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Microbial analysis and detection of Aflatoxin from *Irvingia gabonensis* kernels sold in Oyo Town, Oyo State, Nigeria

Racheal Oluwayemisi Fashogbon[1*], Bukola Margaret Popoola[1], Samuel Aforijiku[2], Ayomide Ezekiel Oyekanmi[1], Osaze Ruth Uloko[1] and Afolake Olanbiwoninu[1]

[1]Department of Biological Sciences, Ajayi Crowther University, Oyo, Oyo State, Nigeria.
[2]Institute of Agricultural Research and Training Ibadan, Oyo State, Nigeria.

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This research work aimed at screening for different microorganisms associated with *Irvingia gabonensis* var. *gabonesis* Kernels, its nutritional value and detection of aflatoxins from some of the infested *I. gabonensis* Kernels sold in Oyo town. A total of 30 different *I. gabonensis* var. *gabonesis* Kernels were randomly purchased from six different points in the five major markets in Oyo town and isolation was done on Nutrient agar (NA), McConkey agar, Eosine Methylene Blue Agar (EMB) and Potato Dextrose Agar (PDA) using pour plate method. The isolates were culturally, morphologically and biochemically characterized. The mineral, proximate and aflatoxin detection of the *I. gabonensis* Kernels with high microbial load and growth of *Aspergillus flavus* was carried out using standard methods. A total of 25 bacteria and 18 fungal were isolated which include *Bacillus* spp*., Staphylococcus* spp., *Aspergillus* spp*., Penicillium* spp., and yeast. *Irvingia gabonensis* seeds with growth of *A. flavus* (OOW1) had the least mineral composition with 5.4% sodium, 20.0 mg/kg vitamin C, 29.4 mg/kg calcium, 0.9 mg/100g iron, 34.4 mg/100 g magnesium and 0.02 mg/100 g zinc. The *I. gabonensis* Kernels with the growth of *A. flavus* (OOW1) had the least mineral composition with 5.5% moisture content, 4.2% crude protein, 45.7% crude fat, 9.1% crude fibre and 1.5% total ash. The *I. gabonensis* Kernels with the growth of *A. flavus* (OOW4, OOJ6 and OOW1) had aflatoxin level of 3.47, 3.69 and 5.10 ppb, respectively. *Irvingia gabonensis* seed with high microbial load and growth of *A. flavus* had low nutritional value making them unsafe for consumption.

Key words: Nutritional value, kernels, mineral composition, microbial load, proximate analysis.

INTRODUCTION

Food is a vital part of the cultural identity of people all over the world. In African countries such as Nigeria, some foods are consumed during religious or cultural festivals, while condiments and soup thickeners such as melon and *Irvingia gabonensis* Kernels (Ogbono) are consumed as a normal culinary practice (Chibundu et al.,

*Corresponding author. E-mail: fashogbonracheal@gmail.com.

2016). *I. gabonensis* commonly known as Dikanut, Africa mango, bush mango or wild mango is an essential product that serve as a source of valuable income to both rural and urban settlers in Africa (Arowosoge, 2017). It is known in South Western part of Nigeria (Yoruba land) as "Apon" and South Eastern part of Nigeria (Igbo land) as "Ogbono".

The tree of *I. gabonensis* grows well in tropical rainforests of Africa. The freshly harvested seeds are sun-dried, grinded and used as recipe for "ogbono" soup (Bamidele et al., 2015). The two known species of *I. gabonensis* Kernels that grow freely are the *I. Gabonensis* var. *Gabonesis* and *I. gabonensis* var. *Excelsa*. The pulp of the *I. gabonesis* var. *Excelsa* is classified as oil seed due to its high fatty matter (54 to 67%) (Bamidele et al., 2015; Akusuand and Kiin-Kabari, 2016). The kernels of *I. gabonensis* contain oil which is sometimes extracted and often used in the production of margarine and drugs. The residue from the extracted kernel is then used as thickening agent in soup (Arowosege, 2017). The pulp of the *I. gabonensis* var. *gabonensis* is sweet, smooth in the mouth and has brittle pulp but its kernel draws less than that of *I. gabonensis* var. *Excelsa* (Akusuand and Kiin-Kabari, 2016). *I. gabonensis* var. *gabonensis* is highly demanded due to its nutritional, economic and medicinal worth. Due to the high moisture content of the African bush mango, the best method of preserving the *I. gabonensis* is by sun-drying the seeds which help to extend the shelf life of the Kernels (Vihotogbé et al., 2019).

However, these Kernels of *I. gabonensis* have been reported to be prone to fungi attack causing the food to become tasteless, loose its thickness, nutritional value and produce mycotoxins (Sanyaolu et al., 2014; Chuku and Aggrey, 2017). Fungi are plant pathogens and major spoilage agents of foods and foodstuffs. During favorable environmental conditions, some fungal strains may release metabolites such as mycotoxins into food hence making it poisonous and unfit for human consumption (Jonathan et al., 2016). Mycotoxins are mainly produced by certain filamentous fungi belonging to *Aspergillus*, *Penicillium* and *Fusarium* genera. The major agro-economic important mycotoxins produce includes aflatoxins, ochratoxins, trichothecenes, zearelenone, fumonisins and tremorgenic. Aflatoxins have been observed as the most toxic because of their highly carcinogenic and hepatotoxic effect, especially *Aspergillus flavus* and *Aspergillus parasiticus* (Ubwa, 2014; Ozer et al., 2012; Menza et al*.,* 2015).

