FOOD CHEMICAL CONTAMINANTS

Fumonisin Levels in Uruguayan Corn Products [1]

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A survey was conducted to evaluate fumonisins FB_1 and FB_2 in Uruguayan corn products. Sixty-four samples of different local brands were purchased from retail stores during a 15-month period and analyzed for FB_1 and FB_2 by methanol–water extraction, cleanup with a 1 mL strong-anion-exchange solid-phase extraction column, and liquid chromatography with *o*-pthaldialdehyde–2-mercaptoethanol derivatization and fluorescence detection. Contamination levels for FB_1 varied from 50 ng/g (detection limit) to 6342 ng/g. Values were highest in feed samples (up to 6342 ng/g), unprocessed corn kernel (up to 3688 ng/g), and milled products, which included polenta (up to 427 ng/g). They were lowest in processed corn kernel (up to 155 ng/g) and snacks (up to 314 ng/g). FB_2 was determined in one-fourth of the total samples and detected at trace levels in only one feed sample. The data demonstrated the natural occurrence of fumonisins in corn products in Uruguay. Feed and polenta that contain fumonisins could be of concern because they are consumed in large amounts and are often the main nutrient source in Uruguay.

Fumonisins are a family of mycotoxins produced by *Fusarium moniliforme*, *F. proliferatum*, and other related species (1, 2) that colonize corn worldwide. Fumonisins have been reported in corn-based human food (3, 4). The toxins produced can accumulate to levels known to be toxic to animals and potentially harmful to humans. The fumonisins cause leukoencephalomalacia (ELEM) in horses (5) and porcine pulmonary edema (PPE) in swine (6). It has been proposed that one of the mechanisms of toxicity of fumonisins is inhibition of the enzyme sphinganine *N*-acyltransferase, which results in a decrease in sphingosine and accumulation of free sphinganine, an intermediate in the biosynthetic pathway for complex sphingolipids (7).

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Esophageal cancer has been linked to one of these toxins, fumonisin B_1 (FB_1; 8, 9). In humans, ingestion of corn infected with *F. moniliforme* has been implicated in the high incidence of esophageal cancer in northeastern Italy (10), the Transkei region in South Africa, and the Linxian region of China (11). Uruguay has a high rate of esophageal cancer that has been associated with drinking "mate," a local herbal infusion sipped very hot through a metal straw. However, this association has never been demonstrated. Because the natural occurrence of fumonisins in Uruguayan corn foods had not been studied, a survey was conducted to evaluate FB_1 and FB_2 in priority corn products.

Experimental

Sample Collection

Sixty-four different local brands of corn products were purchased from retail stores during a 15-month period (1/1995 to 4/1996). Samples included 22 unprocessed corn kernel, 8 polentas, 2 popcorn, 5 snacks, 4 corn starch, 3 breakfast cereals, 4 canned corn, 2 frozen corn, 1 mazamorra (grits), and 13 corn-based feeds. These products were grouped into 5 categories: milled products (I), snacks and breakfast cereals (II), corn-based feeds (III), processed corn kernel (IV), and unprocessed corn kernel (V). The samples were a random selection of the most popular national brands taken from store shelves. A subsample of ca 1 kg of each was ground to uniform consistency and mixed well before analysis.

Reagents and Apparatus

(**a**) *Solvents and reagents.*—Liquid chromatographic (LC) grade acetonitrile and methanol; American Chemical Society (ACS) grade acetic acid, sodium bicarbonate, and sodium tetraborate; *o*-pthaldialdehyde (OPA), Sigma Chemical Co., St. Louis, MO; LC quality water.

(**b**) *Solid-phase extraction (SPE) tubes.*—Strong-anion-exchange (SAX) columns; 1 mL Supelclean LC-SAX, Supelco (Belfonte, PA).

