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Fungal diversity and aflatoxins in cereals and cassava-based foods from Ondo State, Nigeria

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Abstract: Cereals and cassava-based foods serve as major dietary sources for several households in Nigeria. However, these foods are highly prone to contamination by moulds and aflatoxins owing to poor storage and vending practices. We therefore studied the fungal diversity of maize, cassava-based flour (*pupuru*) and rice vended in markets from Ondo state, Nigeria, and assessed the aflatoxin levels of these using Enzyme-Linked Immunosorbent Assay. Fungi were detected in 93 (88 %) of the 106 food samples. Molecular analysis of 65 representative isolates revealed 26 species belonging to 5 genera: *Aspergillus* (80.9 %), *Penicillium* (15.4 %) and *Talaromyces* (1.9 %) in the *Ascomycota*; *Syncephalastrum* (1.2 %) and *Lichtheimia* (0.6%) in *Mucoromycota*. *Aspergillus flavus* was the predominant species in the food samples. Aflatoxins were found in 98 % of the 42 representative food samples and about one half (49.8 %) exceeded the 10 µg/kg threshold adopted in Nigeria for total aflatoxins. Integrated mitigation options, including at post-harvest stages, are suggested to ensure consumer safety.

Keywords: Aflatoxin; Food safety; Maize; Mycology; Nigeria; *Pupuru*; Rice

1. Introduction

Filamentous fungi contaminate food crops worldwide thereby contributing significantly to the problem of food safety and food insecurity [1]. In tropical countries, e.g. Nigeria, favourable warm-to-hot climatic conditions coupled with poor pre- and post-harvest agricultural practices encourage widespread filamentous fungal contamination of cereals such as maize and rice [2–4] and cassava products (e.g. *pupuru*) [5]. Unlike maize and rice that are consumed and available worldwide, *pupuru* is a cassava-based, traditionally processed staple commonly consumed in south-west Nigeria. *Pupuru* processing includes steeping of peeled cassava tubers in water for 4–5 days for submerged fermentation. The wet mash is bagged and dewatered using a mechanical press. The resulting fibres are handpicked from the mash prior to heating in an open fire, dry-milled and sieved into *pupuru* flour [5,6]. Maize, rice and *pupuru* principally contain carbohydrates (74 g, 80 g and 94.6 g) and less amounts of protein (9.4 g, 7.1 g and 2.9 g) and fibre (7.3 g, 1.3 g and 0.9 g) respectively [7]. Together, these foods contribute significantly to the calorie intake of several households in Nigeria, with specificity of *pupuru* to the Ondo and other south-western Nigerian population.

Maize, rice and *pupuru* are commonly locally vended in open markets in Nigeria, such that they are exposed to contamination by fungal spores [8,9]. Specifically, maize and rice are vended in open basins and plastic bowls whereas, *pupuru* is vended in covered basins and plastic bowls. Only a few studies used sequence data for identification of the fungi present on stored cereals in Nigeria. A study of the mycobiota of maize [3] applied a robust polyphasic approach, and an investigation of vended rice used sequences of the internal transcribed spacer (ITS) region [4]. However, ITS sequencing is less suitable for

species-level identification of common food-related genera such as *Aspergillus*, *Penicillium* and *Fusarium* [10]. Both studies were conducted in northern Nigeria, as such, there is paucity of fungal diversity data anchored on molecular techniques from southern Nigeria. Moreover, most of the fungal diversity studies on vended maize, rice and *pupuru* relied on data obtained from morphology and/or microscopy alone [2,5,11–15]. Applying phenotyping methods alone for fungal diversity studies have several drawbacks such as lack of precision and species misidentification [16,17]. Irrespective of the available data, there is currently no genera/species-specific based study on the fungal diversity of *pupuru* anywhere and reliable data on fungal diversity of maize and rice is limited. This creates a major knowledge gap for fungi surveillance database in the country and, therefore, presents a major hurdle to mitigation efforts.

Maize, rice and *pupuru* are stored for long periods, and contamination by toxic fungal metabolites such as aflatoxins can occur when conditions favouring fungal growth are present [18]. Chronic exposure to aflatoxins results in adverse human health effects such as cancers, stunting in children and death [18,19]. Previously, copious amounts of aflatoxins were quantified in maize from Ondo state, Nigeria [11,20], thus, making the state a likely hot-spot for aflatoxin contamination. Yet, there is paucity of data on aflatoxin contents of vended rice and *pupuru* from the state. Therefore, this study was carried out to determine the fungal diversity of maize, rice and *pupuru* using species-specific fungal molecular markers and quantify the aflatoxin levels by ELISA.

2. Materials and Methods

2.1. Study area

Ondo state is a south-western Nigerian state situated in the Derived savannah agroecological zone. The rainfall pattern is a bimodal distribution averaging between 1,000 and 1,300 mm per year and the temperature varies from 26 °C to 38 °C [11,21]. Food crops such as maize and cassava are grown by subsistent farmers in the state [22], whereas rice is mostly imported from other states.

2.2. Sampling of foods

A total of 106 food samples consisting of maize ($n = 46$), *pupuru* ($n = 20$) and rice ($n = 40$) were randomly purchased from major markets (Akure, Ondo, Ore and Owo) in Ondo state, Nigeria. Samples were purchased between December 2019 and January 2020. In each market, 100 g per sample of a food type was randomly collected from three parts of the vending vessel (basin or bowl) into clean polyethylene bags. The maize and rice samples were ground into fine powder using an electric blender (MX-AC400, Panasonic, India), whereas, *pupuru* did not require grinding since it is vended as flour. All food samples were batched in two: batch A for moisture content and mycological analysis and batch B for aflatoxin analysis. Batch A samples were stored at 4 °C and analyzed within 48 hours. For batch B, samples of same food type collected from the same vendor were mixed and stored at -20 °C prior to ELISA analysis. The total composite samples from the batch B samples were 42 (maize = 12; *pupuru* = 10; rice = 20).

2.3. Moisture content analysis of foods

The food samples were subjected to moisture analysis by the oven-drying to constant weight method [23]. Five grams of each sample was weighed and dried in a hot air oven at 105 °C. The weight of the samples was measured every hour until constant weight was achieved. Weight measurements were taken in triplicates per sample.

2.4. Isolation of fungi from foods

Moulds in the food samples were isolated by the dilution plating technique [24]. Ten grams of a food sample was diluted in 90 mL of sterile distilled water. The mixture was then homogenized for 2 min on a vortex mixer prior to spread-plating (100 μ L) on malt extract agar (MEA; Oxoid, UK). All inoculated plates were incubated at 25 °C for 3 days. In order to enumerate fungi in the food samples, fungal colonies on the plates were counted and reported as colony forming units per gram (CFU/g) of the food sample. Furthermore, distinct colonies on the isolation plates were transferred to freshly prepared MEA plates and incubated at 25 °C for 7 days. Thereafter, pure cultures were prepared on MEA slants in 4 mL vials, overlayed with sterile distilled water and stored at 25 °C.

2.5. Characterization of fungal isolates

The isolated moulds were characterized based on morphological characteristics and DNA sequencing. All the strains were cultivated on MEA at 25 °C for 7 days and then assessed for macro- and microscopic characters according to the descriptions in appropriate keys [10,25–28]. Isolates with similar phenotypic characters were grouped and representative isolates from each group were subjected to sequence-based identification.

