Use of Water Slurries in Aflatoxin Analysis

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A method based on the use of water slurries was developed for aflatoxin analysis. This procedure enables aliquots, from kilogram size samples, to be analyzed without an increase in solvent or reagent costs. Slurries were prepared in a 1-gal blender and sample weight to water volume ratios were 1:1.5 for peanut butter, 1:2.25 for whole seeds, 1:4 for commercial meals, and 1:5 for copra. Extraction of 100-g aliquots of slurry with 200 ml of acetone resulted in aflatoxin yields comparable to those obtained by extraction of the dry products by the standard method. Peanuts, peanut butter, peanut meal, cottonseed, cottonseed meal, copra, and corn were successfully analyzed by this slurry technique. The precision of slurry analysis for these products, expressed as coefficient of variation (percent), ranged from 2.6 to 7.8 compared to 5.7 to 20.8 for analyses by the standard procedure. Determinations were made by fluorometric measurements of aflatoxin adsorbed on the Florisil layer in a minicolumn.

The nonuniform distribution of aflatoxin in agricultural products is recognized as a problem in evaluating the true level of contamination in unit lots.

Sampling studies of aflatoxin-contaminated peanuts (Whitaker and Wiser, 1969; Whitaker et al., 1972) and cottonseed (Velasco et al., 1975) have been made by the U.S. Department of Agriculture Research Service for the purpose of providing guidelines for the sampling of these products. The studies showed that the precision of lot analyses, expressed as percent coefficient of variation (standard deviation/mean, × 100), for 29 lots of peanuts ranged from 89 to 292 with an average value of about 162. For 39 lots of cottonseed the percent coefficient of variation ranged from 27 to 272 with an average value of 113.

In the studies, 10 250-g subsamples of peanuts and 20 100-g subsamples of cottonseed were analyzed per lot. A percent coefficient of variation above 100 indicates that the standard deviation is larger than the mean and that estimating the mean level of contamination in a lot with a single or duplicate analysis would be extremely difficult.

Accuracy and precision of aflatoxin determination can be improved by analysis of more or larger subsamples. But a major limiting factor would be the cost of solvents; for example, about 1 gal of solvent is required for the extraction of aflatoxin from 1 kg of subsample. Such a large volume of solvent necessarily restricts the number and size of subsamples that can be routinely analyzed in a quality control operation. For this reason, we investigated the possibility of extracting aflatoxin from water-slurried products.

The use of a water slurry could reduce the variability associated with aflatoxin analysis because (a) distribution of particles is more uniformly achieved with a slurry than with a dry ground product; (b) seeds of high oil content are readily reduced to a fine particle size, whereas only a coarse grind is possible with conventional mills because of clogging.

EXPERIMENTAL SECTION

Most products included in this investigation had been analyzed in previous studies so that contamination levels were approximately known. A twin V shell blender was used to mix comminuted samples to appropriate levels, and all aflatoxin was derived from naturally contaminated products.

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Copra contaminated with aflatoxin (2 kg, ~50 ppb) was coarsely ground in a Bauer mill and finely ground in a Micro mill, and then mixed in a twin shell blender for 20 min. Corn was coarsely ground in a Bauer mill and then finely ground in a Micro mill. One kilogram each of aflatoxin-contaminated (~100 ppb) and noncontaminated ground corn were mixed in a twin shell blender for 20 min. Cottonseed was dehulled in a Bauer mill and then ground in a Dickens mill. Forty-five grams of highly contaminated (>500 ppb) and 4.5 kg of noncontaminated ground cottonseed were mixed in a twin shell blender for 20 min. Cottonseed meal, a commercially prepared product that contained aflatoxin (~80 ppb), was mixed in a twin shell blender for 20 min. Peanuts contaminated with aflatoxin (1 kg, ~25 ppb + 1 kg, ~15 ppb) were sliced, ground in a Dickens mill, and then mixed with 15 g of highly contaminated ground peanut culls in a twin shell blender for 20 min. Peanut butter, noncontaminated (1.5 kg), was hand-stirred with 40 g of highly contaminated peanut culls that had been ground into a paste. Stirring was continued until the mixture appeared uniform. Peanut meal, prepared commercially and containing aflatoxin (1.7 kg, 25 ppb), was mixed with 300 g of more highly contaminated meal (185 ppb) in a twin shell blender for 20 min.

