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Mycotoxins in uncooked and plate-ready household food from rural northern Nigeria

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ABSTRACT

In order to understand the changes in toxic metabolite profiles in uncooked and cooked foods, samples of flour/grain ($n = 40$) and their corresponding plate-ready food ($n = 39$) were collected from 40 households in two states of northern Nigeria. The food samples were analyzed for multiple fungal metabolites by LC-MS/MS and daily intakes of mycotoxins in the diets were estimated and compared to established margin of exposure (MOE) and tolerable daily intake (TDI) values. Both food groups contained 65 fungal and plant metabolites, inclusive of 23 mycotoxins. The mean concentrations of aflatoxin B_1, beauvericin, fumonisin B_1 (FB_1), FB_2, FB_3, hydrolysed FB_1, moniliformin and nivalenol were significantly ($p < 0.05$) higher in flour than in the plate-ready food samples. The levels of several mycotoxins were higher in the flour samples than in plate-ready meals by 129–383%. The dilution effect from proportionate mixing of flour samples with water led to 48–100% reduction in detectable mycotoxins in flour to plate-ready meals. Aflatoxins and fumonisins co-occurred in 36% of the plate-ready foods. Exposures of households to aflatoxins and fumonisins based on 95% CI concentration of mycotoxins in the meals were high, suggesting potential health risks based on calculated low MOE and exceedence of stipulated TDI value, respectively.

1. Introduction

Mycotoxins are toxic secondary metabolites of various fungi that significantly impact global food safety and security, from toxin exposure, economic loss of crops or the salability of said crops. They are a widespread mixture of contaminants in diverse agricultural and food products, with both acute and chronic toxicological effects being a public health concern (IARC, 2015). The established mycotoxins for agriculture and public health concerns are the aflatoxins, fumonisins, ochratoxins, trichothecenes (e.g. deoxynivalenol (DON) and nivalenol (NIV)), zearalenone (ZEN); while much interest is also developing among some emerging (that is, not regulated and not routinely screened for in analytical standard assays) mycotoxins such as alternariol, beauvericin (BEA), enniatins, moniliformin (MON) and tenuazonic acid (IARC, 2015; Karlovsky et al., 2016). Many of the aforementioned toxins have been widely reported to co-contaminate diverse food crops, predominantly maize, but also cassava, millet, rice and sorghum in Nigeria (Abass et al., 2017; Abdus-Salaam et al., 2015; Adetunji et al., 2014b; Chilaka et al., 2016; Ezekiel et al., 2012; Oyedele et al., 2017) and across Africa (Abia et al., 2013, 2017; Ediage et al., 2011; Kamala et al., 2017; Misihairabgwi et al., 2017; Sulyok et al., 2015; Warth et al., 2012). These toxins when ingested are capable of exerting deleterious effects in biological systems and the effects may range from cancers, growth faltering, immune suppressions to cyto- and organ toxicities (Bondy and Pestka, 2000; CAST, 2003; IARC, 2002; Pestka, 2010a; Pestka, 2010b; Turner, 2013; Turner et al., 2003; Turner et al., 2007). Recent research efforts focus on the combined toxicological effects, which may be mediated by these natural contaminant mixtures

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(Vejdovszky et al., 2017), and their role in the context of the exposome concept (Marin et al., 2018; Warth et al., 2017); this necessitates the investigation of household foods from rural settings in Nigeria wherein mycotoxin co-contamination is common (Ezekiel et al., 2014).

Despite the available reports for mycotoxin contamination of crops (e.g. grains and seeds) in Nigeria, there are limited data on home-processed plate-ready meal and none that compare raw grains with plate ready meals at the household level. The use of plate-ready foods offer several benefits in mycotoxin studies as they provide precise measures of type and amounts of food intake, and better estimate of mycotoxin exposure compared to stored grains (Adetunji et al., 2014a, 2017; Ezekiel et al., 2016; Kilonzo et al., 2014; Kimanya et al., 2010, 2014; Murashiki et al., 2017; Oyedele et al., 2017). Paired samples of uncooked grain flours and plate-ready meals are not common in African settings (Kilonzo et al., 2014; Murashiki et al., 2017). Thus this study was designed to:

a) Compare mycotoxin concentrations in uncooked flour with levels in plate-ready food at the household level; thus suggest the potential effects of last-line household processing step(s) on variations in mycotoxin levels.
b) estimate the daily intake and assess the risk of mycotoxins based on the obtained results for plate-ready meals consumed by rural dwellers in two states in northern Nigeria.

2. Materials and methods

2.1. Study design

Two states (Kaduna and Nasarawa) in northern Nigeria were purposely selected for this study based on a previous report of high levels of several distinct mycotoxins in stored maize from these states (Adetunji et al., 2014a, 2014b). Four rural communities whose inhabitants largely depend on own-grown staple crops (especially maize) were selected per state. Five households that were willing to provide a portion of their food samples during a multi-mycotoxin urine biomonitoring study were selected per community (Ezekiel et al., 2014). Overall, the study consisted of 40 households (n = 5 households × 4 communities × 2 states). Each household comprised of three individuals (total = 120: 19 children (aged 1–8 yrs), 20 adolescents (9–19 years) and 81 adults aged > 20 years) who were recruited into the biomonitoring study (*see* Ezekiel et al., 2014) where each family member ate from a common family food pot. In each household, well-structured food frequency questionnaires were administered by trained interviewers to the head of the household to obtain information on food storage, consumption patterns, and socio-economic status. Consumption patterns for the major foods (e.g. maize, millet, rice and sorghum) were obtained from 7-day food recall and weight of the meal consumed the day before sample collection. The head or oldest member of each household provided demography-related responses for the three individuals in their household.

