Laboratory Wet-Milling of Corn Containing High Levels of Aflatoxin and a Survey of Commercial Wet-Milling Products[1]

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ABSTRACT

Corn was inoculated with spores of *Aspergillus flavus* and allowed to incubate until mold growth was observed. This corn was found to contain 638 p.p.b. aflatoxin B_1. This sample and a naturally contaminated sample (120 p.p.b. B_1) were steeped and wet-milled by a laboratory procedure. Aflatoxin was found primarily in steepwater (39–42%) and fiber (30–38%), with the remainder in gluten (14–17%) and germ (6–10%). Increases in concentrations of aflatoxin in the fractions compared with the original corn were steepwater, 4- to 5-fold; fiber, 2.5- to 3-fold; gluten, 1- to 1.5-fold; and germ, 1-fold. The starch fractions had aflatoxin levels of 9.0 and 2.2 p.p.b. and hence contained only about 1% of the toxin originally in the corn. Analyses of 105 samples of commercial corn steepwater produced at 6 individual plants over a period of 1 to 3 months during new corn movement gave negative aflatoxin results in all cases. Assays of a number of production samples of starch, germ, gluten, and gluten feed were all negative. Analytical procedures gave sensitivities of 1 to 3 p.p.b. of aflatoxin B_1.

Previous reports (1,2,3) have presented evidence that commercial corn and grain sorghum of acceptable grade for use by the wet-milling industry are almost entirely free from aflatoxin contamination. Several contaminated commercial samples were found, however, and representatives of the wet-milling industry agreed that two additional studies should be made: 1) a laboratory wet-milling fractionation to determine the probable fate of aflatoxin if contaminated corn should happen to be processed, and 2) a survey of representative samples of finished products from several wet-milling plants.

METHODS AND MATERIALS

Contaminated Corn

Two different lots of corn containing aflatoxin were used for laboratory wet-milling. One (No. 41041) was a naturally contaminated sample found among shipments received by a wet-milling plant (1). The second (No. 333) was artificially infected. The entire contents of a sporulating culture tube of *Aspergillus flavus* NRRL 2999[2] was suspended in water in a Waring Blendor and then mixed with about 4 kg. of sound yellow corn at 21.0% moisture to obtain uniform inoculation. Initial count of mold-damaged kernels was 1%, which would put this corn in grade No. 1. The inoculated 21%-moisture corn (95 to 100% r.h.) was stored at 35°C., a temperature shown to be near optimum for aflatoxin production on peanuts at 97

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[2]This culture has recently been reclassified by NRRL as Aspergillus parasiticus.

to 99% r.h. (4). When vigorous mold growth was noted about 5 days after inoculation, the corn was dried for about 8 hr. in a circulating air oven at 70°C. to a final moisture of 12%.

Aflatoxin was assayed on six 50-g. subsamples of each lot with the following results:

Aflatoxin B_1, p.p.b.

	Laboratory-Inoculated CPC-333	Commercial, Natural Inoculation 41041
	600	57
	710	140
	650	110
	570	120
	650	110
	650	12
Mean:	638 ± 36	91.5 ± 38

Uniformity of results was obtained with the laboratory-inoculated lot, because all kernels were uniformly inoculated. The commercial sample, on the other hand, contained a number of badly infected kernels mixed with a higher proportion of sound kernels; hence a wide variation of assay results was obtained among subsamples. The aflatoxin content used for this sample is the average of the four closest results, i.e., 120 p.p.b.

Aflatoxin

The standard aflatoxin TLC method (5) developed for peanuts works fairly well with corn (1), but is not at all satisfactory for protein-rich co-products from wet-milling because of interfering fluorescent substances. Hence, a more rigorous clean-up procedure (6) was developed for assaying aflatoxin in these products; the TLC step is similar to that of the original FDA method. The new procedure (6) was used for corn and all wet-milling fractions in this study.

Only aflatoxin B_1 was determined. Levels of B_2, G_1, and G_2, when present, were much lower and would probably follow the fractionation that occured with B_1 in wet-milling.

