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# ANALYSIS OF THE NUTRITIONAL, MYCOFLORA AND AFLATOXIN CONTENT OF RAW AND SUN DRIED PLANTAIN

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## ABSTRACT
Plantain is an important food source in Nigeria, and could also be a source of human exposure to microbial toxins, particularly aflatoxin. The current study was therefore aimed at investigating the nutritional, mycoflora and aflatoxin contents of raw and sundried plantain. Total heterotrophic fungi count, enumeration and identification of aflatoxigenic fungi were done using established microbiological procedures. Mineral analysis was done using Jenway digital flame photometer, aflatoxin was detected using Enzyme Linked Immunosorbent Assay (ELISA). The total heterotrophic fungi count recorded for the raw plantain (day 1) was $1.40 \pm 0.20 \times 10^5$ Cfu/g while sundried plantain flour ranged from $2.40 \pm 0.10 \times 10^5$ Cfu/g (day 1) to $12.50 \pm 0.50 \times 10^5$ Cfu/g (day 5). Proximate analysis showed that the raw plantain contained 52.67% moisture, 2.40% ash, 2.30% fat and oil, 0.80% crude fibre, 2.54% crude protein and 39.23% carbohydrates while sundried plantain recorded 26.67% moisture, 3.17 % ash, 0.12 % fat, 5.40% crude protein, 2.01% crude fibre, and 64.63% carbohydrate. Raw plantain recorded the following minerals content 0.28 ppm phosphorous, 1.34 ppm sodium, 104.25 ppm potassium, 0.36 ppm calcium, 5.67 ppm iron and 6 ppm magnesium. Sundried recorded 0.35 ppm phosphorous, 1.42 ppm sodium, 106.20 ppm potassium, 0.10 ppm calcium, 5.00 ppm iron and 0.80 ppm magnesium. Fourty (40) fungi isolates were reported, with the frequency of occurrence and percentage distribution as follows; *Aspergillus niger* 8 (12.50%), *Aspergillus flavus* 6 (9.40%), other *Aspergillus* species. 6 (9.40%), *Penicillium* sp. 7 (11.00%), *Rhizopus* spp. 6 (9.40%) *Mucor spp* 11(17.20%) *Trichoderma* spp10 (15.60%) and *Alternaria* spp 10 (15.60%). Aflatoxin analysis revealed that both sundried and raw plantain recorded very low total aflatoxin level and the aflatoxin B1 and B2 levels were below the limit of detection. The presence of aflatoxin in the sundried plantain flour does not pose any potential health risk to consumers as it was below the maximum permissible limit of 20 ppb.

**KEYWORDS:** Aflatoxin, Plantain, Fungi, *Aspergillus*

## INTRODUCTION

Plantain is a staple crop in Nigeria and the humid tropical zones of Africa, Asia and South America. It is rich in vitamin (A, B and C) as well as minerals such as potassium, magnesium, phosphorous and calcium (Zakpaa *et al*., 2010; Robinson, 1996). Plantain is also rich in crude fibre which helps in regular bowel movement thereby reducing constipation and prevention of colon cancer (Okareh *et al*., 2015). However, despite the nutritional importance of plantain, it is prone to microbial and physiological deterioration, (Agoreyo *et al*., 2003). Report by the Food and Agriculture Organization (2009) stated that more than 2.5 million metric tons of plantain are produced in Nigeria annually; with around 40 to 60% post-harvest. In order to avoid economic loss and optimize the potential nutritional benefits of plantain as well as increase the ease of storage and exportation, the people of West Africa, especially Nigerians have devised ways of processing and preserving plantain to less perishable products such as plantain chips and plantain flour (Okigbo and Nwankamma, 2005).