2.2. Sampling of food

A sample (25 g) each of the uncooked flour/grain and corresponding plate-ready food that was consumed by each of the 40 recruited families on the day prior to the urinary sample collection for biomonitoring (Ezekiel et al., 2014) was obtained. One sample from Nasarawa state was missing resulting in a total of 79 food samples which were distributed as follows: Kaduna (n = 40), Nasarawa (n = 39); Uncooked flour/grain (n = 40), plate-ready meal (n = 39). Based on crop-type, the food samples were categorized into two: a) maize-based [uncooked flour (n = 29), and cooked maize-dough (*tuwo masara*, n = 28)]; b) other foods which consisted of rice flour and grains (n = 8) and cooked rice-dough (*tuwo shinkafa*) and boiled rice (n = 8), and one sample each of millet flour, millet *ogi*, fermented cassava flour, cassava meal (*eba*), yam flour and yam meal (*amala*). The dough (maize and rice) and meal (cassava and yam) were prepared by mixing approximately one part of flour with three parts of boiling water (w/v) to yield a dilution of 25% of mycotoxins in the flour, and stirred with heating from a fire for the dough and yam meal, until the desired consistency was achieved. All food samples were obtained on a single day for each household, with collections occurring during September and October 2012, and immediate cooling until freezing at −20 °C.

2.3. Multi-mycotoxin analysis of food samples

The LC-MS/MS method described by Malachová et al. (2014) was employed to quantify the presence of more than 300 microbial metabolites including mycotoxins in the food samples.

2.3.1. Chemicals

Methanol (LC gradient grade) and glacial acetic acid (p.a) were purchased from Merck (Darmstadt, Germany), acetonitrile (LC gradient grade) from VWR (Leuven, Belgium), and ammonium acetate (MS grade) from Sigma-Aldrich (Vienna, Austria). Mycotoxin standards were obtained from various research groups or purchased from various commercial sources as specified by Abia et al. (2017). Water was purified successively by reverse osmosis with an Elga Purelab ultra analytic system from Veolia Water (Bucks, UK).

2.3.2. Extraction of metabolites and determination of apparent recoveries

Food (uncooked and plate-ready) sample (5 g) was extracted with 20 mL of acetonitrile/water/acetic acid 79:20:1, (v/v/v) in a 50 mL polypropylene tube (Sarstedt, Nümbrecht, Germany). For determination of apparent recoveries, 0.25 g samples of food were spiked with 100 µL of a multi-component stock solution, left overnight to establish equilibrium between sample and spike, and subsequently homogenized with 1 mL of acetonitrile/water/acetic acid 79:20:1, v/v/v. Food and spiked samples were extracted for 90 min on a GFL 3017 rotary shaker (GFL, Burgwedel, Germany), an aliquot of the extract was diluted 1:1 (v/v) with dilution solvent (acetonitrile/water/acetic acid 20:79:1, v/v/v) and injected into the LC-MS/MS instrument (Sulyok et al., 2007).

2.3.3. LC-MS/MS parameters

LC-MS/MS screening of the microbial metabolites was performed with a QTrap 5500 LC-MS/MS System (Applied Biosystem, Foster City, CA, USA) equipped with TurboIonSpray electrospray ionisation (ESI) source and a 1290 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25 °C on a Gemini® C18-column, 150 × 4.6 mm i.d., 5 µm particle size, equipped with a C18 4 × 3 mm i.d. security guard cartridge (Phenomenex, Torrance, CA, USA). The chromatographic method, chromatographic and mass spectrometric parameters are as described by Malachová et al. (2014). ESI-MS/MS was performed in the time-scheduled multiple reaction monitoring (MRM) mode both in positive and negative polarities in two separate chromatographic runs per sample by scanning two fragmentation reactions per analyte. The MRM detection window of each analyte was set to its expected retention time ± 27 and ± 48 s in the positive and the negative modes, respectively.

Confirmation of positive analyte identification was obtained by the acquisition of two MRMs per analyte (with the exception of MON, which exhibited only one fragment ion). This yielded 4.0 identification points according to European Commission decision 2002/657 (EU, 2002). In addition, the LC retention time and the intensity ratio of the two MRM transitions agreed with the related values of an authentic standard within 0.1 min and 30% respectively.

2.3.4. Quantification of mycotoxins

Quantification was based on linear, 1/x weighed calibration using serial dilutions of an external multicomponent stock solution. Concentrations were corrected for apparent recoveries. The accuracy of

Table 1
Demography, food consumption/storage patterns and socio-economic status of study households in rural northern Nigeria.

Parameters	Incidence of parameters across the states		Total (N^a = 40; n^b = 120)
	Kaduna (N^a = 20; n^b = 60)	Nasarawa (N^a = 20; n^b = 60)	
Demographics Male/Female; *Mean Age (yrs)*; Mean body weight Kg (SD)			
Children (range = 1–8 yrs)	6/7; *5.4*; **21.0 (2.4)**	3/3; *4.4*; **21.4 (2.1)**	9/10; *5.0*; **20.1 (2.5)**
Adolescents (range = 9–19 yrs)	4/2; *11.8*; **32.1 (2.4)**	8/6; *13.1*; **34.6 (3.3)**	12/8; *13.0*; **33.1 (3.1)**
Adults (range = 20–80 yrs)	21/20; *39.5*; **64.1 (4.3)**	17/23; *36.1*; **65.0 (8.8)**	38/43; *38.0*; **63.7 (5.4)**
Educational level of participants:			
Unschooled	36.7%	30.0%	33.3%
Primary	43.3%	40.0%	41.7%
High school	20.0%	23.3%	21.7%
Diploma	0.0%	6.7%	3.3%
Household food consumption average frequency:			
Maize	5–7 days/week	5–7 days/week	5–7 days/week
Sorghum	1–2 days/week	1–2 days/week	1–2 days/week
Others (rice, millet, tubers)	3–4 days/week	3–4 days/week	3–4 days/week
Household grain storage duration[c] and (method)[d]:			
≤3 months[c] (bags on bare floor)[d]	60.0%[c] (40.0%)[d]	5.0%[c] (45.0%)[d]	32.5%[c] (42.5%)[d]
4–6 months[c] (silo)[d]	15.0%[c] (10.0%)[d]	30.0%[c] (15.0%)[d]	22.5%[c] (12.5%)[d]
6–12 months[c] ("rhumbu"[e])[d]	25.0%[c] (30.0%)[d]	65.0%[c] (5.0%)[d]	45.0%[c] (17.5%)[d]
> 12 months[c] (kitchen roof)[d]	0.0%[c] (20.0%)[d]	0.0%[c] (35.0%)[d]	0.0%[c] (27.5%)[d]

