Food Control 25 (2012) 374–379

Contents lists available at SciVerse ScienceDirect

Food Control

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

Occurrence of fourteen mycotoxins in tiger-nuts

J. Rubert*, C. Soler, J. Mañes

Departament de Medicina Preventiva, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés, s/n 46100 Burjassot, Spain

ARTICLE INFO

Article history:
Received 21 July 2011
Received in revised form
30 October 2011
Accepted 5 November 2011

Keywords:
LC–MS/MS
Mycotoxins
MSPD
Occurrence
Tiger-nuts

ABSTRACT

A previous developed matrix solid-phase dispersion (MSPD) extraction method was applied for the routine analysis of aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FB_1 and FB_2), beauvericin (BEA), nivalenol (NIV), deoxynivalenol (DON), the toxin T-2 (T-2), toxin HT-2 (HT-2), diacetoxyscirpenol (DAS) and zearalenone (ZEN) in tiger-nuts by liquid chromatography–triple-quadrupole linear ion trap (HPLC–QTRAP®). The extraction solid support used was C_{18}, while the elution solvent was acetonitrile/methanol (50/50, v/v) 1 mM ammonium formate. Using matrix-matched calibration, recoveries and repeatabilities were in the range 67–89% and 2–11% relative standard deviation (RSD), respectively. The method was applied to determine the occurrence of the fourteen selected mycotoxins in a total of 83 tiger-nut samples purchased from different local markets of Valencian Community (Spain) during (March–June 2010 and March–May 2011). DON, OTA, AFs and BEA were detected in 26 samples of the total number of samples.

1. Introduction

Tiger-nuts, or "*chufa*", are consumed as by humans as by animals. In the case of animals, tiger-nuts are transformed in flour and added to the feed. Nevertheless, the main derivate product is "*horchata*" or "*tiger nut milk*". This beverage is a typical product from Valencia (Spain) which has a great national economic importance (Sánchez-Zapata et al., 2009) that makes necessary controls of it quality (Cortés, Esteve, Frıgola, & Torregrosa, 2005). In fact, this tuber has increased its production year to year: the annual production value of tiger-nut is approximately 5 million Euros (Consejo Regulador de la Denominación de Origen Chufa de Valencia, 2009).

These quality controls are important since tiger-nuts can contain physical, chemical and biological contaminants, such as stones, pesticides, bacteria and fungi. Regarding fungi, species such as *Fusarium* spp. and *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium citrinum* and *Rhizopus arrhizus* can growth in this tuber (HACCP guide to elaborate tiger-nut beverage). Moreover, although UE does not establish a regulation of mycotoxins in tiger-nut or its derivates, the presence of AFs and OTA have been demonstrated in tiger-nuts and their beverage in previous works (Rubert, Sebastià, Soriano, Soler, & Mañes, 2011; Sebastià, Soler, Soriano, & Mañes, 2010).

Apart from Valencia area, tiger-nuts are cultivated in Africa (Northern Nigeria, Ghana, and Togo) and India. These countries export several tons of tubers every year to other countries. It is important to keep in mind that mould contamination is strongly related to geographical area and climate; mycotoxins can be developed at various stages and under various conditions. This means that tiger-nuts of different areas can be contaminated by one or several different mycotoxins (Kroes et al., 2002).

The occurrence of such mycotoxins is of great concern because their presence in feeds and foods is often associated with chronic or acute mycotoxicosis in livestock and could threaten human health (Richard, 2007). Moreover, several mycotoxins are remarkably stable during processing and can be found in final products. Concentration may even increase during this processing.

For this reason, the aim of this study was to develop a sensible and specific analytical method expanding up the previous works, to determine AFs, OTA, ZEN, fumonisins FB_1 and FB_2, BEA, type A and B trichothecenes at concentration levels as lower as possible. Validated method was applied on monitoring programme under strict quality assurance: a total of 83 commercialized tiger-nuts were purchased during two years (2010–2011) from different local markets and cooperatives from Valencian Community (Spain).

2. Materials and methods

2.1. Chemical and reagents

HPLC grade acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). Sorbent used for MSPD was octadecy-silica (C_{18}-E) (50 μm) bonded silica from Phenomenex (Torrance, USA).

* Corresponding author. Tel.: +34 96 3543091; fax: +34 96 3544954.
E-mail address: josep.rubert@uv.es (J. Rubert).

0956-7135/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2011.11.004

The certified standards of AFB_1, AFB_2, AFG_1, AFG_2, OTA, ZEN, NIV, DON, DAS, FB_1 and FB_2, BEA were purchased from Sigma Aldrich (Madrid, Spain). T-2 and HT-2 toxin stock solutions (in acetonitrile) were obtained from Biopure referenzsubstanzen GmBH (Tulln, Austria). The individual stock solutions of AFs and OTA at 500 μg ml^{-1} were prepared in acetonitrile and ZEN, NIV, DON, FB_1, FB_2, BEA were prepared at the same concentration in methanol. On the other hand stock solutions of DAS, T-2 and HT-2 at 100 μg ml^{-1} were prepared in acetonitrile. The standards were kept in safety conditions at −20 °C.

All other working standard solutions were prepared immediately before use by diluting the stock solution with methanol/water (50/50, v/v).

2.2. Sampling

Sampling was carried out according to the EU guidance (EU, 2006). Samples of tiger-nuts were purchased from different local markets, supermarkets and cooperatives of Valencian Community (Spain). At the end, a total of 83 tiger-nuts samples were investigated. The samples were recollected during 2010 and 2011 seasons.

