

CLEANING TRIALS FOR CORN CONTAINING AFLATOXIN¹

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ABSTRACT

Physical separation methods were generally ineffective for lowering the aflatoxin content of naturally contaminated corn used in the experimental work. The corn lots tested differed in aflatoxin content (10 to 450 p.p.b. B₁), geographic source, content of broken corn-foreign material, and represented both white and yellow corn. Dry cleaning, wet cleaning, density separation, and preferential fragmentation of the grain were used in the laboratory tests. Aflatoxin was concentrated

in broken corn-foreign material in only 1 of the 10 lots tested. Hand-selected kernels that outwardly appeared sound and free of the bright greenish-yellow fluorescence associated with presence of aflatoxin had the toxin in excess of current guidelines (20 p.p.b.) in six out of seven lots of corn. The hand-selected kernels contained about one-half to as little as one-tenth the level of aflatoxin in the unfractionated lot.

Aflatoxin, a harmful secondary metabolite produced at times by the fungus *Aspergillus flavus*, was first found in peanut meal in a ration that caused the death of a number of turkey poults in England in 1960 (1). Since then, aflatoxin has been found in a number of agricultural commodities, including corn. Seizure by the Food and Drug Administration of a quantity of aflatoxin-contaminated corn (2) and that agency's increased surveillance for possible contamination of corn and corn-based products have focused interest on possible ways for removing aflatoxin from contaminated corn. If successful, physical separation methods normally would be more economical and more readily used than chemical treatments.

Our study was intended to provide information on the effectiveness of several types of physical separation procedures for reducing aflatoxin content of several lots of contaminated corn, with bulk of the grain preferably being maintained in whole kernel form. Machines used in the tests included: simple grain separator (i.e., fanning mill), washer-whizzer, fractionating aspirator, dry gravity table, impact mill, corn breakage tester, and grain scourer. The impact mill and breakage tester were included because Shotwell et al. (3) have indicated that kernel parts with "high" levels of aflatoxin are structurally weakened and crumble easily. More recently, Shotwell³ has indicated that the aflatoxin level must exceed about 80,000 p.p.b. before a kernel will crush easily.

Three of the corn lots used in this study were also used in dry milling tests, and those results will be reported in another paper.

MATERIALS, METHODS, AND EQUIPMENT

Corn

Both white and yellow corn known to be naturally contaminated with aflatoxin in varying amounts, from several geographic sources, and covering all grades, were used. No information was available on the storage or field

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²Agricultural Research Service, U.S. Department of Agriculture. Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

³Shotwell, O. L., personal communication (Jan. 1974).

conditions that led to contamination of the lots used. Each of the 11 lots was blended in a 30-bu. V-shell blender both before and after cleaning in the fanning mill before samples were taken for analyses or for other physical separation tests.

Analytical Methods

Except for a 4.5-lb. sample of cleaned corn from lot E, 50-lb. samples of the blended corn were taken, ground to -20 mesh, blended, and a 2.2-lb. (1 kg.) sample was set aside for analyses. Quantitative aflatoxin determinations were made on 50-g. portions by the method recommended for corn (4,5). Reported values are based on single determinations for the various white corn samples which were of relatively high aflatoxin content. For the yellow corn lots which were of lower aflatoxin content, three determinations were made on uncleaned samples and single determinations on cleaned grain. For a single determination on whole corn, the relative standard error (RSE) has been reported as 37% (6), with decreases to 25% for two determinations and to 20% for three determinations. Aflatoxin values above 50 p.p.b. have been rounded to the nearest multiple of 10, and below 50 to nearest multiple of 5.

Samples from each lot of blended, incoming corn and of corn from a number of the physical separation tests were also hand-picked to determine the weight percentage of kernels and particles exhibiting the bright greenish-yellow (BGY) fluorescence associated with presence of aflatoxin when kernels are examined under a high-intensity, longwave (365 nm.) ultraviolet (UV) light (3,7,8). Unless indicated otherwise, approximately 5-lb. samples were hand-picked. This procedure was employed on some samples to obtain presumptive evidence of incomplete removal of aflatoxin-contaminated corn rather than using the more time-consuming extraction-purification-chromatography method (4,5) referred to above.

Corn samples were graded by the USDA standard procedure (9).