[a] Number of households that provided food consumption and storage pattern data.
[b] Number of participants for demographic information.
[c] Percentage of households based on the specific duration adopted for grain storage.
[d] Percentage of households based on the storage method adopted for grains.
[e] "Rhumbu": local wooden granary.

the method was previously verified by participation in inter-laboratory comparison studies (De Girolamo et al., 2017; Malachová et al., 2015) including a regular scheme organized by BIPEA (Gennevilliers, France). Thus for maize, 160 of the 167 submitted results to BIPEA studies were in the satisfactory range (z-scores between −2 and 2). Based on the related data, a general expanded measurement uncertainty of 50% has been proposed (Stadler et al., 2018). Limits of detection and limits of quantification were determined based on the standard deviation of samples spiked at low concentration levels following the EURACHEM guide (Magnusson and Örnemark, 2014).

2.4. Estimation of mycotoxin exposure and risk from consumption of plate-ready meals

2.4.1. Estimation of exposure to mycotoxins

An estimate of exposure was performed only for mycotoxins where at least 50% of the plate-ready food samples had detectable toxin, except for aflatoxin which is a priority toxin from a public health perspective. For all mycotoxin exposure calculations, samples with non-detects (i.e. less than the limit of detection (< LOD)) were assigned the LOD/2 values. The method described by Kimanya et al. (2010) was adopted with slight modification for the exposure estimate by taking into consideration the adjusted daily meal intake of the participants and the mycotoxin concentration (mean or 95% CI) in the meals consumed. The daily meal intake was adjusted by multiplying the average daily meal consumption of each participant obtained by weighing portions of the specific meal consumed by each household member participating in the study by the weekly frequency (number of days per week) of meal consumption obtained through a 7-day food recall using a food frequency questionnaire. The product was then divided by seven (number of days in a week), in order to obtain a balanced estimate of meal intake per day. The mycotoxin exposure/intake at an individual level (μg/kg bw per day) was then estimated by multiplying the mean or 95% CI mycotoxin concentration in household meal portions (μg/kg) by the individual meal intake (kg/day) and dividing with the individual body weight (kg). The mean and 95% exposures to a mycotoxin per age group were derived by finding the mean values of all individual exposures/intakes to the mycotoxin in each corresponding age group.

2.4.2. Risk characterization for mycotoxins

The margin of exposure (MOE) approach for risk characterization of genotoxic carcinogens (Benford et al., 2010; EFSA, 2007) was adopted in this study for aflatoxins. The MOE for aflatoxins per individual was calculated by dividing the benchmark dose lower limit (BMDL) of 170 ng/kg bw per day (EFSA, 2007) by the aflatoxin exposure of the individual. Risk characterization for DON and fumonisin exposures were performed by comparing each participant's exposure to the tolerable daily intake (TDI) of 1 μg/kg bw per day (FAO/WHO, 2010) and group TDI of 2 μg/kg bw (JECFA, 2011) for DON (and acetylated forms) and fumonisins, respectively. One uncertainty of the risk assessment approach was the adoption of toxicological data, TDIs/HBGVs and reference points outlined for adults to diverse age groups in this study.

2.5. Ethical considerations

The study was approved (under the reference MOH/OFF/237/VOL.1) by the Ethics Committee of the Ministry of Health for the study states in Nigeria (*see* Ezekiel et al., 2014). All participants who gave food samples and responses to the questionnaires provided written informed consent prior to the study. Parents gave consent on behalf of their children and adolescents.

2.6. Statistical analysis

Data obtained in this study were analyzed by SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Simple descriptive statistics were used for data relating to demography and food consumption patterns as well as distribution of mycotoxins/metabolites in the analyzed foods and exposure data. Mycotoxin concentrations in raw and ready-to-eat foods were transformed using the equation $y = \mathrm{Log}_{10}(1 +$ μg/kg of mycotoxin) to create a normal distribution for comparison of toxin levels in both categories of food. The error level was set at 5% for all analyses performed.

3. Results and discussions

3.1. Demographic data and food storage patterns for households

A total of 120 individuals aged 1–80 years from 40 households participated in the study (Table 1). Maize was the dominant food in the diet, with consumption two to three times per day and on average six days per week. The storage duration of the grains employed in making the flour and cooked meals in the households was 1–12 months; 65% of the households in Nasarawa state stored their grains for 6–12 months compared to 25% of the families in Kaduna state. The households adopted mostly grain storage in bags on either the bare floor or on the kitchen roof, rather than in silos and *rhumbu* (a local granary); at least 40% of the families in both states stored their grains in bags on bare floor. The long storage duration and predominant type of storage structure (i.e. bagged grains stored on bare floor) in this study are similar to previous reports for maize grain storage in the agro-ecological zones to which the states belong (Adetunji et al., 2014b; Udoh et al., 2000). Prolonged storage of harvested maize under poor conditions, e.g. limited aeration and high humidity, has been suggested as a contributing factor to an increased production of some mycotoxins in grains (Adetunji et al., 2014b; Kankolongo et al., 2009; Udoh et al., 2000).

