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Mycotoxins and fungal metabolites in groundnut- and maize-based snacks from Nigeria

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This exploratory study was aimed at investigating the spectrum of fungal metabolites in the processed food and snacks. Twenty types of snacks made separately from groundnut ($n = 10$), maize ($n = 8$) and a combination of groundnut and maize ($n = 2$) were analysed for naturally occurring mycotoxins and other fungal metabolites by a liquid chromatography-tandem mass spectrometric multi-mycotoxin method. A total of 18, 21 and 32 metabolites were detected and quantified in the groundnut-, groundnut/maize- and maize-based snacks, respectively. Aflatoxins contaminated 2, 3 and 5 of the groundnut/maize-, groundnut- and maize-based snacks at concentrations up to 14, 1041 and 74 $\mu\text{g kg}^{-1}$, respectively. Thus, the National Agency for Food and Drug Administration and Control (NAFDAC) recommended limit of 20 $\mu\text{g kg}^{-1}$ for aflatoxins was exceeded in 6 of the 20 snacks. Fumonisin contaminated all the maize- and groundnut/maize-based snacks with higher concentrations in the maize-based snacks (mean = 218.7 $\mu\text{g kg}^{-1}$) compared with the groundnut/maize-based snacks (mean = 178.5 $\mu\text{g kg}^{-1}$). Up to 26 different metabolites were found to co-occur in the same samples, thus posing an additional threat to the consumers due to possible additive and/or synergistic effects.

Keywords: aflatoxin; food safety; fumonisin; groundnut; maize; multi-mycotoxin; snacks

Introduction

The quality of staples and their products (e.g. snacks and street foods) that are consumed by humans is imperative considering the global efforts towards increasing food safety, especially in the areas where climatic, poor post-harvest and processing conditions tend to promote mycotoxin contamination of foods. Snacks are quick, portable, less perishable and satisfying foods served commercially as small chops at parties, schools, offices, or as domestic refreshment at home. Usually, snacks are eaten between meals and can be consumed to stave off hunger prior to eating regular meals. In most cases, snacks serve as a meal (especially late hour breakfast) for low income earners who live in high-cost urban/metropolitan cities due to their affordable prices. In Nigeria and across the majority of other African countries, the major groups of snacks are processed from staples such as cassava, groundnut, maize and wheat (Okoruwa 1997; Alabi 2007). In this study, the safety of a variety of snacks processed from maize and groundnut with respect to the presence of chemical contaminants produced by fungi was considered.

Groundnut (also called peanut) is an oil-rich crop which provides high-quality cooking oil and protein-based diets for many households. The high protein and omega-6 fatty acid contents of groundnut make it an ingredient of choice together with cereals (such as sorghum, corn and millets) in the formulation of weaning

foods (Iro et al. 1995). Groundnut and its products also serve as important protein sources in animal rations. In sub-Saharan Africa, groundnut cultivation and its uses in snacks production are second to maize cultivation (Ezekiel et al. 2013). Groundnut and its derivatives such as boiled, roasted, candy, cake and ball are often hawked as snacks on the streets of urban cities in Nigeria (Kayode et al. 2011). Raw and processed groundnuts may be prone to mycotoxin contamination resulting from fungal manifestation on this food due to the presence of the numerous vital nutrients it possesses (Jimoh & Kolapo 2008; Ezekiel et al. 2011). In Nigeria, several reports on groundnut and its products have focused on the microbiological aspects of food safety (Jimoh & Kolapo 2008; Ezekiel et al. 2011, 2013) with just a few that have considered safety in terms of chemical food contaminants (Akano & Atanda 1990; Ezekiel, Sulyok, et al. 2012; Ezekiel et al. 2013). Available reports are limited to groundnut cake (*kulikuli*).

In Africa, maize is the largest cereal crop cultivated for human consumption (mostly as staple food), animal feed and industrial purposes. Maize is highly nutritious and can be processed in various ways (e.g. roasting, boiling, milling, cooking, fermentation or a combination of these). In Nigeria, snacks derived from maize include roasted and boiled corn, popcorn, corn fritter (*kokoro*) and corn cake (*aadun*), as well as *donkwa* which is made by combining maize and groundnut (Okoruwa 1997;

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Ocheme 2009). Regardless of the numerous available maize-based snacks sold across the country, only very few studies have reported the presence of fungal chemical contaminants in these snacks and their parent material (maize) (Udoh et al. 2000; Atehnkeng et al. 2008; Ezekiel, Kayode, et al. 2012).

