A Water Slurry Method of Extracting Aflatoxin from Peanuts[1]

T.B. WHITAKER and J.W. DICKENS, USDA, SEA, AR, North Carolina State University,
and R.J. MONROE, Department of Statistics, North Carolina State University,
Raleigh, NC 27650

ABSTRACT

A water slurry method in which 1100 g of comminuted peanuts was blended with 1500 ml of tap water for 3 min in a blender and the aflatoxin in a 130-g portion of the water slurry was extracted by solvent according to methods similar to those used in Method II of AOAC was compared to the method presently used by the Food Safety and Quality Service, USDA. The proposed water slurry method requires only 180 and 60 ml per sample, respectively, of methanol and hexane compared to the 1650 and 1000 ml, respectively, required by the FSQS method. Blending comminuted peanuts with water reduced the average particle size and distributed the contaminated particles throughout the slurry. Ninety-four percent of the blended particles passed a sieve with 149-μ openings compared to only 66% of the unblended product. Variance among analyses with the FSQS method did not differ significantly from the variance among analyses with the slurry method. However, analyses with the slurry method averaged 16% more aflatoxin than with the FSQS method.

INTRODUCTION

The aflatoxin assay procedure required by the Peanut Administrative Committee for all commercial lots of shelled peanuts produced in the United States is a modification of AOAC Method II by the Food Safety and Quality Service (FSQS) of the U.S. Department of Agriculture (1-3). The FSQS procedure requires solvent-extraction of a 1100-g subsample of comminuted peanut kernels with 1650 ml of methanol, 1350 ml of water, 1000 ml of hexane and 22 g of sodium chloride. In addition to being costly, the solvents are an important energy resource and the used solvents are difficult to dispose of without environmental pollution.

The subsampling error in aflatoxin tests on peanuts is inversely proportional to the weight of the subsample of comminuted peanuts from which the aflatoxin is extracted (4). The ratio of solvents to comminuted peanuts is fixed by requirements for complete aflatoxin extraction and for subsequent analytical procedures. The amount of comminuted peanuts extracted can be reduced without increasing subsampling error by reducing the particle size. Particle size cannot be further reduced with the subsampling mill used presently (5).

A subsample of well-blended peanut paste made from a sample of comminuted peanuts may be used for extraction, but the cost of equipment and the time required for preparing the paste and cleaning the equipment presents problems. Extraction of a subsample of a slurry made by blending a sample of comminuted peanuts in water is a feasible alternative. Blenders presently available in aflatoxin laboratories may be used, cleanup is easy and the blending process reduces the particle size. Use of water slurries in aflatoxin tests has been studied previously (6).

The proposed slurry method consists of extracting aflatoxin with solvent from a 130-g sample of a slurrry formed by blending 1100 g of comminuted peanut kernels, 1500 ml of water and 22 g of sodium chloride in a Waring Blender. In the FSQS method used presently, aflatoxin is extracted from the entire 1100-g subsample. The aflatoxin is probably uniformly distributed throughout the 3000 ml of methanol/water solution; there probably is no sampling error when only 50 ml of the solution is assayed for aflatoxin. However, in the proposed slurry method, aflatoxin is only partially extracted from the peanuts by the water (6) and on the average only 54.5 g of peanut material is in each 130-g sample of slurry. Thus replicated 130-g samples from the same slurry may contain different quantities of aflatoxin. The magnitude of variation in aflatoxin content of the 130-g slurry samples is affected by the size of peanut particles in the slurry (4) and by the differences in total particle weight among equal-weight samples of the slurry.

In this study we measured the size of peanut particles in the slurry and the variation in total particle weight among replicated 130-g samples of the slurry, optimized a procedure for extracting aflatoxin from the slurry, and compared the variance among analyses obtained by the slurry method and by the FSQS method used presently.

PROCEDURE

The proposed slurry method and the FSQS method are outlined in Table I. In step 1 of the slurry method, the ratio of peanut material to water is such that the slurry is fluid enough to blend properly but thick enough to prevent the larger particles in the slurry from settling out of suspension when the slurry is left standing for 5 min. One of the experiments described later determined that the 180 ml of methanol used in step 2 adequately extracts the aflatoxin from 130 g of slurry. The addition of 25 ml of a 10% solution of sodium chloride in step 4 reduces the cloudiness of the chloroform in step 5.

