Nig. J. Biotech. Vol. 36 (1): 194-202 (June 2019)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v36i1.25

Lipid profile of oils from *Irvingia gabonensis* (Baill) seeds and its deterioration by a phytopathogenic fungal species

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Abstract

As a survival strategy, fungi are capable of degrading most organic substances. Fungi associated with diseased *Irvingia gabonensis* (Baill) seeds are suspected to possess the ability to degrade the hydrocarbon substances contained in these seeds. The work seeks to contribute to literature on the physico-chemical nature of oils from healthy and diseased seeds of *I. gabonensis* and to determine the corresponding extent of deterioration in this oil from *Aspergillus oryzae* infected seeds. Using standard laboratory methods, oils from healthy and *A.oryzae* infected seeds of I. gabonensis seed were extracted and subjected to a complete physico-chemical characterization. In addition, a Gas chromatographic profiling of the Fatty Acid Methyl Ester (FAME) from both oils was also done. Results from the study show that oil from I. gabonensis seed is of a high nutritive and industrial quality. Also, A. oryzae significantly (p = 0.05) affected the physico- chemical attributes and fatty acid profile of this oil, thus confirming its ability to deteriorate the quality and usefulness of this oil.

Key words: *Irvingia gabonensis* seed oil, oil deterioration, oil ranciditity, Fatty Acid Methyl Ester (FAME) and phytopathogenic fungi

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Introduction

Fungi are reputed for being good at digesting complex organic compounds that are not generally degraded by other organisms (Covino et al., 2010). This act of degrading substances is not an act of benevolence by these fungal species, rather, it is a strategy for survival. Enzymes produced by fungal species for this purpose are lignin perioxidase, manganese perioxidase and luccase. These enzymes act singly or collectively in breaking down natural or human made materials (Stamets, 2005). In an earlier report, Sanyaolu et al. (2014) identified five pathogenic fungal species associated with *I.gabonenesis* seeds from some markets in Lagos, Nigeria. In this work, they showed the ability of pathogenic fungi to adversely affect the nutritional integrity of the seeds.

Irvingia gabonensis seed is high in fat **(**Giami et al, 1994**).** The seed also relieves dysentery and is also used as a purgative in gastro-intestinal and liver conditions, hernias and urethral discharge (Pischon et al., 2008). The seeds are valuable source of cash income (Tabuna, 1999). *I. gabonensis* seeds show tremendous promise in correcting leptin resistance, thereby promoting weight loss and combating components of metabolic syndrome. It also facilitates the breakdown of body fat by reducing an enzyme (glycerol-3-phosphate dehydrogenase) that enables glucose to be stored as triglycerides in fat cells. Furthermore, it increases the insulin-senitizing hormone, adiponectin, and inhibits amylase digestion

(Pischon et al., 2008). It has been suggested that elevated leptin provokes the growth of certain malignancies, including many form of breast cancer, which helps explain the higher breast cancer risk observed in overweight women and obesity which is also known to increase stroke risk (Soderberg et al., 2003) and promote cardiac hypertrophy (Ren, 2005).

The lipid fraction of a fatty foods contains a complex mixture of different types of molecule. In many foods, the lipid component plays a major role in determining the overall physical characteristics, such as flavor, texture, mouthfeel and appearance. For this reason, it is difficult to develop low-fat alternatives of many food, because once the fat is removed some of the most important physical characteristics are lost. It should be noted that many fats are prone to lipid oxidation, which leads to the formation of off-flavors and potentially harmful products.

Extensive research has been carried out on the physico-chemical characterization, proximate composition and microbial degradation of oils from different seeds such as Melon, Soybean, Cashew, Groundnut, Coconut, butternut etc (Oladimeji and Kolapo, 2007; Essien and Amadi, 2009; Atasiel et al., 2009; Adeleke and Abiodun, 2010 and Mariod et al., 2009); with very scanty corresponding research of the same nature on *I. gabonensis* seed, despite its enormous dietary benefits.

