Food Additives & Contaminants: Part A
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tfac20

Mycotoxin co-occurrence in rice, oat flakes and wheat noodles used as staple foods in Ecuador

Johana Ortiz[ab], John Van Camp[a], Frédéric Mestdagh[a], Silvana Donoso[b] & Bruno De Meulenaer[a]

[a] NutriFOODChem Unit, Department of Food Safety and Food Quality (partner in Food2Know), Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

[b] Faculty of Chemical Sciences, Cuenca University, Cuenca, Ecuador

Accepted author version posted online: 08 Oct 2013.Published online: 07 Dec 2013.

To cite this article: Johana Ortiz, John Van Camp, Frédéric Mestdagh, Silvana Donoso & Bruno De Meulenaer , Food Additives & Contaminants: Part A (2013): Mycotoxin co-occurrence in rice, oat flakes and wheat noodles used as staple foods in Ecuador, Food Additives & Contaminants: Part A, DOI: 10.1080/19440049.2013.853228

To link to this article: http://dx.doi.org/10.1080/19440049.2013.853228

Food Additives & Contaminants: Part A, 2013
http://dx.doi.org/10.1080/19440049.2013.853228

Taylor & Francis
Taylor & Francis Group

Mycotoxin co-occurrence in rice, oat flakes and wheat noodles used as staple foods in Ecuador

Johana Ortiz[a,b], John Van Camp[a], Frédéric Mestdagh[a], Silvana Donoso[b] and Bruno De Meulenaer[a]*

[a]*NutriFOODChem Unit, Department of Food Safety and Food Quality (partner in Food2Know), Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium;* [b]*Faculty of Chemical Sciences, Cuenca University, Cuenca, Ecuador*

(*Received 9 July 2013; accepted 3 October 2013*)

The co-occurrence of aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), ochratoxin A (OTA), deoxynivalenol (DON), fumonisin B_1 (FB_1), zearalenone (ZEN), and HT-2 and T-2 toxins in the main Ecuadorian staple cereals (rice, oat flakes, and yellow and white wheat noodles) was evaluated. A ultra high performance liquid chromatography/time-of-flight mass spectrometry (UHPLC/TOFMS) method was developed and validated to screen for the presence of these mycotoxins in those cereal matrices. Matrix-matched calibration curves were used to compensate for ion suppression and extraction losses and the recovery values were in agreement with the minimum requirements of Regulation 401/2006/EC (70–110%). For most mycotoxins, the LODs obtained allowed detection in compliance with the maximum permitted levels set in Regulation EC/2006/1881, with the exception of OTA in all cereals and AFB1 in yellow noodles. Extra target analysis of OTA in oat flakes and wheat noodles was performed by HPLC with fluorescence detection. High rates of contamination were observed in paddy rice (23% DON, 23% FB_1, 7% AFB_1, 2% AFG_1 and 2% AFG_2), white wheat noodles (33% DON and 5% OTA) and oat flakes (17% DON, 2% OTA and 2% AFB_1), whereas the rates of contamination were lower in polished rice (2% AFG_1 and 4% HT-2 toxin) and yellow noodles (5% DON). Low rates of co-occurrence of several mycotoxins were observed only for white wheat noodles (5%) and paddy rice (7%). White noodles were contaminated with DON and/or OTA, while combinations of AFG_1, AFB_1, DON and FB_1 were found in paddy rice. Yellow noodles were contaminated with DON only; oat flakes contained DON, OTA or AFB_1, and polished rice was contaminated with AFG_1 and HT-2 toxin.

Keywords: mycotoxins; cereals; chromatography – LC/MS

Introduction

Mycotoxins are toxic secondary metabolites produced by different fungal species, growing under a wide range of climatic conditions on agricultural commodities. Contamination may occur throughout the food chain and is considered as a serious worldwide safety problem for the whole agri-food chain (FAO/IAEA 2001; Shephard 2008; Bhat et al. 2010). Hundreds of mycotoxins have been discovered; however, a limited number are significantly threatening food safety. Based on the adverse implications on human health and agricultural productivity, the most important mycotoxins are aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, zearalenone, HT-2 and T-2 toxins (FAO/IAEA 2001; Shephard 2008; Bhat et al. 2010).

Fungal growth is mainly triggered by high water activities (0.80–0.99) and warm temperatures (25–30°C) and often leads to the co-occurrence of several mycotoxins in foodstuffs that could induce synergistic toxicological effects (FAO/IAEA 2001; Desmarchelier et al. 2010). Nowadays, several multi-mycotoxin methods based on LC-MS techniques are being developed to enable sensitive, reliable and fast identification and quantification of mycotoxins (Shephard et al. 2013). LC/TOFMS is a valuable technique for multi-mycotoxin analysis (Tanaka et al. 2006; Senyuva et al. 2008; Zachariasova et al. 2010) with broad detection capabilities that enables retrospective data treatment of non-target compounds (Ojanpera et al. 2006).

Ecuador, a country located at the northeast of South America, could provide favourable conditions for fungi development and mycotoxin production (Pacin et al. 2002). This unique climate diversity is caused by the presence of the Andes mountain range, the influence of the sea and the location of the Equator. Very limited published data are available about the co-occurrence of mycotoxins in staple foods in South American countries in general and in Ecuador in particular (FAO 2004; Sabino 2011). Low occurrences of OTA, DON, T-2 toxin and aflatoxins in Ecuadorian paddy rice and polished broken rice fractions had been formerly reported (Mühlemann et al. 1997a, 1997b), but those products are not intended for human consumption. The main energetic source of the Ecuadorian diet is cereals based (35%) (FAO 2001; Bermudez & Tucker 2003), and staple cereals such as rice, wheat products and oats are known to be prone to mycotoxin contamination (FAO/IAEA 2001; Shephard 2008; Yazar & Omurtag 2008). Ecuador is the major

*Corresponding author. Email: Bruno.DeMeulenaer@UGent.be

consumer of rice amongst the Andean countries (119.2 kg/capita/year) and the third most representative producer as part of the Nations of the Andean Communities (CAN) (INEC 2011a). On the other hand, wheat is one of the most consumed cereals in Ecuador and the national production is not self-sufficient. About 98% of the wheat consumed is mainly imported from Canada and the Unites States (INEC 2011b; USDA 2013). Similar trends of consumption and production are observed for oats, but it is also imported from Chile (FAO 2001).

This paper is the first report on the occurrence of mycotoxins in Ecuadorian staple foods, i.e. polished rice, oat flakes and wheat noodles (white and yellow). The occurrence of mycotoxins in paddy rice is also reported as an indicator of pre-milling contamination. The major mycotoxins of health concern – AFB_1, AFB_2, AFG_1, AFG_2, OTA, DON, FB_1, ZEN, HT-2 and T-2 toxin – were screened by an optimised ultra high performance liquid chromatography/time-of-flight mass spectrometry (UHPLC/TOFMS) method, combined with a posterior target analysis of OTA in oat flakes and wheat noodles using HPLC-FLD. This mycotoxin evaluation in staple cereals and cereal-based products allowed for the prioritisation of the major mycotoxins present in those commodities to support further risk management options.

