FOOD CHEMICAL CONTAMINANTS

Fusarium Mycotoxins in Corn and Corn Products in a High-Risk Area for Gastric Cancer in Shandong Province, China

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Consumption of fermented, but not unfermented, corn pancakes has been linked with elevated stomach cancer mortality rates in rural Linqu County in Shandong Province, China. Previous surveys of fungal contamination of corn in China have detected fumonisins, which are mycotoxins produced by *Fusarium moniliforme*. To determine whether mycotoxins might account for the increased risk of cancer among those consuming fermented pancakes, we obtained specimens of corn, cornmeal, unfermented and fermented pancake batter, and cooked fermented pancakes from each of 16 households in Linqu County for analysis by the U.S. Department of Agriculture. Fumonisins B_1, B_2, and B_3 were detected (≥0.5 μg/g) in 19, 25, and 6% of the corn specimens, respectively, as well as in various corn products. No type A trichothecenes were detected; however, the type B trichothecenes deoxynivalenol and 15-acetyldeoxynivalenol were detected (≥0.5 μg/g) in 58 and 17% of the corn specimens, respectively, and zearalenone was detected (≥0.5 μg/g) in 15% of the cornmeal specimens. The mycotoxins were detected only at low levels (<10 μg/g), which did not increase with fermentation. These findings do not support the hypothesis that mycotoxin contamination increases the risk of gastric cancer among those who consume fermented Chinese pancakes.

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Stomach cancer rates in Linqu County, Shandong Province, China, are exceptionally high (1). A case-control study revealed that the risk of stomach cancer increased by 30% among those who consumed sour (fermented) corn pancakes, a local delicacy, at least daily (2). Corn is commonly contaminated by fungi of the genera *Aspergillus*, which can produce aflatoxins, and *Fusarium*, which can produce fumonisins and trichothecenes (3). Chinese corn contains fumonisins and trichothecenes (4). Chinese fermented pancake batter contains many fungi of the genera *Alternaria, Aspergillus, Aureobasidium, Candida, Fusarium, Geotrichum, Mucor, Penicillium, Phoma, Rhinocladiella, Torula,* and *Trichoderma* (5). Therefore, we analyzed raw corn, cornmeal, unfermented and fermented pancake batter, and cooked sour pancakes from Linqu County for aflatoxin, fumonisins, trichothecenes, zearalenone, and zearalenol.

Experimental

Specimen Collection and Handling

Seven villages were selected at random, representing the 5 townships of Linqu County. In each village, up to 3 households were selected randomly from among those known to be preparing sour pancakes. At each household, 5 specimens were collected in 1996 to represent successive stages of processing: raw corn, corn meal, unfermented batter, fermented batter, and cooked sour pancakes. Specimens were frozen on dry ice and shipped to the U.S. Department of Agriculture (USDA) National Veterinary Services Laboratories (NVSL) in Ames, IA. Three 5 g portions were cut from each frozen specimen; 2 were retained at NVSL for aflatoxin and fumonisin assays, and one was shipped to the Veterinary Sciences Laboratory at North Dakota State University for trichothecene assays. The specimens

Table 1. Fumonisin B_1 contamination of Chinese corn products

Village	Household	Fumonisin B_1, μg/g				
		Raw corn	Cornmeal	Unfermented batter	Fermented batter	Cooked pancake
A	1	**2.4**[a]	**1.0**	**0.6**	**0.6**	**1.1**
A	2	<0.5	**0.6**	**5.7**	**7.2**	**1.0**
B	3	<0.5	<0.5	<0.5	<0.5	<0.5
B	4	<0.5	<0.5	<0.5	<0.5	<0.5
B	5	<0.5	**8.8**	<0.5	<0.5	<0.5
C	6	<0.5	<0.5	<0.5	<0.5	<0.5
C	7	<0.5	<0.5	<0.5	<0.5	<0.5
C	8	0.5	<0.5	<0.5	<0.5	<0.5
D	9	<0.5	**0.8**	<0.5	<0.5	**0.7**
D	10	**2.2**	**0.8**	<0.5	<0.5	<0.5
D	11	<0.5	—[b]	<0.5	<0.5	<0.5
E	12	<0.5	<0.5	<0.5	<0.5	<0.5
E	13	<0.5	**0.7**	<0.5	<0.5	**0.5**
E	14	<0.5	**2.2**	<0.5	<0.5	**0.9**
F	15	<0.5	—	<0.5	<0.5	<0.5
G	16	<0.5	<0.5	**0.9**	**0.8**	**2.2**

