A case report on contamination of zearalenone, fumonisins and 8-ketotrichothecenes possibly including iso-deoxynivalenol in moldy corn harvested in Korea

Hwang-Bae SOHN^{*1}, Yin-Won LEE^{*1}, Moon-Han LEE^{*2}, and Takumi YOSHIZAWA^{*3}

(Received May. 21, 1998; Accepted July. 24, 1998)

孫 煌培^{*1}, 李 仁遠^{*1}, 李 文漢^{*2}, 芳澤 宅實^{*3}: 韓国で収穫されたカビトウモロコシのイソデオキシニバレノールを含む可能性のある8-ケトリコテセン, ゼアラレノン, フモニシンによる汚染例

Summary

Five moldy corn samples were analyzed for *Fusarium* mycotoxins including 8-ketotrichothecenes, zearalenone (ZEA), and fumonisins and found to be heavily contaminated with these toxins. Among 8-ketotrichothecenes, deoxynivalenol (DON), 15-acetyl-DON, and nivalenol (NIV) were the major toxins rather than 3-acetyl-DON and 4-acetyl-NIV. In addition, one moldy corn sample was contaminated with iso-DON, which has the same molecular weight of DON. This is the first report iso-DON and DON as well as other *Fusarium* mycotoxins were occurred in moldy corn from Korea.

Key words: trichothecene, iso-deoxynivalenol, fumonisins, zearalenone, moldy corn

Introduction

Corn is an important crop in Kangwon province, which is located in the mideastern part of Korea. Corn is used not only for animal feedstuffs but also used for a staple food with rice in the Kangwon province. It is sown in the early summer and harvested during the autumn. During the growing period, corn stalk and ear rot is often a serious problem in the Kangwon province. Although incidents of mycotoxicoses due to consumption of corn infected with *Fusarium* species have not been reported in this area, the natural occurrence of *Fusarium* mycotoxins such as deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), nivalenol (NIV), and zearalenone (ZEA) has been reported¹⁾. Several investigators^{2,3)} also reported that *Fusarium* isolates from corn in this area were highly toxic to experimental animals and could produce trichothecenes and

ZEA. A period of prolonged rainy weather during the fall of 1997 resulted in a delayed corn harvest and provided conditions favoring inoculum buildup of *Fusarium* species throughout the Kangwon province. The moldy corn samples were submitted to our laboratory by one farmer to analyze mycotoxins, so the initial research started. Damage by *Fusarium* species was characterized as a reddish pink discoloration. In this paper, we attempted to analyze *Fusarium* mycotoxins including 8-ketotrichothecenes, ZEA and fumonisins in moldy corn samples and we found that one of moldy corn samples was contaminated with iso-deoxynivalenol (iso-DON) with other trichothecenes as well as ZEA and fumonisins.

Materials and Methods

Standards Trichothecene mycotoxins, including DON, NIV, 15-ADON, 3-acetyldeoxynivalenol (3-ADON), 4-acetylnivalenol (4-ANIV), and ZEA were

^{*1} Division of Applied Biology and Chemistry and Research Center for New Biomaterials in Agriculture

^{*2} College of Veterinary Medicine, Seoul National University, Suwon 441-744, Korea

^{*3} Department of Biochemistry and Food Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kita-gun, Kagawa 761-07

purchased from Wako Pure Chemical Industries, Ltd., Osaka. Fumonisin B₁ (FB₁), fumonisin B₂ (FB₂), and fumonisin B₃ (FB₃) were prepared in T. Yoshizawa's laboratory.

Samples Five moldy corn samples of each sample weighing approximately 100 g were submitted to our laboratory to analyze *Fusarium* mycotoxins during the third week of October, 1997 through the Extension Service Center of Seoul National University. All samples were milled to 120-250 mesh with a laboratory mill and at stored -20°C before analyses.