Sun drying is a common method which is used to extend the shelf life of plantain; this method of preservation helps to transform plantain into less perishable product, but may also compromise the nutritional value of the plantain. Sun drying also exposes the plantain to dirt, attack by insects, bacteria, deposition of fungal spores, and a varying degree of other environmental toxicants, depending on the site/location of drying. Adeyanju and Ikotun (1988) reported that plantain was contaminated with moulds and particularly toxigenic species such as *Aspergillus* spp, *Mucur* sp, *Rhizopus* sp*, and Penicillium* sp. Aflatoxins, a known secondary metabolite with potential teratogenic and mutagenic effects have also been reported in plantain flour (Peraica *et al*., 1999). This toxin can suppress the immune system and compromise vaccine efficacy in experimental animals (supply reference). Sun drying is a common method used to prolong the shelf life of plantain, however, this drying method may affect the nutritional content and also expose the plantain to fungi which can release toxins making the plantain unfit for consumption. The aim of this study was to investigate the nutritional, microbial and aflatoxin contents of sun dried and raw plantain.

## MATERIALS AND METHODS

### *Collection and Preparation of Sample*

Unripe plantain were bought from Oba market, in Benin City, Edo State, Nigeria, following which, the unripe plantain were washed with distilled water to remove dirt and other contaminants before weighing. Each plantain finger were hand peeled and chopped into pieces (approximately 2 mm thick) using a sterile stainless steel knife. A portion of the chopped plantain was analyzed immediately and was regarded as raw plantain. The other portion was subjected to sun drying for 4 days, analysis was done every 24hrs.

### *Isolation of Fungi*

Fungi isolation was done using the spread plate method. Briefly, approximately 1g of sample was weighed aseptically into a test tube containing 9ml of sterile peptone water,

the test samples were homogenized, and afterwards a six-fold serial dilution of each sample was made. Then, 1ml each of the appropriate dilutions ($10^2$, $10^3$and $10^5$) were inoculated into Petri dishes containing molten sterile potato dextrose agar (PDA) in which 0.05 mg of streptomycin had been added to suppress bacterial growth.

***Enumeration and Identification of Fungi Isolates***

Fungi which grew on the potato dextrose agar were counted and the mean was reported as the total heterotrophic fungi count. Morphologically distinct fungi colonies were further purified by repeated sub-cultures, and identified as previously described (Alexopoulus *et al*., 1996).

***Proximate Analysis***

***Determination of Moisture Content***

The moisture content of the samples was determined using air oven method (AOAC, 1990). The empty crucibles were weighed ($W_1$), then the crucible with approximately two grams of plantain were weighed ($W_2$) in triplicate, this was dried to constant weight at 105°C for 24 hrs. Upon cooling, the crucible with sample was weighed ($W_3$). The moisture content was calculated as percentage weight loss

$$\% \text{ Moisture Content} = \frac{W2 - W3}{W2 - W1}$$

$W_1$=Initial weight of empty crucible
$W_2$= weight of crucible + sample before drying
$W_3$=final weight of crucible + sample after

***Determination of Ash Content***

Ash content was determined using the furnaces incineration gravimetric method described by AOAC (1990). Approximately 5.0 g of sample was measured into a previously weighed crucible. Afterwards, the sample was burnt to ashes in a muffle furnace at 550°C, it was cooled and weighed, and percentage ash was calculated as follows;

$$\text{Ash} = \frac{\text{Difference in wt of Ash}}{\text{Wt. of sample}} \text{ x } 100$$

Difference in wt. of Ash= $W_3$ -$W_1$
$W_1$= Weight of empty crucible.
$W_2$= Weight of crucible + Sample
$W_3$= Weight of crucible + Ash

