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Health Implication of Mycotoxigenic Fungi and Mycotoxins Analysed from Sun Dried Yam and Potato Chips Sold in Minna Niger State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mycotoxin is toxic secondary metabolite synthesized by filamentous fungi during their stationary phase of growth in agricultural crops, food and feed products. Niger State experienced high temperature and humidity for about three-quarters of the year, and these are some of the conditions that encourage filamentous fungi to produce mycotoxin in food products. Enzyme Linked Immunosorbent Assay (ELISA) was used to dectect mycotoxins. Mycological profile and

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mycotoxins of sun dried yam and potato chips was determined. Total of fifty (50) samples were collected randomly from five markets in Minna and its environs. 179 isolates were recorded. *Aspergillus* species 86(53.1 %) *Mucor* species 27(15.1 %), *yeast* species 25(13.9 %) *Penicillium species* 14(7.8 %), *Fusarium* species 14(7.8 %) *Rhizopus* species 3(1.7 %) and *Microsporum* species 1(0.6 %) were the common fungi. The highest mean fungal counts (12.8 x 10^3 cfu/g) were observed in sweet potato chips bought in Gwada market while the lowest (3.5 x 10^3 cfu/g), was observed in Yam chips from Paiko market. The mean moisture content of 7.41±2.28 % was recorded in potato chips and yam chips had the lowest moisture content of 4.72± 1.66 %. Total aflatoxins, fumonisin and zearalenone contents were detected in 48 (96 %) of the samples. In yam and potato chips, total aflatoxin contamination was 68 and 84 % with mean concentrations of 12.6±7.36 µg/kg and 10.3±13.81 µg/kg respectively. The total aflatoxin concentration range of yam and potato chips were 0-61.84 µg/kg and 0-54.49 µg/kg with 28 and 48 % of the samples below 4µg/kg E.U limit respectively. Fumonisin contamination was 80 and 60% with mean concentrations of 928.123±559.50 µg/kg and 722.64 ± 137.85 µg/kg with a range 0.0-4018.24 µg/kg and 0 – 2055.98 µg/kg and had 52 and 72 % below 1000 µg/kg acceptable limit for yam and potato chips respectively. Zearalenone contamination in yam and potato account for 56 and 68% having mean concentration of 16.33± 9.71 µg/kg and 24.12± 21.03 µg/kg with a range 0 – 45.53 µg/kg and 0 – 83.74 µg/kg respectively. All the samples contaminated with Zearalenone were below the E.U regulatory limit of 100 µg/kg for food stuff intended for direct consumption. Dietary exposure and risk characterisation (EDI and %TDI) were further estimated for total aflatoxins, fumonisin and zearalenone. It was concluded that the level of contamination of these chips with mycotoxins poses a serious threat to public health and there is the need for proactive approach to drastically reduce fungal contamination.

Keywords: Health; mycotoxigenic fungi; mycotoxin; sundried chips.

1. INTRODUCTION

Tuber and root crops such as yam (*Discorea specie*) and Potato (*Ipomoea batatas*) are among the most important food crops consume in Africa and major source of food globally [1,2]. These crops account for about 95 % of the total root and tubers produced in Africa with a production of over 240 million tons annually on 23 million hectares of land [1]. They are essential diet for about 1.2 billion people in developing countries, providing 20 % of world's energy food and protein intake (Makun, et al., 2012). The aggregate value of yam, cassava and sweet potato exceeds all other African staple crops, and is much higher than the value of cereal crops (cereals annually production on average 169 million tons from 108 million hectares of land) [1].

In Nigeria Yam and sweet potato are important food crop grown in nearly all agro-ecological zones. Nigeria is ranked world highest yam production with a total production of 37 million metric tons annually which is sufficient for consumption and export [2]. Sweet potato is ranked second to yam production after cassava. Yam and sweet potato are the cheapest sources of starchy food and the chips are mainly use as flour for food (*tuwo*) and flavor in locally fermented drinks (*kunu zaki*) respectively. The raw sweet potato contains 70–80 % water, 10-30 % carbohydrate (mainly starch but also a little sugar), 1-3 % proteins, 2-3 % fibers and 0.1% fats. It serve as an excellent source of vitamin A, C, E B6, riboflavin, pantothenic acid and folic acid, B-carotene, iron, potassium, copper and several other minerals [3,4]. Dried yam chips have been reported to contain up to 12.3 % moisture and ash content of 2.0%, it contains 80.6 % carbohydrate, which makes the food a good source of energy, Protein and fat have been reported to be 3.2, 0.3 respectively [5].

Fungi are eukaryotic ubiquitous organism that are major contaminants of food and feeds [6]. Fungal contamination of food stuff results in the production of toxic secondary metabolites called mycotoxins which are synthesized during stationary stage of growth when environmental conditions like temperature and moisture are favorable [7-9]. Mycotoxigenic fungi belong to various genera such as *Aspergillus, Pencillium, Fusarium Byssochlamys* and *Mucor* [10].

Mycotoxins of greatest agro-economic importance include Fumonisin, zearalenone, aflatoxins, ochratoxin, citrinin, cyclopiazonic acid, sterigmatocystin, patulin, gliotoxin and different trichothecenes are some of the mycotoxin of greatest agro-economic importance [11]. It is known that almost nothing can be done to remove mycotoxins from food and feed once contaminated, because most mycotoxins are resistant to heat within the normal range of cooking temperature [9].