Extraction and analysis Each sample was analyzed for 8-ketotrichothecenes, ZEA, and fumonisins according to the procedures described previously. Briefly, a ground corn sample was extracted with acetonitrile-water (3:1, v/v) and, following filtration and concentration, applied onto a florisil column for trichothecenes⁴⁾ and ZEA⁵⁾ or onto a strong anion exchange cartridge for fumonisins⁶⁾. A portion of each extract was reacted with trimethylsilylating reagent and analyzed with a Shimadzu QP-5000 gas chromatograph-mass spectrometer (GC-MS) with a selected ion monitoring (SIM) mode. The calculation of trichothecene concentration was based on the average area counts of the fragment ions of each standard toxin by using SIM mode. The analytical conditions were as follows; column, DB-5 fused silica column (30 m × 0.25 mm id, 0.25 μm film thickness, J & W Scientific, CA, U.S.A.), column temperature, 120°C for 5 min and then increased to 270°C at 8°C/min; injector temperature, 280°C; ion source temperature, 200°C, interface temperature, 250°C; ionizing

voltage, 70 eV. The fragment ions for quantitation of trichothecenes were *m/z* 422 and 393 for DON, *m/z* 392 and 350 for 15-ADON, *m/z* 377 and 287 for 3-ADON, *m/z* 379 and 289 for NIV, and *m/z* 480 and 450 for 4-ANIV. Confirmation of trichothecenes was done by scan mode. ZEA was analyzed by high performance liquid chromatography (HPLC) with fluorescence detection. The excitation wavelength and emission wavelength of ZEA were 236 nm and 418 nm, respectively. Fumonisin B₁, FB₂, and FB₃ were derivatized by reacting with o-phthalaldehyde and quantified by HPLC with fluorescence detection. The excitation wavelength and emission wavelength of fumonisins were 335 nm and 440 nm, respectively.

Results and Discussion

All five samples were highly contaminated with 8-ketotrichothecenes, ZEA, and fumonisins. Among trichothecenes, DON, 15-ADON, and NIV were the major toxins at levels ranging from 1,600 to 15,000 ng/g, 140 to 2,700 ng/g, and 94 to 4,300 ng/g, respectively. ZEA and fumonisins including FB₁, FB₂, and FB₃ were coincidentally found at high levels with trichothecenes. Of the 5 samples, 3 corn samples were contaminated with DON and/or FB₁ at levels of over 10 μg/g.

Confirmation of 8-ketotrichothecenes in moldy corn extracts was done by scan mode using the silylated sample extracts. The retention time for each trichothecene was 21.22 min for DON, 22.38 min for 3-ADON, 22.51 min for 4-ANIV, 22.59 min for

Table 1 Occurrence of 8-ketotrichothecenes, zearalenone, and fumonisins in moldy corn

Sample Code	Concentration (ng/g) of								
	DON	15-ADON	3-ADON	NIV	4-ANIV	ZEA	FB ₁	FB ₂	FB ₃
1	12,000	2,700	34	3,400	2.0	570	10,000	4,000	440
2	15,000	1,400	87	4,300	140	7,300	12,000	440	160
3	1,600	140	ND ^{a)}	580	180	400	38,000	21,000	420
4	1,100	1,700	16	94	ND	130	3,400	860	530
5	6,300	30	51	1,400	11	1,500	5,200	700	320

Trichothecenes were quantified by GC-MS with SIM mode, and ZEA and fumonisins were quantified by HPLC with fluorescence detection.

^{a)} Not detected

Fig. 1 Total ion chromatogram of trimethylsilyl (TMS) ethers of moldy corn extract (A) and mass spectra of TMS ethers of DON (B) and iso-DON (C) from the sample extract.

