

Microbial Quality and the Occurrence of Aflatoxins In Plantain/ Yam And Wheat Flours In Ado-Odo Ota

Okafor, S. E.*, and Eni, A. O.

Department of Biological Sciences, College of Science and Technology, Covenant
University, Ota, Ogun State, Nigeria.

Correspondence: Email: nuella954@gmail.com

ABSTRACT

Flours made from various foods including plantain, yam and wheat are a major part of daily diet for millions of people in Nigerian. If these food crops are not dried rapidly and thoroughly prior to milling, fungal growth and mycotoxin production can occur. Aflatoxins, a type of mycotoxin produced by *Aspergillus* species have been classified as Group 1 human carcinogens hence should be monitored in routinely consumed foods as the populace maybe potentially exposed to doses of aflatoxins in their daily diet. This study sought to determine the microbial quality and the occurrence of fungi and aflatoxins in plantain, yam and wheat purchased from four markets (Oja-ota, Sango, Atan and Owode markets) in Ado Odo Local Government Area. The mean microbial count for each sample was determined by plating each sample on nutrient agar and fungi was isolated by plating on Potato Dextrose Agar. The total aflatoxin content of the food samples was determined using the Agra Quant® competitive enzyme linked immunosorbent assay (ELISA) kit. The highest mean microbial count (9.30×10^{13} cfu/g) was observed in a plantain flour bought from Sango market while the lowest (1.16×10^{12} cfu/g), was observed in wheat flour from Oja-Ota market. *Aspergillus flavus* was the predominant (31%) aflatoxigenic fungi isolated compared to *A. niger* (21%). The other fungi isolated include *Rhizopus spp*, *Geotrichium spp*, Yeast, *Penicillium spp* and *Paecilomyces spp*. Aflatoxin was detected in all the food samples tested in this study at concentrations ranging from 0.2 ppb to 5.9 ppb which were all within the CODEX Alimentarius Commission (CAC) aflatoxin acceptable limit of 15 ppb.

Keywords: Aflatoxins, ELISA, *Aspergillus spp*, *Rhizopus spp*, *Geotrichium spp*, Yeast, *Penicillium spp* and *Paecilomyces spp*.

1. INTRODUCTION

The major degradation agents of foods and feedstuffs are fungi which are ubiquitous plant pathogens. Fungal infection of plants results in poor crop yield and quality which translate to economic losses, also toxicity impacts mycotoxins (Makun *et al.*, 2010).

Mycotoxins are secondary metabolites produced by the toxigenic strains of fungi during their stationary phase, which impact food stuffs in pre-harvest or under post-harvest conditions.

Over 400 mycotoxins have been discovered, but scientists pay more attention to those that have proven to be carcinogenic and/or toxic to humans and animals. The mycotoxins that are considered to be the most significant medically and in the food industry include aflatoxins (aflatoxin B₁), ochratoxins (ochratoxin A), fumonisins (fumonisin B1), zearalenone, patulin and trichothecenes (deoxynivalenol) (Huffman *et al.*, 2010).

Aflatoxins are produced mainly by *Aspergillus parasiticus* and *Aspergillus flavus* which are found in many countries, especially in the tropical and subtropical regions where there is optimal condition that supports the growth of fungi and the production of toxin (Rustom,



1997). Aflatoxin B₁ (AFB₁), Aflatoxin B₂ (AFB₂), Aflatoxin G₁ (AFG₁) and Aflatoxin G₂ (AFG₂) are the four naturally occurring types amongst the 18 different types of aflatoxins (Filazi and Sireli, 2013). They were named according to the colour they produce when they fluoresce under ultraviolet light; Aflatoxin B₁ and B₂ produces a strong blue colour, hence the “B”, while Aflatoxin G₁ and G₂ are so named because of their greenish-yellow fluorescence (Kensler *et al.*, 2011).

Ochratoxin A is a secondary metabolite of several *Aspergillus* and *Penicillium* spp (Duarte *et al.*, 2010). Trichothecene was first isolated from *Trichothecium roseum* (Yazar and Omurtag, 2008). Fumonisin is a group of non-fluorescent mycotoxins. They were first isolated from the *Fusarium verticillioides* (Yazar and Omurtag, 2008). Zearalenone is a mycotoxin that is produced primarily by the fungus *Fusarium graminearum* while Patulin is produced by several species of *Aspergillus*, *Penicillium* particularly *Penicillium expansum* (Puel *et al.*, 2010).

Both in man and animals, the effects of mycotoxins can be chronic manifesting in the form of toxicity to the nervous, respiratory, digestive and circulatory systems or death in extreme cases. These toxins have attracted public health concerns owing to their nephrotoxicity, teratogenicity, immunotoxicity, etc. (Bhat and Vasanthi, 2003). Although inhalation and dermal contact can also expose one to mycotoxins most cases of mycotoxicoses in animals and humans occur through ingestion. Aflatoxin exposure to humans can be direct via consumption of plant products, or indirect via consumption of animal products (meat, milk and eggs) (CAST, 2003).

Within normal food processing temperature range (80°C-121°C), there is little or no destruction of these toxins and they are therefore said to be heat-stable. Therefore, under normal cooking conditions, such as frying and boiling or even following pasteurization; these toxins remain active (Milicevic *et al.*, 2010). Some of the effective food processes include physical treatments such as sorting, cleaning and milling, thermal processing done at very high temperature above 150°C such as baking, frying, roasting (Bullerman and Bianchini, 2007).

Aflatoxins have been classified as a Class 1 human carcinogen by the International Agency for Research on Cancer (IARC, 1993) as it leads to a disease called “hepatocellular carcinoma” (liver cancer) when ingested (Williams *et al.*, 2004), is the third-leading cause of cancer worldwide with about 600,000 fresh cases each year.

In relation to animals, aflatoxin contamination has been shown to reduce food intake, increase liver and kidney weights of farm animals, as well as induce immunosuppression and hepatitis in them; all of which contribute to increased mortality in farm animals (Hussein and Brassel, 2001; Zain, 2011).

Yam flour (gbodo), wheat flour and plantain flour (elubo ogede) are major staple foods of Ado-Odo Ota. The residents of these area consume these products because of their nutritional value.

Poor drying during processing or storage conditions below optimal conditions often encourages contamination by fungi such as *Aspergillus*, *Fusarium* and *Penicillium*. This is because of the residual moisture content in the drying products which predisposes them to mould growth.