There is scanty data on the spectrum of fungal metabolites in snacks in Nigeria, especially with respect to the maize-based snacks. This is in contrast to the need to routinely monitor the quality of food available to consumers especially those lacking money to buy high-quality industrial food. In order to bridge this gap, this pilot study was carried out to determine the qualitative and quantitative spectrum of natural chemical contaminants of fungal origin present in routinely consumed ready-to-eat snacks sold in Lagos, Nigeria.

Materials and methods

Chemicals and reagents

Methanol (liquid chromatography (LC) gradient grade) and glacial acetic acid (pro analysis) were purchased from Merck (Darmstadt, Germany), acetonitrile (LC gradient grade) from VWR (Leuven, Belgium) and ammonium acetate (mass spectrometer (MS) grade) from Sigma-Aldrich (Vienna, Austria). Standards of fungal metabolites were obtained either as gifts from various research groups or from commercial sources. Water was purified successively by reverse osmosis and an Elga Purelab ultra analytic system was used from Veolia Water (Bucks, UK).

Samples

Twenty snack samples were purchased from street-traders in Lagos, Nigeria. The snacks included boiled groundnut, burger peanut, corn cake, *donkwa*, *kokoro*, *kulikuli*, peanut candy, popcorn, raw groundnut and roasted groundnut. These snacks were made from either groundnut or maize except for *donkwa* which was produced by combining groundnut and maize. Each sample was collected from several points of trader's trays as three sub-samples (100 g) and mixed together to form a 300-g bulk sample. For samples such as burger peanut and peanut candy which were sold in sealed packs, three parts were purchased and combined to make a bulk sample. The bulky samples were comminuted and 50 g representative sample was obtained from each bulk, and stored at 4°C until analysed.

Sample extraction and determination of matrix effects

Five grams of each representative sample were weighed into a 50-ml polypropylene tube (Sarstedt, Nümbrecht,

Germany) and covered by an extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) in a ratio of 4 ml solvent/g sample. For spiking experiments, 0.25 g sample was extracted. Samples were extracted for 90 min on a GFL 3017 rotary shaker (GFL, Burgwedel, Germany) and diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid 20:79:1, v/v/v). The diluted extracts were injected as described in detail by Sulyok et al. (2007). Centrifugation was not necessary due to sufficient sedimentation by gravity alone. All concentrations of the naturally contaminated samples were corrected by a correction factor (reciprocal of apparent recovery of each analyte). Quantitative standards for averufin (AVER), nidurufin (NID), norsolorinic acid (NOR-AC) and versicolorins A (VER-A) and C (VER-C) were not available. Their identity was unambiguously identified by performing product ion scans and comparing the spectra as well as the retention times to literature data (Shier et al. 2005; Nielsen et al. 2011). Therefore, these compounds were semi-quantitatively determined, using the response factor of averantin (AVRT), a structurally related compound.

LC-MS/MS parameters

Liquid chromatography-tandem mass spectrometric (LC-MS/MS) screening of target microbial metabolites was performed with a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA) equipped with a Turbo IonSpray 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 (all from Phenomenex, Torrance, CA, US). The chromatographic method, chromatographic and MS parameters for 186 of the investigated analytes are as described by Vishwanath et al. (2009). At present, this method has been transferred to another instrument and further expanded to cover 320 metabolites.

ESI-MS/MS was performed both in positive and negative polarities in two separate chromatographic runs per sample by scanning two fragmentation reactions per analyte. The detection window of each analyte was set to its expected retention time ±27 seconds and ±48 seconds in the positive and the negative mode, respectively. Confirmation of positive analyte identification was obtained by the acquisition of two MS/MS transitions per analyte (with the exception of moniliformin (MON) and 3-nitropropionic acid (3-NPA) that exhibit only one fragment ion), which gave 4.0 identification points according to commission decision 2002/657/EC. In addition, LC retention time as well as the intensity ratio of both

monitored MS/MS transitions agreed with the related values of an authentic relative standard within 0.1 min and 30%, respectively.

