Food Control 39 (2014) 192–197

Contents lists available at ScienceDirect

Food Control

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

Co-occurrence of aflatoxins and ochratoxin A in spices commercialized in Italy

Ambra Prelle[a], Davide Spadaro[a,b,*], Angelo Garibaldi[a], Maria Lodovica Gullino[a,b]

[a] *Centre of Competence for the Innovation in the Agro-environmental Sector (AGROINNOVA), University of Turin, Grugliasco, TO, Italy*
[b] *DISAFA, Department of Agricultural, Forestry and Food Sciences, University of Turin, Grugliasco, TO, Italy*

ARTICLE INFO

Article history:
Received 28 June 2013
Received in revised form
4 November 2013
Accepted 9 November 2013

Keywords:
Aflatoxins
Ochratoxin A
Chili
Italy
Pepper

ABSTRACT

A total of 130 spice samples coming from India, China, South America, USA, Northern Africa, Europe and Sub-Saharan Africa were collected in different stores of Northern Italy. They were analysed for aflatoxins (AFs: AFB_1, AFB_2, AFG_1, AFG_2) and ochratoxin A (OTA) content by liquid chromatography with mass spectroscopy and positive electrospray ionization (LC/ESI-MS/MS), and HPLC with fluorescence detector (FLD), respectively. The analysis showed that 20 (15.4%) and 31 (23.8%) out of 130 samples were contaminated with AFs and OTA, respectively. A low level of total AFs contamination was found in the positive samples, the average concentration was 0.64 ng g^{-1}, far below the maximum threshold admitted by the European legislation (5 ng g^{-1} for AFB_1, and 10 ng g^{-1} for total aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2). A higher incidence of OTA was found in chili (60.0%) more than in pepper (13.3%), ranging from 2.16 to 16.35 ng g^{-1}, and from 1.61 to 15.85 ng g^{-1}, respectively. Moreover, three spice samples (2.3%) contaminated by OTA trespassed the threshold admitted by the European Regulation (EC, 2010). The co-occurrence of OTA and AFs in spices was detected in 6 out of 130 samples (4.6%), ranging from 1.61 to 15.85 ng g^{-1} and from 0.57 to 3.19 ng g^{-1}, respectively.

1. Introduction

Spices are widely used all over the world for food preparation, to increase the flavour and aroma, and also in the past as food preservative. Considering the global trade value, the most cultivated spice crops are pepper, capsicum, nutmeg, cumin and cinnamon. Spices are commercially produced in a relatively low number of countries. India is the important spice producer (74% of the world market), followed by Bangladesh (6%), Turkey (5%) and China (5%) (FAOSTAT, 2012).

The fungal growth and development on spices are favoured by environmental conditions, such as temperature and humidity, and by poor manufacturing conditions in the production region. Moulds decrease quality and quantity of food production, and may also create potential risk for human and animal health, due to the production of secondary metabolites, called mycotoxins. Some mycotoxins, like AFs and OTA, are produced by species of *Aspergillus* and *Penicillium*. These mould species can develop in post-harvest, during drying and storage, particularly when good storage practises are not adopted. Aflatoxins (AFs, sum of aflatoxins B_1, B_2, G_1, G_2) and ochratoxin A (OTA) can be found in several types of food commodities, including spices (Wangikar, Dwivedi, Sinha, Sharma, & Telang, 2005).

AFs are difuranocoumarin compounds primarily produced by certain species of *Aspergillus*: *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nomius*, and *Aspergillus tamarri* (Yabe, Nakamura, & Hamasaki, 1999). *A. flavus* and *A. parasiticus* are mainly producers of AFs: aflatoxin B_1 (AFB_1),B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2) (Varga, Frisvad, & Samson, 2011). Occurrence of AFs contaminations on several agricultural products, e.g. maize, wheat, rice, spices, dried fruits and hazelnuts, are worldwide reported (Grajewski, Blajet-Kosicka, Twaruzek, & Kosicki, 2012; Jackson, Kudupoje, & Yiannikouris, 2012; Prelle, Spadaro, Garibaldi, & Gullino, 2012). AFs have been clearly identified as toxic, mutagenic, teratogenic, and carcinogenic compounds. AFB_1 is the most potent carcinogenic compound found in nature (Castells, Marin, Sanchis, & Ramos, 2008). The International Agency for Research of Cancer (IARC, 1993) has classified AFB_1 as a human carcinogen (Group I). OTA is a mycotoxin primarily produced by some strains of *Aspergillus* belonging to the sections *Circumdati* and *Nigri*. In particular, it is produced in warm and tropical climates by *Aspergillus ochraceus*, and in temperate countries by *Penicillium verrucosum*. (Tittlemier,