The growth of molds on the seeds is majorly as a result of poor post-harvest handling, especially during the process of cracking, drying, storage and transportation (Chuku and Aggrey, 2017). An investigation into the aflatoxin content of African bush mango seeds in Nigeria revealed a 35% non-compliance with the European Union standard (Adebayo-Tayo et al., 2006). Due to the infestation of microorganisms in *I. gabonensis* Kernels which has resulted in loss of income and nutritional value of the seeds (Azuonwu et al., 2019), there is need to investigate the different microorganisms associated with *I. gabonensis* Kernels as well as the mineral, proximate and nutritional value of some of the *I. gabonensis* Kernels sold in Oyo town.

MATERIALS AND METHODS

The study was conducted between the months of January to June, 2021. Thirty *I. gabonensis* var. *gabonesis* Kernels were randomly collected from display retailers at six different shops in the five major markets in Atiba Local Government Area of Oyo town, Oyo State. All samples were aseptically packaged and transported to the laboratory for analyses.

Enumeration, isolation and identification of microorganisms

One gram of each samples was weighed and mashed in a stomacher bag containing 9 mL of distilled water using a stomacher machine (Seward STOMACHER® 80 Lab System). One mL aliquot from the stomacher bag was pipetted and transferred into a sterile test tube containing 9 mL of 0.1% peptone water. This process was repeated for each of five sets of test tubes until a dilution of 10^{-6}. 1 mL from the dilution 10^{-5} were plated in duplicate into 15 ml of sterilized and cooled Nutrient Agar (NA), Mac Conkey agar, Eosine Methylene Blue (EMB) agar and Potato Dextrose Agar (PDA). The inverted plates were incubated at 37°C for 24 hours for the bacteria isolates while the PDA plates were inverted and incubated for 5 days at 30°C for the fungal isolates. Distinct colonies were sub-cultured to obtain a pure culture. The inverted plates were incubated for 24 hours in NA, EMB and Mac Conkey agar while the un-inverted PDA plate was incubated for 5 days at 30°C after which the colonies were counted (Kidd et al., 2016). The pure colonies of the bacterial isolates were subjected to gram staining, spore staining, oxidase, catalase and starch hydrolysis. While the pure colonies of the fungal isolates were examined under the microscope after staining with lactophenol cotton blue. The isolates were identified using their morphological characteristics and microscopic structures (Tersoo-Abiem et al., 2020).

Mineral analysis

The mineral analysis of the *I. gabonensis* Kernels with high microbial load and growth of *Aspergillus flavus* were done according to the methods of AOAC (2005). A gram of each sample was digested with 10% HNO_3 after ashing. The sample was filtered after digestion and the filtrate made up to 100 mL with distilled deionized water. Atomic Absorption Spectrometer (Buck Scientific East Norwalk, USA) was used to determine the concentration of iron, magnesium, zinc and calcium while Flame Photometry (Jenway Ltd, Dunmow Essex UK) was used for the determination of Na.

Proximate analysis

Determination of the proximate composition which include moisture, fats, ash, carbohydrate and protein contents of the *I. gabonensis* Kernels with high microbial load and growth of *A. flavus* were done according to the methods of AOAC (2016).

Determination of Moisture content

Moisture content of *I. gabonensis* Kernels samples with high

microbial load and growth of *A. flavus* were analysed using the gravimetric method reported by AOAC, (2016). A 5 g was measured into a previously measured moisture sampler. The sample in the can was allowed to dry by air over a steam bath and then dried in the 105°C for three hours in the oven. It was cooled in a desiccator and weighed. It was then returned to the oven for proper drying. The sample was further dried, cooled and weighed until a regular weight was achieved. Weight of lost moisture content was obtained by difference and calculated as percentage of the weight of sample analyzed.

$$\% \text{ Moisture (\%MC)} = \text{M2-M3/M2-M1} - 100/1$$

where M1 = Measurement of empty moisture can, M2 = Measurement of can + sample before drying and M3 =Measurement of can + sample after drying

Determination of Ash content

Content if the ash in *I. gabonensis* Kernels sample was analyzed by the furnace incineration gravimetric process (AOAC, 2005). A 5 g sample was weighed into previously weighed crucible. It was evaporated to dryness over a steam bath and then burnt in a muffle furnace at 550°C until it becomes grey ash. The ashes in the crucible were carefully removed and chill in a desiccator and weighed again. As the measurement increased, the weight of ash was obtained and expressed as percentage of the sample analyzed and calculated using the formula as shown below.

$$\% \text{ Ash} = \text{W2} - \text{W1} \times 100$$

where, W1 = weight of empty crucible and W2 = weight of crucible + ash.

Determination of Protein content

Protein content was done by Greenfield and Southgate (2003) in which the total nitrogen was obtained and multiplied with the factor 6.38. 10 mL of concentrated H_2SO_4 and 0.5 g of the *I. gabonensis* var. *gabonesis* Kernels was boiled with selenium as a catalyst. Digestion was done in a fume cupboard until a clear mixture was achieved. This breakdown was transferred quantitatively to a standard container and diluted with 100 mL distilled water. 10 mL of the breakdown was mixed with the same volume of 45% NaOH solution and distilled in semi micro- Kjeldahl apparatus. The distillate was transferred into 10% boric acid solution and 3 drops of mixed methyl red and bromocresol green indicator. A total of 50 mL distillate was collected and titrated against 0.02 N H_2SO_4. Titration was done from green colouration to a deep red end- point. Blank was also treated just as described. The Normality (N2) content and protein was calculated as shown below.

$$\text{N2} = \frac{100}{\text{W}} \times 14 \times \frac{\text{N}}{1000} \times \frac{\text{VF}}{\text{Va}} \times \text{T} - \text{Blank}$$

W =weight of sample, N =Normality of titrant, Vf = Total digest volume, Va = Volume of digest analyzed, T = Sample titre, BLK = Reagent Blank titre.

