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A survey of mycotoxins in random street-vended snacks from Lagos, Nigeria, using QuEChERS-HPLC-MS/MS

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ABSTRACT

A survey in African snacks was carried out in order to evaluate the intake of 23 mycotoxins. The African snack samples were purchased from street vendors within Lagos metropolis (Nigeria) and evaluated for the presence of 23 mycotoxins using a modified QuEChERS procedure coupled with liquid chromatography-triple quadrupole linear ion trap mass spectrometer. The snacks included *akara*, baked coconut, coconut candy, *donkwa*, groundnut cake (*kulikuli*), *lafun*, milk curd (*wara*), fresh and dried tiger-nuts, and yam flour. Only three mycotoxins were detected in 23.8% of the studied snacks, and at concentrations ranging from 6 to 54 μg kg^{-1}. The concentrations of aflatoxin B_1 (AFB_1) and AFB_2 reached 23 μg kg^{-1} and 3 μg kg^{-1}, respectively. Moreover a sample of baked coconut contained α-zearalenol (α-ZOL), which was up to 54 μg kg^{-1} in coconut candy. As considers prevalence, aflatoxins and α-ZOL were not detected in *lafun* and groundnut-based snacks (*donkwa* and *kulikuli*), whereas each of the three mycotoxins contaminated 12.5% (1/8) of the coconut-based samples. This is the first report of α-ZOL in cassava and coconut, and their products. AFB_1 and total aflatoxins (TAFs) concentrations exceeded the maximum allowable limit recommended by National Agency for Food and Drug Administration and Control Nigeria (NAFDAC) in one sample of baked coconut ($AFB_1 = 23$ μg kg^{-1} and TAFs = 26 μg kg^{-1}) and *donkwa* ($AFB_1 = 19$ μg kg^{-1} and TAFs = 21 μg kg^{-1}).

1. Introduction

Food contamination by mycotoxins is a continuous concern in food safety. Mycotoxins are able to contaminate a wide-range of food commodities, including raw material or cereal-based products, processed cereals and ready-to-eat (RTE) foods such as snacks (Garrido, Martínez, Romero-González, & Aguilera-Luiz, 2009; Park, Kim, Shon, & Kim, 2002; Roscoe et al., 2008).

Snacks are commonly consumed by all age groups and across social strata in both developed and developing countries. They could be in form of processed cereals, nuts, or even tubers. In Nigeria, and other sub-Saharan African countries, these snack foods can include local delicacies (*akara*, *donkwa*, *kulikuli*, *lafun*, *wara* and yam flour) and common global snacks (biscuits, cookies, coconut candy, fresh and dried tiger-nuts, and sausages). Reports on mycotoxin contamination of these snacks have mainly focused on *Aspergillus* and *Penicillium* mycotoxins, such as aflatoxins and ochratoxin A (Ezekiel, Kayode, Fapohunda, Olorunfemi, & Kponi, 2012; Ezekiel, Sulyok, Warth, Odebode, & Krska, 2012; Fakoor, Beheshti, Asadi, Mihanparast, & Feizy, 2012; Rubert, Sebastià, Soriano, Soler, & Mañes, 2011) with scanty record on some other fungal metabolites including *Fusarium* mycotoxins (Ezekiel, Sulyok, et al., 2012; Rubert, Soler, & Mañes, 2012).

The presence of fungi on snacks and fast foods (Fapohunda & Ogundero, 1990), as well as on regular food items (Fapohunda, Moore, Ganiyu, & Beltz, 2012) in Nigeria is not in doubt since the prevalence of mycotoxins in sub-Saharan Africa has been reported (Fapohunda, 2010). However, there has been dearth of information on multimycotoxin analyses of snacks in developing countries with generous attention only on developed countries (Eskola, Parikka, & Rizzo, 2001). Considering the economic challenge and literacy level of such countries, such as Nigeria, this situation could be alarming.