The molecular analysis was conducted by extracting DNA from the representative isolates grown on MEA at 25 °C for 3–5 days. Parts of the β -tubulin (*BetaA*) and/or calmodulin (*CaM*) genes were amplified and subsequently sequenced for the *Aspergillus*, *Penicillium* and *Talaromyces* isolates and an ITS barcode sequence was generated for the other fungal isolates. Procedures were as previously described in Houbraken et al. [16,29] and Samson et al. [10]. The generated sequences were compared with sequences present in the National Center for Biotechnology Information (NCBI) database and the curated database of Food and Indoor Mycology department (DTO) housed at the Westerdijk Fungal Biodiversity Institute (WI). All molecularly identified isolates are maintained in the DTO working culture housed at the WI. The newly generated sequences were deposited in GenBank under accession number MZ014549–MZ028006. The potential of the isolates belonging to *Aspergillus* section *Flavi* to biosynthesize aflatoxins was tested *in vitro* on neutral red desiccated coconut agar (NRDCA) as described by Ezekiel et al. [30].

2.6. Aflatoxin analysis of foods by Enzyme-Linked Immunosorbent Assay

The concentration of aflatoxins (sum of B and G aflatoxins) present in the food samples was assayed by a quantitative ELISA kit assay (R4701; RIDASCREEN, Inc., GER) according to the manufacturer's instructions, and details on the methodology are given below in short. All reagents and food samples were allowed to reach ambient temperature. Of each sample, 20 g was mixed with 100 mL 70 % methanol extraction solvent. The mixture was homogenized in a shaker (UNISCOPE SM101, England) for 10 min. The supernatant was carefully decanted and filtered through Whatman No. 1 filter paper. The obtained filtrate (100 μ L) was subsequently diluted with distilled water (600 μ L).

Exactly 50 μ L of aflatoxin standards (0, 0.05, 0.15, 0.45, 1.35 and 4.05 μ g/kg) and diluted filtrate was dispensed in duplicates and 50 μ L of conjugate was added into each well. Antibody (50 μ L) was added to each well, mixed gently by shaking the plate manually prior to incubation for 30 min at ambient temperature. After incubation, the content of each well was discarded and the wells were washed thrice with wash buffer (250 μ L) prior to drying on an absorbent paper. Aliquots (100 μ L) of the substrate/chromogen was added to each well prior to mixing by gently shaking the plates manually and incubated for 15 min at ambient temperature. A stop solution (100 μ L) was then added to each well and the optical densities (OD) of the reaction solution in the microtiter plates was read at 450 nm within 30 min using the Microplate reader (LABTRON LMPR-A30, United Kingdom). The corresponding aflatoxin concentration in each well was estimated from standard curve plotted using the percentage binding against the total aflatoxin standards. The recovery and limit of detection (LOD) of the ELISA method were 85 % and 1.75 μ g/kg, respectively.

2.7. Data analysis

All data from this study were analyzed by descriptive statistics using the SPSS Statistics package version 21.0 (SPSS Inc. Chicago, IL, USA). Means for the data on moisture content as well as for the total aflatoxin concentrations in the food types were calculated and tested for significance using the One-way ANOVA ($\alpha = 0.05$).

3. Results

3.1. Moisture contents of foods

The moisture content of the food samples ranged between 3.51 % and 16.1 %. *Pupuru* has the highest mean moisture levels (mean: 11.3 ± 0.35 %; range: 9.09–14.6 %) followed by rice (range: mean: 9.44 ± 0.46 %; 5.08–16.1 %) and then maize (mean: 7.72 ± 0.30 %; range: 3.51–12.3 %).

3.2. Incidence of fungi in foods

3.2.1. Distribution of fungi

The fungal load, expressed as \log_{10} Colony Forming Units (CFU)/g, in the foods ranged $2.0\text{--}4.8 \log_{10}$ CFU/g, with specific ranges and mean values for each food type as: maize ($2.0\text{--}4.8 \log_{10}$ CFU/g; $3.90 \pm 0.11 \log_{10}$ CFU/g), *pupuru* ($2.0\text{--}4.5 \log_{10}$ CFU/g; 2.68 ± 0.20 CFU/g) and rice ($2.0\text{--}4.6 \log_{10}$ CFU/g; $2.64\% \pm 0.10$ CFU/g). In total, 162 fungal isolates were recovered from 93 (87.7%) of the 106 food samples. The foods were predominated by members of the phylum *Ascomycota* to which *Aspergillus* (80.9 %), *Penicillium* (15.4 %) and *Talaromyces* (1.9 %) belong; the other isolates belong to the *Mucoromycota* (*Syncephalastrum* 1.2 %, *Lichtheimia* 0.6 %). The occurrence frequency of the isolated fungi is shown in Figure 1.

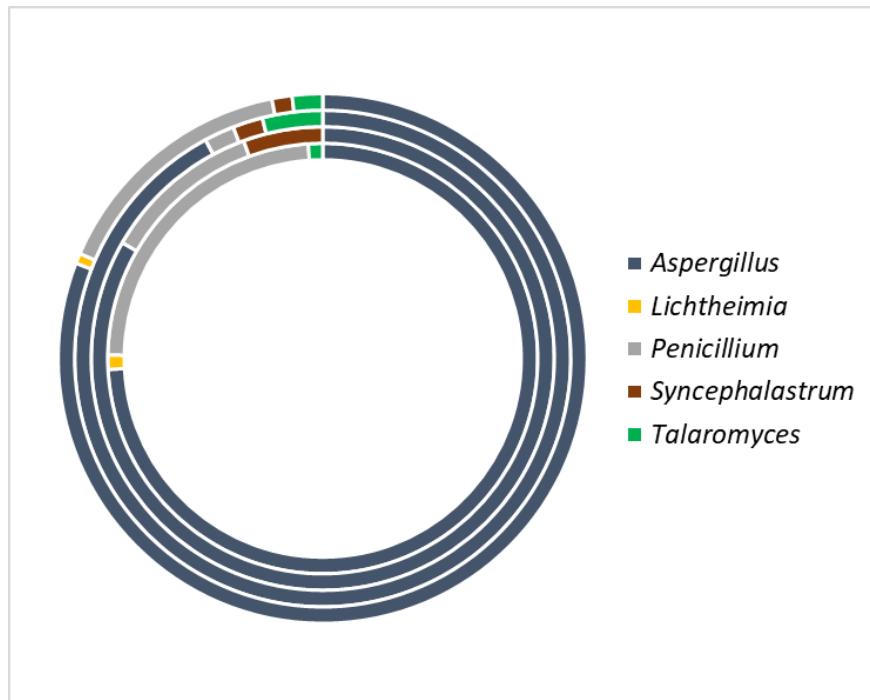


Figure 1. Incidence of fungal genera in foods vended in markets in Ondo state, Nigeria. Inner rings to outer rings represent maize, *pupuru*, rice and all foods.

3.2.2. Species diversity

Overall, 26 species were recovered from the food samples. Maize and rice both contained 13 different fungal species and 10 different fungal species were recovered from pupuru. *Aspergillus* was the predominant genus and the following species were detected (listed per section, according their prevalence): section *Flavi* ($n = 101$; *A. aflatoxiformans*, *A. flavus*, *A. tamarii* and *A. pseudonomiae*), *Nigri* ($n = 7$; *A. brasiliensis*, *A. brunneoviolaceus*, *A. luchuensis*, *A. neoniger*, *A. piperis* and *A. welwitschiae*), *Fumigati* ($n = 3$; *A. fischeri* and *A. fumigatus*), *Clavati* ($n = 2$; *A. giganteus*), *Circumdati* ($n = 1$; *A. melleus*), *Nidulantes* ($n = 10$; *A. sydowii*), *Terrei* ($n = 1$; *A. terreus*), *Candidi* ($n = 2$; *A. tritici*), *Usti* ($n = 2$; *A. calidoustus*) and *Aspergillus* ($n = 2$; *A. chevalieri*). Other species included *Penicillium citrinum* ($n = 24$), *P. cinamopurpureum* ($n = 1$), *Talaromyces funiculosus* ($n = 2$), *Talaromyces sayulitensis* ($n = 1$), *Lichtheimia ramosa* ($n = 1$) and *Syncephalastrum racemosum* ($n = 1$). *Aspergillus flavus* was the predominant species occurring in 50 % of the food samples. The species diversity of filamentous fungi in the foods is shown in Figure 2 and Table S1.