Generally, Consumption of mycotoxins infected food can cause acute to chronic toxic effect characterized by carcinogenic, mutagenic, immunotoxic, hepatotoxic, teratogenic [12], neurotoxic, faetotoxic, hemorrhagic, nephrotoxic, estrogenic and dermotoxic and specifically aflatoxins cause diseases such as aflatoxicosis, hepatocarcinogenicity, encephalopathy and Rrye"s syndrome; whereas ochratoxin cause Balcan Endemic Nephropathy (BEN) and kidney tumors as well as fumonisins cause esophageal cancer, hepatocarcinogenicity, pulmonary edema, leukoencephalomalacia, hepatotoxicity and nephrotoxicity [13]. Moreover, mycotoxins have been linked to birth defects in many animals, nervous system problems (tremors, limb weakness, staggering, and seizures), and tumors of the liver, kidneys, urinary tract, digestive tract, and the lungs [13]. Zearalenone has major effects on reproduction that can lead to hyperestrogenism [14].

In Minna, Niger state, yam and potato chips are sold in the open market with no regulation of quality and high risk of fungi contamination, therefore the need to study and understand the various fungi strains that produce mycotoxins in food and the knowledge about the level of mycotoxins in food is important, identifying the fungal strains responsible for the toxins is better approach in solving mycotoxin related food poisoning. Due to the insidious nature of mycotoxin production and the resulting disease condition which made diagnosis of mycotoxicosis difficult, many cases of both human and animal mycotoxicoses have often not been identified and reported in Nigeria. Not much has been done on the mycological and mycotoxin analysis in yam and potato chips in Niger state and there is paucity of information on mycotoxicoses in the state. Against this backdrop this study seek to analysed mycological and health implication of Aflatoxins, Fumonisins and Zearalenone in yam and potato chips in Minna and its environs.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 50 sun-dried sweet potato and yam chips samples were collected randomly from five market in Minna and its environs, Namely, Gwari, Kure, Chanchaga, Paiko and Gwada markets. Samples collected were packaged and labeled in clean polythen bags and taken to Microbiology laboratory Federal University of Technology Minna, and Central Research and Diagnostic Laboratory Tanke, Illorin Kwara State for mycological and mycotoxin analysis respectively. The collected samples were pulverized aseptically and shared into three portion; one was used to determine moisture content, the second for fungi isolation (mycological analysis) and the third for determination of mycotoxins.

2.2 Moisture Content Determination

Using the method as described by Daniyan et al. [15]; Eziekel and Saning'o [4]. Moisture Content (MC) of sun dried sweet potato and yam chips were determined. Ten gram of each of the chip samples were weighed using digital weighing scale onto a piece of aluminum foil of known weight and then dried to a constant weight at 100^{0}C for 3 hours in an oven. After this, it was cooled in the desiccator for 30 minutes, removed and weighed. The weight of the Aluminum foil was subtracted from the total weight of each of the dried samples, leaving only the weight of each of the dried chip sample. All measurements were in duplicates, using the mean moisture content of the samples (moisture content was determined by the difference between the weight of the samples before and after drying to a constant weight). This was expressed as a percentage of the total weight loss.

$$Moisture\ content\ (MC)\% = \frac{Wt^1 - Wt^2}{Wt_2} X\ 100$$

Where

Wt_1=Initial weight of the sample.
Wt_2=Final weight of the sample.

2.3 Isolation of Fungi

Fungi were isolated and cultured as described by Makun et al [16] and Langat [17]. Ten gram each of potato and yam chip samples were thoroughly mixed, surface sterilized using 3.5 % sodium hypochlorite solution using cotton swab and washed aseptically with 100ml volume of sterile distilled water and pulverized into pieces before it was suspended in 100ml sterile distilled water in a conical flask and was serially diluted in 9ml of sterile distilled water in five replicates (10^{-1} to 10^{-5}). An aliquot of 1.0ml from each test tube was placed and spread on SDA using sterile bent glass rod, incubated at 28°C for 2-4 days. The colonies, which developed on the plates were counted using the colony counter (Model 6399/Stuart Scientific Co. Ltd., Great Britain) and expressed as colony forming units per millilitre (CFU/ml) of sample. Each fungal growth was sub-cultured on a fresh SDA to obtain a pure culture. The pure isolates were inoculated into SDA slant and stored in a refrigerator at 4°c for further analysis.

2.4 Macroscopic Identification of Fungi

Pure cultures were identified on the basis of their cultural morphological and microscopic characteristics using the keys of Pitt and Hockings [18].

Morphological features of molds were studied and the major macroscopic features such as colony diameter, colony color on agar and reverse side and colony texture were used to aid identification. Each morphologically different mold colony from the SDA plates supplemented with chloramphenicol was picked up, transferred to SDA and incubated for 3 days at 30 °C.

2.5 Microscopic Identification

2.5.1 Lactophenol Cotton blue stain

Lactophenol Cotton blue stain was used for staining for microscopic identifications of molds (Forbes et al., 2002). Briefly a drop of Lactophenol Cotton blue stain was placed on the center of a clean slide and a small portion of the colony was picked carefully using mycological pins. This was followed by teasing using pins so that the filaments were well spread and clean sterile cover slips applied gently and viewed under X10 and X40 objective lenses of microscope. Morphological characteristics such as the type and arrangement of spores produced, as well as the mycelial type were identified.

2.6 Sample Extraction for ELISA

Solvent extractions were carried out for total aflatoxins, fumonisins and zearalenone from yam and potato chips based on the procedures recommended in the kits. A known mass (10 g each) of the milled samples were weighed into labelled conical flasks. About 20 ml mixture of methanol and water (70:30) were added to the samples under continuous stirring in a mechanical shaker for 2 hrs. The reaction mixture was filtered through a Whitman No. 2 filter paper. A portion of the filtrate (about 5 ml) was cleaned in a microfilter (5µm pore size), preconditioned with methanol and water in ratio 3:1 v/v. Afterwards, the filtrate was stored at 4 ^{o}C until further analyses. The procedure adopted was based on the manufacturer's guideline (AgraQuant® Total Aflatuxin, AgraQuant® Fumonisin and AgraQuant® Zearalenone).

