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EFFECT OF MYCODETERIORATIVE FUNGI ON THE VEGETABLE OIL FROM KERNELS OF *IRVINGIA GABONENSIS* SOLD IN PARTS OF NIGERIA

Christopher Maduabuchi Duru[1], Sylvia Onyinyechi Anyadoh–Nwadike[2*] and Azuka Keziah Osikwe[3]

[1] Department of Biology, Federal University of Technology, P M B 1526 Owerri, Imo State, Nigeria.

[2]Department of Biotechnology, Federal University of Technology, P M B 1526 Owerri, Imo State, Nigeria.

[3] School of Industrial and Applied Science, Federal Polytechnic Nekede, Owerri. Imo state, Nigeria.

*Correspondence: Email: sylanyad@yahoo.com

ABSTRACT: *Sanitary survey was conducted on the kernels of Irvingia gabonensis sold within the four ecological zones of Nigeria. The aim was to ascertain the effect of mycodeteriorative fungi on the dietary quality of vegetable oil from kernels of Irvingia gabonensis. The study screened for presence of lipid degrading saprotrophs as well as ascertained the quantity of vegetable oil and free fatty acid components using Microbiological and Biochemical methods. Results showed presence of saprotrophs at varying frequencies; Aspergillus flavus, from 17.61±0.25% (Abuja) to 28.33±0.02% (Kebbi); Aspergillus niger, from 14.38±5.07% (Imo) to 23.33±1.05% (Lagos). Fusarium moniliforme, from 13.01±2.89% (Bauchi) to 22.00±2.14% (Lagos). Lasiodiplodia theobromae, from 9.64±1.34% (Bauchi) to 24.00±2.36% (Imo). Penicillium italicum, from 5.00±8.31% (Benue) to 13.21±6.01% (Kebbi). Rhizopus stolonifer, from 11.00±7.32% (Lagos) to 28.35±2.37% (Abuja). These isolates were confirmed to be associated with the lipid degradation of the kernel of Irvingia gabonensis reducing the percentage weight of vegetable oil content from 61.81±0.02(control) to 30.52± 6.14% (study locations) and increasing quantity of free fatty acids from 0.511±10.18 (control) to 6.28±0.05 % (study locations). Strict sanitary supervision of food wares is advocated.Lipid degradation technology of these mycobiota can be exploited by cosmetic industry to improve free fatty acid contents of low fat oils.*

KEY WORDS: Saprotroph, vegetable oil, dietary quality, *Irvingia gabonensis*, mycodeterioration

INTRODUCTION

Mycodeteriorative fungi refer to the saprotrophs, such as *Aspergillus flavus, A niger, Penicillium italicum, Fusarium moniliforme, Lasiodiplodia theobromae* and *Rhizopus stolonifer* associated with post - harvest fungal seed rots of *Arachis hypogea* L.(ground nut), *Cucumeropsis mannii* Naud (melon), etc. Fruit rot of *Psidium guajava* L. (guava), *Persea americana* Mill., *Solanum lycopersicum* L., etc. kernels of *Detarium microcarpum* Guill et Perr., *Irvingia gabonensis* (Aubry – Lecomte ex O'Rorke) Baillon., etc. Tuber crops of *Dioscorea* species, *Ipomoea* species etc. These organisms were known to populate the tropical biome including Nigeria (Duru and Anyadoh, 2009).

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Vegetable oil refers exclusively to vegetable fats which are liquid at room temperature, extracted from seeds or less often from other parts of fruits (Dand, 1999; Thomas, 2002). Many vegetable oil contain admixtures of triglycerides and fatty acids in different proportions depending on the type of plant, the organ and the phytoblast from which it is extracted. A triglyceride is a lipid unit consisting of fatty acid and glycerol subunits. It is synthesized by a condensation reaction between one molecule of glycerol ($C_3H_8O_3$) and three molecules of a fatty acid $CH_3(CH_2)_nCOOH$. Glycerol is monotypic while fatty acids show considerable variations in complexity, implying that the quantity of fatty acids, both saturated and unsaturated, in a natural lipid of plant origin determine the dietary cum industrial quality of the vegetable oil (Ekpe *et al*., 2018; Niyi 2014).