The aim of this work therefore is to contribute to literature on the physico-chemical nature of oils from healthy and diseased seeds of *I. gabonensis* while the specific objectives were to determine the physico-chemical parameters of oil from healthy seeds of *I.gabonensis* and the corresponding extent of deterioration in the oil from *Aspergillus oryzae* infected seeds.

Materials and methods

Collection of samples

Visually diseased and healthy seeds of *I. gabonensis* from which *Aspergillus oryzae* were isolated were collected from four sources namely: Oyingbo, Ajegunle, Bariga and Agege markets, all in Lagos metropolis, Lagos state, Nigeria.

To compare the ability of *A. oryzae* to cause a deterioration in the oil from *I. gabonensis* seed, both the visually healthy and *A.oryzae* infected (diseased) seeds of *I. gabonenesis* had their oils extracted. The extracted oils were subjected to a complete physico-chemical characterization. In addition, a Gas chromatographic profiling of the Fatty Acid Methyl Ester (FAME) from both oils was also done.

Extraction and preparation of oil from Irvingia gabonensis seeds

The soxhlet method of oil extraction and preparation as described by AOAC (1993) was used.

Physico-chemical characterization of the oil extracted from I.gabonensis seed

Standard methods of AOAC (1993) were employed for the determinations of all the parameters such as pH, refractive index, melting point, Saponification value, Unsaponification matter, Iodine number, Peroxide value, Acid value, Percentage free fatty acid (FFA), Cholesterol, Relative density, percentage oil yield and Thiobarbituric acid (TBA).

The FAME analysis consisting of the saturated, monounsaturated and the poly unsaturated fatty acids were carried out by following the modified AOAC (2000) official methods. Here,

50 mg of the oil was saponified (esterified) for 5 mins at 95°C with 3.4 ml of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCl. In addition, 3 ml of 14% boron triflouride in methanol was added. The mixture was heated for 5 mins at the temperature of 90°C to achieve complete methylation process. The FAMEs were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated with 1ml for GC (make: Agilent HP, Model: 6890) analysis and 1µl was injected into the injection port of the GC machine.

The GC model and condition for the FAME analysis are as detailed below:

GC: Agilent HP 6890 powered with HP Chemstation Rev. A 09.01{1206} software.
Injection temperature: Split Injection. Split Ratio: 20:1 Carrier Gas: Nitrogen.

Inlet temperature: 250°C. Column type: HP INNOW ax Column dimensions: 30m × 0.25m × 0.25µm Oven programme: Initial temperature @ 60°C

First Ramping @ 12°C/min for 20min, maintained for 2 min.

Second Ramping @ 15°C/min for 3 min, maintained for 8 min.

Detector: Flame Ionization Detector-FID. Detector temperature: 320°C.

Hydrogen pressure: 22 Pounds per Square inch (psi). Compressed air: 35 psi.

Statistical Analysis of Data

Results obtained in all cases were the mean from 3 replicates. Data so obtained were subjected to statistical analysis using the soft ware package Statistical Analysis System (SAS), 2005 version. Mean separation was done using the Duncan's multiple range test at 5% level of significance.

Results

Physico-chemical characterization of extracted oils

The results as presented in Table 1 show that the mean value for some parameters such as pH, refractive index and percentage yield were significantly ($p = 0.05$) higher in the oils from the healthy seeds than the oils from the diseased seeds. However, in terms of all the parameters that are indicative of deterioration such as saponification value, unsaponifiable matter, acid value, peroxide value and free fatty acid (FFA), the mean values of the oil from the diseased seeds were significantly ($p = 0.05$) higher than those of the healthy seeds. For such parameters as iodine value, cholesterol value and relative density, there was no significant difference in the mean values for both oils; even though the oils from the healthy seeds had a higher mean value for such parameters as iodine value, cholesterol and relative density compared to the oils from the diseased seeds (Table 1).

DETERIORATION OF FAMEs IN BOTH OILS

The results as shown in Table 2 indicate the percentage composition of the individual fatty acids of both types of oil and their corresponding degree of saturation or unsaturation.