Results and discussion

Quality assurance of the LC-MS/MS method

The performance characteristics of the analytical method obtained from spiked blank samples are presented in Table 1. The limits of detection (LODs) ranged between 0.01 $\mu\text{g kg}^{-1}$ for beauvericin (BEAU) and 20 $\mu\text{g kg}^{-1}$ for kojic acid (KA) in both matrices (groundnut and maize). Apparent recoveries were high as expected (Abia et al. 2013) and unlike previous studies performed on another LC-MS/MS instrument where the recoveries of aflatoxins and fumonisins were significantly affected by matrix effects (Ezekiel, Sulyok, et al. 2012; Warth et al. 2012)

recoveries of both toxins were >70%. The accuracy of the method was further verified by participating in a proficiency testing scheme that included (amongst other matrices) maize, maize-based baby food, and peanut cake and paste. Z-scores were in the acceptable range (between -2 and 2) with exception of only one result each for fumonisin B1 (FB1) and ochratoxin A. However, in these cases an unusually high dispersion of results submitted by the participants was reported.

Overview of determined fungal metabolites

A total of 32 fungal metabolites were detected in the snacks at concentrations ranging up to 5357 $\mu\text{g kg}^{-1}$ (Table 2). Five of the metabolites were mycotoxins addressed by regulations (aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), FB1 and fumonisin B2

Table 1. Performance characteristics of the analytical method for 32 analytes found in groundnut and maize from Lagos, Nigeria.

Analyte	Standard	Groundnut		Maize	
	LOD ^a ($\mu\text{g kg}^{-1}$)	LOD ^a ($\mu\text{g kg}^{-1}$)	Apparent recovery ^b (%)	LOD ^a ($\mu\text{g kg}^{-1}$)	Apparent recovery ^b (%)
3-Nitropropionic acid (3-NPA)	0.15	1.5	77.6	1.5	83.2
Aflatoxin B1 (AFB1)	0.02	0.2	86.0	0.15	76.0
Aflatoxin B2 (AFB2)	0.03	0.3	80.0	0.3	78.0
Aflatoxin G1 (AFG1)	0.03	0.3	70.0	0.3	75.0
Aflatoxin M1 (AFM1)	0.04	0.4	77.2	0.4	78.2
Alternariol (AOH)	0.04	0.4	88.4	0.4	82.6
Alternariolmethylether (AME)	0.03	0.3	87.0	0.2	116.0
Averufin (AVER)	- ^c	- ^c	56.0	- ^c	66.0
Beauvericin (BEAU)	0.001	0.01	74.0	0.01	70.0
Brevianamide F (BVD-F)	0.06	0.6	85.0	0.6	85.0
Butenolid (BUT)	0.5	10.0	44.0	6.0	65.0
Chrysophanol (CHRY)	0.2	3.0	54.4	2.0	83.2
Curvularin (CURV)	0.05	0.4	115.0	0.4	113.0
Emodin (EMOD)	0.03	0.2	101.0	0.2	97.0
Enniatin B2 (ENN-B2)	0.01	0.1	66.4	0.1	67.5
Equisetin (EQU)	0.03	0.2	156.0	0.15	184.0
Fumonisin B1 (FB1)	0.4	4.0	82.0	4.5	72.0
Fumonisin B2 (FB2)	0.2	2.0	74.0	2.0	77.0
Fumonisin B3 (FB3)	0.3	3.0	75.0	3.0	86.0
Kojic acid (KA)	2.0	20.0	88.0	20.0	84.0
Macrosporin A (MAC-A)	0.06	0.4	118.0	0.4	111.0
Moniliformin (MON)	0.1	0.7	112.2	0.9	87.6
Monocerin (MONO)	0.01	0.1	87.0	0.1	88.0
Nidurufin (NID)	- ^c	- ^c	115.5	- ^c	88.8
Nivalenol (NIV)	0.1	1.0	84.0	0.8	102.0
Norsolorinic acid (NOR-AC)	- ^c	- ^c	82.9	- ^c	90.4
O-Methylsterigmatocystin (O-STER)	0.01	0.06	77.6	0.05	91.8
Pestalotin (PEST)	0.07	0.7	90.6	0.7	87.4
Radicicol (RAD)	0.05	0.2	221.0	0.2	222.0
Tryptophol (TRPH)	1.2	10.0	93.0	10.0	90.0
Versicolorin A (VER-A)	- ^c	- ^c	177.0	- ^c	161.0
Versicolorin C (VER-C)	- ^c	- ^c	95.0	- ^c	78.0

Notes: ^aLimit of detection (signal-to-noise (S/N) = 3:1) expressed as $\mu\text{g kg}^{-1}$ sample.

