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FATTY ACIDS PROFILE, PHYSICOCHEMICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF UNFERMENTED AND FERMENTED *PARKIA BIGLOBOSA* (AFRICAN LOCUST BEANS) SEED OIL

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ABSTRACT

The African locust bean (*Parkia biglobosa*) seed is one of the major sources of plant protein in African diet. Effect of fermentation on the chemical composition and physicochemical properties of *P. biglobosa* seed oil was determined. The chemical composition of the unfermented and fermented seed oil was determined using Gas-Chromatography-Mass Spectroscopy (GC-MS) and physicochemical parameters were determined using standard methods according to the Association of Official Analytical Chemist (AOAC), while antioxidant activity was determined using the 2, 2-diphenylpicrylhydrazyl radical (DPPH) method. The result revealed that the unfermented seed contained five constituents dominated with 11-octadecenoic acid methyl ester (49.70%) and linoleic acid (16.88%) while the fermented seed contained fifteen constituents with 13-octadecenoic acid methyl ester (25.30%) and oleic acid methyl ester (22.15%) being the major constituents. Palmitic acid methyl ester, methyl stearate and linoleic acid were common to both oils. The proximate compositions (moisture content, saponification acid, peroxide and ash values) of the unfermented seeds are higher than the fermented seeds. 14.383 % and 12.449 % alkaloid, 2.064 % and 1.947% flavonoid content were obtained from the unfermented and fermented seed oils respectively. The seed oils also showed significant activity as antioxidant using the stable radical 2,2-diphenyl picrylhydrazyl (DPPH) with 83.735% and 75.075% inhibition for the unfermented and fermented seed oils respectively. It can be concluded that the fermented seed oil contain more nutritional phytochemicals than the unfermented seeds, thus explained the effect of fermentation and reason for its use as condiment.

Keywords: *Parkia biglobosa*, Palmitic acid methyl ester, methyl stearate, Linoleic acid, phytate, fermentation

INTRODUCTION

Parkia biglobosa (African locust bean), is a genus of flowering plants of the Fabaceae family and is one of the many species of trees which serve as sources of food and medicinal purposes to the indigenous people of Africa. It is a perennial deciduous tree and provides shade for man. It is planted mainly for the food value of its fruit; as a source of mouth wash to relieve toothache, the bean husk (seed coat) are used with indigo dye to improve the luster of fabrics while the tree bark yield a red tannin for dying leather. The leaves are used to treat burns, haemorrhoids, and used as a local soap. The tree is also important in apiculture, being a good source of nectar and suitable for placement of hives. Boiled pods are used to dye pottery black; the ash is applied as a mordant [1-3]. The tree can be found mainly in Africa, especially in the following countries; Nigeria, Benin, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Gambia, Guinea, Guinea-Bissau, Mali, Togo, and Uganda [4-5]. The raw unfermented seeds of *P. biglobosa* are not edible but fermentation improves the nutritional quality and digestibility. The most valuable part of the Locust bean tree is the seed which is high in protein, carbohydrate, lipid and is a good source of fat and calcium [6].

The unfermented seed is known as *Karwa* in Hausa; *Iyere or Igba* in Yoruba; *Soumbala* in Burkina Faso, Mali and Guinea while the fermented seed is called *Iru* in Yoruba, *Ogiri* in Igbo and Dawadawa in Hausa land of Nigeria. They are traditionally used as food condiment for flavouring and are known to be rich in protein and contain easily digestible calcium and edible oil. Medicininal applications include the treatment of parasitic infections, circulatory system disorders, such as arterial hypertension, stroke and disorders of the respiratory system, digestive system and skin. The proximate and mineral composition, phytochemical, antibacterial and termicidal properties of *P. biglobosa* extracts have been investigated [7-10]. The fermented locust bean seed is used in controlling diabetes, cholesterol level; promote good sight and aids digestion. The water and alcoholic extracts of fermented locust bean is used to reduce blood sugar and used in the management of bacterial infections [11-14].

