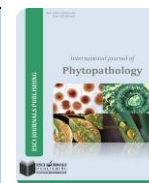




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THE EFFECTS OF POST HARVEST MYCODETERIORATION ON THE PROXIMATE COMPOSITION OF IRVINGIA GABONENSIS SEEDS

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ABSTRACT

Postharvest losses of produce occasioned by microorganisms can be either in quantitative or qualitative terms. Both way, and on a global scale, fungal pathogens have been indicted as one of the most important agents responsible for postharvest losses of crops. Fungi associated with diseased *Irvingia gabonensis* (Baill) seeds were isolated bimonthly from these seeds from four open markets in the Lagos metropolis over a two year period. In terms of number and species abundance, Alayabiagba Market in Ajegunle area had the highest fungal occurrence while *Aspergillus niger* had a higher percentage occurrence from each, and all of the experimental sites combined. Visually healthy seeds of *Irvingia gabonensis* were also inoculated with one of the pathogenic fungal species (*Aspergillus oryzae*) isolated from the diseased seeds and left for between 8-10 days after. Both the visually healthy and *Aspergillus oryzae* infected (diseased) seeds of *Irvingia gabonensis* were subjected to a proximate analysis, considering nutrients such as moisture, fats, ash, protein, crude fibre, carbohydrate and energy. The results from this mycodeterioration studies showed that the *Aspergillus oryzae* caused some significant reduction in the amount of most of the nutritional parameters in the *Irvingia gabonensis* seed. This work is probably a first report on pathogenic fungal species associated with the diseased seeds of *Irvingia gabonensis* in the open market as well as the effect of one of these species on the proximate composition of this seed.

Keywords: *Irvingia gabonensis* seed, fungal pathogen, postharvest deterioration, proximate composition, *Aspergillus* spp., mycodeterioration.

INTRODUCTION

Irvingia gabonensis (family Irvingiaceae) is a tree that grows naturally in Central and Western Africa (Harris, 1996). It is commonly called African Mango tree, bread-tree, bush mango or wild mango in English, Bobo, 'manguier sauvage' (French). 'Biri, goron' (Hausa), 'Oro' (Yoruba), 'Ogbonno' (Ibo) and its trade name is dika nut (Okafor, 1974). The tree grows up to 15 – 40 m in height, its bole is slightly buttressed (Leakey *et al*, 2000). The fruit is an ellipsoid to cylindrical drupe, occasionally nearly spherical, slightly laterally compressed, 4-6.5 cm x 4 – 6.5 cm x 3.5 – 6cm in size, smooth, green when ripe, pulp bright orange, soft, juicy, sweet to slightly bitter, with a few weak fibres, stone woody, single seeded.

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Seeds 2.5 – 4 cm x 1.5 – 2.5 cm in size, seedling with epigeal germination (Harris, 1999). The tree is valued for its dika nut in addition to producing a yellowed fruit. According to reports from Nigeria, it has played a major role in the nutrition, economy and traditional medicine in western and south-western tropical Africa from Nigeria to Angola through several wars during the early 1800s (Burkill, 1994).

Seeds are high in fat, similar to other nuts and seeds and contain an extraordinary fibre content of 14% (Giami *et al*, 1994). It has been prized for its healing properties and now become one of its most exciting discoveries in weight loss industry by United States department of Agriculture products fact sheet (Tabuna, 1999).

I. gabonensis is commonly found in Nigeria, Angola, Cameroon, Central African Republic, Congo, Cote d'ivoire, Democratic Republic of Congo, Equitorial, Guinea,

Guinea Bissau, Liberia, Senegal, Sierra Leone, Sudan, and Uganda (Harris, 1996). Seeds of *I. gabonensis* are known to be infested by the larvae of the merchant grain beetle (*Oryzaephilus mercatus*). Eggs are laid between the testa and cotyledons of the seed or in the cracks in the cotyledon. Prevention of crack prevents infestation (Dudu *et al.*, 1998). However, no disease or pest of *Irvingia gabonensis* tree have been recorded.

