A Method for Determining Kernel Moisture Content and Aflatoxin Concentrations in Peanuts

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ABSTRACT: A method was developed to determine kernel moisture content (KMC) and aflatoxin concentration in discrete peanut samples. Shelled peanuts were weighed to the nearest 0.01 g, and a water slurry was made by blending the peanuts for 2 min with 2.2 mL of water per g of peanuts. The slurry (10 g) was withdrawn and dried at 130°C for 3 h to determine KMC. Methanol was added to the remaining slurry and blended for an additional 1 min, and aflatoxins were quantitated with high-performance liquid chromatography. Comparison of the slurry method with an official peanut moisture method showed good agreement between the two over a range of moisture levels. Recovery of aflatoxin B_1 from spiked samples averaged 97% with an average coefficient of variation of 3.6%. The method enables determination of both KMC and aflatoxin content in peanut samples without degradation of aflatoxin that would occur when using the official moisture method.
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KEY WORDS: Aflatoxin, *Arachis hypogeae, Aspergillus flavus,* groundnuts, liquid chromatography, moisture, mycotoxin, peanuts, water slurry.

Aflatoxins are hepatotoxic, carcinogenic mycotoxins produced by the fungi *Aspergillus flavus* Link and *A. parasiticus* Speare (1). Aflatoxin contamination of food and feed is a serious concern, and great expense is incurred both in monitoring for aflatoxin contamination and in research efforts to manage and/or eliminate it. Several oilseed crops are susceptible to aflatoxin contamination, and these include peanuts, corn, cotton, and tree nuts (1).

The susceptibility of peanuts (*Arachis hypogeae*) to aflatoxin contamination is related to kernel moisture content (KMC) and temperature, whether in the soil while the crop is developing and maturing (preharvest) or in storage (postharvest) (2). If peanuts are properly dried before storage, they must regain moisture during storage for the fungi to grow and produce toxin. This can occur either in poorly ventilated warehouses when condensation forms and drips onto the peanuts or in warehouses with leaky roofs. Thus, prevention of postharvest aflatoxin contamination is a matter of ensuring proper drying of harvested peanuts and maintaining them at a KMC < 8% during the storage period.

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Preharvest aflatoxin contamination of peanuts is associated with drought stress that occurs late in the growing season while the crop is maturing (3–6). In the absence of drought stress or severe insect damage, peanuts maintain a high KMC until the time of harvest and are protected from fungal growth and aflatoxin contamination by natural defense mechanisms, of which phytoalexin production appears to be a part (7–9). However, during late-season drought stress, the KMC of peanuts decreases and the capacity for phytoalexin production is lost. It is under this scenario that preharvest aflatoxin contamination of peanuts occurs (9).

Because preharvest aflatoxin contamination of peanuts and KMC are closely related, research studies aimed at reducing or eliminating aflatoxin contamination may be able to exploit this relationship by identifying germplasm with the capacity to maintain a relatively high KMC during periods of severe drought (10). Central to such studies is the ability to determine the preharvest KMC of peanuts as well as their aflatoxin content. This cannot be done on a particular sample of peanuts because accurate KMC determination involves a complete oven drying of the peanuts, and this process results in some degradation of aflatoxin when present. It could be done by using one sample for moisture and another for aflatoxin; however, that is not desirable when dealing with small samples (such as from individual plants) because of the extremely variable nature of aflatoxin contamination. Therefore, a method was needed whereby both KMC and aflatoxin content could be accurately measured in discrete peanut samples.

The Agricultural Marketing Service of the U.S. Department of Agriculture specifies that a water-slurry method be used in the analysis of raw peanuts for aflatoxin (11,12). In that method, 1100 g of raw peanuts are blended at high speed with 1600 mL of water. One hundred ninety-six grams of that water slurry is then blended with 283 mL of 77:23 methanol–water solution to produce a final extraction mixture that contains 55:45 methanol–water with a solvent/peanut ratio of 5 mL/g. The water slurry is a relatively homogeneous mixture from which a smaller subsample can be removed for aflatoxin analysis. We now report a similar methodology to determine KMC and aflatoxin concentration in freshly harvested peanuts. This method could also be applied to the determination of KMC and aflatoxin content in any sample of peanuts.