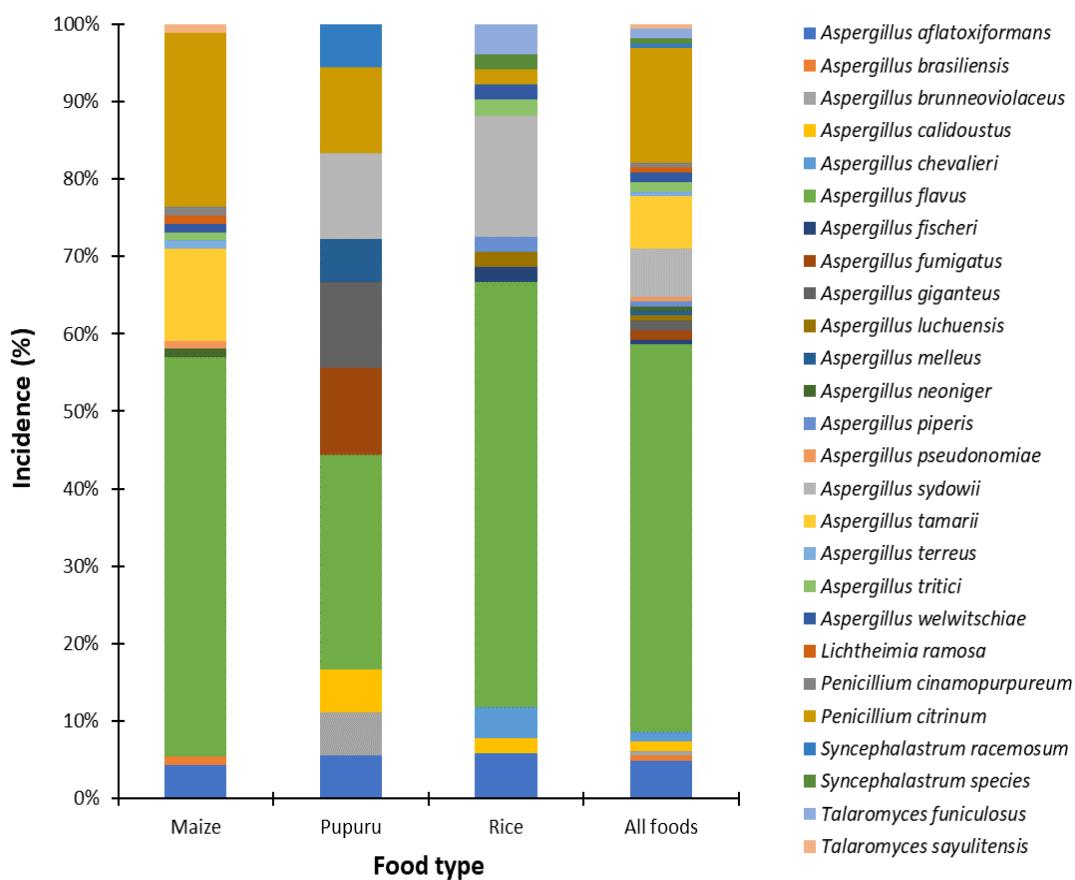


Figure 2. Species diversity of filamentous fungi in foods vended in markets in Ondo state, Nigeria.

3.2.3. Incidence of aflatoxigenic and non-aflatoxigenic species of *Aspergillus* section *Flavi*

Of the 101 isolates belonging to *Aspergillus* section *Flavi*, 80.2 %, 10.9 %, 7.9 % and 1.0 % were *A. flavus*, *A. tamarii*, *A. aflatoxiformans* and *A. pseudonomiae*, respectively. About 59 % of the 101 isolates were toxigenic on NRCA, whereas, 41 % were non-toxigenic. The incidences of aflatoxigenic and non-aflatoxigenic species in the foods are shown in Figure 3 and Table S2.

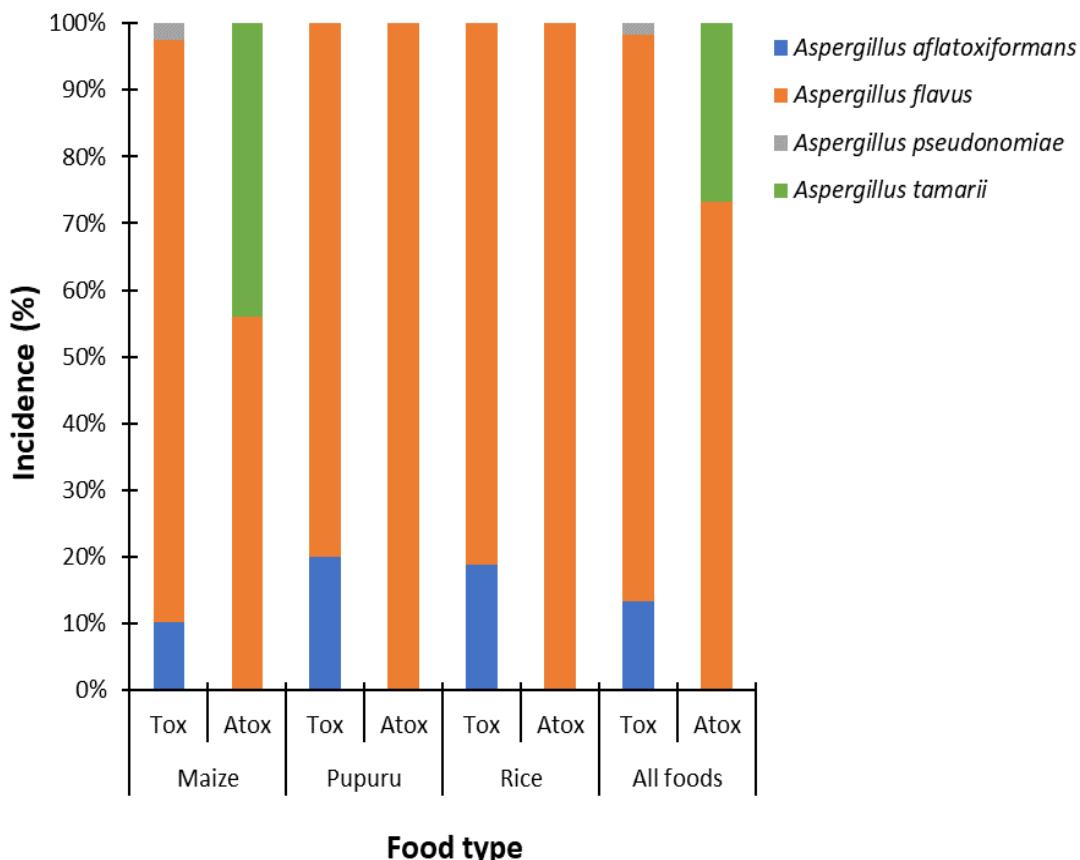


Figure 3. Incidence of aflatoxigenic and non-aflatoxigenic *Aspergillus* section *Flavi* members in foods vended in markets in Ondo state, Nigeria.