The aim of this research is to investigate and also document probably for the first time in literature the fungal species associated with diseased conditions in the *I. gabonensis* oilseeds in an open market in Nigeria. The specific objectives of this research therefore are to isolate pathogenic fungal species from an oilseed – *I. gabonensis* as well as to investigate the biodeterioration potentials of one of these pathogenic fungal species on the nutritional composition of this seed.

MATERIALS AND METHODS

Isolation of fungi from the samples of *I. gabonensis* seed: Isolation of fungi intended for use in this work were carried out from diseased seeds of *I. gabonensis* sampled from four open markets in the Lagos metropolis, namely Oyingbo, Bariga, Alayabiagba in Ajegunle and Agege markets.

The modified method of Adekunle and Oluyode (2005) was employed in isolating fungi from the seed samples. Diseased seeds of *I. gabonensis* (from each of the markets) that had been cut into discs were surface sterilized by leaving them in a solution of common bleach (sodium hypochloride - NaOCl) and sterile distilled water in a ratio of 3:2 for one minute. They were thereafter rinsed in three changes of distilled water, and were placed (using sterile forceps) into already prepared sterile LabM^R PDA plates. The plates were allowed to solidify and thereafter incubated at room temperature (28-31°C) in the incubator and observed daily for fungal growth. To obtain a pure culture, resultant fungal cultures were repeatedly sub cultured into fresh PDA plates until each plate contains only one type of fungal isolate.

Identification of fungi: The first step in the identification of the fungal species was the morphological studies which are hinged on observing the shape, colour, size and texture of fungal species in plates and time taken for each of the fungus to reach the maximum diameter (9cm) in plates. Microscopic slides were prepared by teasing out a little portion of the growth in the plate on to the glass slide, stained with

lactophenol in cotton- blue, and observed under a compound light microscope. The photomicrographs were then compared with the descriptions given by Talbot (1971), Deacon (1980), Domoschet *et al.* (1980) and Bryce (1992) for identification. The final confirmation of the identities of the key fungal species, and a comparison between these key isolates was done using molecular techniques such as PCR and electrophoresis gel analysis and DNA sequencing. In doing this, the CTAB protocol of DNA extraction and PCR (using Universal primers) as described by Thottappilly *et al.* (1999) was adopted for this work. In confirming the presence of DNA from the extracted samples, 1% gel electrophoresis of the extracted DNA samples was prepared using Lambda DNA Hind III Markers.

Pathogenicity test on the diseased *I. gabonensis* seeds: This was done using Koch's postulate. Here freshly prepared sterile plates of Lab M PDA were inoculated with between 2-3 pieces of freshly cut, surface sterilized, diseased *I. gabonensis* seeds. This was left to grow at room temperature and observed daily for fungal growth. Fungal growth at between 4-5 days after inoculation showed multiple growth per plate. After this, each of the distinct fungal pathogen were repeatedly (aseptically) subcultured into freshly prepared sterile LabM PDA until each plate contained only a single fungus.

Spores of about 1×10^5 from each Pure plate were inoculated into plates containing freshly prepared sterile LabM PDA. Between 1-2, surface sterilized, visually healthy whole seeds of *I. gabonensis* were dropped in each of these plates that were inoculated with spores. These were incubated for 5 – 7 days. Following this, a re-isolation was made on the *I. gabonensis* seed, which by now had become infected by the newly introduced spores. The similarities found in the characterization of the new isolates compared to the initial isolates, as well as the symptoms produced on both the initial and the new *I. gabonensis* seed proved the pathogenicity of the initial fungal isolates on *I. gabonensis*.

Mycodeterioration studies: Visually healthy seeds were inoculated with one of the pathogenic fungal species (*A.oryzae*) isolated from the diseased seeds, and left for between 8-10 days. To verify the ability of *Aspergillus oryzae* to cause a deterioration in *I. gabonensis* seeds, both the visually healthy and *A. oryzae* infected (diseased) seeds of *I. gabonensis* were subjected to a proximate analysis. In the proximate

determination, such entities as moisture, fats, ash, protein, crude fibre, carbohydrate and energy were all considered. Results obtained in all cases were the mean for 3 replicates. The data so obtained were subjected to statistical analysis using the software statistical package SAS (2005) at 5% level of significance, and mean separation was done using the Duncan's multiple range test.