Analyses

Starch was determined by a standard CRA polarimetric method in $CaCl_2$; fat was determined by extraction with CCl_4; protein by Kjeldahl. Analytical results obtained with the prepared corn samples are shown in Table I.

Wet-Milling

A conventional laboratory procedure used for wet-milling these samples has been described elsewhere (7). The corn was steeped at 49°C. for 48 hr. in dilute solutions of sulfur dioxide and lactic acid adjusted to pH 4 with sodium hydroxide (8). The steepwater was decanted and the corn ground in fresh water in a Waring Blendor to release the germ. Subsequent separations were: germ, by flotation on liberated starch; fiber, by screening; starch and gluten, by tabling; and middlings, by surface washing of the starch cake on the table. All fractions were washed with

TABLE I. ANALYSIS OF CORN SAMPLES

	Undamaged Corn	Laboratory Inoculated No. 333	Naturally Inoculated No. 41041
Moisture, %	15	12.3	10.9
Protein, (N X 6.25) % d.b.	9.2	10.1	10.0
Starch, % d.b.	71.6	73.3	73.2
Oil, % d.b.	4.7	4.8	4.3
Free fatty acids, as % of oil	1.0	9.4	9.4
Viability (Tetrazolium)	95	24	32
Aflatoxin B_1, p.p.b.	0	638	120

fresh water and dried in a circulating-air oven at 50 to 55°C. All filtrates were combined with steepwater for soluble dry-substance analysis. Because the entire fraction in many cases was used for aflatoxin extraction, fractions were not analyzed for other chemical components. Fractions from the uninoculated sound corn sample were not analyzed for aflatoxin. Distribution of starch, protein, and oil among fractions of sound corn has been reported previously (7).

RESULTS AND DISCUSSION

Laboratory Wet-Milling

Average dry-substance yields and aflatoxin contents of the fractions from duplicate wet-millings of the three corn samples are given in Table II. Excellent total dry-substance recoveries (98.6 to 99.8%) were obtained with all three lots of corn. Dry-substance yields of fractions from the moldy corns were close to normal, but in some instances yields were obviously affected by mold growth. Steepwater yields were higher and steepwater was darker brown in color for the moldy corns, indicating digestive attack of the mold on germ and endosperm. Germ yields from the two moldy-corn samples were lower than from the sound corn.

Unpublished experiments (9) in which oil, protein, and starch recoveries were followed have shown low germ and oil yield from moldy corn. These experiments clearly show that the germ from mold-infected corn is more brittle than normal germ. Consequently, pieces of germ containing some oil are recovered in fiber and gluten. Germ breakage also results in liberation of oil which is absorbed by gluten. Gluten has a high affinity for oil. Oil content of gluten is normally about 6%, but from moldy corn it was 11 to 14%. Breakage of moldy germ not only reduced oil recovery, but also usually increased the level of free fatty acids as a result of the hydrolytic action of mold enzymes. Free fatty acid contents of germ oil were 34.7, 16.8, 14.1, and 3.8% compared with 1.2% for normal germ oil. Refined oil yield is reduced in proportion to the amount of free fatty acids plus an equal quantity of neutral oil included in the soap emulsion.

The higher gluten and middlings yields from the moldy-corn samples probably reflect poor separability of starch and gluten resulting from mold damage of starch granules and gluten particles. The starch content of gluten from moldy corn is usually higher than normal, thereby decreasing the yield of starch. The protein content of starch from moldy corn is usually in the normal range of 0.2 to 0.35% (9).

TABLE II. AFLATOXIN DISTRIBUTION AMONG WET-MILLED CORN FRACTIONS[a]

