GC- MASS SPECTROSCOPIC CHEMICAL CHARACTERIZATION AND PHYSICO-CHEMICAL PROPERTIES OF OIL FROM SEED KERNELS OF FOUR CULTIVARS OF *MAGNIFERA INDICA*

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ABSTRACT

The objective of the study was to evaluate the chemical composition of oil from seed kernels of four cultivars of *Mangifera indica* by Gas Chromatograph -Mass Spectrometry, to study most important physico-chemical parameters, and identify some functional group of the seed oil using FT-IR Spectroscopy. *Mangifera indica* seed oils used for the analysis were obtained by soxhlet extraction method using n-Hexane as solvent. Individual components of the oil were identified by GC-MS. Functional groups of the seed oils were evaluated using Fourier Transform-Infra Red Spectroscopy. From the results of physicochemical analysis; refractive index (1.4528 -1.4600), viscosity (22-42.25Cst), specific gravity (0.86-0.92), free fatty acids (1.41-3.09%.), peroxide value (4.94 - 6.01 meq/kg), acid value (2.81-6.16 mg KOH/g fat), iodine value (48.15 to 50.88 mg I_2/100g,), saponification value (105.72 -125.56mgKOH^{-1}fat) and unsaponifiable matter (1.2-1.56 %). FT-IR spectral obtained showed absorption bands at 3405cm^{-1}, 2923cm^{-1}, 2853 cm^{-1}, 1746 cm^{-1}, 3004.5cm^{-1} -3 005.24 cm^{-1}, and 1656.3cm^{-1}. The results from GC-Mass Spectrometry showed that the major component of *Mangifera indica* seed oil for all the cultivars were cyclohexane (9.423-40.474%), palmitic acid (13.265-24.472%) and 2,5-dimethyl- tetrahydro- Furan (4.742-22.831%). Other compounds identified are Linoleic acid (14.573%), stearic acid (17.152%), and cis-Vaccenic acid (52.052%). It can be concluded that *Mangifera indica* seed oil can be further ultilized domestically and industrially rather than just been discarded as waste.

Keywords: *Mangifera indica*, seed oil, GC-Mass Spectrometry, FT-IR, physico-chemical parameters.

INTRODUCTION

Mango (*Magnifera indica L.*) belongs to the family Anacardiaceae, it is one of the most cultivated fruit in the world and recognized as ‘king of the fruit’ in the Orient (Pott, Marx, Neidhart, Muhlbauer, & Carle, 2003). The edible fleshly portion or pulp of mango fruit is relished to the extent of commercialization. The mango is a very common tropical fruit usually found in Southern Asia, especially in Eastern India, China, Burma, Andaman Islands, Central America and Africa. Its shapes, sizes and color depend on the variety (Nzikou et al., 2010). Mango fruit is mainly used for consumption in South Western Nigeria and it has been processed into juice, jam or dried products. This can ensure consumption of the fruits all-year round. The physico-chemical characteristics of fruits and the qualities of the processed products vary with the variety of mango as some are more suitable than others for specific applications (Doreyappa & Ramanjaneya, 1995). Each part of a mango tree, such as its leaves, flowers, bark, fruit, pulp, peel and seeds contains essential nutrients that can be utilized. It has been well documented that mango fruits are important source of micronutrients, vitamins, and other photochemical. Moreover, mango fruits provide energy, dietary fibre, carbohydrates, proteins, fats and phenolic compounds (Tharanathan, Yashoda, & Prabha, 2006.).

Depending on the cultivars and products made, mango industrial by-products, namely peels and seeds, represent 35-60% of the total weight of the fruit (Larrauri, Rupérez, Borroto, & Saura-Calixto, 1996). These are discarded as waste and create environmental problems.