Kernels of *I. gabonensis* constitutes a staple phytolipid ladden soup thickener, widely consumed in Nigeria and in diaspora. It was found to contain between 34 – 73% oil w/w (Womeni *et al*., 2008; Matos *et al*., 2009; Ekpe *et al*., 2018). A matured plant of *I. gabonensis* is a tree of about forty meters (40m) tall, cultivated as fruit tree crop. The leaves are obovate to elliptical. Inflorescence, an axillary cyme. Flowers are fragrant, green and occur in short clusters. Fruit is a spherical drupe with fleshy edible mesocarp and stony endocarp. The kernels are cotyledoneous and lie within the endocarp. The stony endocarp when broken, the kernel is removed and processed as dietary commodity for market and domestic consumption (Nyananyo, 2006; Iponga *et al* 2018).

The nature of marketing this commodity in Nigeria is such that they are not aseptically carried in bags, display shops and market places. Observations, most of the times reveal rough, spotty and moldy surfaces, suggesting infestation of fungi and associated flora. It therefore becomes necessary, as a health issue to investigate the mycoflora associated with this rough kernel surfaces, with a view to determining the effects on the dietary quality and quantity of the vegetable oil content. This study, therefore aims at screening for the mycoflora infesting these displayed kernels in Nigeria and determine their effects on the dietary quality and quantity of the vegetable oil component, using biochemical techniques.

MATERIALS AND METHODS

STUDY AREA

The study was conducted between the months of June and October of 2018. Within the four ecological zones of Nigeria, namely the Rain forest zone (Rivers, Lagos, Edo and Imo state), Guinea savanna(Benue state), Sudan savanna (Abuja) and Sahel savanna (Bauchi, Kebbi and Sokoto state) (Figure 1).

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Fig. 1: Map of Nigeria Showing Study Location

Legend

Representing the Study Locations

Sample Collection

Kernels of *Irvingia gabonensis* were collected from display shops and marketing steads in the metropolis of Owerri, Imo state (latitude 5^0 28^- and 5^0 30^- N and longitude 7^0 01^- and 7^0 03^- E), Port Harcourt, River state (latitude 4^0 43^- and 0^0 00^- N and longitude 7^0 18^- and 0^0 00^- E), Oshodi, Lagos state (latitude 6^0 27^- N and longitude 3^0 54^- E), Benin city, Edo state (latitude $6^0 33^-$ N and longitude 5^0 37^-E), Jos, Plateau state (latitude 8^0 28^-N and 9^0 43^- N and longitude 8^0 33^- and 9^0 33^- E), Makurdi, Benue state (latitude $7^0 13^-$ and $7^0 30^-$ N and longitude 8^0 30^- and 9^0 00^- E), Argungu, Kebbi state(latitude 10^0 08^- and 13^0 15^- N and longitude 3^0 30^- and 6^0 02^- E), Sokoto, Sokoto state (latitude

13^0 08^- N and longitude 5^0 15^-E), Bauchi, Bauchi state (latitude 9^0 03 $^-$ and 12^0 00^- N and longitude 8^0 50 $^-$ and 11^0 00^- E) and Abuja, Nigeria's capital territory (latitude 8^0 55 $^-$ and 9^0 00 $^-$ N and longitude 7^0 00^- and 7^0 05^- E).

Samples for the control experiments were obtained from the endocarp of fresh fruits harvested from the forestry unit of Agricultural Development Corporation, Nekede, Owerri. Samples collections were made between the months of June and October of 2018. The kernels were collected from each location in separate sterile poly ethane bags and transported to the Biological Science laboratory, Federal University of Technology, Owerri, Imo state, Nigeria; where the assay was conducted.

STERILIZATION

The test kernels from each study location were separately washed several times under running tap water. Then steeped in 0.5% sodium hypochlorite solution for ten (10) minutes. They were then washed in three changes of distilled water. The washed kernels were divided into two parts, one part was used for fungal isolation while the other was for Biochemical evaluation of the oil contents.

EVALUATION OF THE SAPROTROPHS

Blotter method was adopted in the fungal isolation. Some quantities of nine centimeter (9 cm), cellulose papers and distilled water were separately autoclaved at 121 $kgcm^{-3}$ for 15 minutes before use. The filter papers were laid down in three layers per petridish, moist with the sterile distilled water. The kernels were placed on them. The dish complex were incubated at 29^0C, for 14 days.