Physical Separation Procedures

A Pioneer "Hero" fanning mill was used for dry cleaning all but one lot of the corn. Two screenings' fractions were removed in this cleaning operation: pieces of cob and other oversized material retained on a 28/64-in. round-hole-perforated (r.h.p.) sieve, and broken pieces, small kernels, and weed seeds that passed through a sieve, usually 18/64-in. r.h.p. Also, air blast liftings consisting largely of beeswing and dust were collected by means of an auxiliary exhaust blower and cyclone collector attached to the fanning mill. In most instances, 9- to 24-bu. quantities were dry-cleaned.

Only corn that had been dry-cleaned in the fanning mill was used in the tests made with the other machines described below.

A small Miag combined washer, stoner, and whizzer fitted with 3/32-in. r.h.p. screens was used to wash approximately 1-bu. quantities of corn. Typical flow rates were: corn, 20-23 bu./hr.; water, 4-7 gal./bu. corn. Even though the water-recycle valve was open, the results were typical of those obtained with clean water because the washing time was so short. Because some kernels were broken in the whizzer section, the wet corn was screened in a small box sifter to separate essentially whole kernels from the broken pieces passing through a 3.5-mesh sieve.

A 60-lb. sample from lot A (160 p.p.b. aflatoxin B₁) was separated into four

TABLE I
Separations Made on White Corn in Fanning Mill and by Hand

Item	Lot						
	A	B	C	D	E	F	G
Corn to cleaner							
U.S. Grade	1	3	4	4	5	SG	SG
BCFM, ^a %	Tr	3	4	2	2	9	1
DKT, %	1	7	8	7	14	5	18
BGY material, %	0.4	0.8	1.0	1.0	3.3	0.6	1.5
Aflatoxin B ₁ , p.p.b.	140	150	140	140	450	120	120
Corn from cleaner							
Yield, %	99	... ^b	89	91	95 ^c	84	92
U.S. Grade	1		2	3	5	1	SG
BCFM, %	Tr		Tr	0	Tr	Tr	0
DKT, %	Tr		4	5	15	3	19
BGY material, %	NA		NA	NA	2.5	NA	0.9
Aflatoxin B ₁ , p.p.b.	160		160	190	510	150	NA
Hand separation for outwardly sound, non-BGY kernels							
Yield, %	98	84	88	88	83	85	89
Aflatoxin B ₁ content, p.p.b.	15	100	60	70	140	70	250 ^d

^aBCFM = broken corn-foreign material; DKT = damaged kernels, total; BGY = material with bright greenish-yellow fluorescence under ultraviolet (365 nm.) light; NA = not analyzed.

^bNo cleaning test made.

^cScreen with 16/64-in. round-hole perforations (r.h.p.) used to remove fines, corn chips, etc. For other lots, perforations were 18/64-in. diameter.

^dValue from hand separation made by Shotwell et al. (8).

TABLE II
Separations Made on Yellow Corn in Fanning Mill

Item	Lot			
	H	I	J	K
Corn to cleaner				
U.S. Grade	2	SG	SG	SG
BCFM, ^a %	2	6	16	4
DKT, %	3	4	2	16
BGY material, %	0.1	0.1	0.2	0.1
Aflatoxin B ₁ , p.p.b.	15	10	70	25
Corn from cleaner				
Yield, %	93	90	75	90
U.S. Grade	1	1	1	SG ^b
BCFM, %	Tr	Tr	1	Tr
DKT, %	2	2	2	15
Aflatoxin B ₁ , p.p.b.	15	10	15	30

^aSee footnote a, Table I.

^bMusty odor.

fractions with a Carter-Day fractionating aspirator, laboratory model. The adjustable vane was set at an angle of approximately 75°. About 35 lb. of the more dense, cleanest fraction was ground, blended, and sampled for the aflatoxin assay and 500 g. of the whole corn was examined for kernels with BGY fluorescence.

For the test with a dry gravity table (Forsberg model M-10), a 30-lb. sample of lot A was separated into three fractions.

Five-pound portions of lot E, which contained 510 p.p.b. of aflatoxin B₁ and 12% moisture, were subjected to impact at rotor speeds of 1,000 to 2,000 r.p.m. in a series 18 Entoleter Centrimil. The product was separated on a Rotap shaker into fractions retained on the following U.S. Standard Series sieves: 3.5, 4, 5, 6, 8, 12 mesh and pan. Each fraction, 8 mesh and coarser, was then hand-picked to determine content of particles exhibiting BGY fluorescence. Total quantity of +8 mesh material examined for BGY fluorescence for each speed test varied between 460 and 910 g.

