Natural Occurrence of Fumonisins B_1 and B_2 in Domestic Maize of Taiwan

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Samples of maize grown in various districts of Taiwan were collected and analyzed for the presence of fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) using high-performance liquid chromatography. Forty-nine (44.5%) and 2 (1.8%) of 110 samples were found to contain FB_1 (109−1148 ng/g) and FB_2 (222−255 ng/g), respectively. The frequency of detection and also the maximum FB_1 concentration were found in samples from Penton (2/2, 262 ng/g), followed by Chiayi (18/26, 264 ng/g), Tainan (8/16, 160 ng/g), Hualinen (5/14, 1148 ng/g), Taitung (7/20, 109 ng/g), and Yunlin (9/26, 361 ng/g). Of the 110 samples examined, only 2 samples from Hualinen had been detected containing FB_2. During an analysis of the distribution pattern of FB_1, it became apparent that >79% of tested samples had FB_1 concentrations <100 ng/g, whereas 2.7% (or 3 samples) contained FB_1 >300 ng/g. These results clearly illustrated that domestically produced maize for human consumption is frequently contaminated with FB_1.

Keywords: *Fumonisin B_1; fumonisin B_2; maize*

INTRODUCTION

Fumonisins are a group of naturally occurring mycotoxins produced by *Fusarium moniliforme*, *Fusarium proliferatum*, and other related species (Nelson et al., 1992). To date, six different fumonisins have been identified. Of these, fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) are the major toxins, whereas FB_3, FB_4, FA_1, and FA_2 are the minor ones (Cawood et al., 1991). FB_1 and FB_2 are structurally related mycotoxins that are known to be associated with outbreaks of leukoencephalomalacia in horses (Bezuidenhout et al., 1988) and porcine pulmonary edema in swine (Gelderblom et al., 1988), and they are suspected as *F. moniliforme* is associated with human esophageal cancer (Sydenham et al., 1990). It has been proposed that one of the toxicity mechanisms of FB_1 is its inhibition of the enzyme sphinganine *N*-acyltransferase, which results in a decrease in sphingosine and the accumulation of free sphinganine, an intermediate in the biosynthetic pathway of sphingolipids (Riley et al., 1994).

The natural occurrence of fumonisins in corn or corn-based food and feeds has been studied in many instances (Sydenham et al., 1991; Pestka et al., 1994; Schneider et al., 1995). In Taiwan, fumonisin-producing strains of *Fusarium* species, with *F. moniliforme* in particular, have been isolated from cereals (Tseng et al., 1995) and corn-based foodstuffs (Tseng and Liu, 1997). These studies might have explained that corn-based foodstuffs collected from local markets in Taiwan are frequently contaminated with FB_1 and FB_2.

This paper describes for first time the natural occurrence of fumonisins in domestic maize for human consumption in Taiwan.

MATERIALS AND METHODS

Sources of Samples. A total of 110 maize samples of 1996−1997 crops were obtained from eight different districts of Taiwan: Taipei (2), Yunlin (26), Chiayi (26), Tainan (16), Kaohsing (4), Penton (2), Taitung (20), and Hualinen (14) (Figure 1). One kilogram samples of each representative dried maize were collected and stored in paper bags at 4 °C prior to analysis. Fumonisin analyses were performed according to the method of Shephard et al. (1990) with minor modification.