From this Table, it is also evident that the oil from *I.gabonensis* seeds (either healthy or diseased seed) are composed of a total of 12 fatty acids namely Myristic, Palmitic, Palmitoleic, Stearic, Oleic, Linoleic, Linolenic, Arachidic, Arachidonic, Behenic, Erucic and Lignoceric acids in different proportion and that *I.gabonensis* seed are lacking in some types of fatty acids (Figs. 3 and 4) namely caprylic, capric, lauric and margaric acids as their percentage composition in the oils was less than 0.000001.

Also from this Table, it can be seen that the most abundant fatty acid present in both the healthy and diseased (*A.oryzae* infected) seeds of *I.gabonensis* is the polyunsaturated Linoleic acid with a percentage composition of 25.03 and 23.82 respectively, while the least abundant fatty acid in both the healthy and *A.oryzae* infected seeds was the saturated myristic acid with a percentage composition of 0.33 and 0.86 respectively.

The oil from the diseased seeds were shown to have a higher percentage composition of saturated fatty acids than the oil from the healthy seeds, while the oils from the healthy seeds contained a higher proportion of the unsaturated (both the monounsaturated and the polyunsaturated) fatty-acids (Table 3).

Table 1: Physico-chemical parameters of oils extracted from healthy and diseased seeds of *I. gabonensis*

parameter	Healthy seeds.	Diseased seeds.
pH	6.200_b	5.000_a
refractive index (g/100g).	1.516_b	1.458_a
melting point	30.067	30.067
saponification value(mgkoh/g)	224.040_a	236.173_b
unsaponifiable matter (g/kg).	20.963_a	23.327_b
iodine value (g/100g).	39.667_a	37.013_a
peroxide value (meq/kg).	9.333_a	12.667_b
acid value (mg/g)	11.967_a	19.450_b
percentage free fatty acid (%ffa).	6.013_a	9.773_b
cholesterol (mg/100g).	12.310_a	12.190_a
relative density	0.901_a	0.895_a
% oil yield	58.020_b	55.287_a
TBA (µg/g)	16.243_a	18.727_b

*Means on the same row carrying different subscripts are significantly different at p = 0.05.

Table 2: Percentage composition and saturation/unsaturation level of individual Fatty Acids in oils

S/N	Fatty acid and degree of Saturation/unsaturation.	healthy seeds.	diseased seeds.
1	Myristic acid: **C14:0**	0.336766	0.860036
2	Palmitic acid: **C16:0**	11.392863	13.401511
3	Palmitoleic acid: **C16:1**	6.291373	5.582964
4	Stearic acid: **C18:0**	5.954527	8.281806
5	Oleic acid: **C18:1**	21.477837	20.708873
6	Linoleic acid: **C18:2**	25.036503	23.822111
7	Linolenic acid: **C18:3**	23.014590	21.598781
8	Arachidic acid: **C20:0**	0.529693	0.540171
9	Arachidonic acid: **C20:4**	1.892446	1.755522
10	Behenic acid: **C22:0**	0.426655	0.595662
11	Erucic acid: **C22:1**	2.465308	1.861109
12	Lignoceric acid: **C24:0**	1.012433	1.160461

The value of oil components were obtained after triplicate analysis from electronic integration measurements using flame ionization detector

Table 3: Type and percentage composition of FAMEs in oils

Type of fatty acid	Percentage composition healthy seeds	diseased seeds
Saturated	19.821944	24.67064
monounsaturated	30.234518	28.152946
Polyunsaturated	49.943539	47.176414

