

Food Value, Fungi and Aflatoxin Detection in Stored 'Orunla' *Abelmoschus esculentus* L. (Moench) from Ibadan, Nigeria

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ABSTRACT:

Five samples of 'orunla' (sliced dried okra fruits) were purchased from selected markets within Ibadan city, Nigeria. These were wrapped in cellophane paper and stored for 4 months. Laboratory made 'orunla' samples which acts as control were also prepared. Mycoorganisms were isolated from fresh and stored samples. Effect of storage time on proximate and mineral element contents (Fe, Mg, and P) were also determined. Both fresh and stored 'orunla' samples were also screened for possible aflatoxin contamination. The results of the work showed that, biodeteriorating fungi isolated from the market samples and their percentage occurrences include *Aspergillus flavus* (16.88%), *A. niger* (16.23%), *A. fumigatus* (16.23%) and *Rhizopus nigricans* (16.23%) while *Mucor mucedo* (5%), *Candida albicans* (2%) and *Trichoderma* sp. (2%) had the least percentage occurrences. The moisture contents of the samples range from (9.80% to 16.36%), protein (11.27% to 18.74%), ether extract (1.37% to 2.11%), crude fiber (38.18 % 41.27%), ash (11.70% to 12.55%), carbohydrates (31.15% to 31.58%) and dry matter (45.07% to 45.22%). Storage time had significant effect on the nutrient and aflatoxin content of all the samples ($P > 0.05$). Mg and P values were not significantly different from each other except the control. Also, the storage time had effect on the Aflatoxin content in market samples as their aflatoxin content varied and increased with the time of storage. Highest concentration of aflatoxin was detected in Ojaoba samples (10.20 μ /Kg AFB1, 7.30 μ /Kg AFB2, 5.7 μ /Kg AFG1 and 3.22 μ /Kg AFG2), Moniya (8.50 μ /Kg AFB1, 6.3 μ /Kg AFB2, 4.9 μ /Kg AFG1 and 3.17 μ /Kg AFG2) and Apete (9.7 μ /Kg AFB1, 4.4 μ /Kg AFB2, 3.8 μ /Kg AFG1 and 2.9 μ /Kg AFG2) samples while the least aflatoxin was detected in the control (0.0 μ /Kg AFB1, AFG1 AFG2 and 0.13 μ /Kg AFB2) and Ojoo (2.50 μ /Kg AFB1, 2.10 μ /Kg AFB2, 2.1 μ /Kg AFG1 and 1.90 μ /Kg AFG2) after four month of storage. The implication of these results were discussed.

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1.0. Introduction

Okra (*Abelmoschus esculentus* L. Moench) is a flowering plant also known as ladies' finger in some European countries. In Nigeria, it is known as "Ila" in "Yoruba", "Kubewa" in "Hausa" and "Okwale" in "Igbo" tribes (Tindall, 1983; Jonathan *et al.*, 2011a). Okra is a prominent fruit and leafy vegetable grown for domestic consumption with highly nutritious immature leaves and fruits in Nigeria (Farinde *et al.*, 2007, Jonathan *et al.*, 2014). Whole, fresh okra pods also make excellent pickles, it is an annual or perennial plant to about two meters; with origin from Africa. It is cultivated in the tropical, sub-tropical and warm temperate regions around the world. The fruit becomes

fibrous and not suitable for consumption when fully matures (Conway *et al.* 2003; Jonathan *et al.*, 2011a). It is one of the most important vegetable crops in Nigeria, contributing substantially to the Nigerian diet as a main constituent of soup (Hell *et al.*, 2009). Okra is one of the prominent fruit and leafy vegetable grown for domestic consumption in Nigeria. Gakou *et al.* (1994) and Jonathan *et al.*, (2011a) reported that okra consumption provide a valuable contribution to the nutrition of rural and urban populations in West Africa, greenish yellow edible okra oil in the seed is rich in unsaturated fats such as oleic acid and linoleic acid with the content of about 40%. 'Orunla' is commonly used by Yorubas in Western Nigeria and

enjoyed as a good culinary soup ingredient. It is prepared by sun drying (dehydration) of the sliced okra fruits and thereafter grinded into powder, this method is adopted as a preservative method for keeping the fruit for use during the dry season when vegetables are expensive.

However, Okra is a perishable commodity which can deteriorate and this can cause reduction in its quality and quantity if not properly handled during storage processes. According to Gakou *et al.* (1994), despite the wide acceptability and consumption of this dried, powdered okra, little is known regarding the spoilage fungi, their toxigenic properties and nutritional contents. There is scarcity of fresh okra during the dry season as a result of poor storage and preserved method. Okra is a perishable commodity which deteriorates and this can cause reduction in its quality and quantity during handling and storage processes. Traditionally, the fresh okra chips are indiscriminately sun-dried on roof tops, concrete constructions, and along roadsides for some weeks depending on the intensity of sunlight. This unhygienic act inevitably poses the risk of exposing the commodity to direct contamination or indirectly from dust, flies, rodents and even human handlers, which as a result, produce condition for fungal growth. Fungi are the major contaminants of foods in the world and it has been reported that some fungi mostly moulds can produce toxic secondary metabolite under unfavorable environmental conditions (Jonathan and Esho, 2010; Jonathan *et al.*, 2011b).

Mycotoxins, for example Aflatoxin contamination of food have been reported and documented to be the cause of different types of abortion and cancer both in human beings and animals worldwide (Shepherd *et al.*, 2006; Bhat and Miller, 2010; Jonathan *et al.*, 2012). Okra is usually available in large quantities during rainy season and scarce during dry season, there is need to preserve them, therefore there is a need to give important considerations to this food safety and fungal contamination. The aim of this work therefore is to study the nutritional content, fungal and aflatoxin contamination of fresh and stored samples of dried okra ('orunla') samples collected from different markets in Western Nigeria and compare with the standard tolerable limit reported by the International Agency for Research on Cancer (IARC), 2002.

