JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Simultaneous Determination of Masked Deoxynivalenol and Some Important Type B Trichothecenes in Chinese Corn Kernels and Corn-Based Products by Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry

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ABSTRACT: A total of 969 corn kernels and corn-based products collected from 24 provinces in China between 2008 and 2011 were analyzed for deoxynivalenol, deoxynivalenol 3-glucoside, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol by ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Deoxynivalenol was the predominant mycotoxin detected. A total of 29 out of 969 samples (corn kernels: 9/289, mean = 1884 μg/kg; corn-based products: 20/680, mean = 1580 μg/kg) contain deoxynivalenol at the levels exceeding the Chinese regulatory limit of 1000 μg/kg for deoxynivalenol in corn. The average relative concentration ratios (%) for deoxynivalenol 3-glucoside/deoxynivalenol for all four years were 25% ± 5% in corn kernels and 34% ± 4% in corn-based products. The results of this study indicate that it is necessary to include deoxynivalenol 3-glucoside in both risk assessment of deoxynivalenol and its derivatives and development of the tolerance limit for deoxynivalenol in Chinese corn kernels and corn-based products.

KEYWORDS: *deoxynivalenol, deoxynivalenol 3-glucoside, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, corn kernels, corn-based products*

INTRODUCTION

The genus *Fusarium* has been commonly associated with many crop diseases such as ear and kernel rot of corn, scab of wheat and rice, and grain mold infection of sorghum. A number of species of this genus have been reported to produce *Fusarium* mycotoxins. Trichothecenes mainly deoxynivalenol, nivalenol and their esters, and an estrogenic metabolite zearalenone are produced primarily by *F. graminearum* and *F. culmorum* and coincidently occur in cereal crops. The invasion of genus *Fusarium* and contamination with trichothecenes in cereals results in serious economic losses for the grain industry and pose animal and human health problems.[1] The presence of these toxins in foods and feeds is considered to be an important food safety problem. Of great concern is that a number of outbreaks of intoxication in humans and animals have been indicated from consumption of foods or feeds contaminated with these toxins.[2]

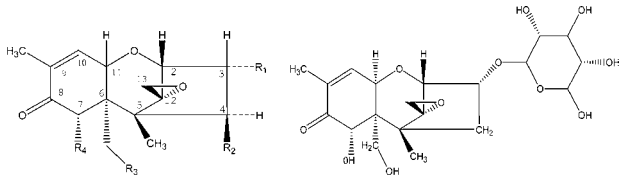
Deoxynivalenol is the most common trichothecenes frequently detected in cereals and the most important causative toxicant responsible for human red mold intoxications. This compound can lead to human food poisoning outbreaks in China with vomiting as the primary symptom associated with *Fusarium*-infested cereals.[3−5] Co-occurrence of deoxynivalenol with its derivatives 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol and other *Fusarium* toxins such as zearalenone, nivalenol, T-2 toxin, and HT-2 toxin in cereals is frequently observed.[7] Many outbreaks of human *Fusarium* mycotoxicoses due to the consumption of foods contaminated with trichothecenes were reported in the former USSR, India, and China.[2] According to the incomplete statistical figures, a total of 53 outbreaks of human food poisoning caused by scabbed cereals occurred and more than 145 000 people were affected during the period between 1960 and 1991 in China.[8] The biggest outbreak of human red mold intoxication, which involved 130 141 victims, occurred in the Anhui province of China in 1991. The most important causative toxicants responsible for these intoxications were trichothecenes, especially deoxynivalenol.[3−5] Epidemiological investigations indicated that clinical observations did not correlate with the low deoxynivalenol levels determined in the corresponding foods and feeds implicated in the human and animal red mold intoxications.[6] This fact suggests that unaltered deoxynivalenol might not be the only source of the health hazard for consumers. Undetected, conjugated forms of mycotoxins that could hydrolyze to the precursor toxins in the digestive tracts of animals may occur in the cereals. The conjugated or masked mycotoxins first received attention in the 1980s.[9,10]

Deoxynivalenol 3-glucoside was first described as a plant conjugate of deoxynivalenol, in the 1980s.[9,10] Studies have

Received: September 6, 2012
Revised: October 31, 2012
Accepted: November 2, 2012
Published: November 2, 2012

dx.doi.org/10.1021/jf3038133 | J. Agric. Food Chem. 2012, 60, 11638−11646

reported that certain intestinal bacteria are able to cleave deoxynivalenol 3-glucoside converting it back to deoxynivalenol.[11] This compound was chemically synthesized by deoxynivalenol-treated corn cell suspension cultures and reported for the first time in naturally contaminated wheat and corn in 2005.[12] Recently, deoxynivalenol 3-glucoside was also found in naturally contaminated barley as well as in barley-based products like malt and beer.[13] Therefore, deoxynivalenol 3-glucoside is regarded as potentially hazardous for human and animal health. Its tentative structure is shown in Figure 1.



Deoxynivalenol, **1**: R_1 = -OH, R_2 = -H, R_3 = -OH, R_4 = OH

3-acetyl-deoxynivalenol, **2**: R_1= -OAc, R_2= -H, R_3= -OH, R_4= -OH

15-acetyl-deoxynivalenol, **3**: R_1= -OH, R_2= -H, R_3= -OAc, R_4= -OH,

Figure 1. Chemical structure of important type B trichothecene and tentative deoxynivalenol 3-glucoside.

A few studies on co-occurrence of parent deoxynivalenol and masked deoxynivalenol in corn-based products have been reported in the world but there are few data from China so far.[11,13,14,18,20−23] The purpose of this study is to evaluate the characterization and distribution of the masked mycotoxin deoxynivalenol 3-glucoside together with some important type B trichothecenes in corn kernels and corn-based products collected in the years 2008−2011 in China. The results obtained from our study will be expected to contribute to ongoing research on the relationship between the consumption of cereals contaminated with deoxynivalenol 3-glucoside and the human red mold intoxications in China. Furthermore, the results will provide some scientific information to include deoxynivalenol 3-glucoside in the group of deoxynivalenol and its derivatives in both risk assessment and development of a tolerance limit for deoxynivalenol in foods.