2.7 Mycotoxin ELISA Procedure

About 100 µL of the standard solution or prepared sample was added into appropriate wells of microtiter plate, 200 µL of HRP-conjugated antibody working solution were added into each well separately. Thereafter, the microtiter plate was sealed with the cover membrane, and incubated for 15 minutes at room temperature. The content was dispensed (by aspiration or dumping) from each dilution well into a corresponding antibody coated microwell and washed repeatedly five times. Washing was done by filling each microwell with de-ionized water using a multi-channel pipette. Complete removal of residual liquid at each step was ensured by striking the plate against an absorbent paper towel. Subsequently, 100 µL of substrate solutions was pipetted into each well. This was mixed gently by shaking the plate manually and incubated for 5 minutes at 25 °C. The reaction was allowed to develop in the dark and the plate was kept away from the drafts and other temperature fluctuations. Lastly, 100 µL of stop solution was added to each well and mixed gently by shaking the plate. After thorough mixing, the optical density of each well was measured using an ELISA micro plate reader set to 450 nm (STAT FAX Elisa Reader MODEL: 303 PLUS). All samples, controls, and standards were assayed in duplicate. The range of quantification and percentage recoveries of the analytes are presented in Table 1.

2.8 Estimation of Exposure and Risk Characterisation

The estimated daily intake (EDI), and percentage tolerable daily intake (% TDI) values were estimated for the staples. The method used by Rodríguez-Carrasco et al. [19] and approved by JECFA was adopted in this study. The "Estimated daily intake" which estimates the amount of toxin that can be ingested daily (µg/kg bw/day) was determined using the formula:

$$Estimated\ Daily\ Intake\ (EDI) = \frac{contamination\ levesl\ x\ consumption\ rate}{Body\ weight\ (kg\ /\ persons)}$$

Table 1. Assay condition

S/N	Parameter	Total aflatoxin	Fumonisin	Zearalenone
1	Extraction solution	Methanol: water (70:30)	Methanol: water (80:20)	Methanol: water (50:50)
2	LOD	2 ppb	200 ppb	19 ppb
3	LOQ	9 ppb	600 ppb	5.7 ppb
4	Range of quantification	4-40 ppb	250-5000 ppb	2-40 ppb
5	Recovery rate	85± 15%	80%	90 ±20%
6	Wavelength	450 nm	450 nm	450 nm

LOD: Limit of Determination, LOQ: Limit of Quantification

Where, Contamination level is the average toxin level found in a certain foodstuff (μg/Kg) and Consumption rate is the amount of the foodstuffs ingested on daily basis (gram/day). The health risk characterisation for the mycotoxin (%TDI) was estimated by dividing the EDI previously calculated with the tolerable daily intake (TDI) (μg/kg bw/day) of the respective mycotoxins as indicated in the formula:

$$\% TDI = \frac{EDI}{TDI} x\ 100$$

The EDI and %TDI was calculated based on the estimated daily consumption outlined in the WHO/GEMS database (2012). According to the database, Nigeria being among the cluster 13 nations consume an average of 23.6 g/day of processed roots and tubers. An average body weight of an adult was determined to be 61 kg. The toxicological guidance value for Fumonisin and zearalenone was set at a provisional maximum tolerable daily intake (PMTDI) value of 2 and 0.5 μg/kg bw/day respectively [11].

2.9 Statistical Analysis

Using the IBM SPSS statistical software version 23.0 and Microsoft office Excel Professional Plus 2010, descriptive statistics and a one-way analysis of Variance (ANOVA) was performed to derive Mean, standard deviation (SD), frequency and range were used to present the results.

3. RESULTS

3.1 Determination of Moisture Content of Sun Dried Sweet Potato and Yam Chips

The moisture contents of sun dried sweet potato and yam chips collected from five markets in Minna and its environs were determined, with potato chips having the highest total mean ±Standard deviation moisture content of 7.41±2.28 % and yam chips had the lowest of 4.72± 1.66 %. The sun dried potato chip samples collected from Gwada Market had the highest moisture content of 8.88±1.82 % followed by that of Gwari market with 8.67±1.67 % while samples collected from Kure Market had the lowest moisture content of 5.97±1.62 %. The yam chips collected from Kure Market have the highest moisture content (6.63±2.33 %) among all the yam chips collected from the five Markets while those of Gwari Market have the lowest moisture of 3.72±0.62 % as shown in Table 2.

3.2 Viable Fungi Cell Count in Potato and Yam Chips collected from five Markets

Viable fungi colony count (CFU/g) in Potato and Yam Chips collected from five Markets in Minna and its environs (Table 3), shows that potato chips in all the market except that of Kure market had the highest viable cell count compared to yam chips. Potato chips collected from Gwada market had the highest viable cell count of 12.8 $x10^3$ cfu/g with samples collected from Paiko Market having the least viable cell count 5.9 x 10^3 cfu/g. Yam chips collected from Kure Market had the highest viable cells (9.3 x 10^3 cfu/g) and also with samples from Paiko Market having the least viable cell count of 3.5 x 10^3 cfu/g.

3.3 Percentage Occurrence of Fungi Spp in Sweet Potato and Yam Chips Marketed in Five Markets in Minna and Its Environs

The percentage occurrence of fungi in potato and yam chips marketed in five Markets in Minna and its environs (Table 4), indicated that, in both yam and potato chips *Aspergillus species* was the most abundant and ranged from 8 to 80 % and 4 to 76 % respectively. *Aspergillus niger* had the highest percentage occurrence of 76 % and 80 % respectively, *Aspergillus terreus* and

Aspergillus nodulan accounted for the least percentage of 4 % each in potato chips.