Oilseed processing expands the use of crops and also brings value to waste products. Vegetable fats and oils derived from plant seeds have been playing vital roles to provide comfort in human lives in various aspects. All oils and fats are made up of a mixture of triglycerides. The characteristic of a particular oil or fat depends on the actual fatty acids present in the individual triglyceride molecules. Some of these fatty acids have longer carbon chains than others, and exist in three forms: saturated, mono-unsaturated and polyunsaturated (Bookock, 1998). Outside the realm of food manufacture, vegetable oils feature in a variety of industrial uses such as in the manufacture of soap, production of paints, skincare products, varnishes, industrial lubricants and plastics. The increase in world population and the increasing demand for oils as well as oil meal has also resulted in tremendous increase in their prices. This increase in prices necessitates the need to investigate new sources of oils, especially among the unconventional and underexploited oilseeds.

The mango seed oil is not yet utilized like the mango fruit, for industrial application in African countries. The underutilization could be due to the limited knowledge of the functional properties of the seed oil and appropriate processing technology. In this article, we characterize the seed oil and studied the physico-chemical properties of oils extracted from the seed kernels of four various cultivars of mango in order to increase the usefulness of the mango seed oil both industrially and domestically.

LITERATURE REVIEW

Extensive research on mango by-products has been performed in the past decade. These researches have revealed that they contain high levels of various health-enhancing substances, such as phenolic compounds, carotenoids, vitamin C and dietary fibre (Ajila, Naidu, Bhat, & Prasada, 2007, Kim, et al 2010, Sogi, Siddiq, Greiby, & Dolan 2013, and Kittiphoom, & Sutasinee, 2013). These compounds contribute to lowering the risk of cancer, Alzheimer's disease, cataracts and Parkinson's disease, among others. The antioxidant and radical scavenging activities of these bioactive compounds have been shown to delay or inhibit the oxidation of DNA, proteins and lipids (Ayala-Zavala et al., 2011). Nzikou et al. (2010) carried out a study on mango seed kernels to clarify their proximate composition and the characteristics of the extracted oil including unsaponifiable matter and fatty acid composition. They concluded that Mango seed kernels contained a considerable unsaponifiable matter and a low amount of crude protein and ash content of 3.2% (with the presence of following minerals: Ca, K, Na, Mg and P). High unsaponifiable matters content (4.58%) guarantees the use the oils in cosmetics industry.

METHODOLOGY

All reagents used in this study were of analytical grade and solutions were prepared from distilled water.

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Oil Extraction

Fruits of four mango (*M. indica*) cultivars, which are commonly known as Ogbomosho, Alphonso, Lippen, and Saigon were bought from the local market, at Ibadan, Oyo State, Nigeria. The ripe mango fruits were washed and peeled. The mango fruit was then depulped and the freshly depulped mango stones were washed in a current of water to free them from adhering pulp, fiber and dirt. The mango seed kernel were then dried and decoated manually. The soft seed obtained had thin outer covers which were peeled off to obtain the seed. These seeds were air dried for weeks, pulverized and stored in air tight container. Oil extraction was carried out according to the method described by Nzikou et al. (2010) using soxhlet extraction technique. The powdered seeds were packed into the extraction chamber and normal hexane poured into the round bottom flask of the soxhlet extractor. The oil in the seeds was leached for 12 –16 hours in each case until all the powdered seed was extracted. An exhaustive oil extraction was considered to be achieved when no more oil was obtained. After the extraction, the solution was concentrated by distillation method. The seed oil was stored in a refrigerator.

Physical and Chemical Properties

The Physiochemical properties of the oils for specific gravity, refractive index, viscosity, iodine value, acid value, Saponification value and peroxide value were determined by AOAC 1990 method. Unsaponifiable matter were carried out using standard analytical methods described by Pena, Anguiano and Arredondo (1992)

Identification of the Constituents of the Oil using Gas Chromatography-Mass Spectrometry (Gc-Ms)

For the analysis of the oil constituent, 7890 A Agilent Technologies with 5975C mass spectroscopy detector (GC-MS) was used. A HP-5 MS capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) was used for the GC system. The temperature program was set up from 50°C to 250°C with 4°C/min, both the injector and detector temperatures were 280°C and Helium was used as carrier gas. The injection volume was 2μL. Ionization energy EI of 70 eV was used for mass spectroscopy detector, with a source temperature of 150°C, scan range 50-300 amu, scan rate s^{-1}. The mass spectra were compared with the NIST/EPA/NIH Mass Spectral Library.