SAMPLE PREPARATION FOR BIOCHEMICAL EVALUATION

The sterilized kernels were dried, pulverized and made into a sludge. The sludge was then warmed to melt the endocytic oil. It was then mechanically agitated to homogenize it.

EXTRACTION OF OIL

This followed the method of Matos *et al.* (2009) sterilized kernels were dried, pulverized and made into a sludge. The sludge was then warmed to melt the endocytic oil. It was then mechanically agitated to homogenize it. Ten grams (10g) of the homogenized sludge were weighed out from each sample into a porcelain dish and placed in a thermostat oven set at 105^0C for four hours (4 hrs), cooled and reweighed. This was repeated for one hour (1 hr) and then for 30 minutes intervals, until constant weights were attained. The residue per samples were transferred into fluted whatman No 1 filter paper and extracted with petroleum ether for six hours at 60 – 80^{o}C in a Soxhlet reflux. The oil extracted were concentrated to constant weights in an oven at 105^{o}C.

The % weight of the oil per sample was estimated using the relation:

$$\% \text{ oil} = \frac{Wo \times 100}{Ws}$$

Where Wo = the weight of oil extracted
Ws = the initial weight of the sludge

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TEST FOR FREE FATTY ACID (FFA)

Ten grams (10 g) of the oil extracted from each state sample were weighed into different conical flask of known weight. Aliquots, 50 ml of hot neutral ethanol was added to each flask. The set up were placed in a hot plate at 40^{o}C and 8 drops of phenophthalin indicator added. The solution, while still hot in the water bath was titrated with 0.1 mol sodium hydroxide (NaOH) solution, swirling vigorously, while titrating. The end point was determined when the colour change persisted for 16 seconds. The free fatty acid content was estimated as oleic acid equivalent, using the relation:

$$FFA = \frac{WT \times M \times V}{\text{gwt of the oil}}$$

Where, FFA = free fatty acid
WT = formula weight of the acid (oleic acid)
M = molarity of NaOH
V = volume of the NaOH.
gwt = gram weight of the oil

Oil depreciation per location is estimated as the % quotient of FFA and the reciprocal value of the oil extracted, using the relation :

$$\text{Depreciation: } \frac{FFA \times 100}{\text{wt oil}}$$

Where, FFA = free fatty acid
WT = weight of the oil extracted.

DETERIORATION ACTIVITY TEST

Fresh kernels from endocarp of fresh fruits of *I. gabonensis* harvested from the forestry unit of Agricultural Development Corporation (ADC), Nekede, Owerri; were used in the investigation. The kernels were surface sterilized in sodium hypochlorite (NaOCl), washed in three changes of distilled water, dispensed, 50 g per conical flask, stoppered with absorbent cotton wool and aluminium foil and autoclaved at 121 kgcm^{2} for 30 minutes. One flask was kept as it was, while sterile distilled water was poured into one flask. The two flasks served as control while the remainder were inoculated with five days old fungi from pure cultures of the fungal isolates of the test samples, in the order of one flask, one fungus. The flasks complex were incubated at 29^{o}C for 14 days. The kernels were washed, dried, pulverized, made into sludge, and oil extracted, using Soxhlet reflux. The free fatty acid (FFA) contents were estimated from the extracted oil, using the above relation. The fatty acids components of the oil was analysed using Gas Chromatography.

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RESULTS

The test samples from the study area showed that the Saprotrophs that infest the kernels were the rot fungi: *Aspergillus flavus, A. niger, Penicillium italicum, Fusarium moniliforme, Lasiodiplodia theobromae* and *Rhizopus stolonifer*. These mycophytes occurred at different frequencies in the study locations as shown in Table 1.

Table 1: Percentage (%) frequency of occurrence of the fungal isolates in the study area.