2.3. Extraction

Sample preparation was performed as described in a previous study (Rubert, Soler, & Mañes, 2011). Tiger-nut samples (200 g) were prepared using an Oster® food processor (Professional Series Blender model BPST02-B00) mixed thoroughly. Representative portions of 1 g (tiger-nut flour) were weighed and placed into a glass mortar (50 ml) and were gently blended with 1 g of C_{18} for 5 min using a pestle, to obtain an homogeneous mixture. The homogeneous mixture was introduced into a 100 mm × 9 mm i.d. glass column, and eluted dropwise with 15 ml of acetonitrile/methanol (50/50, v/v) 1 mM ammonium formate by applying a slight vacuum. Consequently, the extract was transferred to a 25 ml conical tube and evaporated to dryness at 35 °C with a gentle stream of nitrogen using a multi-sample Turbovap LV Evaporator (Zymark, Hopkinton, USA). The residue was reconstituted to a final volume of 1 ml with methanol/water (50/50, v/v) and filtered through a 13 mm/0.22 μm nylon filter purchased from Membrane solutions (Texas, USA).

For the preparation of fortified samples, 1 g of tiger-nut "blank" sample (it was corroborated before the analysis that no analytes were present) was spiked with 0.2 ml of working mixture of the mycotoxins at the appropriate concentration. Then, spiked samples were left to stand 3 h at room temperature before the extraction to allow the evaporation of the solvent and to establish equilibration between the mycotoxins and sample. Ten replicates were prepared for each spiking level.

2.4. Liquid chromatography–mass spectrometry analysis

LC–tandem MS analyses were carried out in 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™ source (ESI) interface. The QTRAP® analyzer combines a fully functional triple-quadrupole and ion trap mass spectrometer within on the same instrument. An extra confirmation tool, Information Dependent Acquisition (IDA), was carried out only for samples that contain the selected mycotoxins since the inclusion of this IDA experiment provides an unequivocal identification of mycotoxins in the matrix (Rubert, Soriano, Mañes, & Soler, 2011).

Separation of analytes was performed using a Gemini C_{18} (Phenomenex, 150 mm × 2 mm, 3 μm of particle size) analytical column preceded by a guard column with the same packing material. The flow rate was set to 0.250 ml min^{-1} and the oven temperature was 35 °C, being eluent A water (mobile phase A) slightly acidified with 0.1% of formic acid with 5 mM ammonium formate, and B (mobile phase B) methanol with 5 mM ammonium formate. The elution gradient started with 10% of eluent B, increasing to 70% in 1.5 min and kept as isocratic during 1.5 min. After this step, B was increased to 80% in 5 min. The last step was to increase 100% B in 10 min. During the further 8 min the column was re-equilibrated to the initial conditions. The volume to injection was of 20 μl.

The analyses were performed using Turbo-V™ source in positive mode. The operation conditions for the analysis in positive ionization mode were the followings: Ion spray voltage 5500 V, curtain gas 15 (arbitrary units), GS1 and GS2, 50 and 60 psi, respectively, probe temperature (TEM) 500 oC. Nitrogen served as nebulizer and collision gas. 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; entrance potential (EP) and collision cell exit potential (CXP) were set at 10 and 4 V, respectively for all analytes. The QTRAP® instrument was operated in SRM mode and with a resolution set to unit resolution for Q1 and Q3. For HPLC–MS/MS analysis, scheduled SRM (sSRM) was used with 60 s of SRM detection window and 1.5 s of target scan time. Analyst® version 1.5.2 software (AB Sciex) was used to control all components of the system and also for data collection and analysis. The MS/MS parameters optimized in this study are summarized in Table 1.

2.5. Validation study

The following parameters were evaluated in order to ensure the quality method: linearity, accuracy, precision, specificity, limits of quantification (LOQ), limits of detection (LOD) and q/Q ratios of the SRM transitions acquired, which were used for confirmation of

Table 1
Product-ions observed in product ion scan mode for selected mycotoxins and SRM optimized parameters.

Mycotoxin	Retention time	Precursor ion (*m/z*)	Product ion	DP (V)	CE (eV)	CEP
NIV	5.90	313.10	175.60^{Q}	50	21	20
		$[M+H]^+$	125.10^{q}		40	
DON	6.60	297.00	175.10^{Q}	36	81	18
		$[M+H]^+$	115.10^{q}		51	
DAS	8.05	384.05	105.00^{Q}	36	53	20
		$[M+NH_4]^+$	115.00^{q}		113	
HT-2	8.90	442.10	215.00^{Q}	31	19	18
		$[M+NH_4]^+$	105.00^{q}		57	
T-2	9.60	484.10	215.00^{Q}	36	23	20
		$[M+NH_4]^+$	185.00^{q}		27	
FB1	8.50	722.30	334.30^{Q}	101	51	26
		$[M+H]^+$	352.30^{q}		45	
FB2	10.50	706.30	336.30^{Q}	131	49	18
		$[M+H]^+$	318.30^{q}		51	
ZEN	10.90	319.10	301.10^{Q}	46	13	20
		$[M+H]^+$	187.10^{q}		25	
AFB_1	8.10	313.10	241.10^{Q}	76	43	22
		$[M+H]^+$	128.00^{q}		87	
AFB_2	7.95	315.10	259.60^{Q}	60	40	32
		$[M+H]^+$	288.60^{q}		40	
AFG_1	7.70	329.08	200.10^{Q}	81	53	22
		$[M+H]^+$	243.10^{q}		35	
AFG_2	7.55	331.10	217.60^{Q}	50	43	20
		$[M+H]^+$	189.60^{q}		43	
OTA	11.35	404.10	239.10^{Q}	60	40	14
		$[M+H]^+$	102.00^{q}		100	
BEA	16.90	801.40	244.20^{Q}	96	35	32
		$[M+NH_4]^+$	262.20^{q}		35	

Q: quantifier q: qualifier.