2.0 Materials And Methods

2.1. Sources and Collection of 'Orunla' Samples

There were two kinds of 'orunla' used for this study, the sundried samples collected from five (5) different markets in Ibadan Nigeria and the oven-dried samples prepared and stored in the laboratory. The markets used sample collection are:

- Bodija at Ibadan North Local Government; Apete at Iddo Local Government.
- Oja-Oba at Ibadan South-East Local Government;
- Ojoo and Moniya Markets both located at Akinyele Local Government, Oyo State.

The samples were separately packed in sterile paper bags, and put in an air tight container until the time of fungal isolation, while a freshly harvested okra sample was prepared into 'orunla' in the laboratory and this served as control. All samples were then brought to Mycology/pathology laboratory, Department of Botany and Microbiology, University of Ibadan for analysis.

2.2. Preparation of Laboratory Sample

The laboratory sample was prepared using oven to dry the sliced fresh okra and then stored for four week in an aseptic insect free container, labeled and kept in the laboratory.

2.3. Isolation of deteriorating Fungi

Two methods were adopted for this purpose:

Direct plating: Ten (10g) of 'Orunla' were examined randomly for their mycoorganism contents. They were surface sterilized with 70% ethanol. With the aid of dissecting forceps, the surface of the stored sun dried okra were scrapped, plated aseptically on Potato dextrose agar (PDA) prepared according to manufacturer's prescription, plated and incubated at $30\pm 2^\circ\text{C}$ at 1.06atm pressure for 5 to 7 days according to the procedures described by Jonathan and Olowofale, (2001). The cultures were examined under microscope for fruiting bodies and hyphae to determine the presence of fungi and pictures taken using a photomicrograph.

Dilution plate method: Ten (10g) of the samples were surface sterilized with 70% ethanol was soaked in 10ml of sterile distilled water (Ayaleen *et al.*, 2006; Jonathan *et al.*, 2015). This was shaken thoroughly, after which 1ml of suspension was pipetted into a sterile test tube containing 9ml of distilled water, and thoroughly mixed together for serial dilution to be achieved; 1ml of aliquots of 10^{-5} and 10^{-6} was added to molten PDA plates. The plates were swirled gently to obtain uniform mixture, and allowed to solidify before incubating at $30\pm 2^\circ\text{C}$ for 5 to 7 days. The fungal colonies were observed every 24hours. Successive hyphae tip were transferred until pure cultures of each of the fungus was obtained.

2.4. Identification of Isolated Fungi

Fungal colonies emerging from symptomatic tissue was picked and transferred to new plates and left to grow for 5 to 7 days after incubation. Pure cultures were then obtained by single spore isolation and maintained on PDA slants for further study. The fungi

were observed under a microscope and identification of the pathogens was carried out using the procedures of Adetunji *et al.*, 2008; Mutegi *et al.*, 2009 and Ngedu, 2009.

2.5. Proximate Analysis

The proximate analysis of both fresh and stored dried 'orunla' samples were carried out at KAPPA Biotechnology laboratory, Ibadan, Oyo State. The parameters determined were; moisture content, crude fibre, crude fat, crude protein and total ash using the method described by AOAC (2005). Also, the mineral content of the samples were carried out using AOAC method (2005).

- The percentage nitrogen in this analysis was calculated using the formula:

$$\% N = \text{Average Titer Value} \times \text{Atomic mass of N} \times \text{Normality of HCl}_2 \text{ used} \times 4$$

$$\text{Or } \% N = \text{Titre Value} \times \text{Normality/Molarity of HCl used} \times \text{Atomic mass of N} \times \text{Volume of flask}$$

$$\text{Crude protein content} = \% N \times 6.25$$

(i. % CP = % N x 6.25)

Where 6.25 is a constant multiplying factor.

- For fat determination, if the initial weight of dry soxhlet flask is W_0 and the final weight of oven dried flask and fat is W_1 , the percentage fat is obtained by the formula:

$$\% \text{ Fat} = \frac{W_1 - W_0}{W_1} \times 100$$

Where W_1 is the final weight of flask + fat
 W_0 is the initial weight of the soxhlet flask.

- Dry Matter and Moisture contents were determined by

$$\% \text{ Moisture} = \frac{W_3 - W_0}{W_1 - W_0} \times 100\%$$

Where: W_0 = Weight of empty crucible
 W_1 = Weight of crucible + sample
 W_3 = Weight of crucible + sample after oven drying.

- The percentage ash was calculated from the formula:

$$\text{Ash Content} = \frac{\text{Weight of Ash}}{\text{Original weight of the sample}} \times 100\%$$

- % Fibre = $\frac{\text{Difference in Weighing}}{\text{Weight of sample}} \times 100\%$

- % Carbohydrate = 100-(%moisture + % crude protein + % crude fat + % ash).

2.6. Determination of Mineral Elements

Powder sample (0.5g) of 'orunla' was mixed with Nitric and perchloric Acid (2.1) and digested for 1 hour until the colour changes to colorless. The digest was made to 25ml with distilled water, and read on atomic absorption spectrophotometer to determine mineral elements.

$$\frac{Y \times 25\text{ml} \times \text{dilution factor}}{5000}$$

2.7. Aflatoxin Analysis

The aflatoxin analysis was carried out in pathology laboratory of International Institute of Tropical Agriculture (IITA), Ibadan. Determination of Aflatoxin B₁, B₂, G₁ levels in the samples were carried out using HPTLC method.