Fermentation is one of the oldest methods of food preservation known to man and the oldest preservation technology in the world. This has been known and practiced by the human race since prehistoric times, long before the scientific underlying principles were understood [15]. The physical, chemical and nutritional characteristics of the African Locust Bean seeds changes immediately after fermentation since the raw African locust beans are nutritionally deficient and unpalatable [16 -18]. Parkouda et al., [19] opined that alkaline-fermented food condiments play an important role in the diets of many people in developing and a few developed countries. *Bacillus subtilis* and *Staphylococcus* spp. were reported to be the microorganisms associated with the fermentation of African Locust beans. They hydrolyze proteins into amino acids and ammonia during fermentation, thereby giving rise to increase in pH during the fermentation process. Most of these fermentations are natural, involve contact with appropriate inocula and are accomplished by the natural temperatures of the tropics. The benefits of fermentation include increased shelf life, removal of toxins, improvement in texture, taste, and flavour as well as increased nutritional value [20-21]. Furthermore, fermented African locust bean seeds provide dietary fiber, energy, minerals, vitamins, thus are used as supplement in the third world countries where the need for protein supplementation is high for both adult and infants. It also serves as good source of protein for animal feeds and livestock. Major benefits of fermentation is the conversion of sugars and other carbohydrates to usable end products, removal of anti-nutritional components, increasing shelf life or storage, reduction in cooking time, detoxification, elimination of beany flavours and improvements in digestibility [22-25]. The objective of this research therefore is to determine the chemical composition, physicochemical properties and antioxidant activity of unfermented and fermented *P. biglobosa* seed oils.

MATERIALS AND METHODS

MATERIALS

Collection and Preparation of Samples

Samples of *Parkia biglobosa* seeds (300 g) were obtained from "Aba Eleshin" in Akinyele Local Government Area of Oyo State, Ibadan, Nigeria in May 2017 and identify by a Taxonomist at the Botany Department, University of Ibadan, Nigeria. The seeds were dehulled and air-dried for five weeks, and kept in desiccator till when needed.

Fermentation Process

Parkia biglobosa seeds (300 g) were boiled for 12 h, dehulled to remove the seed coat by mashing in a mortar. This was subjected to natural solid substrate fermentation without any inoculation for 4 days by wrapping with Banana leaves as it is believed that this will accelerate fermentation of the seeds, while also bringing "an increase in protein and crude fat contents with corresponding decrease in carbohydrate [26].

Reagents: Hexane (C_6H_{14}), methanol (CH_3OH), hydrogen peroxide (H_2O_2), 2,2-diphenyl-1-picrylhydrazyl radical ($C_{18}H_{12}N_5O_6$), potassium ferricyanide $K_3[Fe(CN)_6]$, ferric chloride ($FeCl_3$), sulphuric acid (H_2SO_4), potassium permanganate ($KMnO_4$), ammonium hydroxide (NH_4OH), hydrochloric acid (HCl), ammonium thicoyantae (NH_4SCN), ethyl acetate ($C_4H_8O_2$),

diethyl ether ($(C_2H_5)_2O$), ethanol (C_2H_5OH), phenolphthalein ($C_2H_{14}O_4$) indicator, sodium hydroxide (NaOH), alcoholic solution of potassium hydroxide (KOH), garlic acid ($C_7H_6O_5$), sodium carbonate (Na_2CO_3) and trichloroacetic acid ($C_2HCl_3O_2$) obtained from Sigma-Aldrich, Germany were used.

Equipment

The following apparatus and equipment were used: OAUS Electronic Weighing balance, Buchi Rotary Evaporator fitted with Vacuum pump V-700 and B-490 heating bath was used to concentrate samples. Oven (Carbolite), Ultraviolet-visible (UV-visible) spectrophotometer (Unico1200 & Perkin Elmer lambda 25 model, UK), and Gas Chromatography-Mass (GC-MS) Spectrophotometer (Gas chromatograph/GC-MS (HP 6890), UK), heating mantle, desiccators, syringes, sample bottles and flasks.

Methods

Extraction of Seed Oil

Dried unfermented and fermented *P. biglobosa* seed samples (200 g and 100 g) were transferred separately into a 10 Litres capacity round bottom flask and 680 mL of pure n-hexane was added, stirred and allowed to stay for 72 hours. The mixture was collected using muslin bag. This process was repeated by adding same amount of pure n-hexane to the shaft. The combined filtrate was filtered using Whatman filter paper (1 mm). The filtrate was concentrated with the aid of rotary evaporator set at 35^0C and the concentrate was transferred into a vacuum oven set at 35^0C and 700 mmHg pressure. Fatty acid profile was determined by use of GC-MS spectrometer and physicochemical analyses were carried out for the samples in triplicate in accordance with the Association of Official Analytical Chemist (AOAC) procedures [27]. Antioxidant screening was carried out by use of DPPH method [28].

