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Uncommon toxic microbial metabolite patterns in traditionally home-processed maize dish (*fufu*) consumed in rural Cameroon

Wilfred A. Abia [a, b, *], Benedikt Warth [a, c, **], Chibundu N. Ezekiel [a, d], Bojan Sarkanj [a, e], Paul C. Turner [f], Doris Marko [c], Rudolf Krska [a], Michael Sulyok [a]

[a] Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna (BOKU), Konrad Lorenzstr. 20, A-3430 Tulln, Austria
[b] Laboratory of Pharmacology and Toxicology, Department of Biochemistry, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon
[c] Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Waehringerstr. 38, 1090 Vienna, Austria
[d] Department of Microbiology, Babcock University, Ilishan Remo, Ogun State, Nigeria
[e] Department of Applied Chemistry and Ecology, Faculty of Food Technology, FranjeKuhača 20, Osijek, Croatia
[f] MIAEH, School of Public Health, University of Maryland, 40742 College Park, MD, USA

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ABSTRACT

Toxins of microbial origin frequently contaminate foodstuffs worldwide and pose a serious hazard to humans. This study reports on LCMS/MS quantification of multiple fungal and bacterial toxins, from household sampling of 50 traditionally prepared maize-fufu samples from Bamunka village, western highlands of Cameroon. Seventy-four metabolites including aflatoxin B1 (AFB1) (12/50: mean 0.9, range n.d-1.8 μg kg^{-1}), cereulide (50/50: mean 37; range 1–236 μg kg^{-1}), deoxynivalenol (DON) (50/50: mean 23, range 14–55 μg kg^{-1}), fumonisin B1 (FB1) (50/50: mean: 151, range 48–709 μg kg^{-1}), nivalenol (NIV) (50/50; mean 268, range 116–372 μg kg^{-1}), patulin (PAT) (15/50:mean 105, range 12–890 μg kg^{-1}) and zearalenone (ZEN) (50/50: mean 49, range 5–150) were detected; and of note every sample contained at least 27 toxic compounds. While individual toxin levels were mostly low there is always concern regarding mixtures, for which data are absent or limited. This study reports several novel observations of toxins not previously reported in maize, and the mixture of toxins, e.g. cereulide, PAT and ZEN derivatives (ZEN-cis and ZENsulfate-cis) are reported for the first time in Cameroonian food.

1. Introduction

Maize-*fufu* (also known as *fufu*-corn) is a boiledmaize-doughdish that is consumed year-round as a dietary staple, especially within rural populations of the western highland of Cameroon. Locally prepared maize-*fufu* is usually served with vegetable or groundnut soup. Agricultural products are frequently contaminated with microorganisms capable of producing a diversity of toxins of potential public health concern. While numerous studies have investigated maize grains/flour for a few microbial toxins (Abia et al., 2013a; Adetunji et al., 2014; Shephard et al., 2013; Warth et al., 2012); a detailed analysis of maize-*fufu* for a diverse set of microbial toxins (e.g.mycotoxins and bacterial exotoxins) has not been reported. Such data is valuable in order to better understand the frequency, level and range of exposures that can occur in regular consumers.

A lack of regulation and prolonged crop storage, coupled with favorable conditions for fungal growth and mycotoxin production constitutea major concern within many sub-Saharan regions. Dietary exposures to mycotoxins are a significant public health concern especially in sub-Saharan Africa (reviewed by (International Agency on Cancer Research, IARC, 2012)). The role for aflatoxin B_1 (AFB_1) is best established and risks include acute liver failure, liver cancer and stunting. The epidemiology for other mycotoxins is less developed but suggested health concerns include esophageal cancer, neural tube defects and stunting for fumonisin B_1 (FB_1); renal toxicity for ochratoxin A (OTA); immune suppression for deoxynivalenol (DON); and the estrogenic effects for

* Corresponding author. Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna (BOKU), Konrad Lorenzstr. 20, A-3430 Tulln, Austria.
** Corresponding author. Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Waehringerstr. 38, 1090 Vienna, Austria.
E-mail addresses: abiawilfred@yahoo.com (W.A. Abia), benedikt.warth@univie.ac.at (B. Warth).

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zearalenone (ZEN) (IARC, 2012).

Mycotoxin surveillance studies have shifted focus from detection of single or a few regulated mycotoxins (e.g. AFB_1orFB$_1$) to techniques capable of examining a greater diversity of mycotoxin contaminants (Ediage et al., 2014; Malachova et al., 2014; Sulyok et al., 2007). The application of robust chromatographic methods coupled to highly sensitive mass spectrometry has elucidated unique patterns of co-occurring mycotoxins from various sub-Saharan African countries (Adetunji et al., 2014; Shephard et al., 2013; Warth et al., 2012; Ezekiel et al., 2012; Geary et al., 2016), including Cameroon (Abia et al., 2013a; Ediage et al., 2014; Ngoko et al., 2008; Njobeh et al., 2010; Tchana et al., 2010). In addition to mycotoxins, other microbial metabolites e.g. chloramphenicol and nonactincan occur in maize (Adetunji et al., 2014). However, there are no reports of bacterial toxins such as cereulide in maize or maize based foods, or their co-occurrence with mycotoxins in Africa.

The current study was designed to evaluate the level of fungal secondary metabolites and other potential contaminants (e.g., bacteria toxins) in maize-*fufu* commonly consumed in Bamunka village, in the western highlands zone of Cameroon. The natural contamination level of this locally produced food was evaluated in terms of the spectrum of toxic and potentially toxic metabolites of fungi and bacteria origin.

2. Materials and methods

2.1. Study location and sampling

This study was conducted in Bamunka village in the western highlands (North West) region of Cameroon. Bamunka is located at 1100 m above sea level in the Ndop valley. The rainfall ranges from 1000 to 1500 mm per annum with temperatures from 18 to 35 °C. Bamunka is a rural community solely dependent on subsistence agriculture. The villagers consume their own locally grown and prepared foods, especially locally cultivated maize and additionally rice. Harvesting of maize generally begins in June and spans through late August, which is within the rainy season.