Analysis of the Oil by FT-IR

All infrared spectra were acquired using a Perkin Elmer BX FTIR spectrometer (Perkin-Elmer Norwalk, CT, USA) equipped with a mercury cad detector and KBr plate. Measurements were obtained at 4 cm^{-1} between 4000-600 cm^{-1}. Oil samples were placed on the ZnSe single bounce attenuated total reflectance (ATR) accessory. The spectra were accumulated from 32 scans. The spectrometer was connected to a computer using Perkin-Elmer Spectrum Windows software to manipulate the spectra.

RESULTS

Table 1: The Physico-Chemical Properties of *Mangifera indica* Seed Kernel Oil

Physico-chemical indicators	Alphonso	Ogbomoso	Lippen	Saigon
% oil yield(%)	6.46	6.39	6.78	7.90
Colour	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Appearance	Semi-solid	Solid	Semi –solid	Solid
Refractive index	1.4528	1.4596	1.4600	1.4569
Specific gravity	0.89	0.92	0.86	0.90
Viscosity (Cst)	22	45	31.75	42.25
Saponification value (mg KOH/g fat)	116.41	114.23	125.56	105.72
Free fatty acid (%)	2.26	1.41	3.09	1.98
Iodine value (mg I_2/100g)	48.15	48.86	50.88	48.34
Acid value (mg KOH/g fat)	4.49	2.81	6.16	3.93
Peroxide value (meq/kg fat)	5.77	6.01	4.94	5.54
Unsaponifiable matter (% of total lipid)	1.46	1.20	1.23	1.56

Table 2: Evaluation of the FTIR Spectra

S/N	Frequency (cm^{-1})	Functional group viberation
1	3470.8-3475	O-H stretch of alcohols
2	3004.5	C-H stretching viberation of the cis double bond (=CH)
3	2923.45	CH_3, CH_2 and CH of alkanes
4	2853.18	C-H viberational stretch of the aldehyde (CHO)
5	1746	C=O stretch of esters
6	1656.3 - 1660.5	C=C stretch of the aromatics
7	1465	Bending absorption of the CH_2 aliphatic group
8	1416.9 -1417.76	Rocking vibrations of CH bonds of *cis*-disubstituted alkenes
9	1377	CH_3 deformations of Alkanes
10	1236	C-O stretch of ethers
11	1163	C-O Stretching vibration of esters
12	1117	-CH bending and –CH deformation vibration of fatty acids
13	1097.6	O-C stretch of carboxylic acid
14	721	Overlapping of the CH_2 rocking vibration and the out-of-plane vibration of *cis*-disubstituted alkenes

Table 3: Compounds identified in GC-MS Study of Lippen Seed Kernel Oil.

S/N	Compound	MF	MM (g/mol)	t_R (min)	% Composition
1	Cyclohexane	C_6H_{12}	84	3.237	9.423
2	2,5-dimethyl- tetrahydro- Furan,	$C_6H_{12}O$	100	3.585	4.742
3	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256	3.707	24.472
4	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	40.601	52.052
5	1-Heptatriacotanol	$C_{37}H_{76}O$	536	44.223	4.951
6	3-pentadecyl-Phenol	$C_{21}H_{36}O$	304	49.240	4.359
Total					99.999

Molecular formula (MF), Molecular mass (MM), Retention time (t_R)