Determination of carbohydrate content of the produced *I. Gabonensis* kernels

Carbohydrate was calculated as nitrogen free extractives using the formula described by Greenfield and Southgate (2003).

$$\% \text{ CHO} = 100 - \% \text{ (Protein + ash + fat + moisture content)}$$

Determination of fat content of the produced ogbono samples

Fat content of *I. gabonensis* Kernels was carried out by measuring 0.5 g of the sample into a conical flask. 0.88 mL ammonia solution and 10 mL of 95% ethanol was added to it and mixed properly. 25 mL of diethyl ether was added and mixed properly for 1 minute. 25 mL of petroleum ether was properly mixed with it. Mixture separated into phases and after standing for 1 hour. The fat extract (ether phase) was collected and the sample was re-extracted with the same solvent and the extracts was pooled together. The extract was then transferred to a pre-weighed flask and the solvent recovered. The fat in the container was oven dried at 100°C for 30 minutes. The dried samples were cooled in a dessicator and measured. Dried sample was weighed and fat was assayed. The amount was written as a percentage of the sample analysed. It was calculated as shown below (Greenfield and Southgate, 2003).

$$\% \text{ fat} = \text{W2} - \text{W3} \times 100$$

where W1 =weight of flask alone and W2 =weight of flask and extract

Aflatoxin detection and quantification

Detection of aflatoxin levels from *I. gabonensis* Kernels with high microbial load and growth of *A. flavus* was carried out by Enzyme-Linked Immunosorbent Assay (ELISA) method. 10 g of each sample was extracted with 20 mL methanol: water (70: 30). The residue was dissolved in 1 mL of methanol: water (3:1, v/v) and 200 ml of diluted extract was applied to the enzyme immuno-sorbent assay (ELISA) plate in order to determine the total aflatoxin content. Each one of the samples and standards were applied in duplicates. Testing for total aflatoxin content was carried out on each sample after the extraction process, using AgraQuant assay kit (Romer Labs) according to the manufacturer's instructions in the ELISA kit. The total aflatoxin concentration was read at 450 to 630 nm. The optical densities (ODs) were compared to those of the standards. Total aflatoxin concentration in each sample was expressed in parts per billion (ppb) (Tersoo-Abiem et al., 2020).

RESULTS

Microorganisms were enumerated from *I. gabonensis* Kernels samples obtained from five different markets in Oyo town is shown in Table 1. Regardless of the high dilution factor (10^5) used for the six samples of *I. gabonensis* Kernels randomly purchased from each market, the highest bacterial count was observed in OOJ1 with 2.80 x 10^6 cfu/g on Nutrient agar, 4.8 x 10^6 cfu/g on McMackey agar and 4.4 x 10^4 cfu/g on Eosine. Methylene Blue agar while OOW1 had high fungal count (7.0 x 10^3) on Potato Dextrose Agar. Tables 2 and 3 shows the macroscopic characteristics of isolates on Nutrient agar, Eosine Methylene Blue (EMB) and MacConkey Agar. A total of 25 bacteria were isolated. The bacterial isolates showed various colonial appearances on Nutrient agar ranging from smooth

Table 1. Microbial count of *Irvingia gabonensis* Kernels at 10^5 dilution factor

Sample	NA		Mc Conkey agar		EMB agar		Potato dextrose agar (PDA)	
	No. of colonies	Viable count (cfu/g)	No. of colonies	Viable count (cfu/g)	No. of colonies	Viable count (cfu/g)	No. of colonies	Viable count (cfu/g)
OOW1	245	2.45×10^4	093	6.3×10^4	051	5.1×10^4	07	7.0×10^3
OOW2	034	3.4×10^6	014	1.4×10^6	014	1.4×10^6	02	2.0×10^5
OOW3	016	1.6×10^6	046	2.6×10^6	021	2.1×10^6	02	2.0×10^5
OOW4	230	2.30×10^4	078	7.8×10^4	056	5.6×10^4	06	6.0×10^3
OOW5	140	1.40×10^4	083	1.3×10^4	035	3.5×10^4	02	2.0×10^3
OOW6	109	1.09×10^4	019	1.9×10^4	042	4.2×10^4	02	2.0×10^3
OAJ1	214	2.14×10^4	51	5.1×10^4	011	1.1×10^4	05	5.0×10^3
OAJ2	267	2.67×10^6	042	9.2×10^6	025	7.5×10^6	04	4.0×10^5
OAJ3	183	1.83×10^6	014	1.4×10^6	062	6.2×10^6	-	-
OAJ4	283	2.83×10^6	048	4.8×10^6	018	1.8×10^6	06	6.0×10^3
OAJ5	190	1.90×10^4	012	1.2×10^4	011	1.1×10^4	01	1.0×10^3
OAJ6	083	8.3×10^6	023	2.3×10^6	062	3.1×10^6	-	-
OAK1	120	1.20×10^6	072	5.2×10^6	033	3.3×10^4	-	-
OAK2	279	2.79×10^6	057	8.7×10^6	027	6.7×10^4	06	6.0×10^3
OAK3	187	1.87×10^6	076	2.6×10^4	021	2.1×10^4	01	1.0×10^5
OAK4	129	1.29×10^6	072	4.2×10^6	035	3.5×10^6	04	4.0×10^5
OAK5	192	1.92×10^6	029	2.9×10^6	034	3.4×10^6	-	-
OAK6	192	1.92×10^6	029	2.9×10^6	034	3.4×10^6	-	-
OSB1	110	1.10×10^6	039	3.9×10^4	026	2.6×10^4	-	-
OSB2	182	1.82×10^6	082	3.2×10^4	051	5.1×10^4	05	5.0×10^3
OSB3	119	1.19×10^6	041	4.1×10^4	013	1.3×10^4	-	-
OSB4	153	1.53×10^6	052	5.2×10^4	016	1.6×10^4	-	-
OSB5	129	1.29×10^6	062	6.2×10^6	029	2.9×10^6	-	-
OSB6	192	1.92×10^6	029	2.9×10^6	034	3.4×10^6	06	6.0×10^3
OOJ1	280	2.80×10^6	048	4.8×10^6	044	4.4×10^4	05	5.0×10^3
OOJ2	190	1.90×10^6	029	2.9×10^4	032	3.2×10^6	-	-
OOJ3	125	1.25×10^6	036	3.6×10^4	017	1.7×10^4	02	2.0×10^5
OOJ4	110	1.10×10^6	081	1.1×10^4	029	2.9×10^4	-	-
OOJ5	139	1.39×10^6	077	2.7×10^4	031	3.1×10^4	02	2.0×10^5
OOJ6	192	1.92×10^6	029	2.9×10^6	034	3.4×10^6	05	5.0×10^3

