NOTE

Evaluation of Density Segregation as a Means to Estimate the Degree of Aflatoxin Contamination of Corn[1]

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Mycotoxins are fungal metabolites that are toxic to animals. These compounds pose a constant threat to animal as well as human health through contamination of feed and foodstuffs. Feed manufacturers' concern about mycotoxins in general and aflatoxin in particular has created a need for a reliable, fast, and simple method to evaluate grains before purchase and to monitor the quality of stored grains. One such method is the examination of corn under long-wave (365-mm) ultraviolet irradiation (Shotwell et al 1972). This method is an indirect screening method for aflatoxin and is based on the bright greenish yellow fluorescence (BGYF) presumed to be a plant peroxidase product of kojic acid. The procedure is rapid and simple, and a correlation does exist between the number of BGYF particles found in a sample and the level of aflatoxin contamination (Kwolek and Shotwell 1979, Shotwell and Hesseltine 1981). However, this procedure is only a presumptive test and has a tendency to produce false positive results (Hunt et al 1976, Shotwell et al 1975, Shotwell and Hesseltine 1981). False negative results do occur with this technique (Kwolek and Shotwell 1979, Shotwell and Hesseltine 1981); however, they are infrequent and are usually seen only in samples of low level (<20 ppb) contamination.

In a previous study, Huff (1980) postulated that a difference in density may exist between aflatoxin-contaminated corn and uncontaminated corn. These data suggested that the relative amount of buoyant corn in a given sample might be used to predict the degree of mycotoxin contamination or mold damage in the sample. However, these results were based on a limited number of highly aflatoxin-contaminated corn and were in variance with previous work indicating that physical differences between aflatoxin-contaminated corn and uncontaminated corn were of little significance with respect to detoxification (Brekke et al 1975, Koltun et al 1974). Therefore, the present study was designed to determine whether this difference in density between aflatoxin-contaminated and uncontaminated corn was consistent for a large number of samples and whether the relative amount of buoyant corn would correlate with the degree of aflatoxin contamination of any given corn sample.

MATERIALS AND METHODS

One hundred eighteen corn samples were assayed for aflatoxin B_1 and B_2 by the CB method of analysis (AOAC 1980). A sample of approximately 2 lb of whole corn was examined under high-intensity long-wave (365-nm) ultraviolet irradiation and was designated as BGYF positive if any BGYF material was observed. The density of each corn sample was determined by weighing a known volume of corn (100 ml). Each sample was placed in water and stirred for 2 min, after which the buoyant corn was removed from the surface of the water. The buoyant and nonbuoyant corn segregates were then dried at 80° C for 18 hr and the relative amount of buoyant corn was determined by weight. The fractions were then recombined and a second segregation was performed using an identical procedure except that 30% sucrose, specific gravity 1.112, was used as the suspending liquid. For comparison, the 118 samples segregated with water were divided into two groups of 59 samples. The group designated "lower 50%" represented the 59 samples with the lesser amount of buoyant corn, and the groups designated "upper 50%" represented the 59 samples with the greater amount of buoyant corn. Samples segregated with 30% sucrose were also ranked with respect to the amount of buoyant corn present in a sample and split into two similar groups. Approximately 40 randomly selected corn kernels from the buoyant and nonbuoyant segregates of each sample, using 30% sucrose as the suspending liquid, were shaken in 1% NaOCl solution for 2 min, rinsed in sterile water, and placed (10 per petri dish) onto three separate media. The three media used in these studies were Czapek-Dox agar, malt-7% salt agar, and potato-dextrose agar. The petri dishes were then incubated at 28° C, and the incidence of mold-contaminated corn was recorded daily for five consecutive days.

Statistical analysis of these data was performed with Student's t-test (Bruning and Kintz 1968). All statements of significance are based on the probability level of $P < 0.05$.

RESULTS AND DISCUSSION

Out of the 118 samples, 49 were BGYF positive by visual examination and 54 were aflatoxin positive by the CB method. Twenty samples scored as BGYF positive were found to be aflatoxin negative by the CB method of analysis, and 25 aflatoxin-positive samples were scored as BGYF negative. Therefore, 73

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samples were correctly identified by the BGYF technique, and the calculated accuracy of this procedure was approximately 62%.