Materials and methods

Chemicals and reagents

LC-MS-grade water, acetonitrile, methanol, acetic acid, formic acid, ammonium acetate, sodium hydroxide and isopropanol were supplied by Fluka (Steinheim, Germany). Ochraprep immunoaffinity columns were purchased from R-Biopharm Rhône (Glasgow, UK). Standards, as solid pure extracts, of AFB_1, AFB_2, AFG_1, AFG_2, OTA, DON, FB_1, HT-2 toxin, T-2 toxin, ZEN and zearalenone (ZAN), as well as PBS solution were supplied by Sigma-Aldrich (St. Louis, MO, USA). The standards were reconstituted with acetonitrile, except FB_1 for which acetonitrile/water 1:1, v/v was used. Aliquots of standard solutions were dried under a gentle stream of nitrogen and kept at 4°C, except ZEN, ZAN, HT-2 and T-2 which were kept 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).

Standard solutions

For validation experiments of the multi-mycotoxin method by UHPLC/TOFMS, stock solutions of 1 µg ml^{-1} were prepared by reconstitution of the dried standards with methanol. A mixture of methanol/water (1:1, v/v) containing 5 mM of ammonium acetate with a pH 8.4 was used for further dilutions. A multi-standard stock solution was freshly prepared by mixing suitable amounts of individual standards solutions to cover a concentration level that was roughly the maximum permitted limits in cereals set by Commission Regulation (EC) No. 1881/2006 (European Commission 2006b, 2012). For HT-2 and T-2 toxin, no maximum levels are set and therefore the concentration levels were the same as for other mycotoxins that showed comparable signal intensities at similar concentrations.

For validation experiments of the analysis of OTA by HPLC-FLD, a stock solution of 1 µg ml^{-1} was prepared by reconstitution of the dried standard with acetonitrile. Further dilutions were freshly prepared with a mixture of acetonitrile/water (1:1, v/v).

Sampling and sampling frame

Two sampling plans (bulk and retail) were designed in accordance with Commission Regulation 401/2006/EC (European Commission 2006a) and complemented with relevant information from the Codex Alimentarius (Alimentarius 2008).

Rice samples were collected from the biggest rice mills of the country (called first-category mills) located at the lowlands of the coastal provinces of Guayas and Los Ríos, which are the main rice-producing zones in Ecuador (98.7% of the national production) (INEC 2011a). Both provinces are situated on the Pacific coast and have an average temperature of 25°C. Los Ríos province is located at approximately 6–11 m above sea level and it is subject to tropical monsoons varying from the dry and cool season from June to December to hot and humid conditions from December to June. Guayas province is located at approximately 4–6 m above sea level and its weather ranges from a tropical savannah in the northern to a tropical monsoon in the southern regions. The growth and harvest of the collected rice samples occurred during the rainy season. Incremental samples were collected from different sections of a bulk lot, i.e. 10 times 1 kg, and the gathered aggregate sample of 10 kg was mixed thoroughly. A final amount of 1 kg was taken as a laboratory sample and the remainder was send back to the rice mill (paddy rice) or primary storage place (polished rice). In total, 62 mills in Los Ríos and 61 mills in Guayas were visited and those compiled the rice production of 18 cantons in Guayas and 12 cantons in Los Ríos. A total of 121 samples of paddy rice (60 from Los Ríos and 61 from Guayas) and 125 samples of polished rice (64 from Los Ríos and 61 from Guayas) were gathered from May to July 2010. Both types of samples were collected during the same visit; therefore, those did not belong to the same lot before and after milling. Upon collection, samples of paddy rice were dried using an air oven set at 50°C for 24 h to prevent fungal infestation.

In addition, the supervisor of each mill was surveyed during sampling. The survey comprised questions regarding the use of fertilisers and pesticides in the field, the moisture content of the incoming and polished rice, the

impurities of the incoming rice, storage time before and after the milling process, and management of mould infestation.

Samples of oat flakes, yellow-alkaline and white wheat noodles (Fiocchetti type) were bought at retail in open markets and supermarkets in Cuenca (urban) and Nabon (rural) cantons of Azuay province, in the southern Ecuadorian highlands. The samples were collected in those cantons because this study was part of a larger study on feeding assessment in those areas. Cuenca is located at approximately 2550 m above sea level. Nabon is located at 3000 m above sea level and at 70 km from Cuenca. Incremental samples were either the smallest packages available until completing 1 kg (two to five packages) or enough amounts bought by weight from big sacks. At laboratory level, the content of those packages was thoroughly homogenised. A total of 70 samples of oat flakes, 63 samples of white wheat noodles and 65 samples of yellow wheat noodles were collected during February–March 2010.

Upon collection, samples were kept in dark polyethylene bags at RT and finally ground just before analysis. Half of the samples were randomly selected and shipped to Belgium for multi-mycotoxin analysis.

Sample preparation

Multi-mycotoxin analysis by UHPLC/TOFMS

A total of 2 ml of the solvent mixture acetonitrile/water/acetic acid, 79:20:1 (v/v/v) was added to 0.5 g of homogenised milled sample and the suspension was shaken using a vortex. The mixture was further mixed on a rotary shaker (Labinco, Breda, The Netherlands) for 90 min and then centrifuged for 2 min at 4053*g* (Sigma 4k15, Buckingham, UK). A volume of 750 µl of extract was transferred into microtubes and dried under a gentle stream of nitrogen. The dried extract was redissolved in 1 ml methanol and frozen for 1 h at –24°C. This methanolic extract was subsequently removed and the walls of the microtube were rapidly washed with another 0.5 ml of ice-cold methanol. The combined extract was dried under nitrogen and finally reconstituted in 750 µl of mobile phase A. After vortexing and sonication, the sample was filtered (0.2 µm filter) and a volume of 20 µl was injected.

OTA analysis by HPLC

A volume of 100 ml of the solvent mixture acetonitrile/water, 60:40 (v/v) was added to 25 g of finely milled sample and the suspension was shaken in a horizontal shaker at 300 rpm for 30 min. The mixture was centrifuged for 10 min at 2403*g* (Hettich EBA 20, Tuttlingen, Germany). A volume of 2 ml of extract was diluted with 22 ml of PBS solution. After vigorous shaking, the solution was applied to immunoaffinity clean-up columns (Ochraprep, R-Biopharm Rhône), which were previously brought to RT and conditioned with 3 ml of PBS solution. The diluted extract (24 ml, equivalent to 0.5 g of sample) was passed at a flow rate of 2–3 ml min^{-1}. The column was washed with 10 ml of PBS solution followed by 10 ml of water at a flow rate of 5–6 ml min^{-1}. The column was dried by applying vacuum for 10 s. OTA was eluted from the column with 1.5 ml of the mixture methanol/acetic acid, 98:2 (v/v) passing through by gravity. The eluate was backflushed twice using a syringe and then air was pushed through the column. The collected eluate was finally diluted with 1.5 ml of water, which also passed through the column at a flow rate of 2–3 ml min^{-1}. The sample was filtered (0.45 µm filter) and a volume of 100 µl was injected.

