

## Distribution of *Fusarium* Molds and Fumonisin in Dry-Milled Corn Fractions<sup>1</sup>

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### ABSTRACT

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The distribution of *Fusarium* molds and fumonisins was determined in commercial and experimental dry-milled corn fractions. *Fusarium* infection of the commercial whole corn samples ranged from 10 to 28%; *F. moniliforme* was the predominant species. *Fusarium* counts in corn fractions were <100 colony-forming units (CFU)/g in flaking grits, <100 – 6.4 × 10<sup>4</sup> CFU/g in bran, <100 – 1.6 × 10<sup>4</sup> CFU/g in germ, and <100 – 2.7 × 10<sup>3</sup> CFU/g in flour. Fumonisin concentrations were ≤0.1 μg/g in flaking grits, 0.2–1.1 μg/g in flour, 0.1–2.0 μg/g in germ, and 1.5–3.2 μg/g in bran.

Yellow, blue, and white dent corns naturally contaminated with varying levels of fumonisins (25.4, 3.9, and 0.3 μg of fumonisin B<sub>1</sub> per gram) and *Fusarium* molds (3.9 × 10<sup>6</sup>, 8.0 × 10<sup>5</sup>, and 2.6 × 10<sup>4</sup> CFU/g) were experimentally dry milled with a horizontal drum degermer. Number 5 grits contained significantly lower *Fusarium* counts and fumonisin concentrations than the whole kernel corn. *Fusarium* counts and fumonisins increased as grit size decreased, and high *Fusarium* counts and fumonisin concentrations were found in germ, bran, and fines.

The fumonisins, a group of structurally related mycotoxins, were first isolated from one of the most common fungal contaminants of corn, *Fusarium moniliforme* (Gelderblom et al 1988). Other *Fusarium* species, including *F. proliferatum*, *F. anthophilum*, *F. dlamini*, *F. napiforme*, and *F. nygamai*, also produce fumonisins (Thiel et al 1991, Nelson et al 1992). Of several fumonisins identified, only fumonisins B<sub>1</sub> (FB<sub>1</sub>), B<sub>2</sub> (FB<sub>2</sub>), and B<sub>3</sub> (FB<sub>3</sub>) appear to be produced in significant quantities under both culture and natural conditions (Cawood et al 1991, Sydenham et al 1992). Fungal contamination with *F. moniliforme* and high concentrations of fumonisins in corn have been implicated in the high incidence of human esophageal cancer in certain areas of the world, including the Transkei Region of South Africa, Linxian County in China, northeastern Italy, and the southeastern United States (Cheng et al 1985, Franceschi et al 1990, Rheeder et al 1992, Norred and Voss 1994). Reports also have linked fumonisins to the development of several animal diseases, including leukoencephalomalacia in horses (Kellerman et al 1990), pulmonary edema in swine (Harrison et al 1990), liver cancer in rats (Gelderblom et al 1991), and acute congestive heart failure in baboons and monkeys (Kriek et al 1981a,b; Fincham et al 1992). Recently, the International Agency for Research on Cancer (Lyon, France) classified *F. moniliforme* toxins as potential carcinogens (class 2B carcinogens) to humans (International Agency for Research on Cancer 1993).

Recent surveys of commercial corn-based human foodstuffs from many parts of the world indicate the presence of fumonisins (Bullerman 1996, Pohland 1996, Shephard et al 1996). Even though the surveys were primarily exploratory and far from extensive, the highest concentrations of fumonisins were found in whole kernel

corn and corn products that undergo the mildest forms of processing (i.e., meal, flour, and grits). Corn-based products that were highly processed, such as corn flakes, corn pop cereals, corn chips, tortilla chips, and tortillas, contained either no detectable or only very low amounts of fumonisins.

Dry milling is basically an attempt to separate the anatomical parts of the grain by a physical process (Hoseney 1994) and is not likely to destroy fumonisins. The primary products derived from the corn dry-milling process include grits, bran, germ, meals, and flours (Alexander 1987). In terms of quantity, the largest fraction historically has been brewer's grits; however, the demand for brewer's grits has diminished during recent years. To adjust to this, the dry-milling industry during recent years has increased the production of flaking grits and snack grits, which are processed into many corn-based food products, such as corn flakes and other breakfast cereals, corn chips, extruded snacks, tortillas, and many other snack foods (Leath and Hill 1987).