Proximate analysis of the *Igbonensis* seed flour:

Proximate analysis is a system of analysis of nutrients in which the gross components (proteins, fat, carbohydrate, ash etc) of a food material are determined.

Crude fibre content determination: Crude fibre content in samples was determined using the AOAC (2000) method as detailed below:

Principle: Crude fibre is the organic residue which remains after the material has been treated under standardized conditions with light petroleum, boiling dilute H_2SO_4 , boiling dilute NaOH solution, dilute HCl, alcohol and ether.

PROCEDURE: Three gramme (3.00 g) of the defatted sample was put into a 250 ml beaker, thereafter, two hundred milliliters (200ml) of boiling water was added with 1.25% H_2SO_4 into the 250ml beaker containing the sample. The content in the beaker was heated and kept boiling for 30 minutes during which the mixture was stirred with the glass stirring rod to remove all particles from the sides and top up with boiling water and went down. After this, the mixture was filtered hot with buckner funnel fixed with ashless filter paper. The beaker was rinsed with 50ml boiling water and washed through Buchner funnel to collect the precipitate. The precipitate in the funnel was washed until it was neutral to litmus paper. The residue was scrapped off from the ashless filter paper back into the 250 ml beaker and 200ml boiling water with 2.5% NaOH and was boiled for 30 minutes and during this period, boiling water was added from time to time to make up the 200 ml level and rewashed through Buchner funnel to collect the precipitate, after which the residue was washed with twice with 95% ethanol. This final residue was thereafter dried in an oven to a constant weight and thereafter cooled in a dessicator.

The crude fibre content was calculated using the formula: $\% \text{ crude fibre} = \frac{X_1 - X_2}{X_3} \times 100$

Where

X_1 = weight before, X_2 = weight after,

X_3 = weight of sample

Moisture content: This was done using the AOAC (2000) detailed below:

Principle: This involves the measurement of the weight loss due to the evaporation of water. 3.00 g of the sample was weighed and placed in a crucible of constant weight. The crucible containing the sample was placed in the oven at $105^\circ C$ for about 14 hours. After drying, the weights were measured every one hour until a constant weight was obtained. The loss in weight represents the moisture content.

Calculation: $\% \text{ moisture} = \frac{A-B}{C} \times 100$

Where

A = weight of sample before drying

B = weight of sample after drying

C = weight of sample used

Ash content determination: Ash content in samples was determined using the AOAC (2000) method thus:

Principle: The ash of a foodstuff is the inorganic residue remaining after the organic matter has been burnt away.

Procedure: Exactly three gramme of the sample was weighed into a crucible of known weight and placed in a furnace which was ignited for about 24 hours until grey ash was obtained. The crucible containing the ash sample was removed from the furnace and cooled in the dessicator and then weighed using the analytical balance.

Calculation: $\% \text{ ash} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$

Protein content determination: Protein content was determined in the form of Total nitrogen using the AOAC (2000) as outlined below:

Principle: The kjeldahl method for determining total Nitrogen involves first heating with concentrated H_2SO_4 in a long-necked digestion flask. The reaction rate is accelerated by adding sodium or potassium sulphate which serves as catalyst to raise the boiling point. The oxidation causes the Nitrogen to be converted to ammonia sulfate. After making alkaline with concentrated NaOH solution, the ammonia is distilled into either excess boric acid or standard acid (such as H_2SO_4) and is estimated by titration.