Many of the *Aspergillus* fungi, especially *A. flavus* and *A. parasiticus* produce aflatoxins. Aflatoxins are among the most powerful teratogenic, mutagenic and carcinogenic compounds that occur naturally (Jackson and Al-Taher, 2008) and have a striking association with impaired growth in children (Egal *et al.*, 2005; Gong *et al.*, 2003). The disease that results from the ingestion of aflatoxins is referred to as Aflatoxicosis and one of the largest and most

acute outbreaks of aflatoxicosis ever documented occurred in Kenya in 2004 with 317 cases reported and 125 deaths recorded (CDC, 2004).

There is therefore a need for routine assessment of aflatoxins to ensure these food products are safe for consumption. Hence, this research was aimed to determine the microbial quality and the occurrence of aflatoxins of some foods sold in four different markets in Ota, Ogun State.

1.1 Fungi

Fungi are a group of organisms that have a well-defined nuclei but lack chlorophyll, a characteristic of most other plant (Mavor and Harold, 1966). They are a subdivision of the subkingdom Thallophyta. They are made up of assimilative body which could be ameboid or unicellular in some species. Vascular tissue in these organisms are absent. Fungi are heterotrophs and are also made up of multicellular branching filaments called hyphae reproducing asexually by the means of spores (Talbot, 1971). Fungi are ubiquitous eurythermal organism which can grow in a wide range of habitats (Sancho *et al.*, 2007; Hawksworth, 2006; Mueller and Schmit, 2006).

1.2 Mycotoxins and mycotoxin-producing fungi

Mycotoxins are low-molecular-weight natural products produced during the secondary metabolism of filamentous fungi. These metabolites constitute a toxigenically and chemically heterogenous assemblage which can cause disease and death in humans and other vertebrates (Bennett, 1987). The term mycotoxin originated after an unusual veterinary crisis took place near London, England in the year 1960 during which approximately 100,000 turkey poults died. This mysterious outbreak of turkey X disease was traced to a peanut (groundnut) meal which was contaminated by Aflatoxins, a secondary metabolite produced by *Aspergillus flavus* (Bennett and Klich, 2003). It is important to note that while all mycotoxins are of fungal origin, not all toxic compounds produced by fungi are called mycotoxins. In referring to mycotoxins, the target and the level of concentration is important. Hence, mycotoxins are made by fungi and are toxic to vertebrates and animal groups in low concentrations (Bennett, 1987). Thus, mycotoxins can be classified as hepatotoxins, nephrotoxins, neurotoxins, immunotoxins, and so forth. Organic chemists have attempted to classify them by their chemical structures (e.g., lactones, coumarins); Cell biologists put them into generic groups such as teratogens, mutagens, carcinogens, and allergens.

In fungal growth and development, mycotoxins have no biochemical significance; however, they vary from simple C₄ compounds, e.g. moniliformin, to complex substances such as the phomopsins. Mycotoxins may develop on various foods and feeds at suitable temperature and humidity (Dinis *et al.*, 2007). Mycotoxin exposure to humans may be as a result of exposure to air and dust containing toxins (Jarvis, 2002), consumption of plant-derived foods that are contaminated with toxins and the carry-over of mycotoxins and their metabolites in animal products such as meat and eggs which are then consumed. (CAST, 2003).

There are three major genera of fungi that produce mycotoxins and they include *Aspergillus*, *Fusarium* and *Penicillium*. Currently, more than 300 mycotoxins are known. Some of them are of great public health and agro-economic significance and they include aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins and ergot alkaloids (Hussein and Brassel, 2001).

1.3 Aflatoxins

Aflatoxins are a group of chemically related mycotoxins produced by a large number of *Aspergillus* species, primarily *Aspergillus flavus* and *Aspergillus parasiticus*. Other species of *Aspergillus* that can produce these mycotoxins include *A. nomius*, *A. pseudotamarii*, *A. parvisclerotigenus*, *A. bombycis* of section *Flavi*, *A. ochraceoroseus* and *A. rambellii* from

section *Ochraceorosei* (IARC, 2002; Frisvad *et al.*, 2004). *Aspergillus* is a fungus that grows optimally at 25 °C with a minimum necessary water activity of 0.75. Secondary metabolites are produced by *Aspergillus* at 10-12 °C, but the most toxic ones are produced at 25°C with a water activity of 0.95 (Hesseltine, 1976). *Aspergillus* contaminate a large portion of the world's food, some of which include: maize, rice, groundnut, peanut, barley, wheat and soya (Saleemullah *et al.*, 2006; Masoero *et al.*, 2007).

Approximately eighteen types of aflatoxins have been identified, but there are four major naturally occurring types which includes aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁) and aflatoxin G₂ (AFG₂) (Saleemullah *et al.*, 2006). AFB₁ and AFB₂ are primarily produced by *Aspergillus flavus* while AFG₁ and AFG₂ are primarily produced by *Aspergillus parasiticus* (Goto *et al.*, 2013).

The order of toxicity of these aflatoxins is AFB₁>AFG₁>AFB₂>AFG₂. However AFB₂ and AFG₂ are typically nontoxic except they are metabolized into AFB₁ and AFG₁ respectively inside the cells (Kensler *et al.*, 2011; Filazi and Sireli, 2013). AFB₁ is always genotoxic in vitro and in vivo (EFSA, 2007).