To better assess the extent to which consumers are exposed to fumonisins, more information is needed on the distribution and contamination levels of *Fusarium* and fumonisins in food-grade corn and the fate of *Fusarium* molds and fumonisins during dry milling of corn. Therefore, the objectives of this work were to determine the distribution of *Fusarium* species and fumonisins in: 1) food-grade corn and fractions obtained from commercial dry-milled corn and 2) fractions obtained from experimental dry-milled corn.

### MATERIALS AND METHODS

#### Commercial Dry-Milled Corn

*Corn samples.* Weekly samples, over a period of 12 weeks (January 4 through March 22, 1993), of whole kernel corn and germ, bran, flaking grits, and flour fractions derived from the same corn were obtained from a commercial dry mill. The germ, bran, flaking grits (mostly hard endosperm), and flour (mostly soft endosperm) fractions were chosen for analysis because they represent the major parts of the corn kernel. All of these products were of food-grade quality and of the type purchased by major food-processing companies to manufacture corn flakes, other breakfast cereals, and various snack food items. All the samples were stored at –30°C until analyzed.

*Isolation and identification of Fusarium molds.* *Fusarium* mold infection was determined for the whole kernel corn and its fractions. For whole corn, 100 kernels from each commercial lot (12 total) were surface-sanitized with full-strength household bleach (5.25% NaOCl) for 1 min, rinsed with sterile distilled water three

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times, and dried on sterile paper towels. Surface-sanitized kernels were placed directly on the surface of Czapek-Dox-iprodrone (CZID) agar in petri plates, and the plates were incubated at 25°C for five to seven days (Abildgren et al 1987). CZID is a selective medium for *Fusarium* species and allows formation of large, easily recognizable colonies. In addition, the growth of other molds, such as *Alternaria*, *Epicoccum*, *Penicillium*, and mucoraceous species, is restricted on CZID. Percent infection was determined by counting the number of kernels from which internal mold contaminants grew, and the molds were identified according to the keys described by Samson and van Reenen-Hoekstra (1988).

For corn fractions (germ, bran, flaking grits, and flour), a serial dilution and plate-count technique was used to determine *Fusarium* mold counts. First, all fractions (except flour) were ground to a uniform consistency with a Tecator sample mill (Perstorp, VA) with a 1-mm screen. The ground material (11 g) was suspended in 99 mL (1:10 dilution) of 0.1% sterile peptone water in a sterile stomacher bag. The samples were homogenized for 1 min in a Stomacher lab-blender 400 (Cincinnati, OH). All serial dilutions were prepared in 0.1% sterile peptone water. A surface spread plate technique on CZID was used to determine *Fusarium* counts (colony forming units [CFU] per gram) for all of the weekly corn fractions obtained. The plates were incubated at 25°C for two to five days.

Typical *Fusarium* colonies (isolated only from the whole corn samples) were transferred from CZID plates on 2% water agar plates by the conidial suspension technique described by Nelson et al (1983). Macroconidia were incubated for 24–48 hr to allow germination. Single spores were transferred to carnation leaf agar plates (CLA), and the *Fusarium* molds were allowed to grow for seven to ten days under regulated light (12 hr fluorescent light, 12 hr dark) at 21–22°C. Slide mounts of *Fusarium* grown on CLA were prepared and observed by phase contrast microscopy (Nikon Optiphot, Garden City, NY). Colony morphology, morphology of conidia (microconidia and macroconidia), and conidiophores were compared with descriptions in the key by Nelson et al (1983) to identify *Fusarium* species.

**Fumonisin analysis.** Whole kernel corn samples and dry-milled fractions were sent to the National Veterinary Services Laboratories (Ames, IA) for fumonisin analysis. FB<sub>1</sub>, FB<sub>2</sub>, and FB<sub>3</sub> were

analyzed by the method of Ross et al (1991) with HPLC and reported as total fumonisins.

### Experimental Dry-Milled Corn

**Corn samples.** Three samples of food-grade dent corn (yellow, blue, and white) naturally contaminated with varying amounts of fumonisins were used in the experimental dry-milling study. Yellow and blue corn were obtained from a commercial dry mill during the 1995 crop year and contained FB<sub>1</sub> at 25.4 and 3.9 µg/g, respectively, whereas white corn was obtained from a farm in Nebraska during the 1994 crop year and contained FB<sub>1</sub> at 0.3 µg/g. No information is available regarding whether the fumonisin levels are dependent on corn color. These samples were used because they were the only naturally contaminated samples available and they contained fumonisins in three very different amounts. Yellow corn represented a high level of fumonisin contamination, blue corn represented a much lower level of contamination but still a high level, and white corn represented a low level of contamination. The moisture content of the yellow, blue, and white corn was 12.5, 12.5, and 18%, respectively. The corn samples were stored at –30°C until dry milled.