Instrumental parameters

UHPLC/TOFMS conditions for multi-mycotoxin analysis

UHPLC separation was achieved on an UltiMate 3000 RSLC system (Dionex, Breda, The Netherlands), composed of a vacuum degasser, binary pump, cooled autosampler, column oven (30°C), and equipped with a Zorbax Eclipse XDB C18 column (1.8 µm, 2.1 × 100 mm; Agilent Technologies, Waldbronn, Germany). Mobile phase A consisted of water/methanol/acetic acid 94:5:1 (v/v/v) and mobile phase B of methanol/water/acetic acid 97:2:1 (v/v/v), both containing 5 mM of ammonium acetate with pH 3.25 (eluent A) and pH 5.1 (eluent B). The gradient was: 0–14 min linear increase from 30% to 95% B, 14–14.1 min linear increase to 100% B, followed by re-equilibration of the column, all applying a flow rate of 0.2 ml min^{-1}. The RSLC system contained 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 full width at half maximum (FWHM). It was equipped with an orthogonal ESI operating in positive mode, using a mass range of 50–1000 Da for *m/z* acquisition. The MS method contained four time segments: 0–0.5 min for calibration, 0.5–11.7 min for the detection of DON, AFB_1, AFB_2, AFG_1, AFG_2 and HT-2 toxin, 11.7–12.35 min for the detection of FB_1, and 12.35–14.1 min for the detection of T-2 toxin, OTA and ZEN. MS parameters, common for all segments, were: capillary voltage 6000 V, nebuliser pressure 2 bars, dry gas temperature 200°C and dry gas flow 7 l min^{-1}. The applied capillary exit voltage was 90 V, skimmer 1 voltage 30 V and hexapole RF 250 for segments 1, 2 and 4. For segment 3 (FB_1), those settings were 105, 35 and 600 V, respectively. At the beginning of every run, the MS was calibrated with a sodium acetate calibrant solution.

Quantification was performed using matrix-matched calibration curves (MMCC), which were constructed by spiking the multi-standard solution into testing matrices before extraction at three concentration levels

corresponding to 0.5, 1.0 and 1.5 times an individual concentration of each mycotoxin: 640 μg kg^{-1} for DON; 8 μg kg^{-1} for AFB_1, AFB_2, AFG_1 and AFG_2; 80 μg kg^{-1} g for HT-2 toxin and ZEN, 40 μg kg^{-1} for T-2 toxin and OTA; and 200 μg kg^{-1} for FB_1. For internal quality control purposes, a fixed concentration of 80 μg kg^{-1} of ZAN was added. After spiking, the samples were kept overnight at RT and protected from light to allow the equilibration of the multi-standard working solution with the cereal matrix.

HPLC conditions for OTA analysis

OTA in oat flakes and wheat noodles was analysed on an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of an isocratic pump, a vacuum degasser, an autosampler and a column oven (25°C), and equipped with a Zorbax Eclipse C18 column (5 μm, 4.6 × 250 mm; Agilent Technologies). An isocratic elution was applied with a mobile phase containing a mixture of acetonitrile/water/acetic acid 50:49:1 (v/v/v) at a flow rate of 1 ml min^{-1}. Fluorescence detection was carried out at 247 and 480 nm of excitation and emission wavelengths, respectively. Quantification of OTA was performed by measurement of the peak areas at the OTA retention time and comparing them with a 12-point calibration curve (0.1–250 ng ml^{-1}, $R^2 = 0.9996$). The resultant OTA concentration was finally corrected by the corresponding recovery.

Method performance

For validation experiments, samples of polished rice, wheat noodles and oat flakes taken from the batches of real samples were used as testing matrices to decrease as much as possible the matrix variability in the method performance. Traces of contamination were corrected using the standard addition technique.

Multi-mycotoxin analysis by UHPLC/TOFMS

To assess linearity, calibration curves of multi-standard solutions in pure solvent were plotted at 12 concentration levels: 1.0, 3.0, 5.0, 10, 20, 30, 50, 75, 100, 160, 200 and 240 ng ml^{-1}. LODs and LOQs were assessed by spiking testing samples before extraction at six concentration levels (8, 20, 40, 100, 300 and 400 μg kg^{-1} of DON, and 2, 4, 8, 20, 40 and 80 μg kg^{-1} for the other mycotoxins) in duplicate. The LOD was determined using the equation:

$$\mathrm{LOD} = 3s_{\mathrm{bl}}/S$$

where s_{bl} is the standard deviation (SD) of the intercept; and S is the slope of the respective linear regression calibration curve. The LOD was calculated using (Taverniers et al. 2004):

$$\mathrm{LOQ} = 6s_{\mathrm{bl}}/S$$

Recoveries were calculated according to Desmarchelier et al. (2010) and determined based on six replicates MMCC constructed for each matrix at three concentration levels (detailed above in the section on "UHPLC/TOFMS conditions for multi-mycotoxin analysis"). The intra-day precision was assessed based on the replicates of the recovery experiments, while inter-day precision was performed by spiking one testing sample before extraction at one-fold concentration of the multi-standard solution during 3 consecutive days. The matrix effects were assessed by determining the signal suppression-enhancement (SSE) calculated according to Sulyok et al. (2006) from the comparison of the slope of the calibration curve of extracts spiked just before injection and the slope of the calibration curve of the standard working solutions. The SSE experiments were performed in duplicate at four concentration levels: 25, 50, 75 and 100 μg kg^{-1} for all mycotoxins.

OTA analysis by HPLC

Linearity was assessed by plotting calibration curves of the standard in pure solvent at 12 concentration levels of the standard (0.1, 0.5, 1.0, 2.0, 5.0, 10, 25, 50, 75, 100, 150, 175 and 250 ng ml^{-1}). LODs, LOQs and recovery experiments were performed in duplicate at six concentration levels: 1.5, 6, 12, 30, 60 and 120 μg kg^{-1}. The intra-day precision was assessed by spiking before extraction of a testing sample at 30 μg kg^{-1} of OTA in triplicate, while inter-day precision was performed by spiking a testing sample at the same concentration during 3 consecutive days.

Data evaluation

UHPLC/TOFMS data analysis was performed using the software DataAnalysis version 4.0 SP 2. TargetAnalysis™ software (Bruker Daltonics, Bremen, Germany) was used for generation of extracted ion chromatograms (EICs) of the acquired $[M + H]^+$ ions from the total ion chromatograms (TIC). Identification and distinction between true- and false-positive results were based on the retention time deviation, mass accuracy and SigmaFit™ algorithm, which is a rate for the agreement of the theoretical and measured isotopic patterns (Ojanpera et al. 2006). For all compounds, threshold parameters of detection were: mass accuracy of 5 ppm, *m*/*z* tolerance of 5 ppm, extraction mass window of 15 mDa, *mSigma* of 50 and retention time window of 0.15 min.

Chemstation 3D software (Agilent 1200) was used to control the HPLC system and for single data processing.

Descriptive analyses of rice agricultural practices and comparison tests (analysis of variance (ANOVA)) were

performed in Stata 10.0 (Stata Corporation, College Station, TX, USA). Contamination rates for each mycotoxin and staple cereal were determined together with the standard deviation of a sample proportion (SD_p) calculated according to Uyttendaele et al. (2009).

Results and discussion

UHPLC/TOFMS method performance

The MS parameters were tuned for each mycotoxin by direct infusion of individual standard solutions at a concentration of 1 μg ml^{-1}. Specific MS settings were necessary for FB1 because of its higher molecular mass compared with the other mycotoxins. Positive and negative electrospray conditions were evaluated and all mycotoxins were best detected in the positive mode as $[M + H]^+$ ions for aflatoxins, DON, FB_1, OTA, ZEN and ZAN. HT-2 and T-2 toxins were detected as $[M + NH_4]^+$ ions. The less abundant ions were also detected and used as qualifiers (Table 1). The mobile phases were selected based on previous multi-mycotoxin analytical methods (Sulyok et al. 2006). Both phases were acidified with 1% of acetic acid to improve the peak shape and ionisation (Sulyok et al. 2006; Desmarchelier et al. 2010). A low flow rate was applied in order to change the MS settings along the run and to reduce matrix effects due to co-elution (Songsermsakul & Razzazi-Fazeli 2008). Several chromatographic gradients were evaluated to achieve good peak shapes and separation. A better peak intensity was, however, attained for DON using a different gradient, but this affected the separation of the other mycotoxins eluting later in the chromatogram. Consequently, the applied gradient was considered as the best compromise for a good separation of all mycotoxins, which was established within 14 min (Figure 1).