For tests made in the Stein breakage tester (10), 100-g. portions of corn retained on a 12/64-in. r.h.p. sieve were subjected to impact for 2 min. After separation of the impacted sample by hand-sieving, the +12/64-in. fraction was examined quantitatively for presence of BGY glowing particles.

A 50-lb. sample from lot A was tempered from 10% moisture to 14% for 3.5 hr. and then fed into a S. Howes Eureka grain scourer, size 1, fitted with a 12/64-in. r.h.p. screen. For the first pass the paddles operated at 400 r.p.m., and for the second, 600 r.p.m. After being scoured, the corn was separated on the box sifter into the following fractions: +3.5 mesh, -3.5 mesh +12/64-in. r.h.p., and -12/64-in. r.h.p. Thirty pounds of +3.5 mesh fraction was ground, blended, and sampled for the aflatoxin assay.

As a check on the probable degree of aflatoxin reduction attainable for whole kernel corn, hand separations were made on samples from the seven lots of uncleaned white corn. The samples (960 to 1,300 g. quantities) were shaken on a 9/64-in. r.h.p. sieve to remove fines. Then, oversize material was separated by hand selection under natural or artificial light and under UV light to remove a fraction of outwardly sound, non-BGY kernels. While these kernels were considered to be sound, a closer examination undoubtedly would have revealed some kernels with small or hidden breaks in the hull. The entire fraction from each lot except G was ground and blended and an aflatoxin determination made on a 50-g. portion. No inspection was made for BGY fluorescence after cracking or grinding, although similar studies by Shotwell *et al.* (8) would suggest that some fluorescence would have been found.

RESULTS AND DISCUSSION

Dry Cleaning

Aflatoxin B₁ content of the white corn lots before cleaning varied between 120 and 450 p.p.b. (Table I). For the yellow corn lots the range was considerably lower, 10 to 70 p.p.b. (Table II). The white corn lots represented all grades except No. 2. The yellow corns graded either No. 2 or Sample Grade, and all three of the latter had a musty odor.

From 1 to 16% of the grain was removed as screenings when the white corn was cleaned in the fanning mill, and from 7 to 25% of the yellow corn. Yield of the cleaned white corn usually was 1 to 3 percentage points above the yield of

outwardly sound, non-BGY kernels obtained by hand-sieving and picking. The amount of material removed as screenings was deliberately set high and probably was considerably in excess of that for a typical commercial cleaning operation.

Among the yellow corn lots, J had an excessively high content (16%) of broken corn-foreign material (BCFM)⁴ which was lowered to 1% by the fanning mill. The yield of cleaned corn was only 75%, so appreciable amounts of large broken pieces and small kernels were also removed. The reduction in aflatoxin content indicates that, for this particular lot, much of the aflatoxin was concentrated in the screenings.

Every lot of cleaned white corn that was assayed (one out of seven was not) and one (K) of the four yellow corn lots had an aflatoxin content exceeding that of corn fed to the cleaner. These differences in aflatoxin content of corn to and from the cleaner probably were not significant as they generally were less than the variability in values to be expected from the high relative standard deviation of the assay method (6,11). Screenings from lots D and F (the only screening samples analyzed) assayed 270 and 180 p.p.b. of aflatoxin B₁, respectively, each value exceeding that of the respective lot both before and after cleaning.

Among the white corn lots, the two with highest level of damaged kernels (DKT)⁵ also had highest level of aflatoxin. While lot K of the yellow corns also had a high DKT content, its aflatoxin B₁ content was comparatively low. The dry-cleaning operation removed practically all of the BCFM in each lot, but the DKT content was lowered by only 1 to 4 percentage points. Consequently, the lots with a high DKT content (E, G, and K) usually did not show an improvement in market grade after cleaning, nor was the aflatoxin content lowered for the two lots assayed.