2.7.1. Extraction procedures

Fifty gram of each samples were defatted by extraction with N-hexenesoxhlet-type extractor. The defatted residue was extracted with ethyl acetate (three times, 50ml/each). The extracts were combined, dried over anhydrous sodium sulphate, filtered and then concentrated under vacuum to near dryness, transferred into brown glass vial and evaporated under nitrogen stream. For cleaning up the crude extracts; the crude extract was suspended in 1ml chloroform and applied to 14x0.8cm column containing 2.5 Kiesel gel 60, 70/230 silica gel.

2.7.2. Thin Layer Chromatography (TLC)

For quantitative detection of mycotoxins, thin layer chromatography technique was employed using precoated silica gel plates type 60 F₂₅₄ TLC. Aflatoxin B₁ (Sigma) was used as standard reference. The developing solvent system was ethyl acetate-hexane (v/v, 30:70) and the developed plates were viewed under short wave length UV (252nm) light according to AOAC (2005) and Dorner (1998).

2.7.3. High Performance Liquid chromatography (HPLTC)

HPLTC analysis for aflatoxin detection in the sample was done using Spherisorb 5_{sil} column (250 x 5.6 mm). mobile phase was chloroform-methanol (v/v, 97:3) with flow rate 1.2 mL min⁻¹ for 20 min. the quantitative determination of mycotoxins was carried out compare with standard aflatoxin B₁ (Sigma). Filtration: The samples were filtered into another conical flask using Whatmann filter paper No 1. (Felder *et al.*, 2003; Peroma *et al.*, 2006).

2.7.4. Partition Chromatography

The filtrates were poured into separating funnels held in place by retort stand. The sodium chloride acts as catalyst by speeding up the rate of reaction. (Catalyst) while N-hexane extracted the oil from the sample. As the NaCl₂ solution was poured into the

filtrate, the colour changed because the toxin is not polar and the water forms bond with the polar substances present in the filtrate. As N-hexane was added, it floats due to its lower molecular weight and oil was seen at the top. It was shaken vigorously and allowed to settle for 5 minutes before dispensing the filtrate into conical flask thereby, leaving behind the N-hexane and oil. It was poured back into the separating funnel before dissolving some of the toxins with 25ml of dichloromethane. Dichloromethane acted as the mobile phase, while the filtrate was the stationary phase. The containers containing the dichloromethane and the toxins were taken into the fume hood for evaporation of the dichloromethane. After the dichloromethane has dried off leaving behind the toxins, 1.5ml of dichloromethane was used in dissolving the toxins into Eppendorf tubes. There is no specific amount of dichloromethane but the container containing the toxin must be washed properly and as the tubes were filled, it would stop. They were left for 24 hours in the fume hood.

2.7.5. Calibration

1.5cm was measured along the length of 10 by 20cm HPTLC plate and another 1.5cm was measured towards the width on both sides forming 2 squares on both ends. The sixth spot from the left represent the B standard (high standard) while the eight spot was the I standard (low standard). The plate was calibrated in 18 places in which 2 were for standards and 16 for the unknown samples.

2.7.6. Spotting

The coated and calibrated HPLTC plate with silica gel (stationary phase) was spotted using a

capillary tube of 4ml on each of the spot after mixing with vortex mixer. It was noted that on the B spot, the spitting was done twice with 8ml, while 4ml was spotted once for I.

2.7.7. Development

The spotted HPTLC plate was developed inside a developing chamber containing diethylether (96%), methanol (30%) and distilled water (10%) known as developing solution. After pouring the developing solution inside the developing chamber, the solution was allowed to saturate for about 5 minutes before dropping the HPTLC plate vertically by the side of the chamber. The chamber was covered and left unshaken so as not to alter the mode of travelling of the toxins on the HPTLC plate and preventing the overlap of wavelengths. The plate was removed from the chamber as the mode of travelling reached the maximum level, and was left in the fume hood for the evaporation of the solution to dryness. The Mycotoxins were detected using the use of high performance scanner (Abolude and Ojediran, 2006).

2.8. Statistical Analysis

The experiment was set up in a complete randomized design. A generalized linear model option of the analysis system Minitab version 17 was used to perform the ANOVA and Duncan's Multiple Range Test (DMRT) at $P < 0.05$ level was used for mean separation.

3.0 Results

3.1. Isolated Bio-deteriorating fungi

Table 1: Incidence of fungi species associated with orunla samples obtained from different markets in Ibadan.

| Fungi Isolates | Moniya | Apete | Bodija | Oja Oba | Ojoo | Control | Frequency of Occurrence | %Total Occurrence |
|--------------------------------|-----------|-----------|-----------|------------|-----------|-----------|----------------------------|----------------------|
| <i>Aspergillus flavus</i> | 5 | 5 | 4 | 5 | 5 | 2 | 26 | 16.88 |
| <i>A. fumigates</i> | 5 | 4 | 3 | 5 | 5 | 3 | 25 | 16.23 |
| <i>A. niger</i> | 4 | 4 | 5 | 5 | 5 | 2 | 25 | 16.23 |
| <i>A. tamari</i> | 1 | 0 | 1 | 0 | 2 | 0 | 4 | 2.60 |
| <i>Fusarium oxysporum</i> | 3 | 0 | 2 | 2 | 0 | 0 | 7 | 4.55 |
| <i>Candida albicans</i> | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 1.30 |
| <i>Drechsleraglycines</i> | 0 | 2 | 1 | 3 | 0 | 1 | 7 | 4.55 |
| <i>Penicillium notatum</i> | 1 | 2 | 1 | 2 | 0 | 1 | 7 | 4.55 |
| <i>Penicillium chrysogenum</i> | 1 | 2 | 1 | 2 | 1 | 0 | 7 | 4.55 |
| <i>Muco rmuucedo</i> | 2 | 2 | 1 | 0 | 0 | 0 | 5 | 3.24 |
| <i>Mucor hiemalis</i> | 1 | 1 | 1 | 2 | 1 | 0 | 6 | 3.40 |
| <i>Neurospora crassa</i> | 2 | 1 | 1 | 1 | 1 | 0 | 6 | 3.40 |
| <i>Rhizopus nigrican</i> | 5 | 5 | 5 | 4 | 5 | 1 | 25 | 16.23 |
| <i>Trichoderma</i> sp. | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 1.30 |
| Total fungi | 31 | 28 | 27 | 32 | 26 | 10 | 154 | 100 |