A three-speed, 1-gal Waring Blendor was used to prepare the slurries. Each product and tap water, in the ratio shown in Table I, were slurried at low speed (1550 rpm) for 2 min. The slurry was poured into a low-form pan, and a small beaker was used to scoop out portions from different areas of the pan. A total of 100 g (±0.5 g) was removed and transferred to a 0.5-quart blender cup. Acetone (200 ml) was added and the mixture blended for 3 min at high speed and then filtered through folded filter paper (Watman 2V, 24 cm).

No emulsions were formed by the blending; filtration was rapid, and the filtrates were clear. The acetone extracts were purified and developed on minicolumns according to AOAC Method 26.044 (1975). The following

Table I. Ratio of Sample Weight to Water Volume for Slurry Formation

Product	Sample wt, g	Water vol, ml	Ratio (wt:vol)
Copra	500	2500	1:5
Corn	1000	1500	1:1.5
Cottonseed	1000	2250	1:2.25
Cottonseed meal	500	2000	1:4
Peanuts	1000	2250	1:2.25
Peanut butter	1000	1500	1:1.5
Peanut meal	500	2000	1:4

Table II. Weights and Volumes of Samples and Reagents Used in Aflatoxin Analysis of Water Slurries

| | | | Acetone ext | | | |
Product	Slurry wt, g	Sample wt, g	Total, ml	Aliqt, ml	Gel filt, ml	Residue soln, ml
Copra	100	16	283	100	150	1.8
Corn	100	40	260	90	145	4.0
Cottonseed	100	30.8	270	100	175	4.0
Cottonseed meal	100	20	280	100	175	2.5
Peanuts	100	30.8	270	100	175	4.0
Peanut butter	100	40	260	90	145	4.0
Peanut meal	100	20	280	100	160	2.3

is an outline of the overall procedure: (1) grinding of sample; (2) formation of water slurry; (3) extraction of water slurry with acetone; (4) purification of acetone extract with ferric gel; (5) extraction of aflatoxin from gel filtrate with chloroform; (6) evaporation of chloroform; (7) dissolution of the residue and dilution to volume with chloroform–methanol; (8) development of residue solution on microcolumn containing a layer of Florisil; (9) measurement of fluorescent intensity of aflatoxin on Florisil layer.

Table II lists the weights and volumes of samples and reagents used in the analysis of the various products. The water phase of the slurry was partitioned into the acetone during extraction so that the total extraction volume was 200 ml of acetone plus the water in 100 g of slurry. The volume of the gel solution (acetone extract + ferric gel solution) was adjusted to 200 ml for all samples. Also, concentrations of the residue solutions were adjusted to 2.5 g of dry sample/ml.

The residue solution (1 ml) was added to a minicolumn (20 cm Pyrex glass tubing, 3 mm i.d.) packed with 5–7-mm layers of sand, Florisil, and sand, and with 10–14-mm layers of silica gel and neutral alumina. The column was rinsed with 1 ml of CHCl₃–MeOH (96 + 4). Upon draining and while still wet, the column was placed in a fluorometer and the intensity of the Florisil layer was measured (Velasco, 1975).

Concurrent with slurry analysis was the analysis of the dry products by the standard method. In the standard procedure 50 g of comminuted subsample was extracted with 250 ml of acetone–water (85 + 15). (Although in the sampling studies, aflatoxin was determined on 250 g of peanuts and 100 g of cottonseed, present AOAC (1975) and AOCS (1975) official methods call for analytical samples of 100 and 50 g, respectively.)

RESULTS AND DISCUSSION

The homogenization of products and water in the ratios used (Table I) produced slurries that remained in suspension with minimum separation between the liquid and the solid phases. In general, the amount of water needed to form a near homogenous slurry varied with the texture and the moisture level of the product. We found that copra required the largest volume of water; the weight to volume ratio was 1:5. The next products, in order of weight-to-water requirement, were the defatted, finely ground commercial meals, 1:4, followed by ground whole seeds, 1:2.5; and finally, peanut butter, 1:1.5.