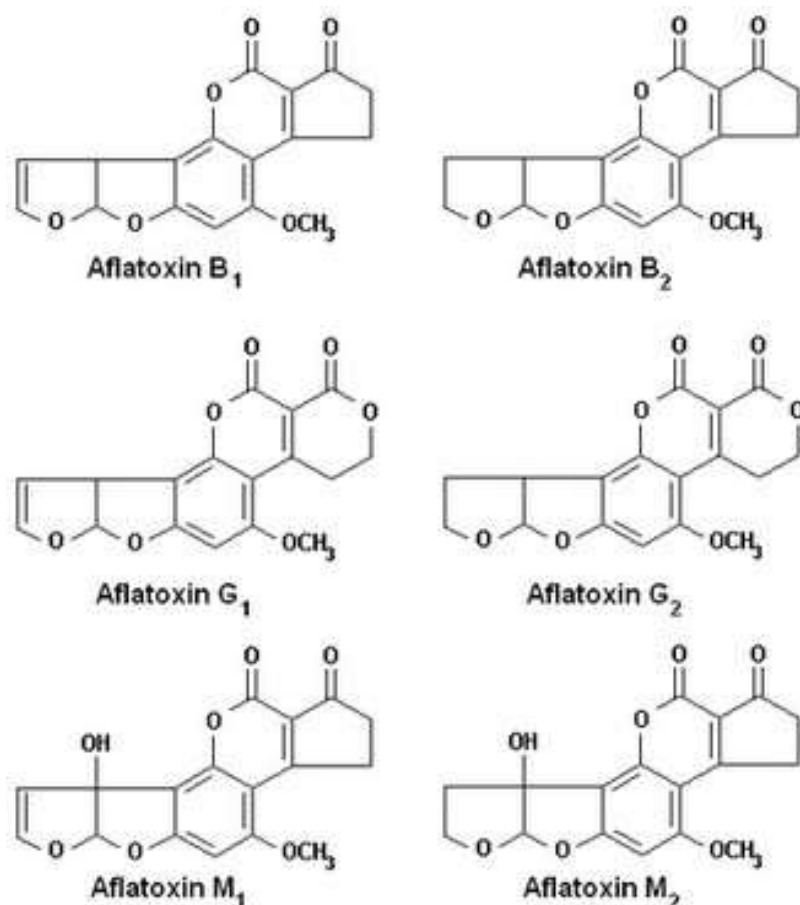


FIGURE 1: Chemical structures of the four major types of aflatoxins (Zain, 2011)

The major types of aflatoxins are named due to blue (B) or green (G) fluorescence under ultraviolet light and their migration patterns during chromatography (Wogan and Busby, 1980; Diekmann and Green, 1992). Since 1987, aflatoxin B₁ has been classified as a group 1

carcinogen (that means carcinogenic to humans) by The International Agency for Research on Cancer (IARC, 1993), and a group 1 carcinogenic agent since 1993 due to the exposure to hepatitis B virus (Castegnaro & McGregor, 1998). The most prevalent aflatoxin usually found in cases of aflatoxicosis is aflatoxin B1 which is responsible for carcinogenicity, acute toxicity, chronic toxicity, immunotoxicity, teratogenicity and genotoxicity. The metabolic derivative of AFB1 is AFM1 while the metabolic derivative of AFB2 is AFM2; both come from the metabolism of some animals, and are normally found in milk and urine (Strosnider *et al.*, 2006).

1.4 Factors influencing the growth of fungi and mycotoxin-producing fungi in food

Mycotoxigenic fungi are those fungi that produce mycotoxins. Members of the genera *Aspergillus*, *Penicillium* and *Fusarium* are the major mycotoxin-producing fungi. Different factors can promote the growth of fungi and the production of mycotoxins in food items. However, the most important factors include:

i.) Temperature: The optimum temperature of *Penicillium* is 25°C to 30°C while that of *Aspergillus* is 30°C to 40°C. The maximum temperature of *Penicillium* is 28°C to 30°C while that of *Aspergillus* is 37°C to 47°C. Various *Fusarium* species can also be regarded as psychrophilic, because of their low optimum temperature of 8°C to 15°C (Moss, 1991).

ii.) Water activity: This is the amount of unbound water in the food which is available for fungal growth. Most storage fungi grow at water activity of less than 0.75 while water activity appreciation varies between 0.61 and 0.91 (Moss, 1991). According to Smith and Moss (1985), moisture content determines whether a substrate can be colonized by microorganisms. These factors enable fungi to degrade complex macromolecular compounds and utilize them for their growth and reproduction; in this process mycotoxins are produced and secreted (Moss, 1996).

iii.) Oxygen: Though oxygen is a necessary factor for the growth of fungi, certain species can grow under anaerobic conditions. The production of mycotoxins is also influenced by oxygen. The growth of *Aspergillus* is restricted to an oxygen concentration of less than 1% (Pitt and Hocking, 1997).

iv.) pH: Fungi compete with bacteria as food spoilers at high water activities (Wheeler *et al.*, 1991). Most fungi are slightly affected by pH over a broad range, commonly 3 to 8 (Wheeler *et al.*, 1991), however, the pH of a medium may exercise important control over a given morphogenic event without remarkably influencing the overall growth of a fungus (Pitt and Hocking, 1997).

Furthermore, poor hygienic practices during transportation and storage may also facilitate fungal growth which will subsequently lead to mycotoxin production in food items.

1.5 Implication of mycotoxin contamination

The consumption of mycotoxin contaminated food is detrimental to both human and livestock as mycotoxicoses can emerge. Mycotoxicoses can generally be categorized into acute and chronic. Acute toxicity is elicited in a short time while chronic toxicity is characterized by low-dose exposure over a long period of time, leading to cancer and other irreversible effects. The consumption of high to moderate amount of mycotoxins results in acute primary mycotoxicoses while the consumption of moderate to low amount of mycotoxins results in chronic primary mycotoxicoses.

Implication on human health

Acute aflatoxicoses, neonatal jaundice, growth retardation, carcinogenicity and immunological suppression in humans are all as a result of aflatoxins. Since aflatoxin has

been classified as a Group 1 carcinogen by IARC, it is of paramount importance, hence its disease pathway in humans (Figure 5).