3.2. Fungal and plant metabolites in uncooked flour and plate-ready meal

3.2.1. Overview of metabolite distribution in flour and plate-ready meal

The method performance parameters for 65 fungal and plant metabolites found in the 79 food samples obtained from 40 households in two states of northern Nigeria are given in Table 2; including individual metabolite limits of detection and analytical recoveries. The recoveries for all metabolites were in expected ranges for this type of multi-analyte assay, with an average of 100% in raw flour and 81% for plate ready foods. Three metabolites significantly contributed to the higher apparent recovery range in raw food (ophiobolin A (6.1%), tenuazonic acid (227.5%) and equisetin (182.5%). The metabolite distribution (occurrences and concentrations) of the 65 metabolites detected in the food samples are given in Table 3 and S1. Uncooked flour contained 59 metabolites, while 46 metabolites were found in the plate-ready foods (Table 3 and S1). The metabolites included 23 emerging and regulated mycotoxins and their metabolites from *Aspergillus*, *Fusarium, Alternaria* and *Penicillium* species (Table 3; 25 mycotoxins are reported in this table due to the inclusion of total aflatoxin (sum of AFB_1, AFB_2, AFG_1 and AFG_2) and total fumonisin (sum of FB_1, FB_2 and FB_3)) as well as 42 other metabolites (mycotoxin precursors and potentially toxic secondary metabolites from plants and other unspecific origins) (Table S1). The most frequently detected metabolites having at least 70% occurrence in the flour samples were 3-nitropropionic acid, BEA, emodin, equisetin, fumonisin B_1 (FB_1), FB_2, FB_3, kojic acid, macrosporin A and MON, while only 3-nitropropionic acid, BEA, FB_1 and kojic acid were prevalent at same level (70% occurrence) in the plate-ready samples (Table 3 and S1). Linamarin and lotaustralin (two plant toxins) were found only in the flour and meal made from cassava (a tuber), at occurrence rates of 40%/26% (flour/meal) and 18%/8% (flour/meal), respectively (Table S1). The metabolite profiles in the uncooked foodstuffs agree with the profiles previously reported in diverse grains (e.g. maize, millet, peanut and rice) (Abdus-Salaam et al., 2015; Adetunji et al., 2014b; Ezekiel et al., 2012; Oyedele et al., 2017) and tuber (e.g. cassava; Abass et al., 2017) from Nigeria whilst the spectra of metabolites in the diets (especially the maize-based meals) are similar to the range of metabolites recently reported in a home-processed maize dish (fufu) in Cameroon (Abia et al., 2017). In view of the scarce data on fungal metabolite contamination of plate-ready household meals in Nigeria, this paper reports for the first time on mycotoxins in household meals in the country.

3.2.2. Variations in mycotoxin levels in flour and plate-ready meal

The concentrations of mycotoxins in the 40 flour and 39 plate-ready food samples are compared in Fig. 1,Table 3 and S1. Of the 25 reported emerging and regulated mycotoxins in Table 3, aflatoxin G_2 (occurrence: 3%, median: 2.2 μg/kg), citrinin (CIT; occurrence: 13%, median: 43.3 μg/kg), diacetoxyscirpenol (occurrence: 50%, median: 2.8 μg/kg),

Table 2
Method performance characteristics of the 65 fungal and plant metabolites identified in the investigated food samples from households in rural northern Nigeria.

Metabolites	Spiking level (μg/kg)	LOD[a] (μg/kg)	App. Rec.[b] (%)	App. Rec.[c] (%)	Metabolites	Spiking level (μg/kg)	LOD[a] (μg/kg)	App. Rec.[b] (%)	App. Rec.[c] (%)	Metabolites	Spiking level (μg/kg)	LOD[a] (μg/kg)	App. Rec.[b] (%)	App. Rec.[c] (%)
3-Nitropropionic acid	480	0.4	82	84	Culmorin	960	1.6	111	73	Macrosporin A	185	0.13	107	80
Aflatoxin B_1	34	0.22	70	77	Curvularin	73	0.4	122	96	Moniliformin	165	1.5	74	96
Aflatoxin B_2	34	0.21	80	78	Cytochalasin B	155	2	96	77	Monoacetoxyscirpenol	90	1.5	90	84
Aflatoxin G_1	34	0.16	77	78	Cytochalasin J	155	2	107	75	Monocerin	80	0.1	100	86
Aflatoxin G_2	34	0.5	77	78	Deoxynivalenol	105	0.8	105	99	Nidurufin	100	0.05	149	84
Aflatoxin M_1	110	0.3	83	84	Diacetoxyscirpenol	105	0.15	88	87	Nivalenol	105	1.1	60	95
α-Zearalenol	200	1	126	81	Emodin	57	0.1	100	80	Norsolorinic acid	100	0.05	92	82
Altenuene	320	0.8	*n.d*	96	Enniatin A	0,16	0.01	103	71	Ochratoxin A	81	0.4	88	79
Alternariol	56	0.1	82	81	Enniatin A1	1,2	0.06	102	78	Ophiobolin A	82	10	6.1	73
Alternariolmethylether	110	0.15	109	81	Enniatin B	1,2	0.012	95	76	Pestalotin	82	1.3	98	85
Andrastin A	64	0.2	100	102	Enniatin B1	3,5	0.03	109	77	Physcion	700	5	128	53
Aspercolorin	22	1	125	82	Equisetin	60	0.7	183	92	Radicicol	70	0.2	108	97
Averantin	104	0.03	85	82	Fumonisin B_1	500	2.4	85	95	Skyrin	57	0.08	77	67
Averufanin	104	0.02	75	75	Fumonisin B_2	500	2	86	78	Sterigmatocystin	57	0.07	104	85
Averufin	64	0.02	61	75	Fumonisin B_3	500	6	91	85	Tentoxin	22	0.1	108	97
Beauvericin	3	0.04	116	85	Fumonisin B_4	500	1.6	86	78	Tenuazonic acid	57	15	228	81
β-Zearalenol	200	1	118	77	Fusaproliferin	60	20	84	65	Typtophol	400	15	78	84
Bikaverin	35	1	126	100	Fusaric acid	52	5	65	63	Versicolorin A	52	0.04	138	75
Brevianamid F	80	0.8	80	83	Hydrolysed FB_1	115	0.2	69	69	Versicolorin C	52	0.04	143	75
Butenolid	490	3	100	81	Kojic acid	1700	20	94	100	Zearalenone (ZEN)	105	0.2	108	79
Chrysophanol	1200	4	126	86	Linamarin	250	2.4	128	49	ZEN-14-sulfate	70	0.03	131	70
Citrinin	170	0.8	42	82	Lotaustralin	1280	1.3	99	102					