Density Separations

In the washer-whizzer tests, yield of the +3.5 mesh fraction (i.e., essentially whole kernels) varied between 91 and 96% except for lot A where excessive breakage reduced the yield to 88%. The latter corn contained only 10% moisture; 90% of the kernels had stress cracks, and presumably lot A was more brittle than the other lots. Yield of the floaters fraction was 0.1 to 0.4% for the white corn lots and 0.4 to 1.4% for the yellow corns. Based upon inspection for BGY material, in no case did the washing operation give a +3.5 mesh fraction that was free of aflatoxin. Weight of BGY fluorescent material found in this fraction ranged between 0.1 and 0.2% for the yellow corns and 0.4 to 1.6% for the white corns, exclusive of lot A. For the latter, the content of BGY material in the +3.5 mesh fraction was 0.1%, and the fraction assayed 100 p.p.b. of aflatoxin B₁ while the -3.5 mesh fraction assayed 180 p.p.b. The +3.5 mesh fraction from lot E assayed 470 p.p.b. of aflatoxin B₁, and had 1.3% of BGY material.

In the fractionating aspirator test on lot A, the corn with highest test weight and kernel weight was recovered in bin 1, equalled 68% of the corn fed, had 0.1% of BGY material, and assayed 160 p.p.b. aflatoxin B₁. Since this aflatoxin content was equal to that of the corn initially and all fractions had BGY material, the separation was ineffective.

Results from the gravity table were equally disappointing.

⁴Very small kernels and pieces of corn and all matter other than corn that will pass readily through 12/64-in. r.h.p. sieve used in grading the corn.

⁵Damaged kernels, total, as determined in USDA grading procedure.

Impact and Abrasion Tests

As speed of the Entoleter rotor was increased from 1,000 to 1,500 to 2,000 r.p.m., the quantity of material retained on a 3.5 mesh sieve (essentially whole kernels) decreased from 85 to 48 to 22%, respectively, and proportion of kernels or particles with BGY fluorescence in this fraction decreased from 1.7 to 1.0 to 0.6%. BGY content of the corn (lot E) fed was 2.5%. While the proportion of BGY material in the coarse fraction decreased as more of the weaker kernels were broken, overall, the reduction was considered inadequate. There was little evidence that kernels containing aflatoxin had been weakened structurally by the mold to the degree required for ready and practical decontamination of the grain by preferential fragmentation. Wichser has stated that corn kernels with 2,000 to 4,000 p.p.b. of aflatoxin usually are hard and will not fragment easily whereas kernels containing 200,000 to 600,000 p.p.b. are very fragile and will fragment with the slightest exerted pressure⁶. Only in exceptional cases, if ever, would one expect aflatoxin in a contaminated lot of corn to be limited to kernels having such high levels of the toxin, with resulting fragility.

Stein breakage tests were made on every lot except G, and again the results indicated that the fragmentation-screening process would not effect a satisfactory reduction in the aflatoxin content.

The grain scourer uses both abrasion and impact. In a test made on lot A, 19% of the corn was removed as fragments and abraded material, but the whole kernel fraction still had 130 p.p.b. of aflatoxin B₁, therefore essentially was unchanged.

Hand-Separated Fraction from White Corn

The fraction of whole, outwardly sound kernels that appeared free of BGY fluorescence constituted a major portion of each sample, as would be expected. These fractions still contained 60 to 140 p.p.b. of aflatoxin B₁ except for lot A which had 15 p.p.b. These levels were about one-half to one-fourth those in the cleaned corn, with the exception of lot A for which the level in the separated fraction was about one-tenth that in the cleaned corn. Although these reductions are noteworthy, they generally were inadequate relative to present FDA guidelines. Consequently, commercial cleaning processes based on photoelectric detection and rejection of BGY-fluorescent kernels and damaged kernels would not be applicable, particularly since such processes would not be expected to duplicate or even approach the separations made by hand.

CONCLUSIONS

None of the physical separation procedures tried, which included dry cleaning, wet cleaning, and hand separation, proved satisfactory for adequately reducing aflatoxin content of naturally contaminated corn. Preferential fragmentation under the impact conditions used followed with separation of the essentially whole kernels by screening also proved unsatisfactory. Even when the outwardly sound, non-BGY kernels did have a low aflatoxin content, none of the conventional separation methods tried were successful. The results obtained with lot A amply demonstrate this point.

The results from our machine separations confirm the conclusions made by Shotwell *et al.* (8) who performed hand separations on samples from a number of the same corn lots.

⁶Wichser, W. R., personal communication (Sept. 1973).

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