Households were included based on their willingness to participate and that their diet regularly included maize-*fufu* (generally prepared as described on Fig. 1 below). A total of 50 households were randomly selected after providing signed informed consent. The study received ethical approval from the National Ethics Committee of Cameroon (AUTORISATION N^{O}. 2014/03/426/L/CNERSH/SP). Samples of ready-to-eat maize-*fufu* ($n = 50$; 10 g each) were collected in September 2015 from the households once during evening meal time. Samples were preserved at −20 °C and couriered on dry ice to the Center for Analytical Chemistry, University of Natural Resources and Life Sciences Vienna (BOKU), Austria, for multi-microbial metabolite analysis.

2.2. Chemicals and reagents

LC gradient grade methanol and acetonitrile, and MS grade ammonium acetate and glacial acetic acid (p.a.) were purchased from Sigma-Aldrich (Vienna, Austria). A Purelab Ultra system (ELGA LabWater, Celle, Germany) was used for further purification of reverse osmosis water.

The different standards of fungal (548) and bacterial (38) metabolites were obtained either as gifts from various research groups or from the following commercial sources: Romer Labs®Inc. (Tulln, Austria), Sigma–Aldrich (Vienna, Austria), BioAustralis (Smithfiled, Australia), AnalyticonDiscovery (Potsdam, Germany), Fermentek (Jerusalem, Israel), Iris Biotech GmbH (Marktredwitz, Germany), Enzo Life Sciences (Lausanne, Switzerland) and LGC Promochem GmbH (Wesel, Germany). Stock solutions of individual or related mixtures were prepared in acetonitrile, and stored at −20 °C. The final combination of analytes "working solution" was freshly prepared prior to spiking experiments.

2.3. Sample preparation

Solid dough of maize-*fufu* samples (100 g each) were ground using an Osterizer blender (Sunbeam Oster Household Products, Fort Lauderdale, Florida, USA). Five grams of each milled sample were extracted with 20 mL extraction solvent (acetonitrile/water/acetic acid, 79:20:1, v/v/v) for 90 min at 180 rpm using a GFL 3017 rotary shaker (GFL 3017, Burgwedel, Germany) and subsequently centrifuged for 2 min at 1500xg on a GS-6 centrifuge (Beckman Coulter Inc., Fullerton, CA). From each extract solvent, 500 μL was transferred into a 1.5 mL glass vial and mixed with an equal volume of the dilution solvent (acetonitrile/water/acetic acid, 20:79:1, v/v/v). 5 μL of the diluted extract was injected into the LC-MS/MS system.

2.4. LC–ESI–MS/MS conditions

The maize-*fufu*extracts were analyzed for the presence of more than 650 microbial metabolites. This included 350 additional metabolites added to the method after (Malachova et al., 2014). Notably, all European Commissions (EC, 2006) regulated mycotoxins were included, as well as the bacterial toxin, cereulide; structures of several are provided on Fig. 2 below. The analyses were based on liquid chromatography coupled with tandem mass spectrometric instrument (LC-MS/MS) using the identical protocol as described by Malachova et al. Regular participation in proficiency testing-schemes confirm that the extension of the range of metabolites is not at the cost of a deteriorated accuracy of the method. In brief, the LC-MS/MS detection process targeting fungal and bacterial metabolites was performed using a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA) equipped with TurboIonSpray electrospray ionization (ESI) source and a 1290 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25 °C on a Gemini® C_{18}-column, 150 × 4.6 mm i.d., 5 μm particle size, equipped with a C_{18} 4 × 3 mm i.d. security guard cartridge (Phenomenex, Torrance, CA, US).

Apparent recoveries have been re-determined for fufu despite validation data for maize was available (Malachova et al., 2014). Five samples of maize-*fufu* were spiked with 100 μL of a multi-analyte standard solution at one concentration level and analyzed as described in section 2.3. A concentration level that was above the regulatory limits in case of some toxins was chosen (e.g. 40 μg/kg for aflatoxins, 160 μg/kg for patulin, 150 μg/kg for cereulide and 1700 μg/kg for fumonisins) as the extent of signal suppression/enhancement was found not to be significantly different on several concentration levels (manuscript in preparation).

2.5. Quantification and identification

Quantification was performed using external calibration based on serial dilution of a multi-component stock solution. Results were corrected for apparent recoveries. The accuracy of the method is verified externally by participation in proficiency testing schemes organized by BIPEA (Paris, France). For maize, 117 satisfactory, 4 questionable and 1 unsatisfactory result have been obtained in the period from 09/2010 to 10/2016. Limits of detection (LOD) and of quantification (LOQ) were estimated at the lowest concentration in spiked samples corresponding to a signal-to-noise ratio (S/N) of 3:1 and 10:1 respectively.

