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Effects of preparation methods and storage on the quality and aflatoxin contamination of Plantain

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Abstract: Plantain is widely cultivated in Nigeria and ranked among the top staple foods. It is commonly prepared in many forms in other to enhance its shelf-life and taste however, care must be taken while handling and storing this product due to fungal and aflatoxin contaminations. This study therefore investigated both the effects of preparation styles and storage on the quality of differently processed plantains in Nigeria. Samples of fresh healthy plantain samples were collected from two different states (Ogun and Oyo States, Nigeria), these samples were then prepared into common chips in Nigeria through different processing methods (such as drying, frying as well as roasting) and then stored for two and four weeks respectively. To assert the effect of preparation style and storage on the samples, both the fresh and stored plantains were analyzed for proximate (carbohydrate, crude protein, fibre, ash and moisture), fungal and aflatoxin (AFB_1, AFB_2, AFG_1 and AFG_2 respectively) compositions. Result showed that the samples generally had higher carbohydrate and protein as compared to fat and ash contents, it was also observed that the fresh samples had better nutrient compositions and less in fungal/aflatoxin compositions as compared to the stored samples. The highest crude protein content was found in fresh plantain samples obtained from Ogun State (1.34±0.01) and was significantly different from that of four weeks stored plantain (1.15±0.00). Also aflatoxin content was found to be much lower in fresh sample but increased with the duration of storage; aflatoxin AFB_1contentfor example was recorded least in Ogun fresh plantain samples and highest in Ogun roasted stored plantain (0.0100±0.000).
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Introduction

Plantain (*Musa* spp.) occupies a strategic position for rapid food production in Nigeria. It has been investigated and ranked third among starchy staples. The country's output for plantain had doubled in the last 20 years. Plantain production, which is mostly concentrated in the Southern part of the country, still remains largely in the hands of small scale farmers who, over the years, have ingeniously integrated it into various cropping systems. In Nigeria, production of plantain is male dominated, while women essentially handle marketing. However, the inadequate knowledge of improved cultural practices of the crop by the farmers, inefficient system of extension services, postharvest spoilage and limit of specialization in areas of research are part of the main challenges plantain production in the country (IITA, 2014).

Spoilage of plantains are usually attributed to fungal pathogens, fungi as an important group of plantain pathogens had been reported to have cause several financial loss during cultivation, harvest and storage of plantains (Mobambo *et al*., 2010). Many of these pathogens such as *Aspergillus flavus, Aspergillus bombycis, Aspergillus chraceoroseus*, *Aspergillus nomius*, and *Aspergillus pseudotamari* are also aflatoxin-producing species, but they are encountered less frequently (Asemoloye et al., 2017; Bennett and Klich, 2000). To avoid fungal spoilage of plantain after harvest, they are usually processed into different forms trough Sun and or oven-drying methods and these had been operated with success (Bowrey *et al.,* 1980; Johnson *et al*., 1998; Demirel & Turhan, 2013). Plantain chips are the most popular plantain products in Nigeria (Fatumbi, 2016). They are prepared by frying round slices of unripened or slightly ripened plantain pulp in vegetable oil. However, care must be taken while storing these dried products due to aflatoxin contaminations.

This study was therefore aimed at investigating the effects of both the preparation styles and storage time on the quality of differently processed plantains in Nigeria.

Materials and Methods

Collection of samples

Fresh samples of Plantains were collected from the major market in two different States in South/West Nigeria (Ogun and Oyo). In each state, freshly harvested healthy plantains were bought in major market viz: in Ogun State, plantain was bought from New Market (Longitude 6°48'55"N and Latitude 3°54'44"E) while in Oyo State, plantain bought from Bodija Market (Longitude 7°43'58"N and Latitude 3°91'92"E) and this was done at different times within two (2) weeks. The fresh samples were collected in glass jar and transported to the Mycology/Pathology Laboratory of Department of Botany, University of Ibadan (Longitude 7°44'17"N and Latitude 3°90'00"E) for further studies.

Sample preparation

The samples were divided into two; the fresh samples were studied immediately while the other part was prepared into chips through sun drying, frying and roasting after which they were stored for two (2) and four (4) weeks consecutively. For the analysis, samples were analyzed for their proximate (carbohydrate, crude protein, fibre, ash and moisture), fungal and aflatoxin (AFB_1, AFB_2, AFG_1 and AFG_2 respectively) compositions.

