Aflatoxins in Hull and Meats of Cottonseed

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Abstract

We have found that when aflatoxins are a contaminant of cottonseed, they may be distributed both in the hulls and in the meats. The concentrations in hulls and meats do not appear to be correlated. Aflatoxins were found in hulls and not in meats of some seed samples, and the reverse situation also was observed. The amounts of toxins were generally much greater in meats, which contained up to 10,200 ppb, than in hulls, which contained up to 390 ppb aflatoxins. Hulls as well as meats from *Aspergillus flavus*-damaged seed represent a potential source of aflatoxin contamination, and both should be analyzed in order to accurately assess the total aflatoxins in seeds.

Introduction

THAT AFLATOXINS CAN OCCUR in cottonseed meats (embryos) damaged by *Aspergillus flavus* Link has been established but information on the toxin content of the hull (seed coat) portion of seeds is limited. A knowledge of the relationship of aflatoxins in hulls and meats is important because hull material is frequently used as a diluent in cottonseed meal, and thus represents a potential source of aflatoxin contamination. Although Pons and Goldblatt (9) reported that about twice as much toxin occurred in hulls containing fine meats as in the large hull-free fraction of a mold-damaged sample, they did not separate the hulls and fine meats prior to analysis. Mayne et al. (6), in laboratory experiments, reported that hull material was a relatively poor substrate, compared to meats, for aflatoxin elaboration by *A. flavus*. Interpretation of these reports is difficult in light of a recent report (2) that describes a normal nontoxic constituent of hulls and fibers which, under certain TLC conditions, is chromatographically similar to aflatoxins B_1 and B_2.

This report describes an improved chromatographic separation of the normal constituent (which occurs in hulls and fibers) from aflatoxins. It also summarizes results of experiments made to establish the possible significance of aflatoxins in hulls and meats, by comparing toxin contents of selected samples of cottonseed hulls and meats. The portion of this study dealing with aflatoxins in hulls and meats does not represent results of a geographical survey for aflatoxins in cottonseed. Instead, it was made to determine the distribution of aflatoxins in the hulls and meats from seed samples selected as ones likely to contain aflatoxins.

Materials and Methods

Sample size varied but all samples were extracted according to the method of Pons and Goldblatt (9). Dried extracts were diluted with chloroform to an appropriate volume and spotted, with and without an internal aflatoxin standard, on glass plates coated with a layer of silica gel (Brinkmann G-HR, 0.4 mm wet thickness). Solvent systems used for development of chromatograms varied, depending upon the objectives of the particular experiments. All chromatograms were developed for approximately 45 min in paper-lined unequilibrated tanks and were evaluated visually with a Blak-Ray, 100 w, 3,660 Å ultraviolet lamp for illumination.

Two general methods were used in attempts to separate a blue fluorescent non-aflatoxin normal constituent of hulls and fibers (2) from aflatoxins, using 30-g samples of hulls. The first method was a silica gel-sodium sulfate-packed column procedure proposed for freeing residues of cottonseed meal extracts from constituents that, when present in sufficient quantities, result in occurrence of toxin-masking streaks in chromatograms (8). In this case, separations of toxins from certain streak-causing constituents of extract residues are made by eluting the undesirable constituents from the column with anhydrous ethyl ether. This solvent does not elute the aflatoxins unless it is contaminated with an appreciable amount of water, or unless it contains ethanol as a preservative. The second method consisted of determining the efficacy of several solvent systems for chromatographically differentiating aflatoxins from the nontoxic normal constituent of hulls and fibers. The following solvent systems were used:

1) 3% methanol in chloroform (4,9).
2) 6% methanol in chloroform, which is similar to a solvent system described by Nesheim (7).
3) 3% methanol in chloroform followed by redevelopment of chromatograms in methanol:chloroform:acetic acid (5:94.5:0.5, v/v/v) (4).
4) Methanol:chloroform:acetic acid (5:94.5:0.5, v/v/v) (4).
5) Chloroform:acetone (85:15, v/v) (10).
6) Chloroform:acetone:2-propanol (82.5:15:2.5, v/v/v) (10).

Determinations of aflatoxins in hulls versus meats were made following selection of a procedure suitable for separating bonafide aflatoxins from the blue fluorescent nontoxin material that occurs in hulls and fibers. Aflatoxin determinations were initially made on a series of 60 hull samples, half of which weighed 30 g each and half of which weighed 50 g each. Direct comparisons were then made between hulls and meats (30-g samples) of 100 seed samples. Seeds were dehulled and separated into paired meats and hull samples. Hulls were ground to pass a 20-mesh screen then shaken on a 40-mesh sieve to remove fine fiber, meats, and hull fragments. Visible meats and fiber fragments were separated by hand from the hull material that remained on the 40-mesh screen.