Many methods have been used for multimycotoxins analysis in food products (Beltrán, Ibañez, Sancho, & Hernández, 2009; Fen, Tao, Pang, Liu & Dong, 2011; Ibañez-Vea, Corcuera Remiro, Murillo-Arbizu, González-Perés, & Lizarraga, 2011; Takino, Tanaka, & Tanaka, 2011). Nowadays, multimycotoxin analyses tend to use

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simpler, cheaper, faster and environmentally friendly extraction systems. With this aim, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) or modified QuEChERS methods have been widely used in the last years, mainly for the extraction of pesticides (Lesueur, Knittl, Gartner, Mentler, & Fuerhacker, 2008), but also for other compounds, such as mycotoxins in eggs and barley (Frenich, Romero-González, Gómez-Pérez, & Vidal, 2011; Rubert, Dzuman, et al., 2012).

Therefore, the main aim of this work was to investigate the presence of 23 mycotoxins using a modified QuEChERS extraction procedure coupled with liquid chromatography-triple quadrupole linear ion trap mass spectrometer (HPLC-QqLIT or QTRAP® detector) for qualifying and quantifying the presence of mycotoxins in snacks from Lagos metropolis (Nigeria).

2. Materials and methods

2.1. Reagents and chemicals

Acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). Deionized water (>18 MΩ cm^{-1} resistivity) was purified using the Milli-Q® SP Reagent water system plus from Millipore Corp. (Bedford, MA, USA). All solvents were passed through a 0.45 μm cellulose filter purchased from Scharlau (Barcelona, Spain). Analytical grade formic acid (purity > 98%), ammonium formate, anhydrous magnesium sulphate, sodium chloride were obtained from Panreac Quimica S.A.U. (Barcelona, Spain).

The standards of aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), ochratoxin A (OTA), sterigmatocystin (STER), α-zearalenol (α-ZOL), zearalenone (ZEN), nivalenol (NIV), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), fusarenon X (FUSX), neosolaniol (NEO), diacetoxyscirpenol (DAS), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and beauvericin (BEA) were purchased from Sigma Aldrich (Madrid, Spain). T-2 and HT-2 toxins, aflatoxin M_1 (AFM_1) and deepoxy-deoxynivalenol (DOM-1) stock solutions (in acetonitrile) were obtained from Biopure referenzsubstanzen GmBH (Tulln, Austria). Fumonisin B_3 (FB_3) was supplied by the PROMEC unit (Programme on Mycotoxins and Experimental Carcinogenesis, Tygerberg, South Africa).

The stock solutions of mycotoxins were prepared according to Rubert, Soler, et al. (2012). All solutions were kept in secure conditions at −20 °C.

2.2. Samples

Twenty-one snack samples were purchased from street-vendors from Lagos metropolis, Nigeria. The snacks included *akara*, baked coconut, coconut candy, *donkwa*, groundnut cake (*kulikuli*), *lafun*, milk curd (*wara*), fresh and dried tiger-nuts, and yam flour. The samples were categorized into seven commodities according to the ingredient base: cassava-based (1 sample), coconut-based (8 samples), cowpea-based (1 sample), groundnut-based (3 samples), milk-based (2 samples), tiger-nut (4 samples) and yam-based (2 samples). Limited samples and uneven sample types were used for this study due to the difficulties encountered during sampling; the major one being the high cost of samples since they were intended to be analysed as composite/bulk samples and not individual retail samples.

Each sample was collected from random points of trader's trays as 3–4 sub samples (50–100 g) and mixed together to form the bulk sample (200–300 g). For samples sold in sealed pre-packed containers, 2–3 parts were purchased and combined to give a bulk sample. The bulk samples were comminuted. Fifty grams representative sample was obtained from each bulk and stored at 4 °C up to be analysed.

2.3. Extraction procedure

Modified QuEChERS procedure was employed to extract mycotoxins from the selected food snacks (Rubert, Dzuman, et al., 2012). Homogenized and representative portions of 2 g were weighed into a 50 mL PTFE centrifuge tube, and then 10 mL of 0.1% formic acid in deionized water was added. The mixture was homogenized for 3 min and allowed to stand for 10 min. After that, 10 mL acetonitrile was added and the mixture was vigorously shaken for 3 min. The next step involved the addition of 4 g $MgSO_4$ and 1 g of NaCl after which the entire mixture was shaken for 3 min. After the extraction, the sample was centrifuged (5 min, 4.500 rpm, 20 °C). Then, an aliquot (1 mL) was filtered through a 22 μm nylon filter before their injection into the LC–MS/MS system.

