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Retention of deoxynivalenol and its derivatives during storage of wheat grain and flour

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ABSTRACT

Fate of deoxynivalenol (DON) and its derivatives—deoxynivalenol-3-glucoside (D3G), 15-acetyldeoxynivalenol (15-AcDON) and 3-acetyldeoxynivalenol (3-AcDON) during six-month storage of wheat grain and flour was investigated. Wheat grain and flour polluted by DON, D3G, 15-AcDON and 3-AcDON were packaged in polyethylene bags, kraft paper bags and cloth bags, and stored at room temperature and 4–6 °C, and mycotoxin levels were analyzed by UPLC-MS/MS after storing for 30, 90 and 180 days. Levels of DON, 15-AcDON and 3-AcDON in wheat grain showed a generally decrease during the storage duration, and DON concentrations averagely decreased by 40–50%. An obviously increase of DON (more than 70%) and decrease of 15-AcDON and 3-AcDON were observed after storage in wheat flour. There were no significant differences of D3G levels in most of wheat grain and wheat flour samples after storage, but some increased dramatically with retention level up to 240%. The results suggested conversion of DON and its derivatives may occur during wheat grain and flour storage.

1. Introduction

Mycotoxins are secondary metabolites produced mainly by molds under certain conditions. It is reported that 25–50% of harvested crops in the world have been contaminated with mycotoxins annually (Ricciardi et al., 2012). Deoxynivalenol (DON) is a naturally occurring trichothecene mycotoxin of cereal grains infected by Fusarium head blight. Contamination of DON in cereal grains and their processed products is a major problem. DON is represented in more than 90% of all mycotoxin-contaminated samples, and its presence is usually a good indicator that other mycotoxins are also present (Sobrova et al., 2010). It is known that DON can be degraded or detoxified into various derivatives by acetylation, oxidation, de-epoxidation or glycosylation (Berthiller, Dall'Asta, et al., 2005; Berthiller, Schuhmacher, Buttinger, & Krska, 2005; Karlovsky, 2011; Kushiro, 2008; Warthet et al., 2012). These derivatives like deoxynivalenol-3-glucoside (D3G) or the acetylated forms 3- and 15-acetyldeoxynivalenol (3-AcDON and 15-AcDON) are of particular importance (Maul et al., 2012). Especially for D3G, the so called masked mycotoxins, which usually escape routine analysis because of their different chemical behaviors in comparison to the parental compounds, has become a concern of researchers worldwide, and D3G has so far been proven the most frequent masked mycotoxin in naturally infected wheat (Berthiller, Dall'Asta, et al., 2005; Desmarchelier & Seefelder, 2011; Kostelanska et al., 2009; Malachova et al., 2011). Although DON derivatives are generally less toxic than DON itself (Kushiro, 2008), the co-occurrence of DON and its derivatives, such as DON and D3G, has made it a hotspot to use DON derivatives as targets for DON detection in cereal crops (Lancova et al., 2008; Nagl et al., 2012).

There are many studies regarding the fate of mycotoxins during storage. Aleš, Andrej, Stanislav, Avrelija, and Mario (2010) reported that levels of DON and nivalenol (NIV) can decrease during organic whole-grain flour storage and aging. Concentrations of DON and NIV at the end of storage were lower (1–29%) than those at the beginning, the degradation of DON and NIV occurs most rapidly when the flour was stored at 25 °C in paper bags with access to the storage atmosphere. Moreover, the level of retention of DON and NIV in flours depended significantly on the type of packaging material, but did not depend on the type of flour or the storage

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temperature. Mycotoxins are very stable compounds that decompose only during long exposure to high temperature processing treatments or after exposure to high concentrations of bases or other aggressive chemicals (Bretz, Beyer, Cramer, Knecht, & Humpf, 2006; Hazel & Patel, 2004; Wolf-Hall, Hanna, & Bullerman, 1999). Mycotoxin retention levels in stored grain are closely related to the kinds and states of the grain and the storage environment. However, currently there is insufficient information about it. Moreover, there has been no published data on the fate of D3G, 3-AcDON and 15-AcDON during the storage of grain.

The aim of this study was to extend the knowledge on the fate of DON, D3G, 3-AcDON and 15-AcDON in wheat grain and wheat flour packaged in different packaging materials and stored in different environments during six-month storage, and establish the influence of wheat state, storage temperature and package material on the fate of DON, D3G, 15-AcDON and 3-AcDON during storage.

2. Materials and methods

2.1. Wheat grain and wheat flour sample preparation

Prior to the start of the experiment, wheat panicles naturally infected with Fusarium fungi were collected from fields in Huai'An city, Jiangsu province (E118°12′~119°36′, N32°43′~34°06′) at Jun. 2012. The panicles were threshed and then partly of the wheat grain were milled for wheat flour, and the milling protocol was performed according to Zhang and Wang (2014). The initial concentrations of DON, D3G, 15-AcDON and 3-AcDON in wheat grain and wheat flour samples were determined by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) assay.