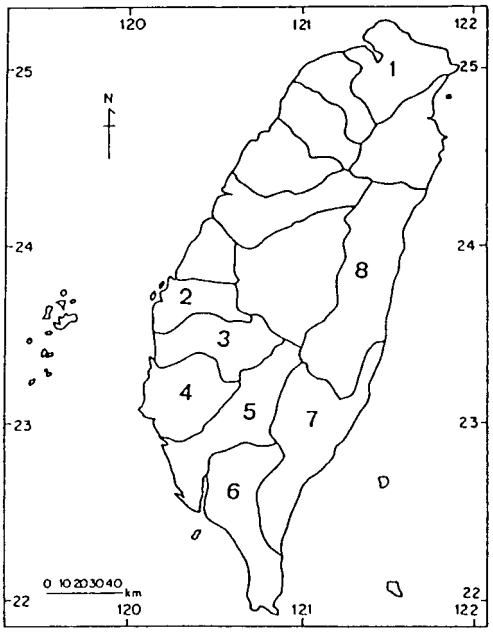

Figure 1. Sampling location of maize in various districts of Taiwan: 1, Taipei (2); 2, Yunlin (26); 3, Chiayi (26); 4, Tainan (16); 5, Kaohsing (4); 6, Penton (2); 7, Taitung (20); 8, Hualinen (14).

Extraction and Cleanup of Samples. Subsamples of ∼200 g were finely ground by a mill (Ye-Shin Iron Factory, Taoyuan, Taiwan) and thoroughly mixed. Twenty-five grams of ground corn was extracted by blending with methanol/water (3:1; 50 mL) in an Ultra-Turrax T 25 mixer (Janke & Gmbh, KG, Germany) for 5 min. The extract was centrifuged at 500*g* for 10 min, and the supernatant was then filtrated through a

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Table 1. Incidence and Levels of Fumonisins in Maize Samples Harvested from Various Districts of Taiwan for Human Consumption in 1996−1997

district	toxin	incidence (positive/total)	range[a] (ng/g)	mean-positives (ng/g) ± SD
Taipei	FB_1	0/2	ND[b]	
	FB_2	0/2	ND	
Yunlin	FB_1	9/26 (34.6%)	0−361	147 ± 20.1
	FB_2	0/26	ND	
Chiayi	FB_1	18/26 (69.2%)	0−264	116.3 ± 22.0
	FB_2	0/26	ND	
Tainan	FB_1	8/16 (50%)	0−160	88.3 ± 12.0
	FB_2	0/16	ND	
Kaohsing	FB_1	0/4	ND	
	FB_2	0/4	ND	
Penton	FB_1	2/2 (100%)	187−262	224.6 ± 47.4
	FB_2	0/2	ND	
Taitung	FB_1	7/20 (35%)	0−109	59.3 ± 6.6
	FB_2	0/20	ND	
Hualinen	FB_1	5/14 (35.7%)	0−1148	749 ± 17.4
	FB_2	2/14 (14.3%)	0−255	238.3 ± 39.2

[a] Values are means of three replicates. [b] ND indicates not detected. Detection limits were approximately 40 ng/g for FB_1 and 80 ng/g for FB_2.

Whatman No. 3 filter paper. A 5 mL volume was applied to a Lichrolut SAX cartridge (500 mg, E. Merck, Darmstadt, Germany) that had been conditioned with 8 mL of methanol followed by methanol/water (3:1; 8 mL). Subsequently, the cartridge was washed successively with methanol/water (3:1; 8 mL) and methanol (3 mL). The toxins were then eluted with 0.5% acetic acid in methanol (14 mL). The eluate was evaporated to dryness at a reduced pressure, and the residue was redissolved in 1 mL of methanol as an extract for analysis.

Analytical Method. The extract was quantitatively determined according to a modified high-performance liquid chromatography (HPLC) method (Shephard et al., 1990). Briefly, *o*-phthaldialdehyde (OPA) reagent was prepared by dissolving OPA (40 mg) in ethanol (1 mL) and adding 5 mL of 0.1 M sodium borate and 50 μL of 2-mercaptoethanol. Derivatives of fumonisins were prepared immediately prior to injection, by the addition of OPA reagent (175 μL) to the sample solution (25 μL). The derivatized samples were analyzed by a reverse phase, isocratic HPLC system consisting of a Waters Associates (Milford, MA) Model 6000 A solvent delivery system and a U6K injector. The analytical column [Lichrosorb RP-18 (10 μm), 250 × 4 mm, Art. 50334] and a precolumn filter [Lichrosorb RP-18 (7 μm)] were purchased from Merck. The detector was a fluorometer (Model FS-970; Schoeffel Instrument, Westwood, NJ). Excitation and emission wavelengths were 335 and 440 nm, respectively. Quantification was achieved by peak height measurement using a Model TR-250 Toricorder (Tokoy Rikokikai Co., Ltd., Japan). The eluate was methanol/0.1 M sodium dihydrogen phosphate (80:20, v/v) adjusted to pH 3.3 with phosphoric acid. The flow rate was 1 mL min^{-1}. Detection limits were approximately 40 ng/g for FB_1 and 80 ng/g for FB_2. Average recoveries of FB_1 and FB_2 from three tests in maize were 85 and 83%, respectively.