Penicillium species and *Fusarium species* in potato chips recorded 36 % each while in yam chips they account for 20 % each. *Sacchromyces cerevisiae* and *Mucor puselluse* recorded 60 and 28 % respectively in potato chips while in yam chips they account for 40 and 80 % respectively.

The incidence of occurrence of fungi species in yam chips marketed in five markets in Minna and its environs is shows that a total of eighty eight (88) fungi isolate was recorded from twenty five (25) samples collected across five (5) markets. The species of fungi isolated are *Aspegillus* species, *fusarium* species*, Penicillium* species, *Microsporum* species, *Mucor* species and *Yeast,* of the total number of 91 isolates recorded, Gwada market recorded the highest incidence of twenty four (24) isolates followed by Gwari Market which recorded Eighten (18) isolates while Chanchaga and Paiko Markets recorded seventeen (17) isolates each. Kure Market recorded the least incidence of fifteen (15) isolates.

The predominant species of fungi isolated from yam chips are *Aspergillus* specie which account for forty five (45) of the isolates. *Aspergillus niger* is the most frequently isolated species of *Aspergillus* followed by *Aspergillus flavus* and *Aspergillus fumigatus,* the least frequently isolated species of *Aspergillus* are *A.terreus and A.parasiticus. Penicilium* specie and *Fusarium* specie both recorded five (5) isolate each and non mycotoxigenic fungi and yeast recorded a total of thirty three (33) isolates.

3.4 Occurrence and Concentration (µg/kg) of Total Aflatoxin (AFT), Fumonisin and Zearalenone in Sweet Potato Chips Marketed in Minna and Its Environs Based on Market Locations

The occurrence and mean concentration of Total aflatoxin, Fumonisin and Zearalenone were analysed in potato chips from all the markets (Table 5). The Total aflatoxins occurrence of sun dried sweet potato chips ranged from 40 to 100 % across the Markets from which samples were collected. Positive samples are all analysed samples with values > Limit of detection (LOD).

Samples collected from Gwari Market had the lowest percentage (40 %) occurrence of mycotoxin contamination while Chanchaga, Gwada and Paiko Markets had the highest percentage contamination (100 %) followed by those collected from Kure market with 80 % contamination.

The potato chips collected from Gwada market had the highest mean concentration ± standard deviation of 20.00±9.39 µg/kg and range 3.95-54.49 µg/kg and lowest percentage safety of 20 % and Gwari market recorded the lowest mean concentration of 1.3±0.82 µg/kg with range 0-3.47 µg/kg and it 100 % safe (concentration below 4 µg/kg). In the overall Total aflatoxin contamination across the Markets, it was recorded that out of the 25 samples collected, 21 (84 %) were contaminated with mean concentration of 10.3±13.81 µg/kg and ranges from 0-54.49 µg/kg.

The occurrence and concentration of Fumonisin in Sun dried Sweet potato had percentage occurrence range from 40% to 80% across the five markets, with Kure and Paiko markets having the highest occurrence of contamination of 80 % followed by Gwada market 60 %, while Chanchaga and Gwari markets had the lowest percentage occurrence of 40 %.

The mean concentration ± S.D 1115.52±144.88 µg/kg of Fumonisin and ranged 0 – 2055.98 µg/kg was the highest and recorded in Paiko market with the least percentage safety of 40 %, followed by samples collected from Kure market with mean concentration ± S.D 1017.107±438.11 µg/kg and concentration range of 0 – 2037 11 µg/kg and percentage safety of 60 %. The lowest mean concentration of 344.40 ± 211.24 µg/kg and ranged 0 – 898.74 µg/kg and 100 % safe.

The overall occurrence, out of 25 samples collected across the five markets 15(60 %) were contaminated with Fumonisin having mean concentration of 722.64 ± 137.85 µg/kg with concentration range of 0 – 2055.98 µg/kg and 72 % safe.

The percentage occurrence of Zearalenone range from 40 % to 100 %, with samples collected from Paiko market having the highest percentage occurrence of 100% and highest mean concentration of 31.90 ± 8.35 µg/kg and range 23.21 - 41.47 µg/kg, followed by samples collected from Kura markets having 80% occurrence, mean concentration of 30.10± 20.09 µg/kg and range 0 – 54.63 µg/kg. The lowest occurrence and concentration of 40% and 12.58

± 5.16 µg/kg respectively were recorded in Gwari market with range 0 -39.11µg/kg.

In the overall, 17 (68 %) out of 25 samples analysed were contaminated with zearalenone having mean concentration of 24.12± 21.03 µg/kg ranging between 0 – 83.74 µg/kg.

All the samples contaminated with Zearalenone are 100 % safe for consumption as they are all below the E.U regulatory limit of 100 µg/kg for food stuffs intended for direct consumption.

3.5 Occurance and Concentration (µg/kg) of Total Aflatoxin (AFT), Fumonisin and Zearalenone in Yam Chips Marketed in Minna and Its Environs Base on Market Locations

The Total aflatoxin occurrence in contamination of Yam chips ranged from 40 % and 100 % marketed across Five Markets in Minna and its environs. Samples collected from Gwari Market had the highest percentage (100 %) Total aflatoxin contamination followed by samples marketed in Gwada and Kure Markets with 80 % occurrence while Chanchaga and Paiko Markets had the lowest percentage contamination of 40 %.

The Yam chips collected from Gwada market has the highest mean concentration ± standard deviation of 23.51±13.49 µg/kg and range 0-61.8 4µg/kg and percentage safety of 40 % and Chanchaga market recorded the lowest mean concentration of 0.93±0.72 µg/kg with range 0-3.73 µg/kg and it 100 % safe (i.e concentration below 4 µg/kg E.U acceptable limit).