2.4. Instrumentation

The analytical method had been previously optimized by Rubert, James, Mañes, and Soler (2012). LC-tandem MS analyses were conducted on a system consisting of a Agilent 1200 chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to a 3200 QTRAP® mass spectrometer (Applied Biosystems, AB Sciex, Foster City, CA, USA) equipped with a Turbo V™ Ion Spray (ESI) interface. Separation of mycotoxins was performed with a reversed-phase analytical column (Gemini C_{18}, 150 mm, 2 mm i.d, 5 μm; Phenomenex) maintained at 35 °C. As mobile phase, 5 mM ammonium formate and 0.1% formic acid in water (A) and 5 mM ammonium formate in methanol (B) were used. The gradient was as follows: at the start 5% of solvent B and after the percentage of solvent B was linearly increased to 95% in 10 min. The percentage of solvent B was kept for 5 min. Finally, the column was equilibrated to initial conditions for 10 min. The flow rate was 200 μl min^{-1} and the injection volume was 10 μl.

The 3200 QTRAP® mass spectrometer (Applied Biosystems, AB Sciex, Foster City, CA, USA) was equipped with a Turbo V™ Ion Spray (ESI) interface. The QTRAP® analyzer combines a fully functional triple-quadrupole and ion trap mass spectrometer within the same instrument. The analyses were performed using Turbo V™ Ion Spray in positive mode. The operation conditions for the analysis in positive ionization mode were the followings: ion spray voltage 5500 V, probe temperature 450 °C, curtain gas 20 (arbitrary units) and GS1 and GS2, 50 and 55 psi, respectively. Nitrogen served as nebulizer and collision gas. Selected reaction monitoring (SRM) experiments were carried out to obtain the maximum sensitivity for the detection of target molecules. The optimization of MS parameters as declustering potential (DP), collision energy (CE) and collision cell entrance potential (CEP) were performed by flow injection analysis for each compound and the values are summarized in a previous work (Rubert, James, et al., 2012), entrance potential (EP) and collision cell exit potential (CXP) were set 10 and 4 V, respectively for all analytes. The mass spectrometer was operated in SRM mode and with a unit resolution for Q1 and Q3. For LC–MS/MS analysis, scheduled SRM (sSRM) was used at 50 s of SRM detection window and 1 s of target scan time, in this form was obtained more than 12 data points for all selected mycotoxins. Analyst® version 1.5.2 software (Applied Biosystems, AB Sciex, Foster City, CA, USA) was used to control and also for data collection and analysis.

2.5. Validation of the method

Performance characteristics of the optimized method were established by a validation procedure with spiked snack samples, studying matrix effect, linearity, trueness, intra-day and inter-day

precision, limits of detection (LOD) and quantification (LOQ) and selectivity.

Linearity was tested by spiking blank extract snacks at six concentration levels within the range of LOQ to 100 times LOQ. The external matrix-matched calibration was compared with standard calibration in solvent in order to evaluate matrix effects. Recovery was studied by spiking blank samples at two-fortification levels 25 and 100 μg kg^{-1}. Precision was evaluated through intra-day and inter-day precision. Intra-day was evaluated at the two concentration levels of the recovery studies, performing five replicates for each level. For inter-day precision, two spiked levels were analysed daily for a period of five days. LODs and LOQs were calculated analysing blank snack samples spiked at decreasing concentration, and they were determined as the lowest concentration of the selected compounds that produce chromatographic peak at signal to noise ratio (S/N) of 3 (LOD) and 10 (LOQ) respectively. These limits were calculated by Analyst version 1.5.2 software (Applied Biosystems, AB Sciex, Foster City, CA, USA).

Finally, selectivity was evaluated by extracting and analysing blank snack samples. Identification of the target mycotoxins was carried out by searching the characteristic transitions of mycotoxins in the appropriate retention time (RT), as well as two SRM transitions and monitoring of SRM ratio was carried out in order to quantify and qualify.