Confirmation of positive analyte identification was obtained by

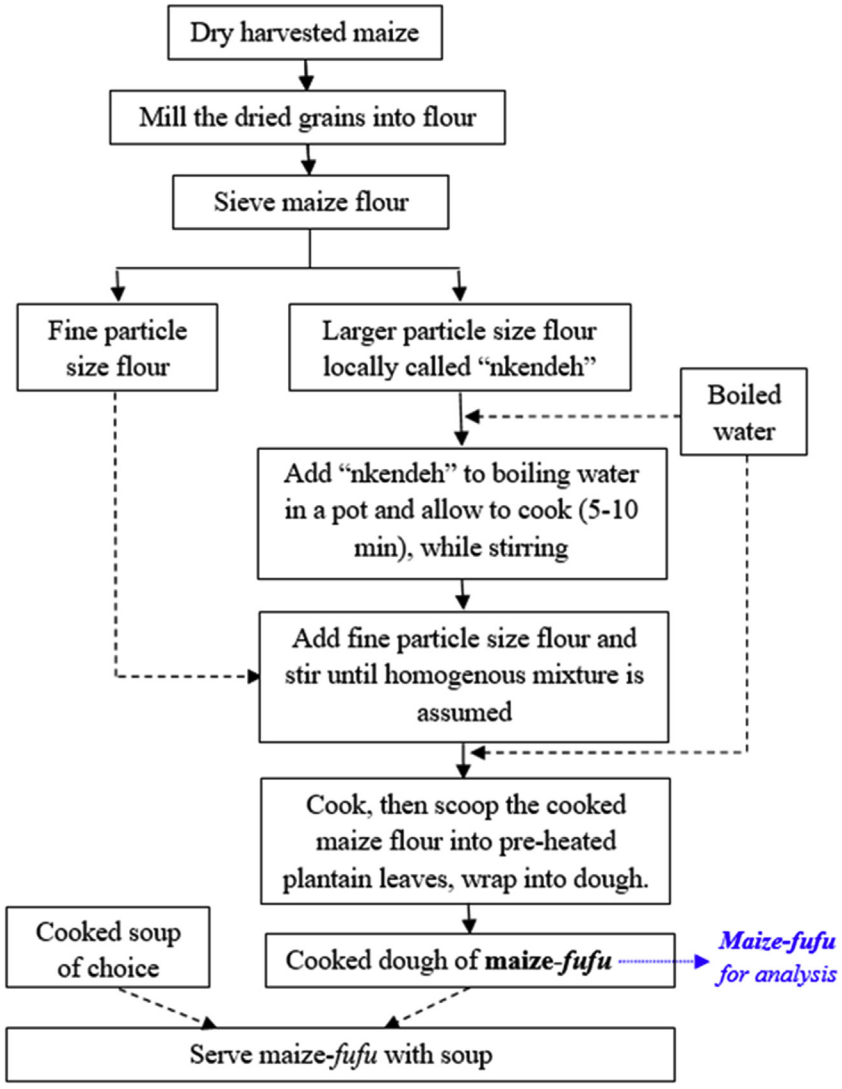

Fig. 1. Traditional workflow of the processing steps involved in preparing maize-*fufu* in Bamunka village, Cameroon.

the acquisition of two time-scheduled multiple reaction monitoring (MRMs) which yielded 4.0 identification points according to the European Commission decision 2002/657 (EC, 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 0.03 min and 30% rel., respectively.

2.6. Data analysis

Descriptive analysis was performed for the data obtained for each detected metabolite using SPSS® (Windows v. 16, Chicago, IL, USA).

3. Results

3.1. Method performance parameters

A total of 74 metabolites were detected in the maize-*fufu* samples. The limit of detection (LOD), limit of quantitation (LOQ), and recovery values for the metabolites after spiking of maize-*fufu* samples are presented in Table 1. Overall, the apparent recoveries for fungal metabolites ranged from 65 to 152% and 81–94% for bacterial toxins.

3.2. Metabolite profiles in maize-fufu

The fungal and bacterial toxin contamination patterns in 50 maize-*fufu* samples analyzed by LC-MS/MS are presented in Fig. 3. The 74 metabolites detected include fungal secondary metabolites, bacterial toxins, plant toxins, and others of undefined origin. A selection of the most essential metabolites is provided on Table 2.

Several mycotoxins of public health concern were observed in the food samples including aflatoxins, fumonisins, patulin (PAT), trichothecenes and ZEN (Table 2, *details for other detected metabolites are on* S1 Table). AFB_1 was found in 12 samples (mean of detectable was 0.9 μg kg^{-1}, range: 0.3–1.8 μg kg^{-1}; median: 0.3 μg kg^{-1}). FB_1 was observed in all the maize-*fufu* samples (mean: 151 μg kg^{-1}; range: 48–709 μg kg^{-1}; median: 112 μg kg^{-1}); while other fumonisins (FB_2, FB_3, FB_4or FA_1)were observed less frequently and at lower concentration.The mean of total fumonisin, whichare regulated ($\sum FB_1$, $FB_2$and FB_3), was 203 μg kg^{-1} (range: 48–978 μg kg^{-1}). FA_1 was only observed in 32% of samples and at lower concentration (mean 8 μg kg^{-1}; range 4–18 μg kg^{-1}). For the trichothecenes, DON and nivalenol (NIV) were detected in all

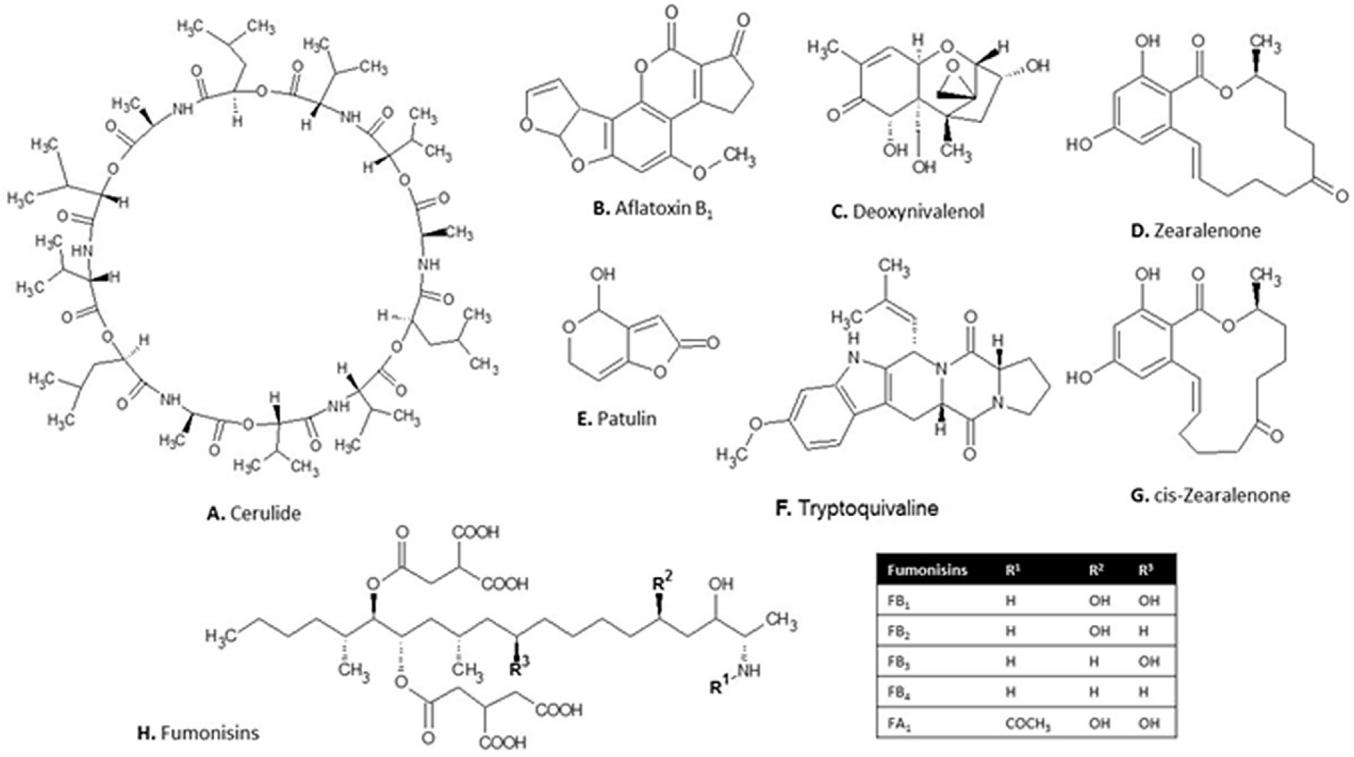

Fig. 2. Chemical structures (A–H) of selected quantified metabolites.