3.3. Aflatoxin levels in food samples

ELISA analysis revealed that 31 (73.8 %) of the 42 composite food samples were contaminated with aflatoxins. Overall, the total aflatoxin levels in the food samples were in the range of 1.75 to 173.3 µg/kg (mean: 43.1 µg/kg). The incidence and levels in each food type were: maize (incidence: 100%; range: 3.50–173.3 µg/kg; mean: 100.6 ± 19.3 µg/kg), pupuru (incidence: 40%; range: 1.75–21 µg/kg; mean: 7.9 ± 4.4 µg/kg) and rice (incidence: 75%; range: 1.75–22.8 µg/kg; mean: 6.5 ± 1.6 µg/kg). Only 29.03 % of the food samples contained aflatoxins below the EU threshold of 4 µg/kg for total aflatoxins, while more than half of the samples exceeded the 10 µg/kg threshold adopted in Nigeria for total aflatoxins.

Table 1. Incidence of aflatoxins in 42 food samples from Ondo state, Nigeria.

Food type	N	%	Incidence (%) of contaminated food samples					Mean (µg/kg) ± SE	Range (µg/kg)	
			≤4 µg/kg*	≤10 µg/kg	≤20 µg/kg**	>20–<100 µg/kg	≥100 µg/kg		Min	Max
Maize	12	100	8.3	16.7	25.0	16.7	58.3	101 ± 19.3	3.5	173.3
Pupuru	10	40	50	75	75.0	25	0.00	7.9 ± 4.4	1.75	21
Rice	20	75	40	86.7	93.3	6.7	0.00	6.5 ± 1.6	1.75	22.8
Total	42	73.8	29.03	58.1	64.5	12.9	58.3	43.1 ± 11.1	1.75	173.3

N = number of samples; % = percentage contaminated samples; *European Union regulatory limit for total aflatoxins in foods; **United States Food and Drug Administration regulatory limit for total aflatoxins in foods.

4. Discussion

Post-harvest fungal growth in food is largely influenced by the moisture levels in the foods [9,31]. Generally, the mean moisture contents of the food samples were low (≤ 11), suggesting they were stored under safe conditions [31,32]. Significantly ($p<0.05$) higher mean moisture levels were reported in the cassava-based food (*pupuru*) compared to the cereals (maize and rice). This observation could be attributed to the high-water content in cassava, (the raw ingredient of *pupuru*), when compared to cereals as well as the *pupuru* production process, which involves addition of water. The mean moisture levels recorded for maize and rice (10.4 % and 12.5 %, respectively) were lower than the values previously reported for maize and rice in Nigeria [2,33] and maize from Ethiopia [34]. Conversely, the mean moisture level in *pupuru* was similar to the value (11.8 %) previously reported in *pupuru* in Nigeria [7]. The low moisture levels in the three food types examined in the present study suggest that the recovery of fungal propagules from the foods is rather influenced by contamination along the production chain and during storage of the foods as well as by open vending of unpackaged grains, and not primarily by moisture level [9,35]. To put into proper context, the food samples were collected from major markets, which are characterized by increased human activities and vehicular movements such that diverse fungal spores become disseminated and carried-over into the foods.

There was a marked difference in the diversity of species in the three food types, with higher species diversity (13 species) occurring in maize and rice compared to 10 species found in *pupuru*. The obvious reason for the lower species diversity in the processed *pupuru* compared to the grains (maize and rice) is the application of heat during *pupuru* processing, which has the capacity to eliminate fungal propagules in foods. *Aspergillus* was the predominant genus in the cereals and cassava-based food product, occurring in more than three quarter of all the food samples examined. This is consistent with previous reports on the *Aspergillus* dominance in Nigerian foods such as maize [21,36], rice [4] and *garri*, a cassava-based food product from Nigeria [35]. These data are in contrast to reports on fungal profiles from maize in South Africa [37] and China [38], where *Fusarium* was the predominant genus. In this study, *Fusarium* was not recovered from any of the food samples, which agrees with findings from two previous studies conducted in Nigeria on rice [2] and *pupuru* [12]. Conversely, this finding from the present study contradicts other available reports [3,34,39,40] that reported several *Fusarium* species in cereals including rice and maize. *Penicillium* was the second most predominant genus in the food samples, occurring in all the foods and mostly in maize and *pupuru*. High incidence of *Penicillium* was previously reported in maize from Ethiopia [34] and *pupuru* from Nigeria [12]. Other fungal genera recovered from the foods include *Talaromyces* (only recovered from the cereals); *Syncephalastrum* (from *pupuru* and rice) and *Lichtheimia* (found only in maize). The latter genus was previously known as *Absidia* and has been reported in Chinese and Brazilian maize [41], but is to the best of our knowledge reported for the first time in Nigerian maize.

In this study, *A. flavus* was the predominant species in the cereals and *pupuru* samples, occurring in 50 % of all the food samples. The predominance of *A. flavus* in the foods is supported by previous reports from Nigeria [4,42,43] and elsewhere [44,45]. *Aspergillus aflatoxiformans* was recovered from all the foods, but mostly in the cereals. This agrees with a previous report from Nigeria, wherein *A. aflatoxiformans* predominated in cereals including maize and rice [3]. *Aspergillus aflatoxiformans* (previously, wrongly classified as *A. parvisclerotigenus*), has been reported in cassava from neighbouring Benin Republic [46], as well as dried mushroom and peanuts from Nigeria [47,48]. But, the occurrence of *A. aflatoxiformans* from any Nigerian cassava-based food has not been documented till now. Two other members of *Aspergillus* section *Flavi* (*A. tamarii* and *A. pseudonomiae*) were only found in maize, which is consistent with previous reports [3,45,49]. Of the *Aspergillus* section *Flavi* isolates tested for toxigenicity *in vitro* on NRCDA, more than one half of the *A. flavus* strains and all of the *A. aflatoxiformans* and *A. pseudonomiae* exhibited toxigenic potential on NRDCA. Aflatoxin production is a chemotaxonomic signature in the *Aspergillus* section *Flavi* group, especially among strains of *A. flavus*, *A. aflatoxiformans* and *A. pseudonomiae* [50]. Consequently, the recovery of toxigenic strains in the present study may

imply that frequent ingestion of these foods is a contributory factor to the high aflatoxin exposure recorded in Nigeria [51–55].

The fungal species belonging to *Aspergillus* section *Nigri* recovered from the foods include *A. neoniger*, *A. brasiliensis*, *A. brunneoviolaceus*, *A. luchuensis*, *A. piperis* and *A. welwitschiae* [56]. *Aspergillus brunneoviolaceus* was only recovered from *pupuru*. This observation agrees with the report on *A. brunneoviolaceus* in *garri*, another cassava-based product, in Nigeria [35] and suggests an association of this species with cassava (products). Other members of the section *Nigri* were recovered only from the cereals. *Aspergillus welwitschiae* was only recovered from maize, which is in agreement with a previous report from USA and Italy [57]. Similarly, *A. brasiliensis* and *A. neoniger* were recovered from maize, whereas *A. luchuensis* and *A. piperis* were found in rice. *Aspergillus fumigatus* (section *Fumigati*), a notorious opportunistic pathogen [58] was only recovered from *pupuru*. The other member of the *Aspergillus* section *Fumigati*, *A. fischeri*, was found in rice. Other members of the *Aspergillus* genera found only in *pupuru* include *A. calidoustus* in *Aspergillus* section *Usti* [59], *A. giganteus* in *Aspergillus* section *Clavati* [60] and *A. melleus* in *Aspergillus* section *Circumdati* [61], which are reported for the first time in Nigerian dried cassava-based food.

