parkia biglobosa*; *Bacillus subtilis*.

1. Introduction

Parkia biglobosa (*P. biglobosa*) is a plant that grows within 7 - 20 m in height [1-3]. *P. biglobosa* tree is an important multipurpose plant in West Africa [4, 5]. The pods have clusters of irregular shape seeds of about 15 on each row [6-9]. This seed was first named by Mungo Park [10] with the botanical name given by a Scottish botanist named Robert Brown in 1826. *P. biglobosa* seed, also known as 'Iru' in Nigeria, is one of the known sources of plant-based protein in some parts of the world, especially in the African diet [11]. Different names were giving to Iru in different countries - Sierra Leone it is called kinda, Nigeria and Ghana as Dawa Dawa, known as Afintin in the Benin Republic, Nététu in Senegal and Soumbala in Burkina Faso, kinema in Nepal and Japan as natto [12-14]. Fermentation biologically converts hard to separate substrates such as sugar or starch into simpler compounds [8]. Food is basically fermented to produce needed body nutrients and get rid of anti-nutrients [15, 16]. This is important to enhance nutrition in most parts of the world, especially in Nigeria. *B. subtilis* and a host of other microorganisms have been reported to have a wide range of usage in food processing and bioremediation of soil or water polluted by microorganisms [17-20]. Several microorganisms have also served as a good source for waste conversion [21-23], such as the bioconversion of waste paper to edible sugar, sweet potatoes peel to bioethanol, orange peel to ethanol and a host of other bioconversion processes. Microorganisms convert available hydrocarbons into organic acids, alcohols, and carbon dioxide [24, 25]. The flavor defines non-volatile compounds while aroma-volatile compounds. Phenolic compounds account for the color. The aroma and flavor of fermented condiments are important not only for the distinction of the condiments but also for evaluating their quality and stability.

2. Materials and Methods

Raw *P. biglobosa* bean seeds were obtained from an open market in Ota, Ogun-State, Nigeria.

2.1. Processing of raw seeds.

Raw seeds were handpicked to remove debris and were processed into soft cotyledon using the method of [7, 26-27].

2.2. Preparation of microorganism used as a starter culture.

Microorganism used was prepared using the method of [7, 11].

2.3. Preparation of samples for fermentation.

Samples to be fermented were pulverized to improve the surface area and proper incorporation of starter culture. 200g of samples were placed inside a 1000 mL flat bottomed flask and fermented for 5 days at various temperatures using a bioreactor. Samples fermented were picked every day (24 hours) and stored in the freezer for further analysis [28-30].

2.4. Preparation of samples for SEM.

Fermented samples were thoroughly degreased and dried to eliminate gas from organic contamination and water. They were cleaned ultrasonically with methanol, and a compressed gas was used to blow it dry and also to remove surface dust. These samples were later dried to remove moisture completely by an oven [31-32].

2.5. Preparation of samples for FTIR.

FTIR spectroscopy of the model Bruker VideoMVP™ Single Reflection ATR Microsampler Spectrometer was used to identify and characterize organic functional groups. Fermented samples were oven-dried at a very low temperature of 70 ºC for 24 hours. Dried samples were ground with a Binatone blender into fine powder. The powder sample was mixed with KBr and ground to reduce the particle size to less than 5mm in diameter. This was done until crystallites can no longer be seen and become paste-like, sticking to the mortar [33-35].

3. Results and Discussion

3.1 African locust beans morphological structure.

Figure 1 shows the morphological structure of unprocessed raw seed of *P. biglobosa*, where only the cotyledon was washed, removed, and the seeds pulverized to increase the surface area. This figure shows an agglomerated/cluster, cohering structure with a coarse corrugated surface. Small pores were noticed at some points with no fissures. Figure 2 shows the structure of the seed after processing, such as washing, dehulling, and boiling. Surface with wider pores (more than Figure 1) also crowded into clusters of dense agglomerated cohering structure; this shows the splitting of larger molecules into smaller ones. Figure 3 (samples fermented for 3 days at 40°C) shows a non-cohering pattern with wider agglomerated fissures, and increased pore size was noticed more than in Figure 2. This figure also shows that the fermentation process has taken place, and carbohydrates were broken down into smaller units.