**Dry milling.** All corn samples were brought to room temperature two days prior to dry milling. Foreign material and broken kernels were removed by hand prior to milling. The moisture content of the yellow and blue corn samples (2,500 g) was first raised to 14% by adding distilled water and holding for 16 hr and then to 18% and holding for 1 hr. Final tempering was done by raising the moisture level to 20% and rotating slowly in a stainless steel drum for 15 min. The moisture content of white corn was raised directly from 18 to 20% and held for 15 min.

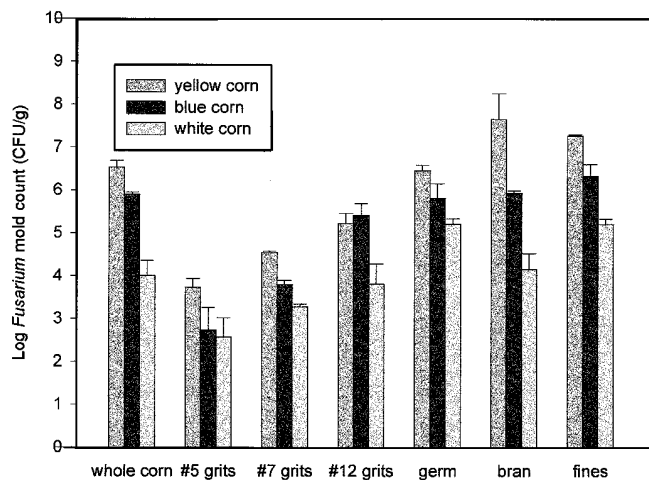


Fig. 1. Distribution of *Fusarium* molds in different fractions of dry-milled corn.

TABLE I  
Fusarium Mold Content of Commercial Food-Grade Corn and Dry-Milled Fractions

Sample	% Fusarium Infection of Whole Corn	Fusarium Counts (CFU/g) in Milled Fractions			
		Bran	Germ	Flaking Grits	Flour
1	25	<100	4.8 × 10 <sup>3</sup>	<100	1.6 × 10 <sup>3</sup>
2	28	2.0 × 10 <sup>3</sup>	8.9 × 10 <sup>3</sup>	<100	<100
3	24	<100	6.0 × 10 <sup>3</sup>	<100	<900
4	21	<100	1.6 × 10 <sup>4</sup>	<100	<100
5	23	1.0 × 10 <sup>3</sup>	<350	<100	<100
6	20	<100	<100	<100	1.3 × 10 <sup>3</sup>
7	20	<100	4.2 × 10 <sup>3</sup>	<100	1.4 × 10 <sup>3</sup>
8	13	<100	<100	<100	<100
9	13	<100	<300	<100	<100
10	17	<100	4.6 × 10 <sup>3</sup>	<100	2.7 × 10 <sup>3</sup>
11	10	<100	2.9 × 10 <sup>3</sup>	<100	<600
12	12	6.4 × 10 <sup>4</sup>	9.4 × 10 <sup>3</sup>	<100	<100

TABLE II  
Fusarium Species Identified in Food-Grade Corn from a Commercial Dry Mill

Fusarium sp. Identified	No. of Isolates <sup>a</sup>	% Occurrence
<i>F. moniliforme</i>	96	42.5
<i>F. subglutinans</i>	65	28.8
<i>F. proliferatum</i>	45	19.9
<i>F. graminearum</i>	20	8.8

<sup>a</sup> Total number of *Fusarium* isolates recovered was 226.

TABLE III  
Total Fumonisin Content<sup>a</sup> (µg/g) in Commercial Food-Grade Corn and Dry-Milled Fractions

Sample	Whole Corn	Bran	Germ	Flaking Grits	Flour
1	0.6	1.8	0.1	0.1	0.5
2	3.3	2.2	1.2	0.1	0.3
3	0.3	1.6	1.0	<0.1	0.3
4	<0.1	2.0	0.9	<0.1	0.3
5	3.2	2.9	0.5	0.1	0.2
6	0.2	3.2	0.6	<0.1	1.1
7	3.5	2.1	0.9	<0.1	0.3
8	0.1	2.1	2.0	0.1	0.2
9	0.3	1.7	0.3	0.1	0.2
10	0.1	1.8	0.7	0.1	0.6
11	3.3	2.0	0.6	0.1	0.3
12	0.7	1.5	0.5	<0.1	0.2

<sup>a</sup> Fumonisin B<sub>1</sub> + B<sub>2</sub> + B<sub>3</sub> = content.