Table 4: Compounds identified in GC-MS study of Alphonso Seed Kernel Oil

S/N	Compound	MF	MM (g/mol)	t_R	% Composition
1	Cyclohexane	C_6H_{12}	84	3.276	40.474
2	2,5-dimethyl- tetrahydro- Furan,	$C_6H_{12}O$	100	3.1615	10.138
3	3,3,6-trimethyl- 5-Heptadien-4-one,	$C_{10}H_{16}O$	92	3.738	1.359
4	Nonanal	$C_9H_{18}O$	88	9.408	1.699
5	Nonanoic acid	$C_9H_{18}O_2$	104	14.048	1.341
6	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256	19.931	13.265
7	9,12-Octadecadienoic acid (Z,Z)-(Linoleic acid)	$C_{18}H_{32}O_2$	280	39.839	14.573
8	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	284	43.939	17.152
Total					99.971

Molecular formula (MF), Molecular mass (MM), Retention time (t_R)

Table 5: Compounds identified in GC-MS study of Saigon Seed Kernel oil

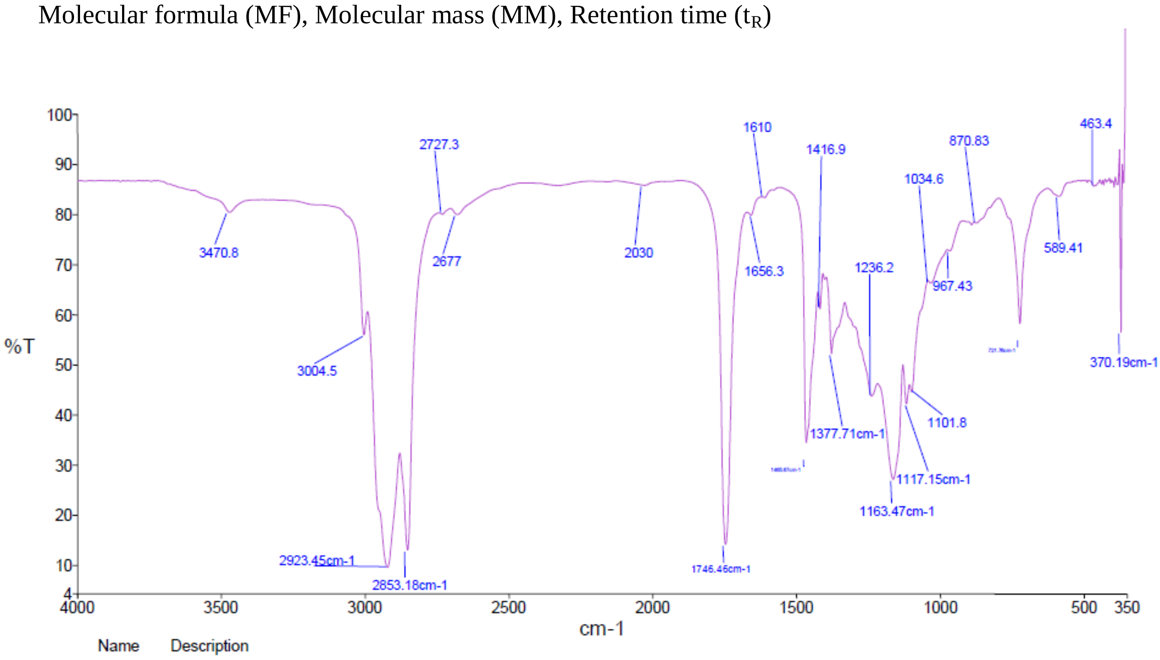
S/N	Compound	MF	MM (g/mol	t_R	% Composition
1	Cyclohexane	C_6H_{12}	84.16	3.350	33.617
2	2,5-dimethyl- tetrahydro- Furan	$C_6H_{12}O$	100	3.368	17.459
3	2,2,4 –trimethyl oxetane	$C_6H_{12}O$	100	3.799	4.661
4	3-methyl-3-Pentanol,	$C_6H_{14}O$	102	4.022	1.514
5	3-hydroxypropyl-Oxirane	$C_5H_{10}O_2$	102	4.062	1.293
6	2-Hexanone	$C_6H_{12}O$	100	4.896	1.421
7	2-methyl-2-Pentanethiol	$C_6H_{14}S$	118	4.615	4.811
8	2,2-dimethyl-Pentanal	$C_7H_{14}O$	112	4.597	1.421
9	1-ethylbutyl Hydroperoxide	$C_6H_{14}O_2$	118	7.597	1.306
10	Nitro-Cyclohexane	$C_6H_{11}NO_2$	129	8.712	7.159
11	Nonanal	$C_8H_{18}O$	130	9.442	1.275
12	8-Heptadecene	$C_{17}H_{34}$	238	14.040	1.476
13	Heptadecane	$C_{17}H_{36}$	240	32.293	2.197
14	n-Hexadecanoic acid (Palmitic acid).	$C_{16}H_{32}O_2$	256	32.898	18.601
Total					98.211

