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Fate of deoxynivalenol and deoxynivalenol-3-glucoside during wheat milling and Chinese steamed bread processing

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ABSTRACT

To investigate the fate of deoxynivalenol (DON) and deoxynivalenol-3-glucoside (D3G) during wheat milling and Chinese steamed bread (CSB) processing, *Fusarium*-contaminated wheat samples containing DON and D3G at five different levels were milled and processed to CSB. Mycotoxin levels in milling fractions, doughs and CSB were determined with UPLC-MS/MS. Concentrations of DON and D3G were 1.2–2.2 times and 2.9–4.4 times higher in bran than that in wheat grain and they were slightly lower in shorts compared to bran. Reductions of 79–90% for DON and 23–39% for D3G in flour were observed, respectively, compared to wheat grain. With respect to wheat grain, the distribution of DON was 35% in bran, 27% in shorts, and 9% in flour, and the distribution of D3G was 77% in bran, 58% in shorts, and 37% in flour. Milling decreased the total amount of DON but increased the amount of D3G, which may result from the binding of DON to starch during the milling process. DON levels approximately doubled when the mixed and fermented dough was processed into CSB. D3G concentrations in mixed and fermented doughs and in CSB did not differ significantly, but were almost 50% lower than in flour. Dough making decreased the amount of D3G and steaming increased the amount of DON. CSB processing may thus release bound DON in flour.

1. Introduction

Fusarium head blight (FHB) or scab, caused by different species of *Fusarium*, is a serious worldwide problem in wheat, barley and other small grain cereals, and generally leads to decreased grain yield and quality, and affects products processed from infected grain (Parry, Jenkinson, & Mcleod, 1995). Moreover, grains can be contaminated with mycotoxins, which are secondary metabolites produced by some *Fusarium* species under certain conditions. It is estimated that 25% of food crops worldwide are affected by mycotoxin-producing fungi, including many basic foods (Köppen et al., 2010). Among a range of mycotoxins, deoxynivalenol (DON) is the predominant one occurring in wheat (Kokkonen, Ojala, Parikka, & Jestoi, 2010). In addition to free DON, masked forms like deoxynivalenol-3-glucoside (D3G) or the acetylated forms 3- and 15-acetyldeoxynivalenol (3-AcDON and 15-AcDON) are of particular importance in contaminated food (Maul et al., 2012). 3-AcDON is converted to DON in mammal metabolism and therefore contributes to the total DON-induced toxicity. The Joint FAO/WHO (World Health Organization) Expert Committee on Food Additives converted the provisional maximum tolerable daily intake (PMTDI) for DON to a group PTMDI of 1 μg/kg bodyweight for DON and its acetylated derivatives; the Expert Committee considered it possible that D3G would be hydrolyzed in the body and that DON would become bioavailable (JECFA, 2011). These mycotoxins can be very stable in food processing (Castelo, Katta, Sumner, Hanna, & Bullerman, 1998; Castelo, Sumner, & Bullerman, 1998; Katta, Jackson, Sumner, Hanna, & Bullerman, 1999; Osborne, 1979; Osborne et al., 1996) and can be found in final products. Studies on the effects of wheat processing covering any physical, chemical or biological process undergone by raw cereals in the formation of food products are fundamental to minimizing the sanitary impact on the production chain. Bullerman and Bianchini (2007) and Hazel and Patel (2004) reviewed the influence of processing on mycotoxin levels. Study on the retention of DON during primary processing (milling) and secondary processing (cooking) is important for risk assessment and management for the people who live on wheat-based food products. Multiple studies in Europe and elsewhere have been conducted on the fate of mycotoxins during food processing in the last 20 years, but mainly

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focused on retention of DON during wheat milling and bread or noodle processing.

Approximately 600 million tonnes of wheat is produced per year worldwide, and 120 million tonnes is produced in China. Much of the wheat is converted to flour for human consumption and processed into various foods, including breads, pastas, noodles and cakes (Pacin, Ciancio Bovier, Cano, Taglieri, & Hernandez Pezzani, 2010). Chinese steamed bread (CSB) is a wheat-based leavened product cooked by steaming to produce a product with a dense crumb and a thin smooth white skin rather than the brown crust of traditional western breads (Huang, 1999; Rubenthaler, Huang, & Pomeranz, 1990; Su, Ding, Li, Su, & Zheng, 2005). Ingredients of CSB are usually simple, generally including only flour, water and yeast, or sour dough. CSB is the most popular wheat product in China, and about 40% of wheat consumption in China is in that form, particularly in northern China (He, Liu, Peña, & Rajaram, 2003). CSB is gaining in popularity in Korea, Japan and some Southeast Asian countries.

No research on the fate of DON and other mycotoxins during CSB processing has been reported. The objective of this study was to evaluate the effect of milling and some processing steps of CSB processing on the fate of DON and D3G.