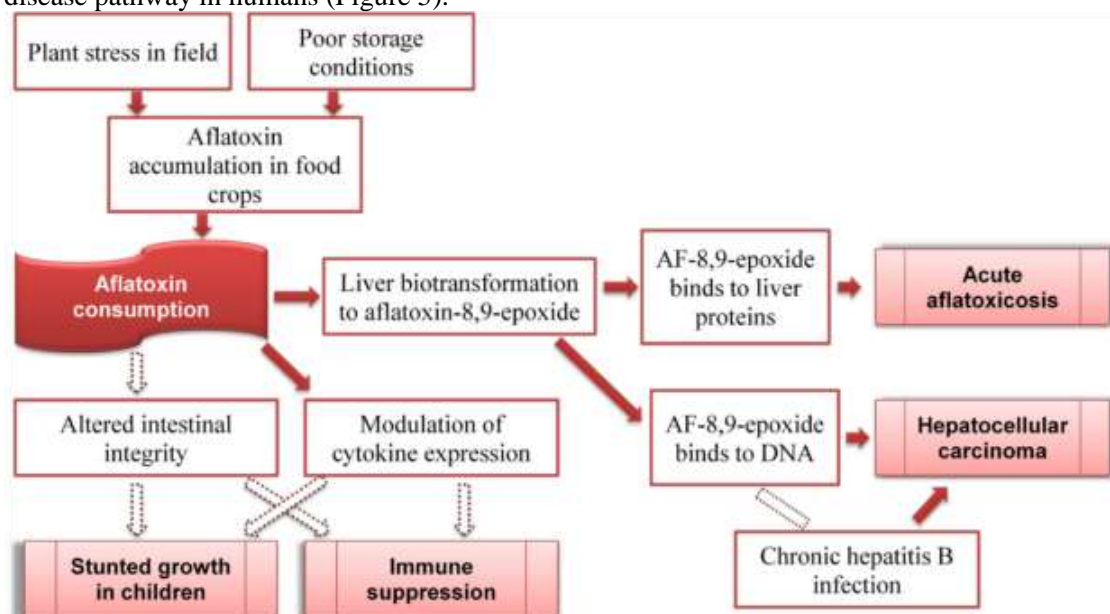


FIGURE 2: Aflatoxin and disease pathways in humans (Wu and Khlangwiset, 2010)

From the figure above, the key predisposing factor in pre-harvest aflatoxin contamination is stress of the host plant in field which could be as a result of drought stress, high temperatures or insect damage while the key predisposing factor in post-harvest aflatoxin contamination is poor storage conditions which can range from pest-related crop damage, excessive heat and moisture to prolonged time of storage.

The toxicity of aflatoxin consumption can be exerted in several ways. The intestinal integrity may be altered (Gong *et al.*, 2008) or the expression of cytokines may be modulated. These effects may result in the suppression of the immune system or stunted growth in children.

Aflatoxin is transformed in the liver to its DNA reactive form aflatoxin-8,9-epoxide by P450 enzymes. This molecule may bind to liver proteins and lead to liver failure which will result in acute aflatoxicoses and it may also bind to DNA which serves as a precursor for aflatoxin-induced hepatocellular carcinoma (liver cancer). Chronic infection with hepatitis B virus can have a synergistic effect with aflatoxins which could result in a significantly higher level of cancer risk.

Acute aflatoxicoses is characterized by haemorrhage, edema, alteration in digestion, acute liver damage (which manifests as severe hepatotoxicity with a case fatality rate of approximately 25%). Low-grade fever, anorexia and malaise are early symptoms hepatotoxicity from aflatoxicoses. Acute exposure to these toxins can progress to potentially lethal hepatitis with jaundice, vomiting, abdominal pain and even death (Strosnider *et al.*, 2006).

The effects of chronic aflatoxicoses are usually subclinical and difficult to recognize. However, some of the common symptoms include impaired food conversion and slower rates of growth with or without the production of an overt aflatoxins syndrome. Chronic aflatoxin exposure causes Hepatocellular Carcinoma (HCC), generally in association with hepatitis B

virus or other predisposing factors. HCC is the sixth most prevalent cancer worldwide (Parkin *et al.*, 2005). It is also important to note that aflatoxicoses can also be as a result of inhalation of the fungal spores as reported in some clinical cases (Dvorackova, 1976).

Implication on animal health

The effects of aflatoxin consumption in all animals is similar, however their susceptibility varies by individual variation, species and age. Anorexia, weight loss, depression, disease, gastrointestinal bleeding, pulmonary edema and liver damage are all symptoms of acute aflatoxicoses (Denli and Pérez, 2006).

Economic Implication of Mycotoxins

Mycotoxin contamination has a far reaching impact on economies. Mycotoxin contamination can also reduce the income of farmers. In respect to livestock production, contamination can lead to mortality which could result in reductions in productivity, weight, feed efficiency, fertility, ability to resist diseases and decrease in the quantity and quality of meat, milk and egg production.

1.6 Mycotoxin levels in food

The consumption of mycotoxin contaminated food will lead to mycotoxicoses when the level of mycotoxins that is not safe for consumption is ingested. Hence, the establishment of toxicological safe limits by different regulatory bodies.

TABLE 1: Toxicological Safe Limits for Mycotoxins (Kibe, 2015)

Mycotoxins	Safe Limit
FB1	2.0 µg/kgbodyweight/day
FB2	2.0 µg/kgbodyweight/day
Total FBS	2.0 µg/kgbodyweight/day
AFB1	1.0 ng/kgbodyweight/day
AFB2	1.0 ng/kgbodyweight/day
AFG2	1.0 ng/kgbodyweight/day
DON	1.0 ng/kgbodyweight/day
T-2	0.06 µg/kgbodyweight/day
ZEN	05 µg/kgbodyweight/day
OTA	5.0 ng/kg body weight/day

2. MATERIALS AND METHOD

2.0 Sample Collection

Yam flour, wheat flour and plantain flour were purchased from four markets (Oja-Ota, Sango, Atan, Owode) in Ado-Ota Ota, Ogun State, Nigeria.

2.1 Sample Preparation

For sample preparation, 10ml of saline solution was dispensed into three test tubes. For each sample, 1g was placed into the first test tube containing 10ml of saline solution making a 10^{-1} serial dilution, the solution was vortexed and left to stand for 30 minutes then 1ml of the supernatant was taken out from the first tube and put into the second test tube with 9 ml of saline solution to make a 10^{-2} dilution. The serial dilution was repeated until 10^{-11} dilution was obtained.

2.2 Microbial Load

To determine the microbial load of each sample, 1ml of each dilution 10^{-1} to 10^{-11} was dispensed into sterile labelled petri dishes and 20ml of NA was added to it using the pour plate method (Van soestbergen and Ching, 1969). The plates were incubated at 37 °C for 24 hours. A colony count of each culture plate was performed to determine the optimal dilution factor. The optimal dilutions (10^{-8} to 10^{-11}) were used and 1ml of each's sample optimal dilutions were plated in duplicates and 20 ml of NA was added to it, swirled gently and left to solidify. The plates were incubated at 37 °C for 24 hours.

2.3 Isolation of fungi

To isolate fungi, 1ml of each's sample optimal dilutions (10^{-5} to 10^{-7}), was inoculated into a petri dish and 20ml of molten PDA was added using the pour plate method (Van soestbergen and Ching, 1969). The plates were swirled gently and left to solidify. The plates were incubated at 25 °C for 5-7 days. To obtain a pure culture, each distinct fungal colony was sub cultured on fresh petri dishes of PDA using grafting method and incubated at 25 °C for 5-7 days for subsequent taxonomic identification.