Means with the same letter (s) in the same rows are not significantly different at $P \geq 0.05$. Occurrence rated on each five samples from the same location and compared to other location as total and percentage

Data on fungi occurrence on five samples from each market was presented in Table 1. From this table,

Ojaoba, Moniya and Apete recorded highest number of fungal species of 32, 31 and 28 respectively while

the control, Ojoo, and Bodija have least occurrence; (10, 26 and 27 respectively). However, the most frequent fungal isolates (percentage total occurrence) from the samples were the *Aspergillus species*; *Aspergillus flavus* (16.88%), *Aspergillus niger* (16.23%), *A. fumigatus* (16.23%) and *Rhizopus*

nigricans (16.23%) while *Mucor mucedo* (5%), *Candida albican* 2%) and *Trichoderma* sp. (2%) occur the least. The morphological and microscopic views of the most occurring fungi are presented in Plates 1 – 4 with their microscopic views respectively).

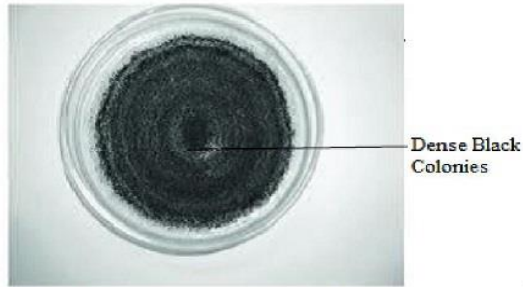


Plate 1a: *Aspergillus niger* (x0.9).

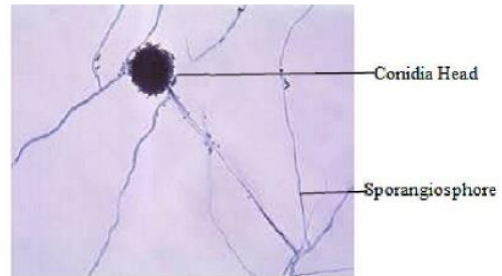


Plate 1b: Micromorphology of *Aspergillus niger* (x40).

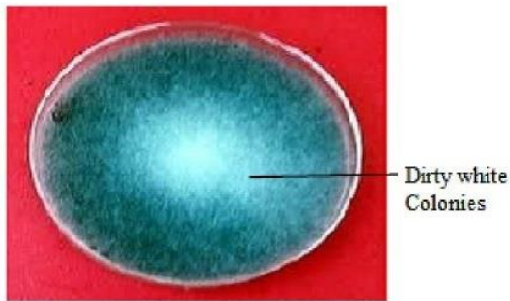


Plate 2a: *Rhizopus nigricans*

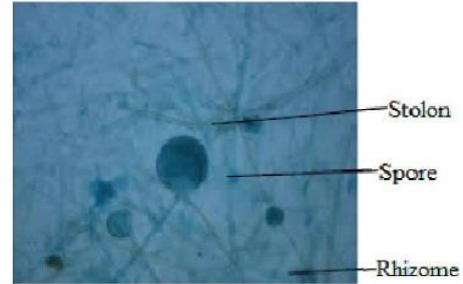


Plate 2b: Micromorphology of *Rhizopus nigricans* (x40).

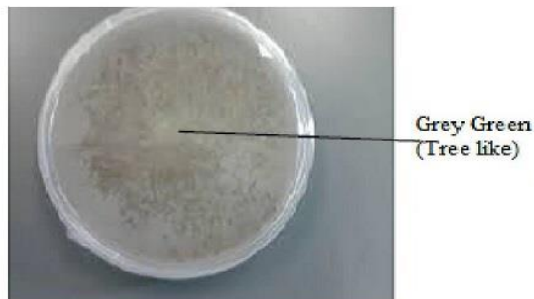


Plate 3a: *Mucor mucedo* (x0.9).

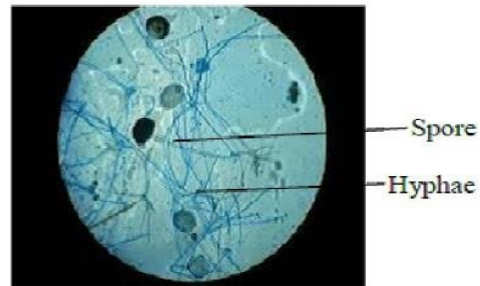


Plate 3b: Micromorphology of *Mucor mucedo* (x40).

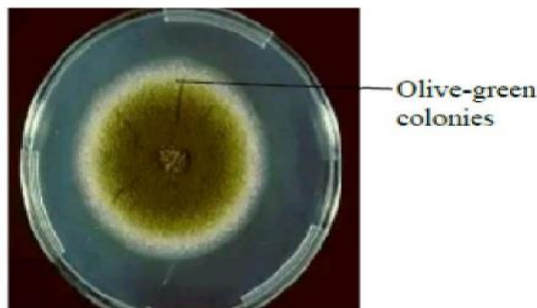


Plate 4a: *Aspergillus flavus* (x0.9).

