Food Control 54 (2015) 208–215

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

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

A R T I C L E I N F O

Article history:
Received 4 November 2014
Received in revised form
29 January 2015
Accepted 3 February 2015
Available online 11 February 2015

Keywords:
Mycotoxins
Maize
Tanzania
LC/MS chromatography
QuEChERS

Chemical compounds studied in this article:
Aflatoxin B_1 (PubChem CID: 14403)
Aflatoxin B_2 (PubChem CID: 2724360)
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)

A B S T R A C T

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.

© 2015 Elsevier Ltd. All rights reserved.

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.

http://dx.doi.org/10.1016/j.foodcont.2015.02.002
0956-7135/© 2015 Elsevier Ltd. All rights reserved.

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

settings of this segment were capillary exit voltage was 105 V, skimmer 1 voltage 35 V and hexapole RF 600. At the beginning of every run, the MS was calibrated with a sodium acetate calibrant solution.

2.5. Quantification of the analytes

Matrix-matched calibration curves (MMCC) were used for quantification of the analytes in order to compensate extraction losses and matrix effects. MMCC were constructed by plotting the peak area against the corresponding concentration (μg kg^{-1}) (Desmarchelier et al., 2010). Ground and homogenized testing matrices were spiked before extraction with the multi-standard working solutions at two concentration ranges: low and high level. For MMCC at low level spiking was done at 6 concentration levels corresponding to 0.5-, 0.75-, 1-, 1.25-, 1.5- and 2 times the individual concentration of: 125 μg kg^{-1} for DON; 4 μg kg^{-1} for AFB_1, AFB_2, AFG_1 and AFG_2; 20 μg kg^{-1} for HT-2 toxin, T-2 toxin, ZEN, OTA and FB_1, 40 μg kg^{-1} for FB_2. For MMCC at high level, spiking was done at 8 concentration levels corresponding to 0.125-, 0.25-, 0.5-, 1-, 2-, 4-, 6- and 8 times the individual concentration of: 500 μg kg^{-1} for DON; 375 μg kg^{-1} for aflatoxins; 250 μg kg^{-1} for HT-2 toxin, T-2 toxin and OTA; 312.5 μg kg^{-1} for ZEN, and 2,500 μg kg^{-1} for FB_1 and FB_2. The spiked testing matrices were kept overnight at room temperature and protected from light to allow the equilibration of the multi-standard working solution with the matrix before extraction.

Testing matrices were composed of healthy kernels sorted from different real samples and did not contain traces of contamination (blank samples).

2.6. Method validation

For validation experiments, the testing matrix was taken from the batches of real samples to decrease the matrix variability in the method performance.

Linearity was evaluated by plotting the MMCC (detailed in *Quantification of the analytes*). Similarly, apparent recoveries were determined in triplicate by constructing MMCC at 6 concentration levels (100–400 μg kg^{-1} for DON, FB_1 and FB_2, 2–8 μg kg^{-1} for aflatoxins, 10–40 μg kg^{-1} for HT-2 toxin, T-2 toxin, OTA and ZEN). Recoveries were calculated as **apparent recovery (%)** = $(((\text{area} - b_{MMCC})/a_{MMCC}) \times 100/C_{spiked})$; where **area** is the peak area of the analyte of the MMCC, $\mathbf{b_{MMCC}}$ is the y-intercept of the MMCC, $\mathbf{a_{MMCC}}$ is the slope of the MMCC, and $\mathbf{C_{spiked}}$ is the spiked concentration (μg kg^{-1}) of the analyte to construct the MMCC (Desmarchelier et al., 2010).

Matrix effects were assessed by determining the signal suppression-enhancement (SSE), which was evaluated in duplicate at 6 concentration levels (25–100 μg L^{-1} for DON, FB_1 and FB_2; 0.5–2 μg L^{-1} for aflatoxins, 2.5–10 μg L^{-1} for HT-2 toxin, T-2 toxin, OTA and ZEN). SSE's were calculated as **SSE (%)** = $((a_{CC.blank\ extracts}/a_{CCsolvent}) \times 100)$; where $\mathbf{a_{CC.blank\ extracts}}$ is the slope of the calibration curve of blank extracts spiked just before analysis, and $\mathbf{a_{CCsolvent}}$ is the slope of the calibration curve of standard solution in pure solvent (Sulyok, Berthiller, Krska, & Schuhmacher, 2006).

Limits of detection (LOD) and limits of quantification (LOQ) were determined based on the recovery experiments, but at 8 concentration levels (25–400 μg kg^{-1} for DON, FB_1 and FB_2, 0.5–8 μg kg^{-1} for aflatoxins, 2.5–40 μg kg^{-1} for HT-2 toxin, T-2 toxin, OTA and ZEN). LOD's were calculated using as **LOD** = $(3s_{bl}/a)$; where $\mathbf{s_{bl}}$ is the standard deviation of the intercept and **a** is the slope of the respective MMCC. The limit of quantification was calculated as 2 × LOD (Taverniers, De Loose, & Van Bockstaele, 2004).

Intra-day precision was determined from the 3 replicates of the recovery experiments. Inter-day precision was determined in triplicate from the analysis of testing matrices spiked before extraction at 3 concentration levels (0.5-, 1- and 1.5 times the middle concentration level used for the recovery experiments) for 3 consecutive days.