formula (MF), Molecular mass (MM), Retention time (t_R)

Table 6: Compounds identified in GC-MS study of Ogbomosho Seed Kernel Oil

S/N	Compound	MF	MM (g/mol)	t_R (min)	% composition
1	Cyclohexane	C_6H_{12}	84	3.313	18.544
2	2,5-dimethyl- tetrahydro- Furan	$C_6H_{12}O$	100	3.646	22.831
3	2,2,4-trimethyl- Oxetane,	$C_6H_{12}O$	100	3.765	5.136
4	2,5-dimethyl-3,4-Hexanediol	$C_8H_{18}O_2$	146	3.991	0.987
5	Toluene	C_7H_8	92	4.031	1.116
6	4,5-dimethyl- 2-Hepten-3-ol	$C_9H_{18}O$	142	4.263	1.593
7	2-Hexanone	$C_6H_{12}O$	100	4.507	1.294
8	3-methyl- Cyclopentanol,	$C_6H_{12}O$	100	4.590	0.698
9	2-methyl- 2-Pentanethiol,	$C_6H_{14}S$	118	4.874	4.260
10	2,2-dimethyl-Pentanal,	$C_7H_{14}O$	114	5.974	2.809
11	1-methylpentyl Hydroperoxide,	$C_6H_{14}O_2$	118	7.584	0.861
12	Cyclohexane, nitro-	$C_6H_{11}NO_2$	129	8.076	7.033
13	Nonanal	$C_9H_{18}O$	142	8.705	1.539
14	2-Decenal, (Z)-	$C_{10}H_{18}O$	154	9.005	1.257
15	2,4-Decadienal, (E,E)-	$C_{10}H_{16}O$	152	9.442	1.020
16	2,4-Decadienal	$C_{10}H_{16}O$	153	14.043	2.938
17	2-Undecenal	$C_{11}H_{20}O$	168	19.561	2.256
18	8-Heptadecene	$C_{17}H_{34}$	238	20.667	1.902
19	Hexadecane	$C_{16}H_{34}$	226	21.431	2.256
20	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256	22.96	20.466
Total					99.0969

Molecular formula (MF), Molecular mass (MM), Retention time (t_R)