Source: Author

surfaces, raised elevation, circular shaped, mucoid colony, pigmented, translucent, opaque, shiny colony, large, medium colonies. Five of the isolates were Gram negative rod while the remaining 20 were Gram positive (4 Gram positive cocci and 21 Gram positive rod). Most of the isolates were indole negative, catalase positive and spore formers. The bacteria isolates were biochemically identified as *Bacillus cereus, Bacillus subtillis, Staphylococcus* spp., *Staphylococcus* spp. and *Salmonella* sp.

Figure 1 shows the percentage frequency of occurrence of bacteria isolated from *I. gabonensis*. Table 4 shows the colonial and morphological characteristics features of fungi isolates on PDA. A total of eighteen fungi were isolated. The fungal isolates showed different cultural appearances such as a velvety and flaky surface with grey to black coloration, White and green variants powdery surface growth, dust-like sporulating surface light brown with smooth border, abundant mycelium with pale brown and dark zonation, white air mycelium with quick differentiation, upper side white color with irregularly smooth and fringed. The fungal isolates were identified as *Aspergillus flavus, Aspergillus niger, Penicillium* sp., and yeast. The percentage frequency of occurrence of fungal isolated from *I. gabonensis* is shown in Figure 2. 17% of the fungal isolates were *A. flavus,* 33% were *A. niger,* 28% were *Penicillium* sp., while yeast had 22%.

Table 2. Macroscopic characteristics of bacteria isolates on Nutrient agar, eosine methylene blue and MacConkey agar.

S/N	Sample	Margin	Colour	Elevation	Texture	Shape
1	OOW1	Entire	Milky	Convex	Shiny	Round
2	OOW2	Entire	Milky	Flat	Shiny	Puntiform
3	OOW3	Lobate	Opaque	Flat	Mucoid	Irregular
4	OOW4	Lobate	Opaque	Flat	Moist	Irregular
5	OOW5	Undulate	Opaque	Flat	Mucoid	Irregular
6	OOW6	Lobate	Opaque	Flat	Mucoid	Filamentous
7	OAJ1	Filamentous	White	Flat	Dry	Filamentous
8	OAJ2	Lobate	White	Flat	Moist	Irregular
9	OAJ3	Undulate	Opaque	Flat	Moist	Irregular
10	OAJ4	Lobate	White	Obonate	Slimy	Irregular
11	OAJ5	Lobate	White	Flat	Moist	Irregular
12	OAK1	Smooth	Opaque	Flat	Moist	Irregular
13	OAK2	Entire	Entire	Milky	Convex	Shiny
14	OAK3	Entire	Milky	Flat	Shiny	Puntiform
15	OAK4	Filamentous	White	Flat	Dry	Filamentous
16	OAK5	Smooth	Opaque	Flat	Moist	Irregular
17	OAK6	Entire	Entire	Milky	Convex	Shiny
18	OSB2	Filamentous	White	Flat	Dry	Filamentous
19	OSB6	Undulate	Opaque	Flat	Mucoid	Irregular
20	OOJ1	Undulate	Opaque	Flat	Mucoid	Irregular
21	OOJ2	Smooth	Opaque	Flat	Moist	Irregular
22	OOJ3	Entire	Milky	Flat	Shiny	Puntiform
23	OOJ4	Smooth	Opaque	Flat	Moist	Irregular
24	OOJ5	Undulate	Opaque	Flat	Mucoid	Irregular
25	OOJ6	Undulate	Opaque	Flat	Mucoid	Irregular

Source: Author

Mineral composition of the *Irvingia gabonensis* Kernels

Mineral composition, proximate analysis and the detection of aflatoxin were determined using three samples of *I. gabonensis* Kernels due to their high microbial load and the presence of *A. flavus* in the karnel. The *I. gabonensis* Kernels without the growth of *A. flavus* (OAJ3) was used as control. Table 5 shows the mineral composition of *I. gabonensis* Kernels. The *I. gabonensis* Kernels without the growth of *A. flavus* (OAJ3) had the highest mineral composition as follows 7.5% sodium, 28.2 ppm vitamin C, 87.4 ppm potassium, 37.9 ppm calcium, 13.4 mg/100g iron, 49.9 mg/100g magnesium and 0.03 mg/100g zinc) compare to *I. gabonensis* Kernels with the growth of *A. flavus*. Sample OOW1 had the least mineral composition with 5.4% sodium, 20.0 ppm vitamin C, 53.6 ppm potassium, 29.4 ppm calcium, 0.9 mg/100g iron, 34.4 mg/100g magnesium and 0.0.2 mg/100g zinc. However, there are no heavy metals present in any of the samples analyzed.

