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SHORT COMMUNICATION

Diminution of Mycotoxins from *Fusarium sp.* in Barley and Wheat through Post-harvest Processing Methods

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Abstract The objective of this study was to analyze mycotoxin contents in Korean barley and wheat infected with *Fusarium sp.* The major contaminant was determine among deoxynivalenol, nivalenol (NIV), and zearalenone, as well as diminution rate of mycotoxin contents by milling, washing, and boiling processes. NIV was found as a major mycotoxin contaminant in Korean barley and wheat, and bran showed higher contamination level than the inner part in whole infected cereal. The results indicate that the milling process of the diseased barley and wheat showed dramatic diminution rate of 84.4%. Furthermore, the washing on barley reduced mycotoxin infection up to 81.0%, and boiling showed 82.7% diminution effect.

Keywords cereals · diminution effect · *Fusarium* mycotoxins · processing methods

Mycotoxins such as deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEA) are groups of secondary metabolites produced by *Fusarium* species which induce mycotoxicosis of human and animal (Ichinoe et al., 1983; Desjardins et al., 1989; Kim et al., 1993). Because the mycotoxins are accumulated in cereals infected with *Fusarium graminearum*, they have high possibility to affect the crop quality (Jelinek et al., 1989). Thus, the contamination monitoring (Park et al., 1996, Ryu et al., 1996, Visconti and Pascale, 2010, Mishra et al., 2013, Pitt et al., 2013) and diminution efforts (Pei et al., 2005, Bullerman and Bianchini, 2007, Meca et al., 2012, Cheli et al., 2013) were highlighted in the food and feed safety in cereals. The objective of the present study was to compare the contamination levels of major mycotoxins and evaluate the diminution effect of mycotoxin contents in cereals by different postharvest processing methods, as well as the relation of mycotoxin contents and milled ratio of grains.

Mycotoxin Analysis in Cereals

The representative samples (10 g) of barley and wheat were homogenized and then extracted with 50 mL of acetonitrile/water (90/10, v/v) mixture for 30 min. The sample was filtered and extracted with EtOAc (40 mL×3). The crude extract was evaporated, and purified with Florisil column chromatography (Stecher et al., 2007). Analysis of DON and NIV was performed by Burlakoti et al. (2008) with gas chromatography (GC) method after trimethylsilyl derivatization with N-trimethylsilylimidazole-trimethylchlorosilane-*n,o*-bis-(trimethylsilyl)trifluoroacetamide-pyridine. DON and NIV concentrations were determined by GC using a DB-5MS column (30 m×0.25 mm, 0.25 mm, J&W Scientific, USA), connected to a mass spectroscopy (MS) (TSQ7000, Finnigan MAT, USA) using a positive electron ionization source and operating in the selected ion monitoring mode. The selected ions were 512 and 422 *m/z* for DON, and 482 and 510 *m/z* for NIV. The ionization energy was 70eV and the temperature of ion source and injector were 185 and 280°C, respectively. The limits of quantizations (LOQ) were determined at 0.30 mg/kg for DON and 0.75 mg/kg for NIV. ZEA analysis was conducted following the method of De Saeger et al. (2003). The concentration was determined by High-performance liquid

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chromatography (HP1100 with HP1046A, Hewlett Packard, US) with a LiChrospher RP18 column (5 mm, 4.6 mm×150 mm, Merck, Germany). The condition of fluorescence detector indicated the wave lengths of excitation and emission were 236 and 418 nm, respectively. Mobile phase was CH_3CN/H_2O (50/50, v/v) and flow rate was 0.8 mL/min. The LOQ was determined to be 0.010 mg/kg for ZEA. Recoveries of NIV, DON and ZEA were 102, 88, and 72% on 0.030 mg/kg and 87, 85, and 74% on 0.300 mg/kg of standard solution, respectively.

Mycotoxins Contamination Rate in Cereals

In the present study, 100 barley and 10 wheat samples were randomly collected, and the samples were grouped by disease severity (normal, 10, 30, and 50%). From the screening result for the three mycotoxins in the cereals, NIV was found to be the major contaminant in both hulled barley and naked barley with a contribution ratio ordered as NIV > DON > ZEA. The mycotoxins concentration was dependent on the disease severity; for example, total mycotoxin contents in 10, 30, and 50% infected hulled barley were 2.581, 8.600 and 16.320 mg/kg, respectively. The total mycotoxin contents in hulled barley were higher than those in naked barley in Korean sample (Table 1).