MATERIALS AND METHODS

Chemicals and Reagents. Stock standard solutions for deoxynivalenol 3-glucoside and deoxynivalenol and its esters, including 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol, in acetonitrile with the concentrations of 49.6 mg/L, 100 mg/L, 101.1 mg/L and 104.0 mg/L, respectively, were purchased from Biopure (Tulln, Austria) and stored at −20 °C. A composite working standard solution of all four analytes at the concentration of 100 μg/L was stored at 4 °C in the dark. All organic solvents including methanol and acetonitrile used for both sample extraction and ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis were of HPLC grade and purchased from Fisher Scientific. Ammonia was of analytical grade and obtained from Beijing Chemical Reagent Company (Beijing, China). Tap water was directly purified into Milli-Q quality water with the conductivity greater than 18.2 MΩ cm at 25 °C by a Millipore water purification system (Millipore, Bedford, MA).

Corn samples. A total of 969 corn kernels and corn-based products (including corn flour, corn grit, and corn flakes) samples were collected between 2008 and 2011 from 24 provinces in China by visiting randomly selected farmers' houses or bought on the retail market. Among these, 203, 404, 215, and 147 samples were obtained in the years 2008, 2009, 2010, and 2011, respectively. Sample information is given in Table 1. Sampling the selected provinces accounts for more than 90% of the population in China, and corn was mainly cultivated and consumed in these regions. All samples were produced locally and either intended (kernels) or directly used (corn-based products) for human consumption. The samples were taken with sampling spears and scoops. Three incremental samples were drawn from each of the top, middle, and bottom layers of the container (five sampling sites for each layer). Multiple subsamples (ca. 500 g) were pooled, mixed thoroughly, and quartered. Opposite quarters were rejected and the remainder remixed to obtain a final reduced sample of 250 g. Samples were maintained in zip lock plastic bags or sterilized kraft paper bags, encoded, and stored at 4 °C until analysis. In the case of corn kernels, half of the entire composite was finely ground, and a 20 g test portion was taken for analysis.

Table 1. Information on Samples Used in the Present Study

	no. of samples		
harvest year	corn kernels	corn-based products[a]	total
2008	203	–	203
2009	20	384	404
2010	60	155	215
2011	6	141	147
Total	289	680	969

[a]Corn-based products include corn flakes, corn flour, and corn grit.

Sample Preparation and Cleanup. The four mycotoxins deoxynivalenol 3-glucoside, deoxynivalenol, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol were extracted and cleaned up by the method described previously.[14] Briefly, 20 g of corn samples were extracted with 80 mL acetonitrile−water (84/16, v/v) for 60 min at 200 rpm/min on an orbital shaker or sonicated for 1 h; both methods gave the same recoveries. The extracts were filtered and divided into two portions. One 10 mL portion was passed through a MycoSep 226 Aflazon+ multifunctional column (Romer Laboratories, Tulln, Austria) and 4 mL of the purified extract was evaporated to dryness under a nitrogen stream. The residue was dissolved in 1 mL of methanol−water (40/60, v/v), centrifuged at 10 000 rpm/min, and analyzed by LC-MS/MS for deoxynivalenol, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol. Another aliquot of 1 mL of filtrate was centrifuged at 10 000 rpm/min followed by filtering through a 0.2 μm nylon membrane filter, ready for deoxynivalenol 3-glucoside analysis by LC-MS/MS.

UPLC Conditions. Detection and quantitation of the four target mycotoxins were performed on a Micromass Quattro Premier XE LC-MS/MS system. The UPLC system was an Acquity ultraperformance liquid chromatography (Waters, Milford, MA). The column used for separation of the four analytes was a 100 × 2.1 mm i.d., 1.7 μm, BEH C_{18} (Waters) thermostatted at 40 °C. The mobile phase included 0.1% aqueous ammonia in water (solvent A) and acetonitrile (solvent B). The linear gradient program started from A/B (95/5, v/v) and reached A/B (55/45, v/v) in 5 min. Afterward, A was linearly decreased to 5% (v/v) within 1 min and maintained at this composition of mobile phase for 1 min. Finally, the initial rate of A and B parts (95/5, v/v) was re-established within 0.1 min followed by a 2.9 min conditioning step. The flow rate was 0.3 mL/min, and the injection volume was 5 μL. The sample temperature was maintained at 5 °C.

MS/MS Conditions. MS/MS was performed on an UltimaTM Micromass-Quattro Pt triple-quadrupole mass spectrometer equipped with an electrospray ionization source (Waters). The mass spectrometer was operated in negative electrospray ionization (ESI^-) mode. The capillary voltage was set at 2.8 kV. The voltages of both RF lens 1 and RF lens 2 were 20 and 1.0 V, respectively; the source block temperature was 100 °C, and the desolvation temperature was 350 °C. Nitrogen was used as a desolvation gas with a flow rate of 550 L/h. The collision gradient was 1.5 and collision gas pressure was 3.0×10^{-3} mbar. The most intense product