Penicillium citrinum, a known citrinin producer [62], was recovered from all the food types. Recent reports found this fungus in cocoa and dried ready-to-eat foods from Nigeria [35,63], with the strains producing copious amounts (up to 372 mg/kg) of citrinin [63]. Furthermore, high levels (16,800 µg/kg and 51,195 µg/kg) of citrinin were previously quantified in Nigerian maize [64,65]. Citrinin is a nephrotoxic mycotoxin [66]. Putting findings from the aforementioned reports together with the high incidence of *P. citrinum* in this study, there is an urgent need for mitigation efforts targeted at reducing *P. citrinum*, which indirectly reduces citrinin levels, in Nigerian foods. Other notable fungal species include *P. cinamopurpureum*, *L. ramosa*, *T. funiculosus* and *T. sayulitensis* recovered only from the cereals and *Syncephalastrum racemosum* found only in *pupuru*. *Talaromyces sayulitensis* is mostly associated with maize [67], which agrees with findings of the present study. *Lichtheimia ramosa* is a soil fungus that causes mucormycosis especially in immunocompromised individuals [68]. *Syncephalastrum racemosum* can cause onychomycosis [69] and this fungus has been previously reported in cocoa seeds in Nigeria [70], but its occurrence in *pupuru* was not yet been documented to date. Major limitations of this study are the use of MEA for fungal isolation and the incubation of fungal culture plates for three days, thus precluding the recovery of slow growing fungi during isolation. However, the main focus was to enumerate mostly toxigenic fungi (*Aspergillus* and *Penicillium*) from the food samples as such data from the study is relevant.

In order to ascertain the aflatoxicological safety of the foods, the levels of aflatoxin in the foods were determined. The focus on aflatoxin determination amidst many other mycotoxins emanated from its status as the most toxicologically important mycotoxin due to its categorization as Class 1 carcinogen [71]. In the present study, 98 % of the examined composite food samples contained aflatoxins; this is consistent with previous reports from Nigeria [20,72] and Kenya [73] that applied ELISA protocols in food analysis and reported aflatoxin incidence > 95 %. The aflatoxin incidence reported for *pupuru* in the present study is higher than the 30 % incidence previously reported [74]. More than one half (54 %) of the food samples exceeded the 4 µg/kg threshold set by the European Union (EU) for total aflatoxins [75]. The proportion of samples exceeding the EU limit is high and calls for more caution and the need to set in place critical intervention measures to limit aflatoxin levels in the foods. This is crucial for foods such as maize and rice, which have the potentials to be aggregated at the local market and exported to the EU. Overall, the high levels of aflatoxins quantified in the food samples agree with the data obtained for the high incidence of toxigenic *Aspergillus* species recovered from the food samples in the present study. It is likely that poor storage practices, which is very common in Nigeria [9], contributed to the high aflatoxin levels in the food samples. Nevertheless, a more robust mycotoxin surveillance study anchored on liquid chromatography-based method is

required to understand the actual extent of aflatoxin contamination in the foods vended in Nigeria.

5. Conclusions

This study provides snapshot data on the fungal diversity and aflatoxin contents of cereals and *pupuru* vended in open markets in Nigeria. Diverse fungal species and high aflatoxin levels were found in the examined foods suggesting the possible influence of poor handling, processing and storage on the contamination of the foods. Urgent mitigation efforts are required to limit toxigenic fungal and aflatoxin contamination of these foods in the country. We therefore recommend proper storage of foods in hermetic devices such as Purdue Improved Crop Storage (PICS) bags and metal silos as detailed in Ayeni et al. [9]. In addition, households are advised to properly sort out bad grains before being applied to make foods.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Numerical values for fungal species diversity in the foods, and Table S2: Numerical values for toxicity of members of *Aspergillus* section *Flavi* in the foods.

Author Contributions: Conceptualization, C.N.E. and K.I.A.; methodology, B.K., D.O.E., K.I.A. and M.M.; software, C.N.E. and J.H.; validation, C.N.E. and J.H.; formal analysis, B.K., D.O.E., K.I.A. and M.M.; investigation, B.K., D.O.E., K.I.A. and M.M.; resources, C.N.E., D.O.E and J.H.; data curation, C.N.E., K.I.A. and J.H.; writing—original draft preparation, D.O.E. and K.I.A.; writing—review and editing, C.N.E and J.H.; visualization, K.I.A., C.N.E and J.H.; supervision, C.N.E.; project administration, C.N.E., K.I.A and J.H.; funding acquisition, C.N.E. and J.H. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Avery, S.V.; Singleton, I.; Magan, N.; Goldman, G.H. The fungal threat to global food security. *Fungal Biol.* **2019**, *123*, 555–557. <https://doi.org/10.1016/j.funbio.2019.03.006>
2. Abdus-Salaam, R.; Atanda, O.; Fanelli, F.; Sulyok, M.; Cozzi, G.; Bavaro, S.; Krska, R.; Logrieco, A.F.; Ezekiel, C.N.; Salami, W.A. Fungal isolates and metabolites in locally processed rice from five agro-ecological zones of Nigeria. *Food Addit. Contam. Part B Surveill.* **2016**, *9*, 281–289. <https://doi.org/10.1080/19393210.2016.1215354>
3. Ezekiel, C.N.; Kraak, B.; Sandoval-Denis, M.; Sulyok, M.; Oyedele, O.A.; Ayeni, K.I.; Makinde, O.M.; Akinyemi, O.M.; Krska, R.; Crous, P.W.; Houbraken, J. Diversity and toxigenicity of fungi and description of *Fusarium madaense* sp. nov. from cereals, legumes and soils in north-central Nigeria. *MycoKeys* **2020a**, *67*, 95–124. <https://doi.org/10.3897/mycokeys.67.52716>
4. Makun, H.A.; Dutton, M.F.; Njobeh, P.B.; Phoku, J.Z.; Yah, C.S. Incidence, phylogeny and mycotoxicogenic potentials of fungi isolated from rice in Niger state, Nigeria. *J. Food Saf.* **2011**, *31*, 334–349. <https://doi.org/10.1111/j.1745-4565.2011.00305.x>
5. Shittu, T.A.; Edema, M.O.; Dada, O.; Atayese, A.O. Microorganisms associated with the spoilage of *pupuru*. *Food Contr.* **2010**, *21*, 203–206. <https://doi.org/10.1016/j.foodcont.2009.05.012>
6. Famurewa, J.; Oluwamukomi, M.; Alaba, J. Effect of different drying methods on the physicochemical characteristics of cassava flour (“*pupuru*”). *Int. J. Biol. Chem. Sci.* **2013**, *7*, 832–839. <https://doi.org/10.4314/ijbcs.v7i2.38>
7. Akinyele, O.F.; Ikujenlola, A.V.; Omobuwajo, T.O. Physico-chemical and sensory properties of *pupuru* and *pupuru* analogues from co-fermented cassava (*Manihot esculenta* Crantz) and breadfruit (*Artocarpus altilis*) blends. *Acta Univ. Sapientiae Aliment.* **2020**, *13*, 32–50.
8. Makinde, O.M.; Ayeni, K.I.; Sulyok, M.; Krska, R.; Adeleke, R.A.; Ezekiel, C.N. Microbiological safety of ready-to-eat foods in low- and middle-income countries: a comprehensive 10-year (2009–2018) review. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*(2), 703–732. <https://doi.org/10.1111/1541-4337.12533>
9. Ayeni, K.I.; Atanda, O.O.; Krska, R.; Ezekiel, C.N. Present status and future perspectives of grains drying and storage practices as a means to reduce mycotoxin exposure in Nigeria. *Food Contr.* **2021**, *126*, 108074. <https://doi.org/10.1016/j.foodcont.2021.108074>
10. Samson, R.A.; Houbraken, J.; Thrane, U.; Frisvad, J.C.; Andersen, B. *Food and Indoor Fungi: CBS Laboratory Manual Series 2*, 2nd ed.; Westerdijk Fungal Biodiversity Institute: Utrecht, The Netherlands, **2019**; pp. 1–472.