Particle Size Distribution

A slurry was prepared by the procedure described in Table I. All of the 2622 g of slurry was then washed through U.S. Standard Sieves (#8, #16, #30, #50 and #100) with a light spray of tap water. The dry weight of material retained on each screen was determined after the material was dried in a forced draft oven at 100 C for 24 hr. For determination of the size distribution before blending into a slurry, a 1100-g sample of comminuted peanuts was also washed through the sieves and treated in a similar manner.

Variability in Weight of Dried Slurry Samples

A slurry was prepared according to the procedure described in Table I. After blending, the slurry was allowed to stand for 5 min in the blender cup. Then samples of slurry were obtained by pouring 130 g of slurry into each of 19 6-inch aluminum pie pans numbered 1 through 19 in the order

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TABLE I

Comparison of the Slurry Method and the FSQS Method

SLURRY METHOD

1. Blend 1100 g of peanuts comminuted in a subsampling mill (5) with 1500 ml of tap water and 22 g of sodium chloride for 3 min at medium speed in a 1-gal blender cup.
2. Blend 130-g of slurry from step 1 with 180 ml of methanol and 60 ml of hexane for 30 sec at high speed in a 1-qt blender.
3. Centrifuge material from step 2 in a 500-ml centrifuge bottle at a rotational centrifugal force of 2500 G for 20 min.
4. Transfer 50 ml of the methanol/water/aflatoxin solution to a 125-ml separatory funnel, add 25 ml of 10% sodium chloride solution, and blend the contents by vigorously shaking the stoppered funnel for 30 sec.
5. Add 50 ml of chloroform to the separatory funnel, shake the stoppered funnel for 30-60 sec, let the fractions separate, and drain the chloroform into a beaker.
6. Complete the assay according to AOAC Method II (2).

FSQS METHOD

1. Blend 1100 g of peanuts comminuted in a subsampling mill (5) with 1650 ml of methanol, 1350 ml of water, 1000 ml of hexane, and 22 g of sodium chloride for 2 min at high speed in a 1-gal blender cup.
2. Centrifuge ca. 500 ml of material from step 1 in a 500-ml centrifuge bottle at a rotational centrifugal force of 2500 G for 20 min.
3. Transfer 50 ml of the methanol/water/aflatoxin solution to a 125-ml separatory funnel and add 50 ml of chloroform.
4. Stopper and shake the separatory funnel for 30-60 sec, let the fraction separate and drain the chloroform into a beaker.
5. Complete the assay according to AOAC Method II (2).

that the samples were poured from the blender. The weights of the samples (comminuted peanuts + salt) were determined after they were oven-dried at ca. 54 C for 72 hr and then at ca. 100 C for 24 hr. This experiment was repeated 3 times. The moisture content of the comminuted peanuts used in this study was determined to be 6.0% wet basis by drying 1200 g in the same manner as the slurry samples. The oven-dried material from each sample of slurry was assumed to consist of peanuts and 1.09 g of sodium chloride. The oven-dry weight of peanuts in each slurry sample was adjusted to reflect the original 6.0% wet-basis moisture content of the comminuted peanuts used to make the slurry.

Optimization of Aflatoxin Extraction

In this experiment, we determined the effect of methanol concentration on aflatoxin extraction from samples of slurry prepared by the procedure already described. Four 130-g samples of the slurry were blended with 90, 135, 180, or 225 ml of methanol and with 60 ml of hexane in a 1-quart blender for 0.5 min. A 50-ml portion of the methanol/water extract was assayed for aflatoxin according to the slurry procedure already described. Quantification of aflatoxin was made for all 4 samples on the same TLC plate. This procedure was repeated until 10 samples of slurry were assayed using each quantity of methanol.