Proximate composition of *I. Gabonensis* Kernels

Table 6 shows the mineral composition of *I. gabonensis* Kernels. The *I. gabonensis* kernel without the growth of *A. flavus* (OAJ3) had the 16.3% carbohydrate content, 5.5% moisture content, 7.8% crude protein, 58.9% crude fat, 10.7% crude fibre and 1.8% total ash, 47.1 kg/100g fatty acids with 2589.0 kg/100g metabolized energy. Sample OOW1 had the least mineral composition with 29.9% carbohydrate, 5.5% moisture content, 4.2% crude protein, 50.7% crude fat, 9.1% crude fibre, 1.5% total ash, 40.6 kg/100 g fatty acids and 2340.3 kg/100 g metabolized energy.

Presence of aflatoxin in the *I. gabonensis* kernels

Aflatoxin level of the *I. gabonensis* Kernels is shown in Table 7. The *I. gabonensis* Kernels without the growth of *A. flavus* (OAJ3) had the no aflatoxin, The *I. gabonensis* Kernels with the growth of *A. flavus* (OOW4, OOJ6 and OOW1) had aflatoxin level of 3.47, 3.69 and 5.10 ppb,

Table 3. Biochemical characterization of the bacterial isolates isolated from *Irvingia gabonensis* Kernels.

S/N	Isolate code	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Motility test	Endospore	Glucose	Sucrose	Lactose	Fructose	Maltose	Probable organism
1.	OOW1	+	Cocci	+	-	-	-	+	+	+	+	-	+	*Staphylococcus* spp.
2.	OOW2	+	Rod	+	-	_	+	+	+	+	-	+	+	*Bacillus cereus*
3.	OOW3	+	Rod	+	-	_	+	+	+	-	-	+	+	*Bacillus cereus*
4.	OOW4	+	Rod	+	+	-	+	+	+	+	-	-	+	*Bacillus subtilis*
5.	OOW5	+	Cocci	+	-	-	-	+	+	+	+	-	+	*Staphylococcus* spp.
6.	OOW6	+	Rod	+	-	_	+	+	+	+	-	+	+	*Bacillus cereus*
7	OAJ1	+	Cocci	-	+	_	-	+	+	+	+	+	+	*Streptococcus* spp
8.	OAJ2	-	Rod	+	-	_	_	+	+	+	-	+	+	*Salmonella* sp.
9.	OAJ3	+	Rod	+	+	-	+	+	+	+	-	-	+	*Bacillus subtilis*
10.	OAJ4	+	Cocci	+	-	-	-	+	+	+	+	-	+	*Staphylococcus* spp.
11.	OAJ5	+	Rod	+	-	_	+	+	+	+	-	+	+	*Bacillus cereus*
12.	OAK1	-	Rod	+	-	_	_	+	+	+	-	+	+	*Salmonella* sp.
13.	OAK2	+	Cocci	+	-	-	-	+	+	+	+	-	+	*Staphylococcus* spp.
14.	OAK3	+	Rod	+	-	_	+	+	+	+	-	+	+	*Bacillus cereus*
15.	OAK4	+	Rod	+	+	-	+	+	+	+	-	-	+	*Bacillus subtilis*
16.	OAK5	-	Rod	+	-	_	_	+	+	+	-	+	+	*Salmonella* sp.
17.	OAK6	+	Cocci	+	-	-	-	+	+	+	+	-	+	*Staphylococcus* spp.
18.	OSB2	+	Rod	+	-	_	+	+	+	-	-	+	+	*Bacillus cereus*
19.	OSB6	+	Rod	+	-	_	+	+	+	+	-	+	+	*Bacillus cereus*
20.	OOJ1	-	Rod	+	-	_	_	+	+	+	-	+	+	*Salmonella* sp.
21.	OOJ2	+	Rod	+	-	_	+	+	+	-	-	+	+	*Bacillus cereus*
22.	OOJ3	-	Rod	+	-	_	_	+	+	+	-	+	+	*Salmonella* sp.
23.	OOJ4	+	Rod	+	+	-	+	+	+	+	-	-	+	*Bacillus subtilis*
24.	OOJ5	+	Rod	+	-	_	+	+	+	+	-	+	+	*Bacillus cereus*
25.	OOJ6	+	Rod	+	-	_	+	+	+	+	-	+	+	*Bacillus cereus*

Source: Author

respectively.

DISCUSSION

The bacterial isolates obtained from this study is similar to the study of Adebayo-Tayo et al. *(*2006) and Adegbehingbe et al. (2014) who isolated similar bacteria from seeds of *I. gabonensis.* The fungal isolated from this research include *Aspergillus flavus*, *Aspergillus niger* and *Penicillum* sp. Which is in line with the work of Chibundu et al. (2016). Tersoo-Abiem et al. (2020) also isolated the similar fungal strains from Ogbono samples that were obtained from different markets in Benue states.

The source of microbial contamination in samples could be due to the environment in which

