Solvent Extraction of Aflatoxins from Contaminated Agricultural Products

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ABSTRACT AND SUMMARY

Solvent extraction of agricultural products has been suggested as an effective means of removing aflatoxins from mold-damaged commodities. The use of various polar solvents such as the azeotrope of acetone-hexane-water and of 2-propanol-water, aqueous acetone, and aqueous ethanol has been reported in the literature. This paper examines the overall aspects of solvent extraction, in particular the use of the azeotrope of 2-propanol-water, to remove aflatoxins from prepress solvent extracted cottonseed meal.

INTRODUCTION

When the problem of aflatoxin contamination in agricultural commodities emerged in 1960, several proposals were examined as possible solutions. Those of major significance were: (a) prevention of *Aspergillus flavus* mold growth in the commodity; (b) developing means to physically separate contaminated products from uncontaminated ones; (c) chemical treatment to inactivate the aflatoxins; and (d) solvent extraction to remove the aflatoxins. Since solvent extraction was an established concept in the oilseed industry, this approach was the focus of much early research.

The removal of aflatoxins from a contaminated product by solvent extraction offers certain advantages over inactivation of aflatoxins. Principally, these are: (a) a suitable solvent used under the proper conditions will eliminate essentially all of the aflatoxins present; (b) removing aflatoxins (as opposed to chemically inactivating them) virtually precludes the possibility of forming other toxic compounds or artifacts in the commodity; and (c) the relatively low processing temperatures used in solvent extraction have a minimal effect on lowering nutritional quality.

Conversely, solvent extraction presents certain difficulties which must be recognized. These include: (a) effective solvent extraction may entail specialized extraction equipment and solvent recovery systems; (b) extraction solvents can remove certain desirable components from the product, as well as aflatoxins; (c) methods must be devised for economic disposal of the aflatoxin-laden solvent extract; and (d) the increased costs added by the additional processing will be reflected in the finished product.

REVIEW OF EARLY SOLVENT EXTRACTION STUDIES

Hexane extraction of oilseed meals was a well-established practice when the problem of mycotoxin contamination emerged in 1960. It was readily recognized that hexane removed practically none of the toxins from these contaminated products, hence the search began for other solvents to extract the toxins. As early as 1961, before the aflatoxins had been characterized, Sargeant et al. (1) and Allcroft et al. (2) reported that contaminated Brazilian groundnut meal, extracted with methanol, produced a toxic residue. This was the initial indication that polar solvents were effective extractants. Building upon this work and that of other researchers (3-5), Hartley et al. (6) chemically characterized the aflatoxins B_1, B_2, G_1, and G_2 in 1963. Characterization of the aflatoxins established that polar compounds were effective solvents, whereas nonpolar ones were not. In the same year, however, Salmon and Newberne (7) found that extracting contaminated peanut meal with hot methanol for 72 hr in a steam-jacketed, continuous extractor did not remove all toxins from the meal. In the light of our present knowledge it seems possible that the extraction may have been impaired by insufficient moisture in the system.

Feuell (8) in 1966 studied the extraction potential of solvents such as methanol, acetone, benzene, chloroform, and water, using a Soxhlet extraction system for a period of 20 hr. With the exception of methanol, Feuell found these polar solvents to be relatively ineffective in extracting aflatoxins from the contaminated meal. Since the returning condensate from a Soxhlet extractor is often well below the boiling point of the solvent used, it is likely that in these experiments temperature, and possibly moisture levels, were not sufficiently controlled to effectively extract the aflatoxins. Feuell concluded from his work that "...with aflatoxin in meals extractability is not the same as solubility." He correctly concluded that moisture plays an important role in the release of aflatoxins in conjunction with appropriate solvents, stating, "Evidently water and hydroxylated solvents like methanol either break down cell barriers or affect their constituents and so facilitate release of the aflatoxin into the extracting solvent."