^bCalculated from spiking a single sample.

^cNo standard available; estimation of concentration based on response and recovery of averantin.

Table 2. Overview of the prevalence of 32 fungal metabolites in the three snack types.

Analyte ^a	Groundnut (<i>n</i> ^b = 10)			Maize (<i>n</i> ^b = 8)			Groundnut–maize (<i>n</i> ^b = 2)		
	<i>Np</i> ^c	Concentration ($\mu\text{g kg}^{-1}$)		<i>Np</i> ^c	Concentration ($\mu\text{g kg}^{-1}$)		<i>Np</i> ^c	Concentration ($\mu\text{g kg}^{-1}$)	
		Range ^d	Median		Range ^d	Median		Range ^d	Median
3-NPA	3	104–150	133	5	2–148	62.4	2	2.9–4.8	4
AFB1	3	52–362	211	5	1–69.5	45	2	9.3–12	11
AFB2	2	559–679	619	3	4.1–5	4.5	2	0.8–1.7	1.3
AFG1	0	<0.3	n.d.	1	3	3	0	<0.3	n.d.
AFM1	2	256–410	333	3	2–2.2	2	0	<0.4	n.d.
AME	1	47	47	8	0.4–45	1	0	n.d.	n.d.
AOH	0	<0.4	n.d.	2	4.8–5.4	5	0	<0.4	n.d.
AVER	5	15–524	73	6	0.8–15	6	2	5.2–6.7	6
BEAU	6	2–84	5	8	0.6–5.2	1.3	2	1.8–1.9	2
BUT	0	<10	n.d.	1	10	10	0	n.d.	n.d.
BVD-F	10	1.1–1892	61	8	10.4–28	21	2	21–22	21
CHRY	0	<3	n.d.	4	4.8–34	15	0	n.d.	n.d.
CURV	0	<0.4	n.d.	2	2.8–6.7	5	0	<0.4	n.d.
EMOD	7	0.4–27	15.5	8	3–52	5.5	2	5–5.5	5.3
ENN-B2	0	<0.1	n.d.	1	0.1	0.1	0	<0.1	n.d.
EQUUS	10	1.2–681	20	8	3.1–15	12	2	83–110.4	97
FB1	0	<4	n.d.	8	44–339	124	2	124–130	127
FB2	0	<2	n.d.	8	18–86	37	2	35–40	38
FB3	0	<3	n.d.	8	4.8–23	11.5	2	12.5–14.4	13.5
KA	6	23–485	54	8	109–5357	345.5	2	194.4–218	206
MAC-A	3	0.6–60	20	8	0.7–9	4	2	5.6–8.6	7.1
MON	0	<0.7	n.d.	6	1.9–11	7	2	68–72	70
MONO	0	<0.1	n.d.	6	1.5–96	4	2	0.7–1.2	1
NID	1	331	331	2	0.2–0.3	0.2	2	0.18–0.23	0.2
NIV	0	<1	n.d.	2	1.8–2.5	2	0	n.d.	n.d.
NOR-AC	2	90–705	397	4	0.03–0.2	0.1	2	0.04–0.07	0.1
O-STER	4	101–1355	878	4	0.1–0.3	0.3	2	0.13–0.19	0.2
PEST	0	<0.7	n.d.	4	0.2–2.3	0.3	2	0.5–0.7	0.6
RAD	0	<0.2	n.d.	2	3.8–12	8	0	<0.2	n.d.
TRPH	4	30–955	45	4	0.5–21	9.4	0	<10	n.d.
VER-A	2	507–872.5	690	3	0.5–0.8	0.6	2	0.4–0.6	0.5
VER-C	4	14–623	214	5	0.4–3.3	2	2	0.6–1.3	1

Notes: n.d., not determined.

^aSee Table 1 for full names.

^bNumber of analysed samples.

^cNumber of positive samples.

^dRange of positive values.