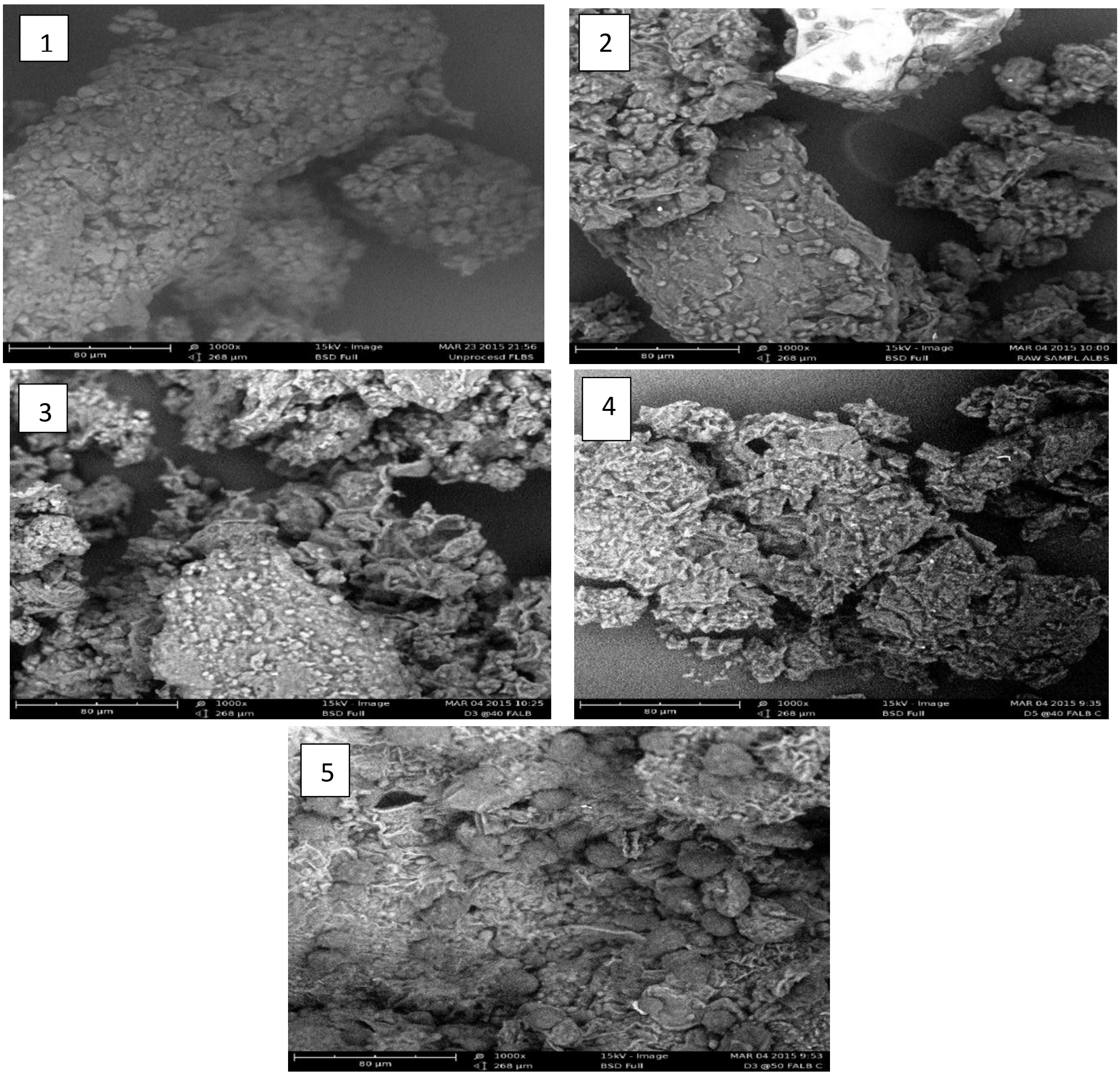

Figure (**1**) Unprocessed *P. biglobosa* seeds [Raw/Unprocessed];(**2**) Processed *P. biglobosa* seeds [Peeled, washed and boiled];(**3**) *P. biglobosa* sample fermented for 3 days at 40 °C; (**4**) *P. biglobosa* sample fermented for 4 days at 40 °C;(**5**) *P. biglobosa* sample fermented for 5 days at 40 °C.