Table 1
The limit of detection (LOD), limit of quantitation (LOQ), and recovery for the major mycotoxins and exotoxin* detected in maize-*fufu* samples from Bamunka, Cameroon.

Toxin type (abbreviation)	Recovery (%)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
Aflatoxin B_1 (AFB_1)	66 ± 6	0.15	0.5
Fumonisin B_1 (FB_1)	65 ± 2	3.2	10
Fumonisin B_2 (FB_2)	71 ± 4	2.4	8
Fumonisin B_3 (FB_3)	75 ± 4	2.4	8
Fumonisin B_4 (FB_4)	71 ± 4	2.4	8
Fumonisin A_1 (FA_1)	71 ± 4	2.4	8
Deoxynivalenol (DON)	100 ± 6	0.8	2.6
Nivalenol (NIV)	73 ± 4	0.8	2.6
Nivalenol-glucoside (NIV-Glc)	73 ± 7	3.2	10
Fusarenon-X (FUS-X)	84 ± 7	3.2	10
Zearalenone (ZEN)	96 ± 13	0.1	0.3
Zearalenone-cis (ZEN-cis)	n.d.	–	
Zearalenone-sulfate (ZEN-S)	96 ± 16	0.1	0.3
Zearalenone-sulfate-cis (ZEN-S-cis)	n.d.	–	
alpha-Zearalenol (α-ZEL)	122 ± 3	0.8	2.6
beta-Zearalenol (β-ZEL)	96 ± 8	1.2	4
Patulin (PAT)	100	5	17
Alternariol (AOH)	88 ± 4	0.3	1
Cereulide (CER)*	81±	0.5	1.7

NB: LOQ is 3.33 times LOD.

samples (mean: 23 μg kg^{-1}; range: 14–55 μg kg^{-} andmean: 268 μg kg^{-1}; range: 116–375 μg kg^{-1}, respectively). NIV-glucoside was detected in all, and on average represented just less than 3% of total NIV present. Additionally, ZEN (mean: 49 μg kg^{-1}; range: 5–150 μg kg^{-1}; incidence: 100%) and some of its derivatives such as ZEN-sulfate (ZEN-S), α-zearalenol and β-zearalenol, as well astwo newly found cis-forms (ZEN-cis and ZEN-sulfate-cis), and PAT (mean: 105 μg kg^{-1}; range: 12–890 μg kg^{-1}; incidence: 30%) produced by *Aspergillus* were quantified.

Besides the major mycotoxins and their derivatives, 57 other-analytes were detected in the maize-*fufu* samples (Fig. 1). From *Aspergillus* molds, kojic acid (mean: 5032 μg kg^{-1}; max: 30251 μg kg^{-1}; 100%) was the most concentratedmetabolites among 11others detected. Additionally, seven aflatoxin precursors were observed: sterigmatocystin (STER), *O*-methylSTER, averufin, versicolorins (VER-A and VER-C), nidurufin and norsoloronic acid. Meanwhile, a product consistent with nigragilin was detected, though a lack of standards restricted our ability to quantify.From *Fusarium*molds, aurofusarin, beauvericin, moniliformin, culmorin (CUL), 15-OH-CUL and chrysogin contaminated all maize-*fufu* samples. 15-OH-CUL had the highest concentration (mean: 178 μg kg^{-1}; max: 295 μg kg^{-1}). From *Penicillium*, questiomycinA was detected in all samples at levels reaching 29 μg kg^{-1} (mean: 18 μg kg^{-1}), while four other metabolites were present in less than 10% of the maize-*fufu* samples. Three *Alternaria* mold metabolites (macrosporin, alternariol (AOH) and AOHmethylether) were found at low concentrations (<3 μg kg^{-1}) in the food samples.

Three bacterial metabolites (cereulide, monactin and nonactin) were detected in the maize-*fufu* samples. Cereulide the most prevalent bacterial metabolite was detected in all samples (mean: 37 μg kg^{-1}; range: 1–236 μg kg^{-1}). Xanthotoxin, a plant toxin, was found in 90% of the samples (mean: 4.9 μg kg^{-1}; range: 0.5–16 μg kg^{-1}).

Additionally, eleven metabolites from unspecified sources were detected.Cyclo (L-Pro-L-Tyr) and asperglaucide had the highest maximum (1082 μg kg^{-1}) and highest mean (271 μg kg^{-1}) levels respectively, with 100% incidence. About 19 of the 57 metabolites were present in all maize-*fufu* samples (100%). In terms of co-occurrence of major toxins of public health concern, AFB_1, cereulide, FB_1, DON, NIV, ZEN were found in all AFB_1 contaminated samples, n = 24 (48% of all samples). Fig. 4 shows the chromatograms for some metabolites co-occurring in the same maize-*fufu* sample in this study.