(FB2)) in cereals and nuts in the European Union (EU), while the other 27 were either regulated in other food commodities or had no existing regulations for their concentration levels in any food commodity. Aflatoxin G2 (AFG2) was not found in any of the snacks. When considering the occurrence of metabolites in the three snack types, 18 metabolites including two regulated mycotoxins contaminated the groundnut-based snacks while 21 metabolites (including four regulated mycotoxins) were found in snacks made from the combination of groundnut and maize. In addition, 32 metabolites including five regulated mycotoxins were detected in the maize-based snacks. The higher occurrence of fungal metabolites in the snacks made from maize, rather than those from groundnut, agrees with recent reports of Warth et al. (2012) and Abia et al. (2013), which have shown that maize is more

prone than groundnuts to contamination by a wide range of fungal metabolites. The concentration levels of metabolites in the snacks reached 218 and 5357 $\mu\text{g kg}^{-1}$ in the case of KA for groundnut/maize- and maize-based snacks, respectively, and 1892 for brevianamide F (BVD-F) in the groundnut-based snacks (Table 2). The distribution of metabolites in the snacks indicated the following order of contamination: *Aspergillus* > *Fusarium* > *Penicillium* = other fungal metabolites > *Alternaria*.

Occurrence of regulated mycotoxins and their derivatives

Regulated mycotoxins that have previously been found to contaminate groundnut, maize and their products include aflatoxins, fumonisins and type B trichothecenes (Kpodo

et al. 2000; Adejumo et al. 2007; Bandyopadhyay et al. 2007; Kpodo & Bankole 2008). Among these, aflatoxins predominate in groundnuts and groundnut-based products (Kpodo et al. 2000; Mutegei et al. 2009; Oliveira et al. 2009; Ediage et al. 2011; Ezekiel, Sulyok, et al. 2012), while maize are more susceptible to fumonisin contamination (Marasas, 2001; Bandyopadhyay et al. 2007; Kpodo & Bankole 2008). In the present study, AFB1 contaminated 2, 3 and 5 of the groundnut/maize-, groundnut- and maize-based snacks at concentrations reaching $362 \mu\text{g kg}^{-1}$ (Table 2). In this small set of samples that were tested, total aflatoxin (AF_{tot} = \sum AFB1, B2, G1 and G2) concentration was much higher in groundnut-based snacks (mean = $620.9 \mu\text{g kg}^{-1}$) than in maize-based (mean = $40.9 \mu\text{g kg}^{-1}$) and groundnut/maize-based snacks (mean = $12 \mu\text{g kg}^{-1}$). Highest aflatoxin concentrations were observed in *kulikuli* ($1041 \mu\text{g kg}^{-1}$), brown *kokoro* ($74 \mu\text{g kg}^{-1}$) and *donkwa* ($14 \mu\text{g kg}^{-1}$) samples, respectively. The present findings of high aflatoxin occurrence in groundnut-based snacks especially groundnut cake (*kulikuli*) agrees well with reports of Ediage et al. (2011) and Ezekiel et al. (2013).

On the other hand, total fumonisins (TF = \sum FB1, B2 and B3) contaminated all maize-based snacks at higher concentrations (mean = $218.7 \mu\text{g kg}^{-1}$) than the groundnut/maize- snacks (mean = $178.5 \mu\text{g kg}^{-1}$). As expected, fumonisins were not detected in any of the groundnut-based snacks. Concentration levels of AF_{tot} reached 183 and $448 \mu\text{g kg}^{-1}$ in *donkwa* (groundnut/maize-based) and corn cake (maize-based) samples, respectively. Nivalenol (NIV), a *Fusarium* toxin, was found in two samples of *kokoro* (maize-based) at minor levels of 1.8 and $2.5 \mu\text{g kg}^{-1}$. The absence of deoxynivalenol and other trichothecenes in the snacks, especially in the maize-based snacks, contradicts the findings of Adejumo et al. (2007), Warth et al. (2012) and Abia et al. (2013), which reported this *Fusarium* toxin in contaminated maize from Nigeria ($n = 40$), Cameroon ($n = 37$) and Mozambique ($n = 2$) up to 745, 435 and $124 \mu\text{g kg}^{-1}$, respectively.