In total, the total aflatoxin contamination of Yam chips across the five Markets as shows that, out of a total of 25 samples collected, 17 (68 %) were contaminated with mean concentration of 12.6±7.36 µg/kg that ranges from 0-61.84 µg/kg and 28 % safe.

The occurrence and concentration of Fumonisin in Yam chips had percentage occurrence range from 20 % to 100% across the five markets, with Kure, Gwari and Paiko markets having the highest occurrence of contamination of 100 % followed by Gwada market having 80 %, while Chanchaga markets recorded the lowest percentage occurrence of 20 %.

The mean concentration ± S.D 1864.74 ± 2111.32 µg/kg of Fumonisin and ranged 1011.95-4018.24 µg/kg was the highest and recorded in Kure market with all the samples (0 %) not safe for consumption (concentration above 1000 ug/kg), followed by samples collected from Paiko market with mean concentration ± S.D 1406.92±1082.16 µg/kg and concentration range of 816.98-1861.01µg/kg and percentage safety of 20%. The lowest mean concentration of 158.365±354.11 µg/kg, range 0.0-791.82 µg/kg and 100 % safe was recorded in Chanchaga Market as shown in Table 6.

The overall total occurrence of Fumonisin contamination shows that 20 (80%) out of 25 samples collected across the five markets were contaminated with Fumonisin with mean concentration of 928.123±559.50 µg/kg, concentration range of 0.0-4018.24 µg/kg and 52 % safe.

The percentage occurrence of Zearalenone contamination of Yam chips range from 40 % to 60 %, with samples collected from Gwari, Kure, Gwada and Paiko markets having the highest percentage occurrence of 60% and highest mean concentration of 17.87±8.49 µg/kg and range 0.0-45.58 µg/kg recorded in samples collected from Kure market, followed by samples collected from Gwari market having mean concentration of 16.95±7.16 µg/kg and range 0 – 34.78 µg/kg, Gwada has mean concentration of 16.50±15.12 µg/kg concentration range of 0- 28.74 µg/kg and Paiko Market recorded 15.59±6.54 µg/kg. The lowest occurrence and concentration of 40 % and 14.75±9.76 µg/kg was recorded in samples collected in Chanchaga market with highest concentration range 0 -48.53 µg/kg.

In the overall, 14 (56 %) out of 25 samples analysed were contaminated with zearalenone having mean concentration of 16.33± 9.71 µg/kg ranging between 0 – 45.53 µg/kg.

All the samples contaminated with Zearalenone are 100% safe for consumption as they are all below the E.U regulatory limit of 100µg/kg for food stuffs intended for direct consumption.

3.6 Occurrence and Concentration (µg/kg) of Total Aflatoxin (AFT), Fumonisin (FUM) and Zearalenone (ZEN) in Sweet Potato Compare with Yam Chips Marketed in Minna and Its Environs

The percentage occurrence and mean concentration (µg/kg) of Total aflatoxin (AFT), Fumonisin (FUM) and Zearalenone (ZEN) in

Sweet Potato compare with Yam Chips marketed in Minna and its environs is shown in table 7. Of the three (3) mycotoxins analysed, total aflatoxin has the highest percentage occurrence of 84 % in Potato chips, followed by Fumonisin with 80 % contamination of Yam chips, zearalenone contamination in potato chips and Total aflatoxin in yam chips has 68 % occurrence each, while the least occurrence of 56 % zearalenone contamination was found in Yam chips.

The mean concentration (µg/kg) ± standard deviation of Total aflatoxin 12.6 ± 13.81 µg/kg and concentration range of 0-61.84 µg/kg is the highest in Yam chips compare to 10.3 ± 7.36 µg/kg kg and concentration range of 0-54.49 µg/kg of potato chips. Potato chips is 48% safe against Yam chips with 28 % which is less safe for consumption.

The mean concentration (µg/kg) ± standard deviation of Fumonisin 928.123 ± 559.50 µg/kg and concentration range of 0-4018.24 µg/kg is higher in Yam chips and 52% safe for consumption compare with 722.64 ± 137.85 µg/kg kg and concentration range of 0-2055.98 µg/kg and 72% safe of potato chips.

The mean concentration (µg/kg) ± standard deviation of Zearalenone 24.12 ± 21.03 µg/kg and concentration range of 0-83.74 µg/kg is higher in potato chips compare with 16.33 ± 9.71 µg/kg kg and concentration range of 0-48.53 µg/kg of Yam chips. Both potato and yam chips were 100% safe for consumption considering the level of Zearalenone contamination of the samples.

3.7 Co-Occurance of the Studied Mycotoxins in Potato and Yam Chips Across Five Markets in Minna and Its Environs

It was recorded that, 100 % and 92 %, 68% and 72% of potato and yam chips were contaminated with at least one and several mycotoxin respectively. Co-occurrence of three mycotoxins (AfT/Fums/Zen) was determined in yam and potato to be 40 and 44 %, respectively, and those of two combinations (AfT/Fums), (AfT/Zen) and (Fums/Zen) in yam and potato chips were (12 %), (4 %), (16 %) and (16 %), (4 %), and (4 %) respectively is shown in Table 8 and 9.

3.8 Estimated Daily Exposure and Risk Characterisation of AfT, Fums and Zen in Yam and Potato Chips

The estimated daily exposure and risk characterisation of the three mycotoxins was evaluated from yam and potato chips in adults (Table 10).The estimated daily intake (EDI) and tolerable daily intake which characterized the risk (%TDI) of aflatoxin and fumonisin was higher in yam chip while zearalenone exposure and risk was higher in potato chip.