11. Adetunji, M.C.; Atanda, O.O.; Ezekiel, C.N.; Dipeolu, A.O.; Uzochukwu, S.; Oyedepo, J.; Chilaka, C. Distribution of mycotoxins and risk assessment of maize consumers in five agro-ecological zones of Nigeria. *Euro. Food Res. Technol.* **2014a**, *239*, 287–296. <https://doi.org/10.1007/s00217-014-2221-0>
12. Aruwa, C.E.; Ogundare, O. Microbiological quality assessment of *pupuru* and plantain flours in an urban market in Akure, Ondo state, Southwestern Nigeria. *Open Access Libr. J.* **2017**, *04*, e3783. <https://doi.org/10.4236/oalib.1103783>
13. Jeff-Agboola, Y.A.; Omosanyin, T.R. Occurrence of toxigenic moulds isolated in maize (*Zea mays*) from Okitipupa metropolis, Ondo State, Nigeria. *Int. J. Food Saf. Nutr. Pub. Health Technol. Coimbatore* **2017**, *9*, 28–37.
14. Makun, H.A.; Gbodi, T.A.; Akanya, O.H.; Salako, E.A.; Ogbadu, G.H. Fungi and some mycotoxins contaminating rice (*Oryza sativa*) in Niger State, Nigeria. *Afr. J. Biotechnol.* **2007**, *6*, 099–108. <https://doi.org/10.4314/ajb.v6i2.56106>
15. Shehu, K.; Salau, I.A.; Salisu, N. Fungal and mycotoxin contamination of stored maize grains in Kebbi state, north-western Nigeria. *J. Adv. Bot. Zool.* **2020**, *8*, 4–8.
16. Houbraken, J.; Frisvad, J.C.; Samson, R.A. Taxonomy of *Penicillium* section *Citrina*. *Stud. Mycol.* **2011**, *70*, 53–138. <https://doi.org/10.3114/sim.2011.70.02>
17. Samson, R.A.; Visagie, C.M.; Houbraken, J.; Hong, S.-B.; Hubka, V.; Klaassen, C.H.W.; Perrone, G.; Seifert, K.A.; Susca, A.; Tanney, J.B.; Varga, J.; Kocsube, S.; Szigeti, G.; Yaguchi, T.; Frisvad, J.C. Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Stud. Mycol.* **2014**, *78*, 141–173. <https://doi.org/10.1016/j.simyco.2014.07.004>
18. International Agency for Research on Cancer (IARC). Mycotoxin control in low- and middle-income countries. In *IARC Working Group Report No. 9*; Wild, C.P., Miller, J.D., Groopman, J.D., Eds.; IARC: Lyon, France, 2015; pp. 1–54.
19. Kamala, A.; Shirima, C.; Jani, B.; Bakari, M.; Sillo, H.; Rusibamayila, N.; De Saeger, S.; Kimanya, M.; Gong, Y.Y.; Simba, A. Outbreak of an acute aflatoxicosis in Tanzania during 2016. *World Mycotox. J.* **2018**, *11*(3), 311–320. <https://doi.org/10.3920/WMJ2018.2344>
20. Ayeni, K.I.; Akinyemi, O.M.; Kovac, T.; Ezekiel, C.N. Aflatoxin contamination of maize vended in Ondo state, Nigeria, and health risk assessment. *Croat. J. Food Sci. Technol.* **2020**, *12*(1), 123–129. <https://doi.org/10.17508/CJFST.2020.12.1.16>
21. Atehnkeng, J.; Ojiambo, P.S.; Donner, M.; Ikotun, T.; Sikora, R.A.; Cotty, P.J.; Bandyopadhyay, R. Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones in Nigeria. *Int. J. Food Microbiol.* **2008**, *122*(1–2), 74–84.
22. Ayodele, O.V.; Akindele, M.O. Extension Activities for Arable Crops Production in Akure South Local Government Area, Ondo State, Nigeria. Proceedings of the Annual Conference of the Agricultural Extension Society of Nigeria, Port Harcourt, Nigeria, 23rd–26th April 2017; University of Port Harcourt Printing Press: Port Harcourt, Nigeria, **2017**.
23. Commission of the European Communities. Commission regulation (EC) No 824/2000 of 19 April 2000 establishing procedures for the taking-over of cereals by intervention agencies and laying down methods of analysis for determining the quality of cereals. *Off. J. Eur. Commun.* **2000**, *L100*, 31–50.
24. Samson, R.A.; Hoekstra, E.S.; Frisvad, J.S.; Filtenborg, O. Methods for the detection and isolation of food-borne fungi. In *Introduction to Foodborne Fungi*; Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O., Eds.; Centraal Bureau voor Schimmel cultuuren: Utretch, The Netherlands, **1995**; pp. 235–242.
25. Frisvad, J.C.; Samson, R.A. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate *Penicillia* and their mycotoxins. *Stud. Mycol.* **2004**, *49*, 1–174.
26. Leslie, J.F.; Summerell, B.A.; *The Fusarium Laboratory Manual*; Blackwell Publishing: Ames, USA, **2006**; 1–388. <https://doi.org/10.1002/9780470278376>
27. Pitt, J.I.; Hocking, A.D.; *Fungi and Food Spoilage*; Springer: London., UK, **2009**; 1–519. <https://doi.org/10.1007/978-0-387-92207-2>
28. Samson, R.A.; Houbraken, J.; Spierenburg, H.; Seifert, K.A.; Peterson, S.W.; Varga, J.; Frisvad, J.C. Phylogeny and nomenclature of the genus *Talaromyces* and taxa accommodated in *Penicillium* subgenus *Biverticillium*. *Stud. Mycol.* **2011**, *70*, 159–189. <https://doi.org/10.3114/sim.2011.70.04>
29. Houbraken, J.; Frisvad, J.C.; Seifert, K.A.; Overy, D.P.; Tuthill, D.M.; Valdez, J.G.; Samson, R.A. New penicillin-producing *Penicillium* species and an overview of section *Chrysogena*. *Persoonia* **2012**, *29*, 78–100. <https://doi.org/10.3767/003158512X660571>
30. Ezekiel, C.N.; Adetunji, M.C.; Atanda, O.O.; Frisvad, J.C.; Houbraken, J.; Samson, R.A. Phenotypic differentiation of species from *Aspergillus* section *Flavi* on neutral red desiccated coconut agar. *World Mycotoxin J.* **2014a**, *7*(3), 335–344. <https://doi.org/10.3920/WMJ2014.1727>

31. Magan, N.; Aldred, D.; Mylona, K.; Lambert, R.J.W. Limiting mycotoxins in stored wheat. *Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess.* **2010**, *27*, 644–650. <https://doi.org/10.1080/19440040903514523>

32. Food Agricultural Organization of the United Nations (FAO). Rural structures in the tropics: Design and development, Food Agricultural Organization: Rome, Italy, **2011**.

