

Mycoflora Distribution in Dry-Milled Fractions of Corn in Argentina

L. E. Broggi,¹ H. H. L. González,^{2,3} S. L. Resnik,⁴⁻⁶ and A. M. Pacin^{4,7}

ABSTRACT

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Corn samples and different commercial dry-milled fractions collected from an industrial mill in Argentina were surveyed for fungal contamination. The percentage of *Fusarium* isolates in whole corn kernels among all fungi recovered was 2.0–97.0%; in corn grits, it was 2.6–50.0%. Maximum levels in the other fractions were 5.2×10^5 colony forming units per gram (CFU/g) in germ and bran, 5.0×10^3 CFU/g in C flour, and 2.7×10^3 CFU/g in corn meal. The high initial contamination from whole corn is reflected in germ and bran, which is destined for animal consumption, but not in corn meal. *F. verticillioides* and *Asper-*

gillus flavus were the most frequent species in the whole corn kernel, but *F. verticillioides* was prevalent in all the other industrial fractions. Other potentially toxigenic fungi that were isolated included *Aspergillus parasiticus*, *Alternaria alternata*, *Penicillium citrinum*, and *P. funiculosum*. In this first report about mold contamination in corn industrial dry-milled fractions in Argentina, the high fungal contamination level observed in the stored corn could indicate the necessity to improve the hybrid quality and the storage conditions to diminish the risk of mycotoxin occurrence.

The Argentinian corn production was 19,362,755 tonnes for the 1998 harvest season, representing 29.2% of the total cereals and oilseeds produced in the same year, and $\approx 50.0\%$ was exported, making Argentina the second largest exporting-country after the United States (Secretaría de Agricultura, Ganadería, Pesca y Alimentación 1999).

There are a number of studies on corn mycoflora in Argentina (Sydenham et al 1993; González et al 1995, 1999), however there is a lack of information regarding the molds present on stored corn in Argentina and in its fractions obtained by industrial dry-milling.

Entre Ríos Province has produced 6.1% (average from 1996 to 2000) of the total Argentinian corn crop (Bolsa de Cereales de Entre Ríos 2000). Most of the corn is destined for human consumption and it is processed in one industrial dry mill located in Gualeguaychú (estimated daily capacity: 24 tonnes per day).

The industrial fractions obtained are corn grits (fractions that do not pass the 2,000 μm screen), C flour (fractions that pass through the 350- μm screen), germ and bran together (fractions that include germ bran and part of the starchy endosperm), and corn meal (fractions that pass the 2,000 μm screen but do not pass through the 250 μm screen). Figure 1 shows a schematic flow of this milling process.

In the United States (Katta et al 1997), the distribution of *Fusarium* molds and fumonisin occurrence were analyzed in a dry mill where the principal fractions obtained were bran, germ, flaking grits, and flour. They determined *Fusarium* infection of whole corn and the *Fusarium* counts in bran (<100 to 6.4×10^4 colony forming units per gram [CFU/g]), in germ (<100 to 1.6×10^4 CFU/g), in flaking grits (<100 CFU/g), and in flour (<100 to 2.7×10^3 CFU/g).

Fungal presence can result in various kinds of damage in stored grains, including a decrease in germinability, discoloration, production of mycotoxins, increased heating, mustiness, and total decay. Low fungal counts of dry-milled corn fractions are essential to maintain food quality for end use products (Vojnovich et al 1972).

The aims of this work were to identify, quantify, and determine the distribution of the contaminant mycoflora in whole stored corn kernels and in the fractions obtained from a corn mill in Argentina and to deduce their mycological quality.

MATERIALS AND METHODS

Sampling

During the 1999 crop season, 75 randomized samples of whole kernel corn (stored for 15 days to eight months), corn grits, germ, and bran, C flour, and corn meal were obtained over a period of nine months (March to November of 1999), from an industrial dry mill in Entre Ríos Province, Argentina. These fractions were chosen for analysis because they represent the major parts of the corn kernel. The sample fractions were withdrawn from the production line in accordance with the procedure published by Apro et al (1987). Subsamples of ≈ 2 kg each were prepared for mycological analysis.