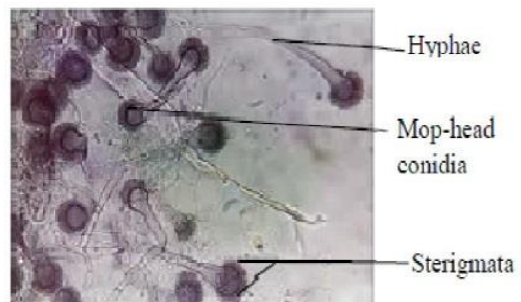


Plate 4b: Micromorphology of *Aspergillus flavus* (x40).

3.2. Result on Aflatoxin Contents in the Samples

The result of this study showed that storage period increase the aflatoxin level as the values recorded for four months are higher than the one months across all the samples (Table 2). Moreover, it was discovered that aflatoxin B1 (AFB1) content of the samples are

generally higher than other aflatoxins and this may be due to highest occurrence of *Aspergillus* fungi observed among the isolated deteriorating fungi as shown in table 1 above. However, highest aflatoxin level AFB1 was recorded in Ojaoba market samples at 10.20.

Table 2: The mean occurrence of aflatoxins in different ‘Orunla’ samples as affected by storage time

| Market | AFBI(μ /Kg) | | AFB2(μ /Kg) | | AFG1(μ /Kg) | | AFG2(μ /Kg) | |
|---------|------------------|--------|------------------|--------|------------------|--------|------------------|--------|
| | 1month | 4month | 1month | 4month | 1month | 4month | 1month | 4month |
| Moniya | 6.51a | 8.50b | 2.30b | 6.30b | 0.30a | 4.90b | 0.31a | 3.17a |
| Apete | 4.25b | 9.70a | 3.40a | 4.40c | 0.12c | 3.80c | 0.29a | 2.90b |
| Bodija | 2.50c | 3.50d | 1.20c | 3.20d | 0.08d | 3.00c | 0.25a | 2.70b |
| Oja Oba | 5.91a | 10.20a | 3.40a | 7.30a | 0.40b | 5.70a | 0.34a | 3.22a |
| Ojoo | 2.15c | 6.40c | 1.30c | 2.10e | 0.09d | 2.10d | 0.23a | 1.92c |
| Control | 0.00d | 0.00e | 0.08d | 0.13f | 0.01d | 0.00e | 0.00b | 0.00d |

*Means with different alphabets in a column are significantly different ($p < 0.05$) by the Duncan’s multiple Range test

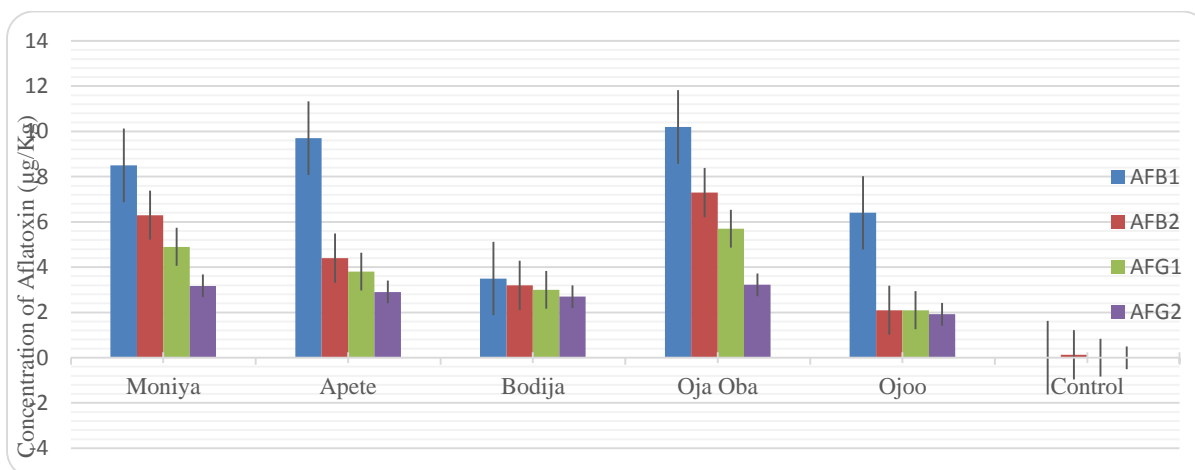


Figure 1: Levels of Aflatoxin contamination on Orunla samples in different markets with Standard Errors (SE)

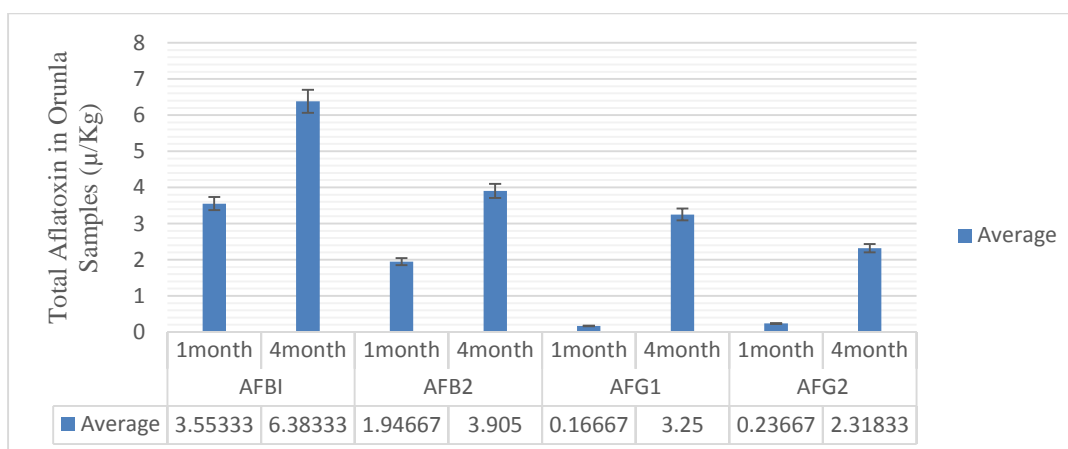


Figure 2: Effect of storage level on total aflatoxin contents of Orunla samples of different markets in Ibadan Nigeria with Standard Error (SE)