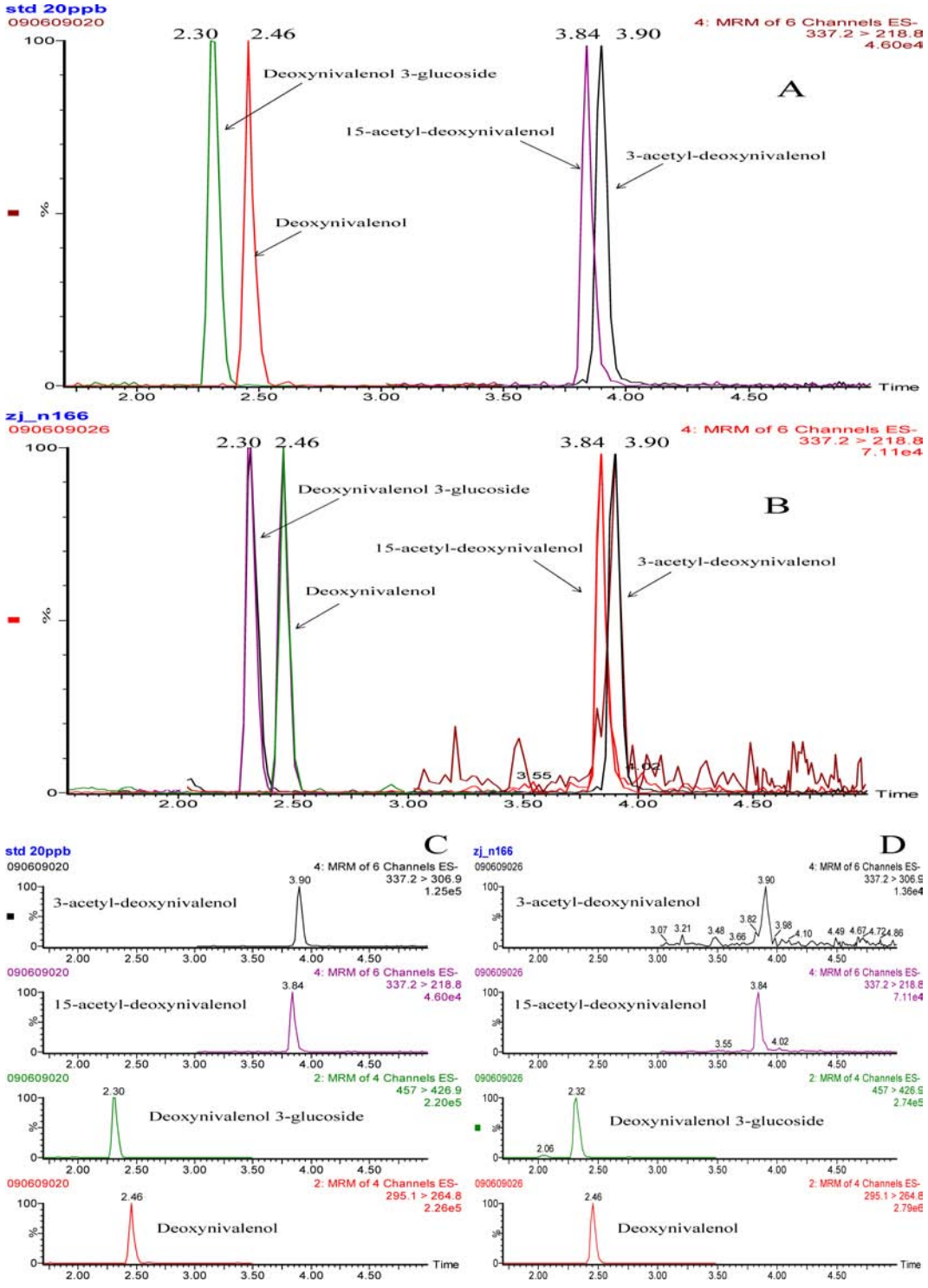


Figure 2. Negative ion total ion chromatograms (TIC) of (A) four trichothecene standards, (B) trichothecene standards in a corn sample, (C) standards at a concentration of 20 μg/kg, and (D) corn samples containing deoxynivalenol 3-glucoside, deoxynivalenol, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol at the respective concentrations of 102, 853, 43, and 145 μg/kg collected from the Zhejiang province in 2009.

ion was employed as the quantifying ion, and the less intense signals were used as qualifying ions for confirmation of toxin identity. In addition, abundance ratios of multiple reaction monitoring (MRM) transitions, as well as the chromatographic retention time, enabled definite confirmation of the toxins presence.

The MS/MS conditions for the determination of four mycotoxins are all in the negative ionization mode. The parent ions (m/z) of deoxynivalenol, deoxynivalenol 3-glucoside, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol are 295.1, 457.4, 337.2, and 337.2, respectively. The quantitative daughter ions, as well as collision energy (eV, put in the parentheses), are 264.8 (10) for deoxynivalenol, 426.9 (13) for deoxynivalenol 3-glucoside, 306.9 (8) for 3-acetyl-deoxynivalenol, and 218.8 (10) for 15-acetyl-deoxynivalenol, respectively, while the qualitative daughter ions (collision energy, eV) are 137.6 (14), 276.9 (14), 172.6 (10), and 277.0 (8), respectively, for deoxynivalenol, deoxynivalenol 3-glucoside, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol.

dx.doi.org/10.1021/jf3038133 | J. Agric. Food Chem. 2012, 60, 11638−11646

Table 2. Natural Occurrence of the Four Mycotoxins in Corn Kernels and Corn-Based Products between 2008 and 2011