2.7. Data evaluation

UHPLC/TOFMS data was treated using the DataAnalysis software version 4.0 SP 2. TargetAnalysis™ software (Bruker Daltonics, Bremen, Germany) was used to generate the extracted ion chromatograms (EICs) of the acquired $[M+H]^+$ and $[M+Na]^+$ ions from the total ion chromatograms (TICs). Identification and distinction between true- and false-positive results was based on retention time deviation (retention time window of 0.25 min), mass accuracy (extraction mass window of 15 mDa, mass accuracy of 5 ppm and m/z tolerance of 5 ppm) and SigmaFit™ algorithm (mSigma of 50) (Ortiz et al., 2013). The contamination of the samples was presented as contamination rate together with the standard deviation of a sample proportion (SD_p) which was calculated according to (Uyttendaele et al., 2009). Pooled intra- and inter-day precision were calculated using the analysis of variance approach and expressed as relative standard deviations (% RSD). Comparison tests (ANOVA) and two-sided *t-tests* were performed in Stata 10.0 (Stata Corporation, College Station, Texas, USA).

3. Results & discussion

3.1. Optimization of sample treatment

To fulfill the purpose of multimycotoxin analysis, the conditions of the sample treatment were optimized to ensure an efficient extraction of all target analytes. For this, a comparison between an aqueous MeCN extraction followed by partition (QuEChERS-based approach) (Yogendrarajah, Van Poucke, De Meulenaer, & De Saeger, 2013; Zachariasova et al., 2010) versus solid–liquid extraction was performed. The QuEChERS-based approaches included soaking of the sample prior extraction which allows a better extraction with the organic solvent (Cunha & Fernandes, 2010; Yogendrarajah et al., 2013), and the acidification of the solvents that facilitates the extraction of more polar mycotoxins (e.g. fumonisins) (Desmarchelier et al., 2010; Koesukwiwat, Sanguankaew, & Leepipatpiboon, 2014; Rasmussen et al., 2010; Zachariasova et al., 2010). Three treatments were tested: i) QuEChERS-based approach using water acidified at 0.1% followed by MeCN acidified at 0.1%; ii) QuEChERS-based approach using water followed by MeCN acidified at 0.5%, and iii) solid–liquid extraction using the solvent mixture MeCN/water/acetic acid, 79:20:1 (v/v/v) (Sulyok et al., 2006). The extracts from all treatments were dried under a gentle stream of nitrogen and reconstituted using mobile phase A. This step reduced considerably the TICs background from co-eluting substances. No significant differences were found amongst the three treatments in terms of apparent recovery ($P = 0.716$). However, lower (slope) sensitivity was obtained using solid–liquid extraction in comparison with the QuEChERS approach acidified at 0.1% ($P = 0.039$) and at 0.5% ($P = 0.010$). Moreover, no significant differences between the tested QuEChERS approaches, i.e. acidified at 0.1% and 0.5% in apparent recoveries ($P = 0.488$) and sensitivity ($P = 0.316$) were observed. Since the fact that acidification of the sample prior extractions could improve the recoveries of polar analytes has been previously suggested (Lacina et al., 2012), the QuEChERS-based approach using water and MeCN, both acidified at 0.1% with acetic acid was chosen as final sample treatment. In addition, a step-by-step addition of the salts

followed by vigorous mixing was adopted since a more efficient recovery of the target analytes due to increase of the polarity of the extraction solvents during the partitioning process has been described (Zachariasova et al., 2010).

3.2. UHPLC/TOFMS optimization

The chromatographic separation of the analytes was established within 13.4 min (Fig. 1). This relative long run allowed managing the different MS settings in each of the time segments, which was particularly important for fumonisins. All mycotoxins were best detected in positive mode. Although the addition of 5 mM ammonium acetate to the eluents should suppress the formation of stable sodium adducts (Sulyok et al., 2006), stable alkali ions could be formed due to the presence of traces of alkali ions coming from the sample preparation. In this study, the ions with the highest intensities detected were $[M+Na]^+$ ions for DON, AFB_1, AFB_2, AFG_1 and AFG_2; $[M+H]^+$ for FB_1, OTA, ZEN and FB_2, and $[M+NH_4]^+$ for HT-2 and T-2 toxins. The less abundant ions were also detected and used as qualifiers (Table 1).

3.3. Method performance

Method performance characteristics are presented in Tables 2–4. Good linearity was obtained when analyzing the multi-standard working solutions in pure solvent ($R^2 > 0.99$) and the MMCC ($R^2 > 0.98$). For most mycotoxins, the proposed method yielded to apparent recoveries (Table 2) in agreement with the regulation 2002/657/EC (70–120%) (European-Commission, 2002). This was not the case for OTA at the lowest spiking level. Remarkably, at the lowest and mid-spiking level, ZEN and FB_2 were not detected.

This pitfall might be caused by the fast shifting of MS settings needed for the specific segment of FB_2 detection at the end of the run. The broad capabilities of extraction of the QuEChERS-based procedure lead as well to the presence of undesired matrix components that cause signal suppression, affecting other performance characteristics like repeatability, detection capability and sensitivity to distinguish false negative results (Antignac et al., 2005; Cunha & Fernandes, 2010; Desmarchelier et al., 2010). The signal suppression or enhancement was dependent on the mycotoxin type (Table 3). Polar mycotoxins (such as fumonisins and OTA) were slightly suppressed, which might be related to their acidic nature. No any suppression pattern was observed for aflatoxins, and AFB_1 was one of the strongest suppressed of the studied mycotoxins. DON was also strongly suppressed and this might be related to the early elution of this mycotoxin (Antignac et al., 2005). On the other hand, enhancement of the signal of HT-2 was observed. In this study, suitable matrix-matched calibration curves were constructed and used for quantification, and to compensate extraction losses and matrix effects (Desmarchelier