PROCEDURE

Digestion of sample: Approximately two gram of the sample was weighed into the kjeldahl's flask and thereafter, 0.1g of $CuSO_4$ and 1.0g Na_2SO_4 was added into the kjeldahl's flask containing the sample. Afterwards, 20 ml of concentrated H_2SO_4 was also added. The flask was then set up in a slanting position on the kjeldahl's

digestion heating mantle and then in the fume cupboard until the colour changes from black to bluish green. The digest on the flask was then removed from the fume cupboard and allowed to cool. This digest was diluted with distilled water and made up to 200 ml. The blank was also prepared using the same procedure above with exception of the sample.

Distillation and titration: Fifty ml of the aliquot of the digest was poured into a distillation flask, and 50ml of 50% NaOH was carefully layered into the solution to make it strongly alkaline. Also, 50ml of 0.1M H₂SO₄ was measured into the receiving flask and two drops of methyl red indicator was added. The flask containing the digest was heated by using the Bunsen burner and the content was distilled into the receiving flask containing the 0.1M H₂SO₄. The distillation was stopped by removing the solution in the receiving flask before the heat was put off. This was done to avoid a drop in pressure. Excess acid was then titrated with 0.1M NaOH from the burette. The blank was set up using the procedure above. Protein content was obtained by multiplying percentage nitrogen by 5.18 the conversion factor for almonds.

$$\% \text{ Nitrogen} = \frac{(B - A) \times \text{Molarity of NaOH} \times 0.014}{W}$$

Where

B = titre value of blank

A = titre value of sample

W = weight of sample used

% protein = % Nitrogen x conversion factor

Standardization of 0.1M sodium hydroxide

Procedure: About 10cm³ of the primary standard of potassium hydrogen phthalate was weighed into a conical flask. Sodium hydroxide was then placed into a burette and two drops of phenolphthalein indicator was added to the evolution in the beaker. The sodium hydroxide was titrated against the primary standard.

Carbohydrate content determination: This was done according to AOAC (2000) as below:

Carbohydrate content was obtained by subtracting the sum of total crude fibre, total lipid content, total protein, ash and moisture from 100.

If A = % protein

B = % fat

C = % fibre

D = % ash

E = % moisture

% carbohydrate

100– (A + B + C + D + E)

RESULTS

Isolation studies: The results of the sampling studies are as summarized in Table 1, while Plates 1A and 1B – 4A and 4B show the culture plates and the photomicrographs of some of the different fungal species encountered in this research.

On a general note however, in terms of number and species abundance, Alayabiagba Market in Ajegunle area had the highest percentage fungal occurrence of 38.85% during the entire period under consideration. In addition, the results from Tables 1 also show that *Aspergillus niger* had the highest percentage occurrence (from each, and all the experimental sites combined) of 63.3%.

Mycodeterioration studies on *Irvingia gabonensis* seeds by *Aspergillus oryzae*: Plates 5A and 5B show the pictures of the representative healthy and diseased seeds respectively of *Irvingia gabonensis* which were grounded into flour from which the proximate composition was determined.

A statistical analysis of the overall mean for each of the following proximate parameter of moisture, ash, fats, proteins, fibre, carbohydrate and energy in the flour of both the healthy and the diseased seeds of *I.gabonensis* showed that the Treatment (i.e. inoculation of the *I.gabonensis* seed with *A.oryzae*) had a significant effect ($P \leq 0.05$) on each of the nutritional parameter examined with the exception of fibre on which the Treatment did not exert a significant effect (Table 2).

A comparison of the individual means (Table 3) for both flours however revealed that for each of the parameters mentioned above [with the exception of fibre where there was no significant difference ($P \leq 0.05$) between the mean values of flour from both the healthy and diseased seeds (3.8567% and 3.3233% respectively) and the mean value for fats, where the diseased seeds had a significantly higher ($P \leq 0.05$) mean value of 56.2700% compared to those from the healthy seeds with 55.4533%]; the mean values for the composition of the other proximate parameters such as moisture, ash, protein, carbohydrate and energy in the healthy seed samples was significantly higher ($P \leq 0.05$) at 5.0733%, 2.6500%, 10.0300%, 24.8000% and 641.0700% respectively than in the diseased seeds with a value of 4.4700%, 2.2700%, 8.8600%, 22.9367% and 631.0670% respectively for each of the afore listed parameters (Table 3).