This is the morphological structure of African locust bean seed fermented for three days above room temperature. The granules noticed adhered more to the surface of the samples. A larger volume of pore spaces indicates an even distribution of phase. A dendritic little flowery pattern to the right was noticed, which suggests that some minerals or compounds were present at that stage. Sample fermented for 4 days at 40 °C (Figure 4) shows a wider but the agglomerated surface pattern with disappearing flowery pattern more than Figures 2 and 3, these probably shows some larger molecules have been broken down from their initial forms, e.g., protein. Agglomerated and cohering structure but with a flowery dendritic pattern which has micro-cracks and minimal pores were noticed in figure 5 (sample fermented for 5 days at 40 °C) with uniform, regular bigger granules, little evidence of disintegration were present, this is different from the trend observed in figure 3 with larger pores. The slow fermentation rate in figure 5 was probably due to the coarse structure.

3.2. Identification of organic functional groups present in P. biglobosa seeds.

Table 1. Unprocessed *P. biglobosa* [Raw].

Functional Organic group	**Vibration type**	**Characteristics Absorptions (cm^{-1})**	**Intensity**
O-H Carboxylic acids, Phenols and Alcohols N-H Amine and Alkynes	Stretching vibration with an H-bond	3272	Strong, sharp and broad
C-H and =C-H Alkenes and Alkanes	Stretching vibration	2925 - 2854	medium but Strong
C=O Carbonyl, Ketones, Esters, and Aldehydes.	Stretching vibration	1744 - 1631	Strong intensity with intense
C=C Alkenes Primary amines NITRO	Bend Stretching vibration Stretching which is Asymmetric	1631 1543	Medium peaks with multiple sharp Strong variable bonds
Alkanes -C-H Alkyl halide C-F	Stretching bend vibration	1392	Strong variable intensity
Alcohols, ethers, C-O, and carboxylic acids. Alkyl halides. C-N C-H wag (-CH_2X) Aliphatic amines.	Stretching Stretching	1238	Strong [Intensity drops within fingerprint region] Absorption is strong Weak-medium
C-N Amines. C-O Ether	Stretching Stretching	1051	Strong [Intensity drops within fingerprint region]

Table 2. Starting Sample (Processed).

Functional group	**Vibration type**	**Characteristics Absorptions (cm^{-1})**	**Intensity**
C-H Alkynes and N-H O-H Phenols, Alcohol, Amine and carboxylic acids	Stretching, H-bonded	3275	Broad, Strong with sharp medium secondary intensity
C-H and =C-H Alkanes	Stretching	2924 - 2855	Strong and medium
NITRO N-O	Stretching but Asymmetric	1536	Strong bonds
C-C (in ring) and C=C -C-H Alkanes	Bend Stretching	1451	Weak-Medium with sharp bond in multiple

Functional group	Vibration type	Characteristics Absorptions (cm^{-1})	Intensity
Alkane, -C-H Alkyl halide, C-F	Stretching Bending	1391	Strong [FP region] variable
C-H wag (-CH2X) Alkyl halides. C-O Carboxylic acids, Alcohol and ethers. C-N	Stretching Stretching	1240	Strong intensity FP
C-H wag ($-CH_2X$) C-O Carboxylic acids, Alcohol, ethers. Aliphatic amines C-N	Stretching Stretching	1160	Strong intensity FP
Aliphatic amines C-N C-O Alcohol. C-O Ester C-F	Stretching Stretching	1058	FP Strong Two bands or more

Table 3. Sample fermented for 3 days at 40°C.