Fraction	Weight g, d.b.	Av. Dev.	% of Grain	Av.[b]	Av. Dev.	Weight γ	% of Sum
Inoculated No. 333							
Original Corn	371.2	±0.0	100	638	±36	238	. . .
Steepwater and solubles	30.6	±0.3	8.15	2450	±650	74	41.5
Germ	22.2	±0.15	5.95	798	±65	18	10
Fiber	33.0	±0.05	8.9	1600	±300	53	30
Gluten	39.6	±0.10	10.65	768	±63	30	17
Middlings	18.0	±1.15	4.8	71	±4	1	0.5
Starch	226.4	±1.95	61.0	9	±0.7	2	1
Total	369.8	±3.70	99.8+0.2			178	100.0
Natural (molded) No. 41041							
Original corn	378.8	±0.0	100	120	±10	45	. . .
Steepwater and solubles	27.3	±0.10	7.2	610	±40	16.5	39.5
Germ	19.8	±0.0	5.2	140	±20	2.5	6
Fiber	47.3	±0.7	12.5	340	±50	16	38
Gluten	40.7	±0.7	10.7	140	±20	5.5	13
Middlings	34.6	±2.2	9.2	25	±0.5	1	2.5
Starch	203.4	±2.0	53.7	2.2	±0.2	0.5	1
Total	373.1	±5.7	98.6			42	100.0
Sound corn							
Original corn	324.0	±0.0	100	. . .		. . .	. . .
Steepwater and solubles	14.7	±0.5	6.0	. . .		. . .	. . .
Germ	20.2	±0.5	6.2	. . .		. . .	. . .
Fiber	25.2	±0.6	9.2	. . .		. . .	. . .
Gluten	23.4	±0.5	7.2	. . .		. . .	. . .
Middlings	8.1	±2.0	2.5	. . .		. . .	. . .
Starch	213.6	±3.5	66.0	. . .		. . .	. . .
Total	310.0		98.1				

[a] Average of duplicate millings.

[b] Average of duplicate analyses on each duplicate.

Each of the duplicate wet-milling fractions was assayed in duplicate for aflatoxin. Average aflatoxin values and their average deviations are given in Table II. Recovery of total aflatoxin in the fractions of inoculated corn was 178 γ compared with the value of 238 γ calculated for the amount of whole corn used for milling. This difference may be due to the high standard deviations of the assay method and is considered nonsignificant. For naturally molded corn, the fractions totaled 42 γ compared with 45 γ calculated for the original corn. While these recoveries are poor in comparison to dry-substance recoveries, they are considered to be very good when the high average deviations of the aflatoxin assay results are taken into account. These deviations result from inaccuracies inherent in reading fluorescent spot intensities on the TLC plates.

Aflatoxin recovered in each fraction is expressed as actual concentration and as percentage of the sum of aflatoxin in the fractions (Table II). In both contaminated corn samples, the steepwater-solubles fractions contained the highest concentration of aflatoxin, i.e., to about 4 times the concentration in the original corn or about 40% of the recovered aflatoxin.

The fiber fractions had aflatoxin concentrations about 2 to 2.5 times higher than the original corn and accounted for about one-third (30 and 38%) of the total aflatoxin. The germ fraction contains an aflatoxin concentration about equal to the original corn and accounts for 6.6 and 9.9% of the total aflatoxin. If it is assumed that no aflatoxin is lost when germ is expelled or extracted for oil recovery, then gluten feed, which is a blend of the steepwater, fiber, and spent germ meal, would account for 81.5 and 83.5% of the aflatoxin from these two corn samples. (Contrary to what the name implies, corn gluten feed usually does not contain free gluten.)

The gluten fraction contained about 1 to 1.5 times the aflatoxin concentration in the original corn and accounted for 17.0 and 13.3% of the total aflatoxin for the two corn samples. Thus, the two feed products accounted for 98.3 and 96.7% of the aflatoxin contained in the two original corn samples.

The largest fraction, and most important product for food purposes, is the starch. In spite of heavy aflatoxin contamination of the original corn, the starch had a very low aflatoxin content (9 and 2.2 p.p.b.) and accounted for only 1.0 and 1.2% of the total aflatoxin. The extremely high levels of aflatoxin contamination of the initial corn were chosen deliberately to facilitate tracing aflatoxin through the process. At lower levels of contamination (5 to 30 p.p.b.), it would be impossible to detect the approximately 1% of original aflatoxin which remained in the starch fraction even though detection limit of aflatoxin in starch is lowest of all fractions (0.5 p.p.b.). The middling fraction is 90% starch and, hence, is low in aflatoxin content.