DISCUSSION

Mould fungi (such as was used in the present studies) has been reported to have caused an improvement in some nutritional parameters in plant produce. Mimawati and Yeti (2013) for example reported that two mould

fungi, *Eupenicilium javanicum* and *Aspegillus niger* which have manannolitic and cellulolytic enzyme can be used to improve the nutritional quality of palm kernel cake. In terms of its nutritional value, the protein level recorded for the healthy seed of *I. gabonensis* was 10.03.

Table 1. Percentage frequency of occurrence of fungal isolates from each of the experimental site over a 30 month period.

Fungal Isolate	1	2	3	4	Average frequency of occurrence of each fungal species in all the sites.
<i>Aspergillus niger</i>	53.3	60.0	73.3	66.6	63.30
<i>Aspergillus oryzae</i>	46.6	6.6	40.0	26.6	29.95
<i>Aspergillus tubingensis</i>	6.6	6.6	40.0	6.6	14.95
<i>Mucor spp.</i>	26.6	13.3	26.6	33.3	24.95
<i>Rhizopus spp.</i>	26.6	20.0	26.6	13.3	21.63
<i>Penicillium spp.</i>	13.3	13.3	26.6	26.6	19.95
Average fungal occurrence/ site	28.83	19.96	38.85	28.83	-

1 = *Irvingia gabonensis* from Oyingbo market, 2 = *Irvingia gabonensis* from Bariga market, 3 = *Irvingia gabonensis* from Ajegunle, 4 = *Irvingia gabonensis* from Agege market

Table 2. Overall means for the proximate composition of the flour from diseased and healthy seeds of *I. gabonensis*.

Sr.	Nutrient	Overall Mean	R-SQ	COV	Root MSE	F-Value	d.f	P-Value
1	Moisture	4.7717	0.995	0.7604	0.0363	414.70	1	0.0024
2	Ash	2.4600	0.9551	2.9172	0.0718	42.06	1	0.0230
3	Fats	55.8617	0.9616	0.2607	0.1456	47.15	1	0.0206
4	Protein	9.4450	0.9091	3.3922	0.3204	20.00	1	0.0465
5	Fibre	3.5900	0.8927	5.2458	0.1883	12.03	1	0.0740
6	Carbohydrate	23.8683	0.9913	0.6356	0.1517	226.27	1	0.0044
7	Energy	636.0683	0.9855	0.1702	1.0829	150.10	1	0.0077

Table 3. A Comparison of the Individual Means for the Proximate Composition of the Flour from Healthy and Diseased Seeds of *I. gabonensis*

Sr.	Nutrient	Mean Healthy	Mean Diseased	Mean Difference	F-value	d.f	P-Value
1	Moisture	5.0733 _b	4.4700 _a	0.6033	414.70	1	0.0024
2	Ash	2.6500 _b	2.2700 _a	0.38	42.06	1	0.0230
3	Fats	55.4533 _a	56.2700 _b	-0.8167	47.15	1	0.0206
4	Protein	10.0300 _b	8.8600 _a	1.17	20.00	1	0.0465
5	Fibre	3.8567 _a	3.3233 _a	0.5334	12.03	1	0.0740
6	Carbohydrate	24.8000 _b	22.9367 _a	1.8633	226.27	1	0.0044
7	Energy	641.0700 _b	631.0670 _a	10.003	150.10	1	0.0077

*Mean values in each row showing different subscripts are significantly different at $P \leq 0.05$.