33. Shitu, S.; Macchido, D.A.; Tijjani, M.B. Detection of aflatoxigenic moulds and aflatoxins in maize and millet's grains marketed in Zaria metropolis. *J. Adv. Microbiol.* **2018**, *13*, 1–9. <https://doi.org/10.9734/jamb/2018/40075>

34. Getachew, A.; Chala, A.; Hofgaard, I.S.; Brurberg, M.B.; Sulyok, M.; Tronsmo, A.M. Multimycotoxin and fungal analysis of maize grains from south and southwestern Ethiopia. *Food Addit. Contam. Part B Surveill.* **2017**, *11*, 64–74. <https://doi.org/10.1080/19393210.2017.1408698>

35. Ezekiel, C.N.; Oyedele, O.A.; Kraak, B.; Ayeni, K.I.; Sulyok, M.; Houbraken, J.; Krska, R. Fungal diversity and mycotoxins in low moisture content ready-to-eat foods in Nigeria. *Front. Microbiol.* **2020b**, *11*, 615. <https://doi.org/10.3389/fmicb.2020.00615>

36. Adetunji, C.; Atanda, O.; Ezekiel, C.N.; Ogara, I.M. The distribution and mycotoxicogenicity of fungal isolates of stored maize grains from five agro-ecological zones of Nigeria. *Mycotoxicol.* **2014b**, *1*, 19–28.

37. Ekwomadu, T.I.; Gopane, R.E.; Mwanza, M. Occurrence of filamentous fungi in maize destined for human consumption in South Africa. *Food Sci. Nutr.* **2017**, *1*–7. <https://doi.org/10.1002/fsn.3561>

38. Xing, F.; Liu, X.; Wang, L.; Selvaraj, J.N.; Jin, N.; Wang, Y.; Zhao, Y.; Liu, Y. Distribution and variation of fungi and major mycotoxins in pre- and post-nature drying maize in North China Plain. *Food Contr.* **2017**, *80*, 244–251. <https://doi.org/10.1016/j.foodcont.2017.03.055>

39. Avila, C.F.; Moreira, G.M.; Nicolli, C.P.; Gomes, L.B.; Abreu, L.M.; Pfenning, L.H.; Haidukowski, M.; Moretti, A.; Logrieco, A.; Del Ponte, E.M. *Fusarium incarnatum-equiseti* species complex associated with Brazilian rice: Phylogeny, morphology and toxicogenic potential. *Int. J. Food Microbiol.* **2019**, *306*, 108267. <https://doi.org/10.1016/j.ijfoodmicro.2019.108267>

40. Nicolli, C.P.; Haidukowski, M.; Susca, A.; Gomes, L.B.; Logrieco, A.; Stea, G.; Del Ponte, E.M.; Moretti, A.; Pfenning, L.H. *Fusarium fujikuroi* species complex in Brazilian rice: Unveiling increased phylogenetic diversity and toxicogenic potential. *Int. J. Food Microbiol.* **2020**, *330*, 108667. <https://doi.org/10.1016/j.ijfoodmicro.2020.108667>

41. Khosravi, A.R.; Mansouri, M.; Bahonar, A.R.; Shokri, H. Mycoflora of maize harvested from Iran and imported maize. *Pak. J. Biol. Sci.* **2007**, *10*(24), 4432–4437.

42. Ezekiel, C.N.; Sulyok, M.; Somorin, Y.; Odutayo, F.I.; Nwabekwe, S.U.; Balogun, A.T.; Krska, R. Mould and mycotoxin exposure assessment of melon and bush mango seeds, two common soup thickeners consumed in Nigeria. *Int. J. Food Microbiol.* **2016**, *237*, 83–91. <https://doi.org/10.1016/j.ijfoodmicro.2016.08.019>

43. Oyedele, O.A.; Ezekiel, C.N.; Sulyok, M.; Adetunji, M.C.; Warth, B.; Atanda, O.O.; Krska, R. Mycotoxin risk assessment for consumers of groundnut in domestic markets in Nigeria. *Int. J. Food Microbiol.* **2017**, *251*, 24–32. <https://doi.org/10.1016/j.ijfoodmicro.2017.03.020>

44. Madbouly, A.K.; Ibrahim, M.I.M.; Sehab, A.F.; Abdel-Wahhab, M.A. Co-occurrence of mycoflora, aflatoxins and fumonisins in maize and rice seeds from markets of different districts in Cairo, Egypt. *Food Addit. Contam. Part B Surveill.* **2012**, *5*, 112–120. <https://doi.org/10.1080/19393210.2012.676078>

45. Probst, C.; Bandyopadhyay, R.; Cotty, P.J. Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa. *Int. J. Food Microbiol.* **2014**, *174*, 113–122. <https://doi.org/10.1016/j.ijfoodmicro.2013.12.010>

46. Adjovi, Y.C.S.; Bailly, S.; Gnonlonfin, B.J.G.; Tadrist, S.; Querin, A.; Sanni, A.; Oswald, I.P.; Puel, O.; Bailly, J.D. Analysis of the contrast between natural occurrence of toxicogenic Aspergilli of the *Flavi* section and aflatoxin B₁ in cassava. *Food Microbiol.* **2014**, *38*, 151–159.

47. Ezekiel, C.N.; Sulyok, M.; Frisvad, J.C.; Somorin, Y.M.; Warth, B.; Houbraken, J.; Samson, R.A.; Krska, R.; Odebone, A.C. Fungal and mycotoxin assessment of dried edible mushroom in Nigeria. *Int. J. Food Microbiol.* **2013**, *162*, 231–236.

48. Frisvad, J.C.; Skouboe, P.; Samson, R.A. Taxonomic comparison of three different groups of aflatoxin producers and a new efficient producer of aflatoxin B₁, sterigmatocystin and 3-O-methylsterigmatocystin, *Aspergillus rambelli* sp. *System. Appl. Microbiol.* **2005**, *28*, 442–453.

49. Iyanyi, N.G.; Ataga, A.E.; Ifegwu, M.K.; Pere, F. Identification of fungal organisms associated with the rhizosphere of maize (*Zea mays* L.): basic molecular techniques. *Niger. Agric. J.* **2020**, *51*, 399–405.

50. Frisvad, J.C.; Hubka, V.; Ezekiel, C.N.; Hong, S-B.; Novakova, A.; Chen, A.J.; Arzanlou, M.; Larsen, T.O.; Sklenar, F.; Mahakamchanakul, W.; Samson, R.A.; Houbraken, J. Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins, ochratoxins and other mycotoxins. *Stud. Mycol.* **2019**, *93*, 1–63. <https://doi.org/10.1016/j.simyco.2018.06.001>

51. Adejumo, O.; Atanda, O.; Raiola, A.; Somorin, Y.; Bandyopadhyay, R.; Ritieni, A. Correlation between aflatoxin M₁ content of breast milk, dietary exposure to aflatoxin B₁ and socioeconomic status of lactating mothers in Ogun State, Nigeria. *Food Chem. Toxicol.* **2013**, *56*, 171–177. <https://doi.org/10.1016/j.fct.2013.02.027>

52. Ezekiel, C.N.; Warth, B.; Ogara, I.M.; Abia, W.A.; Ezekiel, V.C.; Atehnkeng, J.; Sulyok, M.; Turner, P.C.; Tayo, G.O.; Krska, R.; Bandyopadhyay, R. Mycotoxin exposure in rural residents in northern Nigeria: A pilot study using multi-urinary biomarkers. *Environ Int.* **2014b**, *66*, 138–145. <https://doi.org/10.1016/j.envint.2014.02.003>