---

* Corresponding author. DISAFA, Department of Agricultural, Forestry and Food Sciences, University of Turin, Via L. da Vinci 44, I-10095 Grugliasco, TO, Italy. Tel.: +39 011 6708942; fax: +39 011 6709307.
E-mail address: davide.spadaro@unito.it (D. Spadaro).

0956-7135/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2013.11.013

Varga, Scott, & Krska, 2011; Varga, Kevei, Rinyu, Teren, & Kozakiewicz, 1996). OTA is usually found in different foods, such as cereals, spices, coffee, wine and dried fruit (Coronel, Marin, Cano-Sancho, Ramos, & Sanchis, 2012; Spadaro, Lore, Garibaldi, & Gullino, 2010). OTA has been identified as nephrotoxic, hepatotoxic, immunotoxic and teratogenic, and classified in the group 2B by IARC (1993) as a possible carcinogen for humans.

Due to favourable conditions in tropical climates, both mycotoxins can co-contaminate some typologies of spices, not only in the field, but also during drying and storage.

The European Union fixed a maximum admitted level of 5 ng g^{-1} for AFB_1, and 10 ng g^{-1} for total AFs (AFB_1, AFB_2, AFG_1 and AFG_2) intended for chili, chili powder, paprika, white and black pepper, nutmeg, turmeric, ginger and spice mixtures containing one or more of the above-mentioned spices (European Commission, 2010). In the case of OTA, the maximum admitted level for the same spices listed before has been decreased from 30 μg kg^{-1} to 15 μg kg^{-1} since 1/7/2012 (European Commission, 2012).

Despite many papers reported the co-occurrence of AFs and OTA content in spices from different countries, such as Turkey (Ozbey & Kabak, 2012) and Malaysia (Jalili & Jinap, 2012), only one paper reported the AFs content in 28 spice samples marketed in Italy (Romagnoli, Menna, Gruppioni, & Bergamini, 2007).

In Italy, the consumption of spices is low (around 50%), compared to other European countries, such as France, Germany or Spain, but growing quickly, due to the recent immigration from extra-European countries and to the diffusion of ethnical restaurants (Dalpozzo, 2011).

The aim of this study was to analyse the co-occurrence of AFs and OTA in a large number of spice samples collected in different stores of Northern Italy. Two efficient and simple methods of extraction, purification and analysis were validated on several spices: AFs were simultaneous detected by liquid chromatography–tandem mass spectroscopy with electrospray ionization (LC/ESI-MS/MS), and OTA by liquid fluorescence detector (FLD).

2. Materials and methods

2.1. Chemicals and reagents

HPLC grade acetonitrile and LC–MS grade methanol, formic acid and acetic acid were purchased from Sigma–Aldrich (St Louis, MO, USA). AFs (AFB_1, AFB_2, AFG_1, AFG_2) and OTA analytical standards were purchased from Sigma–Aldrich, and dissolved in methanol and acetonitrile, respectively, to prepare working standard solutions at the concentration of 10 μg l^{-1}. All standard solutions were stored in the dark at 4 °C. NaCl, KCl, Na_2HPO_4, KH_2PO_4, polyethylene glycol (PEG), ammonium formate, $NaHCO_3$ and Tween 20 were purchased from Merck (Merck, Darmstadt, Germany). AflaClean select and OtaClean select immunoaffinity columns were obtained from LCTech (Dorfen, Germany). HPLC eluents were degassed for 5 min and filtered through mixed cellulose ester 0.22 μm-filters (Advantec MFS, Pleasanton, CA, USA) before use.

