Food Control 34 (2013) 115–120

Contents lists available at SciVerse ScienceDirect

Food Control

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

Mycobiota and identification of aflatoxin gene cluster in marketed spices in West Africa

G.J.B. Gnonlonfin [a,*], Y.C. Adjovi [b], A.F. Tokpo [b], E.D. Agbekponou [c], Y. Ameyapoh [c], C. de Souza [c], L. Brimer [d], A. Sanni [b]

[a] Biosciences, Eastern and Central Africa, International Livestock Research Institute, P.O. Box 30709, Old Naivasha Road, Nairobi, Kenya
[b] Biochemistry and Molecular Biology Laboratory, Faculty of Sciences and Techniques, University of Abomey-Calavi, 04 P.O. Box 0320, Cotonou, Benin
[c] Microbiology and Food Safety Control Laboratory, High School of Biology and Food Technique, University of Lome, P.O. Box 1515, Lome, Togo
[d] Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Ridebanevej 9, DK-1870 Frederiksberg C, Denmark

ARTICLE INFO

Article history:
Received 17 December 2012
Received in revised form
5 April 2013
Accepted 12 April 2013

Keywords:
Dried milled products
Natural contamination
Fungi
Aflatoxin gene cluster
Aflatoxin B1
West Africa

ABSTRACT

Fungal infection and aflatoxin contamination were evaluated on 114 samples of dried and milled spices such as ginger, garlic and black pepper from southern Benin and Togo collected in November 2008–January 2009. These products are dried to preserve them for lean periods available throughout the year. Fungal contamination was evaluated after plating on selective media with a total of 20 fungal genera identified, ranging from 7 in garlic to 14 in ginger. Ginger and pepper showed high incidence of fungal contamination compared to garlic that had lower levels of fungal contamination. Species of *Aspergillus* were dominant on all marketed dried and milled spices irrespective of country. Gene characterization and amplification analysis showed that most of the *Aspergillus flavus* isolates possess the cluster genes for aflatoxin production. Aflatoxin B1 assessment by Thin Layer Chromatography showed that only garlic (1 sample) and ginger (4 samples) were naturally contaminated with aflatoxin B1 ranging from 390 μg/kg to 1045 μg/kg respectively. Previous reports have mostly highlighted the risk of mycotoxin exposure from staple crops and vegetables in Africa, but such risks now need to be evaluated further for other products such as dried and milled spices.

Crown Copyright © 2013 Published by Elsevier Ltd. All rights reserved.

1. Introduction

Postharvest spoilage by filamentous fungi is one of the most important threats associated with processed and stored food products worldwide. Discolouration, quality deterioration, reduction in commercial value and production of secondary metabolites (mycotoxins) has been linked to mouldy contaminated foods. The warm and humid climates that prevail in Tropical Africa provide favourable conditions for the spread and subsequent establishment of these organisms in numerous foodstuffs (Shephard, Thiel, Stockenstrom, & Sydenham, 1996). The availability of safe food is a prerequisite for the well-being of people and the development of national economies. The low quality and safety of foods in Africa have a significant impact on human and animal health, and are a major constraint to export trade (Manjula, Hell, Fandohan, Abass, & Bandyopadhyay, 2009).

Spices are produced, dried, sold in market and considered as major ingredients in food preparation in West Africa. Drying of foods is practiced in Africa to make the products more durable and preserve them for food insecure periods. Drying is mainly done on an artisanal scale or through small-scale industrial units. Dried products can be infected with fungi already present on the primary product, or contaminating during the drying process that takes place under unhygienic conditions; further spoilage can occur during storage, handling and transport till sale (Mandeel, 2005). Crops or products that are susceptible to fungal growth can also be contaminated with mycotoxins (Bankole & Mabekoje, 2004). Mycotoxin contamination of staple foods in sub-Saharan Africa is detrimental to health, trade and development. Mycotoxins such as aflatoxin are highly carcinogenic, hepatotoxic, mutagenic, teratogenic and are correlated with immunosuppression, reduced nutrient absorption and stunting of infants, and are fatal in high doses (Gong et al., 2002; Rahimi, Bonyadian, Rafei, & Kazemeini, 2010).

In Bahrain red chilli and black pepper were described to show high levels of fungal infestation with 1580 and 1120 CFU/g,

* Corresponding author. Tel.: +254 705593239.
E-mail addresses: bgnonlonfin@yahoo.fr, b.gnonlonfin@cgiar.org (G.J.B. Gnonlonfin).

0956-7135/$ – see front matter Crown Copyright © 2013 Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2013.04.021

respectively (Mandeel, 2005). On dry 'tatase' peppers (*Capsicum annum* L.) seven mould species were isolated with *Aspergillus niger*, *Aspergillus flavus* and *Geotrichum candidum* being the dominant ones, but no aflatoxins were detected (Atanda, Akando, & Afolabi, 1990). Spices are highly susceptible to mycotoxin development with average contamination levels of 0.09, 0.63, 2.88 and 0.03 μg/kg of aflatoxin B1 detected in black pepper, ginger, red paprika and cumin, respectively (McKee, 1995). In red paprika, Zinedine et al. (2006) reported higher levels of 9.68 μg/kg aflatoxin B1.

