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Multiple mycotoxin co-occurrence in maize grown in three agro-ecological zones of Tanzania

Analice Kamala [a, b, 1], Johana Ortiz [a, c, *, 1], Martin Kimanya [d], Geert Haesaert [e], Silvana Donoso [c], Bendantuguka Tiisekwa [f], Bruno De Meulenaer [a]

[a] nutriFOODchem Unit, Department of Food Safety and Food Quality, partner in Food2Know, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium
[b] Tanzania Food and Drugs Authority, P. O. Box 77150, Dar es Salaam, Tanzania
[c] Faculty of Chemical Sciences, Cuenca University, Av. 12 de Abril s/n Cdla. Universitaria, 010201 Cuenca, Ecuador
[d] School of Life Sciences and Bio-Engineering, The Nelson Mandela African Institute of Science and Technology (NM-AIST), P. O. Box 447, Arusha, Tanzania
[e] Department of Applied Biosciences, Faculty of Bioscience Engineering, Ghent University, ValentinVaerwyckweg 1, BE-9000 Ghent, Belgium
[f] Faculty of Agriculture, Sokoine University of Agriculture, P.O. Box 3005, Morogoro, Tanzania

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Aflatoxin G_1 (PubChem CID: 14421)
Aflatoxin G_2 (PubChem CID: 2724362)
Ochratoxin A (PubChem CID: 442530)
Deoxynivalenol (PubChem CID: 442408)
Fumonisin B_1 (PubChem CID: 3431)
Fumonisin B_2 (PubChem CID: 3432)
HT-2 toxin (PubChem CID: 322238)
T-2 toxin (PubChem CID: 5284461)
Zearalenone (PubChem CID: 5281576)

ABSTRACT

In this study, the co-occurrence of multiple mycotoxins in maize kernels collected from 300 households' stores in three agro-ecological zones in Tanzania was evaluated by using ultra high performance liquid chromatography/time-of-flight mass spectrometry (TOFMS) with a QuEChERS-based procedure as sample treatment. This method was validated for the analysis of the main eleven mycotoxins of health concern that can occur in maize: aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), ochratoxin A (OTA), deoxynivalenol (DON), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), HT-2 toxin, T-2 toxin and zearalenone (ZEN). From each zone one major maize producing district for home consumption was chosen and 20 villages for each district were randomly selected for sampling. All mycotoxins of health concern, except for T-2 toxin, were detected in the maize samples. Particularly high levels of AFB_1 (50%; 3–1,081 μg kg^{-1}), FB_1 (73%; 16–18,184 μg kg^{-1}), FB_2 (48%; 178–38,217 μg kg^{-1}) and DON (63%; 68–2,196 μg kg^{-1}) were observed. Some samples exceeded the maximum limits set in Tanzania for aflatoxins or in European regulations for other mycotoxins in unprocessed maize. Eighty seven percent of samples were contaminated with more than one mycotoxin, with 45% of samples co-contaminated by carcinogenic mycotoxins, aflatoxins and fumonisins. Significant differences in contamination pattern were observed among the three agro-ecological zones. The high incidence and at high levels (for some) of these mycotoxins in maize may have serious implications on the health of the consumers since maize constitute the staple food of most Tanzanian population. Effective strategies targeting more than one mycotoxin are encouraged to reduce contamination of maize with mycotoxins.

1. Introduction

Mycotoxins are secondary fungi metabolites that can elicit adverse effects on other organisms (Capriotti et al., 2012). Several mycotoxins are likely to co-occur in foodstuffs under favorable conditions (temperature between 25 and 30 °C and water activity between 0.80 and 0.99) (Bhat, Rai, & Karim, 2010) and emerging evidence suggests that mycotoxins may have synergistic and additive toxicological effects in humans or animals (Berthiller et al.,

* Corresponding author. Faculty of Chemical Sciences, Cuenca University. Av. 12 de Abril s/n Cdla. Universitaria, 010201 Cuenca, Ecuador. Tel.: +593 7 4051000x4124; fax: +593 7 4096526.
E-mail address: johana.ortiz@ucuenca.edu.ec (J. Ortiz).
[1] These authors contributed equally to this manuscript.

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2013; Capriotti et al., 2012). Therefore identification and quantification of multiple mycotoxins is a desire of most food safety control and assurance systems. The evaluation using LC-MS techniques have become essential analytical tools for routine simultaneous analysis of several mycotoxins allowing unambiguous identification and accurate quantification (Senyuva, Gilbert, & Ozturkoglu, 2008; Tanaka, Takino, Sugita-Konishi, & Tanaka, 2006; Zachariasova et al., 2010).

Maize represents the main dietary staple food of the majority of Tanzanians and it is used as main ingredient for complementary foods. Unfortunately, this crop is vulnerable to diverse opportunistic fungi and therefore, maize is potentially vulnerable to mycotoxin contamination (Doko, Rapior, Visconti, & Schjoth, 1995; Yoshizawa, Yamashita, & Chokethaworn, 1996). In Tanzania, the natural occurrence and co-occurrence has been previously described for limited number of mycotoxins, such as fumonisins and zearalenone (Doko et al., 1996), aflatoxins and fumonisins (Kimanya et al., 2008), aflatoxins, deoxynivalenol and fumonisins (Kimanya et al., 2014) by using methods for only single or small group of similar mycotoxins.

The objective of this study was to evaluate the simultaneous contamination, by using a single multi-analyte UHPLC/TOFMS method, of multiple mycotoxins of health concern (AFB_1, AFB_2, AFG_1, AFG_2, OTA, DON, FB_1, FB_2, ZEN, HT-2 and T-2 toxin) potentially present in maize from rural Tanzania. This present work is probably the first comprehensive report on the occurrence of multiple mycotoxins of health concern in Tanzanian maize intended for human consumption.