EXPERIMENTAL PROCEDURES

Method. The method developed for the determination of KMC and aflatoxin in discrete peanut samples is outlined in Figure 1. If the KMC of green (freshly harvested) peanuts is to be determined, the sample must be weighed as soon as possible after it is shelled (initial weight). The actual sample to be used in the analysis should be weighed to the nearest 0.01 g. These peanuts can then be dried for storage until analysis can be done.

At the time of analysis, the sample must be reweighed to the nearest 0.01 g (extraction weight). The extraction weight is the basis weight for the aflatoxin determination, and it is also used in the determination of the KMC. Prepare the slurry by adding 2.2 mL of water/g of peanuts in a blender. The actual amount of water added must also be weighed to the nearest 0.01 g. This is most easily accomplished by pouring the measured volume of water into a tared blender that already contains the peanuts. The water–peanut mixture is homogenized at high speed for 2 min to produce the slurry. Immediately transfer 10 g of the slurry into a preweighed (nearest 0.01 g) aluminum weighing dish (57 mm), and weigh the slurry to the nearest 0.01 g. The dish containing the slurry can then be set aside until all samples are ready to be dried. To proceed with the aflatoxin analysis of the slurry remaining in the blender, a volume of methanol that will result in a solvent to peanut ratio of 5:1 is added to the blender. This volume is determined by the formula:

$$\text{methanol (mL)} = 5\{B - E[\text{B}/(B + C)]\} - \{C - E[C/(B + C)]\} \quad [1]$$

where B = weight of the peanuts at the time of extraction, g; C = weight of the water added to produce the slurry, g; and E = weight of the slurry transferred to the aluminum weighing dish, g.

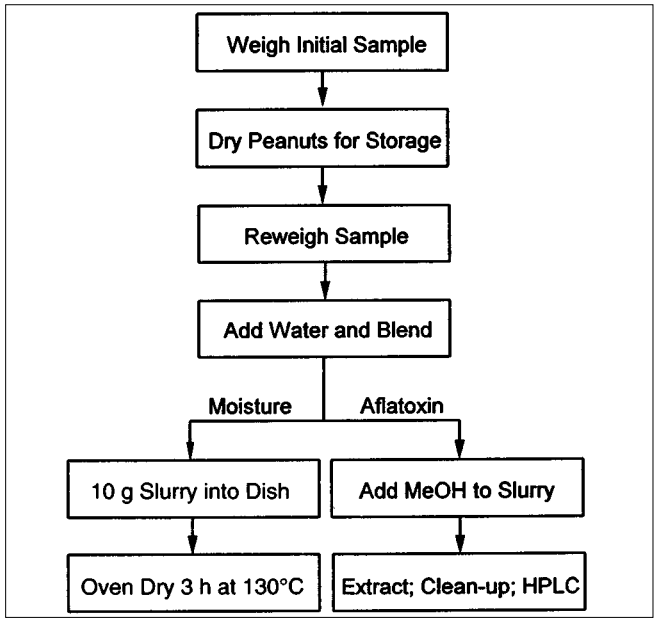

FIG. 1. Flow diagram of the method developed for the determination of kernel moisture content and aflatoxin concentrations in discrete peanut samples.

The final extraction solvent makeup will vary slightly, but it will be approximately 56:44 methanol–water. It will be precisely that makeup when the weight of water added to make the slurry is exactly 2.2 times the peanut weight and exactly 10.00 g of slurry is withdrawn for the moisture determination. More important, however, is that, by weighing the peanuts, water, and slurry and using the above formula, the solvent/peanut ratio will be consistently 5:1, which is necessary for accurate aflatoxin quantitation.