3.3. Effect of Storage period and Location on nutritional contents of 'Orunla' okra

The result from Table 1 shows that the location of sample produced highly significant ($P<0.01$) effect on moisture content, protein, and ether extract contents of 'orunla', but significant for ash, while the crude fibre, carbohydrate and dry matter were not significantly different. On the other hand, crude fibre, ash, moisture content and ether compositions of "Orunla" produced highly significant effect for the

period of storage, while carbohydrate, dry matter and protein contents were significant ($P<0.05$).

3.4. The nutritional compositions of Okra as affected by locations

The ash content of dried okra collected from Ojaoba had the highest value of 16.41, while control (freshly) was the least (Table 2). The moisture contents of control was significantly ($P<0.01$) higher than other locations, but Ojoo, Ojaoba and Bodija were not different from one another.

Table 3: Effect of location on nutritional content of okra during the first month (g/100g)

| Location | Moisture content | Protein | Ether extract | Crude Fibre | Ash | Carbohydrate | Dry matter |
|----------|--------------------|--------------------|-------------------|--------------------|--------------------|--------------------|--------------------|
| Ojoo | 9.80 ^d | 11.27 ^d | 2.11 ^a | 41.27 ^a | 12.55 ^b | 31.58 ^a | 45.22 ^a |
| Ojaoba | 10.03 ^d | 16.36 ^b | 1.46 ^b | 43.06 ^a | 16.41 ^a | 35.37 ^a | 0.00 ^a |
| Bodija | 10.13 ^d | 11.92 ^d | 1.51 ^b | 38.97 ^a | 10.73 ^b | 31.15 ^a | 45.07 ^a |
| Apete | 13.21 ^c | 14.33 ^c | 0.95 ^c | 38.57 ^a | 12.45 ^b | 36.42 ^a | 0.00 ^a |
| Moniya | 16.36 ^b | 18.74 ^a | 1.37 ^b | 38.18 ^a | 11.70 ^b | 31.15 ^a | 0.00 ^a |
| Control | 89.47 ^a | 2.56 ^e | 0.27 ^d | 0.95 ^b | 0.93 ^c | 6.76 ^b | 0.00 ^a |

The means with the same letter are not significantly ($P>0.05$) different from each other across the column

3.5. Effect of location on nutritional content of okra during the fourth month

The moisture content of fresh okra is significantly ($P<0.05$) higher than the sundried from other locations. The sun dried from Moniya is significantly

higher and different from the fresh sample as well as dried from other locations. This is followed by Ojaoba and Apete which were not different from each other.

Table 4: Effect of location on nutritional content of okra during the fourth month

| Location | Moisture content | Crude protein | Crude fat | Crude fibre | Ash | Carbohydrate |
|----------|--------------------|--------------------|-------------------|--------------------|--------------------|--------------------|
| Ojoo | 10.03 ^f | 11.27 ^d | 1.93 ^a | 17.23 ^b | 11.46 ^a | 65.31 ^a |
| Bodija | 10.40 ^e | 13.10 ^c | 0.88 ^c | 13.18 ^d | 9.22 ^c | 62.00 ^a |
| Ojaoba | 10.65 ^d | 14.81 ^b | 1.13 ^b | 22.70 ^a | 10.02 ^b | 63.38 ^a |
| Apete | 14.2 ^c | 14.74 ^b | 0.79 ^d | 15.86 ^c | 9.11 ^d | 61.16 ^a |
| Moniya | 18.36 ^b | 17.94 ^a | 0.88 ^c | 13.18 ^d | 9.22 ^c | 53.65 ^b |
| Control | 89.47 ^a | 2.56 ^e | 0.27 ^e | 0.95 ^e | 0.93 ^e | 6.76 ^c |

The means with the same letter at superscript are not significantly ($P<0.05$) different from each other across the column

3.6. Effect of storage periods on nutritional contents of okra

Moisture content of fresh sample was significantly ($P<0.05$) higher than the dried samples in the first and fourth months of storage. The protein of the dried for the first and fourth months of storage were significantly higher than fresh samples, though not significantly different from each other. The ether

of the dried sample was significantly different from the fresh but the ether differs for the first and fourth month. The moisture content, ether extract, crude fibre and ash of the dried in the (first and fourth months) of storage and fresh were significantly different ($P<0.05$) from one another.

Table 5: Effect of storage periods on nutritional content of okra

| Month | Moisture content | Protein | Ether extract | Crude Fibre | Ash | Carbohydrate | Dry matter |
|-------------------------|--------------------|--------------------|-------------------|--------------------|--------------------|--------------------|--------------------|
| First Month of Storage | 11.08 ^c | 14.67 ^a | 1.84 ^a | 18.92 ^b | 9.10 ^b | 56.65 ^a | 36.11 ^a |
| Fourth Month of Storage | 12.73 ^b | 14.37 ^a | 1.12 ^b | 61.10 ^a | 16.43 ^a | 9.81 ^b | 0.00 ^b |
| Control | 89.47 ^a | 2.56 ^b | 0.27 ^c | 0.95 ^c | 0.93 ^c | 6.76 ^b | 0.00 ^b |

The means with the same letter at superscript are significantly different ($P>0.05$) from each other across the column

3.7. Effect of location on mineral, nutrient of okra at varying storage periods

For first month of storage, for the Ca, first month and second storage has significant effect in the entire sample collected for different locations. The Mg and P of okra collected for Ojoo, Ojaoba, Moniya, Apete, Bodija were non-significantly different from each other but significantly different from control.