Comparison of the Variance among Assays Made with the FSQS Method and with the Slurry Method

This experiment compared the variability of FSQS aflatoxin assays with the variability of assays made with the slurry method. In each case the variance reflects the expected error when a 1100-g sample of comminuted peanuts is analyzed by the respective analytical method. Two estimates of the variability associated with each method were made in this study. In test 1, ca. 44 kg of aflatoxin-contaminated peanuts were comminuted in a subsampling mill. The product was riffle-divided into 40 1100-g samples which were analyzed in pairs: one by the FSQS method and one by the slurry method. The extract from each sample in the pair was spotted 6 times onto the same TLC plate. From the 120 estimates of total aflatoxin per method (6 replications x 20 samples), the variance associated with each method was computed by the analysis of variance.

For test 2, ca. 71 kg of comminuted material was riffle-divided into 64 1100-g samples. Thirty-two pairs of samples were assayed in the manner described for test 1 except that the extract from each sample was spotted 3 times onto the same plate. From the 96 estimates of total aflatoxin per method (3 replications x 32 samples), the variance associated with each method was computed by the analysis of variance.

Comparison of Averaged Assays by the FSQS Method and the Slurry Method

For comparison of the averaged assays by the 2 methods to be meaningful, the average aflatoxin concentration in the samples assayed by each method must be the same. To insure that the averaged aflatoxin concentrations of the samples assayed by each method were the same, large quantities of well-blended and riffle-divided comminuted peanuts were used in each of 4 separate tests. Tests 1 and 2, described in the previous section, along with 2 additional tests (3 and 4) were made to compare the averaged aflatoxin assays of the 2 methods.

In test 3, 64 1100-g samples were prepared in the same way as in test 2. Each of 32 samples were blended in methanol/water/hexane according to the FSQS procedure. Three 250-ml portions of the blended material from each sample were centrifuged and 50-ml quantities of methanol/water from each of all 96 portions were combined. Each of the remaining 32 samples was made into a slurry. Three 130-g portions of slurry from each sample were extracted and centrifuged according to the slurry procedure, and 50 ml of methanol/water from each of all 96 portions were combined.

Twenty 50-ml portions of the methanol/water from the FSQS treatment and twenty 50-ml portions of the methanol/water from the slurry treatment were then assayed for aflatoxin according to the respective procedure. Two 50-ml portions (one from each treatment) were assayed at the same time, and 3 spots of extract from each of the 2 50-ml portions were placed onto the same TLC plates. Totals of 60 estimates of the aflatoxin concentration (20 TLC plates x 3 estimates/plate) in the methanol/water extract from each procedure were thus obtained.

Test 4 was conducted on peanut paste made from aflatoxin-contaminated peanuts with a laboratory peanut-butter mill. The thoroughly blended paste was divided into 8 1100-g samples, and 4 of the samples were each made into a slurry. Ten 130-g portions of slurry from each sample were extracted and centrifuged according to the slurry procedure. Then 50 ml of methanol/water from each of all 40 portions were combined. The remaining 4 samples were carried through step 1 of the FSQS method. Ten 250-ml portions of the blend from each sample were centrifuged according to the FSQS procedure. Then 50 ml of methanol/water from each of all 40 protions were combined.

Twenty-six 50-ml portions of methanol/water from the FSQS treatment and 26 50-ml portions from the slurry treatment were then assayed for aflatoxin according to their respective procedures. Two 50-ml portions (one from each treatment) were assayed at the same time, and 3 spots of extract from each of the 2 50-ml portions were placed onto the same TLC plate. A total of 104 estimates of the aflatoxin concentration (26 TLC plates x 4 estimates/plate) in the methanol/water extract from each procedure was thus obtained.

RESULTS

Proposed Slurry Method

A 1-quart blender is the only additional piece of equipment needed to change from the FSQS procedure to the slurry procedure, because most aflatoxin laboratories have balances suitable for weighing 130- g samples of slurry. The only additional time needed for the slurry procedure is the time required for step 2. The 130 g of slurry contains 54.5 g of peanuts. Only 10.7 g of peanuts is represented in the 50 ml of chloroform extract compared to 18.3 g for the FSQS method. If both methods have the same lower limit for detection of a quantity of extracted aflatoxin, the lowest detectable concentration in peanuts analyzed by the slurry method would be 1.7, i.e., 18.3/10.7 times higher than the lowest detectable concentration in peanuts analyzed by the FSQS method.