Table 1. Occurence of fumonisins B_1 and B_2 in Uruguayan corn-based products

Category	Product	FB_1, ng/g	FB_2, ng/g	Range, positives/ category FB_1, ng/g	Average FB_1, ng/g	Incidence/category FB_1 pos./total, %
I	Polenta	277	—[a]	100–427	105	3/12 (25)
	Polenta	ND[b]	—			
	Polenta	100	—			
	Polenta	ND	—			
	Polenta	ND	ND			
	Polenta	ND	ND			
	Polenta	ND	—			
	Polenta	427	—			
	Corn starch	ND	ND			
	Corn starch	ND	ND			
	Corn starch	ND	ND			
	Corn starch	ND	ND			
II	Pop corn	199	—	152–314	118	4/10 (40)
	Snacks	314	ND			
	Corn flakes	218	—			
	Snacks	152	ND			
	Corn flakes	ND	ND	152–314	118	4/10 (40)
	Pop corn	ND	ND			
	Snacks	ND	ND			
	Snacks	ND	—			
	Snacks	ND	—			
	Corn flakes	ND	—			
III	Feed	3733	—	256–6342	2573	13/13 (100)
	Feed	2800	—			
	Feed	2450	—			
	Feed	2700	—			
	Feed	2390	—			
	Feed	3480	Trace			
	Feed	1848	—			
	Feed	1200	—			
	Feed	5021	—			
	Feed	690	—			
	Feed	540	—			
	Feed	6342	—			
	Feed	256				
IV	Canned cream corn	ND	—	155	65	1/7 (14)
	Canned corn	ND	ND			
	Frozen corn	155	—			
	Mazamorra	ND	—			
	Canned corn	ND	ND			
	Canned corn	ND	—			
	Frozen corn	ND	—			
V	Corn	3153	—	165–3688	963	11/22 (50)
	Corn	ND	—			
	Corn	ND	—			
	Corn	1672	—			
	Corn	513	—			
	Corn	676	—			
	Corn	1877	—			
	Corn	ND	—			
	Corn	ND	—			
	Corn	3688	—			
	Corn	ND	—			
	Corn	5787	—			
	Corn	212	ND			

Table 1. *(continued)*

Category	Product	FB_1, ng/g	FB_2, ng/g	Range, positives/ category FB_1, ng/g	Average FB_1, ng/g	Incidence/category FB_1 pos./total, %
V *(continued)*	Corn	1313	—			
	Corn	ND	—			
	Corn	ND	—			
	Corn	ND	—			
	Corn	1582	—			
	Corn	ND	ND			
	Corn	ND	—			
	Corn	ND	—			
	Corn	165	—			

[a] Not determined.
[b] ND (not detected); equated to detection limit (50 ng/g).

(c) *o-Phthaldialdehyde (OPA) solution.*—Dissolve 40 mg in 1 mL methanol in amber vial; add 5 mL 0.1M sodium tetraborate and 50 µL 2-mercaptoethanol (MCE); prepare daily.

(d) *Fumonisin standards.*—Plant Research Centre, Agriculture and Agri-Food, Ottawa, ON, Canada.

(e) *FB_1 and FB_2 standard solutions.*—Prepare stock solution of 100 µg/mL of each fumonisin in acetonitrile–water (1 + 1, v/v). Dilute 100 µL of each stock solution to 1 mL in acetonitrile–water (1 + 1, v/v) to obtain working solution of 10 µg/mL. Store all standard solutions in freezer.

(f) *Mobile phase.*—Methanol–0.1M NaH_2PO_4 (77 + 23, v/v; pH 3.3) and H_2O.

(g) *LC system.*—Hewlett Packard Model 1050 isocratic system with 20 µL sample loop, 100 × 4.6 mm C_{18} ODS Hypersil column with 5 µm particle size and precolumn; Hewlett Packard fluorescence detector Model 1046A with excitation wavelength of 232 nm and emission wavelength of 425 nm; and Hewlett Packard Model 1050 integrator.

Extraction and Cleanup

A 25 g ground subsample was shaken for 30 min with 250 mL methanol–water (3 + 1, v/v) and then filtered through Whatman No. 50 filter paper. The pH of the extract was adjusted if necessary to pH 5.8–6.5 with 1M NaOH (12). A 1 mL portion containing 0.1 g sample was applied to a 1 mL SAX SPE column previously conditioned with 2 mL methanol followed by 1 mL water (13). The column was washed successively with 500 µL water and 500 µL methanol. All washes were discarded. Fumonisins B_1 and B_2 were eluted with 1 mL methanol–acetic acid (99 + 1, v/v). Washing and elution were accomplished by gravity flow. The eluate was evaporated to dryness under a stream of nitrogen in a water bath (60°C). The residue was redissolved in 200 µL methanol, and portions of this solution were used for derivatization.

Derivatization and LC Analysis

Residues were derivatized immediately prior to injection by addition of 250 µL OPA reagent to 25 µL sample solution. A 20 µL portion of the derivatized samples was analyzed by a reversed-phase, isocratic system with fluorescence detection. Quantitation was by peak height. The flow rate was 1 mL/min. FB_1 and FB_2 standards (10 µ each) were derivatized in the same way. Method recoveries from 6 samples spiked with 100 or 1000 ng FB_1/g ranged from 60 to 110%. Detection limit was 50 ng/g. Results below the level of detection were taken as 50 ng/g for calculations of average.