Researchers investigated the mycoflora and nutritional value of shelled melon seeds (*Citrulus vulgaris* Schard.) in Nigeria and studied potential drying options that will enhance product quality (Bankole, Osho, Joda, & Enikuomehin, 2005). Aflatoxin B1 was detected at levels above 5 μg/kg in 32.2% of the samples, while only 3.5% contained toxin above the 20 μg/kg Nigerian tolerance level for food (Bankole, Ogunsanwo, Osho, & Adewuyi, 2006). Natural occurrence of mycotoxins and fungal contamination on dried vegetables was investigated in many countries (Alghalibi & Shater, 2004; Begum, Lokesh, & Kumar, 2005; Hell, Gnonlonfin, Kodjogbe, Lamboni, & Abdourhamane, 2009; Mandeel, 2005; Nutsugah, Vibeke, Atokple, & Ayensu, 2004).

There is, however, lack of scientific information on the colonized fungi diversity and aflatoxin gene cluster presence in the isolated toxigenic *A. flavus* when it comes to spices. The objectives of this study were to (i) determine the mycoflora and aflatoxin B1 contamination of dried spices from these countries, (ii) to determine the presence of 5 genes from the aflatoxin gene cluster that plays a key role in aflatoxin production in isolated *A. flavus* that contaminate marketed spices from Benin and Togo.

2. Materials and methods

2.1. Samples locations and field methods and tools

The study was conducted in the southern part of Benin and Togo. In Benin samples came from the forest mosaic savannah (Fandohan, Gnonlonfin, Hell, Marasas, & Wingfield, 2005). In this zone dried spices are sold and consumed by the population. Four main markets were randomly selected for the survey and sampling. In Togo samples were randomly collected in the southern economic regions (Hell et al., 2009).

During the survey focus group discussions were conducted. Six focus group sessions were held with 10 sellers in each country. The findings from the focus groups were used to formulate questions used in a questionnaire. Then individual interviews (approximately 10 min) on spices production process, the monthly income generated from the sale of spices and assessed the level of consumption of these products were performed.

The survey was conducted with the help of interpreters where needed. Two types of questions were included in the questionnaire. Fixed choice questions were those where the respondents had to choose one item among several alternatives or questions for which the expected response was "yes" or "no". Examples: Do you produce spice for your family own consumption only? What is your principal activity? Open-ended questions were those which allowed the informants to express their opinion. Examples: How many types of spices do you sale? and their origin?

2.2. Dried spices

Overall 114 samples (1000 g) of dried and milled ginger (39), garlic (35), and black pepper (40) were examined. The latter black pepper fruit, known as a peppercorn when dried contains a single seed. Peppercorns, and the powdered pepper derived from grinding them is described simply as pepper, or more precisely as black pepper (cooked and dried unripe fruit). Dried ground pepper has been used since antiquity for both its flavour and as a medicine. Black pepper is the world's most traded spice, and is produced from the still-green unripe drupes of the pepper plant. The drupes are cooked briefly in hot water, both to clean them and to prepare them for drying. The heat ruptures cell walls in the pepper, speeding the work of browning enzymes during drying. The drupes are dried in the sun for several days, during which the tissue around the seed shrinks and darkens into a thin, wrinkled black layer. Once dried, the spice is called black peppercorn.

Each commodity was collected in Benin and Togo in 2008–2009. All samples were kept in refrigerator at 4 °C till fungal and mycotoxin analysis.

2.3. Chemical and reagents

All reagents and solvents used were of LC grade supplied by Merck (Darmstadt, Germany). LC grade water was obtained by filtering deionized water through a 0.45 μ filter with a Waters Millipore (Milford, MA, USA) system. Solvents and water were degassed for 20 min using ultrasonic bath (Model EIA CP104, Italy). Mix aflatoxin (B1 = G1 = 0.5 μg/mL, B2 = G2 = 0.15 μg/mL) was purchased from Sigma Chemicals (St. Louis, MO). Taq DNA polymerase used for molecular characterization was purchased from Invitrogen (Carlsbad, CA, USA). Agarose gel and primers used for genes determination were purchased from Eurobio (Courtaboeuf, France).

2.4. Moisture content

Moisture content was determined by heating a specific amount of product at 105 °C for 2 h to constant weight and reweighing (AOAC, 1995).

2.5. Isolation of fungi

Fungal genera were enumerated using the dilution plate method described by Pitt and Hocking (1997). Ground samples (10 g each) were thoroughly mixed with 90 mL of sterile water containing 0.1% peptone water to make the 10^{-1} dilution. Further serial dilutions to 10^{-4} dilution were made with 0.1% peptone water. Aliquots (1.0 mL) of each dilution were then transferred to Petri dishes containing Dichloran Chloramphenicol 18% Agar (DG 18) (Oxoid Ltd., Hampshire, UK) and incubated at 25 °C in alternating 12-h periods of fluorescence light and dark during 7 days.

Total number of fungal isolates from each product was recorded after microscopic observation. Both macro and microscopic features of each individual colony were used. A loop full of pure isolates cultured on PDA were sub-cultured on malt extract agar (MEA) (Oxoid Ltd., Hampshire, UK) for identification. The MEA plates were incubated at 25 °C for 7 days. Identification of fungi was based on gross morphology and microscopic features such as spores and fruiting structures using Pitt and Hocking (1997).

2.6. Gene characterization

A. flavus isolates genes were characterized by extracting the DNA and perform a polymerase chain reaction for genes identification using the method described by Melo, Pungartnik, Cascardo, and Brendel (2006). Genes amplification were by using the method described by Rodrigues et al. (2007). Gene markers *aflD*, *aflO*, *aflP*, *aflR*, *aflQ* (Sigma Aldrich, UK) were used and their specifications are as following (Table 2).

Download English Version:

https://daneshyari.com/en/article/6392713

Download Persian Version:

https://daneshyari.com/article/6392713

Daneshyari.com