53. Ezekiel, C.N.; Oyeyemi, O.; Oyedele, O.A.; Ayeni, K.I.; Oyeyemi, I.T.; Nabofa, W.; Chinomso, U. Urinary aflatoxin exposure monitoring in rural and semi-urban populations in Ogun state, Nigeria. *Food Addit. Contam. Part A* **2018**, *35*(8), 1565–1572. <https://doi.org/10.1080/19440049.2018.1475752>

54. Ezekiel, C.N.; Abia, W.A.; Braun, D.; Sarkanj, B.; Ayeni, K.I.; Oyedele, O.A.; Michael-Chikezie, E.C.; Ezekiel, V.C.; Mark, B.; Ahuchaogu, C.P.; Krska, R.; Sulyok, M.; Turner, P.C.; Warth, B. Comprehensive mycotoxin exposure biomonitoring in breastfed and nonexclusively breastfed Nigerian children. *medRxiv* **2020c**, 1–45. <https://doi.org/10.1101/2020.05.28.20115055>

55. Sarkanj, B.; Ezekiel, C.N.; Turner, P.C.; Abia, W.A.; Rychlik, M.; Krska, R.; Sulyok, M.; Warth, B. Ultra-sensitive, stable isotope assisted quantification of multiple urinary mycotoxin exposure biomarkers. *Anal. Chim. Acta* **2018**, *1019*, 84–92. <https://doi.org/10.1016/j.aca.2018.02.036>

56. Houbraken, J.; Kocsube, S.; Visagie, C.M.; Yilmaz, N.; Wang, X.-C.; Meijer, M.; Kraak, B.; Hubka, V.; Samson, R.A.; Frisvad, J.C. Classification of *Aspergillus*, *Penicillium*, *Talaromyces* and related genera (Eurotiales): an overview of families, genera, subgenera, sections, series and species. *Stud. Mycol.* **2020**, *95*, 5–169. <https://doi.org/10.1016/j.simyco.2020.05.002>

57. Susca, A.; Moretti, A.; Stea, G.; Villani, A.; Haidukowski, M.; Logrieco, A.; Munkvold, G. Comparison of species composition and fumonisin production in *Aspergillus* section *Nigri* populations in maize kernels from USA and Italy. *Int. J. Food Microbiol.* **2014**, *188*, 75–82. <https://doi.org/10.1016/j.ijfoodmicro.2014.06.031>

58. Croft, C.A.; Culibrk, L.; Moore, M.M.; Tebbutt, S.J. Interactions of *Aspergillus fumigatus* conidia with airway epithelial cells: a critical review. *Front. Microbiol.* **2016**, *7*, 1–15. <https://doi.org/10.3389/fmicb.2016.00472>

59. Varga, J.; Houbraken, J.; Van Der Lee, H.A.L.; Verweij, P.E.; Samson, R.A. *Aspergillus calidoustus* sp. nov., causative agent of human infections previously assigned to *Aspergillus ustus*. *Eukaryot. Cell* **2008**, *7*(4), 630–638. doi:10.1128/ec.00425-07

60. Varga, J.; Due, M.; Frisvad, J.C.; Samson, R.A.. Taxonomic revision of *Aspergillus* section *Clavati* based on molecular, morphological and physiological data. *Stud. Mycol.* **2007**, *59*, 89–106.

61. Visagie, C.M.; Hirooka, Y.; Tanney, J.B.; Whitfield, E.; Mwange, K.; Meijer, M.; Amend, A.S.; Seifert, K.A.; Samson, R.A. *Aspergillus*, *Penicillium* and *Talaromyces* isolated from house dust samples collected around the world. *Stud. Mycol.* **2014**, *78*, 63–139.

62. Houbraken, J.A.M.P.; Frisvad, J.C.; Samson, R.A. Taxonomy of *Penicillium citrinum* and related species. *Fungal Divers.* **2010**, *44*, 117–133. <https://doi.org/10.1007/s13225-010-0047-z>

63. Akinfala, T.O.; Houbraken, J.; Sulyok, M.; Adedeji, A.R.; Odebode, A.C.; Krska, R.; Ezekiel, C.N. Moulds and their secondary metabolites associated with the fermentation and storage of two cocoa bean hybrids in Nigeria. *Int. J. Food Microbiol.* **2020**, *316*, 108490. <https://doi.org/10.1016/j.ijfoodmicro.2019.108490>

64. Ezekiel, C.N.; Ayeni, K.I.; Akinyemi, M.O.; Sulyok, M.; Oyedele, O.A.; Babalola, D.A.; Ogara, I.M.; Krska, R. Dietary risk assessment and consumer awareness of mycotoxins among household consumers of cereals, nuts and legumes in north-central Nigeria. *Food Control* under review.

65. Okeke, C.A.; Ezekiel, C.N.; Nwangburuka, C.C.; Sulyok, M.; Ezeamagu, C.O.; Adeleke, R.A.; Dike, S.K.; Krska, R. Bacterial diversity and mycotoxin reduction during maize fermentation (steeping) for *ogi* production. *Front. Microbiol.* **2015**, *6*, 1–12. <https://doi.org/10.3389/fmicb.2015.01402>

66. European Food Safety Authority. Scientific opinion on the risks for public and animal health related to the presence of citrinin in food and feed. *EFSA J.* **2012**, *10*, 1–82. <https://doi.org/10.2903/j.efsa.2012.2605>

67. Peterson, S.W.; Jurjevic, Z. The *Talaromyces pinophilus* species complex. *Fungal Biol.* **2019**, *123*, 745–762

68. Neelaveni, V.; Tupaki-Sreepurna, A.; Thanneru, V.; Kindo, A.J. *Lichtheimia ramosa* isolated from a young patient from an infected wound after a road traffic accident. *J. Acad. Clin. Microbiol.* **2017**, *19*, 59–61.

69. Baby, S.; Ramya, T.G.; Geetha, R.K. Onychomycosis by *Syncephalastrum racemosum*: case report from Kerala, India. *Dermatol. Rep.* **2015**, *7*.

70. Fapohunda, S.O.; Moore, G.G.; Aroyeun, S.O.; Ayeni, K.I.; Aduroja, D.E.; Odetunde, S.K. Isolation and characterization of fungi isolated from Nigerian cocoa samples, *Curr. Life Sci.* **2018**, *4*(3), 46–52. <https://doi.org/10.5281/zenodo.1405083>

71. International Agency for Research on Cancer. *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Amines and Mycotoxins*. IARC Monographs on Evaluation of Carcinogenic Risk to Humans. International Agency for Research on Cancer: Lyon, France.
72. Onyedum, S.C.; Adefolalu, F.S.; Muhammad, H.L.; Apeh, D.O.; Agada, M.S.; Imienwanrin, M.R.; Makun, H.A. Occurrence of major mycotoxins and their dietary exposure in North-Central Nigeria staples. *Sci. African* **2019**, *7*, e00188. <https://doi.org/10.1016/j.sciaf.2019.e00188>
73. Nduti, N.N.; Njeru, P.N.; Mwaniki, M.; Reid, G. Aflatoxin variations in maize flour and grains collected from various regions of Kenya. *African J. Food, Agric. Nutr. Dev.* **2017**, *17*, 11743–11756. <https://doi.org/10.18697/ajfand.77.16875>
74. Adeyemo, I.A.; Olaribigbe, O. Incidence of mycoflora and mycotoxin contamination in *pupuru*; a locally fermented cassava flour sold in Okitipupa, Ondo state, Nigeria. *Niger. J. Microbiol.* **2019**, *33*, 4364–4372.
75. European Commission. Commission Decision 2002/657 of 12 August 2002 Implementing Council Directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Results. *Official J. EU* **2002**, *L221*, 8–36. <https://eur-lex.europa.eu/>