15-ADON, and 22.90 min for NIV. Figure 1A shows the total ion chromatogram of trimethylsilyl ethers of one of sample extracts (Sample code No. 2). The agreement of retention times coupled with the presence of diagnostic ions verified the presence of DON, 15-ADON, 3-ADON, NIV, and 4-ANIV in the sample extract. Figure 1B shows the mass spectrum of DON part of chromatogram. The mass spectrum of DON gave the diagnostic ions at m/z 422, 393, 295, 287, 259, 235 and the molecular ion at m/z 512. The GC-MS analysis of the extract also showed the peak other (retention time; 21.91 min) than DON to have a molecular ion at m/z 512 and the major fragment ions were same with those of DON, indicating that

this compound is an isomer of DON (Figure 1C). The level of iso-DON in moldy corn was 1,600 ng/g by extrapolating the calibration of DON. The extract was chromatographed on a preparative thin layer chromatography plate with DON standard and developed in chloroform-methanol (9: 1, v/v). The regions corresponding to DON (R_f ; 0.30) and iso-DON (R_f ; 0.27) were scraped off, eluted with acetone, and concentrated to dryness. The residues were analyzed with a JEOL AX 505 GC-MS with a direct inlet. Figure 2A and 2B show the mass spectra of DON and iso-DON from the moldy corn extract, respectively. Both compounds have a molecular ion at m/z 296.

DON was found from the culture extract of *F.*

Fig. 2 Mass spectra of DON (A) and iso-DON (B) from the sample extract.

roseum and moldy barley⁷⁾ and also reported as vomitoxin as the emetic principle from *Fusarium* infected corn⁸⁾. As for the natural occurrence of DON isomer, Vesonder et al.⁹⁾ isolated DON and DON isomer as the refusal and emetic principle from *Fusarium* infected corn samples. Compared mass spectrum of DON to DON isomer, there was no major ion m/z 248 corresponding to the loss of both water and formaldehyde for the hydroxyl group adjacent to the C-keto group. They proposed DON isomer as DON with the hydroxyl group on the C4 position rather than C7 position. However, there is so far no evidence supporting this structure of 7-hydroxy-DON.

The mass spectrum of iso-DON in this study is somewhat similar to that of DON isomer reported by Vesonder et al.⁹⁾; no major ion at m/z 248 and major ions at m/z 133 and 117 were observed. At this moment, it is not definitely conclusive that both compounds are identical. Further studies could not be carried out to clarify the two compounds because of limited amounts of iso-DON. Mycological survey and production of 8-ketotrichothecenes by *F. graminearum* isolates from corn samples are in progress.

Acknowledgement This work was supported by a grant from Rural Development Administration of Korea.

References

- 1) Kim, J. -C., Kang, H. -J., Lee, D. -H., Lee Y. -W., Yoshizawa, T.: Appl. Environ. Microbiol., **59**, 3798-3802 (1993).
- 2) Kim, K. -H., Lee, Y. -W., Mirocha, C. J., Pawlosky R. J.: Appl. Environ. Microbiol., **56**, 260-263 (1990).
- 3) Lee, Y.-W., Kim, K. -H., Chung, H. -S.: Kor. J. Mycol., **18**, 13-19 (1990).
- 4) Tanaka, T., Hasegawa, A., Matsuki, Y., Ishii, K., Ueno, Y.: Food Addit. Contam., **2**, 125-137 (1985).
- 5) Tanaka, T., Hasegawa, A., Yamamoto, S., Lee, U. -S., Sugiura, Y., Ueno, Y.: J. Agric. Food Chem., **36**, 979-983 (1986).
- 6) Yoshizawa, T., Yamashita, A., Chokethaworn, N.: Food Addit. Contam., **13**, 163-168 (1996).
- 7) Yoshizawa, T., Morooka, N.: Agric. Biol. Chem., **37**, 2933-2934 (1973).
- 8) Vesonder, R. F., Ciegler, A., Jensen, A. H.: Appl. Microbiol., **25**, 1008-1010 (1973).
- 9) Vesonder, R. F., Ciegler, A., Jensen, A. H., Rothwedder, W. K., Weisleder, D.: Appl. Environ. Microbiol., **31**, 280-285 (1976).