The tempered corn samples were dry milled in a laboratory-scale horizontal drum degermer operating at 2,150 rpm (Stroshine et al 1986). The feed rate of the samples was  $450 \pm 10$  g/min. The milled stock was screened over a 3.5-W sieve (Smico Corporation, Oklahoma City, OK) for 30 sec. The product remaining on the sieve was passed for a second time through the degermer operating at 2,250 rpm and combined with the product that passed through the 3.5-W sieve. The combined stock was dried at 40°C to a moisture content of  $17 \pm 1\%$ . The dried stock was separated by sieving over a series of sieves (3.5, 5, 7, and 12 W) for 1 min. The fraction that passed through the 12-W sieve was termed fines. Fines consisted of broken pieces of germ, endosperm, bran, and tip caps. Fractions remaining on the 5-, 7-, and 12-W sieves were aspirated on a Bates laboratory aspirator equipped with an adjustable fan speed control (Rapsilver Supply Co., Brookshire, TX) to remove bran. Germs were hand-picked from grits (5-, 7-, and 12-W sieve fractions). In a commercial mill, germs would be separated from bran with gravity separators.

All the fractions (number 5, 7, and 12 grits, germ, bran, and fines) were dried overnight at 40°C, ground to uniform consistency (except fines), and stored at -30°C until analyzed. Due to the limited availability of naturally contaminated corn, dry milling was carried out only once, but three subsamples were analyzed for *Fusarium* molds and fumonisins.

**Moisture analysis.** Moisture content in corn samples was determined on a 250-g sample with a model 919 Motomco moisture meter (Clark, NJ).

**Isolation and identification of *Fusarium* molds.** Percent internal *Fusarium* infection in whole kernel corn samples and *Fusarium* mold counts in whole kernel corn and different fractions were determined as described earlier. Identification of *Fusarium* molds also was made as previously described.

**Fumonisin analysis.** FB<sub>1</sub> and FB<sub>2</sub> were analyzed with HPLC. FB<sub>3</sub> was not analyzed due to lack of the standard. FB<sub>3</sub> compared to FB<sub>1</sub> and FB<sub>2</sub> occurs at very low levels in nature and is not very toxic. A method described by Rice et al (1995) was adopted with

slight modifications. Briefly, a 10-g sample was extracted with 50 mL of acetonitrile (ACN) and water (1:1) for 60 min with a wrist-action shaker (Burrel, Pittsburgh, PA). A 10- to 25-mL portion of the extract was filtered through Whatman No. 1 filter paper (Maidstone, England). The filtered extract (2 mL) was diluted with 6 mL of 1% potassium chloride and cleaned on a 350-mg C<sub>18</sub> solid-phase extraction column (Waters/Millipore, Milford, MA). Before loading the sample, the C<sub>18</sub> column was conditioned with 2 mL of ACN followed by 2 mL of 1% KCl solution. The sample (2 mL of extract + 6 mL of 1% KCl) was loaded on the column and allowed to flow at a rate of 2 mL/min. The column was washed with 2 mL of 1% KCl followed by 1 mL of ACN and water (15:85). Fumonisin were eluted with 2 mL of ACN and water (70:30). In some cases in which the fumonisin levels were low, 1 mL of the eluted extract was evaporated to complete dryness under a stream of nitrogen at 60°C and redissolved in 250 µL of ACN and water (70:30).

Derivatization of the fumonisins was done by adding 100 µL of the clean extract to a disposable glass vial and was followed by 100 µL of borate buffer (0.05M; pH 8.5), 100 µL of *o*-phthalaldehyde (75 mg of OPA and 100 µL of β-mercaptoethanol in 50 mL of ACN), and 100 µL of water. The reaction mixture was allowed to incubate at room temperature for 10 min, and 5 µL was injected into an HPLC system consisting of a model 510 HPLC pump with a U6K loop injector (Waters/Millipore), a high-speed reverse-phase column (C<sub>18</sub>, 3 µm, 33 × 4.6 mm; Perkin-Elmer Cetus Corp., Norwalk, CT), a model 474 scanning fluorescence detector (Waters/Millipore), and an HP 3395 recorder-integrator (Hewlett-Packard Co., Wilmington, DE). An isocratic mobile phase of 40% ACN and 60% 0.05M KH<sub>2</sub>PO<sub>4</sub> (pH 3.3) was used at a flow rate of 1 mL/min.