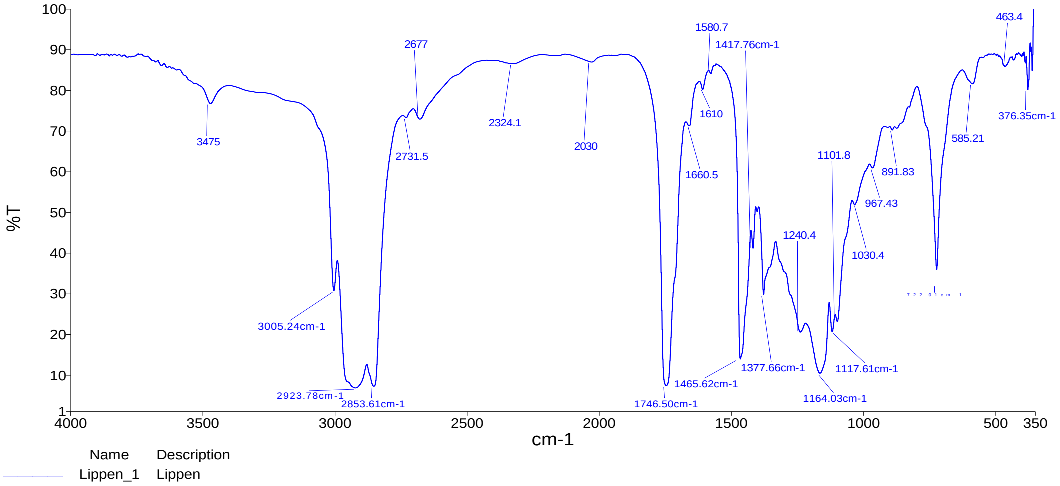


Figure 2: The FT-IR Spectra of Lippen Seed Kernel Oil

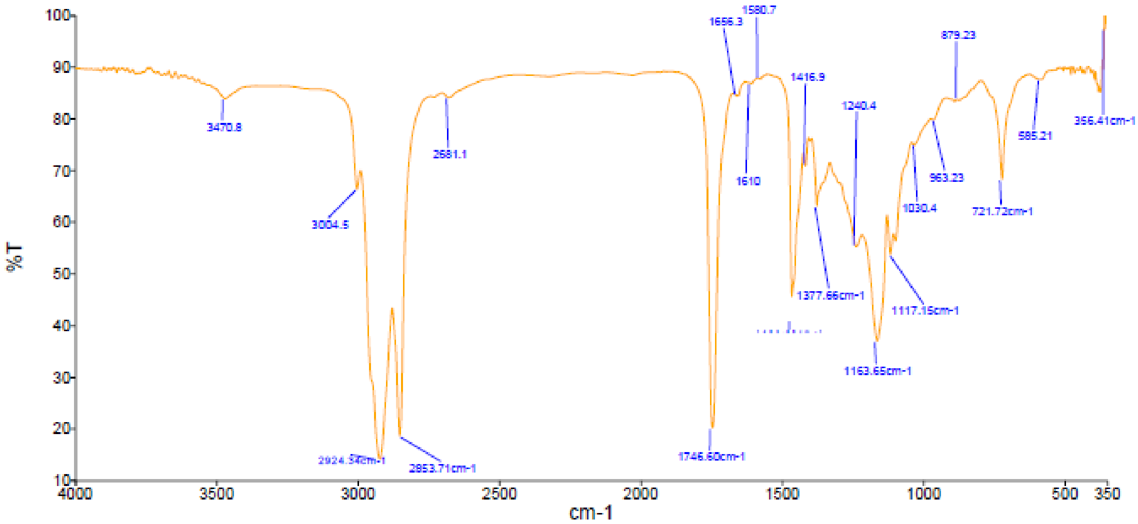


Figure 3: The FT-IR Spectra of Saigon Seed Kernel Oil

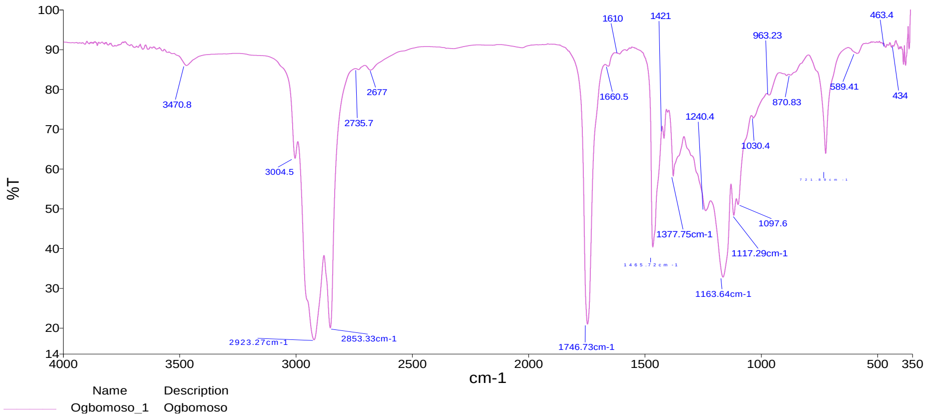


Figure 4: The FT-IR Spectra of Ogbomosho Seed kernel oil

DISCUSSION

The Physico-Chemical Properties of *Mangifera indica* Seed Kernel Oil

The physicochemical parameters of all the seed oils examined are presented in Table1. The oils extracted from the Alphonso and Lippen cultivars were semi-solid, while those from Ogbomosho and Saigon cultivar were solid at room temperature. The percentage yield of the oil obtained from various cultivars ranges from 6.39-7.9%. The Saigon cultivar has the highest yield of 7.9%. The percentage yields of the oils obtained are low but in agreement with those reported in literature (Amel & Eisa, 2015).

The values for the refractive index range from 1.4528 to 1.4600, which are in close agreement with values reported for conventional oils such as soybean (1.468) (Rowe, Sheskey, & Owen, 2006) and palm kernel (1.455) (Sambamurthi, Sundram, & Tan, 2000) The high refractive index of mango seed kernel oil seems to confirm the high number of carbon atoms in their fatty acids (Falade et al., 2008). The viscosity values range from 22 to 42.25Cst. Viscosity increases with molecular weight of the oil and decreases with increasing unsaturation level and temperature. The specific gravity of the extracted oils range from 0.86-0.92. These values are within the range given by FAO/WHO (2009) for edible oil which is 0.9-1.16, but slightly higher than those reported in literatiure (Amel & Eisa, 2015). The peroxide value is one of the most widely used tests for oxidative rancidity in oils and fats, and usually indicates the rancidity level of the oil and thus a measure of the shelf life of the vegetable oil. The results showed that the peroxide values in the extracted oils, range from 6.01 meq/kg fat for Ogbomosho to 4.94 meq/kg fat for lippen cultivar. These values are in line with the standard specified by FAO/WHO (2009) for fresh edible oil which is below 10 mg/kg fat. The results showed that all the extracted oils are in an undegraded state. Iodine values are useful for determination of overall degree of unsaturation of oil. The