Food Control 37 (2014) 177–181

Contents lists available at ScienceDirect

Food Control

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

Fungal and aflatoxin contamination of marketed spices

Walid Hammami [a, 1], Stefano Fiori [b, 1], Roda Al Thani [a], Najet Ali Kali [d], Virgilio Balmas [b, c], Quirico Migheli [b, c, *], Samir Jaoua [a]

[a] Department of Biological & Environmental Sciences, College of Arts and Sciences, Qatar University, P.O. Box 2713, Doha, Qatar
[b] Dipartimento di Agraria, Università degli Studi di Sassari, Viale Italia 39, I-07100 Sassari, Italy
[c] Centro interdisciplinare per lo sviluppo della ricerca biotecnologica e per lo studio della biodiversità della Sardegna e dell'area mediterranea, Viale Italia 39, I-07100 Sassari, Italy
[d] Central Food Laboratory, Ministry of Public Health, P.O. Box 3381, Doha, Qatar

ARTICLE INFO

Article history:
Received 1 July 2013
Received in revised form
29 August 2013
Accepted 14 September 2013

Keywords:
Mycotoxin risk
Food safety
Aspergillus
Penicillium
Aflatoxin biosynthetic genes

ABSTRACT

Fourteen spice samples were collected from local markets in Doha, Qatar, during 2012, and were surveyed for the presence of potentially harmful mycoflora and for contamination with aflatoxins B_1, B_2, G_1, and G_2 by high-performance liquid chromatography (HPLC). Among the tested spice samples, chili powder showed the highest presence of fungal propagules, while ginger, curry and garlic samples did not present any fungal contamination. A total of 120 isolates, mostly belonging to *Aspergillus* and *Penicillium* genera, were collected and 33 representative species were identified by amplification and sequencing of the internal transcribed spacer (ITS) region. *Aspergillus flavus*, *Aspergillus nomius* and *Aspergillus niger* were the most dominant. Thirty-seven *Aspergillus* strains were screened for their potential to produce aflatoxins using biochemical and molecular tools: only 9 *A. flavus* strains showed both fluorescence and amplification with all the three primers targeting *aflP*, *aflM* and *aflR* genes. Aflatoxins were detected in five spices (black pepper, chili, tandoori masala. turmeric and garam masala), and with the exception of garam masala, the tested samples of turmeric, black pepper, tandoori masala and chili powder exceeded B_1 and/or total aflatoxin maximum levels. Our results demonstrate the potential for mycotoxin biosynthesis by fungi contaminating imported spice products.

1. Introduction

A wide array of spices are commonly used as seasoning in Qatar's traditional cuisine. Most of these spices are produced in countries with tropical climates where high temperature, humidity, and rainfall stimulate growth of fungi and contamination by mycotoxins (Martins, Martins, & Bernardo, 2001). Aflatoxins (AFs) are secondary metabolites which are of great concern because of their detrimental effects on human and animal health, including carcinogenic, mutagenic, teratogenic, and immunosuppressive effects (Eaton & Gallagher, 1994). These mycotoxins are produced mainly by *Aspergillus flavus* Link, *Aspergillus parasiticus* Speare and *Aspergillus nomius* Kurtzman, B.W. Horn & Hesselt (Rajasinghe, Abeywickrama, & Jayasekera, 2009).

The four main naturally-occurring aflatoxins are aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2. Among them, aflatoxin B_1 is the most common, as well as the most dangerous for its ability to cause liver cancer in human. Aflatoxins are heat-resistant and can withstand exposure to normal cooking temperatures and microwave treatment (Midio, Campos, & Sabino, 2001).

In recent years, the natural occurrence of aflatoxin in spices has been studied by several teams. A Qatari study on food products showed that chili powder and mixed spices powder contained an aflatoxin level of 69.3 μg/kg and 5.1 μg/kg, respectively (Abdulkadar, Al-Ali, Al-Kildi, & Al-Jedah, 2004). Romagnoli, Menna, Gruppioni, and Bergamini (2007) noted that, out of the 103 samples collected from Italian market and analyzed for aflatoxin content, 7 spice samples resulted positives: 5 chili-peppers, 1 nut meg and 1 cinnamon. Other researchers also detected very high levels of aflatoxin contamination in chilies (Yerneni et al., 2012).