Isolation and Identification of Molds

Contaminant mycoflora was determined for the whole kernel corn and its fractions. For isolation of the internal mycoflora, random subsamples of 600 g of whole corn kernels and corn grits from each sample were surface-disinfected in a commercial 5% aqueous solution of sodium hypochlorite for 1 min, rinsed twice with sterile distilled water, and dried in a laminar flow cabinet. One hundred kernels or corn grits per sample were placed, 20 items per plate, on yeast extract-glucose-chloramphenicol agar (YGCA, Merck No. 16000).

For the other corn fractions (germ and bran, C flour, and corn meal), a serial dilution plate-count technique was used to determine mold counts. The material (10 g) was suspended in 90 mL (1:10 dilution) of sterile-distilled water. The samples were homogenized for 1 min. All serial dilutions were prepared in 9 mL of sterile distilled water (1:100 and 1:1,000 dilution). A surface-spread plate technique on YGCA of 0.1 mL of the samples was used to determine mold counts (CFU/g) for all corn fractions obtained.

The plates were incubated in the dark at 28°C for four to seven days, and the resulting fungal colonies were subcultured on potato dextrose agar (PDA, Merck No. 10130) and identified. Where several fungi were isolated from a single item, all were recorded.

Identification of Fungi

Isolates of fungi were identified according to the following authorities: *Fusarium* spp. according to Nelson et al (1983); *Penicillium* spp., *Aspergillus* spp., and other fungi according to Pitt and Hocking (1997).

The isolation frequency (Fr) in whole corn kernels and in all the industrial fractions as well as the relative density (RD) of species

¹ Facultad de Bromatología, Universidad Nacional de Entre Ríos, Entre Ríos, Argentina.

² Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.

³ Facultad de Ingeniería, Universidad de Buenos Aires, Argentina.

⁴ Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina.

⁵ Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. Present address: Departamento de Industrias, Ciudad Universitaria (C1428DHQ) Núñez, Buenos Aires, Argentina. Fax: 011-4631-1148.

⁶ Corresponding author. E-mail: resnik@di.fcen.uba.ar. Phone/fax: 54 011-4631-1148.

⁷ Centro de Investigación en Micotoxinas, Universidad Nacional de Luján, Argentina.

isolated on the whole kernel and on corn grits were calculated as (González et al 1995):

$$Fr (\%) = \left(\frac{\text{number of samples of occurrences of a species}}{\text{total number of samples}} \right) \times 100$$

$$RD (\%) = \left(\frac{\text{number of isolates of a fungal species}}{\text{total number of fungi or genus isolates}} \right) \times 100$$

Statistical Analysis

Asymptotic tests for equality of proportions were used to compare RD of species isolated from the whole kernel and corn grits (Devore 1987). The Fischer exact test was used to analyze possible differences in the behavior of the Fr of species isolated in all fractions. The analysis was performed by using a software analytical package (Statistix For Windows, Borland International).

