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Distribution and variation of fungi and major mycotoxins in pre- and post-nature drying maize in North China Plain

Fuguo Xing[*, 1], Xiao Liu [1], Limin Wang, Jonathan Nimal Selvaraj, Nuo Jin, Yan Wang, Yueju Zhao, Yang Liu[**]

Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences /Key Laboratory of Agro-Products Processing, Ministry of Agriculture, Beijing 100193, PR China

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ABSTRACT

Forty-four pre- and post-nature drying maize kernels were collected from North China Plain and assessed for fungi infection and mycotoxins contamination. The percentage of fungi infection was significantly higher in pre-nature drying samples than post-nature drying samples except for *Fusariuim graminearum*, which increases from 6.06% to 24.09%. *Fusarium*, *Aspergillus*, *Alternaria* and *Trichoderma* were main genera. *Fusarium verticillioides* (24.77%) and *F. graminearum* (15.08%) were predominant species, followed by *Aspergillus niger* (7.51%) and *Aspergillus flavus* (4.93%). FB_1 and DON were the major mycotoxins presented in the samples, followed by ZEN. All samples showed FB_1 ranging from 16.5 to 315.9 μg/kg. All post-nature drying maize kernels showed DON ranging from 5.8 to 9843.3 μg/kg, while 7 of 22 pre-nature drying samples contaminated with DON ranging from 50.7 to 776.6 μg/kg. The samples contaminated with ZEN in pre- and post-nature drying maize were 3, with the content ranging from 60.5 to 147.6 μg/kg and from 40.7 to 1056.8 μg/kg, respectively. Only 1 sample contaminated with AFB_1 of 148.4 μg/kg. The occurrence of mycotoxins is highly in accordance with the incidence of the corresponding mycotoxin-producing fungi. This is the first comprehensive comparison of fungi infection and mycotoxins contamination between pre- and post-nature drying maize kernels.

1. Introduction

Maize (*Zea mays* L.) is one of the most widely grown cereal crops, which is a staple food in many regions around the world, and large quantity of maize is produced each year than any other grain (International grains council market report, 2013). The United States produces 40% of the world's maize harvest and other top producing countries include China, Brazil, Mexico, Indonesia, India, France and Argentina. Global production was 963 million tons in 2014, which is more than wheat (719 million tons) and rice (495 million tons). In China, the annual maize production was 216 million tons (data from National Bureau of Statistics of China) which was more than rice (206 million tons) and wheat (126 million tons). In China, approximately 70% of produced maize is used as livestock feed. In addition, maize is increasingly used as a raw material for ethanol production and produces a greater quantity of biomass than other cereal plants, which is used for fodder.

The Food and Agriculture Organization (FAO) of the United Nations estimated that approximately 25% of the cereal-based foods produced in the world are contaminated with mycotoxins produced by filamentous fungi as secondary metabolites as they contaminate the foods (Smith et al., 1994). After discovering the hazardous nature of aflatoxin in early 1960s, many countries began conducting surveys on the occurrence of mycotoxins in their agricultural products in last few decades (Cui et al., 2013; Selvaraj et al., 2015). Some key mycotoxins that cause huge health issues and agricultural loss are aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins and ergot alkaloids. These mycotoxins are mainly produced by the *Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria* genera. The simultaneous presence of different mycotoxins in a single commodity produced by different fungal genera is not uncommon (Aresta, Cioffi, Palmisano, & Zambonin, 2003). Among the grains, maize is highly contaminated with many fungi and the subsequent mycotoxins.

* Corresponding author.
** Corresponding author.
E-mail addresses: xingfuguo@caas.cn (F. Xing), liuyang01@caas.cn (Y. Liu).
[1] These authors contributed equally to this work.

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Numerous fungi infect maize, approximately 19 genera of fungal species, among them *Fusarium*, *Aspergillus*, *Alternaria, Penicillium* and *Stenocarpella* were the main genera (Payne, 1999). These fungi produce mycotoxins that can cause toxic and/or carcinogenic effects in humans and animals. *Fusarium* species produce many mycotoxins such as zearalenone, fumonisins and trichothecenes. Occurrences of *Fusarium* contamination on maize in temperate and semi-tropical areas are very common as they cause ear rot, which affects the crop yield to a greater extent. *Aspergillus* species can cause the food to spoil leading to enormous economic loss. Furthermore, some *Aspergillus* species can produce toxic secondary metabolites: like aflatoxins and ochratoxins which affect the food safety (Marín, Ramos, Cano-Sancho, & Sanchis, 2012).