		years						
		2008	2009		2010		2011	
statistical parameters	toxins	corn kernels	corn kernels	corn products	corn kernels	corn products	corn kernels	corn products
range/average/ median (μg/kg)	deoxynivalenol 3-glucoside	3−499/ 66/36	3−93/ 23/13	3−844/ 76/28	3−495/73/22	3−128/ 26/13	40978/6/7	3−39/11/7
	deoxynivalenol	2−4374/ 283/95	0.4−519/ 93/33	0.3−2803/ 249/122	0.3-2149/314/157	0.3−1828/ 170/87	21−376/ 119/77	1−360/ 56/32
	3-acetyl-deoxynivalenol	1−368/ 19/7	0.3−3/1/1	0.3−45/3/2	0.3−59/7/3	0.3−105/7/2	0.3−2/1/1	0.3−16/2/1
	15-acetyl-deoxynivalenol	2−1734/ 156/49	1−95/ 29/18	0.3−831/ 68/32	0.3−465/58/10	0.3−1519/ 50/11	6−65/28/20	1−158/ 24/12
	deoxynivalenol 3-glucoside/ deoxynivalenol (%)[a]	1−700/ 42/19	5−700/ 99/21	2−1600/ 61/27	2−81/16/11	2−1600/ 0.48/0.11	1−13/ 0.07/0.08	3−200/ 0.22/0.13
	deoxynivalenol 3-glucoside/ deoxynivalenol (%)[b]	1−441/ 27/12	3−460/ 64/13	1−1034/ 39/17	1−52/11/7	1−1002/ 31/7	1−9/5/5	2−154/ 14/9
	deoxynivalenol 3-glucoside/4 toxins(%)[c]	1−100/ 13/12	4−50/ 16/14	1−100/ 19/18	2−100/16/9	1−99/10/8	1−11/4/6	3−64/ 12/10
Detection Rate (%)	deoxynivalenol 3-glucoside	33(68/203)	60(12/20)	86(332/384)	65(39/60)	64(99/155)	83(5/6)	54(77/141)
	deoxynivalenol	51 (103/203)	90(18/20)	97(371/384)	82(49/60)	91(141/155)	100(6/6)	95 (134/141)
	3-acetyl-deoxynivalenol	35(72/203)	55(11/20)	73(281/384)	57(34/60)	64(99/155)	33(2/6)	22(31/141)
	15-acetyl-deoxynivalenol	48(97/203)	80(16/20)	92(353/384)	90(54/60)	88(137/155)	67(4/6)	52(74/141)

[a]Deoxynivalenol 3-glucoside absolute to the deoxynivalenol (%) = [(Deoxynivalenol 3-glucoside$_{conc}$)/(Deoxynivalenol$_{conc}$)] × 100. [b]Deoxynivalenol 3-glucoside relative to the deoxynivalenol (mol, %) = [(Deoxynivalenol 3-glucoside$_{conc}$ × $M_{Deoxynivalenol}$)/($M_{Deoxynivalenol\ 3\text{-}glucoside}$× Deoxynivalenol$_{conc}$)] × 100. [c]Deoxynivalenol 3-glucoside/four toxins (%) where the four toxins include deoxynivalenol 3-glucoside, deoxynivalenol, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol.

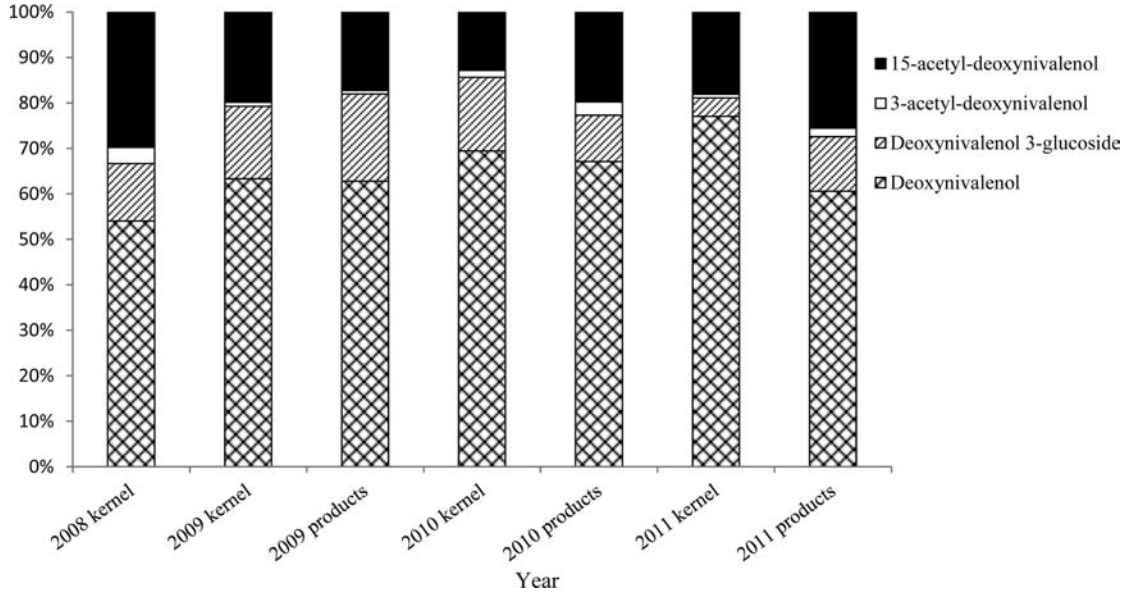


Figure 3. Distribution of the four mycotoxins in corn kernels and corn-based products.

Method Validation. The matrix-matched calibration curve was made in order to minimize the matrix interference. Standard solutions of the four mycotoxins were added to mycotoxin-free corn sample extract residue at levels equivalent to 1, 5, 10, 20, and 50 μg/kg for deoxynivalenol, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol but 5, 10, 100, and 600 μg/kg for deoxynivalenol 3-glucoside, reconstituted with 1 mL of methanol/water (40/60, v/v) and analyzed by LC-MS/MS. The limit of detection (LOD) and the limit of quantitation (LOQ) for these four mycotoxins were determined with signal-to-noise ratios of 3:1 and 10:1, respectively.

The analytical method was validated by spiking corn samples with the four mycotoxins at three different levels with triplicate analyses conducted for each level. Deoxynivalenol, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol at spiking levels of 1, 5, and 20 μg/kg gave recoveries between 89% and 107% for deoxynivalenol, 93% and 113% for 3-acetyl-deoxynivalenol, 89% and 112% for 15-acetyl-deoxynivalenol, with the respective coefficients of variation (CV) between 1.90% and 9.38% for deoxynivalenol, 2.36% and 6.23% for 3-acetyl-deoxynivalenol, and 2.24% and 6.51% for 15-acetyl-deoxynivalenol. However, mean recoveries determined from triplicate analyses of samples spiked with deoxynivalenol 3-glucoside standard at concentrations of 20, 100, and 600 μg/kg were in the range of 70%−90% with CV values of 1.65%−5.93%.