Analysis of the Seed Oil

Gas Chromatography: The oil obtained from the unfermented and fermented *P. biglobosa* seeds was analysed using an HP 6890 Gas Chromatograph powered with ChemStation Rev. A09.01 [1206] Software at the following specifications: column type: HP 5MS, inlet temperature: 150^oC, split ratio: (20:1), with hydrogen as carrier gas. Flow rate: 1.0 mL/min, column dimensions: 30 m x 0.25 mm x 0.25 µm, oven program: initial at 40^oC, ramped at 5^oC/min to 200^oC, and run at 220^oC for 5 minutes [28-29].

Gas Chromatography–Mass Spectrometry: HP 6890 powered with ChemStation Rev. A09.01 [1206] Software was used and GC oven temperature and conditions were as described above. Mass spectra were recorded at 70 eV [30].

Identification of Components: Relative percentage compositions of constituents was obtained from electronic integration measurement using Flame Ionization Detector (FID) set at a temperature of 300^oC while individual components of the oil were identified on the basis of their retention indices determined with reference to a homologous series of n-alkanes and by comparison of their mass spectra fragmentation pattern (NIST0.8 L database/chem. Station system) with data previously reported in literature [31]. The relative percentages of the characterized components and their refractive index values are given in Tables 1 and 2.

Determination of Antioxidant Activity

DPPH Method

The antioxidant activity of both unfermented and fermented *P. biglobosa* seed oil was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method. DPPH radical (3.94 mg) was dissolved in 100 mL of methanol to give a 100 µM solution. The oil extract (0.2 mL) from stock solution of 1g/L was diluted with methanol and 2 mL of DPPH solution (0.5 mM) was added. The decrease in absorption of DPPH at 517 nm was measured in UV spectrophotometer after 30 minutes of incubation for 100 µg/mL sample. Analysis was carried out in triplicates and the average result was recorded [32-33].

Determination of Total Phenolic Compound (TPC)

Procedure: Garlic acid calibration standards: 0.5g of garlic acid was dissolved in 10 mL ethanol and then diluted to 100 mL with water (5 g/L). 1, 2, 5 and 10 mL was diluted to 100 mL with water to create standards with 50, 100, 250 and 500 mg/L concentration respectively. It was stored up to 2 weeks at 4^0C so that standards could retain 98% of their potency as potency is retained for only 5 days at room temperature [27]. Oil sample (0.2 mg) was weighed and poured inside a conical flask and extracted with 10 mL 50% methanol. It was heated in water bath at 80^0C for 30 minutes and allowed to cool. The contents were centrifuged for 5 minutes. 0.2 mL of sample was pipetted and added to 2.8 mL distilled water, 0.25 mL Folin-Ciocalteau reagent, and 1 mL sodium carbonate. It was allowed to stay for 15 minutes and was read in a Spectrophometer at 760 nm. Standard (garlic acid) was treated as above and the reading obtained was used to plot a graph of absorbance where phenolic content was extrapolated. Sample with absorbance reading above 500 mg/L of standard were diluted and re-measured [34].

RESULTS AND DISCUSSION

The oils obtained from both unfermented and fermented seeds were light pink in colour. An average yield of (51.9 ± 0.7% and 61.1± 0.2%) per 100 gm of pulverized seed was obtained respectively. These values are higher than reported values for some other similar oil seeds contents such as water melon seed, 41.32±0.5 per 100 gm (41.32% w/w); Cucumismelo (44.85%) and pumpkin seed (46.03%) oils [28].
The fatty acids methyl ester of the seed oils are presented in Tables 1 and 2 while the results of phytochemical, physicochemical, DPPH and TPC analysis of *P. biglobosa* seed oils are presented in Table 3. Different phytoconsituents like alkaloids, flavanoids, phytate, oxalate, as well as 2,2-Diphenypicrylhydrazyl, total phenolic content are reported. Figure 1 shows the chromatography spectra of unfermented and fermented *P. biglobosa* seed oils.

Table 1: GC-MS Analysis of Unfermented *Parkia biglobosa* seed oil

S/No.	RT (mins)	Compound	Mol. Formula	AI	% Area
1	19.560	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	1915	12.49
2	22.284	Cis-9,12-octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	2097	10.87
3	22.341	11-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	2109	49.70
4	22.776	Methyl stearate	$C_{19}H_{38}O_2$	2130	10.06
5	23.343	Linoleic acid	$C_{18}H_{32}O_2$	2173	16.88
					Total = 100.00

*****Percentages calculated from the flame ionization detection data. RT = Retention Time; AI = Arithmetric Retention Index on HP-5MS column.