Results

Extract residues of hulls were freed from TLC-streaking constituents when chloroform-residue solutions were treated according to the column separation method described by Pons et al. for cottonseed meal (8). It was not satisfactory, however, for removing the blue fluorescent normal constituent of hulls because this material, like aflatoxins, is not eluted from the column with anhydrous ethyl ether. As reported earlier (2), 3% methanol in chloroform failed to

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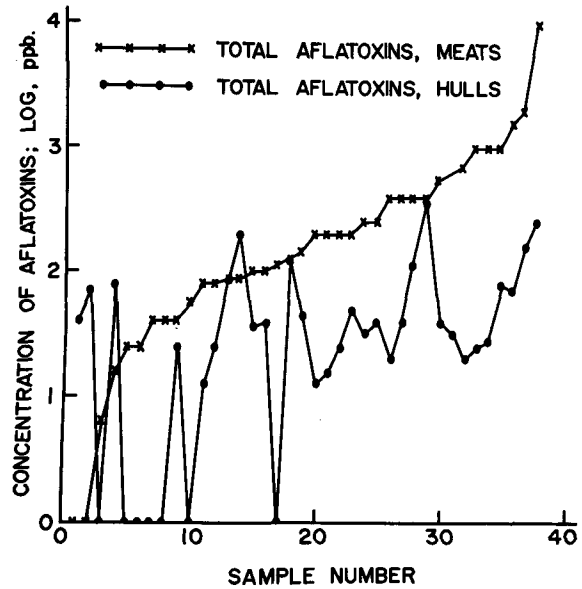

FIG. 1. Relative amounts of aflatoxins (summations of B_1 and B_2) in hulls versus meats of toxic cottonseed.

separate the blue fluorescent constituent of hulls and fibers from aflatoxins chromatographically. Instead, the R_F of the material varied. Depending upon its concentration in particular preparations, it was either between aflatoxins B_1 and B_2, identical with that of B_2, or between B_2 and G_1. However, it was separated from the B toxins in chromatograms developed with chloroform:acetone (85:15). This solvent system had two additional advantages over 3% methanol in chloroform; it eliminated the importance of chromatogram-streaking substances, associated with hull material, because these substances migrated with the solvent front instead of remaining in the area occupied by aflatoxins, and provided better resolution of individual toxins. However, aflatoxin G_1 was masked by the blue-fluorescing substance and the fluorescent intensity of this spot, containing both the blue substance and aflatoxin G_1, made identification of aflatoxin G_2 uncertain. Chromatograms developed with chloroform:acetone:2-propanol (82.5:15:2.5) were similar in appearance to those developed with chloroform:acetone (85:15). The four toxins were separated from the blue fluorescent substance in chromatograms developed with 6% methanol:chloroform and with chloroform:methanol:acetic acid (94.5:5:0.5). The activity of these solvents appeared to be related to their methanol content, because methanol:chloroform solutions containing increasing amounts of methanol resulted in increases in R_F values of the toxins (solutions containing 2 to 9% were tested). The position of the blue-fluorescent nontoxin was considerably lower than the position of aflatoxin G_2 in chromatograms developed with solutions having 5% or more methanol. With these TLC solvents, however, poor separation of aflatoxin B_1 from B_2 and G_1 from G_2 occurred. Therefore, it was necessary to prepare two chromatograms for each sample of hull material, developing one with 6% methanol in chloroform to determine whether aflatoxins G_1 and G_2 occurred; and developing one with chloroform:acetone (85:15) to obtain chromatograms in which the toxins were separated one from the other.

Development of pairs of chromatograms with two different solvent systems, while providing a way to determine whether aflatoxins G_1 and G_2 are present in extract residues of hulls, which have always contained the nonaflatoxin blue fluorescent substance, may not be necessary as a standard practice. This suggestion is based on the following observations. While G_1 and G_2 aflatoxins frequently occur in *A. flavus*-damaged peanuts (5), they have not been observed in 1,325 field-collected cottonseed samples analyzed in our laboratory (1–3). In these samples, when aflatoxins were present, B_1 and B_2 always appeared together, although one or the other type sometimes occurred only as a trace amount. Regardless, in our tests two chromatograms were made for each hull sample residue and one chromatogram was developed in each of the two solvent systems described above so observations could be made for the presence of aflatoxins G_1 and G_2.

Aflatoxins B_1 and B_2, but not G_1 and G_2, were found in some of the samples of cottonseed hulls analyzed in a preliminary experiment. The maximum amount of toxins observed was 118 ppb and the average amount was 45 ppb.

Paired meats and hull samples were then analyzed for aflatoxins to determine the significance of toxins in meats versus hulls. In samples found to contain aflatoxins, again, only aflatoxins B_1 and B_2 were detected. The relative amounts of aflatoxins in hulls versus meats of the toxic samples are illustrated in Fig. 1. The concentrations of toxins in the two types of tissue do not appear to be correlated. As seen in Fig. 1, there were samples in which toxins were not detected in hulls but were detected in the meats and the reverse situation also was observed. In addition, we observed samples with greater toxin concentration in the hulls than in the meats, but the amount of toxins in meats was generally much greater than the amount of toxins in hulls. The maximum amounts of aflatoxins were 10,200 and 390 ppb for meats and hulls, respectively.

These observations support the in vitro culture experiment results of Mayne et al. (6) who reported that hulls were inferior to meats for elaboration of aflatoxins by *A. flavus*. Results of our experiments show, however, that more toxins may occasionally occur in hulls than in meats, under field conditions. They indicate that hulls as well as meats from *A. flavus*-damaged cottonseed should be considered as a potential source of aflatoxin contamination. In addition, the results indicate that hulls, as well as meats, should be analyzed in order to accurately assess the total aflatoxins in seeds.

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