[a] Values in bold face are ≥ 0.5 μg/g.
[b] — = No specimen obtained or specimen quantity insufficient for analysis.

were then shipped, still frozen on dry ice, to the USDA laboratory in Athens, GA, for a rat liver bioassay, which required only 1 g.

Aflatoxin Assay

Aflatoxins were determined by thin-layer chromatography (TLC), based on modification of a previously described method (6). Briefly, a 5 g sample was extracted for 30 min with acetonitrile–water (84 + 16, v/v) and filtered. A portion of the filtrate was eluted through a Mycosep No. 224 mixed solid-phase cleanup column (Romer Labs, Union, MO) to remove interferences. The solvent was evaporated to dryness, the residue was redissolved in toluene–acetonitrile (97 + 3, v/v), and a portion was spotted, along with authentic aflatoxin, on a normal-phase TLC plate. The plate was developed in toluene–acetone (1 + 1, v/v). Detection was by visualization of fluorescence with 365 nm ultraviolet light. The limit of detection was 20 ng/g.

Table 2. Fumonisin B_2 contamination of Chinese corn products

Village	Household	Fumonisin B_2, μg/g				
		Raw corn	Cornmeal	Unfermented batter	Fermented batter	Cooked pancake
A	1	1.0[a]	**0.5**	<0.5	<0.5	<0.5
A	2	<0.5	<0.5	<0.5	<0.5	<0.5
B	3	<0.5	<0.5	<0.5	<0.5	<0.5
B	4	<0.5	<0.5	<0.5	<0.5	<0.5
B	5	<0.5	**2.8**	<0.5	<0.5	<0.5
C	6	<0.5	<0.5	<0.5	<0.5	<0.5
C	7	<0.5	<0.5	<0.5	<0.5	<0.5
C	8	<0.5	<0.5	<0.5	<0.5	<0.5
D	9	<0.5	<0.5	**0.5**	<0.5	<0.5
D	10	**0.9**	<0.5	<0.5	<0.5	<0.5
D	11	**0.6**	—[b]	<0.5	<0.5	**1.1**
E	12	**0.7**	**0.6**	**0.5**	<0.5	<0.5
E	13	<0.5	<0.5	<0.5	<0.5	<0.5
E	14	<0.5	**1.0**	<0.5	<0.5	<0.5
F	15	<0.5	—	<0.5	<0.5	<0.5
F	16	<0.5	<0.5	<0.5	<0.5	**0.7**

[a] Values in boldface are ≥ 0.5 μg/g
[b] — = No specimen obtained or specimen quantity insufficient for analysis.

Table 3. Fumonisin B_3 contamination of Chinese corn products

Village	Household	Fumonisin B_3, μg/g				
		Raw corn	Cornmeal	Unfermented batter	Fermented batter	Cooked pancake
A	1	**0.5**[a]	<0.5	<0.5	<0.5	<0.5
A	2	<0.5	<0.5	<0.5	**0.9**	<0.5
B	3	<0.5	<0.5	<0.5	<0.5	<0.5
B	4	<0.5	<0.5	<0.5	<0.5	<0.5
B	5	<0.5	**0.9**	<0.5	<0.5	<0.5
C	6	<0.5	<0.5	<0.5	<0.5	<0.5
C	7	<0.5	<0.5	<0.5	<0.5	<0.5
C	8	<0.5	<0.5	<0.5	<0.5	<0.5
D	9	<0.5	<0.5	<0.5	<0.5	<0.5
D	10	<0.5	<0.5	<0.5	<0.5	<0.5
D	11	<0.5	—[b]	<0.5	<0.5	**0.6**
E	12	<0.5	<0.5	<0.5	<0.5	<0.5
E	13	<0.5	<0.5	<0.5	<0.5	<0.5
E	14	<0.5	**0.6**	<0.5	<0.5	<0.5
F	15	<0.5	—	<0.5	<0.5	<0.5
G	16	<0.5	<0.5	<0.5	<0.5	**0.7**

[a] Values in boldface are ≥ 0.5 μg/g.
[b] — = No specimen obtained or specimen quantity insufficient for analysis.