2.2. Samples

A total of 130 spices coming from India, China, South America, USA, Northern Africa, Europe and Sub-Saharan area were purchased randomly in North-Western Italy stores, from September 2011 to February 2012. Due to high different typologies of spices, samples were divided in: 30 samples of pepper, 25 of hot pepper and paprika, 21 of mixed spices (such as curry and food seasoning), and 54 other spices including 15 different types of spices (cinnamon, cloves, ginger, juniper, poppy seeds, coriander, fennel, vanilla, mustard, turmeric, nutmeg, sesame, cardamom, anise, dill).

Two hundred mg of each sample were stored in plastic bags, in the dark, at low relative humidity and 4 °C before the analysis. All samples, except powder spices, were pulverized using a food processor, until homogeneous. Spice sampling was done in accordance with sampling provision described on European regulation No 401/2006.

2.3. LC–MS/MS and HPLC apparatus

Liquid chromatography coupled with mass spectrometry was used to detect aflatoxins in spice samples and to confirm OTA absence in spices used for validation method. 1260 Agilent Technologies consisting of binary LC pump and a vacuum degasser; connected with a Varian autosampler Model 410 Prostar (Hansen Way, CA, USA) equipped a 100 μL loop was used as liquid chromatograph and was coupled to a triple quadrupole mass spectrometer Varian 310-MS. The analytical column used for LC separation was a Zorbax Eclipse Plus C18 (100 mm × 4.6 mm, 3.8 μm particle size, Agilent). The chromatographic conditions were: column temperature: 45 °C; mobile phase consisting of eluent A (water with 20 mM ammonium formate buffer at pH 3.35) and eluent B (methanol), using a flow rate of 0.3 ml min^{-1}. A gradient elution was applied as follows: 0–5 min, 50% A/50% B – 30% A/70% B; 5–10 min, 30% A/70% B – 10% A/90% B; 10–11 min, 10% A/90% B, and 11–15 min, 10% A/90% B – 50% A/50% B. Two minutes of post-run was applied. The injection volume was 10 μl.

The triple quadrupole mass spectrometer was operated in the positive electrospray ionization mode (ESI^+). The ionization source conditions were: needle voltage of 2.5 kV, capillary voltage of 60–77 V, source temperature of 50 °C, desolvation temperature of 350 °C, cone gas flow rate of 50 psi, desolvation gas flow rate of 50 psi with nitrogen. Multiple reaction monitoring (MRM) mode of operation was used. The $[M + H]^+$ ions of AFs were used as parent ions. The most intense daughter ions, resulting from collision-induced dissociation with argon, were used to detect and quantify AFs content. The argon pressure was set at 1.8 psi. The most intense daughter ions detected were: m/z 284.9 at 14 eV of collision energy (CE) for AFB_1, m/z 258.9 at 22 eV CE for AFB_2, m/z 242.9 at 18 eV CE for AFG_1, m/z 245 at 24 eV CE for AFG_2, m/z 358 at 18 eV CE and m/z 239 at 30 eV CE for OTA.

OTA was detected with an HPLC apparatus 1100 series Agilent equipped with G1311 quaternary pump, G1379 degasser, G1313A autosampler, G1316A column thermostat and G1321A FLD – Fluorescence Detector. The mobile phase consisted in an isocratic mixture of acetonitrile:water:acetic acid (49:49:2) for 15 min. Sample (30 μL) was injected into the analytical column Synergi 4u Hydro-RP (250 mm × 4.6 mm, Phenomenex) and detected using 333 and 460 nm as wavelengths for excitation and emission, respectively.

2.4. Aflatoxins extraction and clean up

In the first part of this study, concerning the validation of extraction method, two samples for each matrix, which confirmed to be aflatoxin free, were used as follows: one aliquot of the sample was analysed as such, whilst other aliquots were spiked with a known concentration of mycotoxin standard.

The method for AFs extraction from hazelnut, described in a previously published paper (Prelle et al., 2012), was used with slight modification. For 25 g of sample, 5 g of NaCl and 125 ml of extraction solution, methanol:water (80:20) were added, and left for 2 h on a shaker apparatus at 165 rpm. To eliminate the solid phase from the extraction solution, the sample was filtered, first, through a Whatman No. 4 filter paper, and subsequently by using a Whatman CA 0.45 μm syringe filter. A 10 ml aliquot of filtrate was diluted 1:4 in phosphate buffer solution (PBS) and centrifuged at