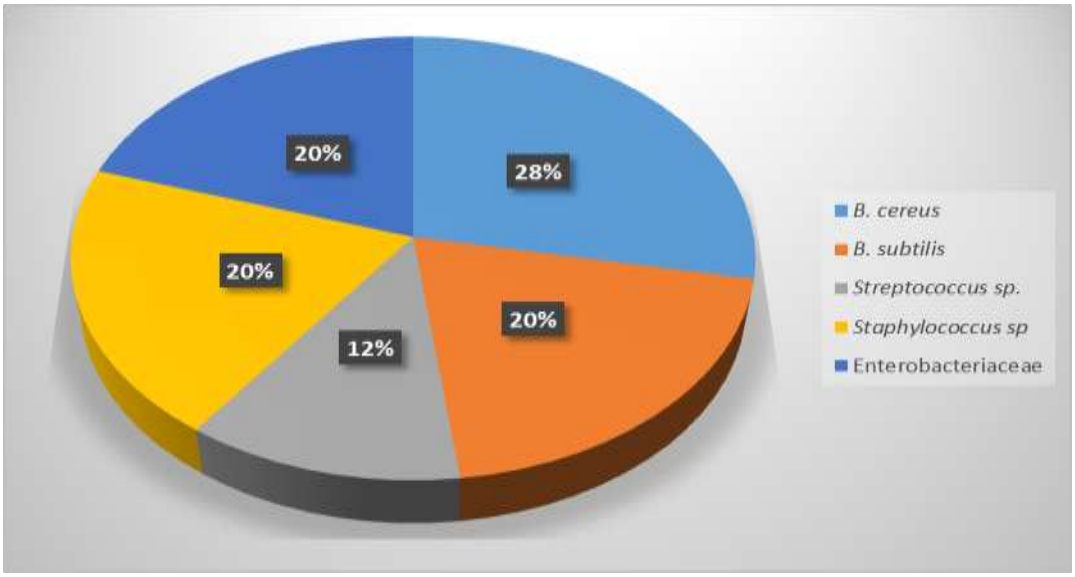

Figure 1. Percentage frequency of occurrence of the bacteria isolated from *Irvingia gabonensis* Kernels.
Source: Author

the Ogbono samples were sold. Ekundayo et al., (2003) isolated some pathogenic microorganisms from his research and he opined that some of the Ogbono samples could have been stored for a very long period before selling. The presence of Staphylococcus aureus and Bacillus sp. Indicate a potential risk and could be harmful to humans when ingested, due to their ability to produce toxins (Saliu, 2008)**.** *Bacillus* sp*.* is normally found in the soil and may have been transported via vegetables. The consumption of these organisms in the Ogbono samples in large numbers could lead to gastrointestinal illness.

The occurrence of *S. aureus* which are Gram positive cocci, catalase-positive, coagulase-positive,oxidase-negative and facultative anaerobes in most of the samples strongly indicated a high level of poor personal hygiene by the sellers. Although *S. aureus* is often associated with the skin and mucous glands (especially in the nose of healthy persons) as commensals (Ibrahim, 2017). Since the market is a busy place, particulate matter carrying microorganisms may have been deposited and unhealthy practices carried out in the market could put the unsuspecting public at massive potential risk with strong public health concern of food poisoning.

Mineral composition

The mineral composition of the ogbono samples without the growth of *Aspergillus flavus* (OAJ3) used as a control had high mineral composition compared to ogbono samples with the growth of *Aspergillus flavus* which is similar to the work of Oseni and Ekperigin (2007). This result is also line with the work of Ibrahim et al. (2017) and Mgbemena et al. (2019) who recorded higher amount of iron and calcium in Ogbono seeds. They also recommended that the seed of Ogbono seed should be consumed due to the high iron content. Mineral content in food is a measure of the amount of specific inorganic components present within the food. Minerals act as co-factors for enzyme reactions. Sodium, calcium and magnesium are required in major quantities. Sodium acts as charge carriers and is a major factor in extra cellular fluid. It also participates in the functioning of muscle nerve (Mgbemena et al*.,* 2016). Sodium and potassium are needed to help maintain the pH of the body so as to regulate muscles and nerves irritability as well as osmotic balance of the body fluids. Iron content in OAJ3 is higher. This is required for blood formation and normal functioning of the central nervous system. Vitamin C is higher in OAJ3 than other samples. It is a fat-soluble vitamin that serves as a good antioxidant, for healthy vision, skins and other tissues in the body (Onojah et al., 2018).

OOW4, OOJ6 and OOW1 had least mineral composition which could be due to the high microbial load, presence of enteric bacteria and toxin producing microorganisms which made it unfit for consumption (Ezekiel et al., 2016).

The proximate composition of *I. gabonensis* Kernels.

Most of the *I. gabonensis* Kernels samples with high microbial load were observed to have high moisture content. The carbohydrates and moisture content ranged from 15.3% to 30.0% and 4.5 to 5.5%. Sample OAJ3

Table 4. Colonial and morphological characteristics features of fungi isolated from *Irvingia gabonensis* Kernels

Isolate	Macroscopy	Microscopy	Probable organism
OOW1	Colonies appears greenish-yellow with white-like borders	Septate hypha with long conidiophores arranged in clusters supporting the phialides. The conidiophores have rough texture and spiny bellow the vesicle.	*Aspergillus flavus*
OOW2	Small to medium colony, round, raised, smooth and colony colour is white to cream	Oval cell shape, multilateral germination pattern, reproduces by budding.	Yeast
OOW3	Velvety and flaky surface due to marked sporulation with grey to green coloration.	Septate hyphae with borne laterally conidiophores and conidia borne in the chain on sterigma. Conidiophores smooth-walled.	*Aspergillus niger*
OOW4	Colonies appears greenish-yellow with white-like borders	Septate hypha with long conidiophores arranged in clusters supporting the phialides. The conidiophores have rough texture and spiny bellow the vesicle.	*Aspergillus flavus*
OOW5	Velvety and flaky surface due to marked sporulation with grey to green coloration.	Septate hyphae with borne laterally conidiophores and conidia borne in the chain on sterigma. Conidial heads are large, globose and dark brown with smooth-walled conidiophores.	*Aspergillus niger*
OOW6	Colonies appear granular, wool-like and yellow to brown	Conidia are rough, smooth or slightly rough and form long chains, conidiospores are long	*Penicillum* sp.
OAJ1	Colonies appear granular, White to yellow fluffy or wool-like.	Conidia are rough, smooth and form long chains, septate hyphae with simple conidiospores.	*Penicillum* sp
OAJ2	Velvety and flaky surface due to marked sporulation with grey to green coloration.	Septate hyphae with borne laterally conidiophores and conidia borne in the chain on sterigma. Conidial heads are large, globose and dark brown with smooth-walled conidiophores.	*Aspergillus niger*
OAJ4	Colonies appear granular, White to yellow fluffy or wool-like.	Conidia are rough, smooth and form long chains, septate hyphae with simple conidiospores.	*Penicillum* sp
OAJ5	Small to medium colony, round, raised, smooth and colony colour is white to cream	Oval cell shape, multilateral germination pattern, reproduces by budding.	Yeast
OAK2	Small to medium colony, round, raised, smooth and colony colour is white to cream	Oval cell shape, multilateral germination pattern, reproduces by budding.	Yeast
OAK3	Velvety and flaky surface due to marked sporulation with grey to green coloration.	Septate hyphae with borne laterally conidiophores and conidia borne in the chain on sterigma. Conidial heads are large, globose and dark brown with smooth-walled conidiophores.	*Aspergillus niger*
OAK4	Velvety and flaky surface due to marked sporulation with grey to green coloration.	Septate hyphae with borne laterally conidiophores and conidia borne in the chain on sterigma. Conidial heads are large, globose and dark brown with smooth-walled conidiophores.	*Aspergillus niger*
OSB2	Velvety and flaky surface due to marked sporulation with grey to green coloration.	Septate hyphae with borne laterally conidiophores and conidia borne in the chain on sterigma. Conidial heads are large, globose and dark brown with smooth-walled conidiophores.	*Aspergillus niger*

