Removal of Aflatoxins From Oilseed Meals by Extraction With Aqueous Isopropanol

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Abstract

Aqueous isopropanol was found to be an effective solvent for removal of aflatoxins from contaminated cottonseed and peanut meals. Extraction with 6 passes of 80% aqueous isopropanol at 60C resulted in complete removal of aflatoxins in both meals, as measured by thin-layer chromatography. Under similar extraction conditions, the isopropanol-water azeotrope, 88% isopropanol by weight, removed 88% of the total aflatoxins in peanut meal, a reduction from 82 to 10 ppb, and 79% of the total aflatoxins in cottonseed meal, a reduction from 214 to 46 ppb. Lower temperatures were less effective with both solvent systems.

Introduction

In recent years, the presence of mycotoxins in food and feeds has attracted increased public attention. It has been observed that some agricultural products may contain toxic metabolic products which are produced by certain strains of the mold *Aspergillus flavus* (1) and other molds. These metabolites, termed aflatoxins, have been characterized chemically (2,3), and feeding experiments have indicated that adverse physiological responses are induced in some laboratory and farm animals if the toxins are fed at sufficiently high levels (4–7). It has been reported that the ingestion of aflatoxins by certain lactating animals results in secretion of similar toxins in the milk (8,9).

Some oilseeds and their corresponding products are subject to aflatoxin contamination (10,11). Aflatoxin contaminated peanuts, for example, can be processed to yield toxin-free refined oil since the oil refining procedures remove these toxins (12), but the meal may retain an aflatoxin content high enough to preclude its use in animal feeds. It has been recommended that such meal be diverted to fertilizer (13). Such diversion, however, results in loss of valuable protein supplements for animals, and the intrinsic economic value of the meal is greatly reduced.

It is of importance, therefore, to develop practical methods for the inactivation or elimination of aflatoxins in contaminated oilseed meals. Previous work has indicated that heat treatments alone, either dry or wet, do not effectively eliminate aflatoxins from contaminated cottonseed or peanut meals (14–17). Autoclaving of wet toxic peanut meals has been reported to reduce aflatoxin content, but the nutritive value of the end product seems questionable (18). More recently, treatment of aqueous peanut meal slurries with hydrogen peroxide has been reported to effect aflatoxin detoxification (19). However, this process appears applicable primarily to production of aflatoxin-free protein isolates. Feuell (18) has summarized the solvent extraction procedures which have been investigated for removal of aflatoxin from contaminated peanut meal.

The present research was undertaken to investigate the efficiency of aqueous isopropanol as a solvent for removing aflatoxins from contaminated cottonseed or peanut meals.

Experimental Procedures

Materials

The cottonseed meal was a selected prepressed solvent extracted sample specially chosen because of its aflatoxin content. It contained 144 ppb (μg/kg) aflatoxin B_1 and 70 ppb aflatoxin B_2. No aflatoxins G could be detected. Meal moisture was 7.2% as received. Other analytical data on the meal include (moisture-free basis): nitrogen, 7.0%; crude fiber, 16.5%; lipids, 1.12%; free gossypol, 0.03%; and total gossypol, 0.75%. The available lysine content was 2.94 g/16 g nitrogen (20).

The peanut meal was a selected prepressed solvent-extracted sample specially chosen because of its aflatoxin content. It contained 54 ppb aflatoxin B_1, 18 ppb aflatoxin B_2, and 10 ppb aflatoxin G_1. No aflatoxin G_2 could be detected. Meal moisture was 7.8% as received. Other analytical data on the meal include (moisture-free basis): nitrogen, 10.0%; crude fiber, 4.8%; lipids, 1.19%. The available lysine content was 2.73 g/16 g nitrogen.

The isopropanol used was Baker Analyzed Reagent Grade, diluted with the calculated quantity of distilled water to prepare the solvent concentrations described.

Equipment

Extractions were carried out in a glass Büchner type funnel having an inside diameter of 9.5 cm and fitted with a coarse fritted glass bottom. A stopcock in the stem portion was used to regulate liquid flow. The funnel was further modified with an insulated metal jacket having inlet and outlet ports to provide for circulation of aqueous glycerol heating fluid around the funnel. To facilitate solvent changes during extractions, a vacuum filtering flask was connected to the funnel stem. During operation, solvent vapors were contained by placing a watch glass covering on the top.

Methods

Cottonseed or peanut meal (200 g) was placed in the extraction funnel and slurried with an equal weight (approx 240 ml) of aqueous isopropanol azeotrope (87.7% isopropanol wt/wt) or 80% (wt/wt) aqueous isopropanol. Both the extractor and solvent were preheated to obtain the desired extraction temperature as rapidly as possible. A 15-min meal-solvent residence time was allowed for each pass. Vacuum was applied to facilitate the removal of miscella between passes.

After completion of the final pass, the meal was drained thoroughly, removed from the extraction funnel, and spread in a glass tray to air-dry at ambient temperature for at least 24 hr before it was assayed for aflatoxin content.