2. Materials and methods

2.1. Wheat milling and Chinese steamed bread processing

2.1.1. Wheat samples

Wheat panicles (cultivars Jimai 22, Aikang 58 and Luomai 24) with and without Fusarium head blight (FHB) were collected from farmer's fields in Xuzhou city, Jiangsu province (E116°22′–118°40′, N33°43′–34°58′), at the end of May 2012. Fusarium-infected panicles were separated manually from Fusarium-free panicles based on color and the degree of grain shriveling in the laboratory. The Fusarium-infected and Fusarium-free panicles were threshed separately. DON and D3G were up to 36,719 μg/kg and 1039 μg/kg, respectively, in the Fusarium-infected wheat kernels and were undetectable in the Fusarium-free panicles. Wheat samples in this research were prepared by mixing the Fusarium-infected wheat kernels with the Fusarium-free wheat kernels proportionately to form wheat samples of 5 mycotoxin levels. Each level was prepared in triplicate.

2.1.2. Milling protocol

The samples were firstly cleaned to remove foreign seeds and materials otherwise unsuitable for milling. After measuring grain moisture content with a Multi-Grain Portable Moisture Meter (DICKEY-John Corp., Auburn, USA), 1 kg cleaned grain was transferred into a polypropylene bucket (max 5 kg) and the required amount of water was added and mixed with the grain for 20 min. The bag was then closed, left for 16–24 h to temper to a moisture content of 16.5% prior to milling. Milling was performed in a MLU 202 Bühler Laboratory Mill with pneumatic product transport (Bühler, Uzwil, Switzerland) equipped with three breaks and three stripping systems. The temperature in the milling room was maintained at 23–25 °C and relative humidity 40–50%. Three milling fractions: bran, shorts, and flour were collected and weighed.

2.1.3. Preparation of Chinese steamed bread

Flour for Chinese steamed bread was prepared by mixing the break flours and the reduction flours. Chinese steamed bread was prepared according to the Chinese Business Standard (procedure 10139-93, Appendix A, 1993). Milled flour samples (100 g) were mixed with yeast solution (1 g dry yeast dispersed in 48 ml water at 38 °C), then fermented for 60 min in a fermentation cabinet (38 °C, 85% RH) after 3 min of mixing. The fermented dough was molded by hand for 3 min into a round dough piece with a smooth surface. After resting in air for 15 min, the doughs were steamed for 20 min in a steaming chamber (100 °C) followed by cooling at room temperature for 40–60 min. Samples were taken at three different stages of the process: at the end of dough preparation (doughs), after the fermentation (fermented doughs), and after steaming (steamed products). Representative subsamples were stored at −20 °C until analyses were performed.

2.2. Chemicals and reagents

Purified water was produced by a Milli-Q system (Millipore Corp., Bedford, MA). DON standard (1000 μg/mL in methanol, certified purity >99.9%) was purchased from Supelco Co. (Bellefonte, PA, USA) and D3G standard (50 μg/mL in acetonitrile, certified purity >99.9%) from LGC (Wesel, Germany). Individual stock solutions of DON and D3G were prepared at concentration of 100 μg/mL in methanol and 10 μg/mL in acetonitrile, respectively, and kept at −20 °C in darkness. Methanol, acetonitrile and formic acid are all HPLC graded and were purchased from Thermo Fisher Scientific (USA). Ammonium acetate came from Sinopharm Chemical Reagent Co., Ltd (Beijing, China).

2.3. Sample treatment and extraction

Ten grams of sample was blended with 50 mL acetonitrile:water (80:20, v/v) at high speed with a T18 ULTRA-TURRAX blender (IKA Co., Staufen, Germany) for 3 min. The mixture was filtered through filter paper. For DON analysis, 8 mL of the filtered extract were cleaned up using multifunctional MycoSep 226 columns (Romer Labs, Inc. Union, MO, USA), 4 mL of the cleaned up extract were evaporated to dryness using N-EVAP in a 50 °C water bath, the residue was dissolved in 1 mL 10 mmol/L ammonium acetate water solution:methanol (1:1, v/v), then filtered through 0.22 μm MICRO PES filter membranes (Membrana, Germany), and finally transferred to a glass vial for analysis. For D3G analysis, the filtered extract was centrifuged at 8870 rpm for 8 min, 1 mL the aliquots were filtered through 0.22 μm filter membranes and transferred to a glass vial for analysis.

2.4. UPLC-MS/MS analysis

DON and D3G analyses were carried out by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS, XEVO-TQ, Waters, USA) equipped with an electrospray ionization source (ESI). For separation of analyte reversed-phase C_{18} stainless columns (50 × 2.1 mm, particle size 1.7 um, Waters, USA) were used. The multiple reaction monitoring (MRM) mode was set, with capillary voltage maintained at 2.5 KV, cone voltage 20 V, and column temperature 26 °C. For DON analysis, the ESI interface was operated in positive-ionization (ESI^+) mode, and the desolvation temperature was 450 °C. Nitrogen was used for desolvation, and its flow rate was 800 L/h. The solvent system consisted of methanol (solvent A) and 10 mmol/L ammonium acetate (solvent B). The gradient applied was a flow rate of 0.3 mL/min for 0–5.5 min, a linear increase from 20 to 85% A, followed by a linear increase from 85 to 100% A for 5.5–5.8 min; a linear decrease from 100 to 20% A for 5.8–6.0 min, followed by an isocratic washout step of 20% A for 2 min. For D3G analysis, the ESI interface was operated in negative-ionization (ESI^-) mode, desolvation temperature was 500 °C and nitrogen flow rate was 1000 L/h. The solvent system consisted of acetonitrile (solvent A) and 0.1% formic acid-water (solvent B), and the following gradient was applied with a