Thus, kilogram quantities of ground whole seeds and of peanut butter can be conveniently slurried in 1-gal blenders, which are commercially available. Although sample size of meals, which require a larger volume of water, must be scaled down to about 500–700 g, kilogram quantities can be conveniently prepared by combining the slurries from two 500-g samples. The quantity of water slurry that can be prepared is limited only by the capacity of the blending or homogenizing equipment available, and can be larger or smaller than the amounts we prepared.

In the extraction of aflatoxin from water slurries, the optimum ratio of acetone volume (milliliters) to slurry weight (gram) was found to be about 2 to 1. The extraction of aflatoxin was incomplete when the ratio was 1 to 1 and not improved when the ratio was 3 to 1 (data not shown).

A comparison of aflatoxin extraction from water-slurried vs. dry products is shown in Table III. The aflatoxin results were computed from five replicates of each of the products and include range, mean, standard deviation, and percent coefficient of variation. The overall average of the means shows that aflatoxin was extracted from water slurries as efficiently as from dry products. The values for standard deviation for slurries were 1.6 as compared to 5.5 for dry products, and values for percent coefficient of variation were 4.1 and 10.3 for slurries and dry products, respectively. Precision was increased about threefold with slurry analysis even though the actual weight of product analyzed was smaller.

The products that were analyzed with the poorest precision were cottonseed and peanuts. The poor precision could have been related to their coarser particle sizes, which in turn appear to be related to high oil content. Seeds of high oil content cannot be ground as finely as seeds of low oil content. Samples of very fine particle size are desirable for analysis because uniformity of subsamples and efficiency of extraction increase with fineness of particles. In the preparation of slurries, seeds of high oil content were ground to finer particles without difficulty; thus, the analyses of these products were improved.

The uniformity of a slurry (1 kg of peanuts + 2.3 l. of water) was determined by analyzing an entire batch over a 4-day period. Peanuts were selected because they showed the widest variation of aflatoxin concentration on analysis. The sample selected was contaminated with about 15 ppb of aflatoxin, a level that is of commercial interest. The results of this test are shown in Table IV. The daily

Table III. Extraction of Aflatoxin from Slurried vs. Dry Products

| | Relative intensity[a] | | | | | | | |
| | Range | | Mean[b] | | Std dev | | Coeff. of variation, % | |
Product	Slurry	Dry	Slurry	Dry	Slurry	Dry	Slurry	Dry
Corn	48–50	49–54	49.8	49.6	1.3	3.8	2.6	7.6
Cottonseed	61–79	57–84	66.4	65.2	3.0	9.6	4.5	14.8
Cottonseed meal	74–81	66–76	75.3	71.9	3.4	4.1	4.5	5.7
Peanuts	44–52	30–53	48.0	40.9	2.5	8.5	5.2	20.8
Peanut butter	49–54	49–56	51.6	51.9	1.5	2.8	2.8	5.4
Peanut meal	61–69	46–63	63.6	52.6	2.8	5.5	4.4	10.5
Copra	48–53	48–58	49.8	53.4	2.2	4.0	4.4	7.5

[a] Relative intensity unit equivalent to 1 ppb of B₁ as measured with an Aminco fluorometer. [b] Determined from five replicates.

Table IV. Uniformity of Aflatoxin Concentration in a Batch of Peanut Slurry (1 kg of Peanuts + 2.3 l. of H_2O)

Rep-li-cate[a]	Rel intensity[b] at day of analysis				
	1	2	3	4	
1	13.0	12.5	13.5	12.5	
2	15.0	12.5	11.0	14.0	
3	16.0	15.0	12.0	12.5	
4	13.0	14.0	14.0	13.0	
5	14.0	13.0	12.5	13.0	
6	13.0	12.5	11.5	14.0	
7	14.0	12.5	13.5	14.5	
8	12.0	12.5	13.0	12.5	
					Over-all av
Mean	13.8	13.1	12.6	13.1	13.2
Std dev	1.28	0.94	1.06	0.67	1.03
Coeff. of variation, %	9.3	7.2	8.4	5.1	7.8

[a] One hundred gram aliquots of slurry. [b] Relative intensity unit equivalent to 1 ppb of B_1 as measured with a Neotec fluorometer.

