SHORT COMMUNICATION

Adsorptive Removal of Aflatoxins[1]

M.S. Kuk*, R.J. Hron, G. Abraham and **P.J. Wan**
Southern Regional Research Center, Agricultural Research Service, USDA, New Orleans, Louisiana 70179

Physical removal of aflatoxins from cottonseed by solvent extraction with ethanol or isopropyl alcohol is technically feasible. These solvents used in the removal process are recycled to extraction systems after regeneration by distillation. However, distillation is costly due to high latent heat of the solvents. Adsorption techniques have been explored as a method to remove aflatoxins from these solvents. Enzyme-linked immunosorbent assays method and a high-performance liquid chromatography method with fluorescence detector were used to determine the toxins in the feed and eluates from the adsorption columns. Experimental data indicate that montmorillonite is highly effective for adsorptive aflatoxin removal. Adsorption data with neutral alumina and silica are also presented. Ethanol and ethanol-based miscellas, obtained from alcoholic cottonseed extractions were spiked with aflatoxins for this investigation.

KEY WORDS: Adsorption, aflatoxins, cottonseed extraction, distillation, ELISA, ethanol, latent heat, miscellas, regeneration, solvent extraction.

Edible oil and a protein concentrate meal are produced from cottonseed *via* a simple operation of solvent extraction with hexane. However, cottonseed can sometimes be contaminated with aflatoxins, the metabolites of *Aspergillus flavus* and *Aspergillus parasiticus*. Because of their potent carcinogenicity, the presence of aflatoxins in food and feed has been a target of strict control. When cottonseed is contaminated with aflatoxins, it would be desirable if the toxins could be completely converted to nontoxic compounds *via* chemical reactions or removed from the cottonseeds by solvent extraction. Nonetheless, the most commonly used oil-extracting agent, hexane, does not have the necessary solvent characteristics for removing these toxins. Ammoniation has been extensively investigated as a chemical detoxification method, but regulatory issues have impeded use of this method (1).

The physical removal by solvent extraction is, therefore, the most likely choice for salvaging contaminated feed materials. Rayner *et al.* (2) reported on batch extraction data, indicating that polar solvents such as neat ethanol or isopropyl alcohol (IPA) are able to remove aflatoxins from cottonseeds. Nevertheless, there has been no follow-up effort to expand the bench data of Rayner *et al.* (2) to a large-scale operation. Use of fresh and pure alcohol, which was indicated by Rayner *et al.* (3), may pose a serious technical or economic problem in actual practice.

In solvent extraction processes, solvents are generally regenerated by distillation and recycled to the extraction unit. In processing cottonseed meal by solvent extraction, it is desirable to concomitantly extract oil with the toxins and other undesirable components from the seed. The ratio of solvent to unit mass of cottonseed in ethanol extraction is higher than that used in hexane extraction. Furthermore, the latent heats of vaporization for both IPA and ethanol are substantially higher than that of hexane. Hence, the energy costs for solvent regeneration by distillation, which has to be included in the alcohol extraction of cottonseed, outweigh economic gains from the simultaneous removal of aflatoxin and oil. Instead of distillation, adsorption was tested for removing aflatoxins from alcohol-based miscellas. Aflatoxin B_1 was used in the adsorption tests. Aflatoxin B_1 is the major toxic component in contaminated cottonseed, and its chemical structure and properties are representative of the aflatoxin varieties G_1, G_2 and B_2 (4). After removing aflatoxins from alcohol-based miscella, solvent recovery may be achieved by a new technique, such as reverse osmosis/ultrafiltration (5), instead of distillation.

MATERIALS AND METHODS

Three types of simulated miscella mixtures were used as the feed materials in the adsorption tests. The first type of feed (type 1) material was prepared by dissolving chemically pure Aflatoxin B_1 (Sigma Chemical, St. Louis, MO) in a miscella mixture produced by extracting cottonseed flakes with 95% ethanol (USI Chemicals, Cincinnati, OH) in a pilot-scale extractor (Crown, Minneapolis, MN). The experimental details for ethanol extraction are given elsewhere (6). The second type of simulated feed material (type 2) was prepared by mixing typical cottonseed triglycerides (Sigma Chemical) with Alatoxin B_1 (Sigma Chemical) and ethanol. To study the solvent effect of IPA and ethanol on adsorption capacity, the third type of feed (type 3) was prepared as type 2, except that IPA was used in place of ethanol. The type 1 feed material contained phosphatides and polysaccharides, which were extracted from cottonseed, while the types 2 and 3 feed lacked such components.