The FB<sub>1</sub> standard was obtained from R. Eppley of the U.S. Food and Drug Administration, Washington, DC. The FB<sub>2</sub> standard was purchased from Sigma Chemical Company (St. Louis, MO). Standard curves were constructed with levels ranging from 0.02 to 2 ng/µL for FB<sub>1</sub> and 0.04 to 2 ng/µL for FB<sub>2</sub>. New standard curves were constructed on each day of analysis, and only standard curves with correlation coefficients >0.999 were accepted. Fumonisin were quantitated by correlating peak areas of the sample extracts to that of the standard curves. The sensitivity of the method was 0.025 and 0.05 µg/g for FB<sub>1</sub> and FB<sub>2</sub>, respectively.

Whole kernel white corn and number 7 grits, germ, and bran fractions obtained from the same corn were spiked with FB<sub>1</sub> and FB<sub>2</sub> standards at 5 µg/g. The ground samples were spiked in duplicate and analyzed as described previously. Percent recoveries were determined after correcting for the initial FB<sub>1</sub> and FB<sub>2</sub> concentrations in different fractions.

**Statistical analysis.** Analysis of variance was performed with SAS software (SAS/STAT User's Guide, version 6, SAS Institute, Cary, NC). *Fusarium* count data were log-transformed before analysis. The data were analyzed separately for each type of corn to compare fumonisin concentrations and *Fusarium* counts in different fractions with that of whole kernel corn. Pearson's correlation coefficients were used to interpret the relationship among FB<sub>1</sub>, FB<sub>2</sub>, and *Fusarium* mold counts. The data from each type of corn also were pooled to determine the correlation coefficients.

## RESULTS AND DISCUSSION

### Distribution in Commercial Dry-Milled Fractions

*Fusarium* infection of whole corn ranged from 10 to 28% (Table I). A total of 226 *Fusarium* isolates was recovered from the 12 commercial lots of food-grade corn. The most prevalent *Fusarium* species isolated from the corn were *F. moniliforme* (42.5%), *F. subglutinans* (28.8%), and *F. proliferatum* (19.9%) (Table II). *Fusarium* contamination of dry-milled corn fractions did not show any particular distribution pattern (Table I); however, mold counts were always lower in flaking grits (<100 CFU/g). The *Fusarium* counts in bran, germ, and flour were <100 -  $6.0 \times 10^4$ ; <100 -  $1.6 \times 10^4$ ; and <100 -  $2.7 \times 10^3$  CFU/g, respectively.

TABLE IV  
Percent Internal Mold-Infection Levels of Corn Samples  
Used in Experimental Dry Milling

Corn Type	Total Infection	<i>Fusarium</i> sp.	Fm <sup>a</sup>	Fp	Fs	Others
Yellow	98	83	77	3	3	17
Blue	76	51	49	2	...	25
White	60	15	19	...	6	45

<sup>a</sup> Fm = *F. moniliforme*; Fp = *F. proliferatum*; Fs = *F. subglutinans*; others = molds belonging to the genera *Acremonium*, *Aspergillus*, *Cladosporium*, *Mucor*, *Rhizopus*, *Penicillium*, and *Trichoderma*.

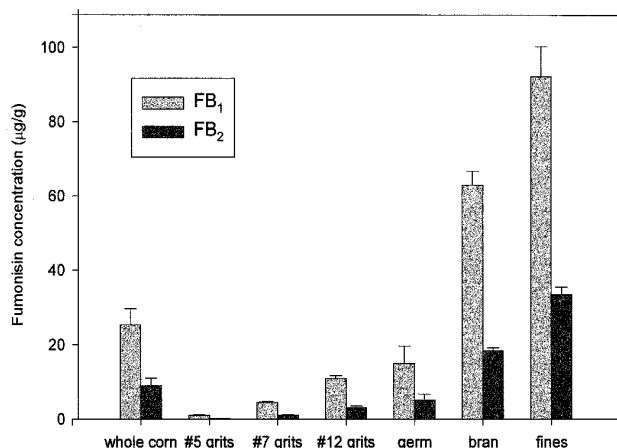


Fig. 2. Levels of fumonisins B<sub>1</sub> and B<sub>2</sub> (FB<sub>1</sub> and FB<sub>2</sub>, respectively) in different fractions of dry-milled yellow corn.