Robertson et al. (9) proposed a mixture of acetone-hexane-water in the proportion of 50:48.5:1.5 (v/v) as the extracting solvent for aflatoxin analysis of peanut products. Goldblatt and Robertson (10) also suggested the possible application of an azeotrope of acetone-hexane-water as a solvent for removing aflatoxin from peanut or other oilseed meals.

Vorster (11) in 1966 reported the effect of various solvent azeotropes on the removal of aflatoxins from contaminated peanut cake, using Soxhlet extractions for 6 and 10 hr. Five azeotropes were examined, hexane-ethanol 79:21; hexane-methanol 73:27; acetone-hexane 59:41; hexane-ethanol-water 85:12:3; and acetone-hexane-water 54.5:44.4:1.1. The latter azeotrope was prepared by arbitrarily adding 3% water to the acetone-hexane 59:41 solvent system, thus providing an excess of water. Although none of the azeotropes removed all of the aflatoxins (possibly due again to poor Soxhlet temperature control), the one containing the excess water was most effective, reducing aflatoxin levels in the peanut cake from 5000 μg/kg (ppb) to 60 ppb.

Thus the three factors which seem essential for extraction of aflatoxins from contaminated products appear to be (a) use of an appropriate polar solvent; (b) adequate moisture in extraction systems to release the aflatoxins; and (c) sufficiently high operating temperatures (usually near the boiling point of the solvent) to effectively solubilize the toxins.

Other researchers using various solvent extraction systems have verified the importance of these factors. Pons and Eaves (12) reported that gossypol, fatty acids, and aflatoxins were removed from cottonseed flakes with acetone containing 25-30% water. Rayner and Dollear (13) indi-

cated that 2-propanol and water (80:20) and 2-propanol-water azeotrope (87.7:12.3) were effective in removing aflatoxins from prepress solvent-extracted cottonseed and peanut meals. Also, Dollear et al. (14) reported that aflatoxins in prepress solvent-extracted peanut meal were extracted by acetone and water (90:10). Gardner et al. (15) significantly reduced the aflatoxins in cottonseed and peanut flakes by extracting with a ternary solvent composed of acetone-hexane-water (54:44:2). It has also been demonstrated that ethanol and water (80:20) and the ethanol-water azeotrope (95:5) were effective in extracting aflatoxins from prepress solvent-extracted cottonseed and peanut meals (16).

Aqueous acetone has been properly cited in many instances as an effective solvent for the extraction of aflatoxins. Certain problems, however, attend the use of this solvent. When oilseed meals are extracted with acetone, they sometimes develop an unpleasant "catty" odor and off-flavor, attributed to compounds formed by reaction of hydrogen sulfide with an acetone condensation product, mesityl oxide (17,18). Mesityl oxide can be generated in the acetone during extraction, and the sulfur-containing amino acids in oilseed meals are available to form compounds such as 4-methyl-4-mercapto-pentan-2-one or other offensive mercaptan derivatives. If odor and flavor are important, extractants other than acetone should be used to remove aflatoxins.

RECENT DEVELOPMENTS

Data from the laboratory extraction work (13,14) on acetone and water (90:10), 2-propanol and water (80:20), and the azeotrope of 2-propanol and water (87.7:12.3), were used for larger scale extractions. The equipment used was a stainless steel basket type extractor, 20 cm square and 60 cm in length (15), equipped with an external spiral steam coil and insulation to provide and maintain heat. A 20-mesh screen near the bottom retained the meal, and the top was fitted with a cover to retard evaporation and cooling. Provisions were available at the outlet to apply a vacuum when needed. A cutaway diagram of the equipment is shown in Figure 1.