Proximate analysis

This was carried out in the Microbiology Laboratory in Institute of Agricultural Research and Training, IAR & T, Apata, Ibadan, Nigeria (Longitude 7°97'00"N and Latitude 3°03'10"E) using the standard protocols of AOAC (2008). The nutrients analyzed included; % Crude Protein, % Crude Fibre, % Fat, % Ash, % Moisture and % Carbohydrate, these were all determine in tree replicates.

Studies on associated spoilage fungi

Fungal isolation was carried out on each of the fresh, dried, roasted and fried samples in the Pathology Laboratory of the Department of Botany, University of Ibadan, Ibadan (Longitude 7°44'17"N and Latitude 3°90'00"E) following the procedures according to Jonathan and Olowolafe (2011). The fungal isolation was carried out in a sterile condition and all laboratory precautions carried out. The Potato Dextrose Agar (PDA) was used as nutrient medium fungal for the culture. The glass wares used were washed and dried in an oven and sterilized in an autoclave at 121°C at 1.06atm.19.5g of PDA was prepared according to the manufacturer's prescription and sterilized in an autoclave at 121^{0}C and 1.03 atm for fifteen (15) minutes. The solution was allowed to cool (till about 45°c) and 25 drops of lactic acid was then added to suppress bacteria growth. The media is then poured in petri dishes and left to solidify. Plantain samples were surface sterilized in 70% ethanol, cut into pieces before placing them on PDA in the petri dish. Fungal mixed cultures were separated on fresh PDA plate and the pure cultures were kept in the laboratory's incubator for 2-7 days at room temperature (30 ± 2°C). The isolated fungi were identified through macro and microscopic characterization and growth pattern (Jonathan *et al.,* 2011a, Jonathan *et al.,* 2011band Jonathan *et al.,* 2013).

The fungal colonies were observed for peculiar characteristic colonial morphology such as the colony appearance, rate of growth followed at regular intervals while the microscopic morphology and type of asexual spores produced were also studied through use of photomicrograph and identified by reference to the compendium of soil fungi (Jonathan *et al*., 2016a).

Aflatoxin Analysis

This was done by following the method developed by Thomas *et al.* (1975) and Jonathan *et al.* (2017; 2016b & c) using Thin Layer Chromatography (TLC). This was carried out in the Microbiology Laboratory of Institute of Agricultural Research and Training, IAR & T, Apata, Ibadan, Nigeria (Longitude 7°97'00"N and Latitude 3°03'10"E). The samples were first grinded. 2g of the power sample was weighed into a 500ml conical flask and 250ml of methanol: water at a ratio of 60:40 v/v, after which the solution was shaken in a mechanical shaker for approximately 30 minutes. The solution was allowed to sediment then filtered through a Whatman filter paper No. 1 into another 500ml conical flask. 125ml of the filtrate was poured in a 250ml separating funnel. 30ml of saturated Sodium-Chloride (NaCl) followed by 50ml hexane was added to the solution in the separating funnel. The solution was then shaken vigorously for 2 minutes and the solution was allowed to separate. The lower methanol water layer is collected in another 250ml separating funnel and 50ml of chloroform is added. The solution is shaken vigorously to give frequent vents.

The chloroform layer was drained in another clean conical flask already containing 5g cupric carbonate, the solution is also shaken and the cupric carbonate is allowed to settle. The solution was filtered through a Whatman filter paper No. 42 having a bed of anhydrous sodium sulphate and the chloroform extract is collected in a beaker (giving the first part of chloroform extract). The cupric carbonate is washed with 25ml chloroform and filtered through the sodium sulphate bed. The filtrate (chloroform extract) is collected in another beaker (to form second part of chloroform extract). The two chloroform

extracts were added together. The chloroform extract is then evaporated in a water bath till dry. The residue obtained is dissolved with chloroform and transferred into a screw cap bottle. The extract in the screw cap bottle is kept for quantitative estimation. The extract which was stored in a screw cap bottle was re-dissolved with 1ml of chloroform and poured into a cuvette of the spectrophotometer where the aflatoxin was read at 250nm wavelength. The intensity of the flourescence produced in spot of the sample was compared with that of the standard aflatoxin spots. The volume of the matching spot with that of the standard was recorded. The concentration of the aflatoxin was calculated using the formular below:

$$\frac{S \times Y \times V}{W \times Z} \quad (1)$$

Where:

S = Volume of each aflatoxin standard in µl of equivalent intensity to Z of sample

Y = Concentration of each of the aflatoxin standard in µg/ml

V = Volume of solvent in µ1 required to dilute final extract

Z =Volume of sample extract in µ1 required to give fluorescence intensity comparable to that of S of each aflatoxin standard.