2.4 Identification of fungi

The isolated fungal colonies were identified on the basis of their micro and macro morphological characteristics.

2.4.1 Microscopy

A drop of mounting fluid, lactophenol cotton blue solution was placed on a grease free slide. A mycelial mat was transferred on fluid using a sterilized and cooled needle. It was pressed gently to enable it mix properly with the stain. A sterile forceps was then used to place a coverslip on the mycelial mat and blotting paper was used to wipe the excess stain. The preparation was examined under low to high power objectives of the microscope.

2.5 Detection of total aflatoxins in food samples using Enzyme Linked Immunosorbent Assay (ELISA)

The total aflatoxin assay was carried out using the The AgraQuant® Total Aflatoxin Assay 1-20 ppb order #: COKAQ1100. It is a direct competitive ELISA that determines a concentration of total aflatoxin present in a sample. Aflatoxins in samples and control standards are allowed to compete with enzyme-conjugated aflatoxin for the antibody binding sites.

2.5.1 Sample preparation/ extraction

Five grams of the samples were weighed and placed in a clean jar. 25ml of 70/30 (v/v) methanol/water extraction solution was added to the jar and sealed properly (the samples were extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively). The mixture was shaken for 3 minutes. The samples were allowed to settle and the top layer of the extract was filtered through a whatman #1 filter and the filtrate was collected.

2.5.2 Assay

Seventeen blue-bordered dilution strips were placed in a microwell strip holder. Seventeen antibody coated microwell strips were also placed in a microwell strip holder. Afterwards, 200 μL of conjugate was dispensed into each blue-bordered dilution well. Then 100 μL of each standard or sample was added into the appropriate dilution well containing the 200 μL of conjugate (a fresh pipette tip was used for each standard or sample). Each well was properly mixed by pipetting up and down 3 times and 100 μL of the contents from each dilution well was immediately transferred into a corresponding antibody coated microwell. The microwell was incubated for 15 minutes at room temperature. The contents of the microwell strips were discarded. The microwells were washed by filling each microwell with distilled water, and then the water was dumped from the microwell strips. The microwells were washed five times. Several absorbent paper towels were layered on a flat surface and the microwell strips were tapped on the towel to expel as much residue water as possible. The bottom of the microwells were also dried with a dry cloth or towel. After which 100 μL of the substrate was pipetted in to each microwell strip and a blue color developed. It was incubated at room temperature for 5 minutes. Then 100 μL of stop solution was pipetted into each microwell strip. The color was changed from blue to yellow (the intensity of the color is inversely proportional to the concentration of aflatoxin present in the sample or standard). The strips were read with a microwell reader at 450 nm filter. The optical density (OD) reading was recorded for each microwell. The OD of the samples were compared to the OD's of the standards and an interpretative result was determined.

3. RESULTS

A total of twelve samples were evaluated in this study and they comprise of three samples each (yam flour (Y), wheat flour (W) and plantain flour (P) from market 1 (Oja-Ota), market 2 (Sango), market 3 (Atan) and market 4 (Owode).

Results of this study showed that the sample with the highest microbial load is plantain flour from Sango at 9.30×10^{13} cfu/g while the sample with the lowest microbial load is wheat flour from Oja-Ota at 1.16×10^{12} cfu/g. From Oja-Ota market, plantain flour had a microbial load of 2.02×10^{12} cfu/g while yam flour had a microbial load of 1.21×10^{12} cfu/g. Wheat flour from Sango market had a microbial load of 4.88×10^{13} cfu/g while yam flour had a microbial load of 1.6×10^{12} cfu/g. From Atan market, yam flour had a microbial load of 7.38×10^{13} cfu/g, wheat flour had a microbial load of 3.51×10^{13} cfu/g while plantain flour had a microbial load of 3.08×10^{12} cfu/g. Plantain flour from Owode market had a microbial load of 3.08×10^{12} cfu/g, wheat flour had a microbial load of 1.94×10^{12} cfu/g and yam flour had a microbial load of 1.27×10^{12} cfu/g (Figure 3).

The result obtained from this study showed that a total number of seven fungi were isolated from the common staple foods purchased from the four different markets in Ado-Odo Ota. The isolated fungi include *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus spp*, *Geotrichum spp*, Yeast, *Penicillium spp* and *Paecilomyces spp*.

Out of the twelve food samples used in this study, *Aspergillus flavus* was isolated from 31% of the food samples, *Aspergillus niger* was isolated from 21% of the food samples, *Rhizopus spp* was isolated from 14% of the food samples, *Paecilomyces spp* was isolated from 10% of the food samples, Yeast was isolated from 10% of the food samples, *Penicillium spp* was isolated from 7% of the food samples and *Geotrichum spp* was isolated from 7% of the food samples (Table 2).

A. flavus, *A. niger*, *Rhizopus spp*, *Geotrichum spp*, Yeast, *Penicillium spp* and *Paecilomyces spp* were isolated from the yam flour samples purchased from the various markets. *A. flavus* had the highest incidence level at 25% while *Geotrichum spp*, Yeast and *Paecilomyces spp* had the lowest incidence level at 8% each. *A. niger*, *Penicillium spp* and *Rhizopus spp* had 17% incidence level each. The fungi isolated from the plantain flour samples purchased from the four different markets include: *A. flavus*, *A. niger*, *Rhizopus spp*, *Paecilomyces spp* and Yeast. *Aspergillus flavus* had the highest incidence level at 38% while *Rhizopus spp*, Yeast and *Paecilomyces spp* had the lowest incidence level at 13% each. *A. niger* had 25% incidence level. The fungi isolated from the wheat flour samples purchased from the four different markets include: *A. flavus*, *A. niger*, *Rhizopus spp*, *Paecilomyces spp*, Yeast and *Geotrichum spp*. *A. flavus* had the highest incidence level at 33% while *Geotrichum spp*, Yeast, *Rhizopus spp* and *Paecilomyces spp* had the lowest incidence level of 11% each. *A. niger* had 22% incidence level (Figure 4).

This research study showed that two strains of *Aspergillus* were isolated from the food samples purchased from the various markets in Ado-Odo Ota, Ogun state and they include: *Aspergillus flavus* and *Aspergillus niger*. *Aspergillus flavus* had a higher incidence level at 31% (112°) while *Aspergillus niger* had an incidence level of 21% (74°) amongst other isolated fungi (Figure 5).