Table2. Moisture contents of sun dried sweet potato and yam chips from

Market (location)	Mean moisture content (%)± S.D			
	Potato chip		Yam chip	
	Range	Mean(%)± S.D	Range	Mean(%)± S.D
Chanchaga Market	4.28-11.11	7.13±3.48	4.17-5.49	4.74±0.50
Gwada Market	6.50-11.11	8.88±1.82	2.99-6.61	4.44±1.44
Gwari Market	6.61-10.38	8.67±1.67	3.09-4.49	3.72±0.62
Kure Market	4.28-8.46	5.97±1.62	4.17-10.01	6.63±2.33
Paiko Market	4.38-7.76	6.40±1.29	2.67-6.05	4.07±1.39
Mean (Total)		7.41±2.28		4.72±1.66

Table 3. Viable fungi cell count in potato and yam chips collected from five markets viable cell count (CFU/g) x10^3

Market (Location)	Potato chips	Yam chips
Chanchaga Market	6.5	5.1
Kure Market	6.2	9.3
Gwari Market	7.7	7.0
Paiko Market	5.9	3.5
Gwada Market	12.8	6.0

Table 4. Percentage occurrence of fungi species in sweet potato and yam chips marketed in minna and its environs

Fungi species	Potato chips (n=25)		Yam chips (n=25)	
	No. of isolate	Prevalence (%)	No. of isolate	Prevalence (%)
A.flavus	15	60	10	40
A,niger	19	76	20	80
A.fumigatus	12	48	10	40
A.parasiticus	2	8	2	8
A.terreus	1	4	3	12
A.nodulan	1	4	-	-
Penicillium spp	9	36	5	20
Fussarium spp	9	36	5	20
S.cerevisae	15	60	10	40
Mucor pusellus	7	28	20	80
Rhizopus spp	-	-	3	12
Microsporium spp	1	4	-	-
Total	91		88	

Table 5. Occurrence and concentration (µg/kg) of total aflatoxin (AFT), fumonisin and zearalenone in sweet potato chips marketed in Minna and its environs base on market locations

		Market (location)					
Mycotoxins		Chanchaga	Gwari	Kure	Gwada	Paiko	pvalue
Total Aflatoxins	Number of samples	5	5	5	5	5	
	Occurrence (%)	100	40	80	100	100	
	Mean± S.D (Concn)	11.54±7.91	1.35±0.82	6.51±3.70	20.00±9.39	12.11±8.75	0.04
	Range	1.05-42.82	0-3.47	0-19.60	3.95-54.49	1.05-23.84	
	Safe (Below4ug/kg)	40%	100%	60%	20%	20%	
Fumonisins	Number of samples	5	5	5	5	5	
	Occurrence (%)	40	40	80	60	80	
	Mean± S.D (Concn)	344.403±211.24	520.50±338.30	1017.107±438.11	615.97±255.66	1115.52±144.88	0.05
	Range	0-898.74	0-1659.75	0-2037.11	0-1194.34	0-2055.98	
	Safe(Below1000ug/kg)	100%	80%	60%	80%	40%	

		Market (location)					
Mycotoxins		**Chanchaga**	**Gwari**	**Kure**	**Gwada**	**Paiko**	**pvalue**
Zearalenone	Number of samples	5	5	5	5	5	
	Occurrence (%)	60	40	80	60	100	
	Mean± S.D (Concn)	19.74±12.12	12.58±5.16	30.10±20.09	26.27±34.30	31.90±8.35	0.01
	Range	0-42.26	0-39.11	0-54.63	0-83.74	23.21-41.47	
	Safe (Below100ug/kg)	100%	100%	100%	100%	100%	

Table 6. Occurrence and concentration (μg/kg) of total aflatoxin (AFT), fumonisin and zearalenone in yam chips marketed in Minna and its environs base on market location

		(Market location)					
Mycotoxins		**Chanchaga**	**Gwari**	**Kure**	**Gwada**	**Paiko**	**pvalue**
Total aflatoxins	Number of samples	5	5	5	5	5	
	Occurrence (%)	40	100	80	80	40	
	Mean± S.D (Concn) ug/kg	0.93±0.72a	11.29±4.57	12.50±5.08	23.51±13.49	14.77±11.85	0.04
	Range	0.0-3.73	1.59-26.08	0.0-30.94	0.0-61.84	0.0-61.12	
	Safe (Below 4ug/kg)	100%	20%	20%	40%	60%	
Fumonisins	Numberof samples	5	5	5	5	5	
	Occurrence (%)	20	100	100	80	100	
	Mean± S.D (Concn) ug/kg	158.365±354.11	424.89±263.22	1864.74±2111.32	785.66±218.90	1406.92±1082.16	0.07
	Range	0.0-791.82	0.0-1188.05	1011.95-4018.24	0.0-1313.84	816.98-1861.01	
	Safe (Below1000 ug/kg)	100%	80%	0%	60%	20%	
Zearalenone	Number of samples	5	5	5	5	5	
	Occurrence (%)	40	60	60	60	60	
	Mean± S.D (Concn) ug/kg	14.75±9.76	16.95±7.16	17.87±8.49	16.50±15.12	15.59±6.54	0.05
	Range	0.0-48.53	0.0-34.78	0.0-45.58	0.0-28.74	0.0-29.11	
	Safe (Below100ug/kg)	100%	100%	100%	100%	100%	

Table 7. Occurrence and concentration (μg/kg) of total aflatoxin (AFT), fumonisin zearalenone in potato compare with yam chips marketed in Minna and its environs