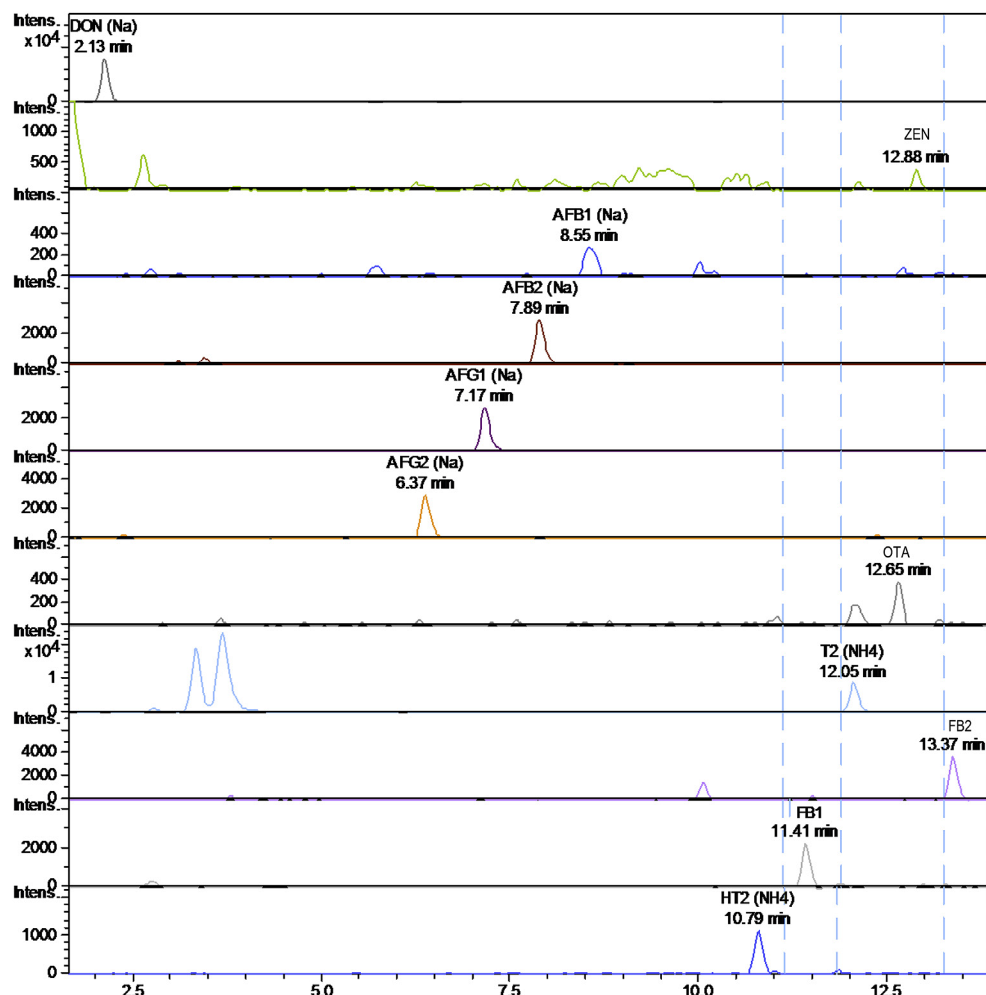

Fig. 1. UHPLC/TOFMS chromatogram for maize kernel spiked at 400 μg kg^{-1} for DON and FB_2; 8 μg kg^{-1} for AFB_1, AFB_2, AFG_1 and AFG_2; and 40 μg kg^{-1} for OTA, FB_1, ZEN, and HT-2 and T-2 toxins.

Table 1
Overview of detected ions, molecular formula, theoretical mass/charge ratio (m/z), retention times (RT) and SigmaFit™ values (mSigma) for the most abundant ion of each mycotoxin.

Mycotoxin	Ion $[M+H]^+$/exact m/z	Ion $[M+Na]^+$/exact m/z	Ion $[M+NH_4]^+$/exact m/z	RT (min)	mSigma
DON	$C_{15}H_{20}O_6$/297.133265	$C_{15}H_{19}O_6Na$[a]/319.115209		2.1	13
AFG_2	$C_{17}H_{14}O_7$/331.081229	$C_{17}H_{13}O_7Na$[a]/353.063174		6.4	8
AFG_1	$C_{17}H_{12}O_7$/329.065579	$C_{17}H_{11}O_7Na$[a]/351.047524		7.1	8
AFB_2	$C_{17}H_{14}O_6$/315.086315	$C_{17}H_{13}O_6Na$[a]/337.068259		7.9	8
AFB_1	$C_{17}H_{12}O_6$/313.070665	$C_{17}H_{11}O_6Na$[a]/335.052609		8.5	6
HT-2	$C_{22}H_{32}O_8$/425.216994		$C_{22}H_{35}O_8N$[a]/442.243544	10.8	11
FB_1	$C_{34}H_{59}NO_{15}$[a]/722.395747	$C_{34}H_{58}NO_{15}Na$/744.377691		11.4	8
T-2	$C_{24}H_{34}O_9$/467.227559		$C_{24}H_{37}O_9N$[a]/484.254108	12.0	9
OTA	$C_{20}H_{18}NO_6Cl$[a]/404.089541	$C_{20}H_{17}NO_6ClNa$/426.071486		12.6	12
ZEN	$C_{18}H_{22}O_5$[a]/319.154000	$C_{18}H_{21}O_5Na$/341.135945		12.8	9
FB_2	$C_{34}H_{59}NO_{14}$[a]/706.406900	$C_{34}H_{58}NO_{14}Na$/728.382777		13.3	8

Notes: DON, deoxynivalenol; AFG_2, aflatoxin G_2; AFG_1, aflatoxin G_1; AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; HT-2, HT-2 toxin; FB_1, fumonisin B_1; T-2, T-2 toxin; OTA, ochratoxin A; ZEN, zearalenone; FB_2, fumonisin B_2.
[a] Most abundant ions.

et al., 2010; Sulyok, Krska, & Schuhmacher, 2007). For most mycotoxins, good intra- and inter-day precision values were achieved (Table 3) considering the maximum percentage of relative standard deviation (% RSD) set in the regulation 2002/657/EC (<20%). This was not the case for AFB_1, ZEN and FB_2. In particular, AFB_1 and ZEN were also strongly affected by matrix effects.