3. Results and discussion

The present study is a combination of two published methods; on the one hand an extraction method based on a modified QuEChERS (Rubert, Dzuman, et al., 2012) for determination of 32 mycotoxins in barley samples. On the other hand, a detection method using HPLC-QqLIT for determining 18 mycotoxins in baby food samples (Rubert, James, et al., 2012). Although these methods have demonstrated their independent capability for analysing these mycotoxins, with the objective of obtaining as much information on contamination and occurrence of these mycotoxins as possible, an investigation of its combination was carried out.

3.1. Method validation data

The presence of matrix components in the extract can affect the ionization of the compounds when ESI is used. For this purpose, eight calibration sets (standard in pure solvent, and matrix-matched standards of cassava-based, coconut-based, cowpea-based, groundnut-based, milk-based, tiger nut-based and yam-based) at six concentration levels, between LOQ and 100 times LOQ were prepared.

Matrix effects for each mycotoxin were calculated for each food commodity. The formula is defined as the percentage of the matrix-matched calibration slope (B) divided by the slope of the standard calibration in solvent (A) multiplied by 100. Table 1 shows the results obtained for coconut-based snack as a model sample. Type A and B trichothecenes, STER and BEA showed slightly signal enhancement, while signal suppression was observed for aflatoxins. Nevertheless, similar matrix effects were observed for cassava-based, cowpea-based, milk-based and yam-based, matrix effects ranged from 59 to 117%. On the other hand, tiger-nuts showed slightly higher signal suppression for aflatoxins in respect of first group. Therefore, external matrix-matched calibration was used for effective quantification for each particular sample.

Linearity was then evaluated as response and good linearity within LOQ and 100 times LOQ, and it was found with determination coefficients higher than 0.9902 in all the cases.

Trueness was evaluated through recovery studies for each snack sample. As an example, recoveries data are shown in Table 1. In this case, coconut-based snack was spiked at two concentration levels. The precision of the method, expressed as relative standard deviation (%RSD), was estimated by the repeated analysis ($n = 5$) of a spiked coconut-based snack at these two levels during the same day (intra-day) and on different five days (inter-day). The results obtained are shown in Table 1.

Table 1
LODs, LOQs, matrix effects (ME) (%), recoveries (REC.) (%) and precision (RSD) (%) obtained in coconut-based snack as model sample.

Compound	ME (%)	r^2	LOQ (ng g^{-1})	LOD (ng g^{-1})	Rec. (%)	RSD (%)	
						Intra-day	Inter-day
NIV	89	0.992	150	60	67	17	19
DON	105	0.991	130	45	71	15	14
3-ADON	110	0.994	13	4	73	14	12
15-ADON	98	0.995	15	5	73	13	15
FUS-X	98	0.992	50	15	81	16	16
DOM-1	114	0.993	35	12	78	15	17
NEO	90	0.997	30	10	83	10	11
DAS	121	0.993	25	8	84	15	13
HT-2	109	0.992	20	6	82	16	15
T-2	107	0.995	4	1.2	78	14	16
FB_1	88	0.995	70	25	95	12	14
FB_2	87	0.997	100	32	93	11	12
FB_3	78	0.991	90	30	95	16	15
ZEN	83	0.994	8	3	92	12	14
ZOL	83	0.993	18	6	94	13	15
BEA	111	0.997	1.8	0.5	102	9	8
AFB_1	67	0.995	0.15	0.05	72	15	10
AFB_2	64	0.996	1	0.3	77	14	15
AFG_1	63	0.993	1	0.25	75	15	14
AFG_2	69	0.994	1	0.35	76	12	11
AFM_1	71	0.996	0.3	0.1	78	17	18
STER	104	0.997	10	3.5	85	10	9
OTA	88	0.998	0.5	0.15	79	12	11

Recoveries obtained for coconut-based sample ranged from 71 to 102% for all mycotoxins assayed at concentration levels evaluated, except for NIV, which was 67%. Acceptable recoveries were therefore obtained throughout the developed QuEChERS method. It can be observed that repeatability, expressed, as RSD was lower than 17% for intra-day experiments and RSDs for inter-day precision were always lower than 19% for two spiked levels. For the other studied snack samples, recoveries ranged from 65 to 99% and RSDs were lower than 20%.