Table 2: GC-MS Analysis of Fermented *Parkia biglobosa* seed oil

S/No.	RT (mins)	Compound	Mol. Formula	AI	% Area
1	19.640	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	1915	10.23
2	20.905	Myristic acid	$C_{14}H_{28}O_2$	1720	3.91
3	21.145	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	2072	2.57
4	22.616	Cis-13-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	2126	25.30
5	22.667	Oleic acid, methyl ester	$C_{19}H_{36}O_2$	2118	22.15
6	22.982	Methyl stearate	$C_{19}H_{38}O_2$	2130	6.71
7	23.966	Linoleic acid	$C_{18}H_{32}O_2$	2173	15.44
8	24.029	Cis-13-octadecenoic acid	$C_{18}H_{34}O_2$	2116	4.26
9	24.07	Octadecanoic acid	$C_{18}H_{36}O_2$	2169	3.39
10	25.574	11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	2333	2.04
11	25.877	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	2312	0.69
12	28.218	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	2531	0.29
13	29.196	Methyl 20-methyl-docosanoate	$C_{23}H_{46}O_2$	2517	0.46
14	30.112	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	2725	0.57
15	30.959	Squalene	$C_{30}H_{50}$	2790	0.58
					Total = 98.59%

*****Percentages calculated from the flame ionization detection data. RT = Retention Time; AI = Arithmetric Retention Index on HP-5MS column.

Table 3: Results of Phytochemical, Physicochemical, DPPH and TPC Analysis of Unfermented and Fermented *Parkia biglobosa* seeds Oils

S/N	Analysis	Unfermented	Fermented
1.	Alkaloids	14.383 %	12.449 %
2.	Flavanoids	2.064 %	1.947 %
3.	Phytate	8.825 %	8.660 %
4.	Oxalate	0.875 mg/g	1.260 mg/g
5.	DPPH	83.735 %	75.075 %
6.	Total Phenolic Compound (TPC)	0.663 %	0.814 %
7.	Acid Value	36.025 mg/kg	17.102 mg/kg
8.	Saponification value	11.950 mg/kg	11.500 mg/kg
9.	Peroxide value	10.450 mEq/kg	6.200 mEq/kg
10.	Ash	29.0%	5.4%
11.	Moisture content	8.6%	56.7%

The moisture content of unfermented *P. biglobosa* seeds was 8.6% but that of the fermented bean increased greatly (56.7%) due to boiling in water during processing. The ash content of unfermented and fermented of *P. biglobosa* seeds were 29.0% and 5.4% respectively. These values agreed favourably well with 30% and 5.1% reported in a previous analysis by Eka [35], but with little difference in amount. There was therefore decrease in ash content after fermentation. Fermentation of African locust bean seeds is nutritionally desirable since the process increases the crude protein [17, 24]. Acid value for the unfermented (36.025 mg/kg) was considerably higher than that of fermented seed oil (17.102 mg/kg). Peroxide value, 10.450 mEq/kg obtained for the unfermented seed oil increased to 17.102 mg/kg for the fermented seed oil. Other parameters like saponification value, total phenolic compound, phytate, oxalate are appreciably the same (Table 3). The saponification value 11.950 and 11.5000 mg/kg obtained for the unfermented and fermented oils respectively justified the molecular weight of the phytochemicals since the saponification value is an index of mean molecular weight of the fatty acids of glycerides, the lower the saponification value, the larger the molecular weight of fatty acids in the glycerol (Roger. and Gadfer, (1999). Also of significance importance is the DPPH free radical scavenging activity of the unfermented seed oil 83.735 %, which was slightly higher than that of fermented seed oil (75.075 %), these values are significant when compared with garlic acid used as standard (85.431%). In another study, it was reported that fermentation increased the nutritive values, free phenolic and antioxidant activity of the African locust bean, making it a potential functional food in addition to its traditional role of dietary protein source [26].