The fresh okra in the first month has significantly higher and different magnesium and phosphorus,

calcium, compared to the dried samples from other locations, but which are not significantly different from one another ($P>0.05$). Similarly, at the end of the fourth month, the values of calcium, and phosphorus significantly reduced. The value of magnesium for fresh 'orunla' increased at the end of the fourth month to 60.07 μ g/g though, but not significantly different from dried samples obtained from other locations.

Table 6: Effect of location on mineral, nutrient of okra at varying storage periods

| Location | First Month | | | Fourth Month | | |
|----------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | Ca | Mg | P | Ca | Mg | P |
| Ojoo | 0.96 ^b | 0.17 ^b | 1.62 ^b | 1.18 ^b | 0.20 ^b | 1.83 ^b |
| Ojaoba | 1.08 ^b | 0.13 ^b | 1.20 ^b | 1.42 ^b | 0.19 ^b | 1.89 ^b |
| Moniya | 1.11 ^b | 0.12 ^b | 1.40 ^b | 1.17 ^b | 0.21 ^b | 1.60 ^b |
| Apete | 1.19 ^b | 0.12 ^b | 0.90 ^b | 1.31 ^b | 0.18 ^b | 1.80 ^b |
| Bodija | 1.26 ^b | 0.19 ^b | 1.37 ^b | 1.32 ^b | 0.24 ^b | 1.72 ^b |
| Control | 69.93 ^a | 60.07 ^a | 61.07 ^a | 80.83 ^a | 56.67 ^a | 63.03 ^a |

The means with the same letter at superscript are significantly different ($P>0.05$) from each other across the column.

3.8. Storage periods at early April and late July

The control which is the fresh sample had the highest Mg, P while the first month after storage had

the least Ca, Mg, P. The early April has the least for all the parameters.

Table 7: Storage periods at early April and late July

| Storage Month | Mineral Nutrients | | |
|---------------|--------------------|--------------------|--------------------|
| | Ca | Mg | P |
| Early April | 14.54 ^b | 0.17 ^b | 1062 ^b |
| Late July | 1.12 ^c | 0.15 ^c | 1.30 ^c |
| Control | 69.93 ^a | 60.07 ^a | 61.07 ^a |

The means with the same letter are not significantly different ($P>0.05$) from each other across the column. Ca – Calcium, Mg – Magnesium and P – Potassium.

3.9. Correlation matrix on the mineral contents of 'Orunla' okra

The result of the correlation matrix presented in table 7 shows highly strong positive association between magnesium and calcium (0.99), while the

observed correlation between phosphorus and calcium (0.99) is highly strong and positive correlation exists between location and calcium (0.66) magnesium (0.65), and phosphorus (0.65).

Table 8: Correlation matrix on the mineral contents of 'Orunla' okra

| | Mg | P | Location |
|----------|--------|--------------------|--------------------|
| Ca | 0.99** | 0.99** | 0.66** |
| Mg | | 1.00 ^{ns} | 0.65** |
| P | | | 1.00 ^{ns} |
| Location | | | |

*P<0.05 = significant, **P<0.01 = highly significant, ns = non-significant. Ca – Calcium, Mg – Magnesium and P – Potassium.

3.10. Correlation matrix on the nutritional content of 'Orunla' okra

The moisture was not positive but highly correlated with protein, ether extract, crude fibre, ash, and dry matter but positive and highly correlated with location, replicate and period of storage. The protein is positive and highly correlated with ether extract, ash, and carbohydrate but not significantly correlated with replicate. The ether extract is positive and highly

correlated with carbohydrate while the crude fibre is positive and highly correlated with ash and period of storage but not positive and non-significantly correlated with dry matter. The carbohydrate is positive but not correlated with carbohydrate, location, dry matter, replicate and period of storage. The location is positive and highly correlated with the replicate.

Table 9: Correlation matrix on the nutritional content of 'Orunla' okra

| | Protein | Ether content | Crude fibre | Ash | Carbohydrate | Location | Dry matter | Month |
|------------------|---------|---------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|
| Moisture content | -0.73** | -0.60** | -0.46** | -0.61** | 0.35 ^{ns} | 0.57** | -0.19** | 0.69** |
| Protein | | 0.27** | 0.35* | 0.50** | 0.29** | -0.16 ^{ns} | -0.26 ^{ns} | -0.54** |
| Ether extract | | | 0.34 ^{ns} | 0.82 ^{ns} | 0.65** | -0.62 ^{ns} | 0.64 ^{ns} | -0.78 ^{ns} |
| Crude fibre | | | | 0.89** | 0.65 ^{ns} | -0.25* | -0.40 ^{ns} | 0.32** |
| Ash | | | | | 0.03 ^{ns} | 0.31 ^{ns} | 0.08 ^{ns} | 0.75 ^{ns} |
| CHO | | | | | | 0.10 ^{ns} | 0.43** | -0.91* |
| Location | | | | | | | -0.52** | 0.36 ^{ns} |
| Dry matter | | | | | | | | -0.47 ^{ns} |
| Replicate | | | | | | | | 0.00 ^{ns} |

*P<0.05 = significant, **P<0.01 = highly significant, ns = non-significant

3.11. Correlation matrix on the nutritional content of 'orunla' okra

The results on Table 9 shows the correlation matrix of the nutritional contents of 'orunla'. Protein had significant (P<0.01) and strong positive correlations with ether content (r = 0.27), crude fibre (r = 0.35), ash content (r = 0.50) and carbohydrate (r =

0.65) but not significant in respect to location (r = -0.16) and dry matter (r = -0.26). Crude fibre is strongly positive and strongly correlated with carbohydrate (r = 0.65) and not significant for ash, location and dry matter. Also, location is strongly positive and strongly correlated with dry matter (r = 0.43).