Table 4. Contd.

OSB6	Colonies appear granular, White to yellow fluffy or wool-like.	Conidia are rough, smooth and form long chains, septate hyphae with simple conidiospores.	*Penicillum* sp.
OOJ1	Colonies appear granular, White to yellow fluffy or wool-like.	Conidia are rough, smooth and form long chains, septate hyphae with simple conidiospores.	*Penicillum* sp.
OOJ3	Small to medium colony, round, raised, smooth and colony colour is white to cream	Oval cell shape, multilateral germination pattern, reproduces by budding.	Yeast
OOJ6	Colonies appears greenish-yellow with white-like borders	Septate hypha with long conidiophores arranged in clusters supporting the phialides. The conidiophores have rough texture and spiny bellow the vesicle.	*Aspergillus flavus*

Source: Author

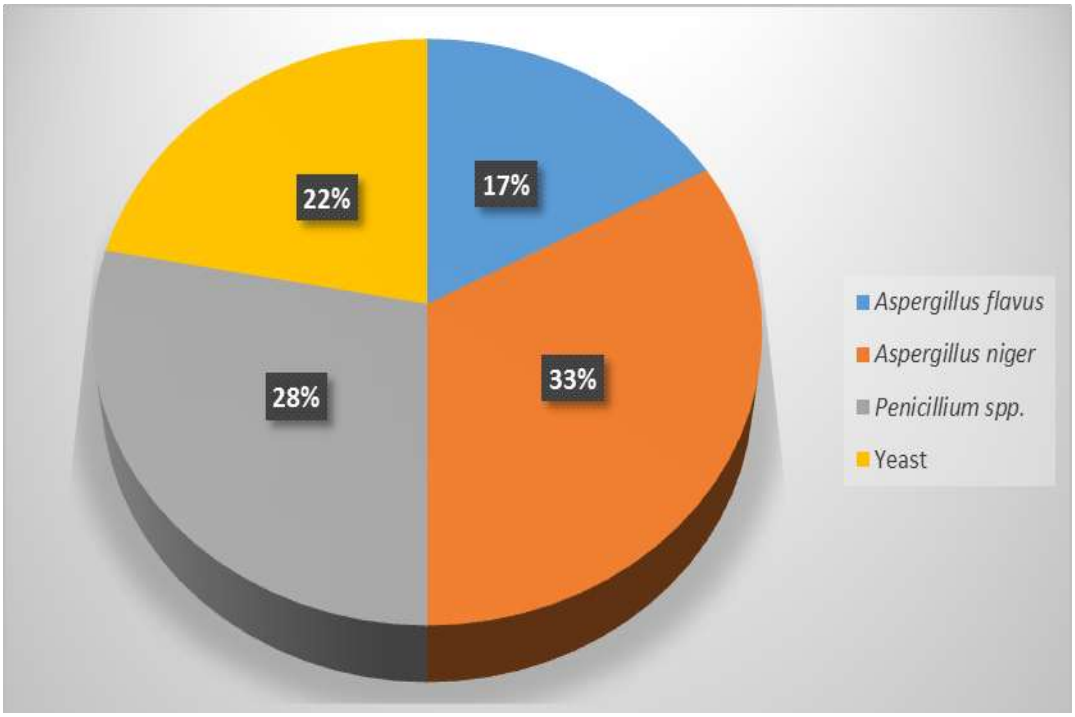

Figure 2. Percentage frequency of Occurrence of Fungi isolated from *Irvingia gabonensis* Kernels
Source: Author

which is the control has the high moisture content compare to OOJ6 and OOW1 which could be the reason why the microbial load is higher. The moisture content is however within the range value of most seeds and contamination.

The crude fibre percentage ranged from 10.7 to 9.1%. Sample OAJ3 had the highest crude fibre while OOW had the least, these results are similar to the report of legumes (Onojah et al., 2018). This is in line with the research of Brooker (2005) who suggested that high moisture content in fruits is an index of its water activity, measure of stability and susceptibility to microbial Onojah et al. (2018) who recorded 10.4% crude fibres from Ogbono samples. It is however lower to the result of Aremu et al. (2005) were 15.2% was reported from Bambara groundnut. The intake of dietary fibre can lower

Table 5. Mineral composition of *Irvingia gabonensis* Kernels.