W = Weight of original sample in g contained in final sample

Statistical data analysis

The data generated for the aflatoxin concentration and proximate composition were subjected to Analysis of Variance (ANOVA) at $P<0.05$ while the Test of Significance was carried out by Duncan Multiple Range Test (DMRT) using SAS (Version 9.1).

Results

The proximate compositions

It was observed that all the samples generally had higher carbohydrate and protein as compared to fat and ash contents. The fresh plantain obtained from Ogun state had the highest crude protein of (1.34±0.01) while the least was detected in the dried stored samples that were collected from Ogun state (1.15±0.00). The highest crude fibre content was found in fresh plantain samples (0.53±0.01) from Oyo State while the least crude fibre content was found in roasted plantain samples (0.27±0.00) from Oyo state (Table 1). The values obtained were significantly different statistically from those obtained from the fresh plantain samples from both States. Analysed crude protein from Ogun and Oyo were 1.34±0.01% and1.29±0.01 % respectively while the crude fibre for Ogun and Oyo samples were 0.50±0.00% and 0.53±0.01% at $P\leq0.05$ respectively.

From the Proximate analysis, crude protein was record highest in the fresh Ogun plantain sample (1.34±0.01) while the lowest crude protein was found in Ogun dried plantain sample after four weeks (1.15±0.00). The highest crude fibre content was found in Oyo state plantain samples (0.53±0.01]), while the least crude fibre content was found in roasted plantain samples collected from Oyo state (0.27±0.00) that have been stored for four (4) weeks (Table 3), this maybe as a result of the long storage period as well as environmental factors. The highest concentration of fat was noticed in fried plantain samples collected from Ogun State after four (4) weeks of preservation (0.55±0.01). The smallest detectable amount of fat was found in roasted plantain samples collected from Ogun State at (4) weeks of preservation (0.24±0.00). This could mean that fat is more possibly found in fried plantain than roasted plantain. Moisture content and carbohydrate composition was highest in fresh plantain samples (66.77±0.10) and those preserved by drying after four (4) weeks (12.75±0.07) collected from Ogun State as recorded in Table 3.

Table 1: The proximate composition of fresh, dried, fried and roasted plantain samples obtained from Ogun and Oyo States, Nigeria

			Proximate Composition of Plantain					
Duration	**Location**	**Plantain Type**	**% Crude Protein**	**% Crude Fibre**	**% Fat**	**% Ash**	**% Moisture Content**	**% Carbohydrate (NFE)**
4weeks	Oyo	Dried	1.17 ± 0.01^{ef}	0.33 ± 0.07^{b}	0.28 ± 0.00^{b}	0.11 ± 0.01^{bc}	14.80 ± 0.00^{d}	98.07 ± 0.02^{a}
4weeks	Oyo	Fried	1.21 ± 0.01^{de}	0.32 ± 0.01^{b}	0.51 ± 0.01^{a}	0.08 ± 0.00^{d}	25.80 ± 0.14^{b}	97.89 ± 0.03^{b}
4weeks	Oyo	Roasted	1.24 ± 0.00^{cd}	0.27 ± 0.00^{b}	0.25 ± 0.00^{b}	0.14 ± 0.00^{a}	22.82 ± 0.03^{c}	98.10 ± 0.00^{a}
4weeks	Ogun	Dried	1.15 ± 0.00^{f}	0.34 ± 0.00^{b}	0.27 ± 0.00^{b}	0.12 ± 0.00^{abc}	12.75 ± 0.07^{d}	98.12 ± 0.00^{a}
4weeks	Ogun	Fried	1.20 ± 0.00^{de}	0.30 ± 0.00^{b}	0.55 ± 0.01^{a}	0.10 ± 0.00^{cd}	26.85 ± 0.07^{b}	97.86 ± 0.01^{bc}
4weeks	Ogun	Roasted	1.22 ± 0.00^{d}	0.31 ± 0.00^{b}	0.24 ± 0.00^{b}	0.14 ± 0.01^{a}	24.50 ± 0.00^{bc}	98.10 ± 0.01^{a}
0weeks	Oyo	Fresh (Control)	1.29 ± 0.01^{bc}	0.53 ± 0.01^{a}	0.31 ± 0.01^{b}	0.12 ± 0.00^{abc}	64.79 ± 0.06^{a}	97.76 ± 0.03^{c}
0weeks	Ogun	Fresh (Control)	1.34 ± 0.01^{a}	0.50 ± 0.00^{a}	0.26 ± 0.01^{b}	0.10 ± 0.00^{cd}	66.77 ± 0.10^{a}	97.81 ± 0.01^{bc}