Fumonisin were detected in all of the whole corn samples, as well as in all of the dry-milled fractions (Table III). Total fumonisin content of the whole corn was <math>0.1\text{--}3.5\ \mu\text{g/g}</math>. Total fumonisin content was <math>\leq 0.1\ \mu\text{g/g}</math> in flaking grits, <math>0.2\text{--}1.1\ \mu\text{g/g}</math> in flour, <math>0.1\text{--}2.0\ \mu\text{g/g}</math> in germ, and <math>1.5\text{--}3.2\ \mu\text{g/g}</math> in bran. In this study, *Fusarium* counts of flaking grits were low, which correlated well with the low fumonisin concentrations. However, in some bran samples higher fumonisin concentrations were found even though *Fusarium* counts were lower. This indicates that *Fusarium* infection levels may not always indicate true contamination levels with fumonisins. Some of the processing steps involved in dry milling, such as drying, may reduce the level of mold contamination but not reduce the level of fumonisins, because they are relatively heat stable (Scott 1993, Jackson et al 1996).

The data presented show that *F. moniliforme*, *F. subglutinans*, and *F. proliferatum* were the major *Fusarium* contaminants found in commercial food-grade corn. *F. moniliforme* and *F. proliferatum* produced fumonisins (Thiel et al 1991, Nelson et al 1992), and low levels of fumonisins were found in the food-grade corn and dry-milled fractions. Variations were observed in the distribution of fumonisins in different fractions. In many samples, higher fumonisin concentrations were found in bran than in the corresponding whole corn samples. However, in some samples the fumonisin concentrations were lower in bran than in the corresponding whole corn. These discrepancies might have been due to errors in the sampling of different fractions. To minimize these errors, controlled dry-milling studies were conducted with naturally contaminated corn.

### Distribution in Experimental Dry-Milled Corn

**Fusarium counts.** All the corn samples used in the experimental dry-milling study had high mold-infection levels (Table IV). The total internal mold-infection levels of yellow, blue, and white corn was 98, 76, and 60%, respectively. Yellow and blue corn were heavily contaminated with *Fusarium* species: the infection levels were 83 and 51%, respectively. The infection level of *Fusarium* species in white corn was only 15%; however, other field fungi, such as *Cladosporium* and *Acremonium*, were prevalent. Most of the *Fusarium* molds identified in the different types of corn belonged to *F. moniliforme*. Other *Fusarium* species identified included *F. proliferatum*, *F. subglutinans*, *F. semitectum*, and *F. acuminatum*. *Fusarium* molds were present in all of the fractions, as determined by *Fusarium* mold counts (Fig. 1). The *Fusarium* count in whole kernel yellow corn was  $3.9 \times 10^6$  CFU/g. The *Fusarium* count of number 5 grits was significantly lower ( $P \leq 0.05$ ) than the count in the whole kernel corn, whereas *Fusarium* counts became higher as the grit size decreased. Higher *Fusarium* counts

also were found in germ, bran, and fines. The *Fusarium* count in whole kernel blue corn was  $8 \times 10^5$  CFU/g. Similar to results with yellow corn, number 5 grits contained the lowest *Fusarium* count, and again, the counts increased as the grit size decreased. Similarly, higher counts also were found in germ, bran, and fines. The *Fusarium* count in white corn was  $2.6 \times 10^4$  CFU/g. Although *Fusarium* counts in different fractions were not significantly different ( $P \leq 0.05$ ) from the whole kernel white corn, the counts were generally lower in larger grits and higher in germ, bran, and fines. These results suggest that in dry milling of corn there tends to be a concentration of *Fusarium* molds in bran, germ, and fines, whereas grits have less contamination.