Fumonisin Assay

Fumonisins were determined by high-performance liquid chromatography (HPLC) with fluorescence detection as previously described (7), with a modification in the mobile phase. Briefly, a 5 g portion of sample was extracted for 30 min with acetonitrile–water (1 + 1, v/v), filtered, diluted with water, and eluted through a Sep-pak C_{18} solid-phase cleanup column (Waters, Milford, MA) to remove interferences. After cleanup, the extract was treated with *o*-phthalaldehyde to form fluorescent derivatives of the fumonisins. A portion of the derivatized extract was injected onto the chromatograph and eluted with ethanol–water (1 + 1, v/v) containing 0.175M acetic acid as the mobile phase. Fumonisins B_1, B_2, and B_3 were quantitated by comparison with authenticated standards. The limit of detection was 0.5 μg/g.

Table 4. Deoxynivalenol contamination of Chinese corn products

Village	Household	Deoxynivalenol, μg/g				
		Raw corn	Cornmeal	Unfermented batter	Fermented batter	Cooked pancake
A	1	—[a]	—	<0.5	<0.5	—
A	2	—	<0.5	<0.5	<0.5	<0.5
B	3	<0.5	**1.6**[b]	<0.5	<0.5	<0.5
B	4	—	<0.5	<0.5	<0.5	<0.5
B	5	<0.5	<0.5	<0.5	<0.5	<0.5
C	6	**1.4**	**1.5**	<0.5	<0.5	**1.1**
C	7	**0.9**	**1.0**	<0.5	<0.5	<0.5
C	8	<0.5	<0.5	<0.5	<0.5	<0.5
D	9	**2.7**	**1.2**	<0.5	**0.5**	**1.5**
D	10	**1.6**	**1.2**	**0.5**	<0.5	<0.5
D	11	**0.9**	—	<0.5	<0.5	**1.1**
E	12	<0.5	**0.5**	<0.5	<0.5	<0.5
E	13	**1.3**	**0.5**	<0.5	<0.5	<0.5
E	14	<0.5	**1.4**	<0.5	<0.5	<0.5
F	15	**1.0**	—	<0.5	<0.5	**0.7**
G	16	—	<0.5	<0.5	<0.5	—

[a] — = No specimen obtained or specimen quantity insufficient for analysis.
[b] Values in bold face are ≥ 0.5 μg/g.

Table 5. 15-Acetyldeoxynivalenol contamination of Chinese corn products

Village	Household	15-Acetyldeoxynivalenol, μg/g				
		Raw corn	Cornmeal	Unfermented batter	Fermented batter	Cooked pancake
A	1	—[a]	—	<0.5	<0.5	—
A	2	—	<0.5	<0.5	<0.5	<0.5
B	3	<0.5	<0.5	<0.5	<0.5	<0.5
B	4	—	<0.5	<0.5	<0.5	<0.5
B	5	<0.5	<0.5	<0.5	<0.5	<0.5
C	6	**0.7**[b]	<0.5	<0.5	<0.5	<0.5
C	7	<0.5	<0.5	<0.5	<0.5	<0.5
C	8	<0.5	<0.5	<0.5	<0.5	<0.5
D	9	<0.5	<0.5	<0.5	<0.5	<0.5
D	10	**0.5**	<0.5	<0.5	<0.5	<0.5
D	11	<0.5	—	<0.5	<0.5	<0.5
E	12	<0.5	<0.5	<0.5	<0.5	<0.5
E	13	<0.5	<0.5	<0.5	<0.5	<0.5
E	14	<0.5	<0.5	<0.5	<0.5	<0.5
F	15	<0.5	—	<0.5	<0.5	<0.5
G	16	—	<0.5	<0.5	<0.5	—

[a] — = No specimen obtained or specimen quantity insufficient for analysis.
[b] Values in bold face are ≥ 0.5 μg/g.