The method performance was assessed in terms of linearity, LOD and LOQ, recovery, signal suppression enhancement, and inter- and intra-day precision. Good linearity for all mycotoxins was achieved both in the multi-standard solutions ($R^2 > 0.99$) and in the MMCC ($R^2 > 0.98$). The obtained values of LOD and LOQ are presented in Table 2. Most of the LODs were similar to previous studies in cereals, in which crude extracts were analysed (Sulyok et al. 2006; Zachariasova et al. 2010). Worldwide, several regulations for maximum levels of mycotoxins in foodstuffs intended for human consumption have been harmonised between countries belonging to some economic/trading communities (FAO 2004; van Egmond et al. 2007). Among all, the European regulations consider the largest amount of mycotoxins in several commodities, and have adopted the lowest permitted levels for human consumption (FAO 2004). Since no regulation is enforced in Ecuador, the obtained LODs and LOQs of this study were compared with the maximum permitted levels established in European Regulation No. 2006/1881/EC. The LODs and LOQs of DON were the highest among all matrices; however, those were low enough for detection and quantification in compliance with this regulation. Similar compliance was achieved for the LODs and LOQs of FB_1 in all cereals; AFB_1 in polished rice, and ZEN in all cereal matrices except polished rice. The obtained LODs of AFB_1 in oat, white noodles and paddy rice, and LODs of ZEN in polished rice allowed only detection, whereas LODs of OTA in all cereals and AFB_1 in yellow noodles were higher than the set levels in the mentioned regulation. For T-2 and HT-2 toxin, no comparison was possible since no maximum permitted levels in cereals are yet established.

Variations in SSE of the different cereal matrices are presented in Table 3. In this study the matrix effects were strongly influenced by the type of matrix and the nature of the analyte as observed in other studies (Songsermsakul & Razzazi-Fazeli 2008; Frenich et al. 2009). Thus, SSE differed significantly amongst different cereal matrices ($p < 0.05$) and different mycotoxins ($p < 0.05$). The matrix effect phenomenon is mainly caused by the presence of

Table 1. Overview of detected ions, molecular formula, theoretical mass–charge ratio (*m/z*), average retention times (RT) and SigmaFit™ (*mSigma*) values 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	$C_{15}H_{20}O_6$[a]/297.133265	$C_{15}H_{19}O_6Na$/319.115209		2.3	13
AFG_2	$C_{17}H_{14}O_7$[a]/331.081229	$C_{17}H_{13}O_7Na$/353.063174		6.8	8
AFG_1	$C_{17}H_{12}O_7$[a]/329.065579	$C_{17}H_{11}O_7Na$/351.047524		7.6	8
AFB_2	$C_{17}H_{14}O_6$[a]/315.086315	$C_{17}H_{13}O_6Na$/337.068259		8.3	8
AFB_1	$C_{17}H_{12}O_6$[a]/313.070665	$C_{17}H_{11}O_6Na$/335.052609		9.0	6
HT-2	$C_{22}H_{32}O_8$/425.216994		$C_{22}H_{35}O_8N$[a]/442.243544	11.3	11
FB_1	$C_{34}H_{59}NO_{15}$[a]/722.395747	$C_{34}H_{58}NO_{15}Na$/744.377691		12.0	8
T-2	$C_{24}H_{34}O_9$/467.227559		$C_{24}H_{37}O_9N$[a]/484.254108	12.5	9
OTA	$C_{20}H_{18}NO_6Cl$[a]/404.089541	$C_{20}H_{17}NO_6ClNa$/426.071486		13.4	12
ZEN	$C_{18}H_{22}O_5$ [a]/319.154000	$C_{18}H_{21}O_5Na$/341.135945		13.7	9

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; and ZEN, zearalenone.
[a]Most abundant ions for all matrices.

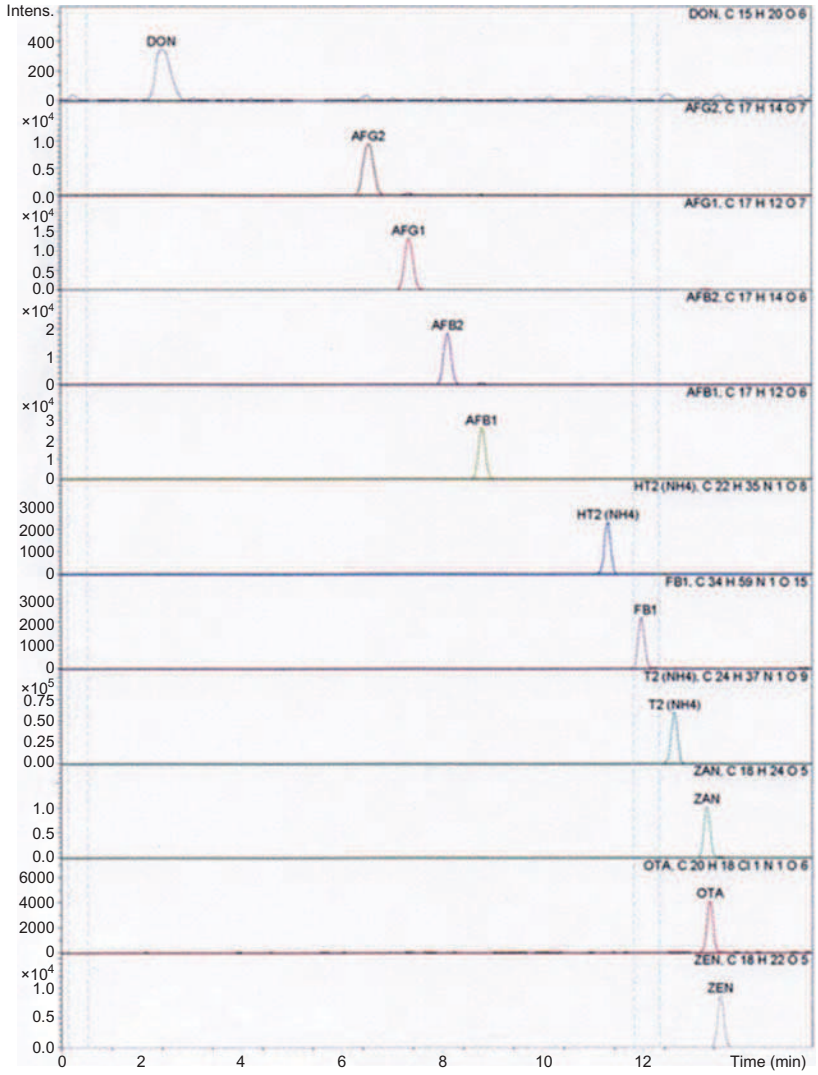

Figure 1. UHPLC/TOFMS chromatograms obtained from a blank sample of milled rice spiked at 80 μg kg^{-1} of all mycotoxins.

Table 2. LODs and LOQs for polished and paddy rice, oat flakes, white wheat noodles and yellow wheat noodles, and maximum levels of contamination allowed in cereals for direct human consumption (all μg kg^{-1}).