Particle Size Distribution

Considerable comminution occurs during preparation of the slurry (Table II). Ninety-four percent of the peanut particles passed through a #100 sieve after blending whereas only 66% passed before blending. As previously mentioned, subsampling error is reduced by comminuting more finely. Comminution is an important aspect of the slurry method because it helps make the 54.5 g of peanut material in the 130-g sample of slurry more representative of the 1100-g sample used to make the slurry.

TABLE II

Percentages of Peanut Material that Passed the Designated Sieves before Blending into a Slurry and after Blending

U.S. standard sieve number	Sieve opening (microns)	Percent material passing sieve before and after blending	
		Before	After
8	2380	100	100
16	1190	98	100
30	590	86	100
50	297	70	97
100	149	66	94

TABLE III

Distribution of the Weights (in g) of Peanut Material among 130-g Samples of Water Slurry

Sample number	Replication			
	1	2	3	Avg
1	54.80	55.20	54.32	54.77
2	54.80	54.30	54.43	54.51
3	54.80	54.46	54.32	54.53
4	55.66	54.34	54.53	54.84
5	54.98	54.46	54.53	54.66
6	54.47	54.46	54.70	54.54
7	53.36	54.46	54.53	54.12
8	53.84	54.40	54.53	54.26
9	55.06	54.40	54.43	54.63
10	54.26	54.51	54.32	54.36
11	54.16	53.63	54.43	54.07
12	54.16	54.35	54.16	54.22
13	54.32	55.10	54.70	54.71
14	54.16	54.46	54.32	54.31
15	53.99	54.34	54.59	54.31
16	55.23	54.24	54.64	54.70
17	54.37	54.35	54.64	54.45
18	54.37	55.10	54.75	54.74
19	54.70	54.89	54.64	54.74
Avg	54.50	54.50	54.50	54.50
SD	0.54	0.36	0.16	0.23
CV(%)	0.99	0.66	0.29	0.43

Variability of Peanut Weight in Slurry Samples

Data from the test on the weight of peanuts in 19 samples of slurry (Table III) indicate that the weights are in good agreement among the samples of slurry. This agreement is important because the calculations of aflatoxin concentrations for the slurry method are based on 54.5 g of peanuts in the sample of slurry. Although there apparently is a slight correlation between sample number and the weight of peanuts in the sample, the differences in the weight of samples poured from the top of the container (low sample numbers) and the weight of samples poured from the bottom of the container (high sample numbers) do not indicate a significant amount of settling during the 5 min the slurry was left standing.

Optimization of Aflatoxin Extraction

The average concentration (ppb) of aflatoxin determined for samples of peanuts when different amounts of methanol were used in step 2 of the slurry method are shown in Table IV. At the 95% level of confidence, significantly less aflatoxin was extracted with 90 ml than with 135, 180 or 225 ml of methanol. Statistically significant differences were not found among the amounts of aflatoxin extracted by the 3 larger quantities of methanol. Based on this test, 180 ml, rather than 135 ml, of methanol was designated in step 2 of the slurry method to give some margin of safety. The effect of blending time in step 2 was also studied. Because this experiment showed no difference in assays between 0.5 and 3.0 min of blending time, the 0.5 min blending time was specified by the authors.

Comparison of the Variance among Assays with the FSQS Method and with the Slurry Method

The results of an analysis of variance on the 120 assays by

TABLE IV

Averages of 10 Determinations of Aflatoxin Concentration in Samples of Peanuts when Different Quantities of Methanol Were Used in Step 2 of the Slurry Method

	Quantity of methanol (ml)			
	90	135	180	225
Methanol:water ratio	55:45	65:35	71:29	75:25
Aflatoxin concentration (ppb)	28.2[a]	43.7[b]	42.0[b]	49.5[b]

$LSD_{.05}$ = 10.97 ppb.

[a,b]Concentrations with no letter in common are significantly different at 5% level.