To determine the within-day method repeatability, the mycotoxin-free corn samples were spiked with the four mycotoxin standards at a concentration of 20 μg/kg each, and extraction, purification, and determination by LC-MS/MS repeated 6 times within a day. Meanwhile, the spiked sample was analyzed once a day for 5

successive days to determine between-day repeatability. The results indicated that the relative standard deviation ranged from 1.82% to 5.42% for intraday and from 4.71% to 11.06% for interday, respectively.

RESULTS AND DISCUSSION

Natural Occurrence of Deoxynivalenol 3-glucoside in Corn Kernels and Corn-Based Products between 2008 and 2011. The extraction and cleanup of deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, and deoxynivalenol 3-glucoside from corn kernels and corn-based products samples resulted in minimal matrix interference. The four toxins were identified by comparing their retention time and the mass spectra with corresponding standards via LC−MS/MS and determined quantitatively. Under LC-MS/MS conditions, very good resolution for the four mycotoxins in corn samples was observed, and their spectra were similar to the corresponding standards (Figure 2). The concentrations of these four mycotoxins in Chinese corn samples analyzed in the present study are given in Table 2.

It was observed in Figure 3 that corn samples were contaminated by deoxynivalenol with the highest detection frequencies of 51% (corn kernels, 103/203) for the year 2008, 90% (corn kernels, 18/20) and 97% (corn-based products, 371/384) for the year 2009, 82% (corn kernels, 49/60) and 91% (corn-based products, 141/155) for the year 2010, and 100% (corn kernels, 6/6) and 95% (corn-based products, 131/141) for the year 2011. Meanwhile, deoxynivalenol 3-glucoside was found along with deoxynivalenol in 68 out of 203 (33%) corn kernels in 2008, 12 out of 20 (60%) corn kernels and 332 out of 384 (86%) corn-based products in 2009, 39 out of 60 (65%) corn kernels and 99 out of 155 (64%) corn-based products in 2010, and 5 out of 6 (83%) corn kernels and 77 out of 141 (65%) corn-based products in 2011.

Additionally, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol were detected at a lower frequency in 2008 corn kernels (3-acetyl-deoxynivalenol: 35%, 15-acetyl-deoxynivalenol: 48%) and 2011 (33% and 67% for corn kernels and 22% and 52% for corn-based products), compared with 2009 (55% and 80% for corn kernels, and 73% and 92% for corn-based products) and 2010 (57% and 90% for corn kernels and 64% and 88% for corn-based products). The average frequencies of deoxynivalenol and 3-acetyl-deoxynivalenol in the four years of samples obtained in this study were higher than those reported previously[15,16] but similar to those reported recently.[17,18] With the exception of samples from 2008, the frequency of deoxynivalenol 3-glucoside in samples collected between 2009 and 2011 was similar to those reported by Berthiller et al.[18]

As for the contamination levels of the four mycotoxins, deoxynivalenol was the predominant toxin detected with the average level of 283 μg/kg in corn kernels of 2008, 93 μg/kg in corn kernels and 249 μg/kg in corn-based products of 2009, 314 μg/kg in corn kernels and 170 μg/kg in corn-based products of 2010, and 119 μg/kg in corn kernels and 56 μg/kg in corn-based products of 2011. Compared with the results from Northern Italy,[19] similar average and maximum concentrations of deoxynivalenol were observed in the four years of samples analyzed in the present study. A total of 29 out of 969 samples (corn kernels: 9/289, mean =1884 μg/kg; corn-based products: 20/680, mean =1580 μg/kg) were positive for deoxynivalenol at the levels exceeding the Chinese regulatory limit of 1000 μg/kg for deoxynivalenol in corn intended for human consumption.

Regarding toxins other than deoxynivalenol, moderate concentration of deoxynivalenol 3-glucoside and 15-acetyl-deoxynivalenol, as well as a lower level of 3-acetyl-deoxynivalenol, were found in corn samples. Deoxynivalenol 3-glucoside was detected at concentrations in the range of 3−499 μg/kg (mean = 66 μg/kg and median = 36 μg/kg) in corn kernels of 2008, 3−93 μg/kg (mean = 23 μg/kg and median = 13 μg/kg) in corn kernels and 3−844 μg/kg (mean = 76 μg/kg and median = 28 μg/kg) in corn-based products of 2009, 3−495 μg/kg (mean = 73 μg/kg and median = 22 μg/kg) in corn kernels and 3−128 μg/kg (mean = 26 μg/kg and median = 13 μg/kg) in corn-based products of 2010, and 3−10 μg/kg (mean = 6 μg/kg and median = 7 μg/kg) in corn kernels and 3−39 μg/kg (mean = 11 μg/kg and median = 7 μg/kg) in corn-based products of 2011. It is worth pointing out that the mean concentration of deoxynivalenol 3-glucoside in samples of 2008, 2009, and 2010, but not 2011, were almost the same as those reported by Li et al.[20] but much lower than those of Berthiller et al.[18] For most of the naturally contaminated samples, the deoxynivalenol 3-glucoside concentration was higher than that of 3-acetyl-deoxynivalenol but similar to or lower than that of 15-acetyl-deoxynivalenol, depending on the harvest year (Table 2). 3-Acetyl-deoxynivalenol was present at the lowest level in comparison with the other three toxins, with the highest concentration of 368 μg/kg in corn kernels of 2008, 3 μg/kg in corn kernels and 45 μg/kg in corn-based products of 2009, 59 μg/kg in corn kernels and 105 μg/kg in corn-based products of 2010, and 2 μg/kg in corn kernels and 16 μg/kg in corn-based products of 2011. As for 15-acetyl-deoxynivalenol, it was detected at the highest level of 1734 μg/kg in corn kernels of 2008, 95 μg/kg in corn kernels and 831 μg/kg in corn-based products of 2009, 465 μg/kg in corn kernels and 1519 μg/kg in corn-based products of 2010, and 65 μg/kg in corn kernels and 158 μg/kg in corn-based products of 2011.