Tested adsorbents were silica of 80–200 mesh (Baker Chemical, Phillipsburg, NJ), neutral alumina with Brockman activity 1 and 80–200 mesh (Fisher Scientific, Fairlawn, NJ), magnesium silicate of 60–100 mesh (Baker Chemical) and a Wyoming montmorillonite of 10–200 mesh (Blackhills, Mills, WY). The physicochemical characteristics of these adsorbents are given in Table 1. These adsorbents were packed in 25 × 400 mm adsorption columns after overnight conditioning *in vacuo* at room temperature. The feed material was percolated through the packed bed by gravity at room temperature, and the eluate fractions were collected for analysis. A nitrogen blanket up to 5 psi was applied to the adsorption columns. The passage of feed materials from the feed reservoir to the eluate collection vessels was protected from light exposure.

Quantitative analysis for aflatoxins was made by a high-performance liquid chromatography (HPLC) method and a fluorescence detector, with excitation set at 363 nm and emission filtered at 440 nm. A C18 column of 15 × 0.46 cm, packed with 5 μ ultrasphere^r (Beckman, Fullerton, CA), was used with methanol/water (50:50) mobile phase with isocratic flow at 1 mL/min. An enzyme-linked immunosorbent assays (ELISA) method (7) was also used

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*To whom correspondence should be addressed at Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

TABLE 1

Typical Physicochemical Properties of Adsorbents

Properties	Montmorillonite[a]	Alumina (F-1)	Magnesium silicate[b]	Silica
SiO_2 (%)	55-65	0.1	84-85	96.5-99.5
Al_2O_3 (%)	15-20	92	—	—
Fe_2O_3 (%)	3-4.5	0.1	—	—
Na_2O (%)	2-4.5	1	—	0-1
MgO (%)	1-2.5	—	14-15	—
CaO (%)	.4-1.5	—	—	—
Surface area (m^2/g)	80 +	100-250	250 +	200 +
pH	8.5-9.5	6.5-8.0	8-9	6-7

[a]Sodium form (Wyoming); K_2O, TiO_2 and volatiles not listed.
[b]Less than 1% Na_2SO_4 present.

for quantitation. Aflatoxin standards (Roemer Lab) in a mixture of CH_3CN and benzene (97:3 vol%) were used for the quantitation.

RESULTS AND DISCUSSION

The adsorption test results from type 2 feed containing ethanol, triglycerides and aflatoxin B_1 and of actual miscellas (type 1) containing phosphatides and polysaccharides are summarized in Table 2. Table 2 clearly indicates that montmorillonite and magnesium silicate had a higher and more selective adsorption capacity than silica and alumina. It appears that montmorillonite and magnesium silicate have a common factor facilitating selective adsorption for aflatoxin B_1 compared with silica and alumina.

The typical chemical and physical properties of these adsorbents are presented in Table 1. As seen in Table 1, the chemical analyses of these adsorbents indicate that, among other cations, magnesium is the common transitional metal present in the oxide form in montmorillonite and magnesium silicate, but that it is absent from alumina and silica. It is quite possible that the five-membered heterocycle moieties of aflatoxins (Scheme 1, aflatoxin B_1 structure) are attracted to magnesium as pyrrole rings of porphyrin are tied to transition metals as in various forms of lipids. Other physical properties, such as the layer axis distance of these minerals, pore size distribution, pore volume, acidity and thermodynamic states, may affect the adsorption capacity and selectivity. It is obvious, however, that based upon present information the adsorption selectivity and capacity appear to be principally determined by the type of cations in the adsorbents.