Mycotoxin	Maximum permitted levels in cereals, 2006/1881/EC	Polished rice		Paddy rice		Oat flakes		White wheat noodles		Yellow wheat noodles	
		LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
DON	750	24	48	29	59	26	53	53	107	84	167
AFG_2	–	1	3	3	5	2	4	6	11	9	18
AFG_1	–	1	1	7	14	1	3	2	4	7	14
AFB_2	–	2	3	4	9	1	3	4	8	6	12
AFt	4 (all cereals); 10 (rice)	–	–	–	–	–	–	–	–	–	–
AFB_1	2 (all cereals); 5 (rice)	1	2	4	8	2	4	2	3	8	15
HT-2	–	20	41	7	15	12	24	5	9	16	31
FB_1	400[a]	16	32	10	19	8	16	15	30	11	22
T-2	–	2	5	6	11	2	3	3	5	7	15
OTA	3	9	18	8	17	8	15	7	14	15	30
ZEN	75	39	77	22	45	3	7	27	54	23	46

Notes: DON, deoxynivalenol; AFG_2, aflatoxin G_2; AFG_1, aflatoxin G_1; AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AFt, total aflatoxins (sum of AFG_2 + AFG_1 + AFB_2 + AFB_1); HT-2, HT-2 toxin; FB_1, fumonisin B_1; T-2, T-2 toxin; OTA, ochratoxin A; and ZEN, zearalenone.
[a]Sum of FB_1 + FB_2, only established for maize-based foods.

Table 3. Variation in signal suppression/enhancement (% SSE) amongst blank extracts of samples of polished rice, oat flakes, white wheat noodles and yellow wheat noodles spiked at four concentration levels in the range of 25–100 μg kg^{-1}.

	% SSE calculated as slope spiked extract/slope liquid standard slope × 100			
Mycotoxin	Polished rice	Oat flakes	White wheat noodles	Yellow wheat noodles
DON	37	55	28	78
AFG_2	67	77	87	82
AFG_1	68	96	102	103
AFB_2	61	60	63	60
AFB_1	70	60	69	65
HT-2	63	85	87	112
FB_1	96	87	98	103
T-2	73	69	84	98
OTA	91	66	79	82
ZEN	76	51	67	68

Note: 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; and ZEN, zearalenone.

co-eluting matrix components in the crude extract, hampering the precision and sensitivity of the analysis (Antignac et al. 2005; Songsermsakul & Razzazi-Fazeli 2008). DON showed the most important ion suppression that could be the cause of the reduced sensitivity in terms of LOD of this mycotoxin. The inclusion of the methanol-freezing step in the sample preparation helped to reduce polar matrix interferences. However, the extraction of water-soluble mycotoxins (i.e. FB_1) could be hampered. By constructing MMCC using representative real samples, matrix effects were substantially corrected. The recovery values determined using MMCC of spiked cereal matrices are presented in Table 4. These recoveries were in agreement with the minimum requirements set in Regulation No. 401/2006/EC (70–110%) (European Commission 2006a). The achieved intra- and inter-day precisions for all cereal matrices are presented in Table 5, and the relative standard deviations (%RSD) were according to the maximum percentages for quantitative methods set in Regulation No. 2002/657/EC (<20%) (European Commission 2002). The validated method was further applied on paddy rice and the LODs and LOQs are also presented in Table 2.

The type of matrix clearly had an impact on the variability of the results, in both precision and recovery. The highest variability was observed for wheat noodles, which could be attributed to their more complex composition, their higher protein and fat content or their higher degree of processing compared with the other matrices, or a combination of these three factors.

HPLC method performance

The calibration curves of OTA standard, both in pure solvent and the cereal matrices, showed good linearity (R^2 > 0.99). The retention time of OTA was 10.7 ± 0.7 min for all cereals. The average recoveries in oat flakes, white and yellow noodles were 88% ± 4%;

Table 4. Recovery (%) ± SD determined using matrix-matched calibration curves of polished rice, oat flakes, white wheat noodles and yellow wheat noodles spiked at three concentration levels.[a]

	Percentage recovery calculated as area-MMCC intercept/MMCC slope × 100/spiked concentration									
Spiking level[a]	DON	AFG_2	AFG_1	AFB_2	AFB_1	HT-2	FB_1	T-2	OTA	ZEN
Polished rice										
0.5	89 ± 8	100 ± 3	99 ± 1	97 ± 4	100 ± 5	97 ± 4	112 ± 10	100 ± 3	94 ± 32	103 ± 7
1	111 ± 4	100 ± 4	101 ± 7	103 ± 3	100 ± 3	103 ± 6	88 ± 10	100 ± 6	96 ± 9	97 ± 14
1.5	96 ± 3	100 ± 5	100 ± 4	99 ± 3	100 ± 2	99 ± 3	96 ± 6	100 ± 2	101 ± 13	101 ± 10
Oat flakes										
0.5	98 ± 4	97 ± 5	97 ± 5	100 ± 6	99 ± 4	111 ± 4	98 ± 9	96 ± 4	95 ± 9	93 ± 8
1	102 ± 6	103 ± 2	103 ± 2	100 ± 3	101 ± 3	97 ± 8	106 ± 3	104 ± 2	105 ± 8	107 ± 7
1.5	99 ± 4	99 ± 3	99 ± 1	100 ± 4	100 ± 2	105 ± 9	98 ± 5	99 ± 2	98 ± 8	98 ± 6
White wheat noodles										
0.5	100 ± 17	113 ± 13	93 ± 27	93 ± 21	97 ± 25	107 ± 15	118 ± 22	109 ± 22	106 ± 13	102 ± 10
1	92 ± 15	93 ± 22	97 ± 21	88 ± 17	94 ± 15	93 ± 24	82 ± 14	85 ± 12	94 ± 21	105 ± 12
1.5	94 ± 10	95 ± 20	93 ± 22	104 ± 22	96 ± 15	102 ± 24	106 ± 14	96 ± 14	98 ± 15	98 ± 11
Yellow wheat noodles										
0.5	94 ± 14	93 ± 10	101 ± 12	109 ± 11	96 ± 10	106 ± 12	116 ± 11	101 ± 12	102 ± 8	99 ± 20
1	106 ± 17	107 ± 22	99 ± 15	91 ± 6	100 ± 16	94 ± 21	81 ± 6	99 ± 17	98 ± 21	95 ± 9
1.5	98 ± 11	98 ± 12	100 ± 11	103 ± 11	99 ± 8	102 ± 18	101 ± 8	100 ± 13	101 ± 8	94 ± 13

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; and ZEN, zearalenone.

[a]Fold-times the individual concentration of 640 μg kg^{-1} for DON; 8 μg kg^{-1} for AFB_1, AFB_2, AFG_1 and AFG_2; 80 μg kg^{-1} for HT-2 toxin and ZEN, 40 μg kg^{-1} for T-2 toxin and OTA, and 200 μg kg^{-1} for FB_1.