Proportion of Deoxynivalenol 3-glucoside to Deoxynivalenol Concentration. As shown in Table 2, the proportion of deoxynivalenol 3-glucoside relative to the deoxynivalenol concentration varied depending on the harvest years and ranged from 1% to 441% (average: 27% ± 7% and median: 12%) in corn kernels of 2008, from 3% to 460% in corn kernels (average: 64% ± 39% and median: 13%) and 1% to 1034% in corn-based products (average: 39% ± 5% and median: 17%) of 2009, from 1% to 52% in corn kernels (average: 11% ± 2% and median: 7%) and 1% to 1002% in corn-based products (average: 31% ± 12% and median: 7%) of 2010, and from 1% to 9% in corn kernels (average: 5% ± 1% and median: 5%), and 2% to 154% in corn-based products (average: 14% ± 3% and median: 9%) of 2011. In total, the average proportion of deoxynivalenol 3-glucoside relative to deoxynivalenol was 25% ± 5% in corn kernels and 34% ± 4% in corn-based products for four-year pooled samples, much higher than those reported in Austria (average: 14% ± 8%).[18] In terms of the samples from each individual harvest year, the highest proportion of deoxynivalenol 3-glucoside relative to deoxynivalenol concentration in corn kernels and corn-based products obtained in this study were much higher than those in Austrian products (46%), with the exception of corn kernel samples from 2010 and 2011. The average relative proportion of both toxins was higher in the 2008 corn kernels, the 2009 corn-based products, and the 2010 samples but almost the same in the 2009 corn kernels and in all of the 2011 samples, in comparison to the Austrian results.[18]

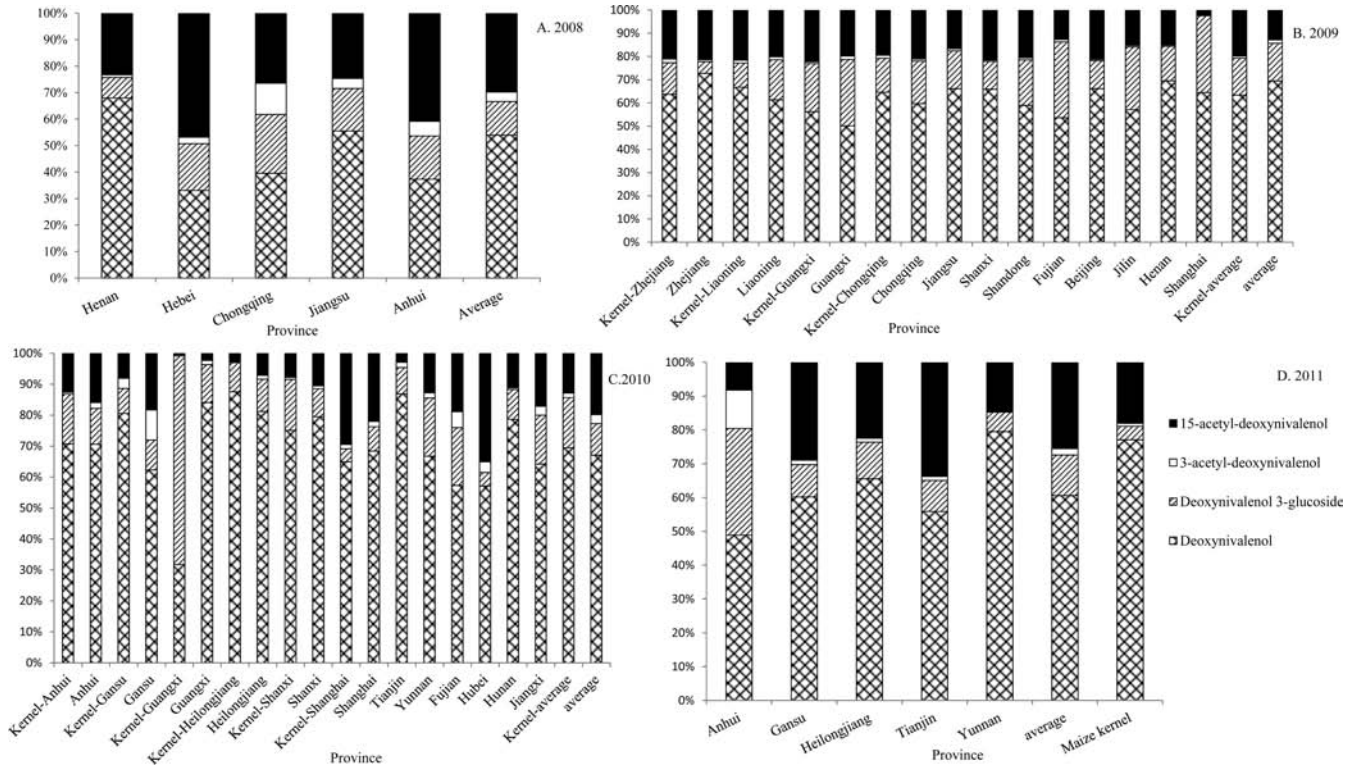


Figure 4. Distribution of the four mycotoxins in corn kernels and corn-based products from different provinces in China.