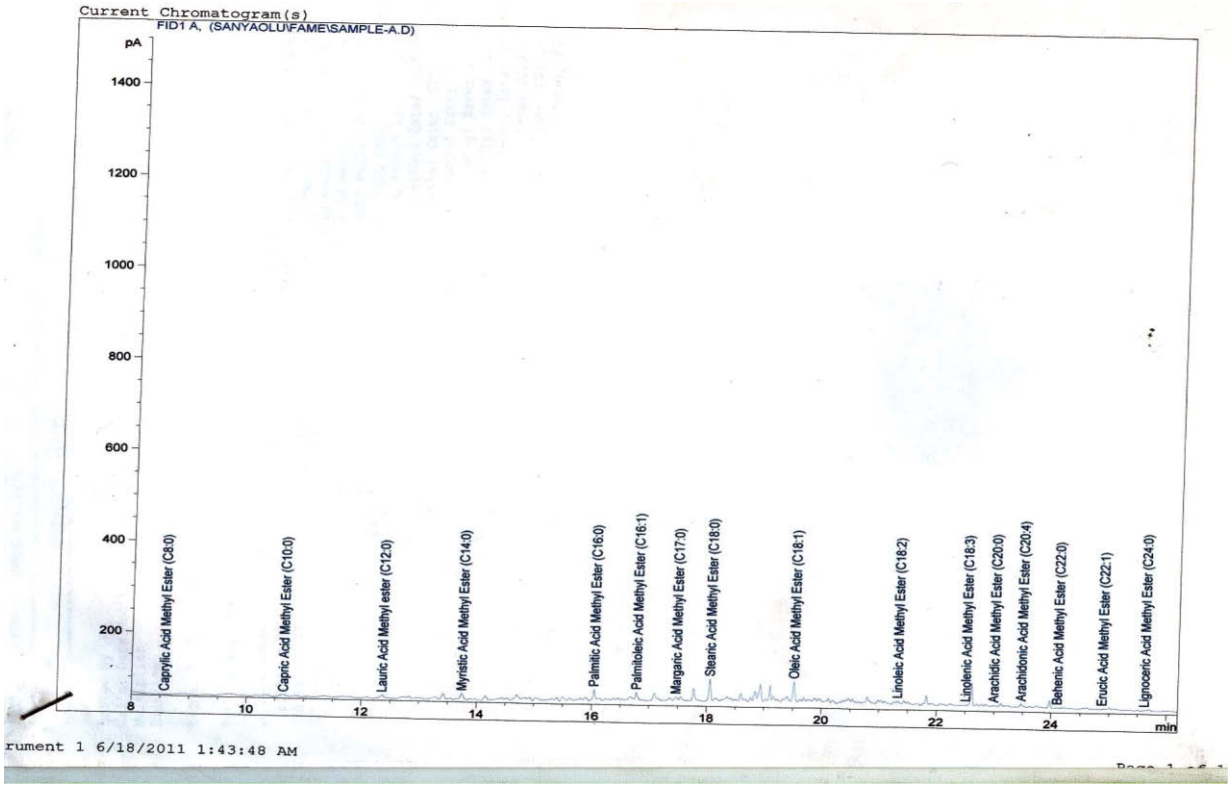

Figure 1: GC chromatograms of the oils from healthy seeds

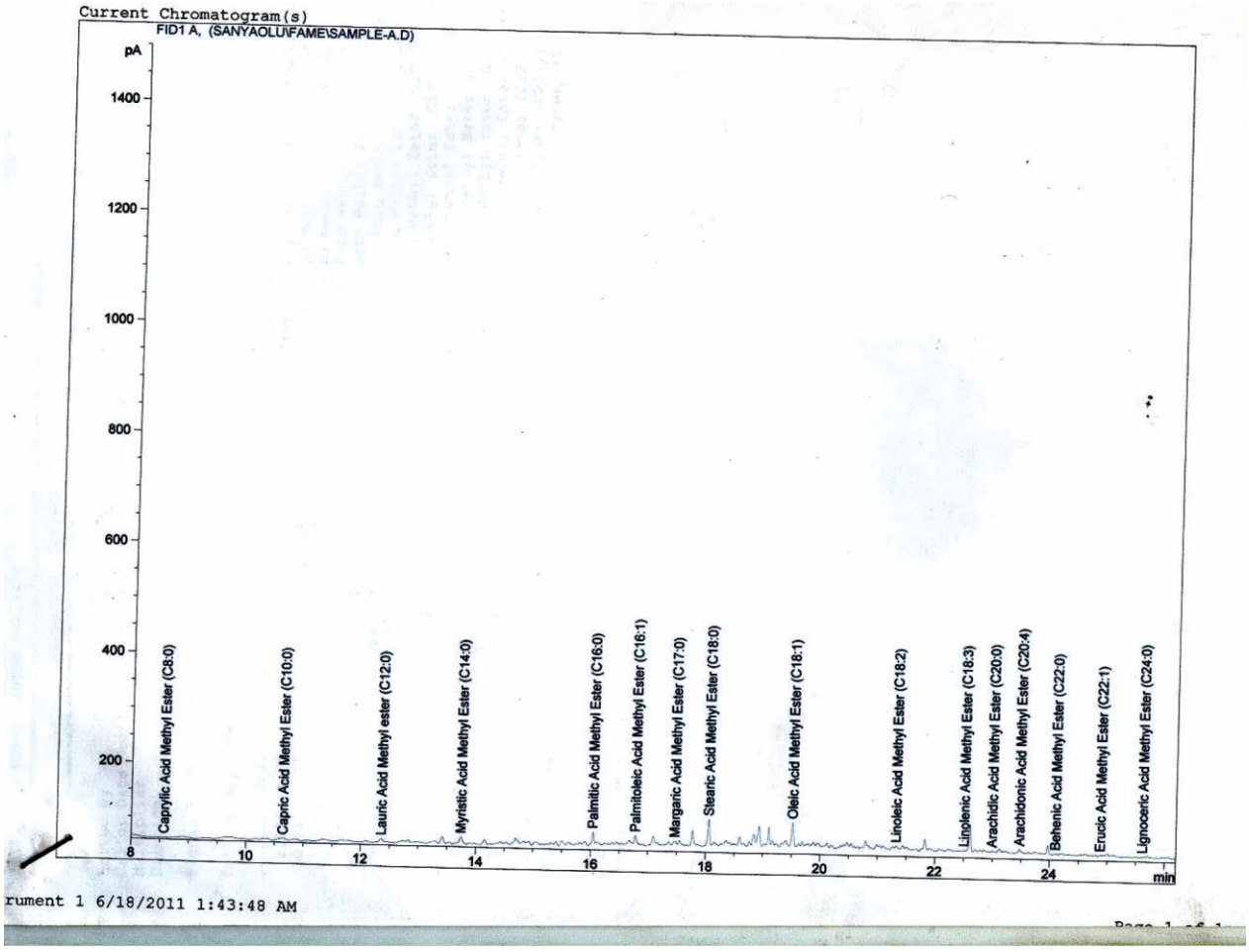

Figure 2: GC chromatograms of the oils from diseased seeds

Discussion

The physico-chemical indices of the expressed oil from the healthy seed of *I. gabonensis* showed that this oil is of comparable quality to the oil from many other oil seeds such as *Bombax glabrum, Arachis hypogaea, Glycine max* and cotton seeds (Adeleke and Abiodun, 2010). The relative density of the oil from healthy and diseased seeds of *I. gabonensis* falls within the Codex recommended range. In addition, the oil expressed from the seeds of *I. gabonensis* showed a higher saponification value than most oil seeds indexed by Codex except palm kernel oil (230-254mgKOH/g), babassu oil (245-256mgKOH/g) and coconut oil (248-265mgKOH/g). This high saponification value thus makes this oil a potentially useful material for soap making business. The iodine value of the oils was also within the Codex recommended range (6.3-150g/100g) for edible oils. The iodine value however has implications for unsaturation and rancidity. The lower the iodine value, the higher the level of unsaturation of the oil, and thus the less likely the possibility of oxidative degradation (rancidity). In this respect, the oil from the seed of *I. gabonensis* appears to be satisfactory. Also, the level of the unsaponifiable matter found in the oils extracted from the seed of *I. gabonensis* falls within the Codex recommended range of 12-28g/kg for edible oils (Ihekoronye and Ngoddy, 1985). In this regard, comparing the acid values of both extracted oils, the ability of this fungus -*A. oryzae*- to secrete lipase enzyme is suspected (which in this case is suspected of causing a deterioration in the quality of the oil). It has previously been reported, that some other pathogenic fungi which were found in association with oil seeds such as melon, soybean and *Detarium senegalense* by Adekunle and Uma (1996), Adekunle and Oluyode (2005) and Adekunle and Adebambo (2007) respectively produced this lipase enzyme. According