RESULTS AND DISSCUSSION

The ranges and means of fumonisin concentrations in maize samples harvested from various districts of Taiwan and their incidences of detection are summarized in Table 1.

Forty-nine (44.5%) of 110 samples were found to be contaminated with FB_1 to a maximum concentration of 109−1148 ng/g. Among the 110 samples, only 2 (1.8%) contained FB_2 toxin in the range 222−255 ng/g. As expected, the levels of FB_1 detected in samples were higher than the corresponding FB_2 levels. Our results are consistent with those reported by Hirooka et al. (1996) and Yoshizawa et al. (1996).

Table 2. Distribution of Fumonisin B_1 Levels in Maize Grown in Taiwan for Human Consumption

fumonisin concn (ng/g)	no. of samples	sample (%)
<100	87	79.1
101−200	16	14.6
201−300	4	3.6
>300	3	2.7
total	110	100.0

The highest frequency of FB_1 detection was encountered in samples from Penton district (100%) followed by Chiayi (69.2%), Tainan (50%), Hualinen (35.7%), Taitung (35%), and Yunlin (34.6%). The highest FB_1 concentration was found in a sample from Hualinen district [1148 ng/g (Table 1)].

Nine samples from the Yunlin district (34.6%) had an average of 147 ng/g of FB_1. Eighteen Chiayi samples (69.2%) were contaminated with an average of 116 ng/g of FB_1. Of the 16 samples from Tainan, 8 had FB_1 at an average of 88 ng/g. There was a lower and a reduced incidence of fumonisin contamination in samples of Taitung. Only 5 and 2 of 14 samples from Hualinen had an average of 749 and 238 ng/g of FB_1 and FB_2, respectively. Fumonisins (FB_1 and FB_2) were not detected in any of the samples from Taipei and Kaohsing districts (Table 1). The contamination levels and the frequency of occurrence in Taiwan were considerably lower than those reported in similar surveys in Costa Rica (Viquez et al., 1996), South Africa (Shephard et al., 1996), and other Asian countries (Ueno et al., 1993).

Table 2 displays the distribution pattern for FB_1 levels determined in 110 maize samples intended for human consumption. More than 79% of tested samples had total fumonisin concentration <100 ng/g, and 2.7% (or three samples) of these contained toxin at >300 ng/g.

In conclusion, the current survey clearly indicates that maize grown in Taiwan for human consumption is contaminated to various degrees with FB_1 and FB_2. Further investigation is need to clarify the difference in fungal infestation and fumonisin production in maize of each district of Taiwan.

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LITERATURE CITED

Bezuidenhout, S. C.; Gelderblom, W. C. A.; Gorst-Allman, C. P.; Horak, R. M.; Marasas, W. F. O.; Spiteller, G.; Vleggaar, R. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *J. Chem. Soc., Chem. Commun.* **1988**, 743−745.

Cawood, M. E.; Gelderblom, W. C. A.; Vleggaar, R.; Behrend, Y.; Thiel, P. G.; Marasas, W. F. O. Isolation of the fumonisin mycotoxins: A quantitative approach. *J. Agric. Food Chem.* **1991**, *39*, 1958−1962.

Gelderblom, W. C. A.; Jaskiewicz, K.; Marasas, W. F. O.; Thiel, P. G.; Horak, R. M.; Vleggaar, R.; Kriek, N. P. J. Fumonisins—novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* **1988**, *54*, 1806−1811.