Table 10: Correlation matrix on the nutritional content of 'Orunla' okra

| | Crude protein | Crude fat | Crude fibre | Ash | CHO | Location |
|------------------|---------------|-----------|---------------------|--------|---------|---------------------|
| Moisture content | -0.87** | -0.67** | -0.89 ^{ns} | -0.98* | -0.99** | 0.56** |
| Crude Protein | | 0.36* | 0.76** | 0.82** | 0.82** | -0.51** |
| Crude fat | | | 0.68** | 0.80** | 0.70** | 0.39 ^{ns} |
| Crude fibre | | | | 0.90** | 0.90** | -0.17 ^{ns} |
| Ash | | | | | 0.98** | 0.01 ^{ns} |
| CHO | | | | | | -0.01 ^{ns} |
| Replicate | | | | | | 1.00 ^{ns} |
| Location | | | | | | |

*P<0.05 = significant, **P<0.01 = highly significant, ns = non-significant. CHO – Carbohydrate

4.0 Discussion

In this study, the most frequent fungi isolated from 'orunla' samples from different locations in Ibadan were found in the genera of *Aspergillus*, *Rhizopus* and *Mucor*. *Aspergillus flavus*, *A. niger* and *A. fumigatus* and *R. nigricans* have higher percentages of occurrence in all the samples except in the laboratory prepared 'orunla'. Similar mycoorganisms were isolated by Hell *et al.*, (2009), on some selected Benin vegetables; Simon-Oke *et al.* (2014) and by Jonathan and Esho (2010), on stored *Pluerotus ostreatus* and *P. pulmonarius* (edible Nigerian fungi). The results obtained from the present study deviated from the observation of Jonathan and Olowolafe (2001) on 'dodo ikire'. The difference observation may be due to different substrates used in respect to their moisture contents. It was also observed that the findings of these studies is in agreement with that of Faride, (2001); Kumar *et al.* (2008); Olayiwola *et al.* (2013) and Jonathan *et al.* (2011b, 2015) who isolated other related fungi as a bio-deteriorating organisms from spices and some other street sold foods (details of these are presented in Table 1, Figure 1 and 2).

This could be due to moisture content in the samples which predispose them to microbial infection (Braide *et al.* 2015 and Nwachukwu *et al.*, 2015). The moisture content observed in the samples could be due to humid environment during storage. Moreover, some fungi associated with food have been reported to release chemicals that are hazardous to man and animals (Coronel *et al.*, 2010; Makunet *al* , 2009 and Alemuet *al*; 2008; Jonathan and Olowolafe, 2001). Consumption of excessive amount of these compounds can cause illness or fatality. The toxins produced by fungi in normal okra plant have been reported by Youssef and Palmateer (2008) and to include aflatoxin B₁, B₂, G₁, and G₂, zearalenone and diacet oxyscir phenol.

From this study, it was observed that the level of Aflatoxin B₁ was higher when compared with other toxins. This probably corroborated the high percentage occurrence of *A. flavus* in the samples. The location effect in this study was highly significant (P<0.01) on concentrations of the aflatoxin B₁ in the samples and this indicates that the locations are determinant factors for the source of the food products. Storage time has significant effect on the concentrations of aflatoxins as shown on Figure 2. The highest concentration of Aflatoxin B₁ in Moniya sample compared to control could be attributed to the increase in moisture content of 'orunla' during storage. (Braide *et al.* 2012). These pathogens possess the ability to produce extracellular hydrolytic enzymes that are capable of breaking down stored food products (Moss, 2008; Gelineau-van *et al.*, 2009; Fapohunda *et al.*, 2008 and Essono *et al.*, 2008; Diaz *et al.*, 2005). Although the Aflatoxin contents in the studied samples were generally below the

tolerance limit, but may increase above the limit if samples kept for longer period considering the increase in the concentration level from one month to four month. The moisture content of 'orunla' is significantly (P<0.05) higher than the dried samples. The location effect was found to support the highest concentration of aflatoxin found in Moniya and the lowest concentrations found in Oja-oba.

The reduction in nutritional content in Tables 2 and 3 suggests that the fungi isolated utilized these nutrients for their successful establishment, cellular growth, reproduction and survival in 'orunla'. This was similarly observed by Fagbohun *et al.* (2011). Nutritional quality should be of priority in Nigeria especially among the low income group to avert problem of dietary toxicoses (Tothil *et al.*, 2006).

The presence of fungi in food could lead to depletion of nutrients and the metabolite produced by them poses dietary toxicity to the populace especially when consumed in amount above tolerable level (Djadouni and Larrea, 2016). Attention should therefore be given to campaign on aflatoxin contamination in food because it is a threat to human survival (Risk Assessment Studies, 2001). This study revealed fungal invasion and deterioration in relation to nutrient composition of 'Orunla' for human public health.

5.0. Conclusion and Recommendation

Continuous education and campaign should be given on food hygiene and safety to the populace by government and all other stakeholders. Periodically quality and toxigenicity check should be carried out on food commodities in our markets.

Storage facilities should be subsidized and more skilled labour should be put in place to handle food. This will help to avoid economic and health risk crisis in our food products. There should be campaign to discourage unhygienic method of drying by providing small and medium scale farmer storage facilities at subsidized rate.

Hazard analysis critical control point (HACCP) should be sited at every food commodity market in Nigeria. Mycotoxin regulation should be enforced in Nigeria.

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