**Fumonisin content.** Good recovery of FB<sub>1</sub> and FB<sub>2</sub> was observed from all of the fractions. Recovery of FB<sub>1</sub> from whole kernel corn, number 7 grits, germ, and bran was  $109.7 \pm 8.9$ ,  $99.3 \pm 9.3$ ,  $97.4 \pm 12.8$ , and  $101.3 \pm 11.5$ , respectively. Recovery of FB<sub>2</sub> from the same fractions was  $84.7 \pm 3.4$ ,  $89.0 \pm 3.0$ ,  $92.6 \pm 8.5$ , and  $74.0 \pm 11.0$ , respectively. Similar recovery of fumonisins from whole kernel corn was reported by Murphy et al (1993) and Rice et al (1995). Scott and Lawrence (1994) reported lower recovery of fumonisins from corn bran flour when methanol and water (3:1) was used as an extraction solvent, but higher recovery was reported when methanol and borate buffer (3:1) (pH 9.2) was used as an extraction solvent. Scott and Lawrence (1994) also reported that methanol and water was sufficient to obtain a good recovery of fumonisins from one brand of breakfast cereal but not from another brand. Rice et al (1995) reported higher recovery of fumonisins from *F. proliferatum* culture material when ACN and water (50:50) was used as an extraction solvent rather than methanol and water (3:1). Controversies in the literature regarding which extraction procedures obtain good recoveries of fumonisins (Usleber et al 1994) indicate the need for comprehensive studies to determine fumonisin recoveries in different matrices when extracted and analyzed by different procedures.

The average FB<sub>1</sub> and FB<sub>2</sub> concentrations in whole kernel yellow corn were 25.4 and 8.9  $\mu\text{g/g}$ , respectively. The FB<sub>1</sub> and FB<sub>2</sub> concentrations were significantly lower ( $P \leq 0.05$ ) in the number 5, 7, and 12 grits than in the whole kernel corn, whereas the concentrations increased with the decreasing grit size of the remaining fractions (Fig. 2). Significantly higher ( $P \leq 0.05$ ) fumonisin concentrations were found in the bran and fine fractions. Fumonisin concentrations in germ were not significantly different ( $P \leq 0.05$ ) from those in whole kernel corn.

The average FB<sub>1</sub> and FB<sub>2</sub> levels in whole kernel blue corn were 3.9 and 1.6  $\mu\text{g/g}$ , respectively. Distribution of fumonisins in milled fractions of blue corn were similar to those observed with yellow corn, grits containing the lowest levels of fumonisins, and bran

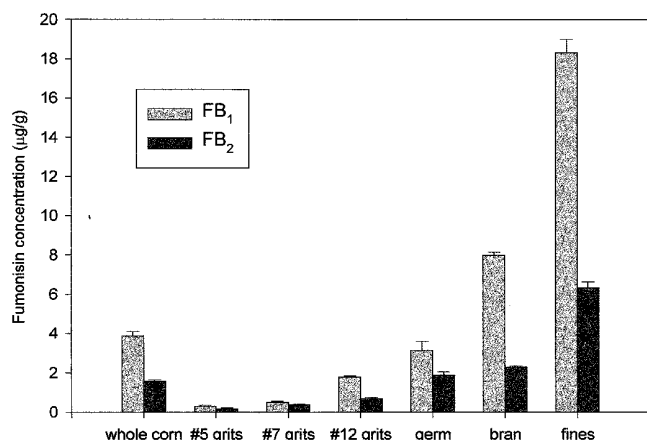


Fig. 3. Levels of fumonisins B<sub>1</sub> and B<sub>2</sub> (FB<sub>1</sub> and FB<sub>2</sub>, respectively) in different fractions of dry-milled blue corn.

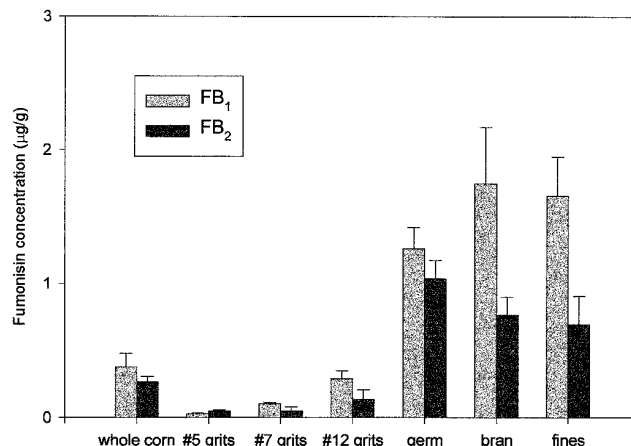


Fig. 4. Levels of fumonisins B<sub>1</sub> and B<sub>2</sub> (FB<sub>1</sub> and FB<sub>2</sub>, respectively) in different fractions of dry-milled white corn.