The obtained LOD's and LOQ's are presented in Table 4. With the exception of OTA, the proposed method allowed the quantification of the major mycotoxins of health concern in agreement with the maximum permitted levels of mycotoxins in unprocessed maize outlined in the European regulations (European-Commission, 2013, 2014) and Tanzania Bureau of Standards (TBS, 2004). Those results are comparable to those obtained in other studies in maize that employ QuEChERS-like extraction combined with LC-MS/MS for the multimycotoxin analysis (Desmarchelier et al., 2010; Rasmussen et al., 2010) or UHPLC/TOFMS for the analysis of Fusarium toxins (Zachariasova et al., 2010).

3.4. Mycotoxin co-occurrence in maize

The developed method was applied for the analysis of 60 samples from three agro-ecological zones representing the major maize growing areas in Tanzania. The results reveal that maize grown and consumed in Tanzania is contaminated with multiple mycotoxins at important levels (Table 5). The most frequently occurring mycotoxins were FB_1 (73%), FB_2 (48%), DON (63%) and AFB_1 (50%).

Co-occurrence of FB_1 and FB_2 was found in 43% of all samples. In general, 15% of contaminated samples with fumonisins exceeded the maximum limit set by European regulations (EC/1881/2006). The observed occurrence and levels of contamination with fumonisins were higher than reported in the previous investigations (Kimanya et al., 2008, 2009, 2014). This could be related to geographic and seasonal variations, as well as the low detection limit of the method set in this study for FB_1. Remarkably, in some samples the levels of FB_2 were higher than FB_1 as previously observed in maize hybrids in Argentina (Ramirez et al., 1996). Furthermore, the occurrence of FB_2 alone in some samples was also observed. This unusual finding has also been described in Argentina for some strains of *Fusarium proliferatum* (Sydenham et al., 1993). Studies on factors that influence accumulation of higher levels of FB_2 than FB_1 in maize from Tanzania are needed.

On the other hand, DON occurred in 63% of the samples and 5% was above the maximum limit set by European regulations (EC/1881/2006). The incidence and levels of DON contamination found in this study were higher than previous reported in Tanzania (Kimanya et al., 2014) and other African countries (Abia et al., 2013; Adejumo, Hettwer, & Karlovsky, 2007). The difference could be due to nature of the samples. While samples tested in this study were maize kernels intended for human consumption subject for further processing, the samples analyzed in previous studies were commercially processed ready-to-use maize flour.

In general, 50% of all samples were contaminated with at least one of the studied aflatoxins. Co-occurrence of AFB_1, AFB_2, AFG_1 and AFG_2 was observed in only one of the samples. In Tanzania, the maximum limits for AFB_1 and aflatoxins (total) are the same as European regulations, i.e. 5 and 10 $\mu g\ kg^{-1}$ respectively (TBS, 2004). Contamination levels above those maximum limits were observed in 28% for AFB_1 and 8% for the total amount of aflatoxins. The study verifies a previous report that Tanzanian maize is contaminated with unacceptable levels of aflatoxins (Kimanya et al., 2008). Such high incidence and levels have been also reported in other regions of Africa (Mwihia et al., 2008).

Table 2
Apparent recovery values (%) ± standard deviations determined based on matrix-matched calibration curves in maize kernels spiked at 6 concentration levels.

Spiking level[a]	Apparent recovery (%)										
	DON	AFG_2	AFG_1	AFB_2	AFB_1	HT_2	FB_1	T-2	OTA	ZEN	FB_2
0.5	77 ± 20	90 ± 21	108 ± 18	108 ± 14	95 ± 4	98 ± 3	116 ± 17	99 ± 15	127 ± 18	n.d.	n.d.
0.75	110 ± 21	103 ± 6	98 ± 7	99 ± 5	106 ± 6	92 ± 12	107 ± 14	104 ± 6	91 ± 12	105 ± 3	104 ± 10
1	112 ± 3	96 ± 12	89 ± 11	94 ± 11	93 ± 7	103 ± 5	98 ± 4	98 ± 13	100 ± 17	95 ± 3	97 ± 9
1.25	98 ± 2	103 ± 13	105 ± 3	92 ± 17	103 ± 15	109 ± 6	89 ± 7	97 ± 10	98 ± 9	n.d.	n.d.
1.5	102 ± 3	111 ± 4	108 ± 7	114 ± 2	101 ± 1	104 ± 2	95 ± 6	102 ± 3	92 ± 13	n.d.	100 ± 4
2	98 ± 1	96 ± 4	98 ± 1	99 ± 3	99 ± 4	95 ± 4	106 ± 1	100 ± 3	105 ± 7	100 ± 0.3	100 ± 1

Notes: DON, deoxynivalenol; AFG_2, aflatoxin G_2; AFG_1, aflatoxin G_1; AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; HT-2, HT-2 toxin; FB_1, fumonisin B_1; T-2, T-2 toxin; OTA, ochratoxin A; ZEN, zearalenone; FB_2, fumonisin B_2.
n.d. = Signal not detected.
[a] Fold-times the individual concentration of 200 $\mu g\ kg^{-1}$ for DON, FB_1 and FB_2 4 $\mu g\ kg^{-1}$ for AFB_1, AFB_2, AFG_1 and AFG_2; and 20 $\mu g\ kg^{-1}$ for OTA, HT-2 toxin, T-2 toxin and ZEN.

