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Fate of mycotoxins in two popular traditional cereal-based beverages (*kunu-zaki* and *pito*) from rural Nigeria

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Beauvericin (PubChem CID: 105014)
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Enniatin A (PubChem CID: 57369704)
Enniatin B (PubChem CID: 164754)
Fumonisin B1 (PubChem CID: 3431)
Fumonisin B2 (PubChem CID: 2733489)
Fumonisin B3 (PubChem CID: 3034751)
Fusaproliferin (PubChem CID: 6506535)
Moniliformin (PubChem CID: 40452)
Zearalenone (PubChem CID: 5281576)

ABSTRACT

Mycotoxins frequently contaminate cereals and their fermented products, however, the carry–over profiles and concentrations of mycotoxins in the final product depend on the processing techniques employed. Therefore, the fate of mycotoxins in two popularly consumed Nigerian cereal-based beverages, *kunu-zaki* and *pito*, were studied using the raw cereals, their malted forms and the final drinks. Liquid chromatography tandem mass spectrometric analysis revealed 24 and 33 fungal metabolites in *kunu-zaki* and *pito* respectively, and their cereal inputs. Among the metabolites were 12 mycotoxins (alternariol, alternariolmethylether, beauvericin, deoxynivalenol, enniatin A and B, fumonisin B_1 (FB_1), FB_2 and FB_3, fusaproliferin, moniliformin and zearalenone). Mycotoxin levels were higher in the maize-based *kunu-zaki* (<LOQ–123 μg/kg) and its cereal ingredients (0.1–31,200 μg/kg) than in the sorghum-based *pito* (<LOQ–5 μg/kg) and its cereal base (1.2–85 μg/kg) respectively. Processing drastically reduced concentrations of all mycotoxins in *kunu-zaki* (range: 76.2–99.9%) and *pito* (59.3–94.8%). Both drinks therefore represent a safe food in the local setting.

1. Introduction

Traditional cereal-based fermented drinks especially those derived from maize and sorghum are among the commonest and widely consumed traditional foods in sub-Saharan Africa and other (sub)-tropical regions (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003). In Nigeria, traditional beverages are widely consumed and mostly preferred to commercial soft drinks by those from low income settings due to lower cost of production and assumed higher nutritional content of the fermented beverages. The beverages are usually produced through a series of processes involving cleaning, washing, steeping and wet milling of grains, sieving of

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slurry, mixing of filtrate with slurry from ground malted grains, cooking of mixture, cooling, addition of sweeteners (e.g. sugar and spices) to obtain the tasty liquor (Adejuyitan, Adelakun, Olaniyan, & Popoola, 2008; Makinde & Oyeleke, 2012). Steeping initiates fermentation which is further promoted by mixing the filtrate with ground malted grains and depending on the extent of fermentation, the drinks could either be classified as non alcoholic or alcoholic beverages (Blandino et al., 2003). The non alcoholics are clear and sweet while the traditional beers are often sour, do not contain any hops and are consumed unrefined (Haggblade & Holzapfel, 1993).

Kunu-zaki drinks which are usually produced by single or mixed grains of maize, millet and sorghum (Adeleke, Olaitan, & Olubile, 2004; Gaffa & Ayo, 2002) have been reported to purge the bowels and relieve flatulent conditions (Omakwu, 1980). *Kunu-zaki* is non alcoholic; hence, the health benefits of consuming this fermented beverage, its cheap preparation and accessibility in local markets and stores underscore its importance among the rural communities. *Pito* is a traditional alcoholic beverage usually produced by the single fermentation of sorghum grains. The single fermentation of sorghum grains makes this beverage a low alcoholic drink (Iwuoha & Eke, 1996; Uzogara, Agu, & Uzogara, 1990).

Mycotoxins, toxic fungal secondary metabolites, are known to contaminate diverse agricultural commodities including cereals (e.g. maize) and other grains (e.g. sorghum) and their products (Abia et al., 2013; Adetunji et al., 2014; Warth et al., 2012). When ingested through contaminated foodstuffs, they cause a range of harmful effects including diverse types of cancers, immune system suppression and target organ toxicities in humans and animals, and effects could be acute or chronic (Bondy & Pestka, 2000; CAST, 2003). Several mycotoxins including aflatoxins, deoxynivalenol (DON), fumonisins, ochratoxins and zearalenone (ZEN) have previously been reported in beers from Africa and Europe (Abia et al., 2013; Bertuzzi, Rastelli, Mulazzi, Donadini, & Pietri, 2011; Matumba, Monjerezi, Khonga, & Lakudzala, 2011; Matumba et al., 2014; Odhav & Naicker, 2002). In spite of rich literature on mycotoxins in cereals and beers (traditional and refined), little or no information is available with respect to mycotoxin presence in the two beverages (*kunu-zaki* and *pito*) let alone reports on the increase or loss of mycotoxins during processing of the drinks. Considering the large population of rural residents who consume one or both of these drinks and the health risks posed by mycotoxins to man, this study aimed at investigating the extent to which processing would reduce mycotoxin levels in the aforementioned traditional fermented beverages.