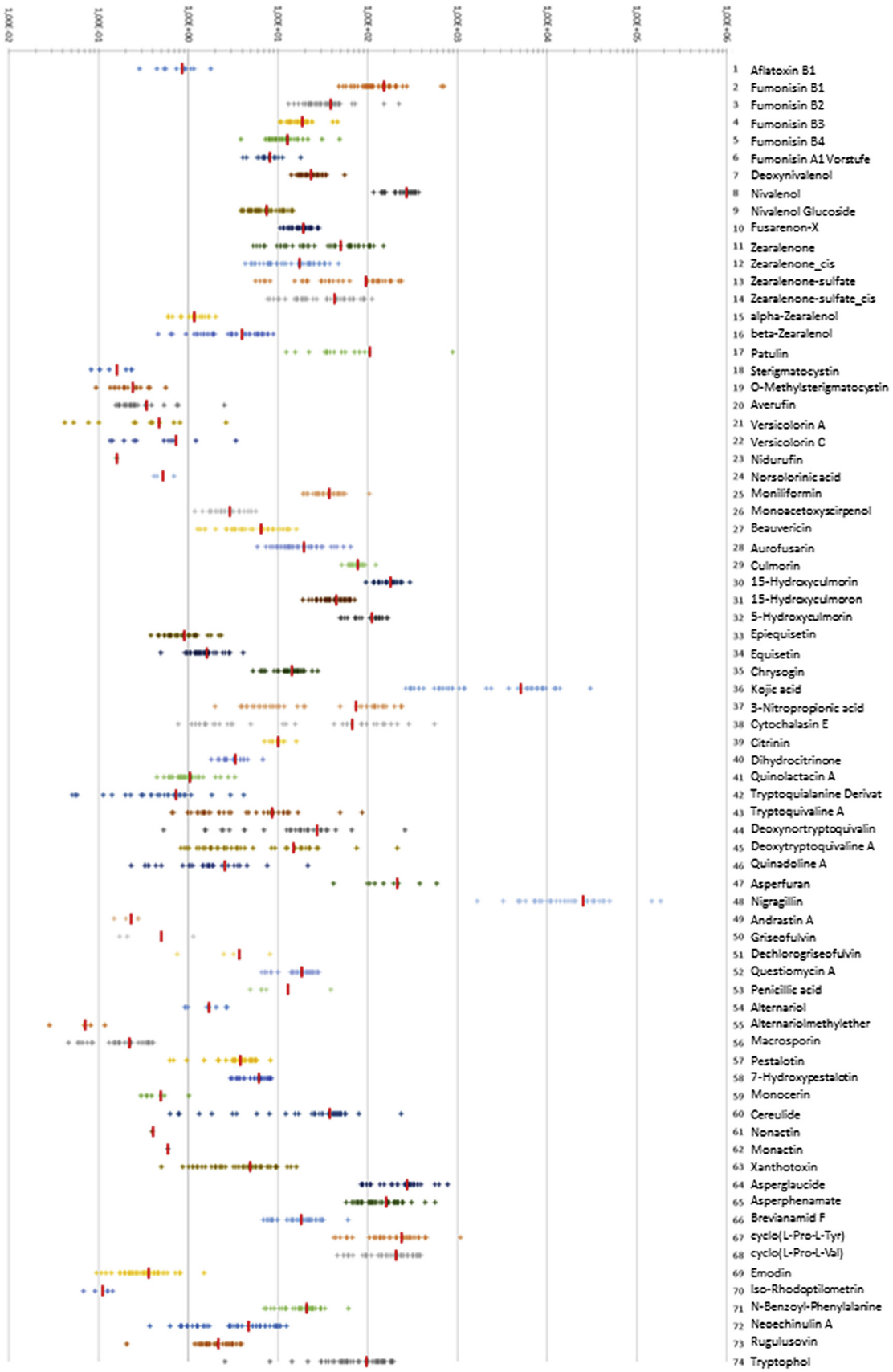

Fig. 3. Metabolites (N = 74) of microbial and plant origin detected in traditional maize porridge (*fufu*) from Bamunka, Cameroon.

Table 2
Occurrence frequencies and contamination levels of major mycotoxins and exotoxin* in maize-*fufu* samples from Bamunka, Cameroon.

Toxin type (abbreviation)	Positive Samples (%)	Mean ± SD (μg kg^{-1})	Median (Range) (μg kg^{-1})
Aflatoxin B_1 (AFB_1)	12 (24)	0.9 ± 0.4	0.9 (0.3–1.8)
Fumonisin B_1 (FB_1)	50 (100)	151 ± 122	112 (48–709)
Fumonisin B_2 (FB_2)	47 (94)	39 ± 35	31 (13–223)
Fumonisin B_3 (FB_3)	42 (84)	19 ± 7	18 (10–46)
Fumonisin B_4 (FB_4)	39 (78)	13 ± 8	11 (4–49)
Fumonisin A_1 (FA_1)	16 (32)	8 ± 3	7 (4–18)
Deoxynivalenol (DON)	50 (100)	23 ± 7	21 (14–55)
Nivalenol (NIV)	50 (100)	268 ± 70	295 (116–372)
Nivalenol-glucoside (NIV-Glc)	50 (100)	8 ± 3	7 (4–15)
Fusarenon-X (FUS-X)	49 (98)	19 ± 5	19 (11–29)
Zearalenone (ZEN)	50 (100)	49 ± 38	43 (5–150)
Zearalenone-cis (ZEN-cis)	50 (100)	17 ± 10[a]	18 (4–47)[a]
Zearalenone-sulfate (ZEN-S)	50 (100)	95 ± 75	100 (6–237)
Zearalenone-sulfate-cis (ZEN-S-cis)	50 (100)	43 ± 29[a]	37 (8–112)[a]
alpha-Zearalenol (α-ZEL)	20 (40)	1.2 ± 0.4	1.2 (0.6–2.0)
beta-Zearalenol (β-ZEL)	45 (90)	3.9 ± 2.0	3.5 (0.5–8.9)
Patulin (PAT)	15 (30)	105 ± 219	42 (12–890)
Alternariol (AOH)	8 (16)	1.7 ± 0.7	1.7 (0.9–2.7)
Cereulide (CER)*	50 (100)	37 ± 34	41 (1–236)

[a] Quantified based on the response factor of ZEN and ZEN-S, respectively.

4. Discussion

A number of recent reports covering sub-Saharan Africa including Cameroon have posited that food safety from a mycotoxin perspective is a major issue requiring priority attention (Abia et al., 2013a; Shephard et al., 2013; Warth et al., 2012; Ediage et al., 2014; Ezekiel et al., 2012; Geary et al., 2016; Abia et al., 2013b; Ezekiel et al., 2014; IARC, 2015; Kimanya, 2015). The actual food safety scenario may be even more complex when a comprehensive picture of multi-contaminant profiles including toxic bacterial metabolites (e.g. the emetic cereulide of *B. cereus*) is considered (Anonymous, 2009, 2015). In the present study, an array of regulated mycotoxins, a potent exotoxin (cereulide) and several non-regulated potentially toxic fungal metabolites were quantified in locally prepared and consumed maize-*fufu* in Bamunka, Cameroon. The metabolite profiles of the maize-*fufu* samples basically reflect lapses in the adoption of good agricultural and food processing (handling and storage) practices at the household level. These practices are meant to limit fungal and bacterial contamination of food and contribute to safer food production as well as safeguarding consumer health.