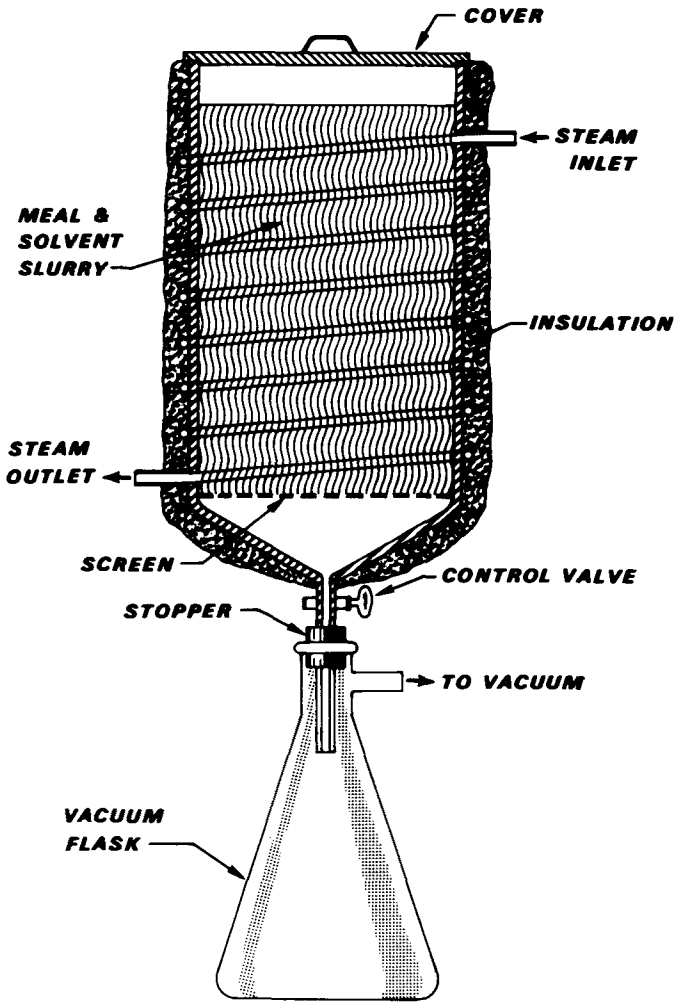

FIG. 1. Diagram of basket type extractor used in batch extractions.

In application, a quantity of solvent ten times the weight of meal was heated to near boiling in a reservoir located above the extractor. With the control valve in the closed position, 7-9 kg of contaminated meal was introduced into the extractor simultaneously with sufficient heated solvent to make a slurry. Steam was applied to maintain temperature, and the mixture was allowed to steep for 45 min. With the control valve opened, the solvent was then drained from the container and a slight vacuum applied to facilitate draining. This process was repeated twice with fresh, heated solvent, but steeping times for the second and third solvent contacts were reduced to 15 min. When the third extraction was complete, the solvent remaining in the reservoir was allowed to percolate through the meal by gravity until the reservoir was empty. Vacuum was applied to the extractor to drain the meal charge completely. The extracted product was spread out in trays and dried in a mechanical convection oven overnight without added heat.

The results of extracting aflatoxin-contaminated, prepress solvent-extracted cottonseed meal with various solvents are shown in Table I.

Extraction with acetone-water (90:10) clearly provides excellent reduction in total aflatoxin levels, from 519 ppb to 3 ppb. Also, the quantity of soluble components extracted (4.4%) is the lowest for the three solvent systems compared. The potential odor and flavor problems associated with acetone extraction, however, outweigh these desirable results, and the suitability of this solvent remains doubtful.

Using the procedure described above, aflatoxin reduction with 2-propanol-water (80:20) was similar to that achieved with acetone-water (90;10). The soluble components extracted with 2-propanol-water, however, were

TABLE I

Batch type Solvent Extraction of Aflatoxin Contaminated Cottonseed Meal

Solvent	Aflatoxin content, ppb			Soluble components extracted, %
	B_1	B_2	Total	
None	448	71	519	–
Acetone, water (90:10)	3	ND[a]	3	4.4
2-propanol, water azeotrope (87.7:12.3)	10	2	12	7.7
2-propanol, water (80:20)	3	ND	3	11.3

[a]None detected.