The range of the percent of buoyant corn, mean aflatoxin level, density, and percent aflatoxin-positive samples for the upper and lower 50% groups segregated with either water or 30% sucrose are presented in Table I. The mean aflatoxin level, density, and the incidence of aflatoxin-positive samples did not significantly differ between the upper 50% and lower 50% groups when water was used as the suspending liquid. Density was the only variable in which a significant difference was noted between the lower and upper 50% groups segregated by 30% sucrose. The levels of aflatoxin-positive samples in the lower 50% groups were 37.29 and 47.46% with water and 30% sucrose, respectively, and for the upper 50% group, 54.24 and 44.07%, respectively.

These data indicate that density segregation is a poor estimate of the degree of aflatoxin contamination of corn samples. From data reported in an earlier study (Huff 1980), we postulated that a greater proportion of the aflatoxin-positive samples should be found in the 59 samples containing the greatest amount of buoyant corn. However, only approximately 50% of the samples that contained the greatest amount of buoyant material were indeed aflatoxin positive. The BGYF method used in this study was a more accurate index of aflatoxin contamination than density segregation, even though the BGYF technique was hindered by sample size and was performed on whole rather than cracked corn, owing to the need to preserve sample integrity for further evaluations.

These data indicate that if density segregation did separate aflatoxin-contaminated corn from aflatoxin-free corn, then it lacked specificity, and one or several additional factors contributed to the amount of buoyant corn in any given sample. The ability of density segregation to separate aflatoxin-contaminated corn from aflatoxin-free corn was confirmed by analyzing the nonbuoyant segregate of the aflatoxin-positive samples using 30% sucrose as the suspending liquid. These analyses indicated that of the 54 aflatoxin-positive samples, only four remained aflatoxin positive when the buoyant corn was removed from the sample. One factor that might contribute to the nonspecificity of density segregation is contamination of corn with fungi that do not produce aflatoxin but damage the corn kernel in a way that alters its density. However, when the corn from the buoyant and nonbuoyant segregates of each sample, using 30% sucrose as the suspending liquid, was plated on various media and the plates read over several days, the nonbuoyant corn had a significantly higher incidence of mold contamination. These results on mold contamination may reflect a higher incidence of cracked kernels in buoyant corn, which although not apparent upon inspection, allowed the surface disinfectant to penetrate the kernel.

These data demonstrate that density segregation, due to a lack of specificity, is not a reliable method to predict aflatoxin contamination or the level of such contamination. The nonspecific nature of density segregation suggests that additional factors probably contribute to the density of corn kernels. However, these data as well as a previous report (Huff 1980) do demonstrate that aflatoxin-contaminated corn can be segregated from aflatoxin-free corn based on density. Furthermore, because density segregation isolates and concentrates aflatoxin-contaminated corn into the buoyant segregate, this procedure may increase the probability of detecting aflatoxin-positive samples. Additional research is needed to evaluate this procedure for mycotoxin-contaminated corn in general.

TABLE I
Range of Buoyant Corn, Mean Aflatoxin Level, Density, and Percentage of Aflatoxin-Positive Samples Segregated with Water or with 30% Sucrose[a]

Segregation Method	Range of Buoyant Corn (%)	Mean Aflatoxin Level (ppb)	Density (g/100 ml)	Aflatoxin Positive (%)
Water Segregation				
Lower 50%	0.09–0.51	39 a	69.58 ± 0.18 a	37.29 ± 6.35 a
Upper 50%	0.52–2.32	66 a	69.43 ± 0.20 a	54.24 ± 6.53 a
30% Sucrose Segregation				
Lower 50%	2.71–13.66	40 a	69.98 ± 0.16 a	47.46 ± 6.55 a
Upper 50%	13.80–54.16	72 a	69.01 ± 0.20 b	44.07 ± 6.51 a

[a] Within treatments and within columns, values followed by different letters differ significantly ($P < 0.05$). Values represent the mean ± SEM of 59 samples.

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