Recent studies show that change in climatic trends may pose longer-term impacts on the distribution of fungi, the occurrence of mycotoxins and host crop plants (Wu et al., 2011). However, apart from weather parameters, agronomic factors such as drying methods and time may affect the nature and degree of mycobiota contamination and the occurrence of mycotoxins in the grains (Storm, Sørensen, Rasmussen, Nielsen, & Thrane, 2008). Drying makes it easy for farmers to bring down the moisture content to a safe level as a result, the produce, with sufficiently low water content restrains fungal invasion, spore germination and fungal growth.

The aim of this study was to evaluate the mycoflora and the occurrence of main mycotoxins in maize before and after drying. As fungal variation mainly occurs during drying process, it was assumed that the effective control of fungi must be taken, which influences the growth of toxigenic fungi and the production of mycotoxins during post-harvest period.

2. Materials and methods

2.1. Chemicals

High performance liquid chromatography (HPLC) grade methanol and acetonitrile were purchased from Fisher Scientific (Fisher Chemicals HPLC, USA). Aflatoxin B_1 (AFB_1), Zearalenone (ZEN), Deoxynivanol (DON) and Fumonisin B_1 (FB_1) standards were procured from Sigma-Aldrich Chemicals (USA). ToxinFast immunoaffinity columns for AFB_1 and ZEN were purchased from HuaanMagnech Bio-tech (Beijing, China); Mycosep™ 227 columns were from Romer Labs, Inc., (USA).

2.2. Sample collection and preparation

A total of 44 maize samples were collected from the local farmers of three major maize production provinces (Shandong, Hebei and Henan) in North China Plain in the autumn of 2014. The samples were taken from eight areas such as Southwestern (Nanyang), Southeastern (Linyi), Central (Liaocheng, Heze, Zibo, Xingtai and Handan), and Northern (Langfang) regions of North China Plain. The amount of samples from each place is 2 or 3 according to the maize yield. The detailed samples information was presented in Table 1. Of 44 maize samples, 22 samples of fresh maize without drying treatment in the October were collected, which were named as pre-drying samples. After 2 months, other 22 samples were collected again from the same group of farmers. For each sample, at least ten incremental samples of 100 g each were taken and combined, according to EC 401/2006 norms (European Commission, 2006). All samples were taken up immediately for analyzing the mycobiota. Each 200 g of a sample was ground to a fine powder and stored at −20 °C until further detection of four major mycotoxins.

Table 1
The detailed samples information of maize.

Sample	City	Province
1	Heze	Shandong
2		
3	Liaocheng	Shandong
4		
5		
6	Zibo	Shandong
7		
8		
9	Xingtai	Hebei
10		
11		
12	Handan	Hebei
13		
14		
15	Langfang	Hebei
16		
17		
18	Nanyang	Henan
19		
20		
21	Linyi	Shandong
22		

2.3. Determination of water activity (a_w) and moisture content

The a_w and moisture content of kernels of maize were determined by automatic analysis, using Aqualab 4TE (Decagon Devices, Pullman, WA, USA).

2.4. Recovery, identification and enumeration of the mycoflora from kernels of maize

From each sample, approximately 30 g subsamples of maize kernels was disinfected by immersion in 1% sodium hypochlorite solution for 3 min, followed by three rinses with sterile distilled water. After disinfection, 60 kernels were randomly separated and directly seeded onto 5 Petri dishes (bottom diameter: 90 mm, 6 kernels per dish) containing Potato Dextrose Agar (PDA) and 5 Petri dishes (6 kernels per dish) containing Dichloran 18% Glycerol Agar (DG18) for the isolation of internal mycoflora (Pitt & Hocking, 2009). The plates were incubated at 25 °C for 5 days, but the observations were made daily. The fungi grew from maize kernels internal. The average percentage of kernels infected with fungi was then calculated.

Speciation of isolated fungal strains was performed according to Tournas, Rivera Calo, and Sapp (2013) with minor modifications. Recovered isolates was purified by re-culturing on PDA and identified to genus or species level using the conventional methods and keys described in “Fungi and Food Spoilage” (Pitt & Hocking, 2009), “Introduction to Food- and Airborne Fungi” (Samson, Hoekstra, & Frisvad, 2004, pp. 283–297), “Identification of Common *Aspergillus* species” (Klich, 2002), “*Fusarium* Species: An Illustrated Manual for Identification” (Nelson, Touson, & Marasas, 1983) and “A Laboratory Guide to Common *Penicillium* Species” (Pitt, 1985). Isolates which are not identified at the species level by different conventional plating methods were identified using molecular method as described below.

2.5. Identification of isolated fungal strains using molecular method

To extract genomic DNA, fungal isolates were grown in YES liquid medium inoculated with 1×10^5 spores. After cultivating at 200 rpm (28 °C) for 5 days, mycelia were collected by filtration through Whatman filter paper, and washed with distilled water.