Table V. Water Content of Acetone Extracts[a]

Slurry product	Found, %	Expect-ed, %	Found/expected
Copra	30.9	29.4	1.05
Corn	27.2	23.1	1.18
Cottonseed	29.0	25.9	1.12
Cottonseed meal	30.7	28.6	1.07
Peanuts	29.7	25.9	1.15
Peanut meal	31.6	28.6	1.10
Peanut butter	26.4	23.1	1.14

[a] Water was determined by distillation and density measurements of distillates.

standard deviation of eight replicates ranged from 0.67 to 1.28, with an overall average of 0.99, and the percent coefficient of variation ranged from 5.1 to 9.3 with an average of 7.8.

In the extraction of slurries with acetone, we determined the amount of water partitioned into the acetone to ascertain the total volume of extract solution. The determination was necessary since we did not know to what degree the water phase might have become saturated during the slurry formation and, hence, what its solubility was in the acetone. The water content in each extract (Table V), as determined by distillation and density measurements, exceeded the calculated amount present in the 100-g slurry aliquots. Apparently, the moisture level of the samples is reflected in the water content figures. For this reason we assumed that the entire water phase was partitioned into the acetone and that the total volume of extracting solution was the volume of acetone plus the volume of water in the slurry aliquot.

In another test, we determined the amount of aflatoxin extracted by the water phase during slurry formation. Centrifugation of the slurry and analysis of the supernatant water layer showed its aflatoxin content to be about 5%. A peanut meal sample contaminated with about 50 ppb of aflatoxin was used in this test.

Because of the more uniform distribution of aflatoxin in slurries than in the dry products, and also because water slurries present no undue problem in mailing, their use in

Table VI. Storage of Treated Water Slurries Containing Aflatoxin

Slurry[a] aliquot	Treatment	Rel intensity[b] after storage (weeks)				
		0	1	2	4	8
Peanut meal	Acetone, 10% (v/v)	37	30	28	25	24
	Sterilization[c]	Unsatisfactory				
	NaCl, 16.7% (w/v)	38	42	35	39	36

[a] Slurry ratio 1:4 (sample:water). [b] Relative intensity unit equivalent to 1 ppb of B_1 as measured with an Aminco fluorometer. [c] Autoclave temperature 121°C for 15 min at 15 psi.

interlaboratory studies should have some appeal. A drawback to water slurry samples, however, is the biological decomposition that can take place if the slurry is not held at low temperature. Three methods were evaluated as a means of retarding decomposition: (1) addition of organic solvent; (2) sterilization by means of autoclaving; and (3) addition of salt.

Table VI shows the results of this experiment. The addition of 10% acetone retarded decomposition; however, aflatoxin levels decreased by about 30%. The decrease signified the probable decomposition of aflatoxin. Sterilization by autoclaving proved unsatisfactory due to formation of a cooked mass which decreased the homogeneity of the slurry. Only the addition of salt, 25% to the water phase, satisfied both the biological and chemical stability of the slurry system. The level of aflatoxin in the slurry remained within the experimental error of the procedure during the 8 weeks of storage. Ambient temperatures reached 33°C during the months of storage, which were June and July. An attractive feature of using a salt solution is that it increases the density of the liquid phase which in turn improves the suspension of particles in the slurry.

The simplicity and economical aspects of water slurries should enable larger size samples to be analyzed for aflatoxin. This will help reduce the risk of incorrectly assessing the contamination level of sample lots in commercial markets. In addition, further improvement in slurry analysis, from that presented here, can be achieved by using mills designed specifically for slurry formation. Such mills should produce a finer size particle which in turn should improve particle distribution and particle extraction efficiency.

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