iodine values obtained for fat range from 48.15 to 50.88 mg I_2/100g, with lippen cultivar having the highest value. The acid value of oil may be used as a measure of quality. It is affected by the duration and conditions of storage of the oil. The acid values obtained for the oil range from 2.81mg KOH/g fat to 6.16 mg KOH/g fat. The low acid value indicates that the oil is not rancid (Roger, Rebecca, Georges, & Mathias 2010). The saponification values were found to be high but lower than 190 mg/g for all the cultivars. The values obtained range from 105.72mgKOH/g fat to 125.56mgKOH/g fat. High saponification values are recommended in detergents manufacturing and possibly provide some cleansing, thus it could be used in the production of soaps, shampoos and lather shaving creams and some other cosmetics (Auwal, Edward, & Abdulhamid, 2010). The free fatty acid values obtained range from 1.41%-3.09%. Low free fatty acid content shows that the oil would not easily go rancid when properly stored. The mango seed kernel oil contains low unsaponifiable matter (1.2-1.56%), which are known to affect physical properties of oils such as melting-points(Abayeh, Garba, Adamu, & Abayeh 2013).

FT-IR Spectral Analysis of *Mangifera indica* seed kernel oil

Representative IR spectra for various cultivars of *Mangifera indica* seed kernel oils are shown in Figure 1, 2, 3, and 4. The assignments of peaks and the functional groups present are given in Table 2. The IR spectra showed a typical characteristic absorption bands for common triglyceride and also fatty acid. The FTIR spectra for the various mango cultivars were very much similar. However, some differences were detected in the intensity and position of some bands. These variations are due to the difference in length and degree of unsaturation of the acyl group (Kristin, Dinesh, & Kalpana 2012)

Compounds Identified in GC-MS Study of the Various Cultivars of *Mangifera indica* Seed Kernel Oil

The chemical compounds identified for the various cultivar of the oil, with their percentage yeids, retention time, molecular formula and molecular mass are given in table 3, 4, 5, and 6. The results showed that cyclohexane (9.423-40.47%), 2,5-dimethyl- tetrahydro- Furan (4.742-22.831%), and palmitic (13.265-24.47) acid were present in all the samples.