TABLE II

Continuous Extraction of Aflatoxin Contaminated Cottonseed Meats and Meal With 2-Propanol-Water Azeotrope

Conditions	Run no. 1 Meats	Run no. 2 Meats	Run no. 3 Meal	Run no. 4 Meal
Initial moisture, %	6.0	6.0	7.8	7.8
Final moisture, %	6.0	6.0	4.0	4.6
Retention time, min	60	90	60	90
Feed rate, kg/hr	20	15	45	30
Total initial aflatoxins, ppb	353	353	294	294
Total final aflatoxins, ppb	3	6	9	12

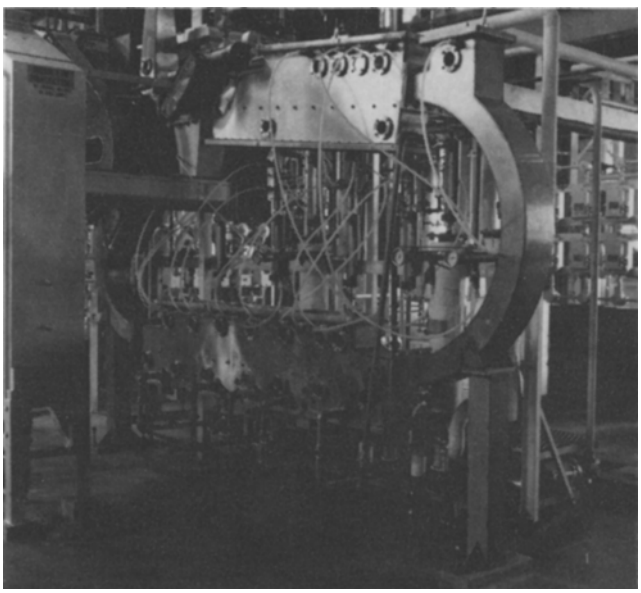

FIG. 2. Continuous pilot-scale extractor installed at the Southern Regional Research Center.

nearly three times greater than those extracted with acetone-water.

The azeotrope of 2-propanol and water offers an acceptable compromise between the two other solvents. With this solvent system the aflatoxins are reduced from 519 ppb to 12 ppb, and the soluble components extracted are retained at a reasonable 7.7%.

This solvent was chosen for further investigative work, using the pilot plant Crown Solvent Extractor at the Crown Iron Works, Minneapolis, MN. Essentially, this equipment is a vertical loop continuous extractor, which employs both concurrent and countercurrent extraction to enhance efficiency. Cottonseed meats, flaked to 0.012 in. thickness, and prepress solvent-extracted cottonseed meal, screened on a 30-mesh screen to remove excess fines and flaked to 0.010 in. thickness, were extracted for 60 and 90 min each, using the azeotrope of 2-propanol and water. The solvent to meal ratio was approximately 2.5:1, and extraction temperature was 77 C. Table II shows the results of these extractions. in general, it appears that the aflatoxins are removed more readily from full-fat flaked meats, being reduced from 353 ppb to 6 ppb or less by this procedure. In the prepress solvent-extracted cottonseed meal, the slightly lower initial aflatoxin content of 294 ppb was reduced to 12 ppb or less.

The Southern Regional Research Center, ARS, USDA has installed a pilot-plant scale Crown Extractor, shown in Figure 2, and a prepress solvent-extracted cottonseed meal containing 300 ppb total aflatoxins was extracted with the azeotrope of 2-propanol and water, using a solvent-to-meal ratio of 2.5:1. With an average temperature of about 77 C and a retention time of 30 min, the aflatoxin content was lowered to a level of 2 ppb.

It appears that with efficient extraction queipment, the azeotrope of 2-propanol and water effectively removes aflatoxins from contaminated cottonseed meal. The constant boiling point of this binary solvent facilitates solvent recovery and reuse, and no off-flavors or odors are imparted to the extracted product.

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