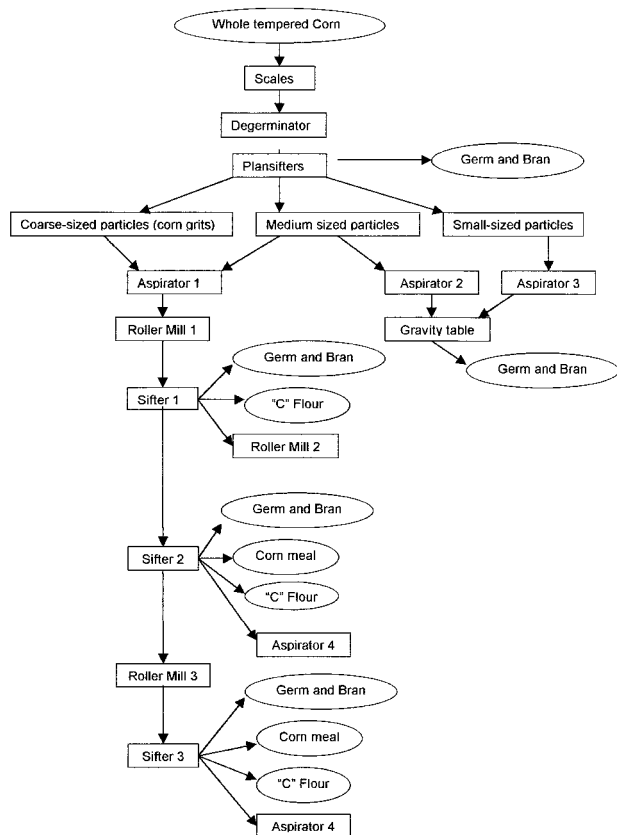


Fig. 1. Schematic flow of the industrial dry mill in Entre Ríos Province, Argentina.

RESULTS AND DISCUSSION

Mold Distribution in Whole Corn and Commercial Dry-Milled Fractions

The mycoflora associated with corn kernels and their industrial fractions obtained by dry-milling are shown in Table I. *F. verticillioides* (= *F. moniliforme*) and *Aspergillus flavus* Link were the most frequent fungal species in the whole corn kernel, but *F. verticillioides* was the most prevalent fungus in all the other fractions.

The predominant *Aspergillus* spp. isolated among internal mycoflora was *A. flavus* (Table I). This fungus was isolated as a contaminant in the whole corn and in all the fractions, and it was highest in the whole corn (80.0%) along with *F. verticillioides*. The other *Aspergillus* spp. identified was *A. niger*, *A. parasiticus*, and *A. penicillioides*.

For the *Penicillium* species isolated from corn kernels and its fractions (Table I), *P. funiculosum* was recovered from all fractions, mainly in the whole kernel (Fr = 53.3%) and in the germ and bran fraction (Fr = 40.0%). Hesseltine et al (1981) found that 28.6% of corn kernels samples harvested in North Carolina were infected by *Penicillium* spp., and 1.6% with *P. funiculosum*.

Additional fungal species identified included *Rhizopus stolonifer*, *Mucor racemosus*, *Trichoderma harzianum*, and *Epicoccum nigrum*. Some isolates of dematiaceous fungi such as *Curvularia lunata*, *Cladosporium cladosporioides*, and *Acremonium strictum* were also identified as components of the internal mycoflora. Other less prevalent isolated fungi included *Moniliella suaveolens*, *Endomyces fibuliger*, *Chrysonilia sitophila*, *Phoma glomerata*, and *P. sorghina*. Based on the observed Fr, *Curvularia lunata*, *Epicoccum nigrum*, and *Trichoderma harzianum* were recorded only in whole corn. *Cladosporium cladosporioides*, *Mucor racemosus*, *Rhizopus stolonifer*,

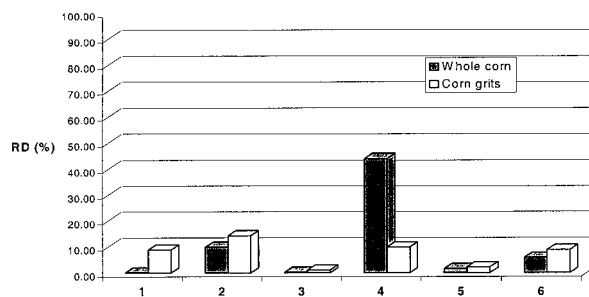


Fig. 2. Relative density of prevalent toxigenic molds in whole and corn grits in Entre Ríos province, Argentina (1999). 1: *Alternaria alternata*; 2: *Aspergillus flavus*; 3: *Aspergillus parasiticus*; 4: *Fusarium verticillioides*; 5: *Penicillium citrinum*; 6: *Penicillium funiculosum*.