Out of the twelve food samples purchased from the four markets, *Aspergillus flavus* was isolated from nine food samples. *Aspergillus flavus* was isolated from yam flour, wheat flour and plantain flour purchased from Oja-Ota, Sango and Atan markets. However, the food samples (yam flour, wheat flour and plantain flour) purchased from Owode market contained no *Aspergillus flavus*. Aflatoxins were detected in the twelve food samples purchased from the four markets (Table 3).

Total aflatoxins: Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2) were detected in the twelve food samples purchased from Oja-Ota, Sango, Atan and Owode markets but were within the aflatoxin acceptable limit of 15 ppb established by Codex Alimentarius Commission (Table 4).

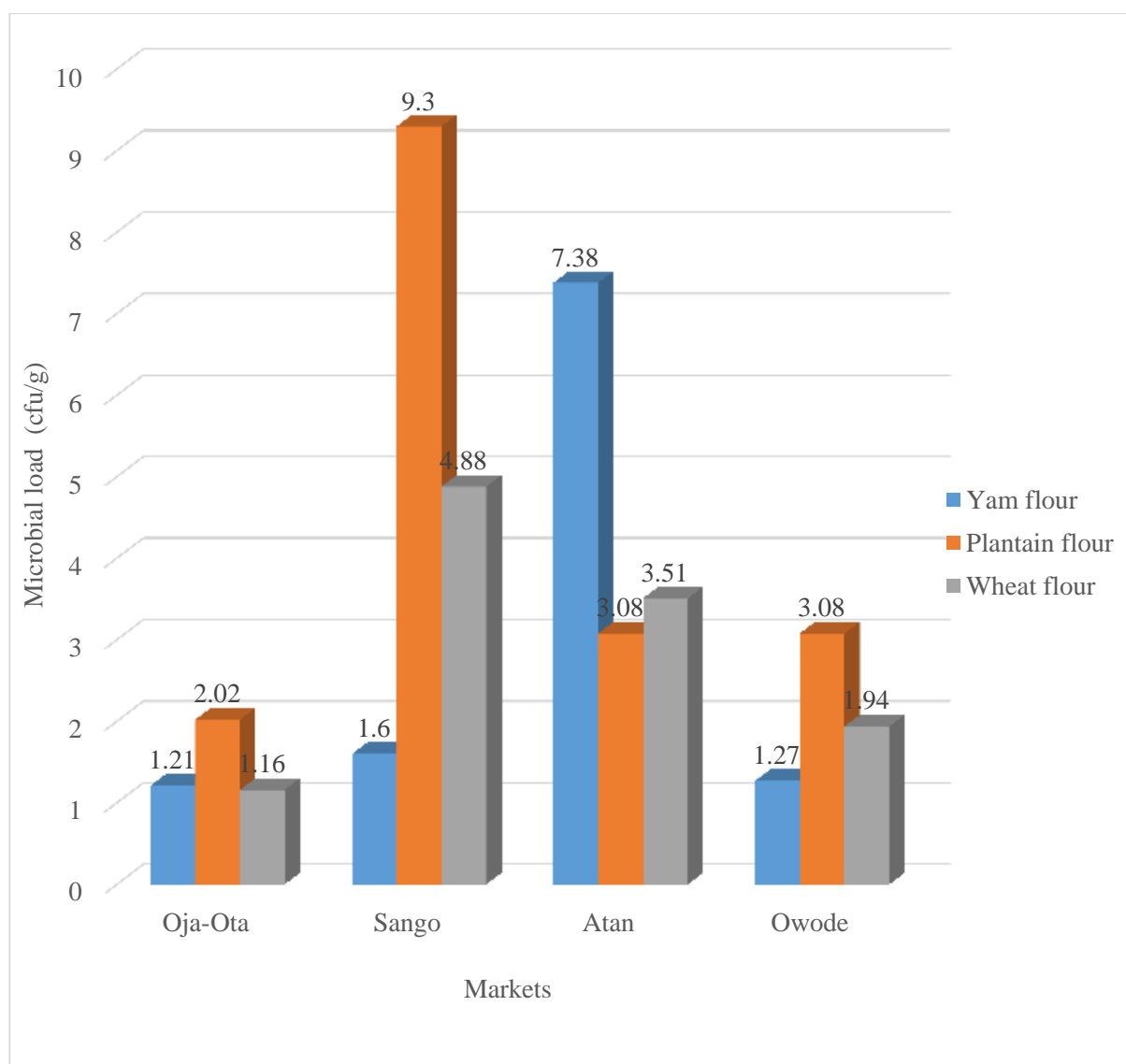


FIGURE 3: Microbial load of organisms isolated from common staple foods purchased from four markets in Ado-Odo Ota.

TABLE 2: Fungi isolated from common staple foods purchased from four markets in Ado-Odo Ota

Sample		Isolated fungal species			
S/N	ID				
1	M1	<i>Aspergillus</i>	<i>Aspergillus</i>	<i>Penicillium</i>	
	Yam	<i>flavus</i>	<i>niger</i>	<i>spp</i>	
2	M1	<i>Aspergillus</i>	<i>Aspergillus</i>		
	Plantain	<i>flavus</i>	<i>niger</i>		
3	M1	<i>Aspergillus</i>	<i>Aspergillus</i>	<i>Geotrichum</i>	
	Wheat	<i>flavus</i>	<i>niger</i>	<i>spp</i>	
4	M2	<i>Aspergillus</i>	<i>Aspergillus</i>	<i>Rhizopus</i>	<i>Penicillium</i>
	Yam	<i>flavus</i>	<i>niger</i>	<i>spp</i>	<i>spp</i>
5	M2	<i>Aspergillus</i>	<i>Aspergillus</i>	<i>Rhizopus</i>	
	Plantain	<i>flavus</i>	<i>niger</i>	<i>spp</i>	
6	M2	<i>Aspergillus</i>	<i>Aspergillus</i>		
	Wheat	<i>flavus</i>	<i>niger</i>	<i>Yeast</i>	
7	M3	<i>Aspergillus</i>			
	Wheat	<i>flavus</i>	<i>Rhizopus spp</i>		
8	M3	<i>Aspergillus</i>		<i>Geotrichum</i>	
	Yam	<i>flavus</i>	<i>Rhizopus spp</i>	<i>spp</i>	
9	M3	<i>Aspergillus</i>			
	Plantain	<i>flavus</i>			
10	M4		<i>Paecilomyces</i>		
	Plantain	<i>Yeast</i>	<i>spp</i>		
11	M4		<i>Paecilomyces</i>		
	Yam	<i>Yeast</i>	<i>spp</i>		
12	M4	<i>Paecilomyces</i>			
	Wheat	<i>spp</i>			