Mycotoxins		Potato chips	Yam chips
Total Aflatoxins	Number of samples	25	25
	Occurrence (%)	84	68
	Mean± S.D (Concn)	10.3±13.81	12.6±7.36
	Range	0.0-54.49	0.0-61.84
	Safe(Below 4ug/kg)	48%	28%
Fumonisins	Number of samples	25	25
	Occurrence (%)	60	80
	Mean± S.D (Concn)	722.64±137.85	928.123±559.50
	Range	0.0-2055.98	0.0-4018.24
	Safe(Below1000ug/kg)	72%	52%
Zearalenone	Number of samples	25	25
	Occurrence (%)	68	56
	Mean± S.D (Concn)	24.12±21.03	16.33±9.71
	Range	0.0-83.74	0.0-48.53
	Safe (%) (Below100ug/kg)	100%	100%

Table 8. Chain of co-occurring mycotoxins in potato chip sold in Minna and its environs

No of co-occurring toxins	Co-occurring toxins	Number of samples	% co-occurrence
Two toxins	AFLT/ZEN	4	16
	AFLT/FUM	1	4
	FUM/ZEN	1	4
Three toxins	AFLT/FUM/ZEN	11	44

Table 9. Chain of co-occurring mycotoxins in yam chip sold in Minna and its environs

No of co-occuring toxins	Co-occurring toxins	Number of samples	% co-occurrence
Two toxins	AFLT/ZEN	1	4
	AFLT/FUM	3	12
	FUM/ZEN	4	16
Three toxins	AFLT/FUM/ZEN	10	40

4. DISCUSSION

The result of this study has revealed that sweet potato and yam chips have wide spread of fungi contamination, with all the samples contaminated with at least one fungus species. Suleiman et al [20] started that, Minna markets, where movement of people and vehicles are in confined spaces may result in high dust and potential microbial spore formation with little aeration that would accelerate fungi and mycotoxins development. Similarly, the samples analysed in this work were found to be contaminated with aflatoxins, fumonisins and zearalenone. Djeri et al [21] stated that contamination of chips could be due to the production, drying and preservation conditions as well as the insalubrity existing at storage places.

Currently, there are no national regulations in place that deal with mycotoxins in foodstuffs in Nigeria. Therefore, the maximum levels permitted in the EC [22] for mycotoxins in cereals and derived products intended for direct human consumption were used as reference levels in the present study. The samples analyzed contained total aflatoxin and fumonisins at levels unacceptable by national and international mycotoxin regulatory bodies while zearalenone concentrations were within acceptable tolerable range. This is an indication that these food commodities that are intended for direct human consumption in Minna, Niger state are of very low quality and could constitute health hazard to the human populace.

Table 10. Estimated daily exposure and risk characterisation of AFT, fums and zen in yam and potato chips

Mycotoxin	Chip	Mean conc. (g/kg)	Average daily intake (g/day)	Adult weight (kg)	Estimated daily Intake (EDI) (g/kg bw/day)	Tolerable daily intake TDI (g/kg bw/day)	Risk characterization (%TDI)
Total Aflatoxin	Yam chip	12.6	23.6	61	4.87	NA	NA
	Potato chip	10.3	23.6	61	3.98	NA	NA
Fumonisin	Yam chip	928.12	23.6	61	359.08	2.0	17.95
	Potato chip	722.64	23.6	61	279.59	2.0	13.98
Zearalenone	Yam chip	16.33	23.6	61	6.32	0.5	1.26
	Potato chip	24.12	23.6	61	9.33	0.5	1.87

NA = Not Applicable

In this study, several fungi with varied frequencies of occurrence were isolated from sun dried yam and potato chips sold in open markets. Some of these fungi were *Aspergillus spp, Fusarium spp, Penicillium spp, Mucor spp* and *yeast* have been reported previously by Okigbo et al [23], Djeri et al [21] in Togo, Bankole and Mabekoje [24] and Ekundayo [25] from the South Western region of Nigeria in yam flour. The fungal species isolated in present study were known to cause spoilage of food products such as Cassava [26], Garri [27] and Cocoyam chips [28]. Jimoh and Kolapo [26], stated that some species of *Aspergillus* and *Mucor* produce toxins which can harm man and other animals when foods containing them are consumed.

The incidence of fungal contamination of yam chips, observed in the samples was higher than that which was earlier reported by Makun et al [16] in Niger state, similar reports of high fungal contamination have been reported by Bankole and Mabekoje [24], in Oyo and Ogun South Western Nigeria. The warm and humid condition in rainy season could facilitate the growth of fungi and thereby the high incidence of various molds encountered.

The most important factors responsible for fungal growth and mycotoxin production are high moisture content (20 to 25%), high relative humidity (70% and above), and warm temperature (20 to 30 ^{o}C). The production of mycotoxins during storage and transportation of food products can be linked to poor hygienic circumstances, high temperatures and moisture content. These are often the circumstances experienced in Africa [8].

The moisture content of 5.76 -7.41 % of potato chips and 3.72 – 6.62 % of yam chips in the present study is responsible for mold growth and toxin production [26]. Higher moisture content of yam and potato chips have been reported by Bankole and Adebanjo [29], Okungbowa and Osagie [30] and Ezekiel & Saning'o [4] The moisture content of yam chips as well as the humidity, high temperatures and substrate composition favored the colonization by these fungi. Bankole et al [31], Jimoh and Kolapo [26] suggested that species of *Mucor* and *Aspergillus* produce toxins which can harm man and other animals. Total aflatoxins, fumonisins and zearalenone contamination depended on several parameters including moisture content, temperature, processing practices and storage facilities Ezekiel and Saning'o [4].