2. Materials and methods

2.1. Chemicals and reagents

LC-MS grade water, acetonitrile (MeCN), methanol, acetic acid, ammonium acetate; sodium hydroxide and isopropanol were purchased from Fluka (Steinheim, Germany). Analytical grade sodium chloride and anhydrous magnesium sulfate were purchased from Merck KGaA (Darmstadt, Germany). Solid pure standards of AFB_1, AFB_2, AFG_1, AFG_2, OTA, DON, FB_1, FB_2, ZEN, and HT-2 and T-2 toxins were purchased from Sigma–Aldrich (St. Louis, MO, USA). The standards of FB_1 and FB_2 were reconstituted with a mixture of MeCN/water 1:1 v/v, while pure acetonitrile was used for the other standards. Aliquots of standard solutions were dried under a gentle stream of nitrogen and stored at 4 °C, except ZEN, OTA, HT-2 and T-2 that were stored at −20 °C. For MS calibration, a sodium acetate solution was prepared by mixing 0.1% acetic acid and 1% 1 M NaOH in water/isopropanol mixture (1:1).

Individual stock solutions of 1 μg mL^{-1} were prepared reconstituting the dried standards solutions with methanol and a mixture of methanol/water (1:1, v/v) containing 5 mM of ammonium acetate with a pH 8.4 was used for following dilutions. A multi-standard stock solution was freshly prepared by mixing individual standards solutions at different concentration levels considering the maximum permitted limits in unprocessed maize set by the European Commission (EC) N°1881/2006 and N°165/2013 (European-Commission, 2013, 2014).

2.2. Sampling

Maize kernels intended for human consumption were sampled according to (Kimanya et al., 2008) from 300 households of three agro-ecological zones (Hanang' district in the Northern highlands area; Kilosa district in the Eastern lowland area and Rungwe district in South-Western highlands area) representing the major maize growing areas in Tanzania. In each zone, 20 villages were randomly selected and, for each village five samples were collected from different households. The five samples were mixed to obtain a composite sample of at least 1 kg for each village. In total 60 samples were collected for laboratory analysis. The samples were packaged in paper bags, sealed and then transported to the Tanzania Food and Drugs Authority laboratory in Dar es Salaam. The samples were shipped to Belgium and maize kernels were finally ground before analysis.

2.3. Sample treatment

Mycotoxins were extracted using an extraction procedure defined as quick, easy, cheap, effective, rugged and safe (QuEChERS) (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003) which was originally developed for analysis of pesticide residues and is also being utilized widely to extract diverse compounds like mycotoxins from cereals or cereal-based food, allowing high sample throughput (Cunha & Fernandes, 2010; Desmarchelier et al., 2010; Rasmussen, Storm, Rasmussen, Smedsgaard, & Nielsen, 2010; Rubert et al., 2013; Vaclavik, Zachariasova, Hrbek, & Hajslova, 2010; Zachariasova et al., 2010).

A total amount of 1 ± 0.05 g of ground and well homogenized sample was weighted into a 50 mL conic tube and 2 mL of water acidified with acetic acid 0.1% (v/v) were added and mixed with a vortex for 30 s. The complete extraction was achieved with the addition of 2 mL of MeCN acidified with acetic acid 0.1% (v/v). The suspension was mixed for 1 min with a vortex and then was thoroughly mixed using a rotary shaker (Labinco, Breda, The Netherlands) for 2 min. Phase partitioning was achieved with the addition of 0.4 ± 0.01 g of NaCl and 1.6 ± 0.01 g of anhydrous $MgSO_4$ followed by vigorously shaking by hand after each addition. Finally the mixture was centrifuged for 5 min at 4053 g (Sigma 4k15. Buckingham, England). An aliquot of 0.75 mL of the supernatant organic layer was dried under a gentle stream of nitrogen. The dried extract was reconstituted with 0.75 mL of mobile phase A. After mixing with a vortex and with sonication for 5 min, the extract was filtered (0.2 μm filter) and a volume of 20 μL was used for analysis. Samples were further diluted as necessary if their concentration did not fit into the appropriate calibration range for a given analyte.

2.4. Instrumental parameters

UHPLC/TOFMS conditions were slightly modified from (Ortiz, Van Camp, Mestdagh, Donoso, & De Meulenaer, 2013). UHPLC separation was achieved on an UltiMate 3000 RSLC system (Dionex, The Netherlands), consisting of a vacuum degasser, binary pump, cooled autosampler and column oven (37 °C). The system was equipped with a Zorbax Eclipse XDB C_{18} column RRHD (1.8 μm, 2.1 × 100 mm) (Agilent Technologies, Waldbronn, Germany). Mobile phase A consisted of water/methanol/acetic acid 94:5:1 and mobile phase B of methanol/water/acetic acid 97:2:1, both containing 5 mM of ammonium acetate with pH 3.25 (mobile phase A) and pH 5.1 (mobile phase B). A binary gradient was applied with flow rate of 0.2 mL min^{-1}: 0–0.5 min. 30% B, 0.5–13 min linear increase from 30 to 95% B, 13–13.1 min linear increase to 100% B and kept until 13.8 min, followed by re-equilibration of the column for 10 min. The UHPLC was coupled with a splitless interface to a time-of-flight mass spectrometer (micrOTOF II, Bruker Daltonics, Bremen, Germany) with a resolving power of 16,500–18,000 FWHM. It was equipped with an orthogonal electrospray ionization source (ESI) operating in positive mode, using a mass range of 50–1,000 Da for m/z acquisition.

TOFMS settings were in accordance with the procedure described previously by our laboratory (Ortiz et al., 2013), with the inclusion of an additional segment for detection of FB_2 at 13.1–13.8 min. The MS