Natural Cooccurrence of Deoxynivalenol and Nivalenol, Trichothecene Mycotoxins, in Commercial Foods*

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1. Introduction

During cultivation of wheat and barley in a crop field, field fungi, *Fusarium graminearum* (the imperfect stage of *Gibberella zeae*), often invade the plants, resulting in contamination of the harvested cereals by Fusaria mycotoxins. In the previous papers¹⁻⁴⁾, the authors described the frequent natural occurrence of trichothecene mycotoxins, deoxynivalenol and nivalenol, in freshly harvested wheat and barley grains in Japan, and it was also proposed that these trichothecenes found in the cereals may be produced before harvest by different strains of *Fusarium graminearum* producing deoxynivalenol or nivalenol⁵⁾.

It is likely that these trichothecenes contaminate certain foodstuffs made from the above cereals. However, the natural contamination of trichothecenes in foods has not been investigated. In this paper, we wish to describe the chemical analysis of deoxynivalenol and nivalenol in commercial parched-barley flours ("Mugi-kogashi" or "Hattaiko" in Japanese).

2. Materials and Methods

Commercial samples of parched-barley flours were purchased at local markets in Kagawa, Ehime and Kumamoto prefectures.

A sample (50 g) was extracted with methanol-water (150+50 ml) followed by filtration. The filtrate was defatted with *n*-hexane, concentrated and chromatographed successively

on XAD-2 and Florisil columns according to Kamimura's method⁶⁾ with some modifications. Thereafter, the extract was applied to a Sep-Pak C₁₈ cartridge and eluted with 2 ml of 30% methanol in water. After concentration of the eluate, an aliquot of the residue was treated with a mixture of trimethylsilylimidazole-bis (trimethylsilyl)acetamide-trimethylchlorosilane (3:3:2) and quantitated by mass fragmentography using a Hitachi M-80A mass spectrometer. Analytical conditions were as follows: 1 m×3 mm id. glass column packed with 3% OV-17 on 80-100 mesh Chromosorb W (AW-DMCS), column temperature 270°C, helium flow rate 28 ml/min, ion source temperature 185°C, ionizing voltage 20 eV, total emission 110 μ A, multiplier gain 1.4 kV, scan rate m/z 1-1500 for 8 sec, vacuum 3×10^{-7} torr. Fragment ions monitored were m/z 512 (M^+), and 497 ($M^+ - CH_3$ for deoxynivalenol, and m/z 482 ($M^+ - 110$) and 413 for nivalenol.

3. Results and Discussion

The cleanup procedure using XAD resin and Florisil columns has been adopted successfully for the analysis of naturally occurring trichothecenes in several cereal grains^{4,7,8)}. After the extraction and cleanup of parched-barley flours, we observed interference with the deoxynivalenol peak on gas liquid chromatography with electron capture detector (GLC-EC) by a concomitant peak, and hence the lower limit of detection by GLC-EC was estimated to be around 100 ppb. On the other hand, the nivalenol peak was unaffected

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Fig. 1. GC-MS (mass fragmentography) at m/z 512 and 497 for deoxynivalenol trimethylsilyl ether (A), and at m/z 482 and 413 for nivalenol trimethylsilyl ether (B) from the extract of naturally contaminated parched-barley flour

by any interfering peak. The percentage recovery of these mycotoxins ranged from 75 to 80% at a level of 100 ppb, as measured by GLC-EC.

Fig. 1 shows a typical mass-fragmentogram of the extract of parched-barley flour sample. The fragment ions monitored at m/z 512 and 497 for the trimethylsilyl ether of deoxynivalenol (t_R , 6.9 min) and at m/z 482 and 413 for that of nivalenol (t_R , 8.0 min) gave satisfactory results. By this method, as little as 10 ppb of the toxins can be detected.

Table 1 shows the concentrations of deoxynivalenol and nivalenol found in the parched-barley flours, as measured by mass fragmentography. Both trichothecenes were detected in all samples tested at levels ranging from 27 to 85 ppb for deoxynivalenol, and from 37 to 190 ppb for nivalenol. In sample numbers 46, 47, 53 and 54, these toxins were also detected by GLC-EC. With both these toxins, the values at the two ions were similar, and the coefficients of variation ranged from 6.4 to 9.4% for deoxynivalenol and from 3.8 to 12.3% for nivalenol.

In order to confirm the presence of these trichothecenes, the mass spectrum was taken at the peak corresponding to the individual toxin. As shown in Fig. 2, each spectrum shows diagnostic fragments due to the trimethylsilyl ether of deoxynivalenol or nivalenol.

To our knowledge, this is the first report on the natural cooccurrence of trichothecene mycotoxins, deoxynivalenol and nivalenol, in commercial foodstuffs. From the viewpoint of potential risk to human health, further extensive studies are required to clarify the mycotoxins contamination level in various foodstuffs made from domestic wheat and

Table 1. GC-MS Analysis of Deoxynivalenol and Nivalenol in Commercial Samples of Parched-barley Flours

Sample No.	Trichothecene level (ppb) analyzed at the indicated ion					
	Deoxynivalenol			Nivalenol		
	m/z 512	m/z 497	Avg.	m/z 482	m/z 413	Avg.
45	25	29	27	41	32	37
46	66	75	71	150	139	145
47	59	70	65	74	88	81
51	25	29	27	47	41	44
53	77	93	85	199	181	190
54	44	51	48	84	94	89

Fig. 2. Mass spectra of trimethylsilyl ethers of deoxynivalenol (A) and nivalenol (B) found in naturally contaminated parched-barley flour
Fragment ions in parentheses were derived from impurities.

barley.

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References

- 1) Morooka, N., Uratsuji, N., Yoshizawa, T., Yamamoto, H.: *J. Food Hyg. Soc. Japan* **13**, 368 (1972).
- 2) Yoshizawa, T., Tsuchiya, Y., Teraura, M., Morooka, N.: *Proc. Jpn. Assoc. Mycotoxicol.* **2**, 30 (1976).
- 3) Yoshizawa, T.: *ibid.* **8**, 6 (1978).
- 4) Yoshizawa, T.: "Trichothecenes-Chemical, Biological and Toxicological Aspects", edited by Ueno, Y., p. 197 (1983), Kodansha Ltd., Tokyo, Japan.
- 5) Yoshizawa, T., Matsuura, Y., Tsuchiya, Y., Morooka, N., Ichinoe, M., Kurata, H.: *J. Food Hyg. Soc. Japan* **20**, 21 (1979).
- 6) Kamimura, H., Nishijima, M., Saito, K., Takahashi, S., Ibe, A., Ochiai, S., Naoi, Y.: *ibid.* **19**, 443 (1978).
- 7) Kuroda, H., Mori, T., Nishioka, C., Okasaki, H., Takagi, M.: *ibid.* **20**, 137 (1979).
- 8) Kamimura, H., Nishijima, M., Yasuda, K., Saito, K., Ibe, A., Nagayama, T., Ushiyama, H., Naoi, Y.: *J. Assoc. Off. Anal. Chem.* **64**, 1067 (1981).