SL	**Fungal isolates**					
	A. flavus	***A. niger***	***F. moniliforme***	***L. theobromae***	***P. italicum***	***R. Stolonifer***
Ab	17.61±0.25	17.00±0.05	16.00±1.06	12.58±1.12	6.41±6.27	28.55±2.37
Ba	23.23±8.69	21.87±6.13	13.01±2.89	9.64±1.34	7.00±4.14	26.00±1.46
Be	20.48±4.21	19.01±2.87	20.56±0.89	17.00±1.87	5.00±8.31	16.56±1.36
Ed	20.56±4.26	19.32±1.12	19.63±0.16	15.57±1.63	6.26±2.89	20.13±4.12
Im	17.77±1.87	14.38±5.07	21.88±0.14	24.00±2.36	9.23 ±2.12	22.98±6.85
Ke	28.33±0.02	19.61±0.04	17.00± 0.74	16.86±1.18	13.21±6.01	17.04±1.41
La	24.66±0.02	23.33±1.05	22.00±2.14	12.68 ±1.14	6.00±0.05	11.00±7.32
Pl	19.00±0.85	17.00±0.00	16.81±10.06	13.04±2.78	7.84±1.89	26.87±7.14
Rv	27.87±1.16	20.32±8.18	17.56±4.89	17.00±3.21	12.54±1.45	16.45±0.05
Sk	26.68±1.18	21.00± 6.04	17.48±0.89	17.32±7.21	12.89± 3.14	17.13±8.29

Legend

SL…….. Sample Location
Ab. ……Abuja
Ba …… Bauchi state
Be…….. Benue state
Ed …….. Edo state
Im …….. Imo state
Ke……. Kebbi state
La…… lagos state
Pl…….. Plateau state
Rv…….. River state
Sk…… Sokoto state.

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The gravimetric estimation of oil and free fatty acids per location samples is shown Table II below.

Table II: Percentage (%) weight of oil and free fatty acid contents per location samples of the study area

Location	sludge (g)	% wt oil	%wt FFA	Depreciation (%)
Abuja	10	32.41±0.06	6.69±1.23	20. 63±0.08
Bauchi	10	31.65±0.12	6.49±0.02	20.51±0.14
Benue	10	32.26±1.38	6.63±0.02	20.57±0.54
Edo	10	31.34±2.02	6.44 ±2.06	20.57 ±0.02
Imo	10	28.85 ±0.00	5.93 ±1.04	20.56±0.18
Kebbi	10	28.38 ±0.04	5.83±1.18	20.56±0.08
Lagos	10	31.91±2.07	6.56± 0.00	20.56±2.00
Plateau	10	31.62 ±0.34	6.49±0.04	20.56±0.05
Rivers	10	28.46±4.00	5.85±0.05	20.57±0.05
Sokoto	10	28.34±3.04	5.81±3.09	20.51±0.00

The free fatty acid (FFA) contents were used to demonstrate the vegetable oil dietary quality

The pathogenicity test is shown in Table III

Table III: Percentage (%) weight of oil and free fatty acid contents of kernels of *I. gabonensis* infested with the test fungi, after 14 days of incubation.

Fungal isolates	sludge (g)	% wt oil	%wt FFA	Deviation	
				Oil	FFA
Control	10	61.81±0.02	0.511±1.24	**0.00**	**0.00**
A. flavus	10	57.54±0.05	9.27 ±1.05	4.27±2.87	8.76±1.16
A. niger	10	55.99±1.14	9.266±6.00	5.82±4.12	8.75±0.05
F. moniliforme	10	44.01±0.00	7.82±3.14	17.80±0.04	7.30±0.09
L. theobromae	10	53.56±0.09	15.36±10.39	8.25±0.05	14.84±1.24
P. italicum	10	54.62±2.09	12.97±0.05	7.19±1.02	12.46±1.09
R. stolonifer	10	54.12±0.00	10.41±3.89	7.69±0.00	9.89±1.07

The gravimetric estimation of the oil and free fatty acid per sample sludge, per organism from the pathogenicity test result, showed a steep deviation from the yield from the wild (study locations),(Tables II and III).

Extracted oil from the control experiment, which was found to be 61.81±0.05 % w/w (Table III) of the kernel sludge was analyzed for its fatty acid composition using Gas chromatography. The result is shown in Table IV.