Results and Discussion

Naturally occuring levels of FB_1 in Uruguayan corn and corn-based products varied from 50 ng/g (detection limit) to 6342 ng/g (Table 1). Values were highest in feed samples (average, 2573; range, 256–6342 ng/g); unprocessed corn kernel (average, 963; range, ND–3688 ng/g); and milled products, including polenta (average, 105; range, ND–427 ng/g). They were lowest in snacks and breakfast cereals (average, 118; range, ND–314 ng/g) and processed corn kernel (average, 65; range, ND–155 ng/g).

FB_1 incidence was 100% in feed samples and a minimum of 14% in processed corn kernel. The distribution of FB_1 levels for all 4 categories as percent of total samples is shown in Figure 1. Feeds also gave the highest frequency of positive samples. The feed sample wtih the highest FB_1 value (6342 ng/g) originated from an incident involving a horse with fatal ELEM symptoms.

FB_2 was determined at random in one-fourth of the total samples and detected at trace levels in only one feed sample (Table 1).

The data presented are the first report of the natural occurrence of fumonisins in corn products in Uruguay. Feed and polenta that contain fumonisins could be of concern because they are consumed in high amounts and are often the main nutrient source for animals and humans, respectively.

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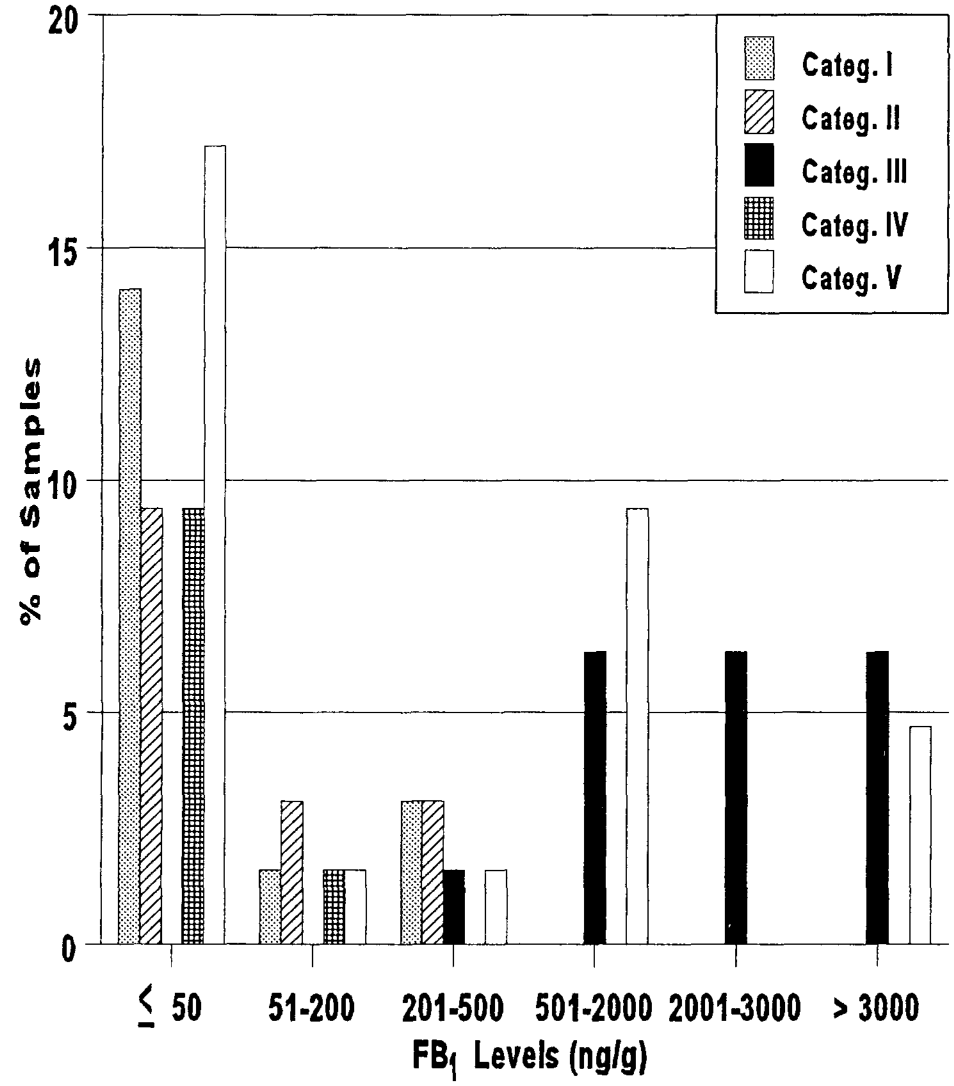

Figure 1. Frequency distribution of FB_1 levels.

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