

Effect of Preparation Styles and Storage Time on Nutrient Value and Aflatoxin Contamination of Plantain

Gbolagade S Jonathan, Tobi P Animasaun and Michael D Asemoloye*

Mycology/Fungal Biotechnology Unit, Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria

***Corresponding Author:** Michael D Asemoloye, Mycology/Fungal Biotechnology Unit, Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria.

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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 such as dried or fried plantain chips in order to enhance its shelf-life and taste. However, care must be taken while processing, handling and storing these plantain products due to fungal and aflatoxin contaminations. This study therefore investigated both the effects of preparation styles and storage on the quality of differently processed plantain chips 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 (AFB₁, AFB₂, AFG₁ and AFG₂ 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 AFB₁ content for example was recorded least in fresh plantain chips and highest in roasted stored plantain (0.0100 ± 0.000) at four weeks of storage.

Keywords: *Plantain; Chips; Shelf-life; Preparation Style; Storage*

Introduction

Plantain (*Musa* spp.) occupies a strategic position for rapid food production in Nigeria [1]. It has been investigated and ranked third among starchy staples and the country's output for plantain had doubled in the last 20 years [2]. 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 [3]. 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 [4,5].

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 [6-8]. 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 [9]. To avoid fungal spoilage of plantain after harvest, they are usually processed into different forms through Sun and or oven-drying methods and these had been operated with success [10,11]. Plantain chips are the most popular plantain products in Nigeria [12-15]. 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.

Aim of the Study

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 three major markets in two different States in South/West Nigeria (Ogun and Oyo). In each state, freshly harvested healthy plantains were bought in three major markets viz: in Ogun State, plantain was bought from these markets (Longitude 6°48'55"N and Latitude 3°54'44"E, Longitude 7°48'55"N and Latitude 3°64'44"E and Longitude 6°78'55"N and Latitude 3°04'44"E) while in Oyo State, plantain bought from these locations (Longitude 7°43'58"N and Latitude 3°91'92"E, Longitude 7°13'58"N and Latitude 3°21'92"E and Longitude 7°93'58"N and Latitude 3°21'92"E) and these were done 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 first parts were studied immediately while the other parts were prepared into chips through sun drying, frying and roasting and stored for two (2) and four (4) weeks consecutively. These samples were all analysed for their proximate (carbohydrate, crude protein, fibre, ash and moisture), fungal and aflatoxin (AFB₁, AFB₂, AFG₁ and AFG₂ 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 [16] and Jonathan., *et al* [17]. 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., *et al* [18,19]. 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 1.06 atm. 19.5g of PDA was prepared according to the manufacturer's prescription and sterilized in an autoclave at 121°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 [20].

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 [20,21].

Aflatoxin analysis

This was done by following the method used by Oluwafemi and Ibeh [22] and Jonathan., *et al.* [20] using High Performance Liquid Chromatography (HPLC) method. The aflatoxins detected included Aflatoxins B₁, B₂, G₁ and G₂ which are the most common aflatoxins in the developing countries [22,23]. 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 HPLC is made up of LDC, with Milton Roy, Constametric 1 pump, and a Lichrosorb RP-18 column (Merck Hibar) with particle size of 5 µm, length of 125 mm, and inside diameter of 4 mm. The pump pressure is 60MPa and the injector was of an automatic type (Rheotype Gilson Abimed Model 231).

The detector had a fluorescence spectrophotometer (Shimadzu RF 535, gamma excitation 365 nm, and gamma emission 444 nm) and the flow rate was 1mL per minute and the injection volume was 50 µL with the use of mobile phase containing water/acetonitrile (75 : 25) with flow rate 1.2 mLmin⁻¹ for 20 minutes. 50 gram of each sample of "Suya spice" was defatted by extraction with N-hexene Soxhlet-type extractor and the defatted residue was extracted with ethyl acetate (three times, 50 mL/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 14 × 0.8 cm column containing 2.5 Kiesel gel 60 and 70/230 silica gel. The aflatoxin analysis was done using Lichrosorb RP-18 column. The quantitative determination of the aflatoxins was carried out compared with standard aflatoxin B₁, B₂, G₁ and G₂ (Sigma).

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 9.2).

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% and 1.29 ± 0.01% respectively while the crude fibre for Ogun and Oyo samples were 0.50 ± 0.00% and 0.53 ± 0.01% at P ≤ 0.05 respectively.

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). 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.

Duration	Location	Plantain Type	Proximate Composition of Plantain					
			% 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}

Table 1: The proximate composition of fresh, dried, fried and roasted plantain samples obtained from Ogun and Oyo States, Nigeria. Means with the same alphabets down the column are not significantly different at $P \leq 0.05$ using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as “Means ± SD” only.

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 2). 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 2).