Previous reports were focused on the fungal contamination in spices. *Aspergillus* (25 species) and *Penicillium* (7 species) were the predominant genera in 120 samples of 24 kinds of spices in Egypt (El-Kady, El-Maraghy, & Mostafa, 1992). *A. flavus*, *Aspergillus niger* Tiegh., *Aspergillus ochraceus* G. Wilh., *Aspergillus fumigatus* Fresen., *A. flavus* var. *columnaris* Raper & Fennell, *Aspergillus terreus* Thom, *Penicillium chrysogenum* Thom and *Penicillium corylophilum* Dierckx

* Corresponding author. Dipartimento di Agraria, Università degli Studi di Sassari, Viale Italia 39, 07100 Sassari, Italy. Tel.: +39 (0)79 229295; fax: +39 (0)79 229316.
E-mail address: qmigheli@uniss.it (Q. Migheli).
[1] The first two authors have equally contributed to the present work.

0956-7135/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2013.09.027

were the most common species. Ahene, Odamtten, and Owusu (2011) reported that *A. flavus* was the most frequently isolated species in all the spice products marketed in Ghana. *Aspergillus alutaceus* Berk. & M.A. Curtis (an ochratoxin producer) and *Fusarium verticillioides* (Sacc.) Nirenberg were also isolated. Most of these studies were carried out by using traditional methods, such as the observation of macro- or microscopic features, and the ability to grow on specific media, hence possibly leading to over- or underestimation of fungal species.

In the present study, we aimed at investigating the presence of fungal contamination in different spices available in the Qatari markets. A polymerase chain reaction (PCR)-based approach targeting both the Internal Transcribed Spacer (ITSI-5.8S-ITS2; ITS) region of the nuclear rRNA genes and three genes in the aflatoxin biosynthesis pathway was adopted in order to understand the relationship between fungal population and aflatoxin content.

Our results further emphasize the need to monitor the levels of mycotoxin contamination in susceptible commodities and to evaluate the health risk related to the consumption of food products that are largely used in domestic cooking.

2. Materials and methods

2.1. Sampling

The fourteen powdered spices used in this study were obtained from three Qatari local markets. These were: chili, kashmiri chili hot, kashmiri chili mild, basil, oregano, ginger, curry, cumin, turmeric, tandoori masala, garam masala, black pepper, garlic and coriander. One kilogram of each spice was sampled and stored at 4 °C until analysis.

2.2. Mycological examination

Total fungal counts were determined according to Samson, Hocking, Pitt, and King (1992). Ten grams of each spice were homogenized in 90 ml of sterile water for 2 min. Aliquots (100 μl) were plated onto sterile plastic Petri dishes containing solidified dichloran rose bengal chloramphenicol (DRBC) agar (Sigma, USA) and incubated at 28 °C for 5 days. After monospore isolation on potato dextrose agar (PDA) (Himedia, India), fungal cultures were preserved in sterile water at 4 °C until molecular identification (Nakasone, Peterson, & Jong, 2004).

2.3. Fungal genomic DNA extraction

To perform genomic DNA extraction, fungi were grown for 3–7 days at 28 °C in potato dextrose broth (PDB; Becton, India). Fungal mycelia were collected and ground in a mortar using liquid nitrogen. The DNA extraction was carried out with DNeasy Plant Mini Kit (QIAGEN, California, USA) according to the manufacturer's instructions. Five μl of each DNA sample were checked on a 1% agarose gel containing ethidium bromide (0.5 μg/ml) and visualized under ultraviolet light.

2.4. Fungal identification

Isolated strains were preliminarily grouped according to their morphological differences and one or two representatives from each of these groups was further analyzed molecularly by PCR and sequencing of the ITS region as described below.