Meal samples were assayed for aflatoxin content

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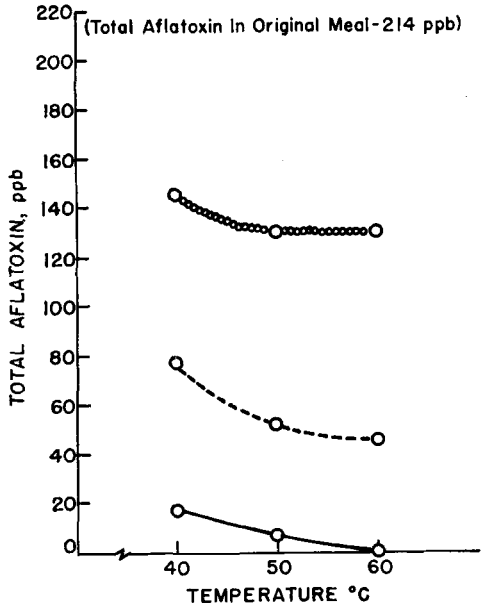

FIG. 1. Effect of solvent and temperature on extraction of aflatoxins from cottonseed meal using 6, 15-min solvent passes. ooooooooo, 100% isopropanol; --------, isopropanol-water azeotrope; ———, 80% aqueous isopropanol.

by the method of Pons et al. (21). Briefly, the procedure involved extraction of the aflatoxins from the meal with acetone:water (70:30 v/v), purification by treatment with lead acetate, partitioning of aflatoxins into chloroform, purification of the extract on a silica gel column, separation of aflatoxins on TLC plates coated with Silica Gel GHR, and visual comparison of the intensity of the fluorescence under ultraviolet light of sample aliquots and appropriate aflatoxin standards. The visual procedure employed allows estimation of aflatoxin values in a range of ±20% to 30% of the amounts present on a TLC plate. As little as 1 ppb aflatoxins can be detected.

The percentage of meal solids extracted in each procedure was determined by stripping solvent from

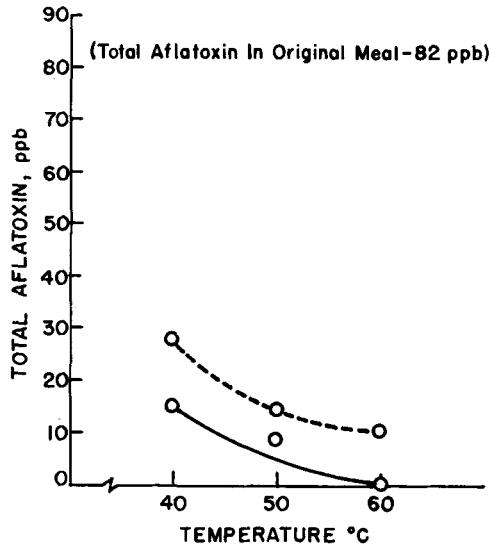

FIG. 2. Effect of solvent and temperature on extraction of aflatoxins from peanut meal using 6, 15-min solvent passes. --------, isopropanol-water azeotrope; ———, 80% aqueous isopropanol.

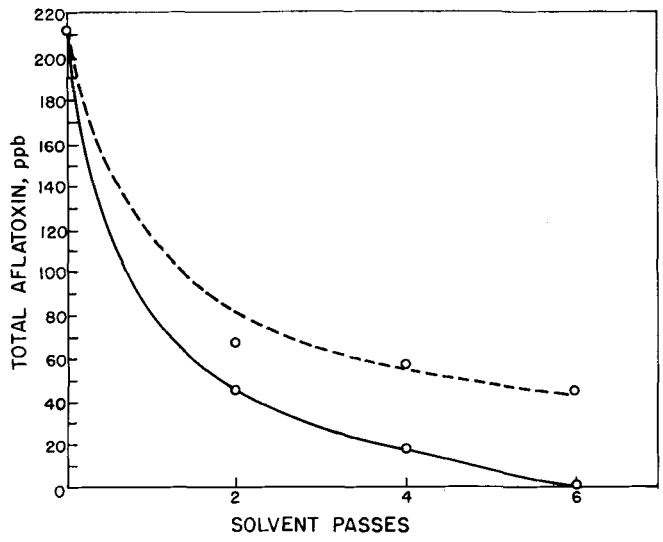

FIG. 3. Effect of solvent passes on extraction of aflatoxins from cottonseed meal at 60C; 15-min residence time per pass. --------, isopropanol-water azeotrope; ———, 80% aqueous isopropanol.

the miscella in a rotary evaporator under reduced pressure (1 mm Hg) at 100C until a constant weight was attained.

Results and Discussion

Effect of Solvent

Eighty per cent aqueous isopropanol was a more effective solvent for extracting aflatoxins from cottonseed or peanut meal than was the isopropanol-water azeotrope. This is illustrated in Fig. 1 for cottonseed meal, extracted with 6 solvent passes at various temperatures. At 60C for example, the isopropanol-water azeotrope lowered the aflatoxins from 214 to 46 ppb, a reduction of 79%; whereas, under similar conditions, the 80% aqueous isopropanol reduced aflatoxins to a level below the sensitivity of the TLC assay method employed.