Plate 1A. Photograph of *Aspergillus oryzae* (Ajegunle) X 0.5

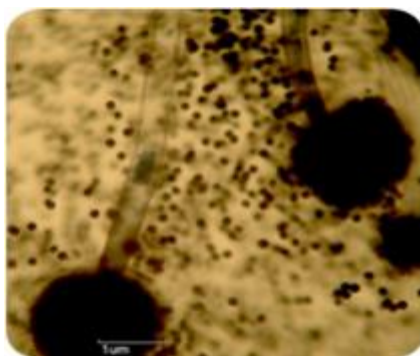


Plate 1B. Photomicrograph of *Aspergillus oryzae* x100



Plate 3A. Photograph of *Aspergillus tubingensis* X 0.5



Plate 3B. Photomicrograph of *A. tubingensis* X 100



Plate 4A. Photograph of *Aspergillus niger* X 0.5

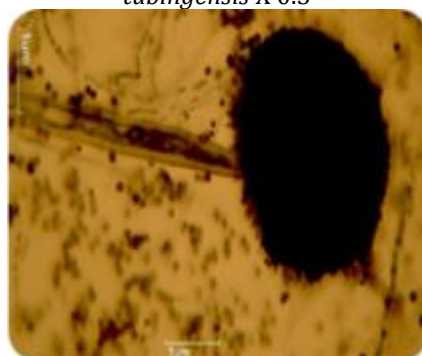


Plate 4B. Photomicrograph of *A. niger* X 120



Plate 5a. Healthy Seeds of *I. gabonensis* X 0.8



Plate 5b. Diseased Seed of *I. gabonensis* X 0.8

a value that falls within the range 10.23 recorded for *Bombax glabrum* (Adeleke and Abiodun, 2010), but lower than recorded for *M.oliefera* seeds, 29.36% (Anwar and Rashid, 2007), and *Glycine max*, 40.13% (Maidala *et al.*, 2013). Furthermore, *I. gabonensis* can be said to be a rich source of carbohydrate as the carbohydrate level in both the healthy (24.8000) and diseased (22.9367) seeds of *I. gabonensis* were higher than what was obtained for other oilseeds such as *B. glabrum*, 16.60% (Adeleke and Abiodun, 2010) and *Arachis hypogaeae*, 1.81% (Atasie *et al.*, 2009). Fat is important in diets as it promotes the absorption of fat soluble vitamins. Also, fat is a high energy nutrient and does not add to the bulk of the diet (Atasie *et al.*, 2009).

The fat (55.4533 and 56.2700) and fiber content (3.8567 and 3.3233) obtained respectively for both the healthy and diseased seeds of *I. gabonensis* in this work falls within the range of what was obtained from some other oilseeds such as *A.hypogaeae* (Atasie *et al.*, 2009) and *B.glabrum* (Adeleke and Abiodun, 2010), but higher than the value of 12.27% obtained for *G. max* (Maidala *et al.*, 2013). The crude fibre in this result indicates the ability of *I. gabonensis* seed to maintain a healthy physiological grip for a normal peristaltic movement of the intestinal tract. Diets low in crude fibre is undesirable as it could cause constipation and such diets have been associated with diseases of colon like piles, appendicitis and cancer (Atasie *et al.*, 2009). Generally, in terms of the changes in

the nutritional value of this oilseed *I. gabonensis*, the findings in this work generally agrees with the findings of Oladimeji and Kolapo (2008), where different microorganisms that were inclusive of fungi were shown to cause a reduction in the nutritional worth of some oilseeds in Nigeria. In terms of their ability to cause a deterioration, fungal species generally are known to have caused a rapid deterioration in the nutritional quality of seeds by causing a discoloration, contributes to heating and losses in dry matter through the utilization of carbohydrate as their source of energy, degrades and or alter the digestibility of proteins and fats (Magan and Aldred, 2007). In the present study, *A. oryzae* infection of *I. gabonensis* seeds caused a significant deterioration in the nutritional quality of this seed.

Further, the spores of some fungi are known to have caused some respiratory disease in some exposed workers (Lacey and Crook, 1988). Apart from the deterioration in the nutritional worth of produce, the production of mycotoxins, especially of some types, which are newly being reported with some species/strains of fungi in some places (Darnetty and Salleh, 2013), further accentuates the human and animal health and safety concerns associated with the consumption of food materials that have suffered from fungal attack. This research is probably the first to report on pathogenic fungal species associated with the diseased seeds of *I. gabonensis* in the open market.

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