Occurrence of other fungal metabolites

Further metabolites found include those produced by several species of *Alternaria*, *Aspergillus*, *Chaetomium*, *Fusarium*, *Penicillium* and other fungi. This indicates a widespread contamination of snacks and their ingredients by these moulds. All additional fungal metabolites found in the snacks have previously been reported to contaminate a wide range of food including cereals, nuts and their products (Sulyok et al. 2010; Ezekiel, Sulyok, et al. 2012; Warth et al. 2012; Abia et al. 2013). In terms of prevalence, equisetin (EQU) and BVD-F occurred in all 20 snacks (Table 2). The concentrations of EQU ranged from $1.2 \mu\text{g kg}^{-1}$ in raw and roasted groundnuts to $681 \mu\text{g kg}^{-1}$ in *kulikuli*, while BVD-F occurred at $1.1 \mu\text{g kg}^{-1}$ in raw groundnut to $1892 \mu\text{g kg}^{-1}$ in roasted groundnut. Although toxicity of these two

metabolites is not yet established in humans, a public health threat cannot completely be ruled out due to their widespread co-occurrence with other mycotoxins and fungal metabolites in the snacks.

Further consideration is given to metabolites without any existing regulation despite their established toxicity. These include alternariolmethylether (AME), BEAU, emodin (EMOD), Enniatin B2 (ENN-B2) and MON. BEAU, Enniatins and MON, which are regarded as emerging *Fusarium* mycotoxins (Jestoi 2008). In this study, BEAU was the most prevalent emerging mycotoxin, occurring in 16 out of the 20 snacks (mean = $8.9 \mu\text{g kg}^{-1}$), followed by MON which contaminated 8 of 20 samples (mean = $22.3 \mu\text{g kg}^{-1}$). ENN-B2 was detected in the *kokoro* sample at $0.1 \mu\text{g kg}^{-1}$. The genotoxic EMOD occurred in 17 of 20 snacks (mean = $13.2 \mu\text{g kg}^{-1}$), while AME was found in 10 of 20 (range = $0.4\text{--}47 \mu\text{g kg}^{-1}$; mean = $14.1 \mu\text{g kg}^{-1}$) samples. The concentrations found in this study were significantly lower than those found to be toxic in the above-mentioned study. Though reports are not yet available for the synergistic interactions of these non-regulated metabolites, effects of independent administrations have been reported. For example, BEAU is genotoxic to human lymphocytes (Celik et al. 2010) while MON and the enniatins can induce cytotoxicity in many mammalian systems (Jestoi 2008; Tan et al. 2011). AME, a notable *Alternaria* toxin, is fetotoxic and teratogenic in mammals (Barkai-Golan 2008; European Food Safety Authority 2011). Therefore, this study may provide useful data for future toxicological studies focusing on interactive effects of multiple fungal metabolites.

Impact of mycotoxins in the snacks on public health

Public health concern raised by food contaminants is usually due to occurrence of toxins in food (regulated by limits or non-regulated) and co-occurrence with other fungal metabolites, so it is therefore imperative to consider the compliance of snacks to the maximum limits (MLs) of NAFDAC, coupled with existing data on the toxicology. In Nigeria, only AFB1 and AF_{tot} are regulated in food and the limits are 10 and $20 \mu\text{g kg}^{-1}$ respectively, thus 7 and 6 of 20 snacks samples exceeded the MLs. Snacks with AF_{tot} levels above the ML were boiled groundnut ($n = 1$), brown *kokoro* ($n = 2$), *kulikuli* ($n = 2$) and white *kokoro* ($n = 1$), which corresponded to 60% and 100% of maize- and groundnut-based snacks, respectively. Considering the highly toxic potency of AFB1 and its classification as a Group 1 human carcinogen (International Agency for Research on Cancer 2002; Wild & Turner 2002) as well as its co-occurrence with other aflatoxins and regulated toxins in the snacks, consumers of these, who are mostly school-aged children and

young adults, may be at risk to mycotoxicoses (Whitlow et al. 2000; Ezekiel et al. 2013).

Noteworthy also is the co-occurrence of 3–15, 11–26 and 21 metabolites in the groundnut-, maize- and groundnut/maize-based snacks, respectively. *Donkwa*, *kulikuli* and all maize-based snacks contained cocktails of more than 10 metabolites. Several metabolite combinations of toxicological importance in the snacks include: (1) AFB1/AFB2/FB1/FB2/FB3/MON/BEAU/EMOD in *donkwa*, (2) AFB1/AFB2/AFM1/BEAU/EMOD in *kulikuli* and (3) FB1/FB2/FB3/BEAU/AME/EMOD in all maize-based snacks, mostly additional to AFB1/AFB2/MON in this type of snack. Regardless the concentrations of the individual metabolites in the snacks, it should be emphasised that synergistic or additive damaging effects may be induced in vital organs of consumers who ingest these snacks. Also chronic intoxication might be possible due to constant daily exposures to low doses over a longer period of time, which is likely in case of these snacks.