Table 3
Signal suppression/enhancement (SSE) expressed as percentage (%), intra-day precision and inter-day precision expressed as relative standard deviation (% RSD).

	SSE (%)	Intra-day (% RSD)	Inter-day (% RSD)
DON	20	10	9
AFG_2	65	8	8
AFG_1	75	7	7
AFB_2	87	8	9
AFB_1	28	30	16
HT-2	116	12	25
FB_1	89	13	3
T-2	80	8	6
OTA	80	23	12
ZEN	56	36	31
FB_2	83	31	18

Notes: DON, deoxynivalenol; AFG_2, aflatoxin G_2; AFG_1, aflatoxin G_1; AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; HT-2, HT-2 toxin; FB_1, fumonisin B_1; T-2, T-2 toxin; OTA, ochratoxin A; ZEN, zearalenone; FB_2, fumonisin B_2.

In addition, HT-2 toxin, ZEN and OTA occurred in 25%, 10%, and 3% of all samples, respectively. No contamination with T-2 toxin was observed. This study is the first report of occurrence of HT-2 toxin and OTA in Tanzanian maize. On the other hand, the occurrence of ZEN in maize has been previously described in Tanzania and in some other African countries (Abia et al., 2013; Doko et al., 1996; Shephard et al., 2013). Three (100%) and 2 (66%) samples of contaminated samples with OTA and ZEN respectively exceeded the maximum limit set in the European regulations, while this was not the case for HT-2 toxin. The occurrence of multiple mycotoxins is an indication of the toxigenic capacities of *Fusarium* and *Aspergillus* species contaminating Tanzanian maize besides aflatoxins and fumonisins that are commonly reported to contaminate maize worldwide (Logrieco, Bottalico, Mulé, Moretti, & Perrone, 2003).

Contamination with more than one mycotoxin was observed in 87% (52/60) of the samples. Co-occurrence of the carcinogenic mycotoxins, aflatoxins and fumonisins, was observed in 45% of the samples (Fig. 2), co-occurrence of aflatoxins with OTA in 3% of the samples, and 2% of the samples were co-contaminated with aflatoxins, fumonisins and OTA. The co-occurrence of aflatoxins and fumonisins in Tanzanian maize has been described in previous studies (Kimanya et al., 2008, 2014). Simultaneous contamination with these toxins is particularly alarming since there is evidence that FB_1 synergistically promotes liver tumors initiated by AFB_1 (Gelderblom et al., 2002) and may possibly enhance immunosuppression (Speijers & Speijers, 2004). Similarly, the presence of more than one mycotoxin within the same commodity can increase mycotoxin production as well as potentiate some synergistic interactions in causing toxicity (Grenier & Oswald, 2011).

Table 4
Limits of detection (LOD) and limits of quantification (LOQ) of the analytical method, and maximum contamination levels allowed in unprocessed maize according to Tanzania Bureau of Standards (aflatoxins); European regulations EC/165/2013 (HT-2 and T-2 toxins) and EC/1881/2006 (other mycotoxins), all expressed in $\mu g\ kg^{-1}$.

	Maximum permitted levels	LOD	LOQ
DON	1,750	38	75
AFG_2	–	0.6	1.2
AFG_1	–	0.4	0.7
AFB_2	–	0.6	1.3
AFB_1	5	0.8	1.6
AF_{total}	10	–	–
FB_1	–	4	8
FB_2	–	86	172
FB_{total}	4,000	–	–
HT-2	–	0.6	1
T-2	–	2	4
HT-2 + T-2	200	–	–
OTA	5	6	12
ZEN	350	30	60

Notes: DON, deoxynivalenol; AFG_2, aflatoxin G_2; AFG_1, aflatoxin G_1; AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AF_{total}, sum of $AFG_2 + AFG_1 + AFB_2 + AFB_1$; HT-2, FB_1, fumonisin B_1; FB_2, fumonisin B_2; FB_{total}, sum of $FB_1 + FB_2$; HT-2 toxin; T-2, T-2 toxin; OTA, ochratoxin A; ZEN, zearalenone.

Regarding *Fusarium* toxins, fumonisins co-occurred with DON in 30 samples (50%), DON with HT-2 toxin in 10 samples (17%), fumonisins with HT-2 toxin in 13 samples (22%), while DON and ZEN co-occurred in 2 samples (3%). This contamination pattern was in accordance with opinion of the Joint FAO/WHO Expert Committee on Food Additives which states that those Fusarium mycotoxins are the most abundant in cereals and are widely distributed (JECFA, 2001; Soriano & Dragacci, 2004).

Occurrence of the studied mycotoxins differed significantly among the three Tanzanian agro-ecological zones. The trend was particularly observed for AFB_1, FB_1, FB_2 and HT-2. In general, the agro-ecological zone of Kilosa showed significantly higher occurrence rates for AFB_1 than did Hanang' ($P = 0.001$) and Rungwe ($P < 0.001$). The occurrence of FB_1 and FB_2 in Kilosa was also higher than in Hanang' ($P = 0.013$ and $P < 0.001$, respectively). The occurrence of FB_2 in Rungwe was also significantly higher than in Hanang' ($P < 0.001$). In contrast, most maize kernels samples contaminated with HT-2 were from Rungwe (80%) and the occurrence rate in this district was significantly higher than in Hanang' ($P = 0.001$) and in Kilosa ($P < 0.001$). Based on the contamination levels and occurrence, aflatoxins contamination did not represent a problem in Rungwe, neither in Hanang', while fumonisins contamination was not a problem in Hanang'.

