A Physical Method for the Segregation of Aflatoxin-Contaminated Corn

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ABSTRACT

Two lots of corn differing in the degree of aflatoxin contamination (527 and 3,317 ppb) were used to evaluate a process to separate aflatoxin-contaminated from uncontaminated corn. The two lots were segregated into buoyant and nonbuoyant portions in water and in three sucrose solutions (20, 30, and 40%). Removing corn buoyant in water resulted in a 60% reduction in the level of aflatoxin through the removal of 22% of the corn. Improved ability to segregate aflatoxin-contaminated from uncontaminated corn was exhibited through the graded increase in densities of the sucrose solutions. Removing 53% of the corn with 40% sucrose reduced the aflatoxin level by approximately 90%. These data show that aflatoxin-contaminated and uncontaminated corn differ in density. This difference in density may have practical application in aflatoxin detection and in separation of contaminated from uncontaminated corn.

"Aflatoxin" designates a group of mycotoxins produced by fungi in the *flavus-parasiticus* group of the genus *Aspergillus* (Diener and Davis 1969). These ubiquitous fungi can invade and produce aflatoxin on a seemingly endless variety of food and feedstuffs consumed by humans and animals (Scott 1978). Although aflatoxin B_1, B_2, G_1, and G_2 are the four major metabolites of these fungi, aflatoxin B_1 is predominant and considered to be the principal toxic element. Aflatoxin is primarily a hepatotoxin; however, its toxicity can be expressed through a variety of pathologies including impaired coagulation (Doerr et al 1976), interaction with nutritional requirements (Hamilton 1977), impaired immunity (Richard et al 1975), and decreased renal function (Tung et al 1973). Furthermore, aflatoxin is a potent carcinogen in trout (Halver 1969) and rats (Wogan et al 1974), and a growing volume of epidemiological evidence showing a high correlation between the incidence of human hepatomas and aflatoxin consumption (Wilson 1978) clearly indicates the carcinogenic potential of aflatoxin to man. The toxicity, ubiquity, carcinogenicity, and documented outbreaks in swine (Loosemore and Harding 1961), cattle (Loosemore and Markson 1961), and poultry (Hamilton 1971) emphasize the threat from this mycotoxin to both animal and human health.

Because of the need to salvage aflatoxin-contaminated food and feedstuffs, several methods designed to detoxify aflatoxin-contaminated commodities have been investigated (Dollear 1969). Probably the most efficient detoxification method is the exposure of aflatoxin-contaminated materials to various forms of ammonia under elevated temperatures and pressures (Gardner 1971, Marsi et al 1969, McKinney et al 1973, Prevot 1974). A practical ammoniation process was shown by Brekke et al (1978) to detoxify 99% of the aflatoxin in corn. Although the ammoniation procedure is efficient, its effectiveness is limited to a small number of mycotoxins. Koltun et al (1974) found a correlation between density and aflatoxin contamination in cottonseed. The present study was therefore initiated to determine whether a similar difference in density exists between aflatoxin-contaminated and uncontaminated corn. If such a physical difference exists, it could provide a way to segregate aflatoxin-contaminated corn from uncontaminated corn.

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MATERIALS AND METHODS

Three lots of corn, designated lots 1, 2, and 3 were used in these studies. Lot 1 was purchased as aflatoxin-free corn and was confirmed aflatoxin-free through analysis of three 800-g subsamples that were ground and then assayed in triplicate (nine analyses). Corn lots 2 and 3, assayed identically to lot 1, were found to be naturally contaminated with aflatoxin at levels of 527 ± 26 and 3,317 ± 152 ppb, respectively. Duplicate samples of approximately 4,000 g were taken from each lot of corn and placed in a vessel containing either water or sucrose solution (20, 30, or 40%), with specific gravities of 1.000, 1.075, 1.112, and 1.145, respectively. The solutions were stirred, and the buoyant corn was removed, rinsed, and dried at 80°C for 18 hr; the nonbuoyant corn was collected and processed identically. After drying, the buoyant and nonbuoyant segregates were weighed, and the percentage of buoyant corn was calculated. The density of these segregates was determined by weighing a known volume (100 ml) of corn from each segregate. Each segregate was then separated into damaged (cracked or insect-damaged) and whole corn, and the percentage of damaged corn was determined by weight. The segregates of corn from the three lots were then ground to a powder, stored at 4°C, and assayed in triplicate for the presence of aflatoxin B_1.