Table 5. RSD (%) of intra- and inter-day precision for polished rice, oat, white wheat noodles and yellow wheat noodles spiked at three concentration levels.[a]

	Polished rice		Oat flakes		White wheat noodles		Yellow wheat noodles	
Mycotoxin	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
DON	4	7	5	10	16	20	15	19
AFG_2	4	5	3	13	15	12	11	20
AFG_1	4	16	2	16	18	15	16	19
AFB_2	3	2	4	3	17	19	10	19
AFB_1	3	6	3	13	15	17	12	18
HT-2	4	8	12	16	15	14	15	20
FB_1	11	17	4	8	14	11	15	19
T-2	4	11	3	7	16	10	16	19
OTA	12	9	8	1	15	20	11	19
ZEN	9	8	7	4	10	4	14	16

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; and ZEN, zearalenone.
[a]Spiked at 0.5, 1.0 and 1.5 times the individual concentration of 640 μg kg^{-1} for DON; 8 μg kg^{-1} for AFB_1, AFB_2, AFG_1 and AFG_2; 80 μg kg^{-1} for HT-2 toxin and ZEN, 40 μg kg^{-1} for T-2 toxin and OTA, and 200 μg kg^{-1} for FB_1.

97% ± 8% and 103% ± 2%, respectively. Good average intra-day (%RSD = 5.1) and inter-day (%RSD = 5.2) precision were achieved. Both recovery and precision values were also in agreement with European Union criteria. The LODs and LOQs were 1.5 and 3 μg kg^{-1} for oat flakes and 1.9 and 3.8 μg kg^{-1} for wheat noodles.

Mycotoxin co-occurrence in staple cereals in Ecuador

Rice

Contamination rates, SD_p, means, SD and ranges of all mycotoxins are presented in Table 6. Of the 43 analysed samples of paddy rice and 46 of polished rice, 21 (49%) and three (7%) samples, respectively, were contaminated at least with one mycotoxin. Polished rice was contaminated with AFG_1 or HT-2 toxin. Mycotoxin co-occurrence was found only in three samples of paddy rice (7%) (Guayas 67%, Los Ríos 33%). The mycotoxin combinations found in this crop were: (1) AFG_1, AFB_1 and FB_1 (33%) and (2) DON and FB_1 (67%) (data not shown). The levels of AFB_1 and FB_1 exceeded the established maximum limits for human consumption; however, paddy rice is not consumed as such. This contamination pattern was in agreement with the possible mycotoxigenicity of the isolated fungal species of *Aspergillus* and *Fusarium* that had been previously described in Ecuadorian paddy rice (Pacin et al. 2002). Moreover, low occurrences of DON and aflatoxins in Ecuadorian paddy rice had been formerly reported (Mühlemann et al. 1997a, 1997b).

In general, the rates of contamination in polished rice were considerably lower than in paddy rice, suggesting a substantial reduction of mycotoxin contamination with the milling process, as described in other studies (Sales & Yoshizawa 2005; Ok et al. 2009). On the other hand, the contamination with *Fusarium* toxins (FB_1, DON and HT-2) was higher for the rice from Los Ríos than from Guayas province. Although the findings suggested that the detected mycotoxins were mostly field produced, the differences between Los Ríos and Guayas might be also associated with the period between harvest and the milling process. The analysed rice was harvested during the rainy season and the high humidity of paddy rice might lead to the growth of storage fungi, or the proliferation of field fungi, and subsequently the rapid accumulation of mycotoxins (Sales & Yoshizawa 2005). According to the survey applied during sampling, in both provinces similar frequencies of use of pesticides (58.3% for Los Ríos, 56.5% for Guayas) and fertilisers (73.3% for Los Ríos, 74.2% for Guayas) were reported. Furthermore, it was reported that the storage of paddy rice before milling was shorter in Guayas, with a minimum storage time of 1–5 days (70%) up to longer than 3 months (5%). In Los Ríos the minimum storage time of 1–5 days was less frequently reported (53.1%) and, conversely, a storage time longer than 3 months was more frequent in this province (24%). Regarding management of mould infestation on the rice before milling, in Los Ríos the major action reported was superficial mould cleaning before milling (38%), while disposal of the product (26%) and also superficial mould cleaning (23%) were the most common practices in Guayas province.

According to UNA (the Ecuadorian National Storage Unit), the mill should accept fresh-harvested paddy rice (a maximum of 2 days old), with a maximum of 20% of moisture content and 5% of impurities (stones, insects, etc.). According to the survey, most supervisors stated that the incoming rice is usually accepted with a higher moisture content (61% in Los Ríos, 62% in Guayas) and impurities (65% in Los Ríos, 46% in Guayas) than permitted. The reported average moisture content (Los Ríos 21% ± 6%; Guayas 22% ± 5%) and percentage of impurities (Los Ríos

Table 6. Contamination rates, standard deviation of a sample proportion (SD_p), means, SD and ranges of all mycotoxins in Ecuadorian staple cereals: polished and paddy rice, oat flakes, white wheat noodles and yellow wheat noodles, according to the region of sample collection.

Mycotoxin	Contamination rate (%, SD_p)	Region	Positive/total	Mean (μg kg^{-1})	SD	Range (μg kg^{-1})
Paddy rice (n = 43)						
DON	23%, SD_p = 6.4%	Guayas	2/20	62.4	0.6	62–62.8
		Los Ríos	8/23	79.9	21.5	43.9–102.4
AFG_2	2%, SD_p = 2.3%	Guayas	0/20	–	–	–
		Los Ríos	1/23	–	–	3.3[a]
AFG_1	2%, SD_p = 2.3%	Guayas	1/20	–	–	63.7[a]
		Los Ríos	0/23	–	–	–
AFB_1	7%, SD_p = 3.9%	Guayas	3/20	20.6	23.3	4.9–47.4
		Los Ríos	0/23	–	–	–
FB_1	23%, SD_p = 6.4%	Guayas	3/20	40.4	16.3	22.6–54.3
		Los Ríos	7/23	277	399.1	17.9–1146.4
Polished rice (n = 46)						
AFG_1	2%, SD_p = 2.2%	Guayas	0/20	–	–	–
		Los Ríos	1/26	–	–	2[a]
HT-2	4%, SD_p = 3.1%	Guayas	0/20	–	–	–
		Los Ríos	2/26	32.8	9.6	26–39.5
Oat flakes (n = 42)						
DON	17%, SD_p = 5.8%	Cuenca	4/30	50	17.2	32.2–69
		Nabon	3/12	76.8	64.7	41.2–151.5
AFB_1	2%, SD_p = 2.4%	Cuenca	1/30	–	–	2.7[a]
		Nabon	0/12	–	–	–
OTA	2%, SD_p = 2.4%	Cuenca	0/30	–	–	–
		Nabon	1/12	–	–	161.6[a]
White wheat noodles (n = 43)						
DON	33%, SD_p = 7.1%	Cuenca	9/25	95.4	29.1	57.6–142.8
		Nabon	5/18	120.5	58.2	87.4–224.2
OTA	5%, SD_p = 3.2%	Cuenca	0/25	–	–	–
		Nabon	2/18	60.8	45.7	28.5–93.1
Yellow wheat noodles (n = 37)						
DON	5%, SD_p = 3.7%	Cuenca	2/27	85.6	1.9	84.2–86.9
		Nabon	0/10	–	–	–

Notes: DON, deoxynivalenol; AFG_2, aflatoxin G_2; AFG_1, aflatoxin G_1; AFB_1, aflatoxin B_1; HT-2, HT-2 toxin; FB_1, fumonisin B_1; and OTA, ochratoxin A.
[a]Unique values.

8% ± 4%; Guayas 8% ± 5%) exceeded the permitted limits in both provinces without differing significantly (p = 0.33 and 0.76, respectively), which is in agreement with the higher mycotoxin occurrence observed in paddy rice. Hence, more severe quality controls of the incoming rice at the mill must be enforced to prevent post-harvest contamination in both paddy and polished rice.