2.2. Storage conditions

The experiment was designed as a three-factorial design that was completed with three replications. The studied factors were: wheat states (wheat grain and wheat flour), storage environments (room temperature, 18–27 °C, 28–60 r.h. and 4–6 °C in a refrigerator), package materials (polyethylene bag, kraft paper bag and cloth bag). Wheat grain and flour samples were packaged in three different materials and stored for 180 days in two different storage environments. The experiment sustained from Jul. 2012 to Jan. 2013. DON, D3G, 15-AcDON and 3-AcDON levels in all samples were analyzed by UPLC-MS/MS when they were stored for 30, 90 and 180 days.

2.3. Chemicals and reagents

Purified water was produced by a Milli-Q system (Millipore Corp., Bedford, MA). DON standard (1000 μg mL^{-1} in methanol, certified purity >99.9%) was purchased from Supelco Co. (Bellefonte, PA, USA), 15-AcDON and 3-AcDON standard (both 100 μg mL^{-1} in acetonitrile, certified purity >99.9%) from Enzo Biochem, Inc. (Farmingdale, New York, USA), and D3G standard (50 μg mL^{-1} in acetonitrile, certified purity >99.9%) from LGC (Wesel, Germany). Methanol, acetonitrile and formic acid were all HPLC graded and were purchased from Thermo Fisher Scientific (USA). Ammonium acetate came from Sinopharm Chemical Reagent Co., Ltd (Beijing, China).

2.4. Sample treatment and UPLC-MS/MS analysis

DON and D3G assay was performed according to Zhang and Wang (2014), and 3-AcDON and 15-AcDON detection were conducted like DON analysis. The retention time of 3-AcDON and 15-AcDON were 2.15 min and 2.16 min, respectively, and the accurate masses of 3-AcDON and 15-AcDON were 213.3 and 261.2, respectively.

2.5. Method validation

The recovery rate of the method herein was performed by spiking with three different concentrations for DON, 15-AcDON and 3-AcDON (20, 50 and 200 μg kg^{-1}), and for D3G (50, 100, and 500 μg kg^{-1}) in wheat grain and flour. Mycotoxins were extracted from spiked samples and their concentrations were determined by UPLC-MS/MS as described above. Triplicates of each concentration were analyzed. Average of recovery data, triplicate of each level, and for each analyte and matrix are shown in Table 1. The limit of detection (LOD) and limit of quantification (LOQ) for each analyte were assessed at a ratio of signal/noise (S/N) = 3 and signal/noise (S/N) = 10, respectively. LOD of DON in wheat grain and flour was 0.5 μg kg^{-1}, and LOQ was 1.5 μg kg^{-1}, LOD of 15-AcDON and 3-AcDON in wheat grain and wheat flour were both 1.0 μg kg^{-1}, and LOQ were 3.0 μg kg^{-1}, and for D3G, LOD in wheat grain and wheat flour was 3.0 μg kg^{-1}, and LOQ was 10.0 μg kg^{-1}.

3. Results

3.1. Changes of DON concentrations during storage

Mean concentrations of DON before storage and at different storage time for each specific combination of the trial factors are presented in Table 2. A generally continuous decrease trend of DON concentrations in wheat grain packaged in three different packaging materials and stored at room temperature and at 4 °C were observed, which averagely decreased by 40–50% after 180-days storage comparing to the initial levels, and wheat grain packaged in cloth bag and stored at room temperature showed the greatest DON concentration decrease (35.5% retention level after storage). The results also showed that DON concentrations in wheat grain decreased dramatically from the 30–90 days storage, which decreased averagely more than 40%. While DON concentration in all wheat flour samples in the whole storage duration showed a generally continuous increase, which increased more than 70% after storage, and the results was completely contrary to that of wheat grain. The ANOVA results (Table 6) demonstrated that DON retention levels influenced by the state of wheat and storage temperature. Wheat grain and flour stored in polyethylene bag,

Table 1
Recoveries of DON, D3G, 15-AcDON and 3-AcDON in wheat grain and wheat flour.

Mycotoxin	Sample matrix	Average recovery (%) and standard deviations		
		Spiking level (μg kg^{-1})		
		20	50	200
DON	Wheat grain	103 ± 4.3	108 ± 4.6	111 ± 3.4
	Wheat flour	93 ± 1.6	93 ± 2.6	113 ± 4.4
15-AcDON	Wheat grain	107 ± 0.9	116 ± 1.2	92 ± 0.5
	Wheat flour	95 ± 0.2	101 ± 0.2	111 ± 0.1
3-AcDON	Wheat grain	94 ± 0.9	88 ± 0.6	101 ± 1.6
	Wheat flour	113 ± 0.7	95 ± 0.3	113 ± 0.0
Mycotoxin	Sample matrix	Average recovery (%) and standard deviations		
		Spiking level (μg kg^{-1})		
		50	100	500
D3G	Wheat grain	72 ± 1.9	75 ± 1.6	99 ± 0.2
	Wheat flour	77 ± 4.2	80 ± 4.2	92 ± 1.3