Functional group	Vibration type	Characteristics Absorptions (cm^{-1})	Intensity
Amine. O-H Phenols, carboxylic acids, Alcohol, C-H Alkynes	Stretching, H-bonded	3271	Strong, broad, sharp and Medium secondary intensity
=C-H Alkanes, C-H and alkenes	Stretching	2923 - 2853	Medium but Strong intensity
C=C Alkenes	Stretching	1625	Variable
C=C Aromatic N-O Nitro compounds.	Stretching but asymmetric	1536	Strong bands. Weak-Medium, sharp, multiple bands.
Alkanes -C-H C-C, C=C (in ring) Aromatic and	Stretching Bend	1444 - 1401	Weak-Medium, sharp, multiple bands. FP
Alcohol C-O, carboxylic acids. C-H wag ($-CH_2X$) C-N Aliphatic amines.	Stretching Stretching	1240	Strong
Alcohol C-O carboxylic acids. C-H wag ($-CH_2X$)	Stretching	1159	Strong FP

Functional group	Vibration type	Characteristics Absorptions (cm^{-1})	Intensity
Aliphatic amines.	Stretching		
Aliphatic amines C-N. C-O Alcohol, carboxylic acids, ethers. Alkyl halide C-F. C-O Ester	Stretching Stretching	1058	Strong medium FP Strong Two bands

Table 4. Sample fermented for 4 days at 40°C.

Functional group	Vibration type	Characteristics Absorptions (cm^{-1})	Intensity
Aliphatic amines C-N. Alcohol C-O, ethers, carboxylic acids. Alkyl halides. Ester C-O.	Stretching Stretching	1060	Strong medium FP Two Strong bands
Alkyl halides C-CI. C-Br Alkyl halides.	Stretching Stretching	5890	Strong

Table 5. Sample fermented for 5 days at 40°C.

Functional group	Vibration type	Characteristics Absorptions (cm^{-1})	Intensity
O-H Alcohol, carboxylic acids and phenols. C-H Alkynes	Stretching, H-bonded	3329	Sharp, broad and Strong
N-H Amine	Stretching	3266	Secondary and medium
C-H Alkanes and =C-H alkenes	Stretching	2923	Medium and strong
C=C Alkenes	Stretching	1631	Variable intensity
Aromatics C-C Aromatics C=C Alkanes -C-H	Stretching Stretching bend	1412	Weak-Medium multiple bands
Alcohol C-O, ethers, carboxylic acids. Alkyl halides. Aliphatic amines C-N. Alkyl halide C-F	Stretching Stretching Stretching	1098	Strong-medium FP Strong
Aliphatic amines C-N. Alcohol C-O, carboxylic acids, ethers. Alkyl halide C-F. C-O	Stretching	1033	Strong-medium FP Strong

Functional group	Vibration type	Characteristics Absorptions (cm^{-1})	Intensity
	Stretching		Two bands

FTIR revealed (Tables 1 – 5) strong stretching characteristics spectrum broad bond of Phenols, hydrogen-bonded Alcohol, O-H, and carboxylic acids compound were noticed. Vibration stretching of C=H, Alkynes which have a sharp and strong intensity, was recorded at the same absorption frequency in all the samples. Similarly, a secondary band N-H medium stretching broad and amine was also noticed. Alkene =C-H and alkane C-H band with stretching frequency and medium with strong intensity were noticed in the entire spectrum. All analyzed samples revealed similar properties and characteristics as reported above but at absorption frequency which was not the same. This might probably be due to the similarity in aroma noticed during and after fermentation. Samples at different stages can be distinguished by the difference in the intensity of each compound. This research identified different volatile compounds in fermented *P. biglobosa,* such as carbonyls, esters, and alcohols.

4. Conclusions

This research identified different volatile compounds in fermented *P. biglobosa*, such as carbonyls, esters, and alcohols. The morphological study revealed the progress of fermentation at different stages.

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Conflicts of Interest

The authors declare no conflict of interest.

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