TABLE I
Isolation Frequencies (%) of Fungal Species Belonging to the Most Important Toxigenic Genera

Species	Whole Kernel	Corn Grits	C Flour	Corn Meal	Germ and Bran
<i>Alternaria alternata</i>	6.7	10.0	7.7	13.3	—
<i>Aspergillus candidus</i>	—	—	7.7	—	6.7
<i>A. flavipes</i>	—	—	7.7	—	—
<i>A. flavus</i>	80.0	40.0	23.1	6.7	13.3
<i>A. fumigatus</i>	6.7	—	—	—	—
<i>A. niger</i>	40.0	10.0	—	6.7	6.7
<i>A. parasiticus</i>	6.7	10.0	—	—	—
<i>A. penicillioides</i>	—	10.0	7.7	6.7	20.0
<i>A. restrictus</i>	6.7	—	—	—	—
<i>A. wentii</i>	—	10.0	—	—	—
<i>Eurotium chevalieri</i>	20.0	10.0	53.9	26.7	46.7
<i>Fusarium verticillioides</i>	80.0	50.0	76.9	40.0	73.3
<i>Penicillium glabrum</i>	6.7	30.0	23.1	26.7	20.0
<i>P. variabile</i>	20.0	30.0	38.5	26.7	26.7
<i>P. citrinum</i>	6.7	20.0	15.4	13.3	—
<i>P. funiculosum</i>	53.3	10.0	30.8	6.7	40.0
<i>P. solitum</i>	—	—	7.7	—	—

and *Phoma glomerata* were isolated from the whole corn kernel as well as from all fractions. *Alternaria alternata* isolates were recovered from the whole corn kernel and all fractions, except germ and bran samples. *Moniliella suaveolens*, *Endomyces fibuliger*, and *Phoma sorghina* were recovered from C flour samples, while *Chrysonilia sitophila* and *Acremonium strictum* were isolated only on broken corn samples. Fungi involved in dry ear rots (cob, kernels, and stalk rot), *Nigrospora oryzae* (Shurtleff et al 1998) were isolated only in the whole kernel.

The prevalence of *F. verticillioides* was observed taking into account the RD in the whole kernel (Fig. 2), followed by *Aspergillus flavus*, which was predominant in corn grits closely followed by *F. verticillioides*, *Alternaria alternata*, and *Penicillium funiculosum*.

Statistical analysis (Table II) showed significant ($P < 0.05$) and highly significant differences ($P < 0.01$) when comparing frequencies of isolation between whole corn and all the milled fractions for *Aspergillus flavus*. Highly significant difference was observed in the RD comparison between whole corn and corn grits for *F. verticillioides*.

F. verticillioides was the only *Fusarium* species identified in all categories of samples in this work (Table III). *Fusarium* infection in the RD of whole corn was 2.0–97.0%. *Fusarium* counts were always lower in corn meals (<100 to 2.7×10^3 CFU/g). Katta et al (1997) recorded lower percentages of *Fusarium* infection of whole corn (10.0–28.0% occurrence) than observed in this work. The corn

meal counts were similar in both works, although they had lowest counts in the flaking grits (<100 CFU/g).

In a previous study in the United States (Katta et al 1997), *F. subglutinans*, *F. proliferatum*, and *F. graminearum* were isolated along with *F. verticillioides*, which corresponded to the 42.5% of all *Fusarium* species. The prevalence of *F. verticillioides* as internally seedborne fungus in the whole corn kernels in Entre Ríos is similar to that reported in the United States, Canada, Australia, and Italy (Miller et al 1983; Blaney et al 1986; Foster et al 1986; Logrieco and Bottalico 1988; Wicklow 1988) and to those observed in freshly harvested Argentinean corn samples from Buenos Aires, Córdoba, and Santa Fe provinces (Sydenham et al 1993; González et al 1995, 1999) in popcorn samples from Buenos Aires Province (de Souza et al 2000) and in Flint corn collected in Salta, Tucumán, Catamarca and Jujuy provinces (González et al 2000).