2. Materials and methods

2.1. Samples

Samples of raw and malted cereals (maize and sorghum grains) and beverages produced from the grains were collected from medium-scale local producers of *kunu-zaki* and *pito* in a rural community of Nasarawa State, Nigeria. Three categories of samples were collected per drink and they included: (1) *kunu-zaki* – raw maize grains, malted maize grains and fermented drink; (2) *pito* – raw sorghum grains, malted sorghum grains and fermented drink. The malted maize grains were visibly bad/dirty grains showing discolorations, cracks and holes from insect infestation, while all other maize and sorghum grains were physically clean. All samples were collected at the points of beverage production during September 2012. Information on the processing techniques of each drink was obtained from the producers by interview. About 100 g of grains per sample was collected in clean Ziploc® bags while 50 mL of the beverage was collected in Falcon tubes (Sarstedt, Nümbrecht, Germany). Each 100 g sample of grains was quartered to give four 25 g subsamples. A representative 25 g subsample was then taken, ground to fine powder in a high-speed blender (Waring Commercial Blender 8010BU, Model HGBTWT, Connecticut, USA), and packaged in clean Ziploc® bags. All beverage and representative grain samples were immediately frozen at −20 °C in Nigeria and sent on dry ice to IFA-Tulln, Austria for multi-mycotoxin analysis.

2.2. Chemicals and reagents

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) was obtained from Sigma–Aldrich (Vienna, Austria). Water was purified successively to 18.2 MΩ by reverse osmosis and an Elga Purelab ultra analytic system from Veolia Water (Bucks, UK). Standards of fungal metabolites (including mycotoxins addressed by regulatory limits) were obtained from commercial sources and a few additional metabolites were obtained as gifts from other research groups (Malachova, Sulyok, Beltrán, Berthiller, & Krska, 2014). No quantitative standard was available for averufin (AVER), averufanin (AVFN), versicolorins A (VER-A) and C (VER-C); hence, they were semi-quantified using the response factor of averantin. Absolute quantification of Andrastin A (AND-A) and siccanol (SICC) was not feasible due to lack of a quantitative standard. Relative quantification based on peak area was done for comparison between the samples.

2.3. Multi-mycotoxin analysis of samples

All grain and beverage samples were analyzed for the presence of 295 microbial metabolites (Malachova et al., 2014) including 17 commonly investigated mycotoxins: aflatoxin B_1 (AFB_1), AFB_2, AFG_1, AFG_2, alternariolmethylether (AME), alternariol (AOH), beauvericin (BEAU), DON, enniatins (ENNs), fumonisin B_1 (FB_1), FB_2, FB_3, fusaproliferin (FP), moniliformin (MON), nivalenol (NIV), ochratoxin A (OTA) and ZEN. Multi-microbial metabolite analysis was carried out using liquid chromatography equipment coupled to a tandem mass spectrometric instrument (LC-MS/MS).

Mycotoxins were extracted from grains and beverages using acetonitrile/water/acetic acid (79:20:1, v/v/v) as extraction solvent. For the grains, 5 g of each ground sample was extracted with 20 mL of solvent in a 50 mL polypropylene tube (Sarstedt, Nümbrecht, Germany) for 90 min using a GFL 3017 rotary shaker (GFL 3017, Burgwedel, Germany). On the other hand, mycotoxins were extracted from 2.5 mL of the beverage samples in 15 mL polypropylene tubes containing 7.5 mL of extraction solvent and centrifuged at 10,000 rpm for 3 min at ambient temperature. Centrifugation was not necessary for grain samples due to sufficient sedimentation by gravity. Extracts were diluted with acetonitrile/water/acetic acid (20:79:1, v/v/v) solvent and injected into the LC system as described in detail by Sulyok, Berthiller, Krska, and Schuhmacher (2006). LC-MS/MS screening of target fungal metabolites was performed using a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA) equipped with TurboIonSpray electrospray ionization 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). Confirmation of positive analyte identification was obtained by the acquisition of two time-scheduled multiple reaction monitoring (MRMs) which yielded 4.0 identification points according to the European Commission decision 2002/657. 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% rel., respectively.

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