***Determination of Crude Lipid***

Crude lipid was determined by ether extract method using Soxhlet apparatus. Approximately 1 g of moisture free sample was wrapped in filter paper; this was then placed in fat free thimble and then introduced in the extraction tube. Afterwards, weighed, cleaned and dried receiving beaker was filled with petroleum ether and fitted into the apparatus. Water and heater was then turned on to start extraction. After 4 - 6 siphoning, the ether was allowed to evaporate, afterwards the beaker was then disconnected before the last siphoning. Extract was then transferred into clean glass dish with ether. Afterwards the dish was placed in an oven at 105$^o$C for 2 hrs and cooled in a desiccator. The percent crude fat was determined by using the following formula;

$$\% \text{ Crude Fat} = \frac{\text{Wt. of ether extract}}{\text{Wt. of sample}} \text{x } 100$$

***Determination of Crude Fibre***

The crude fibre was done according to the method of James (1995). Approximately 5.0 g of sample was boiled in 150 mL of 1.25 % $H_2SO_4$ solution for 30 min under reflux. Sample was then washed in several portions of hot water, and then a two-fold cloth was used to trap the particles. This was returned to the flask and boiled again in 150 mL of 1.25 % NaOH for 30 min under same condition. After washing, sample was allowed to drain dry, before being transferred to a weighed crucible and dried in the oven at 105$^o$C until a constant weight was achieved. Then, it was transferred to a muffle furnace where it was burnt until only ash was left .Afterwards the fibre content was calculated as follows;

$$\% \text{ Crude fibre} = \frac{W2-W3}{\text{Wt.of sample}} \times 100$$

$W_2$ = weight of crucible + sample after washing, boiling and drying

$W_3$= Weight of Crucible + Sample Ash

***Determination of Crude Protein***

This was done by Kjeldahl method described by Chang (2003).The total nitrogen was determined and multiplied with factor 6.25 to obtain protein content. Sample (0.5 g) was mixed with 10 mL of concentrated $H_2SO_4$ in digestion flask. A tablet of selenium catalyst was added to it before it was heated under a fume cup board until a clear solution was obtained (the digest). The digest was diluted to 100 mL in a volumetric flask and used for the analysis. The 10 mL of the digest was mixed with equal volume of 45% NaOH solution in a Kjeldahl distillation apparatus. The mixture was distilled into 10 mL of 40% boric acid containing 3 drops of mixed indicator (bromo cressol green/methyl red). A total of 50 mL of distillates was collected and titrated against 0.02 N EDTA from green to a deep red end point. A reagent blank was also digested, distilled and titrated. The nitrogen content and hence the protein content was calculated using the formula below:

$$\% \text{ Ash} = \frac{\text{Difference in wt of Ash}}{\text{Wt. of sample}} \times 100$$

1 mL of 1 N $H_2SO_4$ = 14 mg
Protein (%) = $N_2$ (%) x6.2

W= Weight of sample (0.5g)
N= Normality of titrant 0.02 ($NH_2SO_4$)
Vt= Total digest volume (100ml)
Va= Volume of digest analyzed (10ml)
T = Simple titre value
B= Blank titre value

***Carbohydrate Value***

The total carbohydrate content (%) in the samples was calculated by difference method, this is subtraction of the summation of other constituents in the food (protein, fat, water, fiber and ash) from the total weight of the food (AOAC, 1990).

% Total Carbohydrate = [100 – % (Protein + Fat + Moisture + Ash + Fiber)

***Mineral Analysis***

Sodium and potassium contents were determined using a Jenway digital flame photometer. This was done by digesting the ash with perchloric acid and nitric acid (Bonire *et al*., 1990); afterwards readings were taken using the digital flame photometer. Phosphorous was determined by the molybdovanadate method, while calcium, magnesium, and iron were determined using atomic absorption spectrophotometer (Buck Scientific, Norwalk United Kingdom) as described by Essien *et al*., 1992. Absorptions obtained were compared with the absorption standards of respective minerals spectrophotometer.

***Aflatoxin Determination***

Aflatoxin level was determined according to the method described by Shruti (2016), using an Enzyme Linked Immunosorbent Assay (ELISA) reader (Multiskan™ FC Microplate Photometer). Aflatoxin test kit (Romer Lab$^{sR}$ Inc, USA) was used to determine the aflatoxin content, according to the manufacturer's instruction. The absorbance (at 450 nm) and optical densities obtained for each sample were then extrapolated against a standard curve to obtain the aflatoxin content.