Six (6) compounds were identified for the Lippen cultivar representing a total of 99.999% of the oil (Table 3). Cis vaccenic acid had the highest percentage composition (52.052%), this cultivar had the highest percentage of palmitic acid (24.472%), but least amount of Cylcohexane (9.423%) and 2,5-terahydro-Furan(4.742%). 3-Pentadecyl-Phenol (4.359%) and 1-Heptatriacotanol (4.951%) were also present. cis-Vaccenic acid, also known as (*E*)-octadec-11-enoic acid can be converted into rumenic acid by mammals, a conjugated linoleic acid (. Banni et al, 2001), that it shows anticarcinogenic properties (Lock, Corl, Barbano , & Bauman). Thus this oil can be use in food and pharmaceutical industries. The palmitic acid present also make this oil very good for soap production.

Eight compounds were identified in the study of Alphonso cultivar representing a total of 99.971% of the oil (Table 4). This cultivar had the highest percentage of cyclohexane (40.474%). Linoleic acid (14.573%), palmitic acid (13.265%), 2,5-dimethyl tetrahydro-furan(10.138%) and stearic (17.152%), were found in this cultivar. The presence of stearic acids and palmitic acids make this oil fit for the manufacturing of candles, soap, cosmetics, shaving soaps, lubricants, and pharmaceuticals. Research points to linoleic acid's anti-inflammatory, acne reductive, skin-lightening and moisture retentive properties when applied topically on the skin (Diezel, Schulz, Skanks, & Heise, 1993, Letawe, Boone, & Pierard, 1998, Ando, Ryu, Hashimoto, & Oka, 1998). Linolic acid reduces the incidence of tumor and also inhibits carcinogenesis. Hence this oil can be ultilized in food, cosmetic and pharmaceutical industry. This oil will also be good for biofuel.

The GC-MS study for the Saigon cultivar revealed 14 compounds representing 98.211% of the oil (Table 5). The major fatty acid present was palmitic acid (18.601%). Cyclohexane (33.617%), 2,5-dimethyltetrahydro-furan(17.459%), 1-ethylbutylhydroperoxide (7.159%), 2,2,4 –trimethyloxetane (4.661%) and 2-methyl-2-Pentanethiol(4.811%), were also present. This oil will be good for biofuel and in soap production.

Twenty compounds were identified in the study of Ogbomosho cultivar (Table 6), representing a total of 99.0967% of the oil. Palmitic acid (20.466%), cyclohaxane (18.544%), 2,5-dimethyl tetrahydro-furan (22.831%), 2,2,4 –trimethyloxetane (5.136%) and 2-methyl-2-Pentanethiol (4.260%) were also present. This oil will be good for soap production and also as biofuel.

This study identified hydrocarbon compounds and fatty acids in high percentage. In other studies in the literature the compounds mostly identified are fatty acid esters, this might be because the studies are done using a gas chromatography with a flame ionization detector using Fatty acid methyl ester (FAME) as the sample. For this research the compounds were identified with a Gas Chromatography –Mass Spectrometer with the oil extract as sample.

CONCLUSION

The main constituents of *Mangifera indica* seed kernel oils were fatty acids and hydrocarbons. The low peroxide value, acid value and % free fatty acid shows that all the extracted oils are in an undegraded state and have low susceptibility to oxidative rancidity and deterioration. High saponification values of oil make them recommended for the production of soaps, shampoos and lather shaving creams and some other cleansing agent. Hence *Mangifera indica* seed kernel oil should be further utilized in the food, cosmetics and pharmaceutical industries, rather than just being discarded as waste.

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