Conclusion

Because of the very small number of samples investigated in this study, conclusions can only be drawn with much uncertainty. This study indicates the usefulness of further studies to generate a large set of data. This study also shows the presence of a wide range of fungal metabolites in routinely consumed snacks at concentrations capable of inducing toxic effects to humans. The variety of fungal metabolites quantified may point to deficiencies in good agricultural practices employed for the raw materials (maize and groundnut) and processing conditions of these snacks. Co-occurrence of metabolites in various snacks may pose additional threats due to possible additive and/or synergistic effects.

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References

- Abia WA, Warth B, Sulyok M, Krska R, Tchana AN, Njobeh PB, Dutton MF, Moundipa PF. 2013. Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). *Food Cont.* 31:438–453.
- Adejumo TO, Hettwer U, Karlovsky P. 2007. Occurrence of *Fusarium* species and trichothecenes in Nigerian maize. *Int J Food Microbiol.* 116:350–357.
- Akano DA, Atanda OO. 1990. The present level of aflatoxin in Nigerian groundnut cake (“kulikuli”). *Lett Appl Microbiol.* 10:187–189.
- Alabi MO. 2007. Nutrition processing and utilization of selected food crops in Nigeria. Gboko: Peace House Publishers; p. 1–138.
- Atehnkeng J, Ojiambo PS, Donner M, Ikotun T, Sikora RA, Cotty PJ, Bandyopadhyay R. 2008. Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones in Nigeria. *Int J Food Microbiol.* 122:74–84.
- Bandyopadhyay R, Kumar M, Leslie JF. 2007. Relative severity of aflatoxin contamination of cereal crops in West Africa. *Food Addit Contam.* 24:1109–1114.
- Barkai-Golan R. 2008. *Alternaria* mycotoxins. In: Barkai-Golan R, Nachman P, editors. *Mycotoxins in fruits and vegetables*. San Diego (CA): Academic Press; p. 185–203.
- Celik M, Aksoy H, Yilmaz S. 2010. Evaluation of beauvericin genotoxicity with the chromosomal aberrations, sister-chromatid exchanges and micronucleus assays. *Ecotoxicol Environ Safety.* 73:553–1557.
- Eidiage EN, Di Mavungu JD, Monbaliu S, Peteghem CV, De Saeger S. 2011. A validated multi-analyte LC–MS/MS method for the quantification of 25 mycotoxins in cassava flour, peanut cake and maize samples. *J Agric Food Chem.* 59:5173–5180.
- European Food Safety Authority. 2011. Scientific opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. *EFSA J.* 9:2407.
- Ezekiel CN, Anokwuru CP, Fari A, Olorunfemi MF, Fadairo O, Ekeh HA, Ajoku K, Gbuzue N, Akinsanmi F. 2011. Microbiological quality and proximate composition of peanut cake (*Kulikuli*) in Nigerian markets. *Acad Arena.* 3:103–111.
- Ezekiel CN, Kayode FO, Fapohunda SO, Olorunfemi MF, Kponi BT. 2012. Aflatoxigenic moulds and aflatoxins in street-vended snacks in Lagos, Nigeria. *Internet J Food Safety.* 14:83–88.
- Ezekiel CN, Sulyok M, Babalola DA, Warth B, Ezekiel VC, Krska R. 2013. Incidence and consumer awareness of toxigenic *Aspergillus* section *Flavi* and aflatoxin B1 in peanut cake from Nigeria. *Food Cont.* 30:596–601.
- Ezekiel CN, Sulyok M, Warth B, Odebo AC, Krska R. 2012. Natural occurrence of mycotoxins in peanut cake from Nigeria. *Food Cont.* 27:338–342.
- International Agency for Research on Cancer. 2002. Traditional herbal medicines, some mycotoxins, naphthalene, and styrene. *IARC Monogr Eval Carcinog Risk Chem Hum.* 82:1–556.
- Iro N, Iliyas A, Tato A. 1995. Studies on the preparation and nutrient composition of Kunu-Gyado: a traditional Nigerian-groundnut-cereal based weaning food. *Food Nutr Bullet.* 16:238–240.
- Jestoi M. 2008. Emerging *Fusarium* –mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin—a review. *Crit Rev Food Sci Nutr.* 48:21–49.
- Jimoh KO, Kolapo AL. 2008. Mycoflora and aflatoxin production in market samples of some selected Nigerian foodstuffs. *Res J Microbiol.* 3:169–174.
- Kayode FO, Oguntona CRB, Elemo GN, Bankole AO, Erukainure OL. 2011. Chemical properties and microbial quality of peanut (*Arachis hypogaea*) snacks in Lagos metropolis. *Ferment Technol Bioeng.* 1:77–87.
- Kpodo K, Thrane U, Hald B. 2000. *Fusaria* and fumonisins in maize from Ghana and their co-occurrence with aflatoxins. *Int J Food Microbiol.* 61:147–157.
- Kpodo KA, Bankole SA. 2008. Mycotoxin contamination in foods in West and Central Africa. In: Leslie JF, Bandyopadhyay R, Visconti A, editors. *Mycotoxins: detection methods, management, public health and agricultural trade*. Wallingford: CABI Publishing; p. 103–116.