## RESULTS

Table 1 shows the total heterotrophic fungi count obtained from raw and sun dried plantain. Raw plantain recorded 1.40 ± 0.20 x $10^5$ cfu/g while the sundried plantain recorded 2.40 x $10^5$Cfu/g (day 1), 4.30 x $10^5$ cfu/g (day 2), 17.20 x $10^5$ cfu/g (day 3), 11.20 x $10^5$ cfu/g (day 4), before reducing to 12.50 x $10^5$ cfu/g (day 5).

Table 1: Total heterotrophic fungi count obtained from plantain

| | **Mean Count x $10^5$ cfu/g ± SD** | |
|---|---|---|
| **Days** | **Raw plantain** | **Sun dried plantain** |
| 0 | 1.40 ± 0.20 | ND |
| 1 | | 2.40 ± 0.10 |
| 2 | | 4.30 ± 0.30 |
| 3 | | 17.20 ± 2.60 |
| 4 | | 11.20 ± 2.00 |
| 5 | | 12.50 ± 0.50 |

Table 2 shows the proximate composition analysis obtained from the raw plantain and sundried plantain, moisture content recorded for the raw plantain was 52.67 % (day 0), after sun drying this reduced steadily in the following days to 42.83% (day 1), 42.30% (day 2) 30.70 % (day 3) and 26.70 % (day 4). Raw plantain recorded lipid content of 2.30% (day 1), while sun dried plantain ranged from 0.12 % (day 4) to 2.30 % (day 1). Ash content recorded for raw plantain is 2.40% while sun dried ranged from 2.21 %

(day 2) to 3.170 % on (day 4). The crude protein content recorded for raw plantain was 2.54% (day 0), sun dried ranged from and 2.00 % (day 3) to 2.40% (day 1). Crude fibre result recorded for the raw plantain flour was 0.80%, sun dried ranged from 0.80%, (day 1) to 5.40 % (day 4). The carbohydrate content recorded for raw plantain was 39.23% (day 0) while sun dried recorded carbohydrate content which ranged from 49.20% 3(day 3) to 64.31% (day 5).

Table 2: Proximate analysis of Sun dried plantain

| **Parameter** | **Days** | **Means ± standard deviation (%)** **Sun Dried** |
|---|---|---|
| Moisture | 0 | 52.67 ± 2.00 |
| | 1 | 42.83 ± 2.30 |
| | 2 | 42.30 ± 2.50 |
| | 3 | 23.30 ± 1.50 |
| | 4 | 26.70 ± 2.90 |
| Lipids | 0 | 2.30 ± 0.04 |
| | 1 | 2.30 ± 0.01 |
| | 2 | 1.40 ± 0.03 |
| | 3 | 1.00 ± 0.20 |
| | 4 | 0.12 ± 0.06 |
| Ash | 0 | 2.40 ± 0.16 |
| | 1 | 2.50 ± 0.30 |
| | 2 | 2.21 ± 0.30 |
| | 3 | 2.25 ± 0.30 |
| | 4 | 3.170 ± 0.30 |
| Protein | 0 | 2.54 ±0.30 |
| | 1 | 2.40 ±0.30 |
| | 2 | 2.10 ±0.10 |
| | 3 | 2.00 ±0.20 |
| | 4 | 2.01 ±0.02 |
| Fibre | 0 | 0.80 ± 0.0 |
| | 1 | 0.80 ± 0.02 |
| | 2 | 2.40 ± 0.01 |
| | 3 | 3.40 ± 0.01 |
| | 4 | 5.40 ± 0.00 |
| Carbohydrate | 0 | 39.29 ± 0.00 |
| | 1 | 49.20 ± 0.01 |
| | 2 | 49.60 ± 0.02 |
| | 3 | 54.00 ± 0.02 |
| | 4 | 64.63 ± 0.01 |