Additionally, the recommended maximum moisture content of polished rice is 14%. According to the survey, the moisture content of the final product is low enough to warrant safety storage for extended periods of time. The average moisture content of the final product reported in Los Ríos (11.5% ± 3%) did not differ significantly from the reported in Guayas (11% ± 2%) (p = 0.22).

Oat flakes

Contamination rates, SD_p, means, SD and ranges of all mycotoxins are presented in Table 6. Of the 42 analysed samples (30 from Cuenca and 12 from Nabon), nine samples (21%) were contaminated with one mycotoxin, being DON, OTA or AFB_1. The levels of AFB_1 and OTA exceeded the established maximum limits. No mycotoxin co-occurrence was observed in oat flakes. Since the LODs of OTA of the multi-mycotoxin analysis by UHPLC/TOFMS were higher than the maximum permitted limits, extra target analyses were performed by HPLC. Of the 35 analysed samples (31 from Cuenca and four from Nabon), two (6%) samples (one from Cuenca and one from Nabon) were contaminated with OTA (mean = 3.4 ± 0.3 μg kg^{-1}).

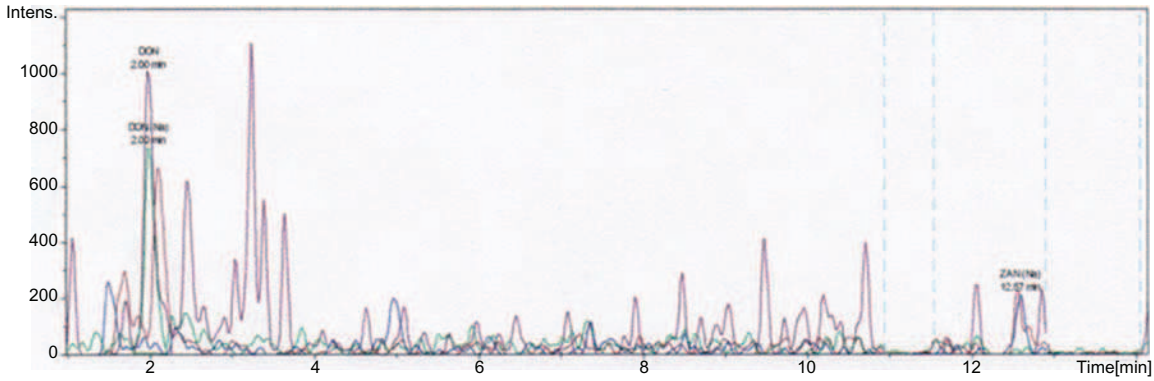

Figure 2. UHPLC/TOFMS chromatograms of natural contamination of DON in white noodles, spiked with ZAN for internal quality control.

No data on mycotoxin occurrence in oat flakes are available in the Ecuadorian context. The low contamination rate reported in this study could be related to the fact that most of the oat that is consumed in Ecuador is imported and the incoming oat should comply with the regulations enforced by the exporting countries, although in Ecuador no such controls are applied. Interestingly, it should be mentioned that 73% of the contaminated samples were those bought by weight from big sacks.

Wheat noodles

Contamination rates, SD_p, means, SD and ranges of all mycotoxins are presented in Table 6. Of the 43 samples of white noodles (25 from Cuenca and 18 from Nabon), 15 samples (35%) were contaminated with DON and/or OTA (Figure 2). Co-occurrence of those mycotoxins was observed in two samples (5%) (data not shown). The levels of OTA exceeded largely the established maximum limits. Regarding yellow noodles, of the 37 samples (27 from Cuenca and 10 from Nabon), two samples (5%) were contaminated with DON only. Additional target analyses of OTA were also performed by HPLC (29 samples of white and 30 samples of yellow noodles) and no contamination was detected in any of the samples.

The contamination of wheat-based products with DON and OTA is considered as a worldwide problem (Kushiro 2008; Zaied et al. 2009). However, the effect of processing into pasta largely contributes to the reduction of those mycotoxins (Kushiro 2008; Duarte et al. 2010; Gonzalez-Osnaya et al. 2011). Furthermore, the manufacturing process of yellow noodles requires the inclusion of alkaline salts and these might reduce the mycotoxin content, similar to the reduction of aflatoxins and *Fusarium* toxins during nixtamalisation of maize products. This could explain the lower mycotoxin occurrence observed in yellow noodles compared with white ones. On the other hand, noodles in Ecuador are locally produced, but most of the wheat (as durum wheat or flour) is imported from countries were mycotoxin regulations are enforced. As well as for oat flakes, most of the contaminated samples (up to 80%) were those bought by weight from big sacks, a situation that might contribute to fungal growth and subsequent mycotoxin production. Inadequate storage conditions could favour factors such as moisture uptake and insect infestation; however, further research is necessary to identify the storage factors implicated in mycotoxin production at the temperate climate of the places where the samples were collected.

Conclusions

This study reports the co-occurrence of mycotoxins with the major health concerns in the most important Ecuadorian staple cereals (polished rice, oat flakes and wheat noodles). The analyses were performed by a reliable and sensitive UHPLC/TOFMS method that was developed and validated for those cereal matrices. In addition, extra target analysis of OTA in oat flakes and wheat noodles were performed by HPLC-FLD. Since no mycotoxin regulations are enforced in Ecuador, the obtained LODs and LOQs were compared with the European maximum permitted limits (Regulation No. 2006/1881/EC) and only the LODs of OTA in all cereals and AFB1 in yellow wheat noodles were higher than those limits. High rates of contamination were observed in white wheat noodles (33% DON, 5% OTA) and oat flakes (17% DON, 2% OTA and 2% AFB_1), and lower rates in polished rice (2% AFG_1 and 4% HT-2 toxin) and yellow noodles (5% DON). Although paddy rice is not consumed as such, it was also analysed to evaluate pre-milling contamination (23% DON, 23% FB_1, 7% AFB_1, 2% AFG_1 and 2% AFG_2). Low rates of mycotoxin co-occurrence were observed only for white wheat noodles (5%) and paddy rice (7%). Although the levels of contamination of the studied mycotoxins were rather low, their occurrence in the assessed staple cereals may pose a hazard to public health considering their important role in the Ecuadorian diet. For follow-up studies, daily intake of

those staple cereals must be estimated to support further risk exposure assessments. Furthermore, a retrospective analysis of emerging mycotoxins will be performed using the full spectral information generated by TOFMS.

Acknowledgments

The authors thank the authorities of the Minister of Agriculture, Livestock, Aquaculture and Fisheries (MAGAP) for their support during sampling of the rice, as well as to the supervisors of the mills. The authors are also very grateful to all members of the project "Food, Nutrition and Health", especially Eng. Juana Cabrera, for their invaluable collaboration during sample collection.

Funding

The authors are grateful to the Flemish Interuniversity Council – Institutional University cooperation (VLIR-UOS) for its financial support, within the cooperation between Cuenca University (Ecuador) and Ghent University (Belgium). Frédéric Mestdagh was a postdoctoral researcher funded by the Research Foundation – Flanders (FWO-Vlaanderen).

References

Antignac JP, 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. Anal Chim Acta. 529:129–136.

Bermudez O, Tucker K. 2003. Trends in dietary patterns of Latin American populations. Cad Saúde Pública. 19:S87–S99.

Bhat R, Rai RV, Karim AA. 2010. Mycotoxins in food and feed: present status and future concerns. Compr Rev Food Sci Food Saf. 9:57–81.