The detection of many of the mycotoxins (and derivatives) in this maize dish was not surprising considering previous reports on maize and maize-based products such as maize-beer, from the same study community in Cameroon (Abia et al., 2013a). The level of AFB_1 (range0.3–1.8 μg kg^{-1}) found in the maize-*fufu* samples is similar to the AFB_1 level in traditionally fermented and cooked opaque maize beer (mean: 1.8 μg kg^{-1}; range: 0.7–3.0 μg kg^{-1}) (Abia et al., 2013a) but lower than the levels reported in unprocessed maize grains in 2009 (mean: 35 μg kg^{-1}; range: 6–345 μg kg^{-1}) and 2010/2011 (mean: 81 μg kg^{-1}; range: 6–645 μg kg^{-1}) previously sampled from Cameroon (Ediage et al., 2014). Factors such as processing, variation in study location, sampling time, seasonal and annual variations and climate may be associated with the relatively low levels of AFB_1 observed in this current study. The presence of AFB_1 rather than G-type aflatoxins in the maize-*fufu* samples corresponds with previous reports of only B-type aflatoxins in maize from Cameroon (Abia et al., 2013a; Ediage et al., 2014); this can be best attributed to the lack of diversity of aflatoxigenic *Aspergillus* species in this environment. Probst et al. (2014) reported high dominance of *A. flavus* L strain (90.9%) responsible for only B-aflatoxin production and very insignificant (2.2%) presence of *A. parasiticus* (producer of Band G-type aflatoxins) compared to some parts of West Africa where B and G-type aflatoxin producers other than *A. parasiticus* occur in higher numbers (Diedhiou et al., 2011). Notably, the detected level of $AFB_1$was below the maximum tolerable limits (MTL) of 2 μg kg^{-1} specified for ready-to-eat maize in the European Union (EC, 2006) in all samples analyzed.

The upper level of FB_1 found in the maize-*fufu* samples is similar to that previously reported in maize-beer (max: 741 μg kg^{-1}, (Abia et al., 2013a)). The mean levels of FB_1, FB_2, and FB_3 in this study were at least three times lower than the respective levels previously reported in Cameroonian maize beer and maize flour (Abia et al., 2013a) and 20 times lower than levels in maize grains from two sampling years also in Cameroon (Ediage et al., 2014). We report for the first time, FB_4 and FA_1 in maize or maize products from Cameroon, whereby FA_1 is reported for the first time in food from sub-Saharan Africa. The sum of FB_1, FB_2 and FB_3 levels in all samples were lower than the MTL of 1000 μg kg^{-1} in maize-based products for direct human consumption (EC, 2006). The observed variations in maize-*fufu*, relative to the previously studied maize-beer, may in part reflect variations described above for AFB1, but may also be due to the hand sorting and discarding of visibly spoiled grains prior to milling for maize-*fufu* preparation. There are also known to be significant inter-village and annual variations in levels of fumonisins in rural settings (Nikiema et al., 2008; Nikièma et al., 2004). There may also be some decrease caused by the multi-step cooking procedure, and include loss into the water.

Besides aflatoxins and fumonisins, thetrichothecenes DON and NIV (plus NIV-Glc) and the ZEN family (parent ZEN and five derivatives) were found in the maize-*fufu* samples. An unusual DON:NIV concentration ratio of approximately 1:10 was observed, with both toxins contaminating all the food samples. Turner, (2010) previously reviewed that the frequency and levels of DON in food are usually higher relative to NIV; a fact observed in reverse order in this study. The presence of each of DON, NIV, ZEN in all and culmorin in 50% of the maize-*fufu* samples strongly suggest contamination of maize by *Fusarium culmorum*in addition to *F. graminearum*. About 18% of the maize-*fufu* samples contained ZEN at levels exceeding the MTL of 100 μg kg^{-1} specified for maize intended for direct human consumption (EC, 2006). The ZEN derivatives had relatively high occurrence frequencies in the samples; though their concentrations varied. ZEN-S, α- and β-zearalenol