n.d: not determined.
[a] Limit of detection [expressed as μg/kg sample;].
[b] Apparent recovery from spiking uncooked food (n = 5).
[c] Apparent recovery from spiking of plate-ready meals (n = 5).

Table 3
Distribution of 25 emerging and regulated mycotoxins and their metabolites in household food in rural northern Nigeria.

Mycotoxins	Uncooked flour (n^a = 40; N^b = 23)				Plate-ready meal (n^a = 39; N^b = 19)				Flour *vs* Plate-ready food	
	N[c] (%)	Concentrations (μg/kg)			N[c] (%)	Concentrations (μg/kg)			% detect flour - % detect meal	(Median flour/Median meal) × 100
		Min	Max	Median		Min	Max	Median		
Aflatoxin B_1	18 (45)	0.3	37.3	2.2	15 (38.5)	0.5	8.3	1.7	6.5	129
Aflatoxin B_2	7 (17.5)	0.4	2.3	1.6	4 (10.3)	0.3	0.5	0.5	7.2	320
Aflatoxin G_1	10 (25)	0.2	29.3	1.4	2 (5.1)	1.6	4.7	3.1	19.9	45
Aflatoxin G_2	1 (2.5)	2.2	2.2	2.2	*n.d*	*n.d*	*n.d*	*n.d*	2.5	–
Total aflatoxin	18 (45)	0.3	63.7	3	15 (38.5)	0.5	12.5	2.1	6.5	143
Aflatoxin M_1	4 (10)	0.6	2	1.2	*n.d*	*n.d*	*n.d*	*n.d*	10	–
Beauvericin	40 (100)	0.02	435	2.2	32 (82.1)	0.1	105	1.6	17.9	138
Citrinin	5 (12.5)	12.9	177	43.3	*n.d*	*n.d*	*n.d*	*n.d*	12.5	–
Deoxynivalenol	5 (12.5)	1.3	19.4	5.5	5 (12.8)	2.1	8	2.8	0	196
Diacetoxyscirpenol	20 (50)	0.9	15.1	2.8	*n.d*	*n.d*	*n.d*	*n.d*	50	–
Fumonisin B_1	32 (80)	2.7	7,387	437	29 (74.4)	2.8	1,590	118	5.6	370
Fumonisin B_2	29 (72.5)	1.2	2,583	112	27 (69.2)	4.5	610	32.1	3.3	349
Fumonisin B_3	28 (70)	6	934	71.4	27 (69.2)	0.8	213	26.2	0	273
Fumonisin B_4	*n.d*	*n.d*	*n.d*	*n.d*	24 (61.5)	5.2	145	13.4	−61.5	–
Hydrolysed FB_1	14 (35)	2.5	153	6.9	3 (7.7)	0.6	2.6	1.8	27.3	383
Total fumonisin	32 (80)	2.7	10,904	601	29 (74.4)	3.6	2,557	188	5.6	320
Moniliformin	30 (75)	2.7	2,636	71.7	23 (59)	1.6	196	21.3	16	337
Monoacetoxyscirpenol	24 (60)	0.7	46.5	3.1	*n.d*	*n.d*	*n.d*	*n.d*	60	–
Nivalenol	19 (47.5)	1.7	36.9	6.7	4 (10.3)	1.8	3.1	2.2	37.2	305
Ochratoxin A	*n.d*	*n.d*	*n.d*	*n.d*	1 (2.6)	1.5	1.5	1.5	−2.6	–
Tenuazonic acid	4 (10)	1.8	1,290	25.9	*n.d*	*n.d*	*n.d*	*n.d*	10	–
Zearalenone (ZEN)	12 (30)	0.3	438	0.9	5 (12.8)	0.8	222	2.6	17.2	35
ZEN-14-sulfate	3 (7.5)	0.03	8.3	0.1	4 (10.3)	0.1	11	0.1	−2.8	100
α-Zearalenol	1 (2.5)	5.3	5.3	5.3	1 (2.6)	3.9	3.9	3.9	0	136
β-Zearalenol	1 (2.5)	8.9	8.9	8.9	1 (2.6)	8.5	8.5	8.5	0	105

n.d. not determined.
[a] Number of samples analyzed.
[b] Number of metabolites.
[c] Number (percentage) of positive samples.