Trichothecene Assay

Five grams of ground sample was extracted with 20 mL acetonitrile–water (84 + 16, v/v) for 1 h on a horizontal shaker. A 6 mL portion of the supernatant was filtered through 1 g of a 1 + 1 (w/w) mixture of neutral aluminum oxide (EM Science, Gibbstown, NJ, No. 1077) and C_{18} packing material (Analtech, Newark, DE, No. 78050 RP bonded modified silica gel 150 A, pore 35–75 μm, 14% C), and 2 mL of the eluate was evaporated with nitrogen at 55°C for 30 min. The evaporated aliquot was equivalent to 0.5 g sample. The residue was derivatized with a mixture of trimethylchlorosilane, *N*-trimethylsilylimidazole, *N,O*-bis[trimethylsilyl] trifluoroacetamide, and pyridine to form trimethylsilyl (TMS) derivatives of 10 type A trichothecenes (neosolaniol, scirpentriol, 15-acetoxyscirpenol, diacetoxyscirpenol, HT2 toxin, T2 toxin, iso-T2 toxin, acetyl-T2 toxin, T2-triol, and T2-tetraol), 5 type B trichothecenes (nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, and fusarenon-X), and 2 estrogens (zearalenol and zearalenone).

The TMS derivatives were assayed by gas chromatography and selected-ion monitoring on a Finnigan Incos 50 mass spectrometer, using 3–4 ion fragments for identification and quantitation of each mycotoxin. For each batch of samples, a corn blank was spiked with 1 μg/g (sample equivalent) of deoxynivalenol, 15-acetyldeoxynivalenol, HT2 toxin, T2 toxin, and zearalenone and put through the full procedure. The response of this spiked blank response was then used as the reference point for the test samples and control pool. This approach monitors the recovery of type A and B trichothecenes as well as zearalenone. General recoveries of the 17 mycotoxin additions to cornmeal ranged from 80 to 100%. The limit of detection for all 17 mycotoxins was 0.5 μg/g.

Rat Liver Bioassay

The rat liver bioassay was performed as previously described (8). Briefly, each 1 g portion of corn or corn product was extracted with 10 mL acetonitrile–water (1 + 1, v/v). The extracts were filtered (Whatman No. 2), and 2.0 mL was dried under reduced pressure and resuspended in 0.2 mL dimethyl sulfoxide. Precision-cut slices (8 × 0.25 mm) of liver were prepared from previously healthy male Sprague-Dawley rats fed a fumonisin-free diet. Each slice was dosed with 50 μL of the extract at 37°C for 48 h. After incubation, the slices were rinsed with saline, blotted dry, weighed, and frozen at –80°C pending analysis.

Levels of free sphinganine and sphingosine in the rat liver slices were determined by a modification of a method previously published (9). Briefly, each slice was ground, sonicated, incubated, and hydrolyzed with base and then extracted with chloroform and water under alkaline conditions. The aqueous supernatant was discarded, and the hydrophobic fraction was retained for analysis by HPLC. For each specimen, the ratio of sphinganine (Sa) to sphingosine (So) was determined; an elevated Sa/So ratio (>0.35) was interpreted as evidence of contamination by fumonisin B_1.

Results

We obtained specimens from 16 households, all of which supplied samples of raw corn, batter (before and after fermentation), and cooked pancakes. All but 2 of the households also supplied cornmeal samples, for a total of 78 specimens.