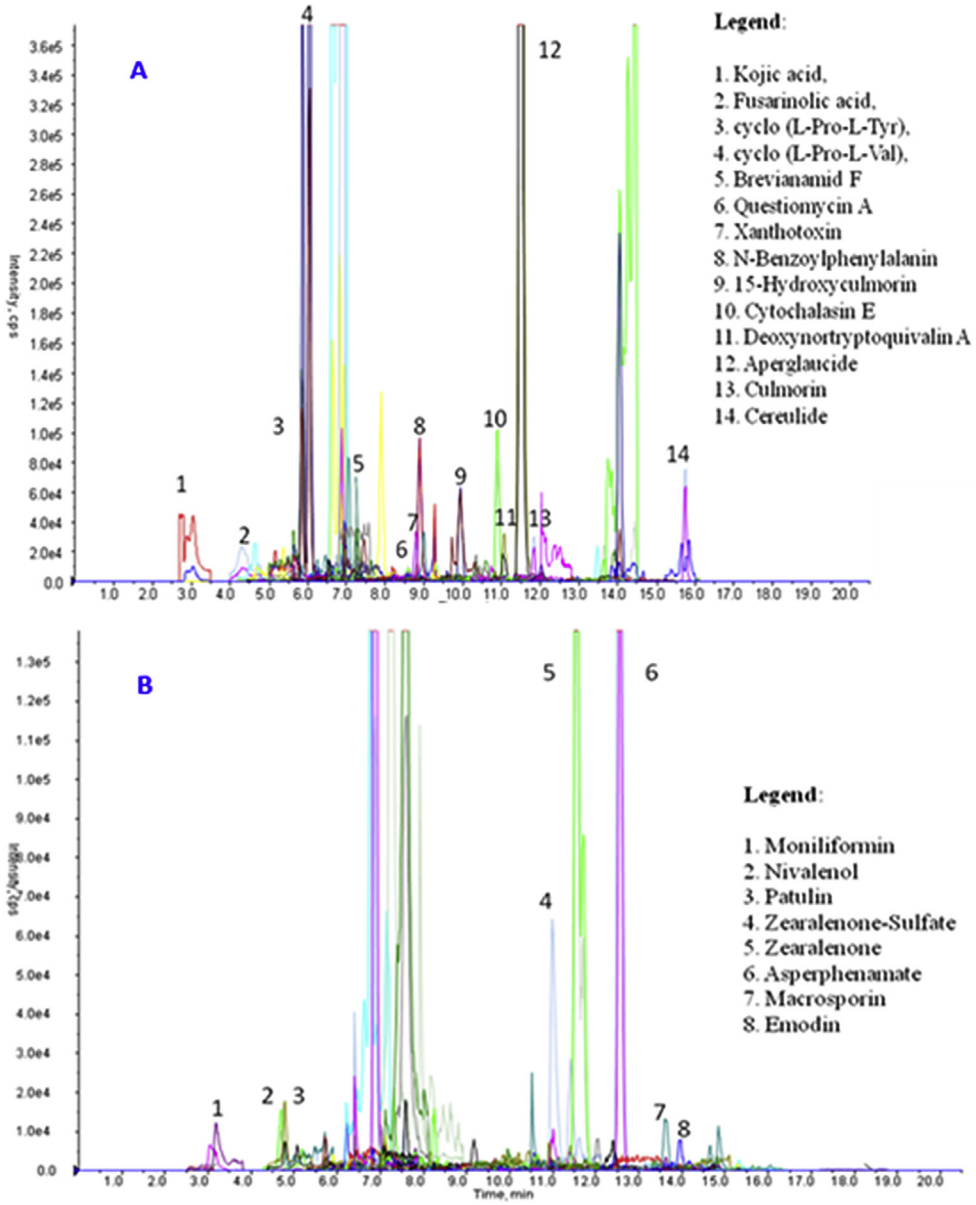

Fig. 4. Chromatograms for some metabolites co-occurring in a same maize-*fufu* sample (positive [A], and negative [B] electrospray ionization modes) and chromatograms for selected metabolites.

have been previously reported in opaque maize beer from the same community (Abia et al., 2013a).

PAT, a common contaminant of fruits, whose regulations are limited to fruits only (EC, 2006; FAO, 2004), is reported for the first time in maize-based samples from sub-Saharan Africa. Its source in these samples is unclear, but could possibly reflect *Aspergillus clavatus* infestation of damaged maize, as both cytochalasin E and tryptoquivalines were also detected. The presence of PAT in maize-based product, maize-*fufu* corroborates with the finding of Mansfield et al. (2008), who revealed presence of PAT in fresh and ensiled maize from Pennsylvania, USA. The frequency, mean concentration and range of PAT (30%, 105 μg kg^{-1}, max: 890 μg kg^{-1}) in this current study was similar to that reported by Mansfield et al. (2008), (23%, 80 μg kg^{-1}; max: 1210 μg kg^{-1}), although the fungal species differ. In fruits PAT levels have been reported to increase during storage (20 °C) even for short periods of time (Morales et al., 2007), though no data is available for maize storage. There are no guidelines on acceptable levels of PAT in cereals for human consumption, though given that the mean level of PAT detected in maize-*fufu* exceeded regulatory limits in fruit juices (50 μg L^{-1} (EC, 2006)), its presence in these food samples presents a potential concern.

In this study, an additional 57 metabolites of fungal and bacterial origin were quantified. Among these, the presence of the bacterial toxin, cereulide, is striking mainly due to its toxicological relevance in food safety and widespread occurrence in the samples. Its presence is indicative of certain strains of endospore forming *B. cereus* in themaize or its flour. Cereulide a heat stable and proteolytic stable toxin has been previously reported to contaminate an array of food, often starchy e.g. rice, pasta, vegetables, but can include egg, meat and milk (Ceuppens et al., 2013).This is the first reported occurrence of cereulidein food from Cameroon. Cereulide