monoacetoxyscirpenol (occurrence: 60%, median: 3.1 μg/kg) and tenuazonic acid (occurrence: 10%, median: 25.9 μg/kg) were only found in the flour while FB_4 (occurrence: 62%, median: 13.4 μg/kg), ochratoxin A (occurrence: 3%, median: 1.5 μg/kg) and other four metabolites of those given in Table S1 occurred in only prepared meals. Overall, the concentrations of 70% of the metabolites were higher in the flour than in the corresponding prepared meal (Table 3 and S1). With respect to variations in the mycotoxin concentrations observed in the two categories of food samples (Fig. 1), the mean levels of AFB_1, total aflatoxin, BEA, FB_1, FB_2, FB_3, HFB_1, total fumonisin, MON and NIV were all significantly ($p < 0.05$) higher in the flour samples than the plate-ready meal samples. The median values of mycotoxin occurrence data points in flour and plate-ready meals were considered for estimating the proportion of the mycotoxin levels in the flour and plate-ready meal (Table 3). The levels of the various mycotoxins in the flour were 129–383% (i.e. 4 folds) higher than the levels in the plate-ready

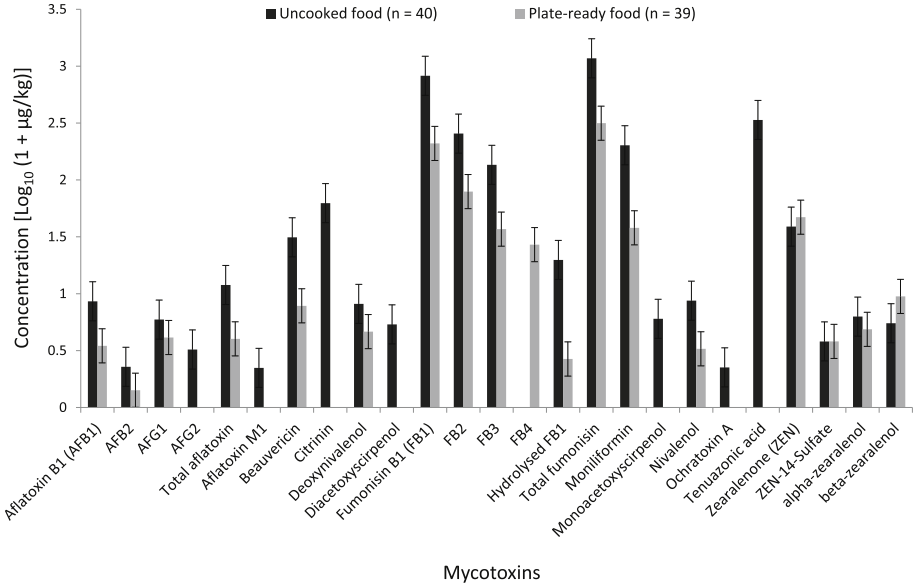

Fig. 1. Mean levels of 25 emerging and regulated mycotoxins and their metabolites in raw (uncooked) food and ready-to-eat household meals from rural northern Nigeria. Uncooked flour is not consumed in households.

meals, except for ZEN and AFG_1 where the levels in the meals were higher than in the flour. In order to estimate the percentage reduction of detectable mycotoxins due to processing, only mycotoxins with at least 50% data points in flour and corresponding meal samples were considered (data not shown). The non-detects (< LOD) in the meal samples where data existed for flour samples were assigned LOD/2 value and the mean percentage reductions of the detectable mycotoxins obtained were 48, 52, 72, 73, 73 and 81% for BEA, AFB_1, FB_1, total fumonisin, MON and total aflatoxin, respectively.

Multiple mycotoxin contamination of diverse crops including cassava, maize, millet, rice, sorghum and their processed foods have been previously reported in Nigeria (Abass et al., 2017; Abdus-Salaam et al., 2015; Adetunji et al., 2014b; Chilaka et al., 2016; Ezekiel et al., 2012; Oyedele et al., 2017) and across Africa (Abia et al., 2013, 2017; Ediage et al., 2011; Kamala et al., 2017; Sulyok et al., 2015; Warth et al., 2012). In this study, the mycotoxin levels we found in the uncooked flour and plate-ready meals were similar to the levels reported in maize flour and meals from neighboring Cameroon (Abia et al., 2013, 2017) but lower than the concentrations in maize flour and maize-based meal from Tanzania (Kamala et al., 2017; Kimanya et al., 2014) and Zimbabwe (Murashiki et al., 2017). In Malawi, however, aflatoxins were not detected in 15 locally processed dehulled maize flours (Matumba et al., 2014). The disparities observed in the toxin levels across the regions may be due to several factors including climate, agricultural and storage practices, season and annual variations.

Furthermore, several mycotoxins (e.g. CIT, diacetoxyscirpenol, monoacetoxyscirpenol and tenuazonic acid) considered to be emerging mycotoxins in this study, due to their sparse/unusual occurrence in food items in Nigeria and Africa at-large and/or limited toxicological data, were found at relatively high levels only in the uncooked maize flour. This agrees with the recent reports on the high contamination of Nigerian maize (Ogara et al., 2017; Okeke et al., 2015) and maize from other African countries (Abia et al., 2013; Warth et al., 2012) by one of these toxins (CIT), though the toxin is yet to be regulated in this crop by the European Union or Nigerian regulatory agencies. One sample each of rice flour and meal contained < 4 μg/kg of aflatoxins and fumonisins, thus corroborating the previous report of fumonisin levels in rice from Nigeria (Abdus-Salaam et al., 2015); the aflatoxin level, however, cannot be considered as low due to the carcinogenic potential of this mycotoxin (IARC, 2015).