2.4.1. ITS1-ITS4 amplification

The 5.8S ribosomal DNA region (600 bp) of the selected isolates was amplified using universal primers ITS1 and ITS4 (White, Bruns, Lee, & Taylor, 1990). Each PCR reaction was performed using 12.5 μl of Taq PCR Master Mix 2X (QIAGEN, California, USA), 0.5 μl of both the forward and reverse primers (10 pmol), 5–10 ng DNA template and ultrapure H_2O up to 25 μl. The thermocycler was set as follows: initial denaturation at 94 °C for 5 min; 35 cycles of 30 s at 94 °C for denaturation, 30 s at 54 °C for annealing and 30 s at 72 °C for elongation, followed by a final elongation step of 5 min at 72 °C. To check the amplified DNA, 5 μl of each reaction were loaded on a 1% agarose gel containing ethidium bromide (0.5 μg/ml) and visualized under ultraviolet light.

2.4.2. PCR product purification and sequencing

PCR products were purified with Pure-Link PCR Purification Kit (Life Technologies California, USA) according to the manufacturer's instructions, and their concentration was then determined with a Qubit® 2.0 Fluorometer (Life Technologies California, USA). Twenty ng of DNA template and 6.4 pmol of either ITS-1 or ITS-4 primer were collected into sequencing tubes, dried in the themocycler at 60 °C and sent to BMR-Genomics company of the University of Padova (Italy) for the sequencing service. Sequences were then blasted in the NCBI database to provide species identification.

2.5. Determination of aflatoxin levels in spices

Aflatoxin contamination level was determined in the Central Food Laboratory (Ministry of Public Health, Doha, Qatar) using a high performance liquid chromatography (HPLC). Forty g of spice powder were mixed with 150 ml of acetonitrile: water (60:40) with a high speed laboratory blender for 3 min. After filtration with Whatman No. 1 filter, 2 ml of the filtrate were diluted with 48 ml of phosphate buffer saline (PBS, pH 7.4). The solution was injected into the immuno-affinity column (R-Biopharm RHONE, Glasgow, Scotland) which is prior condition by 15 ml phosphate buffer saline, at a flow rate of 0.5 ml/min. The column was then washed with 10 ml of distilled water, dried in an air stream and, finally, elution was carried out with 1.5 ml of methanol passing slowly through the immuno-affinity column. Methanol was then evaporated on a water bath (at about 70° C) under steam of nitrogen until dried completely. The residue was resuspended with 200 μl of hexane and 50 μl of trifluoroacetic acid in a 4 ml vial and the solution was vortexed for 30 s. After the addition of 1.95 ml of acetonitrile: water (1:9), reaction was allowed by mixing for 30 s and then 50 μl of the sample solution (aqueous layer) were injected on HPLC after passing through 0.22 μm syringe filter. The mobile phase consisted of acetonitrile: water : methanol (180:640:180) while the flow rate was set at 1 ml/min. The HPLC column used was a Nova pack C18: 4 μm, 150 × 3.9 mm (Waters, Milford, USA). Mycotoxin standards (aflatoxin B_1, B_2, G_1 and G_2) were purchased from Sigma, Germany. From the stock solution (1000 ppb for B_1 and B_2; 300 ppb for G_1 and G_2), a mixed standard solution was prepared by serial dilutions of water: acetonitrile (9:1) aiming to get the concentration of 0.5–50 ppb of B_1 and G_1 and 0.15–15 ppb of B_2 and G_2 from an initial stock concentrations of 1000 ppb for B_1 and G_1 and 300 ppb for B_2 and G_2.

2.6. Fluorescence detection

Coconut agar medium (CAM) was used for rapid detection of aflatoxin synthesis (Lin & Dianese, 1976). Briefly, one hundred g of shredded coconut were homogenized for 5 min with 300 ml of hot distilled water. The homogenate was filtered through four layers of cheesecloth, and the pH of the clear filtrate was adjusted to pH 7 with 2 N NaOH. After agar addition, the medium was autoclaved. The plates were inoculated with PDA plugs of *Aspergillus* strains and then incubated at 28 °C for 3–5 days. The reverse side of the plates