to Adeleke and Abiodun (2010), the maximum recommended peroxide level in edible vegetable oil by Codex is 10meq/kg. The perioxide value in the oil from the healthy seeds of *I. gabonensis* falls within the Codex limit. However, the peroxide value of the oil from the diseased seeds of *I. gabonensis* is above the Codex limit in edible vegetable oils. This is important because peroxide level is an indication of the deterioration of fats; meaning therefore that the action of *A. oryzae* on the seed of *I. gabonensis* clearly resulted into the spoilage of the seeds. Another indicator of deterioration of the oil from the diseased seed of *I. gabonensis* is the higher level of free fatty acid compared with the oil from the healthy seeds. Free fatty acid as a parameter indicates the presence of fatty acids in the oil, and the higher the free fatty acid, the higher the fatty acid content of the oil, and thus the higher the possibility of oxidative deterioration (Okpokwasili and Molokwu, 1996; Atasie et al., 2009). In other words, the higher the fatty acid composition of an oil, the lower the stability and the lower the shelf life of the oil (Enemuor et al., 2012). Another important parameter of deterioration attributable to the action of *A. oryzae* on the seed of *I. gabonensis* is the significant increase in the TBA value compared to the amount for the oil from the healthy seeds. A higher TBA value is a clear indication of deterioration. Although the action of *A. oryzae* significantly reduced the oil yield in the seed of *I. gabonensis*. This notwithstanding, *I. gabonensis* seed are a very rich source of oil. This is because the percentage oil yield from this seed was higher than obtained from many other oil seeds {*Jugulans cinerea*, 46.15% (Essien and Amadi, 2009), *Moringa oleifera*, 34.80% (Anwar and Rashid, 2007), *Monechma ciliatum* (black mahlab seeds), 13.5% and *Prunus mahaleb* (white mahlab seeds), 30.95% (Mariod et al., 2009).

