A Water Slurry Method of Extracting Aflatoxin from Peanuts

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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 slurry 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 presently used, 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 presently.

PROCEDURE

The proposed slurry method and the FSQS method are outlined in Table 1. 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 1. 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 1. 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
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 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 portions 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.