Such variations probably could be contributed to the relatively different climate characteristics. Kilosa experiences two rainy seasons, with early rains from November till January and main rains from March to June, average annual rainfall is 800 mm and temperatures range from 18 °C in the hills to 30 °C in the lowlands. Hanang' has daily average temperature of about 28.5 °C and the area experiences a bimodal rainfall season, with short rains during from September to October and the main rains from December to April. Annual rainfall varies from 700 mm to 900 mm. While Rungwe is characterized by rainfall throughout the year, ranging from an average of 900 to 2,700 mm and cool temperatures ranging from −6 to 25 °C. Fog and mist are also common in Rungwe (Timiza, 2011). These prevailing conditions may have an influence on the fungal species, as well as production, harvesting and storage period. Furthermore, agronomic and postharvest practices probably expose maize to fungal infection and subsequent mycotoxins contamination. The results encourage a study on local applied pre and postharvest practices and fungal species contaminating maize in Tanzania with a view to understand their geographical distribution and potential to produce toxins in food so as to formulate strategies that can target more than one type of mycotoxins.

4. Conclusions

The present study shows the simultaneous co-occurrence of two carcinogenic mycotoxins, aflatoxins and fumonisins, together with the other *Aspergillus* toxin, OTA and *Fusarium* toxins, DON, HT-2 and ZEN in maize intended for human consumption, which is an important indication of the pattern of multiple mycotoxins contamination in Tanzania. Although HT-2, OTA and ZEN were not found in a large proportion of analyzed food samples, its persistent co-occurrence with other significant mycotoxins could raise serious public health concerns as their interactions may be synergistic or additive in causing toxicity in humans. The observed contamination levels and mycotoxin diversity in Tanzanian maize, particularly in the agro-ecological zone of Kilosa, was alarming. Therefore, further studies on fungal species contaminating maize with a view to

Table 5
Contamination rates, standard deviation of the proportion of positive samples (SD_p), frequency of occurrence, means of positive samples, standard deviations (SD) and ranges of multiple mycotoxins in Tanzanian maize, according to location of sample collection (each area n = 20; overall n = 60).

Mycotoxin	Contamination rate, SD_p (%)	Location	Frequency	Mean (μg kg^{-1})	SD	Range (μg kg^{-1})
AFB_1	50%, SD_p 6.5%	Kilosa	18	106	286	3–1,081
		Hanang'	8	4	1	3–5
		Rungwe	4	5	3	2–8
		Overall	30/60	65	225	
AFB_2	7%, SD_p 3.2%	Kilosa	3	93	82	12–177
		Hanang'	0	–	–	–
		Rungwe	1	–	–	3[a]
		Overall	4/60	70	81	
AFG_1	5%, SD_p 2.8	Kilosa	3	15	20	2.7–39
		Hanang'	0	–	–	–
		Rungwe	0	–	–	–
		Overall	3/60	15	20	
AFG_2	2%, SD_p 1.7%	Kilosa	1	–	–	3[a]
		Hanang'	0	–	–	–
		Rungwe	0	–	–	–
		Overall	1/60	3[a]	–	
FB_1	73%, SD_p 5.7%	Kilosa	18	1,535	2,572	44–10,569
		Hanang'	11	131	152	19–444
		Rungwe	15	2,053	4,722	16–18,184
		Overall	44/60	1,361	3,232	
FB_2	48%, SD_p 6.5%	Kilosa	13	1,971	2,142	187–5,902
		Hanang'	2	240	88	178–302
		Rungwe	14	4,187	9,873	322–38,217
		Overall	29/60	2,921	6,997	
DON	63%, SD_p 6%	Kilosa	11	532	714	68–2,196
		Hanang'	13	515	598	79–1,925
		Rungwe	14	433	567	84–1,931
		Overall	38/60	490	607	
HT-2	25%, SD_p 5.6%	Kilosa	1	–	–	23[a]
		Hanang'	2	18	0.2	18–19
		Rungwe	12	19	3	15–25
		Overall	15/60	20	3	
OTA	3%, SD_p 2.3%	Kilosa	1	–	–	73[a]
		Hanang'	1	–	–	16[a]
		Rungwe	0	–	–	–
		Overall	2/60	45	40	
ZEN	10%, SD_p 3.9%	Kilosa	1	–	–	73[a]
		Hanang'	0	–	–	–
		Rungwe	2	1,057	575	651–1,464
		Overall	3/60	729	699	

[a] Unique values.

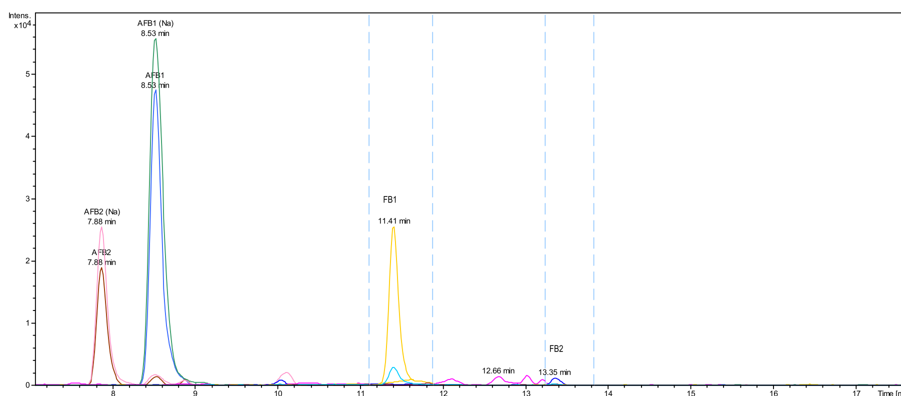

Fig. 2. UHPLC/TOFMS chromatogram for natural contamination of AFB_1 (1018 μg kg^{-1}), AFB_2 (90 μg kg^{-1}), FB_1 (1097 μg kg^{-1}) and FB_2 (282 μg kg^{-1}) in maize kernel.

understand their geographical distribution and local practices for handling maize deserve attention to formulate pre- and post-harvest strategies to tackle this agro-food problem.