Parameter	OAJ3	OOW4	OOJ6	OOW1
Sodium %	7.5	6.0	6.2	5.4
Vitamin C mg/kg	28.2	23.5	22.4	20.0
Potassium	87.4	78.1	72.5	53.6
Calcium mg/kg	37	33.4	30.2	29.4
Iron mg/kg	13.4	13.3	11.4	09.1
Magnesium mg/100g	49.9	41.7	40.8	34.4
Zinc mg/100g	0.03	0.03	0.02	0.05
Heavy metal (pb, As) ppm	NIL	NIL	NIL	NIL

Source: Author

Table 6. Proximate analysis of the ogbono sample.

Proximate composition	OAJ3	OOW4	OOJ6	OOW1
%Carbohydrate content	16.3±0.55	20.5±0.44	22.6±0.16	34.0±0.58
%Moisture content	4.5±0.51	5.2±0.15	4.8±0.10	5.5±0.25
%Crude protein	7.8±0.43	6.9±0.46	5.4±0.56	4.2±0.47
%Crude fat	58.9±0.55	55.3±0.37	55.9±0.25	45.7±0.184
%Crude fibre	10.7±0.26	10.4±0.22	9.8±0.39	9.1±0.10
%Total ash	1.8±0.61	1.7±0.14	1.5±0.13	1.5±0.52
Fatty acids (kg/100 g)	47.1±0.36	44.2±0.09	44.7±0.66	36.6±0.05
Metabolized energy (kg/100 g)	2589.0±0.38	2511.9±0.49	2544.3±0.42	2340.3±0.50

Source: Author

Table 7. Presence of aflatoxin in the *Irvingia gabonensis* Kernels.

Proximate composition	Level of aflatoxin (ppb)
OAJ3	1.05±0.64
OOW4	3.47±0.21
OOJ6	3.69±0.28
OOW1	5.10±0.07

Source: Author

cholesterol level, risk of coronary heart disease, diabetes and hypertension (Ramola and Raw, 2003).

The total ash content of *I. gabonensis* Kernels ranged from 1.5 to 1.8%. Sample OAJ3 was higher than OOW4, OOJ6 and OOW1. The result obtained is related to the work of Efosa et al. (2017). Ash content in food is the inorganic residue left after the removal of moisture and organic matter. It provides the measure of the total amount of minerals within a food. Crude fiber contains indigestible cellulose which helps to absorb water, provide roughage and better functioning of the alimentary system.

The crude fat content of I*. gabonensis* Kernels ranged from 58.9 to 45.7%. The fat in sample OAJ3 was higher than that of OOW1. This could be due to the infestation of microorganisms in the Kernels. The high value of the crude fat in OOJ3 suggests that the Kernels may be a source of vegetable oil for industrial uses.

The crude protein in *I. gabonensis* Kernels samples ranged from 4.2 to 7.8%. The crude protein value for Sample OAJ3 is higher than the ogbono samples obtained from OOW4, OOJ6 and OOW1. The crude protein value of OOW1 is low compared to some commonly consumed plant protein in Nigeria and this does not qualify the seed as a protein rich food. The lowvalue obtained could also be as a result of the long storage period before been purchased for this research (Onojah et al., 2018). Protein contents contribute positively to the requirement for biomolecules needed for repair and maintenance of the body tissues as well as

synthesis of vital hormones for the body (Soetan et al., 2010).

The calculated fatty acid value for *I. gabonensis* Kernels ranged from 47.1 to 36.6 kg/100g. The results suggest that oil gotten from samples OAJ3, OOW4 and OOJ6 are suitable as edible oil and can be used for industrial purposes. Sample OOW1 had lower fatty acid value due to the presence of some pathogenic microorganisms in the Kernels.

The calculated metabolized energy ranged from 2589.0 to 2340.3 kg/100g which shows that the sample have good concentration of energy.

The detection of aflatoxins in the *I. gabonensis* Kernels reveals the production of toxins by *A. flavus*. Aspergillus species such as *A. flavus* and *A. parasiticus, these doubles* as the most notorious fungi commonly isolated from *I. gabonensis* seed due to their high potential for producing aflatoxins (Osibona et al., 2018). Several factors such as moisture contents, high relative humidity, temperature, substrate composition and the presence of competing microorganisms influenced mold growth on the seeds (Adebayo-Tayo et al., 2006). The environmental conditions in some part of Nigeria favours the growth of fungi and aflatoxin production in foods. Some measures of precautions should be taken when handling and processing dry foods. The growth of molds on *I. gabonensis* seeds is a pointer to the potential health risk associated with its consumption (Osibona et al., 2018). In Nigeria, several foods including nuts, cereals, dry fish, spices, and melon seeds among other food substances, are susceptible to contamination with aflatoxins due to the critical conditions such as temperature and humidity which is known to favour the growth of aflatoxin-producing molds (Ubwa et al., 2014, Chigoziri and Ekefan, 2013).

Conclusion

This study revealed different microorganisms associated with *I. gabonensis* Kernels sold in different markets within Oyo Town, Oyo State, Nigeria. Microorganisms isolated include *Bacillus cereus, Bacillus subtillis, Staphylococcus* spp. *Staphylococcus* spp., Enterobacteriaceae, *Aspergillus flavus, Aspergillus niger, Penicillium* spp and yeast. The *I. gabonensis* seeds with the growth of *A. flavus* had low mineral composition and proximate value. Although, the total aflatoxin levels of the samples analysed were below the maximum acceptable limits specified by International Regulatory Agencies in food and agricultural products (20 ppb), frequent and prolonged intake of the *I. gabonensis* Kernels could result in health hazards and reduced the economic value of the food. This study showed that some of the *I. gabonensis* Kernels samples used had been stored for a longer period. Inadequate elimination of moisture and exposure to dirty environment (markets), made them lose some of their nutrients as well as minerals.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

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