Table 1 also gives LODs and LOQs for target mycotoxins in coconut-based snack sample. The LODs ranged from 0.1 to 60 μg kg^{-1} and LOQs ranged from 0.25 to 150 μg kg^{-1} for AFB_1 and NIV, respectively. In all other snack samples, LOQ ranged from 0.2 to 180 μg kg^{-1} for AFB_1 and NIV, respectively.

Based on these obtained results, the QuEChERS-HPLC-QTRAP® method allowed the compliance of all the mycotoxins and snack matrices with the Commission Regulation No. 1881/2006 (EC, 2006) to be assessed, and the method was successfully validated according to the criteria specified in Commission Decision 2002/657/EC for quantitative confirmation method (EC, 2002). Furthermore, the specificity of the methods was demonstrated by the analysis of blank of each snack sample and spiked snack samples.

3.2. Occurrence of mycotoxins in commercialized snack samples

At the end, three mycotoxins could be detected; AFB_1, AFB_2 and α-ZOL. They were detected in 23.8% of the snack samples; the results are shown in Table 2. The samples that were positive for these mycotoxins belonged to three snack categories: cassava-based (*lafun*), groundnut-based (*donkwa* and *kulikuli*) and coconut-based (baked coconut and coconut candy).

In the *lafun* sample, only α-ZOL was detected at 11 μg kg^{-1}. This is the first time this metabolite or masked mycotoxin from ZEN is

Table 2
Frequency and concentration (μg kg^{-1}) of mycotoxins in street-vended snacks from Lagos, Nigeria.

	Snack type					
	Cassava-based ($n = 1$)		Coconut-based ($n = 3$)		Groundnut-based ($n = 8$)	
	Positives	Range	Positives	Range	Positives	Range
Aflatoxin B_1	n.d.	n.d.	1	<LOD–23	2	<LOD–19
Aflatoxin B_2	n.d.	n.d.	1	<LOD–3	1	<LOD–2
α-zearalenol	1	11	1	<LOD–54	n.d.	n.d.

n.d. Not detected.

detected in this food commodity; however ZEN has been detected in cereals and cereal-derivatives. For example, Njumbe Ediage, Di Mavungu, Goryacheva, Van Peteghem, and De Saeger (2012) detected ZEN in cassava flour at concentration levels lower than 12 μg kg^{-1}. The explanation may be that the mycotoxin has been metabolized during the processing or storage of the *lafun*. The absence of aflatoxins and fumonisins in the cassava-based snack is in accordance with previous report of Gnonlonfin, Hell, Fandohan, and Siame (2008, 2012), which showed that these mycotoxins did not contaminate cassava chips from Benin.

In this study, high levels of aflatoxins (26 μg kg^{-1}) were detected in baked coconut and α-ZOL (54 μg kg^{-1}) was detected in coconut candy. These results demonstrated that coconut and its product might be susceptible to mycotoxin contamination. Our data may therefore be useful in cases where desiccated coconut agar serves as the conventional medium for the detection of aflatoxigenic fungi and direct visual determination of aflatoxins. False positive results may be obtained if the coconut base is not properly screened for mycotoxin contamination.

Regarding tiger-nuts contamination, only one sample was contaminated with AFB_1 and AFB_2 at trace concentration level. These results are in line with previous works that evaluated the presence of mycotoxins in tiger-nut samples (Rubert et al., 2011; Sebastià et al., 2010).

The most contaminated samples were groundnuts. Groundnut is an important legume crop in many tropical and subtropical areas of the world, and mycotoxin contamination causes economic losses in its processing and exportation. Specifically, aflatoxins are the major mycotoxins that contaminate groundnuts and can affect their quality and that of theirs products (Juan, Zinedine, Moltó, Idrissi, & Mañes, 2008). All the studied groundnut-based samples ($n = 3$) were contaminated with AFB_1 at concentration levels ranging from <LOQ to 19 μg kg^{-1}. In 1988, AFB_1 was listed as a group I carcinogen by the International Agency for Research on Cancer (IARC) as it is a cause of human primary hepatocellular carcinoma (IARC, 2002). Two samples were contaminated also with AFB_2 (concentration levels lower than 2 μg kg^{-1}) and one of them contained STER, which is considered as the precursor of aflatoxins (Yabe & Nakajima, 2004).