With respect to differences in toxin levels in the uncooked and cooked foods, higher concentrations of mycotoxins were found in the uncooked foods than in the prepared meals. This finding agrees well with a previous report on higher aflatoxin levels in 20 maize grains (occurrence: 45%; range: 18–480 μg/kg; mean: 53 μg/kg) than in the corresponding maize meals (occurrence: 35%; range: 6–30 μg/kg; mean: 6 μg/kg) from households in Kibwezi District of Makueni County in Eastern Kenya (Kilonzo et al., 2014). Due to the limited reports on comparison of mycotoxin levels in uncooked flour and prepared meals, this study provides some insight of the role of "last-line" food processing prior to consumption on mycotoxin reduction. To prepare the meals from each of the grain-based flour (maize, millet, rice and sorghum) samples, flour is usually mixed/diluted first in water at ambient temperature and then the mixture is cooked (boiled) on fire at about 100 °C for 10–15 min by stirring the mixture into a meal. For cassava, the flour is added to boiling water and retained without cooking for 5–10 min. It is therefore evident that dilution effect from proportionate mixing of flour and water, which is relevant for both water and non-water soluble toxins, may be responsible for the reduction of 48–81% of the detectable mycotoxins in flour to plate-ready meals recorded in this study. This fact, though from a different concept, agrees with previous suggestion on mycotoxin release into wash or boil water by some pre-cooking steps (e.g. cleaning/washing and soaking) applied to maize, consequently lowering consumer exposure (Karlovsky et al., 2016; Kaushik, 2015; Kilonzo et al., 2014; Matumba et al., 2015). However, it is noteworthy to mention that in the latter case (extraction of toxin into wash/boil water) the liquid containing the toxin is often discarded as a mycotoxin control step; this did not happen in the case of the present study, thus indicating the detectable mycotoxin reduction was due to dilution effect. Furthermore, considerations on cooking as a step for toxin reduction in the plate-ready samples, based on previous suggestions from Trivede et al. (1992) and Karlovsky et al. (2016), may not be valid for our study due to the relatively low temperature (< 150 °C) and short cooking time adopted for the foods reported herein. However, since CIT, diacetoxyscirpenol, monoacetoxyscirpenol and tenuazonic acid occurred at undetectable levels in the maize porridge (*tuwo masara*), the impact of household cooking practices (temperature and time) on the fate of these toxins in the foods needs to be investigated by further studies. An additional point of note is the presence of selected mycotoxins (FB_4 and ochratoxin A) and other metabolites (e.g. sterigmatocystin) in plate-ready meals but not in the uncooked flour. It is possible that the dilution and cooking steps enhanced the accessibility of these toxins in the plate-ready meals. In the case of FB_4, this may have been formed by the rearrangement or modification of other forms of fumonisin present in the uncooked flour whose concentrations reduced in the plate-ready meal (Dall'Asta and Battilani, 2016).

3.3. Mycotoxin levels and co-occurrence in household meals

The mycotoxin concentrations quantified in the plate-ready meal samples obtained from Kaduna and Nasarawa states are indicated in Table 4. Mean levels for total aflatoxin, BEA, ZEN/total zearalenone and MON differed significantly ($p < 0.05$) between the states, with respective higher values of 3.5, 10.1, 57.4/60.2 and 61.4 μg/kg in samples from Nasarawa state compared to 1.9, 2.6, 0.8/0.8 and 14.5 μg/kg in samples from Kaduna state. The levels of total fumonisin also differed significantly ($p < 0.05$) between the states but with higher mean values in samples from Kaduna state (446 ± 634 μg/kg) than in those from Nasarawa state (173 ± 179 μg/kg). The variations observed for mycotoxin concentrations in the plate-ready meals correspond to the mycotoxin patterns observed in stored maize grains from both states (Adetunji et al., 2014a, 2014b). This difference was additionally reflected when metabolites in urine samples from the participants were examined (Ezekiel et al., 2014).

With respect to co-occurrence of mycotoxins in the household meals (Table 5), 36% (predominantly maize-based meal/*tuwo masara*) of the 39 meal samples contained both aflatoxins and fumonisins at concentrations ranging 0.5–12.5 μg/kg and 3.6–711 μg/kg, respectively. Similar high prevalence of aflatoxin/fumonisin combination was also reported in maize grains from Nigeria (Adetunji et al., 2014b) and maize flour from Tanzania (Kimanya et al., 2014). The only millet *ogi* sample also contained both toxins (total aflatoxin: 0.5 μg/kg; total fumonisin: 46 μg/kg) while the only sample of *eba* (cassava meal) had 3.7 μg/kg total fumonisin. Aflatoxins and fumonisins have both been recently reported in cassava-based products from Nigeria albeit at similar low concentrations (Abass et al., 2017). In this present study, 25% and 7% of the maize meal samples contained AFB_1 and fumonisin levels in excess of the recommended EU limits of 2 and 1,000 μg/kg, respectively, for processed foods. Co-occurring mycotoxins in foods continue to arouse the interests of food safety experts due to potential combinatory effects that may arise due to toxin interactions; some of which have been reported recently (Alassane-Kpembi et al., 2016; Vejdovszky et al., 2017).

3.4. Estimates of mycotoxin intake

The individual exposures in households due to consumption of the mycotoxin contaminated plate-ready meals were estimated based on average and 95% CI mycotoxin concentrations in the meals. Mycotoxin exposures were variable in the age groups to which individuals in the households were assigned, with children being more exposed than adolescents and adults. At 95% CI mycotoxin concentrations, the means

Table 4
Geographical differences between mycotoxin levels in plate-ready household meals of rural dwellers in two northern Nigerian states.