Regarding the proportion of deoxynivalenol 3-glucoside absolute to actual deoxynivalenol concentration, it differed from year to year at the average of 42% (range: 1%−700% and median: 19%) in corn kernels of 2008, 99% (range: 5%−700% and median: 21%) in corn kernels and 61% (range: 2%−1600% and median: 27%) in corn-based products of 2009, 16% (range: 2%−81% and median: 11%) in corn kernels and 48% (range: 2%−1600% and median: 11%) in corn-based products of 2010, and 7% (range: 1%−13% and median: 8%) in corn kernels and 22% (range: 3%−200% and median: 13%) in corn-based products of 2011. It should be noted that the proportion of conjugated deoxynivalenol absolute to free deoxynivalenol concentration was less than 1 in most samples analyzed, but higher values of deoxynivalenol 3-glucoside/deoxynivalenol ≥ 1 in 36 samples (4, 2, 21, 5, and 4 samples from 2008 corn kernels, 2009 corn kernels, 2009 corn-based products, 2010 corn-based products, and 2011 corn-based products, respectively) were observed. These results are consistent with those reported by Sasanya[21] but different from those previously described elsewhere.[18,22]

Distribution of Deoxynivalenol and Its Derivatives in Four-Year Samples from Different Regions. Concerning the contamination of deoxynivalenol, deoxynivalenol 3-glucoside, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol in the four-year samples examined, deoxynivalenol was the predominant toxin detected in all of the samples with an average concentration accounting for 54%, 64% (63%), 69% (67%), and 77% (60%) of the total four toxins in the 2008 corn kernels, 2009 corn kernels (corn-based products), 2010 corn kernels (corn-based products), and 2011 corn kernels (corn-based products), respectively, followed by 15-acetyl-deoxynivalenol and deoxynivalenol 3-glucoside at 30% and 13% for 2008 corn kernels; 20% and 16% for 2009 corn kernels, 17% and 19% for 2009 corn-based products; 13% and 16% for 2010 corn kernels, 20% and 10% for 2010 corn-based products; and 18% and 4% for 2011 corn kernels, 26% and 12% for 2011 corn-based products. 3-Acetyl-deoxynivalenol accounted for only a small fraction of the total four toxins (within a range of 1% to 4%, Figures 3 and 4). A total of 619 samples (120 corn kernels and 499 corn-based products) were positive for both deoxynivalenol and deoxynivalenol 3-glucoside. Among these, deoxynivalenol 3-glucoside was coincidentally detected along with deoxynivalenol at both lower frequency and level in 583 (114 corn kernels and 469 corn-based products) samples analyzed. On average, the deoxynivalenol 3-glucoside concentration was 3 times (in the range between 1 and 7) higher than that of deoxynivalenol in 6 out of 289 (2%) corn kernels and 5 times higher (in the range between 1 and 16 times) than that of deoxynivalenol in 30 out of 680 (4%) corn-based product samples. With exception, 2 samples of deoxynivalenol-free corn kernel samples from Guangxi contained deoxynivalenol 3-glucoside at high levels of 222 μg/kg and 268 μg/kg. Both 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol in these two samples were negative. In terms of the geographical distribution of mycotixins, levels of the four mycotoxins in corn kernels and corn-based product samples from South China (such as Guangxi, Chongqing, Anhui, Jiangsu, Fujian, etc.) where high temperature, humidity, and precipitation were observed, were higher than those from North China.

It should be emphasized that the natural occurrence of deoxynivalenol 3-glucoside was positively correlated with that of deoxynivalenol in the 4 years of corn samples with correlation coefficients of 0.339, 0.994 (0.791), 0.452 (0.541), and 0.438 for 2008 corn kernels, 2009 corn kernels (corn-based products), 2010 corn kernels (corn-based products), and 2011 corn-based products (Figure 5), respectively. It is worth pointing out that a positive correlation between deoxynivalenol and deoxynivalenol 3-glucoside levels showed that the relative