Aflatoxin was extracted from the samples by the aqueous acetone method of Pons et al (1966). The extracts were reduced to dryness and stored under nitrogen before analysis. They were brought to a known volume with chloroform/methanol (9:1), and small portions (1 or 2 μl) were spotted on 100-μm precoated silica gel chromatographic plates (Eastman Kodak, Rochester, NY). The chromatograms were developed in unlined chambers with a solvent system of benzene/methanol/acetic acid (90:5:2.5) and were presented to the investigator in a double blind fashion. The amount of aflatoxin B_1 was determined through visual comparison of the

TABLE I
Results of Segregation of Three Lots of Corn by Water

Corn	Damaged Corn[a,b] (%)	Percent of Total Corn[a,b]	Aflatoxin Level[b] (ppb)	Aflatoxin Removed (%)
Lot 1				
Untreated	12.93 ± 2.40 a		0	
Treated				
Nonbuoyant	16.73 ± 1.97		0	
Buoyant	...	2.26 ± 0.27 a	0	...
Lot 2				
Untreated	33.03 ± 0.92 b		527 ± 26 a	
Treated				
Nonbuoyant	25.25 ± 2.40		175 ± 26 b	
Buoyant	...	9.61 ± 0.57 b	5,500 ± 921	66.76 ± 4.96
Lot 3				
Untreated	20.79 ± 1.72 c		3,317 ± 152 a	
Treated				
Nonbuoyant	16.97 ± 1.35		1,422 ± 184 b	
Buoyant	...	21.50 ± 1.22 c	4,149 ± 233	57.25 ± 5.59

[a] Values represent the mean ± SEM of duplicate samples and triplicate assays.
[b] Values with different letters within columns differ significantly ($P < 0.05$).

unknowns with simultaneously developed reference standards. Crude confirmation was obtained by spraying the plates with 15% sulfuric acid in water, and positive confirmation was obtained through the formation of the water adduct (aflatoxin B_{2A}) and a mixture of epimeric acetate derivatives by the method of Pohland et al (1970).

The data were subjected to an analysis of variance in which an F-ratio was calculated. If the F-ratios were significant ($P < 0.05$), the least significant difference among treatment means was calculated. The data, expressed as percentages, were subjected to a logarithmic transformation for statistical analysis, and a Student's *t*-test was applied where appropriate (Bruning and Kintz 1968).

RESULTS

The results of segregating buoyant from nonbuoyant corn by water are presented in Table I. The aflatoxin level was significantly ($P < 0.05$) reduced by removing the buoyant corn in lots 2 and 3. Aflatoxin levels were reduced from 527 to 175 ppb in lot 2 and from 3,317 to 1,422 ppb in lot 3. Those of lot 2 were reduced 67% through removal of 9% of the corn and of lot 3, 57% through removal of 22% of the corn. Lot 1, which was aflatoxin negative, contained only 2% buoyant corn. Percentages of damaged corn did not correlate with either the level of aflatoxin or the amount of buoyant corn.

A third segregate apparent in water, semibuoyant corn, was either standing on end or suspended between the bottom of the vessel and the surface of the water. This third segregate was isolated and data was collected with respect to the other segregates (Table II). Density of both semibuoyant and buoyant corn was significantly ($P < 0.05$) less than that of nonbuoyant corn. A large amount of aflatoxin was present in the semibuoyant corn. Furthermore, the level of aflatoxin found in the buoyant corn represents a significant ($P < 0.05$) increase over the level of aflatoxin found in the untreated corn.

The apparent difference in density between aflatoxin-contaminated and uncontaminated corn appears to be a mechanism by which contaminated and uncontaminated corn could be segregated. Increasing the density of the liquid used to segregate uncontaminated corn from contaminated corn should improve the process. In experiments to evaluate this hypothesis, corn was segregated with several sucrose solutions (20, 30, and 40% in water); the results of these experiments are presented in Table III. An increase in the specific gravity of the solutions increased the ability to remove contaminated corn. With 40% sucrose, 87% of the aflatoxin was removed from lot 2 and 90% from 3; 53% of the corn in each of these two lots was removed.