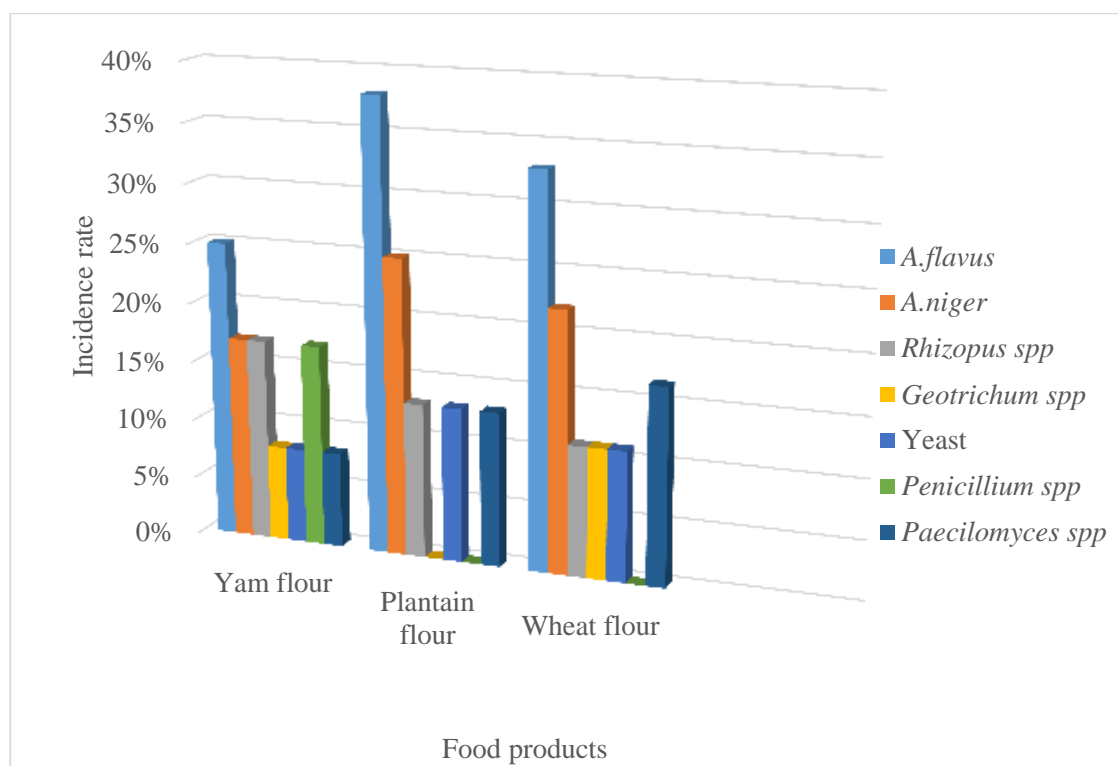


FIGURE 4: Incidence of seven fungi isolated from common staple foods purchased from four markets in Ado-Odo Ota.

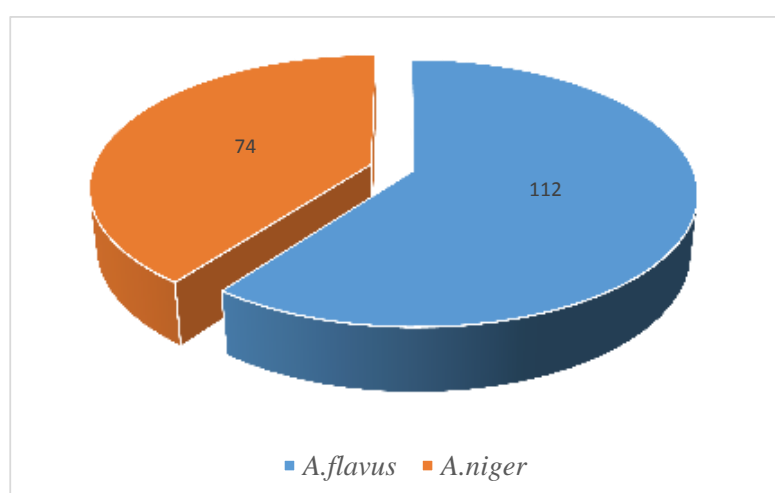


FIGURE 5: Incidence of the different isolated *Aspergillus spp* from common staple foods purchased from four markets in Ado-Odo Ota.

TABLE 3: The comparison of isolated *A. flavus* from common staple foods to the detection of total aflatoxin using ELISA

S/N	Sample Source	Sample Name	<i>A. flavus</i> Isolated	Aflatoxin Detected
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1	Oja-Ota	Yam	Yes	Yes
2	Oja-Ota	Plantain	Yes	Yes
3	Oja-Ota	Wheat	Yes	Yes
4	Sango	Yam	Yes	Yes
5	Sango	Plantain	Yes	Yes
6	Sango	Wheat	Yes	Yes
7	Atan	Wheat	Yes	Yes
8	Atan	Yam	Yes	Yes
9	Atan	Plantain	Yes	Yes
10	Owode	Plantain	No	Yes
11	Owode	Yam	No	Yes
12	Owode	Wheat	No	Yes

TABLE 4: Summary of ELISA result and total concentration of aflatoxin in common staple foods purchased from four markets in Ado-Odo Ota

S/N	Sample Source	Sample Name	O.D	Ppb	Remark
1	Oja-Ota	Yam	1.015	2.1	Passed
2	Oja-Ota	Plantain	1.142	0.9	Passed
3	Oja-Ota	Wheat	1.508	>REF	Passed
4	Sango	Yam	0.743	5.9	Passed
5	Sango	Plantain	1.099	1.3	Passed
6	Sango	Wheat	1.24	0.2	Passed
7	Atan	Wheat	1.203	0.5	Passed
8	Atan	Yam	0.934	3	Passed
9	Atan	Plantain	1.11	1.2	Passed
10	Owode	Plantain	1.002	2.2	Passed
11	Owode	Yam	0.933	3	Passed
12	Owode	Wheat	1.067	1.5	Passed

4. DISCUSSION

The result from this study showed that the sample with the highest microbial load was plantain flour purchased from Sango with 9.30×10^{13} cfu/g. The samples purchased from Oja-Ota and Owode markets had low microbial count with Oja-Ota having the lowest microbial count values while the food samples purchased from Sango and Atan markets had high microbial counts. The three food samples (yam flour, wheat flour and plantain flour) purchased from Oja-Ota market all had low microbial load compared to other markets. Wheat flour had a microbial load of 1.16×10^{12} cfu/g, yam flour had a microbial load of 1.21×10^{12} cfu/g while plantain flour had a microbial load 2.02×10^{12} cfu/g. The high microbial load from Sango and Atan markets maybe due to poor storage conditions which encouraged proliferation of microorganisms. Enquiries made from the market vendors in Owode market reported that fresh food products are bought from a wholesales person, this may be the reason for a low microbial count in all the food samples purchased from Owode.