Survey of Commercial Wet-Milling Products

Although the corn supply used by the wet-milling industry contained no detectable aflatoxin (1), representative commercial products derived from corn in 1965 to 1967 also were assayed.

Starch. Starch constitutes the largest portion of the corn kernel and is a major food material. As an additional check on product safety, one company assayed unmodified food-grade starch from its production during the first 12 weeks of 1967. All starch samples tested were free of aflatoxin B_1. The detection limit of aflatoxin in starch was 0.5 p.p.b., the lowest of any of the products tested because corn starch contains a much lower concentration of interfering substances.

Corn Germ. Commercial corn germ, the source of corn oil for human consumption, also was examined for possible aflatoxin contamination. Six cars of dried germ, comprising two cars shipped by each of three wet-millers in September 1965, were found to be free from aflatoxin.

Corn Oil. The discovery that germ from corn purposely contaminated with aflatoxin contains aflatoxin B_1 at about the same level as the original grain led to laboratory investigation of what happens to the toxic material during oil extraction, refining, and bleaching. This work has been reported previously by Parker and Melnick (10). It was learned that if aflatoxin were present in corn germ, some of it would pass into the crude oil during solvent extraction. Subsequent studies indicated that somewhat less aflatoxin would carry over into expelled crude oil than into solvent-extracted crude oil. However, the process of alkali refining and bleaching renders the refined oil completely free of aflatoxin. It was determined also that much of the aflatoxin remains in the spent germ after solvent extraction; hexane extraction of whole germ which contained 425 p.p.b. of aflatoxin B_1 yielded spent germ which contained 750 p.p.b. of aflatoxin.

Gluten and Gluten Feed. The experimental milling of corn deliberately contaminated with very high levels of aflatoxin indicated that gluten might be expected to contain aflatoxin at about the same p.p.b. level as the corn from which it was derived (Table II). As part of the program of surveying product wholesomeness, one company assayed ten samples each of gluten and gluten feed produced in its plants during the final 2 months of 1966. None of the samples contained aflatoxin. The lower limit of detection in these materials, 5 p.p.b. of aflatoxin B_1, is due to the high level of extraneous fluorescing substances.

Steepwater. The laboratory wet-milling studies described above showed that steepwater from infected corn contained about 4 times higher concentration of aflatoxin than the original corn. Therefore, it was reasoned that analysis of commercial steepwater for aflatoxin would be the most sensitive way to detect aflatoxin contamination of commercial corn. Samples would be most homogeneous and would represent many hundreds of bushels because of the countercurrent nature of the steeping batteries. Heavy steepwater from six separate wet-milling plants was assayed for aflatoxin B_1 over a 1- to 3-month period at the end of the 1966 harvest season. Of 105 samples analyzed, 104 showed no aflatoxin within the sensitivity of the method. Sensitivity for this material is 3 p.p.b. One sample was reported to contain 29 p.p.b. aflatoxin B_1. However, when reassayed by means of a two-dimensional chromatographic technique, the suspected fluorescent spot was found not to be aflatoxin B_1. These negative results are especially interesting because the period covered by the sampling was during the new crop movement when corn is at high moisture and most vulnerable to mold infection.

Other Products. Another group of spot-sampled corn and milo products was checked for aflatoxin content and all were found to be negative. These products included starches, syrups, dextrose, germ, spent germ, oil cake, oil, gluten, and gluten feed.

CONCLUSIONS

Corn contaminated with aflatoxin by growth of *Aspergillus flavus* when wet-milled yields fractions which are all contaminated with aflatoxin, but to different extents. Aflatoxin contained in the original corn is distributed as follows: steepwater, 40%; fiber, 30 to 38%; gluten, 14 to 17%; germ, 6 to 10%; and starch, 1.0 to 1.2%. Gluten feed (steepwater, fiber, and spent germ) would contain 80 to 90% of the total aflatoxin.

A survey of starch and major co-products produced in six wet-milling plants over a 1-year period failed to show presence of any aflatoxin. This confirms a previous report (1) in which a survey of corn used in commercial wet-milling showed that significant levels could not be detected.

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