TABLE II
Effect[a] of Further Segregation of Lot 3

Corn	Density (g/100 ml)	Aflatoxin (ppb)
Untreated	57.25 ± 0.74 a	3,317 ± 152 a
Treated		
Nonbuoyant	59.09 ± 0.60 b	1,069 ± 156 b
Semibuoyant	57.26 ± 0.45 a	1,625 ± 55 b
Buoyant	48.17 ± 0.41 c	4,032 ± 374 c

[a] Values represent the means ± SEM of duplicate samples and triplicate assays. Values with different letters within columns differ significantly ($P < 0.05$).

TABLE III
Effect[a] of Segregation of Corn by 20, 30, and 40% Sucrose Solutions

Corn Lot	Sucrose (%)	Buoyant Corn (%)	Aflatoxin Removed by Removal of Buoyant Corn (%)	Aflatoxin in Nonbuoyant Corn (ppb)
2	20	44.38 ± 2.11 a	72.86 ± 2.29 a	143 ± 12 a
	30	49.20 ± 0.97 ab	87.86 ± 2.14 b	64 ± 11 b
	40	53.87 ± 2.33 bc	87.54 ± 1.88 b	66 ± 10 b
3	20	33.97 ± 1.61 a	70.84 ± 2.63 a	967 ± 87 a
	30	40.19 ± 1.03 b	80.58 ± 1.91 b	644 ± 63 b
	40	53.17 ± 1.68 c	90.74 ± 0.91 c	307 ± 30 c

[a] Values within lots represent the mean ± SEM of duplicate samples and triplicate assays. Values within lots and columns with different letters differ significantly ($P < 0.05$).

DISCUSSION

These data indicate that a physical difference exists between aflatoxin-contaminated and uncontaminated corn. Brekke et al (1975) studied several methods designed to segregate corn by physical means and found these methods to be ineffective in reducing the aflatoxin level in corn. The methods used by Brekke et al differed from those used in this study, which may account for the apparent discrepancy between Brekke's results and the ones presented here. The decreased density of the aflatoxin-contaminated corn in the two lots used in this study provided the means to decrease the aflatoxin levels by as much as 60% by removing the corn buoyant in water. Increasing the density of the solution used to segregate corn further reduced the aflatoxin level in nonbuoyant corn; the reduction could be as much as 90%. The

finding that aflatoxin-contaminated corn is less dense than uncontaminated corn cannot simply be attributed to the amount of damaged corn present because this variable did not correlate with the amount of buoyant corn. The change in density could result, however, from fungal utilization of the nutrient-rich endocarp of corn kernels and consequent denaturation of this nutrient source.

The greater level of aflatoxin in the buoyant segregate demonstrates the selectivity of this procedure for aflatoxin-contaminated corn. The procedure can both isolate and concentrate aflatoxin-contaminated corn, which improves the probability of detecting the presence of aflatoxin. Furthermore, only 2% of the kernels in the aflatoxin-free corn were buoyant in water, whereas 9 and 22% of the kernels in lots 2 and 3, respectively, were buoyant in water. Therefore, the amount of buoyant corn present in a sample may provide reason to suspect that the corn may be mycotoxin and/or fungal damaged and may approximate the amount of damage.

Aflatoxin-contaminated corn, because of its difference in density, is buoyant in various liquids and therefore can be separated from aflatoxin-free corn. The added cost of drying the nonbuoyant corn left after the buoyant corn is removed may render this an impractical method of detoxification. However, the difference in density of aflatoxin-contaminated corn provides the basis for designing various segregation processes. One possible process is to pass corn through a jet of air to separate the light, aflatoxin-contaminated kernels from the heavier kernels, which are less likely to be aflatoxin-contaminated. This difference in density of corn may prove to be indicative of fungal damage in general and therefore separate mycotoxin-damaged corn without being specific for any one mycotoxin out of the more than 200 that exist. Certainly, further evaluation of physical differences between mycotoxin-contaminated and uncontaminated corn is justified because of the practical applications of such differences for both removing mycotoxins from corn and improving the detection of mycotoxin-contaminated corn.

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