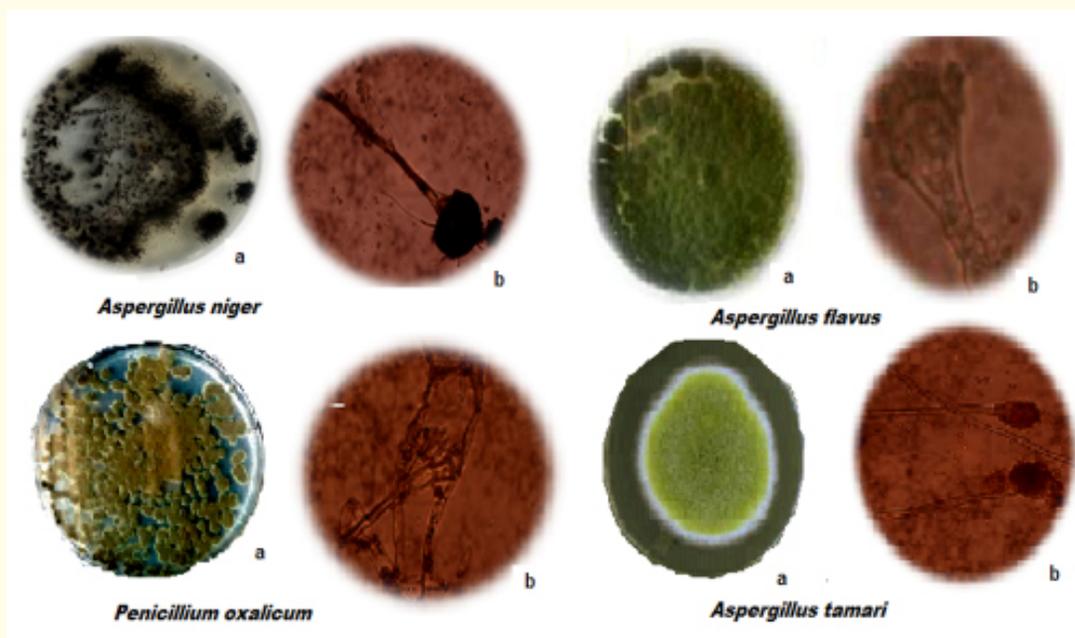


Plate 1: Associated spoilage fungi with the plantain samples (a) plate macroscopic and (b) microscopic views.

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

Table 2: The fungal load (spore count) calculated in differently processed plantain samples at different storage time. Means with the same alphabets down the column are not significantly different at $P \leq 0.05$ using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD".

Aflatoxin compositions

The aflatoxin compositions of the fresh and processed stored samples is presented in table 3. The samples also contained varying amount of aflatoxins but not more that the tolerance limit of 5 µgkg⁻¹ according to the European Union specification. It was observed that the fresh samples generally had very low aflatoxin content while the processed stored plantain chips had higher aflatoxin contents. It was also observed that the processing methods and storage time had significant effects on the aflatoxin composition of the plantain chips (Table 3). The highest aflatoxin B1 of 0.01 µgkg⁻¹ was recorded for stored roasted samples from Ogun at weeks 4 and 2 respectively while the least aflatoxin B1 of 0.003 µgkg⁻¹ was recorded for stored fried samples from Oyo at weeks 4 and 2 respectively among the stored samples.

The highest aflatoxin B1 of 0.0111 µgkg⁻¹ was recorded for stored roasted samples from Oyo at weeks 4 and 2 respectively while the least aflatoxin B2 of 0.005 µgkg⁻¹ was recorded for stored fried samples from Oyo and Ogun at weeks 4 and 2 respectively among the stored samples. Similar results were recorded for the aflatoxin G1 and G2 as presented in table 3.

Discussion

Fungi have 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 four fungal species were found to be predominantly associated with plantain samples. They were identified as *Aspergillus flavus*, *A. niger*, *A. tamari*, and *Penicillium oxalicum*. Similar fungal species have been well associated with several other street foods [3,17-20] while *A. flavus* have been well established as aflatoxigenic fungi due to its ability to produce fungal toxin called aflatoxin. It is therefore suggested that 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.

The results obtained from this study are similar to the observation of Jonathan, *et al.* [19,20] on 'Attiekie' and 'suya spices' that the processing, storage time and fungal composition greatly affect the nutrient composition of food products. It was observed in this study from

Duration	Location	Plantain Type	Aflatoxin Content (gkg ⁻¹)			
			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

Table 3: The aflatoxin content of fresh, dried, fried and roasted plantain samples obtained from Ogun and Oyo States, Nigeria. Means with the same alphabets down the column are not significantly different at $P \leq 0.05$ using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means \pm SD" only.

the proximate analysis that crude protein was record highest in the fresh Ogun plantain sample while the lowest crude protein was found in Ogun dried plantain sample after four weeks. This 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.

The highest crude fibre content was found in fresh samples, while the least crude fibre content was found in roasted plantain samples that have been stored for four (4) weeks, this maybe as a result of the long storage period as well as environmental factors. Likewise, the highest concentration of fat among the processed stored samples was noticed in fried plantain samples while the smallest detectable amount of fat was found in roasted plantain samples at (4) weeks of preservation. These could mean that fat is more possibly found in fried plantain than roasted plantain. Also, the moisture content and carbohydrate composition was highest in fresh plantain samples and those preserved by drying after four (4) weeks, this maybe 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 [21]. It was also observed that this study is in agreement with that of [22-28] who isolated other related fungi as a bio-deteriorating organisms from spices and some other foods.

The highest level of aflatoxin in this study was observed in Ogun (roasted plantain) preserved for two (2) weeks and four (4) weeks respectively, this could also be linked to be due to the high moisture content in these stored plantain samples which implies that the higher the moisture content, the higher the microbial growth as similarly observed by Braide, *et al.* [29] and Nwachukwu, *et al.* [30]. 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 [31-33]. The fungi could have been introduced during exposure and direct contact of these agricultural products in the market according to [34-36] and 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 [37].

Aspergillus species are the 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 [22]. 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 4 weeks. 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 [35,38,39].

Conclusion and Recommendation

It is obvious from this study that the longer the period of storage, the higher the aflatoxin produced in plantain. Also, elevated moisture content of stored fried and roasted plantain chips during storage increases fungal activity hence the level of aflatoxin. 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. Out of the three method of preservation considered in this work, the dried method 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 for this food product. Long storage period should also be discouraged before consumption of plantain chips. Basis for threat assessment in food products should also focus on fungi and mycotoxins.

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