Means with the same alphabets down the column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only

The highest ash content was observed in stored roasted plantain samples collected from Oyo state after four (4) weeks (0.14±0.00) as well as roasted plantain from Ogun state after four (4) weeks (0.14±0.00). while the least ash content was observed in fried plantain samples from Oyo state after four (4) weeks of storage. Moisture content and carbohydrate composition was highest in fresh plantain samples (66.77±0.10) and those preserved by drying after four (4) weeks (12.75±0.07) collected from Ogun State as recorded in Table 1.

Associated spoilage fungi

The fungi which were commonly isolated from the samples were identified as *Aspergillus flavus, Aspergillus niger, A. tamari*and *Penicillium oxalicum* (Plate 1). It was also observed that the fungal load in the samples increased with the storage time (Table 4). The fungal load was recorded highest at week four (4) of storage, it was also observed that dried samples tend to contain more fungal load than the fried samples (Table 4).

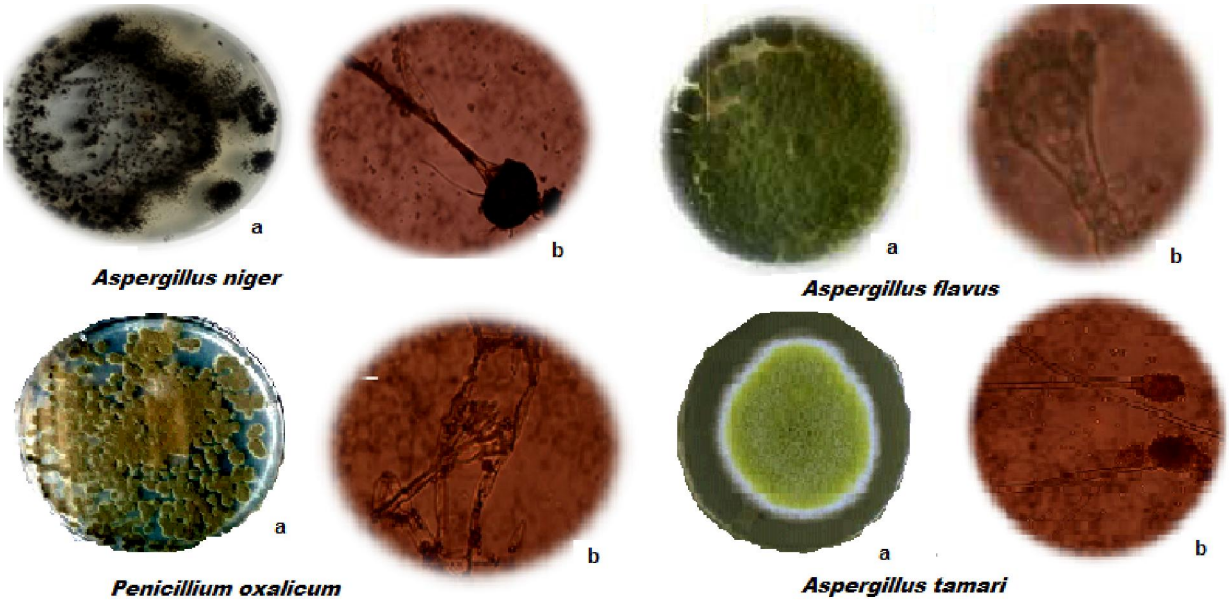

Plate 1: Associated spoilage fungi with the plantain samples (a) plate macroscopic and (b) microscopic views

Table 4: The fungal load (spore count) calculated in differently processed plantain samples at different storage time