TABLE V

Results of Analysis of Variance and Related Statistical Data for Tests 1 and 2

Source	Test 1		Test 2	
	FSQS	Slurry	FSQS	Slurry
Variance	1763	2240	182	252
Degrees of freedom	107	107	94	94
Standard deviation (PPB)	40	46	12	15
Mean assay (PPB)	159	179	58	66
Coefficient of variation	26	26	23	24

Null Hypothesis: Slurry variance = FSQS variance.
F test for test 1: F = 2240/1763 = 1.27; P = 0.110.
F test for test 2: F = 252/182 = 1.38; P = 0.060.

TABLE VI

Comparison of Assays with the FSQS Method and with the Slurry Method

Test number	Assay method	Aflatoxin (ppb)				
		B1	B2	G1	G2	Total
1	FSQS	72.6	16.1	56.6	13.3	158.6
	Slurry	87.7	17.5	57.9	15.5	178.6
	% Diff[a]	20.8	8.7	2.3	16.5	12.6
2	FSQS	36.1	5.8	12.8	3.0	57.7
	Slurry	43.7	6.2	13.4	3.2	66.5
	% Diff	21.1	6.9	4.7	6.7	15.3
3	FSQS	40.3	6.3	16.0	4.9	67.5
	Slurry	44.4	6.8	18.1	6.4	75.3
	% Diff	10.2	7.9	13.1	30.6	11.6
4	FSQS	17.9	3.8	10.3	2.7	34.7
	Slurry	21.2	4.6	12.7	4.3	42.8
	% Diff	18.4	21.1	23.3	59.3	23.3
Avg	FSQS	41.7	8.0	23.9	6.0	79.6
	Slurry	49.3	8.8	25.5	7.4	90.8
	% Diff	17.6	11.2	10.9	28.3	15.7

[a]% Diff = 100 (slurry-FSQS)/FSQS.

the slurry method and by the FSQS method in test 1 and on the 96 assays by each method in test 2 are given in Table V. The null hypotheses, that the variance among the slurry assays and the variance among the FSQS assays are equal, cannot be rejected by the F test for test 1 (F = 1.27) or for test 2 (F = 1.38). Failure of the experiment to show a statistically significant difference between variances for the 2 methods indicates that the error introduced by using the 130-g sample of slurry (54.5 g of peanuts) is negligible.

Comparison of Averaged Assays by the FSQS Method and the Slurry Method

For all tests the average aflatoxin values were higher for the slurry method than for the FSQS method (Table VI). The analysis of variance showed that all of these differences were significant at the 95% confidence level. Since a large number of 1100-g samples that were riffle-divided from comminuted peanuts were used in the tests, the true average aflatoxin concentration in the samples assayed by the 2 methods probably was about the same.

According to previous variance studies (4) the average aflatoxin concentration in the samples analyzed by the FSQS and slurry methods were predicted not to differ by more than 10 ppb in test 1, 4.8 ppb in test 2 and 5.6 ppb in test 3, 95% of the time. The difference in the average aflatoxin concentrations of the 4 1100-g samples of peanut paste used for each method in test 4 probably was also very small because of the homogeneity of the paste. In addition, there is only a 6% chance that the aflatoxin concentration in the samples chosen for assay by the slurry method in all 4 tests would be higher than in the samples chosen for assay by the FSQS method. Further studies are needed to determine the cause of the differences in the averages of the assays by the 2 methods.

REFERENCES

1. Dickens, J.W., JAOCS 54:225A (1977).
2. AOAC: Natural Poisons, Chapter 26 from Official Methods of Analysis. 12th Edition, Association of Official Analytical Chemists, Washington, DC, 1975, p. 7,16.020
3. United States Department of Agriculture Inspector's Instructions for Aflatoxin, USDA Food Safety and Quality Service, Washington, DC 1975.
4. Whitaker, T.B., J.W. Dickens and R.J. Monroe, JAOCS 51: 214 (1974).
5. Dickens, J.W., and J.B. Satterwhite, Food Technol. 23:950 (1969).
6. Velasco, J., and S.L. Morris. J. Agric. Food Chem. 24:86 (1976).

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