The presence of fumonisins in one groundnut-based sample, although at trace concentration levels, was curious and should attract further investigations. Maize, rather than groundnut, has been established as the cereal most probably associated with fumonisins; however, there is also evidence that fumonisins occur in other crops. In this study, the presence of fumonisins in peanuts was may be a result of inadvertent interference (cross contamination) with other snacks during handling or transit.

4. Conclusion

This survey has shown that street-vended snacks in Lagos, Nigeria, especially the cassava-, coconut- and groundnut-based types are mainly contaminated by aflatoxins. This may pose a toxicological threat to consumers. Moreover, this manuscript has reported for the first time the occurrence of α-ZOL in cassava and coconut, and their derived-products.

Generally, the simultaneous extraction of many mycotoxins from different snacks could be challenging. However, the present approach using a modified QuEChERS has allowed 23 mycotoxins to be extracted simultaneously. The developed analytical method could extract target mycotoxins at low cost and low time consuming with increasing throughput; thus demonstrating the applicability and effectiveness of modified QuEChERS. Conclusively, a combined method involving a modified QuEChERS and HPLC-QTRAP® was a successful analytical method for the detection and quantitation of 23 mycotoxins from Nigerian snack samples.

References

Beltrán, E., Ibañez, M., Sancho, J. V., & Hernández, F. (2009). Determination of mycotoxins in different food commodities by ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry. *Rapid Communications in Mass Spectrometry, 23*, 1801–1809.

Eskola, M., Parikka, P., & Rizzo, A. (2001). Trichothecenes, ochratoxin A and zearalenone contamination and Fusarium infection in Finnish cereal samples in 1998. *Food Additives and Contaminants, 18*, 707–718.

EU. (2002). Commission decision 2002/657/EC of 12 of August 2002, implementing council directive (EC) 96/23 concerning the performance of analytical methods and the interpretation of the results (text with EEA relevance). *Official Journal of the European Communities, L221*(2002).

EU. (2006). Commission regulation (EC) no 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Official Journal of the European Communities, L70*(2006), 12–34.

Ezekiel, C. N., Kayode, F. O., Fapohunda, S. O., Olorunfemi, M. F., & Kponi, B. T. (2012). Aflatoxigenic moulds and aflatoxins in street-vended snacks in Lagos, Nigeria. *Internet Journal of Food Safety, 14*, 88–92.

Ezekiel, C. N., Sulyok, M., Warth, B., Odebode, A. C., & Krska, R. (2012). Natural occurrence of mycotoxins in peanut cake from Nigeria. *Food Control, 27*(2), 338–342.

Fakoor, J., Beheshti, S. S., Asadi, H. R., Mihanparast, M., & Feizy, S. (2012). Preliminary survey of aflatoxins and ochratoxin A in dried fruits from Iran. *Bulletin of Environmental Contamination and Toxicology, 88*, 391–395.

Fapohunda, S. O., & Ogundero, V. W. (1990). Physiology of fungi associated with fast foods in Nigeria. *International Biodeterioration, 26*, 23–32.

Fapohunda, S. O. (2010). *Impact of mycotoxins on sub-Saharan Africa: Nigeria as a case study*. http://www.mycotoxins.org/factsheet Accessed 20.11.12.

Fapohunda, S. O., Moore, G., Ganiyu, O. T., & Beltz, S. S. (2012). Toxigenic *Aspergillus flavus* and other fungi of public health concern in foods and organic matter in south west Nigeria Mycology. *An International Journal of Fungal Biology, 3*(3), 210–219.

Feng, Y., Tao, B., Pang, M., Liu, Y., & Dong, J. (2011). Occurrence of major mycotoxins in maize from Hebei Province, China. *Frontiers of Agriculture in China, 5*(4), 497–503.

Frenich, A. G., Romero-González, R., Gómez-Pérez, M. L., & Vidal, J. L. M. (2011). Multi-mycotoxin analysis in eggs using a QuEChERS-based extraction procedure and ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry. *Journal of Chromatography A, 1218*, 4349–4356.

Garrido Frenich, A., Martínez Vidal, J. L., Romero-González, R., & Aguilera-Luiz, M. M. (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 Chemistry, 117*, 705–712.