Figure 1 shows the mineral composition obtained from the plantain flour. The fresh plantain flour recorded the following values for the minerals P (0.28ppm), Na (1.34ppm), K (104.25ppm), Ca (0.36ppm), Fe (5.67ppm) and Mg (0.96ppm). Furthermore the sundried plantain flour recorded the following; P (0.35ppm), Na (1.42ppm), K (106.20ppm), Ca (0.10ppm), Fe (5.00ppm) and Mg (1.80ppm). The mineral composition observed for the oven plantain flour was; P (0.20ppm), Na (1.40ppm), K (106.50ppm), Ca (0.20ppm), Fe (4.07ppm) and Mg (0.680%).

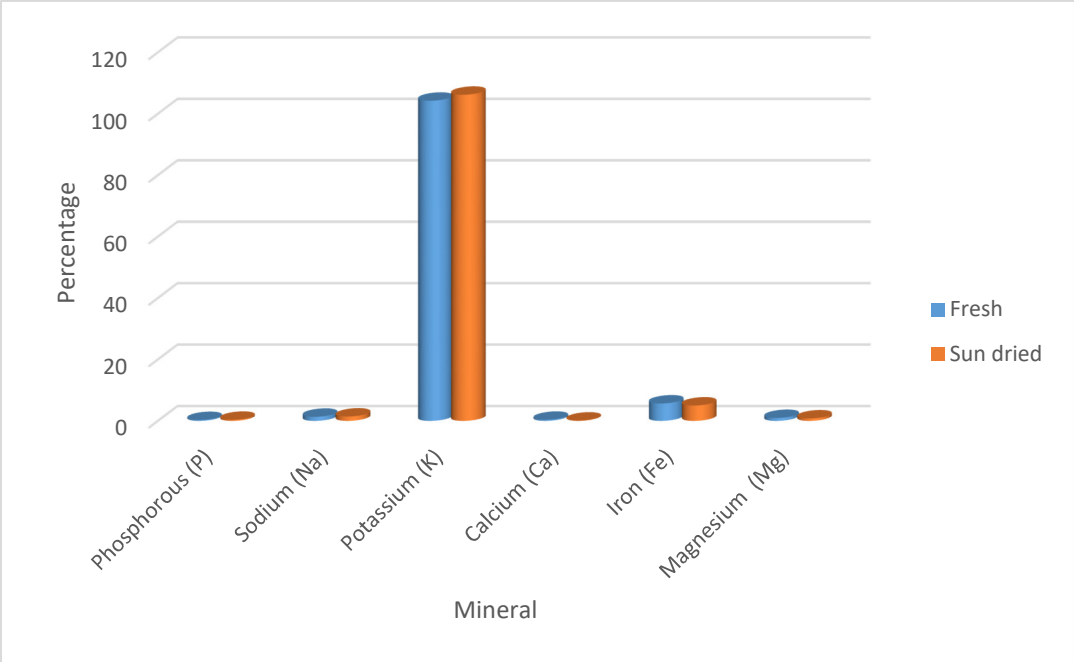


Fig. 1: Mineral Analysis of raw and sundried plantain

Figure 2 shows the percentage frequency of the fungi species isolated from sundried plantain. Fourty (40) fungi isolates were recorded, they included *Aspergillus* spp 10 (25.00%), *Penicillium* spp. 4 (10.0%), *Rhizopus* spp. 4 (10.0%), *Mucor* spp 8(20.0%), *Trichoderma* spp 7 (17.50%), *Alternaria* spp 7 (17.50%).