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Table IV: Fatty acid component of the kernel oil of *Irvingia gabonensis* as shown by Gas Chromatography

FATTY ACID		PERCENTAGE WEIGHT
Myristic acid	(C14 :O)	51.00±0.26%
Lauric acid	(C12:O)	36. 92±1.38%
Palmitic acid	(C16:O)	7.08±0.00%
Stearic acid	(C18: O)	1.05±0.02%
Oleic acid	(C18:1)	2.08±1.12%
Linoleic acid	(C18:2)	1.08±0.04%
Linolenic acid	(C18:3)	0.63±0.05%

DISCUSSION

A total of six fungi, *Aspergillus flavus, A niger, Penicillium italicum, Fusarium moniliforme, Lasiodiplodia theobromae* and *R stolonifer,* were isolated as shown in Table 1.This agreed with the earlier report of Duru *et al*. (2009) on the rot fungi of *Irvingia gabonensis*. In this study, these saprotrophs were found to depreciate the oil content of *I gabonensis* kernel, both in quantity and in dietary quality (Tables II and III). This concur with the findings of Ekundayo et al (2013) on the microbial fermentation of *Irvingia gabonensis* juice. These fungi occured in all the locations investigated with the frequencies of between the ranges of 5.00±2.14 in Benue state to 27.87±1.07 in River state. This was based on the integer value of isolates, from 200 kernels per test sample; Table 1. This report also tally with that of Duru *et al.* (2012) on roll back aflatoxicosis

The obvious dietary implications of food and other edible materials contamination and degradation, no dought has some obvious health and industrial implication. This is implied in this study, and the reports of Adamson *et al*. (1990) and Bassey et al (2018) concur with it. The biochemical analysis from the pathogenicity test result showed that the lipid of the kernel contain seven fatty acids: Myristic acid (51.00±0.26%) Lauric acid (36.92±1.38%) Palmitic acid (7.08±0.00%) Stearic acid (1.05±0.02%) Oleic acid (2.08±1.12%) Linoleic acid (1.08±0.04%) Linolenic acid (0.63±0.05%) this agreed with the range found by Ekpe *et al.* (2018), Adeyeye *et al.*, (2013) and Kieran and David (2017).

Myristic acid had been implicated in the reduction of arthritic pains and other inflammations in human. In cosmetics industries, they are used as lubricants. Palmitic acid is applied in soap making, and in treatment of insect bites. Oleic acid had been implicated in the lowering of heart attack and prevention of cancer. They are also useful agent in the manufacturing of butter and flavor in food candy. Stearic acids are known as emulsifying agents linoleic acid and linolenic acids are usually, called essential oils (Farvid *et al*. 2014; Shomonov – Wagner 2015; Amy 2017; Chembaline and Depeters 2017; Atli 2019). Therefore any organic hydrolysis that alter the proportion of these molecules as provided by nature is bound to affect the health of the consumer or his industrial need.

The quantification of the oil and its fatty acids contents of the staple soup thickener, *I. gabonensis* from the four major ecological zones of Nigeria is an empirical assessment of dietary depreciation in the face of Nigerian health and food safety standard with regards to consumption of unprotected commodities. The lipid contents per location within the study period in the field and in the laboratory are shown in Tables II and III. The quantities of oil obtained from the field were far less than the range from the laboratory; 28.34±3.04% in Sokoto, to 32.41±0.06 % in Abuja, compared to the laboratory yield of between 53.56±0.09 to 61.81±0.02%. This showed that from these study locations, out of every 10 g of *I. gabonensis* consumed, the consumer is short changed by the range of 47.08% to 47.56% of the oil quantity.

The depreciation indices per organism as shown in the deterioration activity test result (Table III) indicated that the fatty acid contents increased astronomically while the quantity of oil decreased. The increase in the quantity of free fatty acid is as a result of catabolic activities of the saprotrophs which hydrolyzed the lipids into fatty acids and glycerol. The degradation range from 7.30±0.09 % by *F. moniliforme* to 14.84±1.24 % by *L. theobromae,* within the laboratory period is an indication of the activity velocity of the saprotrophs.

CONCLUSION

The results of this study showed that the rough necrotic rot surfaces of the traded commodity, kernels of *I. gabonensis* were associated with the *Saprotrophs*; *Aspergillus flavus, A. niger, Penicillium italicum, Fusarium moniliforme, Lasiodiplodia theobromae* and *R stolonifer.* These fungi occur at different frequencies and were found to be responsible for the myco-deterioration of the vegetable oil component of the kernels of the *I. gabonensis.* They reduce the vegetable oil in quantity by decreasing the oil content and in quality by increasing the fatty acid component of the lipid. The decrease in both quality and quantity may be due to the metabolic activities of these associated saprotrophs. These may not only decrease the quality of nutrients expected from the test plant but may as well be deleterious to public health.

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