TABLE V  
Correlation Coefficients among Fumonisin B<sub>1</sub> and B<sub>2</sub>  
(FB<sub>1</sub> and FB<sub>2</sub>, respectively) and *Fusarium* Mold Count  
in Dry-Milled Corn Fractions<sup>a</sup>

Correlation Coefficient	Corn Type			Pooled Data
	Yellow	Blue	White	
FB <sub>1</sub> × FB <sub>2</sub>	0.99*	0.98*	0.77*	0.99*
FB <sub>1</sub> × mold count	0.75*	0.68*	0.59**	0.60*
FB <sub>2</sub> × mold count	0.73*	0.71*	0.56**	0.59*

<sup>a</sup> \* = Significant at  $P \leq 0.001$ ; \*\* = significant at  $P \leq 0.01$

and fines containing the highest levels of fumonisins (Fig. 3). The whole kernel white corn contained much lower levels of FB<sub>1</sub> and FB<sub>2</sub> (0.3 and 0.27 µg/g, respectively). As with yellow and blue corn, fumonisin concentrations were significantly lower in grits and higher in germ, bran, and fines ( $P \leq 0.05$ ) (Fig. 4).

Although the distribution pattern varied slightly in different types of corn with different levels of contamination, in general, the fumonisin concentrations were lower in grits and higher in germ, bran, and fines. Although a commercial corn dry mill can have as many as 20 fractions, depending on the desired product, this study indicates which of the mill fractions are likely to be contaminated. The higher *Fusarium* counts and fumonisin concentrations in germ, bran, and fines may be due to localization of the fungus in the areas of tip cap and germ just beneath the pericarp. The increase in *Fusarium* counts and fumonisin concentrations with decreasing grit size may be due to contamination of grits with germ. Corn grits sometimes can be contaminated with germ fractions, depending on dry-milling conditions (Brekke et al 1973). In the current experiment, germs were handpicked from grits, and increased contamination of grits was observed with decreasing grit size.

The correlation coefficients among FB<sub>1</sub>, FB<sub>2</sub>, and *Fusarium* mold counts are presented in Table V. In general, FB<sub>1</sub> and FB<sub>2</sub> were strongly correlated. High correlations between FB<sub>1</sub> and FB<sub>2</sub> also were reported by Murphy et al (1993). FB<sub>1</sub> and FB<sub>2</sub> levels also were significantly correlated with *Fusarium* counts. However, as mentioned earlier, *Fusarium* counts refer only to viable mold propagules. These numbers may be used a predictor of product storage quality but have little meaning in terms of levels or types of molds or mycotoxins present.

Results from the current study on the distribution of fumonisins in dry-milled corn fractions agree with findings on zearalenone (ZEN) in corn and deoxynivalenol (DON) or ZEN in wheat in other studies. In a study of dry milling of corn naturally contaminated with ZEN, Bennett et al (1976) reported high concentrations of ZEN in the hull and high fat fractions and low concentrations in grits, low-fat meal, and flour. Abbas et al (1985) studied the effect of wheat milling on DON and found that DON was recovered throughout all of the milling fractions (bran, shorts, reduction flour, and break flour), with the highest concentration found in bran, followed by shorts, reduction flour, and break flour. Seitz et al (1986) observed similar results when hard red winter wheat containing DON was milled. Concentrations of DON were generally lower in flours and higher in bran. Nowicki et al (1988) studied the effect of milling on DON in naturally contaminated samples of Canadian western red spring wheat and Canadian amber durum wheat. DON concentrations were highest in bran for both wheats and lower in break flours. Recently, Trigo-Stockli et al (1996) reported that DON and ZEN concentrations were highest in bran and lowest in flour from experimental dry-milled wheat.

## CONCLUSIONS

Dry milling of corn resulted in a concentration of fumonisins in certain fractions, including germ, bran, and fines. These fractions are widely used in the production of animal feeds and, thus, pose potential health risks to domestic animals. On the other hand,

flaking grits, which are widely used in breakfast cereals and snack foods, contained low concentrations of fumonisins, lessening the potential risks to humans. However, depending on the efficiency of the dry-milling process in achieving a clean separation, the smaller grits can be contaminated with *Fusarium* molds and fumonisins. Moreover, the bran that is sometimes used in certain breakfast cereals may be highly contaminated with *Fusarium* molds and fumonisins. Thus, monitoring corn-based ingredients for fumonisins may be necessary to avoid potential contamination.

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