Lipids are a major source of energy and provide essential fatty acids (EFAs). EFAs are fatty acids that humans need to consume in their diets because the body requires them for its wellbeing but is incapable of producing same. Notwithstanding the above, excessive consumption of certain fat components can be inimical to health e.g. cholesterol and saturated fats. The results of the free fatty acid profiles of the oil expressed from *I .gabonensis* seeds show this oil to be rich in a complex of fatty acids, particularly the unsaturated fatty acids which have been generally acclaimed as being good for human health (Mozaffarian et. al., 2004). The oils extracted from healthy seeds of *I. gabonensis* had a lower amount of saturated fatty acids

compared to the oil from the *A. oryzae* infected seeds.

This reduction in the amount of the unsaturated fatty acids observed in the *A. oryzae* infected seeds of *I. gabonensis* may be because this group of fatty acids are believed to be more susceptible to oxidative degradation than their saturated counterparts (Leibovitz et al., 1990). Polyunsaturated fatty acids have been indicated in the protection of mammals against cardiac arrhythmias and a lowered resistance to insulin resistance while the monounsaturated fatty acids have also been reported as having a negative correlation with coronary atherosclerosis (Storlien et al., 1996). As reported by Gebhardt and Thomas (2002), the oil expressed from the seed of *I. gabonensis* is of higher quality compared to butter (66.1% saturated fatty acid, 30.3% monounsaturated fatty acid and 3.7% polyunsaturated fatty acid) and margarine (20.4% saturated fatty acid, 43.6% monounsaturated fatty acid and 33.3% polyunsaturated fatty acid).

Conclusion

Findings from the physico-chemical characterization of the oils expressed from *I. gabonensis* seeds proved this oil to be of a high nutritional and Industrial value. *A. oryzae* found associated with diseased seeds of *I. gabonensis* caused a deterioration in the quality of the oil. The fatty acid composition of the oils from both the healthy and *A. oryzae* infected seeds of *I. gabonensis* were determined and their respective concentrations in these seeds have thus been documented.

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