- Marasas WF. 2001. Discovery and occurrence of the fumonisins: a historical perspective. *Environ Health Perspect.* 109:239–243.
- Mutegi CK, Ngugi HK, Hendriks SL, Jones RB. 2009. Prevalence and factors associated with aflatoxin contamination of peanuts from Western Kenya. *Int J Food Microbiol.* 130:27–34.
- Nielsen KF, Månsson M, Rank J, Frisvad JC, Larsen TO. 2011. Dereplication of microbial natural products by LC-DAD-TOFMS: experiences gained from an inhouse database of 719 mycotoxins and fungal metabolites. *J Nat Prod.* 74:2338–2348.
- Ocheme OB. 2009. Comparative analysis of the proximate composition and sensory properties of Dokuwa produced in Niger state. *Nig Food J.* 27:59–65.
- Okoruwa AE. 1997. Utilization and processing of maize. IITA Research Guide 35. Ibadan: IITA.
- Oliveira CAF, Goncalves NB, Rosim RE, Fernandes AM. 2009. Determination of aflatoxins in peanut products in the north-east region of Sao Paulo, Brazil. *Int J Mol Sci.* 10:174–183.
- Shier WT, Lao Y, Steele TW, Abbas HK. 2005. Yellow pigments used in rapid identification of aflatoxin-producing *Aspergillus* strains are anthraquinones associated with the aflatoxin biosynthetic pathway. *Bioorg Chem.* 33:426–38.
- Sulyok M, Krska R, Schuhmacher R. 2007. A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. *Anal Bioanal Chem.* 389:1505–1523.
- Sulyok M, Krska R, Schuhmacher R. 2010. Application of an LC-MS/MS based multi-mycotoxin method for the semi-quantitative determination of mycotoxins occurring in different types of food infected by moulds. *Food Chem.* 119:408–416.
- Tan DC, Flematti GR, Ghisalberti EL, Sivasithamparam K, Barbetti MJ. 2011. Toxigenicity of enniatins from western Australian *Fusarium* species to brine shrimp (*Artemia franciscana*). *Toxicon.* 57:817–825.
- Udoh JM, Cardwell KE, Ikotun T. 2000. Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. *J Stored Prod Res.* 36:187–201.
- Vishwanath V, Sulyok M, Labuda R, Bicker W, Krska R. 2009. Simultaneous determination of 186 fungal and bacterial metabolites in indoor matrices by liquid chromatography/tandem mass spectrometry. *Anal Bioanal Chem.* 395:1355–1372.
- Warth B, Parich A, Atehnkeng J, Bandyopadhyay R, Schuhmacher R, Sulyok M, Krska R. 2012. Quantitation of mycotoxins in food and feed from Burkina Faso and Mozambique using a modern LC-MS/MS multitoxin method. *J Agric Food Chem.* 60:9352–9363.
- Whitlow LW, Diaz DE, Hopkins BA, Hagler WM. 2000. Mycotoxins and milk safety: the potential of block transfer to milk. In: Lyons TP, Jacques KA, editors. *Biotechnology in the feed industry*, Proceedings of Alltech's 16th Annual Symposium. Sheffield: Nottingham University Press; p. 391–408.
- Wild CP, Turner PC. 2002. The toxicology of aflatoxins as a basis for public health decisions. *Mutagen.* 17: 471–481.