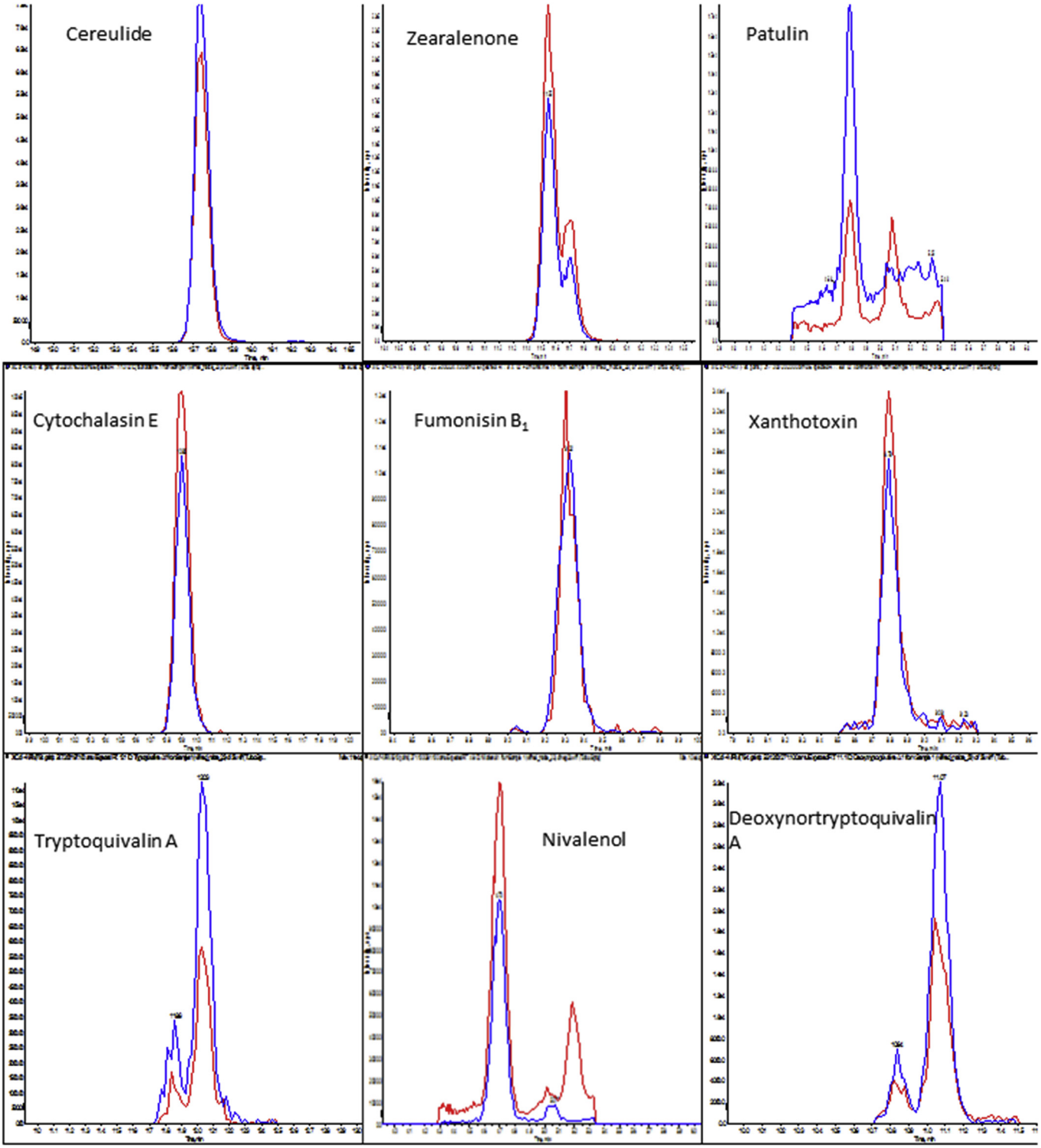

Fig. 4. (*continued*).

causes emetic food poisoning globally though the incidence of adverse events is thought to be underreported and often not confirmed with biological tests. In one family incident a mother and two infants were affected with symptoms related to contaminated rice. Notably in the more severely affected infants, one of whom died from acute toxicity, cereulide in low μg L^{-1} concentrations was detected in biofluids (serum, urine) but was below the LOD in the mothers samples (Shiota et al., 2010). The precise concentrations for adverse health effects remain poorly defined and may depend on many host factors. The concentration of cereulide in our study (mean 37 μg kg^{-1}; range 1–236 μg kg^{-1}) overlaps with the amounts in 13 of 14 commercially purchased Japanese food samples, where those foods were implicated in vomiting-type food poisoning cases ranged from 10 to 1280 μg kg^{-1} (Agata et al., 2002).

Thus our data raise a concern, especially for the young. The most likely source is grain/flour contact with soil which is very common with harvesting, handling/processing and storage practices in the rural setting in Africa.

The cereulide challenge to food safety appears similar to those of mycotoxins. It is heat stable, similar to aflatoxin, and additionally is acid resistant, so undergoes limited reduction during food processing, or once in the stomach (Rajkovic et al., 2014). In sub-emetic concentrations, cereulide has been shown to impair the cell layer of differentiated Caco-2 (Rajkovic et al., 2014), raising the question on potential consequences for the toxicity of co-occurring mycotoxins. Given the postulate role of cereulide in gastrointestinal tract (gut) toxicity, and on the basis of animal studies (Grenier and Applegate, 2013; Enongene et al., 2000, 2002), there may be particular concerns when co-exposure to toxins such as AFB_1 and FB_1occur.

The potential impact on health from these complex and varying "cocktails" of mycotoxins and metabolites, for which scarce or no toxicological data exist, remains an issue. Risk assessment typically relies on toxicological characterization of individual toxins; thus while most of the regulated toxins were observed at levels just below "safe levels", understanding of multiple types of complex mixtures requires much work (Alassane-Kpembi et al., 2016). Considering the spectrum of fungal metabolites detected in these maize-*fufu* samples, studies of mixtures may need to include those beyond the established "main culprits" such as AFB_1, FB_1 and DON. For example, aurofusarin, found in all maize-*fufu*, has recently been shown to possess cytotoxic properties (Vejdovszky et al., 2016). Recent studies indicated that also so called "emerging mycotoxins" such as beauvericin, moniliformin or the enniatins perhaps also should not be neglected in risk assessment studies (Gruber-Dorninger et al., 2016).

On the bases of the mean levels of regulated mycotoxins (AFB1, total FB, DON and ZEN), the average weight of fufu intake per day (adult males: 1480 g; adult females: 1270 g) and the average body weights of the studied adult populations (males: 74.15 Kg; females: 64.45 Kg), the calculated average daily exposures to these mycotoxins were lower than 50% MTL for each respective mycotoxin. Also there was no significant difference across sex. Notwithstanding, this paper for the first time in Cameroon has provided data on co-occurrence of several EU legislated mycotoxins (including AFs, FBs, DON, and ZEN), other fungal secondary metabolites and plant toxins (e.g. cereulide). Considering the wide consumption and popularity of maize-*fufu* in the North West region of Cameroon and beyond, caution is suggested at not dismissing reports of modest levels of microbial toxins, where complex mixture are involved, especially where populations may be burdened with other exposures or limited nutritional diversity.

5. Conclusion

The LC-MS/MS based multi-analyte method has demonstrated a wide range of microbial toxins of an important dietary staple, maize-*fufu*. Among the regulated mycotoxins found in the samples, only ZEN levels exceeded the MTL, suggesting that from a legislated mycotoxins perspective the relative safe nature. However, the co-occurrence of dozens of toxins, including PAT (for which no legal limit in cereal-based foods exists) and the bacterial toxin (cereulide) in all maize-*fufu* samples raises some concern, especially as gut toxicity is a potential mechanism of stunting for some of the established mycotoxins e.g. AFB_1 and FB_1 (Rajkovic et al., 2014). This study was limited to a small sample size and only measured one food item from the community, but despite its size reports novel data on large spectrum of toxic microbial metabolites within a dietary staple in this region. It may be useful for epidemiological studies that are currently being established to restrict aflatoxin exposure in infants, to consider other confounders where their concentration and toxicological profile merit such attention. An important aspect is that such studies attempt to cryo-preserve biological specimens for later analysis as our understanding of the risk of these complex mixtures improves.

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Authors contributions

BW conceived the original idea. BW, CNE, PCT, MS, RK, WAA designed the experiments.MS, BS, WAA, CNE performed the experiments. WAA, CNE analyzed the data.WAA, CNE, WB, MS, KR, PCT, BS, DM wrote and reviewed the paper.

Conflict of interest

Authors do not have any potential conflicting interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fct.2017.06.011.

Transparency document

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