From this report, a total of seven fungi (*Aspergillus flavus*, *Aspergillus niger*, *Rhizopus spp*, *Geotrichum spp*, Yeast, *Penicillium spp* and *Paecilomyces spp*) were isolated from the three types of flour samples purchased. *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus spp*, *Geotrichum spp*, and *Penicillium spp* were also isolated from flour samples in similar studies (Abulude and Ojediran, 2006; Padonou *et al.*, 2009). *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus spp* were also isolated from all flour samples and this result is similar that of Jonathan *et al.* (2011).

The aflatoxin bearing fungi that grew on the flour samples must have been present in the surrounding air in form of spores following fermentation as a result of the sun drying and

storage of the yam and plantation chips prior to milling. Fungi could have been introduced during exposure to and direct contact with agricultural products in the market (Ekundayo, 1986; Aboaba and Amisike, 1991; Okigbo, 2003).

Aspergillus spp are the common fungi isolated in this study and this report is similar to that of Jonathan *et al.* (2011) which reported *Aspergillus spp* as the common isolated fungi. *Aspergillus flavus* was the most prevalent *Aspergillus spp* with 31% incidence rate followed by *Aspergillus niger* with 21% incidence rate amongst others. The report of this study contradicts the report of (Ajayi and Olorundare, 2014) which stated that *Aspergillus niger* was the most prevalent with 50% incidence rate followed by *Aspergillus flavus* with 6.25% incidence rate amongst others. The high incidence rate of *Aspergillus flavus* in these flour samples may make them unfit for human consumption because of the toxic metabolites (aflatoxins) they produce.

The report of this study showed that aflatoxins were detected in the twelve food samples purchased from Oja-Ota, Sango, Atan and Owode markets. Amongst the fungi isolated in this study, *Aspergillus flavus* is the only fungus that produces aflatoxins (Goto *et al.*, 2013). However, *Aspergillus flavus* was isolated from only nine samples out of the twelve samples used in this study. Yam flour, wheat flour and plantain flour purchased from Owode market contained no *Aspergillus flavus* and this leads us to the question of how aflatoxins were detected from these food samples. *Aspergillus flavus* may have been present in these food samples initially but not during assay in the laboratory because of stress from prolonged storage and very high temperature condition. Also, the presence of aflatoxin in the food samples that contained no *A. flavus* may be a result of contamination from other food products during milling.

Total aflatoxins (AFB1, AFB2, AFG1 and AFG2) were detected in all flour samples (i.e. twelve food samples) used in this study. This study also showed that total aflatoxins were detected in all yam flours and plantain flours purchased and this report corroborates that of Jonathan *et al.* (2011) which reported the detection of total aflatoxins in yam flour and plantain flour.

Due to the toxigenicity of aflatoxins, International agencies have restricted the level of aflatoxins in food and this level varies from country to country and across regulatory bodies. The level of total aflatoxin content in all flour samples in this study ranged from 0.2 to 5.9 ppb and this is in accordance with Codex Alimentarius Commission (CAC), whose aflatoxin limit is 15 ug/kg or 15 ppb; this implies that the food products are safe for human consumption. However, this report is not in agreement with Jonathan *et al.* (2011) but corroborates the report of the study conducted in Malawi and Zambia in the year 2014.

The total aflatoxin content in the wheat flour samples from this study range from 0 to 1.5 ppb and did not exceed the maximum level of aflatoxins allowed in wheat and wheat by-products established by the European Commission (4.0 ug/kg or 4 ppb) and the Brazilian legislation (5.0 ug/kg or 5 ppb). With this result it is safe to say that the wheat flour sold in these four markets are safe for human consumption as it met the established aflatoxin level of three regulatory bodies.

The low level of aflatoxin content in these food products may be because of predominant atoxigenic strains of *A. flavus*. Research has shown that not all strains of *A. flavus* produces aflatoxins. The International Institute of Tropical Agriculture (IITA) in collaboration with Agriculture Research Service of the United States Department of Agriculture (USDA-ARS), AATF, University of Bonn and University of Ibadan developed an indigenous biological control product named AflaSafe. AflaSafe contains a mixture of four atoxigenic strains of *A. flavus* originating from Nigeria on sorghum grain as a carrier.

The strains of *A. flavus* used in the production of AflaSafe cannot produce aflatoxins because they have inherent defects in one or more of the 26 genes in the aflatoxin biosynthetic

pathway. These strains cannot also be transformed to the toxigenic strains since the selected atoxigenic strains belong to genetic groups that possess only atoxigenic strains. It is important to note that the application of aflasafe does not increase the total number of *Aspergillus spp* in the environment but rather it shifts the strain profile from toxigenic to atoxigenic strains of aflasafe. The protection conferred by aflasafe is a long-lasting one as it extends from field to store thus protecting crops along the entire value chain (from field to fork).

5. CONCLUSION

The overall low aflatoxin concentration of the food samples used in this study are below the aflatoxin limit established by Codex Alimentarius Commission (CAC). This implies that the persons in Ado-Odo Ota are consuming safe wheat flour, yam flour and plantain flour products that are free from aflatoxins' contamination. However, it could also be possible that other mycotoxins that were not tested for may be present. There is therefore a need to conduct further intensive studies on other mycotoxins in these food products to ensure the well-being of the inhabitants.

It is recommended that farmers should adopt the application of AflaSafe to mitigate aflatoxin contamination of their agricultural products. Proper hygiene should be observed by the market vendors and careful measures should be taken in the application of physical methods like cleaning before and after milling each food product especially in the market places as these methods help in the reduction of aflatoxin contamination but not its total elimination.

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