In this study total aflatoxin contamination of yam and potato chips was 68 % and 84 % occurrence, mean concentration of 12.6 ug/kg and 10.3 ug/kg with a range 0-61.84 ug/kg and 0-54.49 ug/kg respectively. There has not been a report of aflatoxin contamination of yam and potato chips in Minna Niger State, but there are few reports in other parts of Nigeria. Total Aflatoxin contamination of Yam chip 22 %, 51 %, 97.5%, 98% and 100% occurrence was reported by Bankole et al. [31] in Ogun and Oyo, Bankole and Adebanjo [29] in South West, Abiala et al, [32] in Oyo State, Bassa et al. [33] in Benin. Total aflatoxin concentration of 12.15 µg/kg and 190 µg/kg in yam chips were reported by Bankole and Adebanjo [29] and Abiala et al. [32] respectively.

Ezekiel and Saning'o [4], reported total aflatoxins contamination of sun dried sweet potato with 36% occurrence, mean concentration of 40.31 µg/kg with a range 10.49 - 77.12 µg/kg. There is very little information regarding zearalenone (ZEN) in yam and potato chips in Nigeria. Thieu et al. [34] in Sweden detected zearalenone in 8 % cassava chip, with average concentrations of 10.0 µg/kg, and high incidences of aflatoxins and zearalenone in feedstuffs and pig feeds (83.3 - 100 %) were detected. Chilaka et al. [2], reported that incidence rate and maximum levels of zearalenone in Garri, '*Lafun*' and '*Amala*' were 17 % (17 ug/kg), 6 % (16 ug/kg), and 9 % (19 ug/kg), respectively.

Adejumo et al. [35] also reported zearalenone as one of the major mycotoxins contaminating agricultural products in Nigeria. The occurrence of zearalenone in other tuber crop products such as potato products has been reported [36]. In contrast, Somorin et al. [37] did not detect zearalenone in amala (yam flour) samples in their study. The variation in the results may be attributed to the sampling methods used. Somorin et al. [37] sampled yam flour. Fungi have easy accessibility to yam chips than yam flour because of the large surface area of the yam chips against the small particle size of yam flour. Zearalenone is stable during storage and milling and processing or cooking of food and does not degrade at high temperatures.

The concentrations of zearalenone in this study is below the EU limits for ZEN in cereals intended for direct human consumption, however there is still a safety problem because of the co-occurrence of multiple mycotoxins in these food products, as observed in tables 8 and 9. The frequency of mycotoxin co-occurrence in the samples suggests that a large proportion of the population, may be exposed to these mycotoxins through daily consumption of these food products. The occurrence of mycotoxins in these products may be attributed to the crude method of processing, poor storage, and marketing practices used for these products. The co-occurrence of these mycotoxins can affect both the level of mycotoxin production and the toxicity of the chips. Exposure to more than one mycotoxin may have potentiation, synergistic, antagonistic or additive effects on their toxicity. Radully et al. [12] stated that multiple mycotoxicoses may also occur because the human diet is a complex mixture of various ingredients. Simultaneous spoiling of food by several toxigenic fungi has been reported by several workers [11,12]. Moreover, some fungi are able to produce a broad spectrum of mycotoxins, and it is confirmed that combined physiological effects of mycotoxins are as relevant as the toxicity of a single mycotoxin. The harmful effects of simultaneous exposures to mycotoxins cannot be predicted solely relying on their individual toxicities. Chronic-combined effects of mycotoxins could be when they target the same physiological pathways. Complex biological systems, like the immune system, where every aspect of the mechanisms is essential and strictly regulated, are very sensitive to multiple mycotoxin exposures.

Rational limit for combined mycotoxin exposures, the exact concentrations of co-occurring mycotoxins should be determined, even when the individual concentrations are in the sub-toxic levels. This should be an important goal for further research in this field because people may consume mycotoxins in low toxic concentrations without any detectable symptoms, but the combinations of these low level toxin exposures may be detrimental [38-43].

This study shows that potato and yam chips in general represent a mycological and mycotoxin risk for consumers. The raising cases of liver cirrhosis may be due partly consumption from foods contaminated by mycotoxigenic fungi [12].

5. CONCLUSION

The study reports the occurrence of fungi and mycotoxin contamination of yam and potato chips sold in Minna Niger State. The highest moisture content of 7.41±2.28 % was recorded in potato chips and yam chips had the least moisture content of 4.72± 1.66 %. The fungal species isolated were *Aspergillus, Fusarium, Penicillium, Mucor* and *yeast. Aspergillus* species were the most commonly isolated fungi (55.2 %) while *Penicillium* spp were the least encountered fungi (5.7 %).

From this study, sun dried yam and potato chip products sold in Minna and its environs were contaminated with moulds and consequently, aflatoxin, fumonisin and zearalenone. Mycotoxin was detected in 48 (96 %) of the total samples. In yam and potato chips, total aflatoxin contamination was 68 and 84 % with mean concentrations of 12.6±7.36 µg/kg and 10.3±13.81 µg/kg respectively. Fumonisin contamination was 80 and 60 % with mean concentrations of 928.123±559.50 µg/kg and 722.64 ± 137.85 µg/kg for yam and potato chips respectively. Zearalenone contamination in yam and potato accounted for 56 and 68 % and had mean concentration of 16.33± 9.71 µg/kg and 24.12± 21.03 µg/kg respectively. Out of fifty samples analysed for total aflatoxins, fumonisin and zearealenone, 31(62 %), 19 (38 %) and 0 (0 %) respectively were unsafe for consumption based on unacceptable level of mycotoxins. The difference in contamination levels between yam and potato chips was statistically not significant ($P > 0.05$).

The estimated daily intake (EDI) and tolerable daily intake (%TDI) of aflatoxin and fumonisin was higher in yam chip (4.87 and 359.08 g/kg/bw/day) while zearalenone exposure and risk was higher in potato chips (1.87 %).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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