Mycotoxins	Kaduna (n^a = 20)			Nasarawa (n^a = 19)		
	P^b	Range (μg/kg)	Mean (μg/kg) ± SD	P^b	Range (μg/kg)	Mean (μg/kg) ± SD
Total aflatoxin[c]	25	0.5–4.2	1.9 ± 1.5b	52.6	0.5–12.5	3.5 ± 4.0a
Total fumonisin[d]	75	3.7–2,410	446 ± 634a	73.7	3.6–711	173 ± 179b
Beauvercin	70	0.2–9.6	2.6 ± 2.6b	94.7	0.1–105	10.1 ± 24.8a
Deoxynivalenol	20	2.1–8	3.9 ± 2.8a	5.3	2.8	2.8a
Moniliformin	60	1.6–52.6	14.5 ± 15b	57.9	4.5–196	61.4 ± 57.1a
Nivalenol	20	1.8–3	2.3 ± 0.5	0	*n.d*	*n.d*
Ochratoxin A	5	1.5	1.5	0	*n.d*	*n.d*
Zearalenone	5	0.8	0.8b	21.1	1.6–222	57.4 ± 110a
Total zearalenone[e]	5	0.8	0.8b	26.3	0.1–233	60.2 ± 115a

Note.
n.d: not detected in samples.
Means with similar alphabets in a row are not significantly different at α = 0.05.
[a] Number of analyzed samples.
[b] Percentage of contaminated samples.
[c] Total aflatoxin: Σ (aflatoxin B_1 (AFB_1), AFB_2, AFG_1 and AFG_2).
[d] Total fumonisin: Σ (fumonisin B_1 (FB_1), FB_2 and FB_3).
[e] Total zearalenone: Σ (ZEN and ZEN-14-sulfate).

of individual exposures to total aflatoxin were 0.061, 0.052 and 0.045 μg/kg bw per day for the children, adolescents and adult age groups, resulting in MOEs of 2,787, 3,269 and 3,778, respectively. Means of individual exposures at 95% CI concentrations for total fumonisin (8.7, 7.4 and 6.4 μg/kg bw per day for children, adolescents and adults, respectively) were at least three times higher than the 2 μg/kg bw per day TDI health-based guidance value for group of fumonisins while the exposures to DON and its acetylated forms did not exceed the established TDI reference point of 1 μg/kg bw per day in any age group (WHO, 2011a; WHO, 2011b). Individual exposures to BEA and MON at 95% CI ranged 0.1–0.14 μg/kg bw per day and 0.82–1.11 μg/kg bw per day, respectively, for the age groups.

For mycotoxins where we previously reported biomarker data (Ezekiel et al., 2014), individual exposure estimates in this paper were lower than the estimates obtained during the urinary biomonitoring study. Specifically, the estimates for aflatoxin and fumonisin intakes based on meals were lower than the 0.67 μg/kg bw per day and 35 μg/kg bw per day reported based on urinary AFM_1 and FB_1 biomarkers, respectively. The variances in the food *vs* urine exposure data may have resulted from several factors including different limits of detection for analytical methods of both matrices, heterogeneity of meal samples resulting in mycotoxin detection bias in food samples, or the fact that higher urinary mycotoxin levels were a product of consumption of several food items in one day in contrast to the utilization of food contamination data from only one food item per household for exposure estimates.

Since biomarkers reflect exposure by all potential routes, estimations are best performed using human biomonitoring rather than food consumption data (IARC, 2015; Warth et al., 2013). The fact that the latter approach may significantly underestimate exposure is highlighted once again by the study at hand. Despite the variances in exposure estimations (food *vs* urine), it is best to adopt good agricultural, storage and food handling/processing practices which favor the reduction or elimination of mycotoxins in the food system and ensure low toxin exposures in consumers. Considering that the JECFA does not allocate a provisional TDI for aflatoxins but recommends that its level in foods be reduced to "As Low As Reasonably Achievable (ALARA)" in view of the remarkable genotoxic carcinogenic potential of this toxin, the MOEs obtained in our study which were lower than 10,000 in each age group strongly indicate a public health concern. Aflatoxins as potent human carcinogens (IARC, 2002) have been linked to growth faltering in children, protein malnutrition, liver cancer and immune suppression (Gong et al., 2002, 2004, 2012; Tchana et al., 2010; Turner et al., 2003, 2007); therefore, every attempt to prevent and minimize their concentrations in foods should be prioritized.

4. Conclusion

Mycotoxin contamination of foods remains a global challenge and the risk is increased with the occurrence of multiple mycotoxins in same food matrix/sample. In this study we have shown, for the first time in Nigerian foods, a comparison of toxin profiles and levels in paired samples of uncooked flour and served meal at the household level. Simple dilution of flour with water prior to cooking and the cooking of raw foods into plate-ready meals were capable of substantial reduction of detectable mycotoxins in the served meals. For the first time, we also report that CIT, diacetoxyscirpenol, monoacetoxyscirpenol and tenuazonic acid were completely undetectable in the served meals due to influence of "last-line" steps during maize flour processing into porridge (*tuwo masara*). Future studies may screen for potential breakdown or conjugation products and evaluate their toxicological potentials. Through a comparison between food-based and biomarker-based exposure assessment in the same households, we could further demonstrate that the latter is more comprehensive as shown in recent human biomonitoring studies from Nigeria (Braun et al., 2018; Ezekiel et al., 2014). This study further revealed high aflatoxin intake across all age groups (children, adolescents and adults) based on the assessed plate-ready meals in this study, highlighting the potential risks of liver cancer in addition to other health effects due to exposures from several co-occurring toxins. It is therefore suggested that households adopt good agricultural practices, harvest mature crops timely, dry the crops to safe moisture levels on clean drying platforms, and then store crops under the best conditions in order to minimize fungal colonization and toxin accumulation in the crops.

Conflicts of interest

The authors declare they have no competing financial interests.

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Table 5
Co-occurring mycotoxins in 39 plate-ready household meals in rural northern Nigeria.

Food categories	Number of samples analyzed	Samples (%) with aflatoxins + fumonisins	Percentage samples exceeding various European Union limits			
			AFB_1 (2 μg/kg)	Total aflatoxin (4 μg/kg)	Fumonisin (1,000 μg/kg)	Zearalenone (100 μg/kg)
Maize meal (*tuwo masara*)	28	46	25	14	7.1	0
Others (millet, rice and tubers)	11	9.1	0	0	0	9.1
All samples	39	36	18	10	5.1	2.6

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.04.002.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.04.002

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