Duration	Location	Plantain Type	Total spore count (x 10^5 cfu g^{-1})
4weeks	Oyo	Dried	1.25 ± 0.07^{b}
4weeks	Oyo	Fried	0.80 ± 0.00^{d}
4weeks	Oyo	Roasted	1.45 ± 0.07^{a}
4weeks	Ogun	Dried	1.35 ± 0.07^{ab}
4weeks	Ogun	Fried	1.00 ± 0.00^{c}
4weeks	Ogun	Roasted	1.25 ± 0.07^{b}
2weeks	Oyo	Dried	0.60 ± 0.14^{e}
2weeks	Oyo	Fried	0.45 ± 0.07^{e}
2weeks	Oyo	Roasted	0.55 ± 0.07^{e}
2weeks	Ogun	Dried	0.80 ± 0.00^{d}
2weeks	Ogun	Fried	0.50 ± 0.00^{e}
2weeks	Ogun	Roasted	0.25 ± 0.07^{f}
0weeks	Oyo	Fresh (Control)	0.20 ± 0.00^{f}
0weeks	Ogun	Fresh (Control)	0.25 ± 0.07^{f}

Means with the same alphabets down the column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only

Aflatoxin compositions

It was observed the stored plantain chips had more aflatoxin contents as compared to fresh plantain, and also the dried chips had more aflatoxin contaminations as compared to others (Table 5); for example fresh plantain samples collected from Oyo and Ogun had no detectible aflatoxin AFG2 content. The highest level of aflatoxin was observed in Ogun (roasted plantain) preserved for two (2) weeks (0.0135±0.001) and four (4) weeks (0.0135±0.001) respectively.

Table 5: The aflatoxin content of fresh, dried, fried and roasted plantain samples obtained from Ogun and Oyo States, Nigeria

			Aflatoxin Content (□gkg^{-1})			
Duration	**Location**	**Plantain Type**	**Aflatoxin B_1**	**Aflatoxin B_2**	**Aflatoxin G_1**	**Aflatoxin G_2**
4 weeks	Oyo	Dried	0.0050±0.000^{c}	0.0075±0.001^{d}	0.0040±0.000^{cd}	0.0075±0.001^{c}
4 weeks	Oyo	Fried	0.0030±0.000^{d}	0.0050±0.000^{e}	0.0060±0.000^{ab}	0.0040±0.000^{d}
4 weeks	Oyo	Roasted	0.0055±0.001^{c}	0.0110±0.000^{b}	0.0050±0.000^{bc}	0.0105±0.001^{b}
4 weeks	Ogun	Dried	0.0075±0.001^{b}	0.0100±0.000^{c}	0.0065±0.001^{a}	0.0080±0.000^{c}
4 weeks	Ogun	Fried	0.0045±0.001^{c}	0.0050±0.000^{e}	0.0035±0.001^{d}	0.0045±0.001^{d}
4 weeks	Ogun	Roasted	0.0100±0.000^{a}	0.0135±0.001^{a}	0.0060±0.000^{ab}	0.0135±0.001^{a}
2 weeks	Oyo	Dried	0.0050±0.000^{c}	0.0075±0.001^{d}	0.0040±0.000^{cd}	0.0075±0.001^{c}
2 weeks	Oyo	Fried	0.0030±0.000^{d}	0.0050±0.000^{e}	0.0060±0.000^{ab}	0.0040±0.000^{d}
2 weeks	Oyo	Roasted	0.0055±0.001^{c}	0.0110±0.000^{b}	0.0050±0.000^{bc}	0.0105±0.001^{b}
2 weeks	Ogun	Dried	0.0075±0.001^{b}	0.0100±0.000^{c}	0.0065±0.001^{a}	0.0080±0.000^{c}
2 weeks	Ogun	Fried	0.0045±0.001^{c}	0.0050±0.000^{e}	0.0035±0.001^{d}	0.0045±0.001^{d}
2 weeks	Ogun	Roasted	0.0100±0.000^{a}	0.0135±0.001^{a}	0.0060±0.000^{ab}	0.0135±0.001^{a}
0 weeks	Oyo	Fresh (Control)	0.0010±0.000^{e}	0.0015±0.001^{f}	0.0005±0.001^{e}	0.0000±0.000^{e}
0 weeks	Ogun	Fresh (Control)	0.0005±0.001^{e}	0.0010±0.000^{f}	0.0005±0.001^{e}	0.0000±0.000^{e}
0 weeks	WHO	Fresh (Control)	0.00005±0.00^{e}	0.00005±0.00^{f}	0.00005±0.00^{e}	0.00005±0.00^{e}