Acknowledgments

The authors are grateful to the Flemish Interuniversity Council-Institutional University cooperation (VLIR-UOS) (reference number: ZIUS 2007AP2016) for their financial support. In addition, the authors acknowledge Cuenca University (Ecuador), Ghent University (Belgium) and Tanzania Food and Drugs Authority (Tanzania) for the valuable support.

References

Abia, W. A., Warth, B., Sulyok, M., Krska, R., Tchana, A. N., Njobeh, P. B., et al. (2013). Determination of multi-mycotoxin occurrence in cereals, nuts and their

products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). *Food Control, 31*(2), 438–453.

Adejumo, T. O., Hettwer, U., & Karlovsky, P. (2007). Occurrence of Fusarium species and trichothecenes in Nigerian maize. *International Journal of Food Microbiology, 116*(3), 350–357.

Anastassiades, M., Lehotay, S. J., Stajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International, 86*(2), 412–431.

Antignac, J. P., de Wasch, K., Monteau, F., De Brabander, H., Andre, F., & Le Bizec, B. (2005). The ion suppression phenomenon in liquid chromatography–mass spectrometry and its consequences in the field of residue. *Analytica Chimica Acta, 529*(1–2), 129–136.

Berthiller, F., Crews, C., Dall'Asta, C., De Saeger, S., Haesaert, G., Karlovsky, P., et al. (2013). Masked mycotoxins: a review. *Molecular Nutriton & Food Research, 57*(1), 165–186.

Bhat, R., Rai, R. V., & Karim, A. A. (2010). Mycotoxins in food and feed: present status and future concerns. *Comprehensive Reviews in Food Science and Food Safety, 9*(1), 57–81.

Capriotti, A. L., Caruso, G., Cavaliere, C., Foglia, P., Samperi, R., & Lagana, A. (2012). Multiclass mycotoxin analysis in food, environmental and biological matrices with chromatography/mass spectrometry. *Mass Spectrometry Reviews, 31*(4), 466–503.

Cunha, S., & Fernandes, J. (2010). Development and validation of a method based on a QuEChERS procedure and heart-cutting GC-MS for determination of five mycotoxins in cereal products. *Journal of Separation Science, 33*(4-5), 600–609.

Desmarchelier, A., Oberson, J. M., Tella, P., Gremaud, E., Seefelder, W., & Mottier, P. (2010). Development and comparison of two multiresidue methods for the analysis of 17 mycotoxins in cereals by liquid chromatography electrospray ionization tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 58*(13), 7510–7519.

Doko, M. B., Canet, C., Brown, N., Sydenham, E. W., Mpuchane, S., & Siame, B. A. (1996). Natural co-occurrence of fumonisins and zearalenone in cereals and cereal-based foods from Eastern and Southern Africa. *Journal of Agricultural and Food Chemistry, 44*(10), 3240–3243.

Doko, M. B., Rapior, S., Visconti, A., & Schjoth, J. E. (1995). Incidence and levels of fumonisin contamination in maize genotypes grown in Europe and Africa. *Journal of Agricultural and Food Chemistry, 43*(2), 429–434.

European-Commission. (2002). Commission decision (EC) N° 2002/657. Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of European Commission, L 221*, 8–36 (Brussels).

European-Commission. (2013). Commission Recommendation (EC). No. 165/2013. On the presence of T-2 and HT-2 toxin in cereals and cereal products. *Official Journal of European Union, L 91*, 12–15 (Brussels).

European-Commission. (2014). Commission Regulation (EC). No. 1881/2006. Setting maximum levels for certain contaminants in foodstuffs. Amended by Jan. 2014. *Official Journal of European Union, 010.001*, 1–35 (Brussels).

Gelderblom, W., Marasas, W., Lebepe-Mazur, S., Swanevelder, S., Vessey, C., & De la M Hall, P. (2002). Interaction of fumonisin B1 and aflatoxin B1 in a short-term carcinogenesis model in rat liver. *Toxicology, 171*(2), 161–173.

Grenier, B., & Oswald, I. (2011). Mycotoxin co-contamination of food and feed: meta-analysis of publications describing toxicological interactions. *World Mycotoxin Journal, 4*(3), 285–313.

JECFA. (2001). Safety evaluation of certain mycotoxins in food. In *WHO food additives SERIES 47 (Vol. FAO food and nutrition paper 74)*. Geneva: Bernan Assoc.

Kimanya, M. E., De Meulenaer, B., Tiisekwa, B., Ndomondo-Sigonda, M., Devlieghere, F., Van Camp, J., et al. (2008). Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages of Tanzania. *Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 25*(11), 1353–1364.

Kimanya, M. E., De Meulenaer, B., Tiisekwa, B., Ugullum, C., Devlieghere, F., Van Camp, J., et al. (2009). Fumonisins exposure from freshly harvested and stored maize and its relationship with traditional agronomic practices in Rombo district, Tanzania. *Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 26*(8), 1199–1208.

Kimanya, M. E., Shirima, C., Magoha, H., Shewiyo, D., De Meulenaer, B., Kolsteren, P., et al. (2014). Co-exposures of aflatoxins with deoxynivalenol and fumonisins from maize based complementary foods in Rombo, Northern Tanzania. *Food Control, 41*, 76–81.

Koesukwiwat, U., Sanguankaew, K., & Leepipatpiboon, N. (2014). Evaluation of a modified QuEChERS method for analysis of mycotoxins in rice. *Food Chemistry, 153*, 44–51.