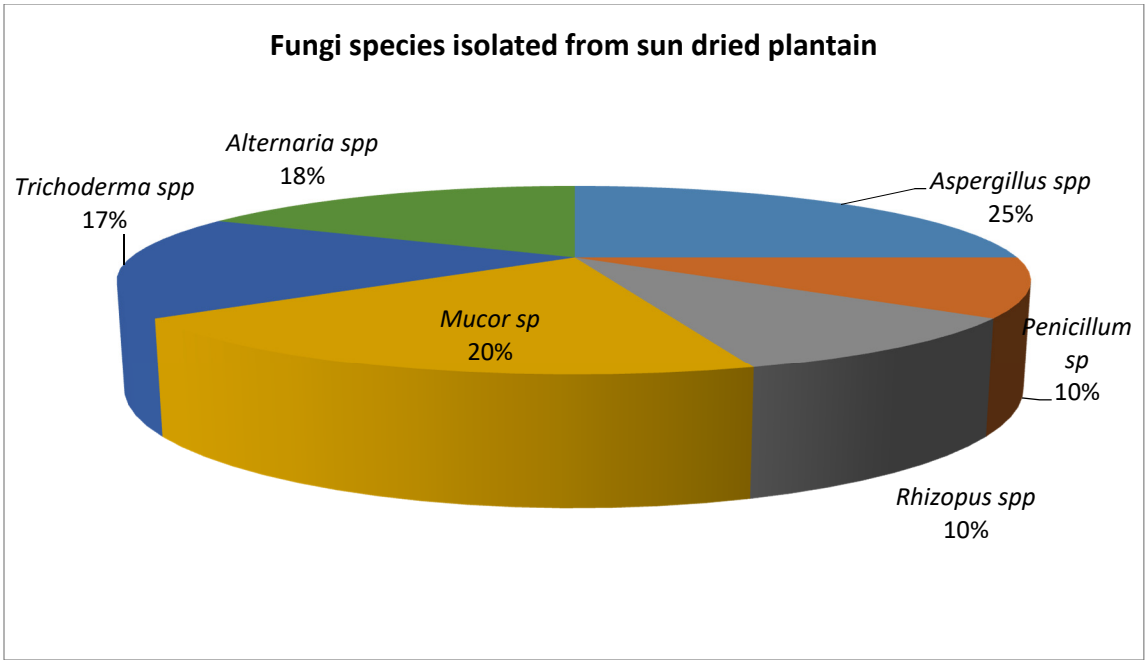


Fig. 2: Frequency of occurrence and percentage distribution of the fungi species isolated from sun dried plantain

Table 3 shows that the aflatoxin content of sun dried plantain was 1ppb, while the aflatoxin B1 and B2 levels were below the limit of detection.

Table 3: Aflatoxins Levels of Sundried and Raw Plantain

| Plantain | Total Aflatoxin (ppb) | Aflatoxin B1 (ppb) | Aflatoxin B2 (ppb) |
|---|---|---|---|
| Raw | ≤ LOD | ≤ LOD | ≤ LOD |
| Sundried | 1 | ≤ LOD | ≤ LOD |

Limit of Detection (LOD): ≥ 0.5

## DISCUSSION

Plantain is a poorly delineated source of human exposure to aflatoxins and pathogenic fungi. The current study was therefore aimed at investigating the effect of sun drying on the nutritional, mycoflora and aflatoxin contents of plantain. The proximate analysis showed that the moisture, lipid and protein contents of plantain flour were reduced after drying under the sun. The moisture content recorded for the raw plantain was 52.70%, Agoyero *et al.* (2011) has reported moisture contents of 59.40%. Moisture content give an indication of anticipated shelf life of a food, as low moisture content is a requirement for a longer shelf life. Fat/oils, indicating the total lipid content of the plantain were shown to reduce during drying; similar findings have been reported by Agoyero *et al.* (2011) who stated that unripe plantain gives values of 2.75% (fresh) and 1.38% (after sun drying). Solar radiations mediated oxidation of the composite lipids, especially the unsaturated fatty acids decreases overall crude lipids

content, lipid oxidation is known to be increased by many factors such as heat, sun light and radiations (Savage *et al*., 2002).