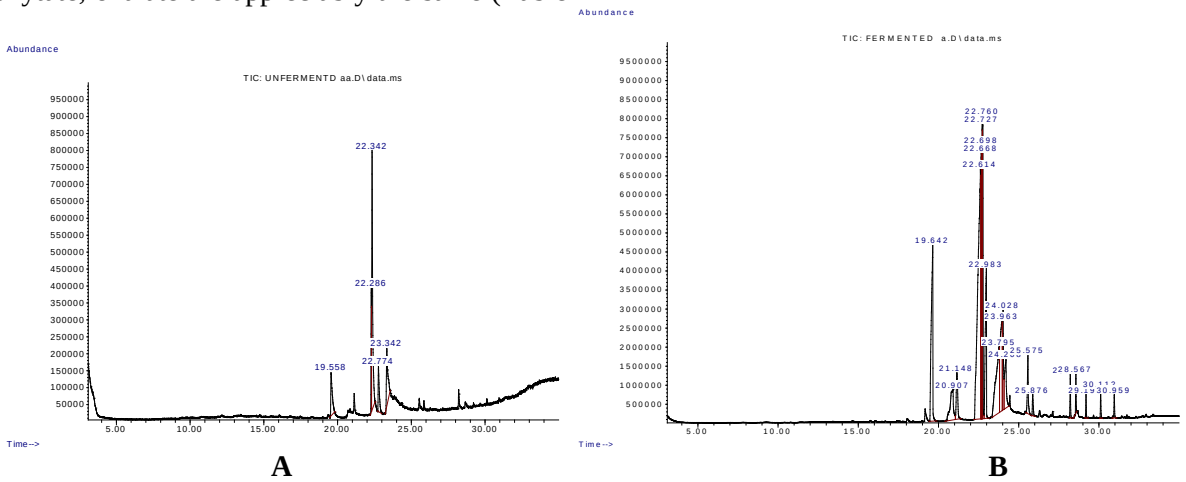

Figure 1: Gas chromatography Spectra of Unfermented (A) and Fermented (B) *P. biglobosa*

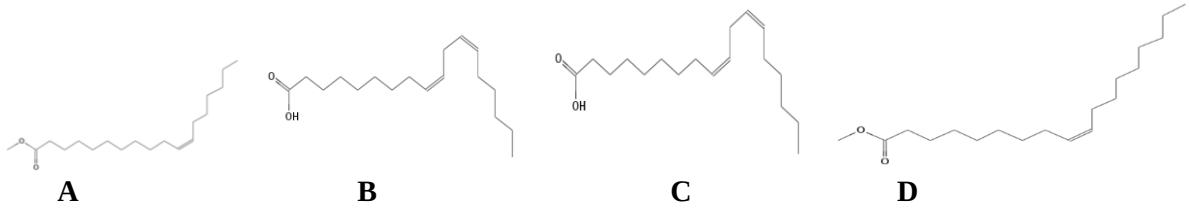

Figure 2: Structures of the most abundant compounds, 11-Octadecenoic acid, methyl ester (49.70%) **(A),** Linoleic acid (16.88%) **(B)**, Cis-13-octadecenoic acid, methyl ester (25.30%) **(C),** Oleic acid, methyl ester (22.15%) **(D)** obtained from Unfermented and Fermented *P. biglobosa* seed oils.

Oxygenated terpenes, fatty acids and esters were the major constituents of both the unfermented and fermented *P. biglobosa* seed oils. The GC Spectrum of the seed oils are shown in Figure 1 with the fermented seed oil having more peaks (constituents) than the unfermented seed oil.

Five constituents totalling 100% were identified in the GC-MS analysis of unfermented *P. biglobosa* seed oil (Table 1) and was dominated with 11-octadecenoic acid, methyl ester(49.70%) followed by Linoleic acid (16.88%). Fifteen constituents were identified in the fermented *P. biglobosa* seed oil, totalling 98.59% with cis-13-octadecenoic acid, methyl ester (25.30%) and oleic acid, methyl ester (22.15%) as the major constituents. This shows the effect of fermentation of the beans. Palmitic acid, methyl ester, methyl stearate and linoleic acid were identified in both oils. Figure 2 shows the structures of the major constituents obtained from the seed oils. The polyunsaturated essential fatty acid found in the seed oils occur widely in plant glycosides. They are essential fatty acid in mammalian nutrition and are used in the biosynthesis of prostaglandins and cell membranes. They are permitted as a natural additive in organic products, as flavoring agents and thickners in food, detergents and cosmetics [5,20,27].

CONCLUSION

Fermented *Parkia biglobosa* seed is used as condiment in Africa and is believed to have many health benefits. This present study aimed at determining the fatty acid constituents of the unfermented and fermented seed oil using Gas-Chromatography- Mass Spectrometry. Results showed that they contain mainly esters of fatty acids. The unfermented seed oil was dominated with 11-octadecenoic acid, methyl ester and linoleic acid while the fermented seed oil contained cis-13-octadecenoic acid, methyl ester and oleic acid, methyl ester as the major constituents. The seed oils also showed significant activity as antioxidant using the stable radical 2,2-diphenyl picrylhydrazyl (DPPH) radical. Also, fermentation of African locust bean seeds is nutritionally desirable since the process increases the crude protein.

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