Codex Alimentarius. 2008. Proposed draft sampling plans for aflatoxin contamination in almonds, brazil nuts, hazelnuts and pistachios (N07-2004). FAO/WHO: The Netherlands; p. 19.

Desmarchelier A, Oberson JM, 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. J Agric Food Chem. 58:7510–7519.

Duarte SC, Pena A, Lino CM. 2010. A review on ochratoxin A occurrence and effects of processing of cereal and cereal derived food products. Food Microbiol. 27:187–198.

European Commission. 2002. Commission decision (EC) No. 2002/657. Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Off J Eur Union. L221:8–36.

European Commission. 2006a. Commission Regulation (EC) No. 401/2006. Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Off J Eur Union. L70:12–34.

European Commission. 2006b. Commission Regulation (EC). No. 1881/2006. Setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union. L364:5–24.

European Commission. 2012. Commission Regulation (EC). No. 1881/2006. Setting maximum levels for certain contaminants in foodstuffs. Amended by Sept. 2012. Off J Eur Union. 2006R1881:1–34.

FAO. 2001. FAO-Perfiles Nutricionales por Países-Ecuador, Organización de las Naciones Unidad para la Agricultura y la Alimentación (FAO)/Servicio de Planificación, Estimación y Evaluación (ESNA), Roma; [Internet]. [cited 2013 Jan 30]. Available from: ftp://ftp.fao.org/es/esn/nutrition/ncp/ecumap.pdf

FAO. 2004. Worldwide regulations for mycotoxins in food and feed in 2003 [Internet]. Rome; p. 165. [cited 2012 Apr 30]. Available from: http://www.fao.org/docrep/007/y5499e/y5499e00.htm

FAO/IAEA. 2001. Manual on the application of the HACCP system in mycotoxin prevention and control [Internet]. Rome; p. 124. [cited 2013 Jan 30]. Available from: http://www.fao.org/docrep/005/y1390e/y1390e00.htm.

Frenich AG, Vidal JLM, Romero-Gonzalez R, Aguilera-Luiz MD. 2009. Simple and high-throughput method for the multimycotoxin analysis in cereals and related foods by ultra-high performance liquid chromatography/tandem mass spectrometry. Food Chem. 117:705–712.

Gonzalez-Osnaya L, Cortes C, Soriano JM, Molto JC, Manes J. 2011. Occurrence of deoxynivalenol and T-2 toxin in bread and pasta commercialised in Spain. Food Chem. 124:156–161.

INEC. 2011a. Análisis del Sistema Agroalimentario del Arroz en el Ecuador, Instituto Nacional de Estadística y Censos, Ecuador; [Internet]. [cited 2013 Apr 30]. Available from: http://www.ecuadorencifras.com/sistagroalim/pdf/Arroz.pdf

INEC. 2011b. Boletín agropecuario mensual N°14: Producción de trigo & cacao en el Ecuador, Instituto Nacional de Estadística y Censos, Ecuador; [Internet]. [cited 2013 Apr 30]. Available from: http://www.ecuadorencifras.com/cifras-inec/pdfs/agro14.pdf

Kushiro M. 2008. Effects of milling and cooking processes on the deoxynivalenol content in wheat. Int J Mol Sci. 9: 2127–2145.

Mühlemann M, Lüthy J, Hübner P. 1997a. Mycotoxin contamination of food in Ecuador: A: aflatoxins. Mitt Gebiete Lebensm Hyg. 88:474–496.

Mühlemann M, Lüthy J, Hübner P. 1997b. Mycotoxin contamination of food in Ecuador: B: ochratoxin A, deoxynivalenol, T-2 toxin and fumonisin. Mitt Gebiete Lebensm Hyg. 88:593–612.

Ojanpera S, Pelander A, Pelzing M, Krebs I, Vuori E, Ojanpera I. 2006. Isotopic pattern and accurate mass determination in urine drug screening by liquid chromatography/time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 20:1161–1167.

Ok HE, Chang HJ, Choi SW, Cho TY, Oh KS, Chun HS. 2009. Occurrence and intake of deoxynivalenol in cereal-based products marketed in Korea during 2007–2008. Food Addit Contam. 2:154–161.

Pacin AM, Gonzalez HHL, Etcheverry M, Resnik SL, Vivas L, Espin S. 2002. Fungi associated with food and feed commodities from Ecuador. Mycopathologia. 156:87–92.

Sabino M. 2011. Latin America: advances and challenges in mycotoxins. In: Ramírez M, Barros G, Chulze S, editors. Proceeding of the conference strategies to reduce the impact of mycotoxins in Latin America in a global context. 1st ed.; Nov 15–18; Mendoza; p. 6; ISBN 978-950-665-689-8.

Sales AC, Yoshizawa T. 2005. Updated profile of aflatoxin and Aspergillus section Flavi contamination in rice and its byproducts from the Philippines. Food Addit Contam. 22:429–436.

Senyuva HZ, Gilbert J, Ozturkoglu S. 2008. Rapid analysis of fungal cultures and dried figs for secondary metabolites by LC/TOF-MS. Anal Chim Acta. 617:97–106.

Shephard GS. 2008. Determination of mycotoxins in human foods. Chem Soc Rev. 37:2468–2477.

Shephard GS, Berthiller F, Burdaspal PA, Crews C, Jonker MA, Krska R, Lattanzio VMT, MacDonald S, Malone RJ, Maragos C, et al. 2013. Developments in mycotoxin analysis: an update for 2011–2012. World Mycotoxin J. 6:3–30.

Songsermsakul P, Razzazi-Fazeli E. 2008. A review of recent trends in applications of liquid chromatography-mass spectrometry for determination of mycotoxins. J Liq Chromatogr Relat Technol. 31:1641–1686.

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 Commun Mass Spectrom. 20:2649–2659.

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 Commun Mass Spectrom. 20:1422–1428.

Taverniers I, De Loose M, Van Bockstaele E. 2004. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. Trends Anal Chem. 23:535–552.

USDA. 2013. Grain and feed annual: Ecuador wheat corn rice production consumption exports imports forecast 2013, USDA Foreign Agricultural Service, Global Agriculture Information Network Ecuador; [Internet]. [cited 2013 Apr 30]. Available from: http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Grain%20and%20Feed%20Annual_Quito_Ecua dor_3-14-2013.pdf

Uyttendaele M, Busschaert P, Valero A, Geeraerd AH, Vermeulen A, Jacxsens L, Goh KK, De Loy A, Van Impe JF, Devlieghere F, 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. Int J Food Microbiol. 133:94–104.

Van Egmond HP, Schothorst RC, Jonker MA. 2007. Regulations relating to mycotoxins in food. Anal Bioanal Chem. 389:147–157.

Yazar S, Omurtag GZ. 2008. Fumonisins, trichothecenes and zearalenone in Cereals. Int J Mol Sci. 9:2062–2090.

Zachariasova M, Lacina O, Malachova A, Kostelanska M, Poustka J, Godula M, Hajslova J. 2010. Novel approaches in analysis of Fusarium mycotoxins in cereals employing ultra performance liquid chromatography coupled with high resolution mass spectrometry. Anal Chim Acta. 662:51–61.

Zaied C, Abid S, Zorgui L, Bouaziz C, Chouchane S, Jomaa M, Bacha H. 2009. Natural occurrence of ochratoxin A in Tunisian cereals. Food Control. 20:218–222.