Lacina, O., Zachariasova, M., Urbanova, J., Vaclavikova, M., Cajka, T., & Hajslova, J. (2012). Critical assessment of extraction methods for the simultaneous determination of pesticide residues and mycotoxins in fruits, cereals, spices and oil seeds employing ultra-high performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A, 1262*, 8–18.

Logrieco, A., Bottalico, A., Mulé, G., Moretti, A., & Perrone, G. (2003). Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. *European Journal of Plant Pathology, 109*(7), 645–667.

Mwihia, J., Straetmans, M., Ibrahim, A., Njau, J., Muhenje, O., Guracha, A., et al. (2008). Aflatoxin levels in locally grown maize from Makueni District, Kenya. *East African Medical Journal, 85*(7), 311–317.

Ortiz, J., Van Camp, J., Mestdagh, F., Donoso, S., & De Meulenaer, B. (2013). Mycotoxin co-occurrence in rice, oat flakes and wheat noodles used as staple foods in Ecuador. *Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 30*(12), 2165–2176.

Ramirez, M. L., Pascale, M., Chulze, S., Reynoso, M. M., March, G., & Visconti, A. (1996). Natural occurrence of fumonisins and their correlation to Fusarium contamination in commercial corn hybrids growth in Argentina. *Mycopathologia, 135*(1), 29–34.

Rasmussen, R. R., Storm, I. M. L. D., Rasmussen, P. H., Smedsgaard, J., & Nielsen, K. F. (2010). Multi-mycotoxin analysis of maize silage by LC-MS/MS. *Analytical and Bioanalytical Chemistry, 397*(2), 765–776.

Rubert, J., Fapohunda, S. O., Soler, C., Ezekiel, C. N., Manes, J., & Kayode, F. (2013). A survey of mycotoxins in random street-vended snacks from Lagos, Nigeria, using QuEChERS-HPLC-MS/MS. *Food Control, 32*(2), 673–677.

Senyuva, H. Z., Gilbert, J., & Ozturkoglu, S. (2008). Rapid analysis of fungal cultures and dried figs for secondary metabolites by LC/TOF-MS. *Analytica Chimica Acta, 617*(1–2), 97–106.

Shephard, G. S., Burger, H.-M., Gambacorta, L., Krska, R., Powers, S. P., Rheeder, J. P., et al. (2013). Mycological analysis and multimycotoxins in maize from rural subsistence farmers in the former Transkei, South Africa. *Journal of Agricultural and Food Chemistry, 61*(34), 8232–8240.

Soriano, J. M., & Dragacci, S. (2004). Occurrence of fumonisins in foods. *Food Research International, 37*(10), 985–1000.

Speijers, G. J. A., & Speijers, M. H. M. (2004). Combined toxic effects of mycotoxins. *Toxicology Letters, 153*(1), 91–98.

Sulyok, M., Berthiller, F., Krska, R., & Schuhmacher, R. (2006). Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Communications in Mass Spectrometry: RCM, 20*(18), 2649–2659.

Sulyok, M., Krska, R., & Schuhmacher, R. (2007). Application of a liquid chromatography-tandem mass spectrometric method to multi-mycotoxin determination in raw cereals and evaluation of matrix effects. *Food Additives and Contaminants, 24*(10), 1184–1195.

Sydenham, E. W., Shephard, G. S., Thiel, P. G., Marasas, W. F., Rheeder, J. P., Peralta Sanhueza, C. E., et al. (1993). Fumonisins in Argentinian field-trial corn. *Journal of Agricultural and Food Chemistry, 41*(6), 891–895.

Tanaka, H., Takino, M., Sugita-Konishi, Y., & Tanaka, T. (2006). Development of a liquid chromatography/time-of-flight mass spectrometric method for the simultaneous determination of trichothecenes, zearalenone and aflatoxins in foodstuffs. *Rapid Communications in Mass Spectrometry: RCM, 20*(9), 1422–1428.

Taverniers, I., De Loose, M., & Van Bockstaele, E. (2004). Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. *Trends in Analytical Chemistry, 23*(8), 535–552.

TBS. (2004). *Rice specification*. TZS 592. Dar es Salaam, Tanzania: Tanzania Bureau of Standards.

Timiza, W. (2011). *Climate variability and satellite – observed vegetation responses in Tanzania*. Master thesis Physical Geography and Ecosystem Analysis. Lund University. Seminar series 205.

Uyttendaele, M., Busschaert, P., Valero, A., Geeraerd, A. H., Vermeulen, A., Jacxsens, L., et al. (2009). Prevalence and challenge tests of Listeria monocytogenes in Belgian produced and retailed mayonnaise-based deli-salads, cooked meat products and smoked fish between 2005 and 2007. *International Journal of Food Microbiology, 133*(1–2), 94–104.

Vaclavik, L., Zachariasova, M., Hrbek, V., & Hajslova, J. (2010). Analysis of multiple mycotoxins in cereals under ambient conditions using direct analysis in real time (DART) ionization coupled to high resolution mass spectrometry. *Talanta, 82*(5), 1950–1957.

Yogendrarajah, P., Van Poucke, C., De Meulenaer, B., & De Saeger, S. (2013). Development and validation of a QuEChERS based liquid chromatography tandem mass spectrometry method for the determination of multiple mycotoxins in spices. *Journal of Chromatography A, 1297*, 1–11.

Yoshizawa, T., Yamashita, A., & Chokethaworn, N. (1996). Occurrence of fumonisins and aflatoxins in corn from Thailand. *Food Additives and Contaminants, 13*(2), 163–168.

Zachariasova, M., Lacina, O., Malachova, A., Kostelanska, M., Poustka, J., Godula, M., et al. (2010). Novel approaches in analysis of Fusarium mycotoxins in cereals employing ultra performance liquid chromatography coupled with high resolution mass spectrometry. *Analytica Chimica Acta, 662*(1), 51–61.