The distribution study of total mycotoxin was evaluated with 1.045 mg/kg of the total mycotoxin in whole wheat grain. Concentration of the bran, inner bran and flour were 5.123, 2.531, and 0.910 mg/kg, respectively. Similarly, the bran showed higher concentration than any other edible part both on the hulled barley and naked barley. These mycotoxin distribution patterns of each part were similar to the results of Zheng et al. (2014).

Diminution Effects of Post-harvest Methods in Cereals

In order to investigate the effects of the three different post-harvest processing methods on the diminution of mycotoxin contents in cereals, milling, washing, boiling and tempering methods were applied to barley and wheat.

Milling. Results of the present study confirmed the diminishing effect of the three contaminated mycotoxins based on milling ratio of Korean barley and wheat. From the experiments, the diminishing effect of the total mycotoxins was dependent on the milling ratio and showed very similar pattern with those of DON and NIV. However, ZEA was not reduced to below 30% with 60% milling ratio. The correlations of diminishing effect with milling ratio were best fitted with single exponent equation, and their correlations (r^2) ranged from 0.95 to 0.99 (Fig. 1). Additionally, the diminishing effect of wet and dry milling methods on wheat sample was identified. After dry and wet milling process, the residual total mycotoxins were 0.117 and 0.202 mg/kg, and the reduction rates were 84.4 and 73.1%, respectively. From the quantization of the different parts of wheat after milling, most of the residual mycotoxins were detected on the bran.

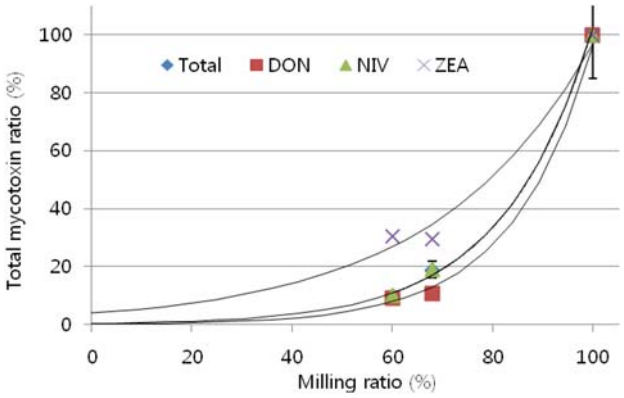

Fig. 1 Diminution rate of mycotoxins by milling ratio in hulled barley.

Fig. 2 Diminution rates of total mycotoxin on barley after 1st and 2nd water washing and boiling treatments.

Washing. The 60% milled hulled barleys (0.347 mg/kg as the total mycotoxin) were washed with water, stirred with glass plug, and soaked in water for 1 h. The results of analysis of mycotoxins after water-washed treatment showed the total residual amounts of mycotoxins were remarkably removed (Fig. 2).

Results showed the average diminishing ratios of the total contents after 1st and 2nd washings were 68.6 and 81.0%, respectively. These diminution effects might have been caused by removal of the mill dust. Water solubility of the mycotoxins could also cause the diminution effect, because DON and NIV are known as hydrophilic contaminants, whereas ZEA is known as hydrophobic mycotoxin (Zheng et al., 2014).

Boiling. Boiling of barley for 1 h has 82.7% diminution effect on the total mycotoxins including NIV as the main contaminant, and this diminishing ratio was comparable with 2nd washing effect. Hazel and Patel (2004) reported DON was reduced by around 50% after boiling. Thus, it is expected that DON and NIV would be reduced to at least 50% after washing and boiling process.

The present study reports on the main *Fusarium* mycotoxin, NIV, in Korean barely, and DON, NIV, and ZEA in wheat.

Mycotoxin contamination level was highly dependent on the disease severity. Considering that the major contamination part on barley and wheat is the bran, dry and wet milling process can reduce contamination effectively. Furthermore, DON and NIV would be removed by over 50% by washing and/or boiling process of the cereal.

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