Aflatoxin and Fumonisin Assay

Aflatoxin B_1 was not detectable (<20 ng/g) in any specimen. Fumonisin B_1 was detected (≥0.5 μg/g) in 24 specimens (Ta-

Table 6. Zearalenone contamination of Chinese corn products

Village	Household	Zearalenone, μg/g				
		Raw corn	Cornmeal	Unfermented batter	Fermented batter	Cooked pancake
A	1	—[a]	—	<0.5	<0.5	—
A	2	—	<0.5	<0.5	<0.5	<0.5
B	3	<0.5	**0.5**[b]	<0.5	<0.5	<0.5
B	4	—	<0.5	<0.5	<0.5	<0.5
B	5	<0.5	<0.5	<0.5	<0.5	<0.5
C	6	<0.5	<0.5	<0.5	<0.5	<0.5
C	7	<0.5	<0.5	<0.5	<0.5	<0.5
C	8	<0.5	<0.5	<0.5	<0.5	<0.5
D	9	<0.5	<0.5	<0.5	<0.5	<0.5
D	10	<0.5	<0.5	<0.5	<0.5	<0.5
D	11	<0.5	—	<0.5	<0.5	<0.5
E	12	<0.5	<0.5	<0.5	<0.5	<0.5
E	13	<0.5	<0.5	<0.5	<0.5	<0.5
E	14	<0.5	**0.5**	<0.5	<0.5	<0.5
F	15	<0.5	—	<0.5	<0.5	<0.5
G	16	—	<0.5	<0.5	<0.5	—

[a] — = No specimen obtained or specimen quantity insufficient for analysis.
[b] Values in bold face are ≥ 0.5 μg/g.

ble 1), including 3 samples of raw corn, 7 samples of cornmeal, 3 samples of unfermented batter, 3 samples of fermented batter, and 7 samples of cooked pancake. The maximum concentration was 8.8 μg/g. Fumonisin B_2 was detected (≥0.5 μg/g) in 12 specimens (Table 2), including 4 samples of raw corn, 4 samples of cornmeal, 2 samples of unfermented batter, and 2 samples of cooked pancake. The maximum concentration was 2.8 μg/g. Fumonisin B_3 was detected (≥0.5 μg/g) in 6 specimens (Table 3), including 1 sample of raw corn, 2 samples of cornmeal, 1 samples of fermented batter, and 2 samples of cooked pancake. The maximum concentration was 0.9 μg/g.

Trichothecene Assay

Seventy-two specimens were of sufficient quantity for analysis of zearalenone, zearalenol, and trichothecenes. Of these, 21 had detectable (≥0.5 μg/g) deoxynivalenol (Table 4), including 7 samples of raw corn, 8 samples of cornmeal, 1 sample of unfermented batter, 1 sample of fermented batter, and 4 samples of cooked pancake. Two samples of raw corn (Table 5) had 15-acetyldeoxynivalenol (≥0.5 μg/g), and 2 samples of cornmeal (Table 6) had zearalenone (≥0.5 μg/g). No other type B trichothecenes (nivalenol, neosolaniol, or fusarenon-X) were detected (<0.5 μg/g). No type A trichothecenes (scirpentriol, 15-acetylscirpenol, diacetoxyscirpenol, T2 toxin, iso-T2 toxin, acetyl-T2 toxin, T2-tetraol, or T2-triol) were detected (<0.5 μg/g). No zearalenol was detected (<0.5 μg/g).

Rat Liver Bioassay

Any specimen with Sa/So > 0.35 was deemed positive for fumonisin activity in the rat liver bioassay (Table 7). There were 9 such specimens, including 2 of 16 specimens of raw corn (13%), 4 of 14 specimens of cornmeal (29%), 1 of 16 specimens of unfermented batter (6%), 1 of 16 specimens of fermented batter (6%), and 1 of 16 specimens of cooked pancake (6%). Each of the specimens found positive by the rat liver bioassay contained fumonisin B_1 above the limit of detection; however, the bioassay was negative for 6 other specimens with detectable fumonisin B_1 concentrations, ranging from 0.5 to 7.2 μg/g.