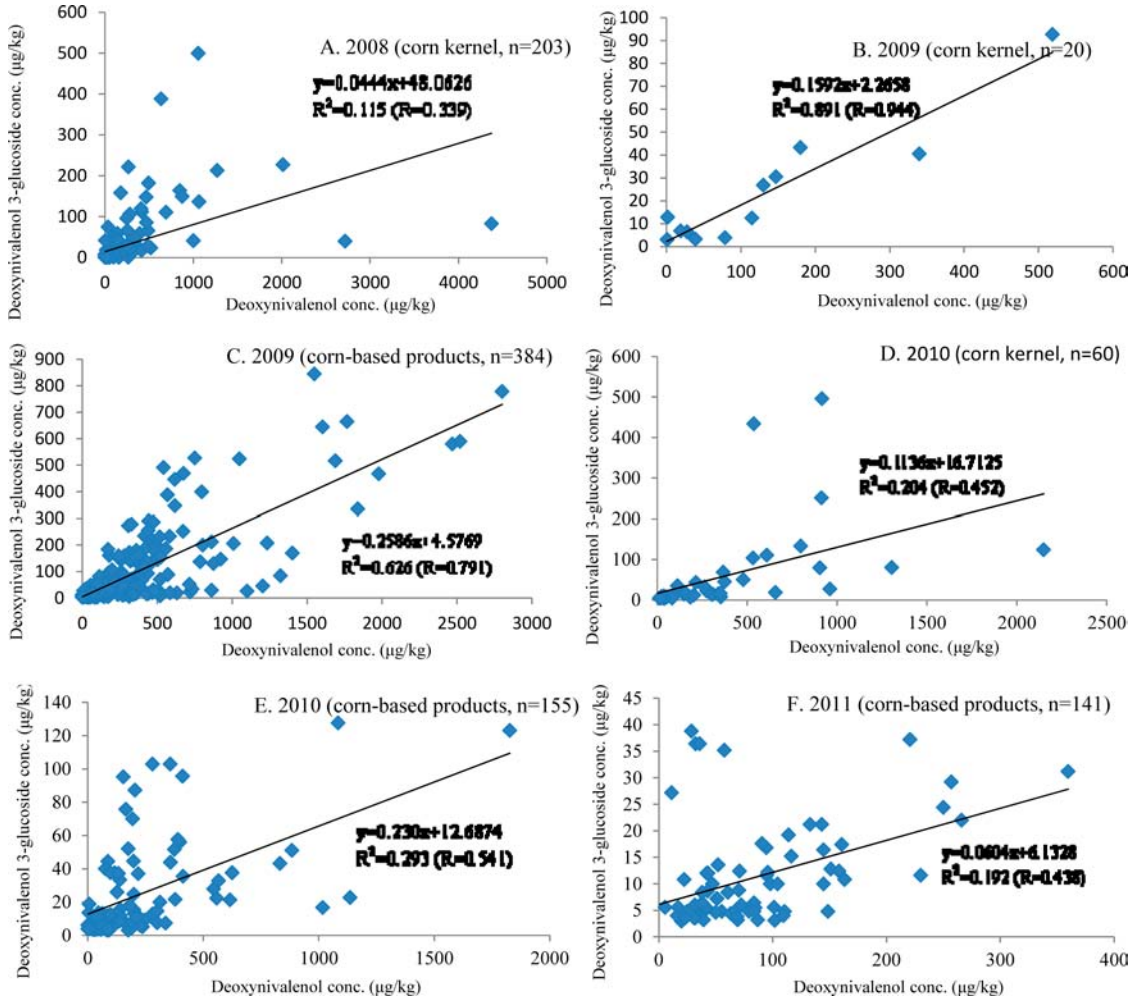


Figure 5. Correlation between deoxynivalenol and deoxynivalenol 3-glucoside levels in corn kernels and corn-based products between 2008 and 2011 in China.

proportion of deoxynivalenol 3-glucoside in relation to deoxynivalenol is rather low and stable, about 25% ± 5% (corn kernels) and 34% ± 4% (corn-based products) in the 4 years of samples.

The predominant *Fusarium* toxin in Chinese corn kernels and corn-based products described previously and involved in human red-mold intoxication in China so far is deoxynivalenol. Deoxynivalenol 3-glucoside is formed from deoxynivalenol in *Fusarium*-infected plants and stored in the vacuole.[24] This compound has been found in naturally contaminated cereals such as wheat, maize, oats, barley as well as in malt and beer made worldwide.[12,18,20,22,23]

It has been shown that deoxynivalenol 3-glucoside was detected at a considerable level in Chinese corn kernels and corn-based products for four successive years for the first time. Although the fate of deoxynivalenol 3-glucoside after digestion by mammals is largely unknown, the concern is that this compound may be cleaved to deoxynivalenol and glucose. In a fed digestion model conducted by De Nijs et al., there was no evidence of release of deoxynivalenol from deoxynivalenol 3-glucoside, spiked at a deoxynivalenol 3-glucoside level of 2778 μg/kg food. This shows that the conditions in the gastrointestinal tract do not result in hydrolysis of this glucoside into the original mycotoxin.[26] It is known that 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol can be converted into deoxynivalenol in vivo. Deoxynivalenol 3-glucoside, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol therefore contribute to the total deoxynivalenol concentration and deoxynivalenol-induced toxicity.[18] Accordingly, the Joint FAO/WHO Expert Committee on Food Additives decided to convert the provisional maximum tolerable daily intake (PMTDI) for deoxynivalenol to a group PMTDI of 1 μg/kg body weight for deoxynivalenol and its acetylated derivatives (3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol) at the seventy-second meeting held in 2010.[27] In this regard, the committee considered the toxicity of the acetylated derivatives equal to that of deoxynivalenol, but the committee concluded that there was insufficient information to include deoxynivalenol 3-glucoside in the group PMTDI at that time. Worldwide regulations for deoxynivalenol do not consider its conjugates thus far.[26]

According to a previous report,[18] over 30% of the extractable total deoxynivalenol can be present as deoxynivalenol 3-glucoside in maize. The occurrence of deoxynivalenol 3-glucoside was confirmed after identification in Chinese wheat and maize samples in the same concentration range as deoxynivalenol described previously and obtained in the present and previous studies.[20] Therefore, there is a need for routine analysis of food samples for masked mycotoxins along with deoxynivalenol and to add deoxynivalenol 3-glucoside to

the list of contaminants monitored by China National Food Contaminant Surveillance Network. The results of the present study might also be of some interest for ongoing discussions on setting the maximum tolerance limits for group deoxynivalenol in cereals, especially as this substance occurs in levels comparable to or even higher than the levels of 3-acetyl-deoxynivalenol or 15-acetyl-deoxynivalenol in Chinese corn kernels and corn-based products.

Although deoxynivalenol 3-glucoside and other masked mycotoxins in foods being consumed by the victims in any of the human red mold intoxication episodes in the past decades in China were not analyzed at the time the outbreaks happened, their potential toxic effect may not be ruled out, based on the results for deoxynivalenol that were below the Chinese regulatory limit of 1000 μg/kg (from 16 to 599 μg/kg) in some left-over food samples.[4] Hence, the authors assume that the total deoxynivalenol intake by the victims through the causative foods might be significantly underestimated, owing to the possible hydrolysis of the deoxynivalenol 3-glucoside conjugate back to its toxic precursor mycotoxin deoxynivalenol during digestion. Therefore, potential toxicity of this toxin remains a threat. Additional studies about bioavailability, further metabolism of deoxynivalenol 3-glucoside, and a comprehensive risk assessment for deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, and deoxynivalenol 3-glucoside are required.

Corn and winter wheat are widely intercropped in China. Corn is sown in late spring and harvested in the autumn, whereas winter wheat is sown in late autumn and harvested in the following early summer. Wheat stalk has never been gathered after harvest in some areas of China. The wheat straw left in the field provides a favorable condition for *Fusarium* species surviving, and the fungal spores will propagate in large quantities, if the environmental conditions are suitable for their growth.[25] Thus, abundant pathogens in combination with high humidity, much precipitation, and less sunlight led to the prevalence of ear rotting of corn in China. Further research is needed to investigate the infection of wheat straw by the *Fusarium* species along with the *Fusarium* infection of intercropped corn to elucidate the role of wheat straw in the prevalence of ear rotting of corn.

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Funding

This study was financially supported by the National High-tech R&D Program (863 Program), the Ministry of Science and Technology of the People's Republic of China (Grant 2012AA101603).

Notes

The authors declare no competing financial interest.

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