The actual miscellas contained *ca.* 30 ppm of phosphorus (6) and a small amount of free sugars, which were detected as shown in a chromatogram given in Figure 1. Comparing the adsorption performance of actual miscellas to that obtained with the simulated feed of type 2 and type 3 (Table 2), it is seen that phosphatides and polysaccharides compete for the free adsorption sites and retard aflatoxin adsorption. This competition for the free adsorption sites among aflatoxin B_1, phosphatides and free sugars was expected. Phosphatides and saccharides are known to be attracted to adsorbents. Solvent effects of IPA and ethanol on adsorption capacity and selectivity were not observed either for montmorillonite and magnesium silicates (Table 2). In a separate experiment, hexane-extracted cottonseed flakes were tested as adsorbents. Aflatoxin analysis of the eluates from the defatted cottonseed indicated that the amount of aflatoxin B_1 adsorbed by the flakes was limited to the solvent retained by flakes and was insignificant compared to that adsorbed by montmorillonite and magnesium silicate. Less than 20% of the toxins in the feed was adsorbed by the defatted flakes. Nevertheless, reverse migration of aflatoxins to the defatted flakes may not be insignificant when the toxin concentration in the feed to the adsorption unit is higher by several orders than the test feed. Aflatoxin build-up in miscellas has to be avoided, not only to maintain the extraction efficiency of toxins but also to prevent the possible reverse migration of the toxins. The aflatoxin build-up problem can be avoided by incorporating adsorption columns with the oilseed extraction unit.

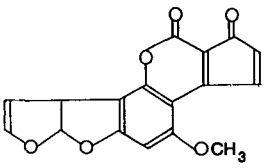
Because magnesium oxides exist either in calcium or sodium montmorillonites, with minor variations in their

TABLE 2

Aflatoxin B_1 Concentration (ppb) in Eluates After Adsorption from Three Simulated Feeds (P = 1 atm, T = 25°C)

	Feed		
Adsorbent	Type 1[a]	Type 2	Type 3
Feed	37	35	34
Silica	18	18	*b*
Alumina	6	3	*b*
Magnesium silicate	≈0	≈0	≈0
Montmorillonite	≈0	≈0	≈0

[a]Contained cottonseed phosphatides and polysaccharides.
[b]Because of the low adsorption capacity for Aflatoxin B_1 compared to the other tested adsorbents, these data were not taken.



SCHEME 1

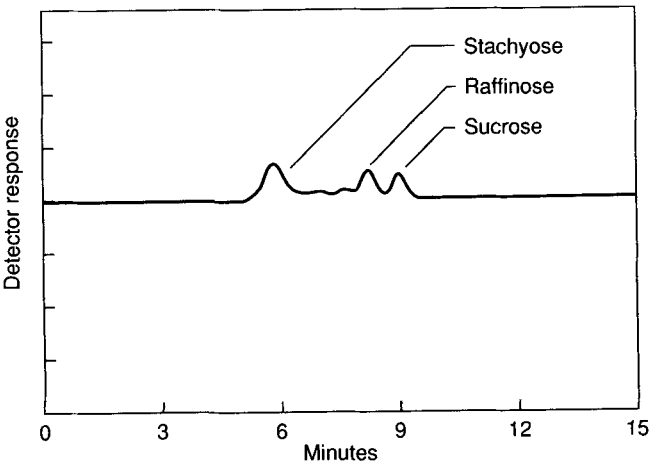


FIG. 1. HPLC chromatogram of free sugars extracted from cottonseed with ethanol (mobile phase - deionized H_2O @ 1mL/min; Refractive Index detector with an Aminex^r column).

compositions, it is expected that most montmorillonites, regardless of the locality of their origin, should behave in a similar manner. Although the experimental investigation was performed with aflatoxin B_1, it is expected that the results can be extended to other aflatoxins.

Alkalis have been reported to be capable of destroying aflatoxins (8). The spent adsorbents were regenerated by washing them with acetone and sodium hypochlorite (5 vol%) at room temperature, followed by drying with air. Such washing was effective for regenerating the contaminated adsorbents in this study. The amounts of aflatoxin in the contaminated adsorbents were of the same order as those originally present in the tested miscellas. However, this regeneration method may not be effective when the spent adsorbents are excessively contaminated with aflatoxins. As seen in Table 2, eluates from montmorillonite and magnesium silicate did not contain aflatoxin B_1 at all. These data indicate that montmorillonite and magnesium silicate may have a higher adsorption capacity for aflatoxin than that contained in the tested miscellas. To determine the adsorption capacity of these minerals, magnesium silicate and montmorillonites, and to establish the proposed regeneration method in detail, the adsorption isotherms and their desorption characteristics are to be further investigated in a separate study.

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