Discussion

With a detection limit of 20 ng/g, no aflatoxins were found in any of the specimens. Fumonisin B_1 was detected (≥0.5 μg/g) in 19% of raw corn specimens from Linqu County, versus 47, 27, and 32% of raw corn specimens obtained in Nebraska, Iowa, and Illinois, respectively, and analyzed in the same laboratory in 1996 (10). Fumonisin B_1 levels exceeded 5.0 μg/g in 3.9% of the raw corn specimens in the USDA survey (10) but in none of the Chinese specimens. Fumonisin B_1 was detected in all 9 specimens for which the rat liver slice bioassay was positive; however, the bioassay was negative for 6 of 15 specimens (40%) with detectable fumonisin B_1. Thus, it appears that the bioassay, while highly specific, is relatively insensitive. A positive bioassay indicates the presence of toxins that block ceramide synthase, a key enzyme in sphingolipid biosynthesis. This enzyme inhibition is believed to be the mechanism of toxicity of fumonisins, and it has been shown that mycotoxins with structures unlike fumonisins, including aflatoxin and trichothecenes, produce negative results in the bioassay (11).

Deoxynivalenol was found in 58% of raw corn specimens from Linqu County, versus 30, 18, and 13% of corn specimens obtained in Nebraska, Iowa, and Illinois, respectively, and ana-

Table 7. Rat liver bioassay for fumonisin contamination of Chinese corn products

Village	Household	Ratio of Sphinganine to Sphingosine				
		Raw corn	Cornmeal	Unfermented batter	Fermented batter	Cooked pancake
A	1	**1.97**[a]	**0.55**	**0.65**	**1.17**	**1.60**
A	2	0.17	**0.38**	0.14	0.11	—[b]
B	3	0.13	0.19	0.12	—	0.13
B	4	0.12	—	0.09	0.05	0.08
B	5	0.17	**2.74**	0.14	0.13	0.06
C	6	0.14	0.09	0.08	0.07	0.05
C	7	0.14	0.18	0.13	0.09	0.05
C	8	0.10	0.16	0.15	0.13	—
D	9	0.16	—	0.29	0.15	—
D	10	**2.33**	0.27	0.07	0.07	0.14
D	11	0.12	—	0.06	0.07	0.05
E	12	0.11	0.13	0.09	0.22	0.29
E	13	0.16	0.14	0.11	0.08	—
E	14	0.13	**3.24**	0.21	0.24	0.12
F	15	0.12	—	0.13	0.07	—
G	16	0.11	0.20	0.12	0.09	—

[a] Values in bold face signify a ratio ≥ 0.35 μg/g.
[b] — = No specimen obtained or specimen quantity insufficient for analysis.

lyzed in the same laboratory in 1996 (10). Deoxynivalenol concentrations exceeded 1.0 μg/g in 33% of the Chinese specimens, versus only 6.8% in the USDA survey (10).

Although deoxynivalenol contamination was somewhat more widespread in Shandong Province than in the American Midwest, the opposite was true for fumonisin B_1, and even the maximum levels of these mycotoxins were below the toxic range. The present study found no evidence that any of the target mycotoxins increased during the fermentation process. Our findings do not support the hypothesis that these mycotoxins account for the increased risk of gastric cancer associated with consumption of fermented (sour) versus unfermented (sweet) corn pancakes in Linqu County.

A previously published survey (4) of raw corn in Linxian County, China (a high-risk area for adenocarcinoma of the gastric cardia and squamous cell carcinoma of the esophagus), detected much higher levels of fumonisin B_1 (range, 18–155 μg/g), total type A trichothecene levels of up to 2 μg/g, and total type B trichothecenes levels of up to 6 μg/g, while aflatoxin levels ranged from 0.7 to 38.4 ng/g. The Linxian survey used an immunoassay, which may have been more sensitive, to determine concentrations of trichothecenes and aflatoxin, but fumonisin analysis in the Linxian study, as in our study, was by HPLC. Moreover, in the Linxian County study, *Fusarium moniliforme* isolated from Chinese corn was found to produce high levels of nitrosamines in vitro (4), and nitrosamines were detected in a previous analysis of Chinese fermented pancake batter (5). We are currently assessing nitrosamine levels in additional specimens of fermented pancake batter from Linqu County.

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