CONCLUSIONS

F. verticillioides was recorded as being the most prevalent fungus in all fractions, and it was also the predominant fungus in whole corn kernels, based on its relative density. These results indicate high probability of fumonisin contamination. In a previous work on freshly harvested corn collected in this province, it was observed that this cereal grain was contaminated with fumonisin (Pacín et al 2001), which means that all fractions could be contaminated.

Other fungi associated with corn and its fractions in Argentina, such as *Aspergillus flavus*, *Aspergillus parasiticus*, *Penicillium funiculosum*, *Penicillium citrinum*, and *Alternaria alternata*, should be of concern because of their toxigenic potential.

The high fungal contamination found on storage corn compared with the other studies mentioned here suggests that it is necessary on one hand to improve the hybrid quality trying to diminish the initial fungal contamination in whole corn and also to improve the storage conditions. Note that the corn meal obtained in the dry-milling process in Argentina has low fungal counts similar to less contaminated corn processed in other countries but fungal counts were high in C flour destined for human consumption. The highest fungal counts were observed in the germ and bran samples (maximum 5.7×10^5 CFU/g), used exclusively to prepare animal feeds.

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TABLE II
P Values for the Comparison of Contaminant Mycoflora in Whole Corn Kernels and in Different Fractions Obtained by Dry-Milling^a

Species	RD ^b	Fr ^b	Fr ^c	Fr ^d	Fr ^e
<i>Aspergillus flavus</i>	—	*	**	**	**
<i>A. fumigatus</i>	**	—	—	—	—
<i>A. niger</i>	—	—	*	—	—
<i>Cephalospora</i> spp.	*	—	—	—	—
<i>Cladosporium cladosporioides</i>	**	*	*	**	—
<i>Curvularia lunata</i>	**	**	—	—	—
<i>Fusarium verticillioides</i>	**	—	—	—	—
<i>Mucor racemosus</i>	**	**	—	**	**
<i>Nigrospora oryzae</i>	**	—	—	—	—
<i>Penicillium variable</i>	**	—	—	—	—
<i>P. funiculosum</i>	—	*	—	*	—
<i>Rhizopus stolonifer</i>	—	—	**	**	**
<i>Trichoderma harzianum</i>	**	—	—	—	—

^a RD, relative density; Fr, isolation frequency; * $P < 0.05$; ** $P < 0.01$.

^b Comparison between whole corn and corn grits.

^c Comparison between whole corn and C flour.

^d Comparison between whole corn and corn meal.

^e Comparison between whole corn and germ and bran.

TABLE III
Fusarium Content of Commercial Food-Grade Corn and Dry-Milled Fractions^a

Samples	RD (%) <i>Fusarium</i> Infection		<i>Fusarium</i> Counts (CFU/g) in Milled Fractions		
	Whole Corn	Corn Grits	Germ and Bran	C Flour	Corn Meal
1	55.0	2.6	6.2×10^4	3.6×10^3	<100
2	3.0	—	4.0×10^5	730	300
3	84.0	6.1	5.7×10^5	na	2.7×10^3
4	97.0	16.7	5.2×10^5	2.0×10^4	400
5	93.0	50.0	1.2×10^5	na	100
6	11.1	20.0	9.0×10^3	540	320
7	31.8	—	<100	5.0×10^3	<100
8	—	—	1.1×10^3	<100	<100
9	5.6	—	1.1×10^3	<100	<100
10	2.0	—	<100	<100	<100
11	10.0	na	<100	<100	<100
12	—	—	2.9×10^3	840	<100
13	—	—	<100	<100	<100
14	—	2.7	1.1×10^4	500	<100
15	80.0	23.1	1.9×10^5	4.5×10^3	<100

^a RD, relative density; na, not analyzed.

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