Protein is important in immune functions, transport of oxygen and repair of worn out tissues "crude protein" measures the level of protein in food. Crude protein reduced after sun drying; this implies that sun drying does not conserve crude protein. In the presence of atmospheric oxygen, proteins contained in exposed tissues tend to react, forming several intermediates which make the amino group of the amino acids non bio-available. Decrease in protein content in this analysis probably occurred as a result of Millard reaction; which results between carbohydrates and protein (Wiriya *et al*., 2009). Similar losses of crude protein by the application of heat have been reported (Enomfon-Akpan and Umoh, 2004; Morris *et al*., 2004). Ash is the inorganic residue after the water and organic matter have been removed by burning a food sample; it gives an indication of the total amount of minerals in a food. The increase in the ash content could be as a result of the removal of moisture which tends to increase the concentration of nutrients (Morris *et al*., 2004). Increase in ash content of *Musa paradisiaca*, *Discorea rotundata* and *Colocasia esculenta* after drying have been reported by Agoreyo *et al*. (2011) in Benin City. Crude fiber measures the cellulose, hemicellulose and lignin content of food; this was determined to be 0.80 % (fresh), previous studies have recorded 0.98% and 0.90% (Zakpaa *et al*., 2010) for fresh plantain while sun dried plantain recorded 5.40%. Crude fibre enhances the transit time through the bowels, facilitates bowels movement thus reducing the risk of colon cancer.

Minerals are inorganic and have low volatility compared to other food components. The amount of mineral elements recorded in this analysis was generally low, this findings does not concur with the results of Baturh and Ruth (2015) who investigated the effect of drying methods on the nutritional importance of plantain in Benue. The disparity observed in the mineral contents may be as result of the plantain cultivar, different conditions of experimental analysis as well as the soil type (Zakpaa *et al*., 2010). The decrease in the Fe, Mg and Ca after sun drying could be due to the anti-nutritional factors, oxalate and phytate (unfortunately these were not determined in the present study) present in the sample, thereby making the mineral unavailable by forming complexes with them, as reported by Enomfon-Akpan and Umoh, (2004). Furthermore, Alinnor and Akalezi (2010) reported the decrease in Fe of cocoyam tuber, white yam and coco yam chips respectively after drying. Sun drying has also been reported to reduce calcium and magnesium content of all the tropical food crops except cocoyam chips in Benin City (Lawal *et al*., 2012).

The fungi species *Penicillum* sp, *Mucor* sp., *Aspergillus flavus Aspergillus niger, Rhizopus spp. Penicillum* sp. *Aspergillus spp, Trichoderma spp* and *Alternaria spp* isolated in this study have be reported by Fagbohun *et al*., (2010) in Ado-Ekiti, when they investigated the mycoflora and nutrient content of sundried cassava chips (*Manihot*

*esculenta*) during storage. The presence of these fungi species maybe due to their ability to secrete extracellular digestive enzymes (Lawal *et al*., 2012) and their ability to tolerate the drying condition. Inadequate processing and handling practices such as spreading on the floor to enhance drying may facilitate contamination.

It was observed that the sun dried plantain recorded a very low total aflatoxin level (1ppb), aflatoxin B1 and B2 levels were below the limit of detection despite the fact that *Aspergillus flavus* was found on the sun dried plantain. Ige *et al*. (2012) stated that the presence of *Aspergillus flavus* in a sample does not necessarily indicate the presence of aflatoxin. In Tanzania, 18 samples of cassava products processed by small holder farmers using sun drying and solid state fermentation showed no aflatoxins contamination (Muzanila *et al*., 2000). This asserts that this plantain does not pose any public health risk. However, the absence of aflatoxins in mouldy sun dried samples may suggest possible prevalence of other mycotoxins that were not tested in this study.

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