In comparable extractions, also shown in Fig. 1, 100% isopropanol was least effective in extracting aflatoxins from cottonseed meal. A reduction of only 39%, or 214 to 130 ppb total aflatoxins was accomplished.

Extraction of peanut meal with six passes of isopropanol-water azeotrope at 60C reduced the

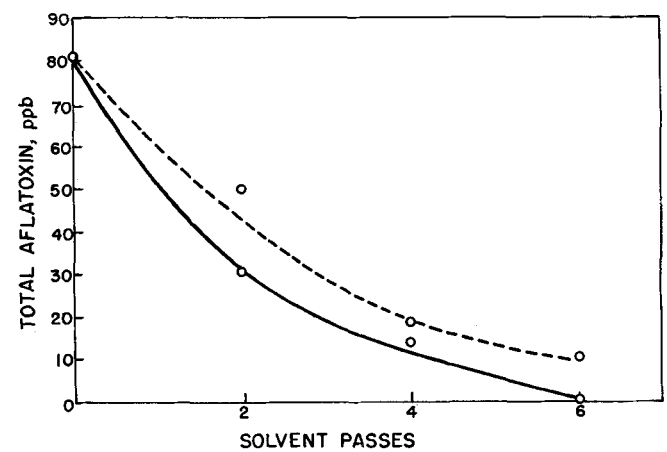

FIG. 4. Effect of solvent passes on extraction of aflatoxins from peanut meal at 60C; 15-min residence time per pass. --------, isopropanol-water azeotrope; ———, 80% aqueous isopropanol.

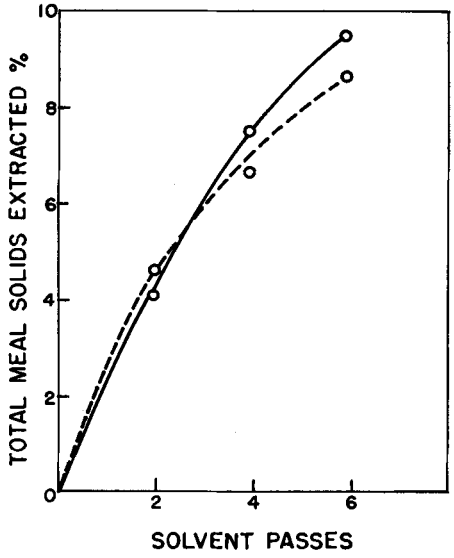

FIG. 5. Extraction of meal solids at 60C with 80% aqueous isopropanol. --------, cottonseed meal; ———, peanut meal.

aflatoxins from 82 to 10 ppb (88%); whereas, similar extraction with the 80% aqueous isopropanol lowered aflatoxin concentrations to below the level detectable by TLC, as shown in Fig. 2.

Effects of Temperature

The effects of temperature on the extraction of aflatoxins from cottonseed meal with isopropanol-water azeotrope or 80% aqueous isopropanol are also shown in Fig. 1. For both solvent systems, higher extraction temperatures favored reduction in aflatoxin levels. Extraction at 60C with 6 solvent passes reduced aflatoxin levels from 214 to 46 ppb with the azeotrope, a reduction of 79%, and similar extractions with the 80% aqueous isopropanol resulted in complete elimination of the aflatoxins, as measured by TLC. Lower extraction temperatures were generally less effective.

A similar effect of temperature on the extraction of aflatoxins from peanut meal using isopropanol-water, azeotrope or 80% aqueous isopropanol is shown in Fig. 2. Extraction at 60C with 6 solvent passes reduced aflatoxin levels to 10 ppb with the azeotrope, a reduction of 88%, and similar extractions at 60C with the 80% aqueous isopropanol reduced aflatoxin content to below the levels measurable by TLC. As noted previously for cottonseed, lower extraction temperatures were less effective with both solvent systems.

Effect of Solvent Passes

The effectiveness of the isopropanol-water azeotrope or 80% aqueous isopropanol in removing aflatoxins from either cottonseed or peanut meals increased, as expected, with the number of solvent passes. As shown in Fig. 3 and 4, 6 passes with 80% aqueous isopropanol at 60C effectively reduced aflatoxin levels in both cottonseed and peanut meals to below the limits detectable by the TLC assay employed.

Extractable Solids

The effective removal of aflatoxins from cottonseed or peanut meals with 80% aqueous isopropanol is accomplished by the extraction of some soluble meal components. Fig. 5 shows this relationship for cottonseed and peanut meals. At 60C, 6 passes with 80% aqueous isopropanol removes 8.7% of the total solids from cottonseed meal and 9.5% of the total solids from peanut meal.

ACKNOWLEDGMENTS

Many helpful suggestions and encouragement came from Dr. Godfrey E. Mann and Dr. Leo A. Goldblatt. Experimental assistance was given by William R. Turnipseed and L. P. Codifer, Jr.

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[Received April 2, 1968]