Means with the same alphabets down the column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only

Discussion

Fungi has been well associated with deterioration of many food products due to their wide range diversities and saprophytic mode of nutrition, some have also been reported to cause plantain rotting and postharvest spoilage. In this study, a total of eight fungal species were found to be predominantly associated with plantain samples. They were identified as *Aspergillus flavus, A. niger, A. parasiticus, A. tamari, F. oxysporum, P. oxalicum, Penicillium chrysogenum, Rhizopus stolonifer*. The biodeteriorating and aflatoxigenic fungal species that colonized Plantain must have been present in the atmosphere in form of spores after the sun drying, frying and roasting.

Varying level of aflatoxin in fresh and stored samples may be due to the alternating moisture content in the stored plantain sample which supports the fungal growth differently. This could also be due to the fact that moisture content in the samples play some roles in predisposing them to microbial infection (Braide *et al.* 2015 and Nwachukwu *et al.*, 2015). Alternating moisture content in the samples could be due to humid environment as previously observed that fungi associated with food usually release chemicals that are hazardous to man and animals known as mycotoxins of which aflatoxin is novel excample (Coronel *et al*., 2010; Makun *et al.,* 2009 and Alemu*et al*; 2008; Jonathan and Olowolafe, 2001). The fungi could have been introduced during exposure and direct contact of these agricultural products in the market according to (Ekundayo,1986; Aboaba and Amisike, 1991; Okigbo, 2003).

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). *Aspergillus* species are the most common fungi isolated in this study. The prevalence of *Aspergillus* spp in these stored food products may be the factors responsible for the high level of aflatoxin detected in them as it has been reported that aflatoxin is majorly produced by *Aspergillus flavus*, *A. nominus* and *A. parasiticus* species of fungi (Mushtaq *et al.,* 2012; Jonathan et al., 2016a). Aflatoxin B_1, B_2 and aflatoxin G_2 were found

to be the highest after the analysis. The maximum aflatoxin B_1 concentrations allowed for human consumption ranged from 5 to 50 ppb. This level varies from country to country. Most countries limit aflatoxin in food to 20 ug/kg (Bankole *et al*, 2014). Although, the Aflatoxin contents in the studied samples were generally below the tolerance limit, but may increase above the limit if samples are kept for longer period considering the increase in the concentration level from 0 to 4weeks. The maximum acceptable aflatoxin level of 4-20 mL/kg by the European food safety authority is much higher than what was detected in this work but storage for a longer period may increase the aflatoxin level (USDA, 2010).

The results obtained from this study are similar to the observation of Jonathan and Olowolafe (2011) on 'dodo ikire, alternating nutrient levels I the samples could be due to exposure to sun drying thereby reducing the nutritional value. It also infers that the longer the period of storage, the higher the risk of the plantain becoming unhygienic for consumption due to fungal incidences. This may also be as a result of time of storage leading to accumulation of moisture, environmental factors which implies that long period of storage and preservation can lead to plantain spoilage. All storage attempts to prolong the shelf life of mature green plantain should be preceded by an economic analysis of the system in place: network type, stage of harvest, market value and price after conservation (N'da Adopo *et al.*, 1996). It was also observed that the findings of these studies is in agreement with that of Abiala *et al*. (201); 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 foods.

Conclusion and Recommendation

It is obvious from this study that the longer the period of storage, the higher the aflatoxin produced in plantain. Also, exposure to high moisture content during storage period increases fungal activity which was observed in the level of aflatoxin in Fried and roasted plantain compared to that of Dried due to reduced moisture content in dried Plantain sample. Avoidance of moisture is therefore needed to be ensured in the preparation of dried Plantain which is processed to 'Plantain flour' to avoid mould development. Of the three method of preservation considered in this work, only the dried is considered the best and the dried Plantain samples must be placed in dry environment in order to limit the tendency of the growth of aflatoxigenic fungi after a long storage period. A proper storage method is therefore needed to be ensured. In the preparation of plantain, long storage period, before consumption should also be discouraged. Also the produce to be consumed needs to be evaluated to serve as a basis for threat assessment.

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