Gnonlonfin, G. J. B., Adjovi, C. S. Y., Katerere, D. R., Shephard, G. S., Sanni, A., & Brimer, L. (2012). Mycoflora and absence of aflatoxin contamination of commercialized cassava chips in Benin, West Africa. *Food Control, 23*, 333–337.

Gnonlonfin, G. J. B., Hell, K., Fandohan, P., & Siame, A. B. (2008). Mycoflora and natural occurrence of aflatoxins and fumonisin B1 in cassava and yam chips from Benin, West Africa. *International Journal of Food Microbiology, 122*, 140–147.

IARC. (2002). Some naturally occurring substances; food items and constituents, heterocyclic aromatic amines and mycotoxins. In IARC. (Ed.). *IARC (International Agency for Research on Cancer) Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 56 1993* (pp. 489–521), Geneve.

Ibañez-Vea, M., Corcuera, L. A., Remiro, R., Murillo-Arbizu, M. T., González-Perés, E., & Lizarraga, E. (2011). Validation of a UHPLC-FLD method for the simultaneous quantification of aflatoxins, ochratoxin A and zearalenone in barley. *Food Chemistry, 127*, 351–358.

Juan, C., Zinedine, A., Moltó, J. C., Idrissi, L., & Mañes, J. (2008). Aflatoxins levels in dried fruits and nuts from Rabat-Salé area, Morocco. *Food Control, 19*, 849–853.

Lesueur, C., Knittl, P., Gartner, M., Mentler, A., & Fuerhacker, M. (2008). Analysis of 140 pesticides from conventional farming foodstuff samples after extraction with the modified QuECheRS method. *Food Control, 19*, 906–914.

Njumbe Ediage, E., Di Mavungu, J. D., Goryacheva, I. Y., Van Peteghem, C., & De Saeger, S. (2012). Multiplex flow-through immunoassay formats for screening of mycotoxins in a variety of food matrices. *Analytical and Bioanalytical Chemistry, 403*, 265–278.

Park, J. W., Kim, E. K., Shon, D. H., & Kim, Y. B. (2002). Natural co-occurrence of aflatoxin B1, fumonisin B1 and ochratoxin A in barley and corn foods from Korea. *Food Additives and Contaminants, 19*, 1073–1080.

Roscoe, V., Lombaert, G. A., Huzel, V., Neumann, G., Melietio, J., Kitchen, D., et al. (2008). Mycotoxins in breakfast cereals from the Canadian retail market: a 3-year survey. *Food Additives and Contaminants, 25*, 347–355.

Rubert, J., Sebastià, N., Soriano, J. M., Soler, C., & Mañes, J. (2011). One-year monitoring of aflatoxins and ochratoxin A in tiger-nuts and their beverages. *Food Chemistry, 127*, 822–826.

Rubert, J., Dzuman, Z., Vaclavikova, M., Zachariasova, M., Soler, C., & Hajslova, J. (2012). Analysis of mycotoxins in barley using ultra high liquid chromatography high-resolution mass spectrometry: comparison of efficiency and efficacy of different extraction procedures. *Talanta, 99*, 712–719.

Rubert, J., James, K. J., Mañes, J., & Soler, C. (2012). Applicability of hybrid linear ion trap-high resolution mass spectrometry and quadrupole-linear ion trap-mass spectrometry for mycotoxin analysis in baby food. *Journal of Chromatography A, 1223*, 84–92.

Rubert, J., Soler, C., & Mañes, J. (2012). Occurrence of fourteen mycotoxins in tiger-nuts. *Food Control, 25*, 374–379.

Sebastià, N., Soler, C., Soriano, J. M., & Mañes, J. (2010). Occurrence of aflatoxins in tigernuts and their beverages commercialized in Spain. *Journal of Agricultural and Food Chemistry, 58*, 2609–2612.

Takino, M., Tanaka, H., & Tanaka, T. (2011). Multi mycotoxin analysis in food products using immunoaffinity extraction. *Methods in Molecular Biology, 747*, 259–266.

Yabe, K., & Nakajima, H. (2004). Enzyme reactions and genes in aflatoxin biosynthesis. *Applied Microbiology and Biotechnology, 64*(6), 745–755.