Hirooka, E. Y.; Yamaguchi, M. M.; Aoyama, S.; Sugiura, Y.; Ueno, Y. The natural occurrence of fumonisins in Brazilian corn kernels. *Food. Addit. Contam.* **1996**, *13*, 173−183.

Nelson, P. E.; Plattner, R. D.; Shackelford, D. D.; Desjardins, A. E. Fumonisin B_1 production by *Fusarium* species other than *F. moniliforme* in section Liseola and some related species. *Appl. Environ. Microbiol.* **1992**, *58*, 984−989.

Pestka, J. J.; Azcona-Olivera, J. I.; Plattner, R. D.; Minervini, F.; Doko, M. B.; Visconti, A. Comparative assessment of fumonisin in grain-based foods by ELISA, GC-MS and HPLC. *J. Food Prot.* **1994**, *57*, 169−172.

Riley, R. T.; Wang, E.; Merrill, A. H. Liquid chromatographic determination of sphinganine and sphingosine: Use of the free sphinganine-to-sphingosine ratio as a biomarker for consumption of fumonisins. *J. Assoc. Off. Anal. Chem.* **1994**, *77*, 533−540.

Schneider, E.; Usleber, E.; Martlbauer, E. Rapid detection of fumonisins B_1 in corn-based food by competitive direct dipstick enzyme immunoassay/enzyme-linked immunofiltration assay with integrated negative control reaction. *J. Agric. Food Chem.* **1995**, *43*, 2548−2552.

Shephard, G. S.; Sydenham, E. W.; Thiel, P. G.; Gelderblom, W. C. A. Quantitative determination of fumonisins B_1 and B_2 by high-performance liquid chromatography with fluorescence detection. *J. Liq. Chromatogr.* **1990**, *13*, 2077−2087.

Shephard, G. S.; Thiel, P. G.; Stockenstrom, S.; Sydenham, E. W. Worldwide survey of fumonisin contamination of corn and corn-based products. *J. Assoc. Off. Anal. Chem.* **1996**, *79*, 671−687.

Sydenham, E. W.; Thiel, P. G.; Marasas, W. F. O.; Shephard, G. S.; Van Schalkwyk, D. J.; Koch, K. R. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, Southern Africa. *J. Agric. Food Chem.* **1990**, *38*, 1900−1903.

Sydenham, E. W.; Shephard, G. S.; Thiel, P. G.; Marasas, W. F. O.; Stockenstrom, S. Fumonisin contamination of commercial corn-based human foodstuffs. *J. Agric. Food Chem.* **1991**, *25*, 767−771.

Tseng, T. C.; Liu, C. Y. Occurrence of fumonisin B_1 and B_2 in corn-based foodstuffs in Taiwan market. *Mycopathologia* **1997**, *137*, 57−61.

Tseng, T. C.; Lee, K. L.; Deng, T. S.; Liu, C. Y.; Huang, J. W. Production of fumonisins by *Fusarium* species of Taiwan. *Mycopathologia* **1995**, *130*, 117−121.

Ueno, Y.; Aoyama, S.; Sugiura, Y.; Wang, D. S.; Lee, U. S.; Hirooka, E. Y.; Hara, S.; Karki, T.; Chem, G.; Yu, S. Z. A Limit Survey of Fumonisin in Corn and Corn-based Products in Asian Countries. *Mycotoxin Res.* **1993**, *9*, 27−34.

Viquez, O. M.; Castell-Perez, M. E.; Shelby, R. A. Occurrence of Fumonisin B_1 in Maize Grown in Costa Rica. *J. Agric. Food Chem.* **1996**, *44*, 2789−2791.

Yoshizawa, T.; Yamashita, A.; Chokethaworn, N. Occurrence of fumonisins and aflatoxins in corn from Thailand. *Food. Addit. Contam.* **1996**, *13*, 163−168.

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