To proceed with the aflatoxin analysis, add the methanol and sodium chloride, approximating 10% of the peanut weight, to the slurry and blend for 1 min. At this point, any number of quantitative aflatoxin methods could be used. The method used in our laboratory is a modification of the method of Dorner and Cole (13). Filter the extract through Whatman 4 filter paper (Maidstone, England) into a 20 × 150 mm disposable test tube and collect 15 mL. Add 15 mL of a salt solution (150 g sodium chloride, 150 g zinc acetate, 3.75 mL acetic acid, 1 L water), shake vigorously, and filter through glass fiber filter paper. Partition 10 mL of the filtrate against 4 mL chloroform. Collect 2.0 mL of the chloroform layer, evaporate under a stream of nitrogen, and redissolve in 2.0 mL of high-performance liquid chromatography (HPLC) injection solvent (62:38 methanol–water plus 0.1% acetic acid). Inject 20 µL into an HPLC system that consists of a reverse-phase column (Waters Nova PAK C_{18}, 3.9 × 150 mm, 4 µ; Waters Chromatography, Watertown, MA), mobile phase of 70:35:1.2 water–methanol–butanol (0.8 mL/min), a photochemical reactor (PHRED with a KRC 25-25 reactor coil; Aura Industries, Staten Island, NY) for postcolumn derivatization of aflatoxins B_1 and G_1, and fluorescence detection (Shimadzu RF-551; ex: 365 mm; em: 440 mm; Kyoto, Japan). External standard quantitation is achieved by comparing peak areas of samples with those of a standard that contains 5 ng/mL of B_1 and G_1 and 1.5 ng/mL of B_2 and G_2. Each 20-µL injection of standard contains 0.1 ng of B_1 and G_1 and 0.03 ng of B_2 and G_2, which corresponds to 20 ng/g and 6 ng/g, respectively, of the toxins in the peanuts (based on extraction weight).

To proceed with the determination of KMC, place the dishes, containing approximately 10 g of slurry, in a forced-air draft oven and dry at 130 ± 3°C for 3 h. Remove the dishes and place them in a desiccator to cool to room temperature. Weigh to the nearest 0.01 g and calculate %KMC from the formula:

$$\%\text{KMC} = [\{A - [(F - D)(B + C)/E]\}/A] \times 100 \quad [2]$$

where A = initial weight of kernels, g; B = weight of kernels at time of extraction, g; C = weight of water added to make slurry, g; D = weight of weighing dish, g; E = weight of slurry transferred to weighing dish, g; F = weight of dried slurry (including weighing dish), g.

TABLE 1
Comparison of Mean Kernel Moisture Contents (KMC) and Coefficients of Variation (CV) as Determined with an Official Peanut Moisture Method (Ref. 14) and the Proposed Slurry Method[a]

		Official method		Slurry method	
	Sample	KMC (%)[b]	CV (%)	KMC (%)[b]	CV (%)
Test 1	1	39.41[a]	2.8	39.61[a]	2.4
	2	31.24[a]	2.2	31.04[a]	2.4
	3	26.95[a]	2.0	26.96[a]	1.8
	4	17.98[a]	1.7	17.88[a]	2.6
	5	3.99[a]	1.3	3.22[b]	10.2
Test 2	1	33.99[a]	2.7	33.88[a]	1.8
	2	26.63[a]	1.3	26.62[a]	1.1
	3	22.22[a]	1.5	21.65[b]	1.7
	4	19.64[a]	1.1	19.55[a]	2.1
	5	12.30[a]	1.1	11.93[b]	3.0

[a]Values are the means of eight determinations.
[b]Means in a row followed by the same superscript letter are not significantly different ($P \leq 0.05$).

Comparison of the proposed slurry method with an official method for moisture determination. Two experiments were conducted to compare the accuracy of the proposed moisture method with an official method (14). Approximately 14 kg of mature Florunner peanuts (Georgia Seed Development Commission, Plains, GA) were harvested and cleaned. A sample of approximately 1500 g of the green peanuts (sample 1) was taken from the lot, and the remainder was put in a mesh bag and placed in a peanut drier box to begin drying. These peanuts were then sampled at various times during the drying process to obtain five samples that covered a wide range of moisture (Table 1). Each sample was shelled and riffle-divided to produce 16 subsamples of approximately the same size. The ASAE standard (14) for moisture measurement in peanuts was used to determine the %KMC in eight of the subsamples. Briefly, the samples were oven-dried at 130 ± 3°C for 6 h and %KMC was determined as:

$$(\text{mass of kernels lost/initial mass of kernels}) \times 100 \qquad [3]$$

The %KMC in the other eight subsamples was determined by the proposed slurry method. The entire experiment was repeated on a second lot of freshly harvested peanuts.

The mean %KMC, as determined by each method for each sample, was calculated, and differences in means at $P \leq 0.05$ were determined with a *t*-test.

Evaluation of aflatoxin determination. To determine the recovery of aflatoxins with the slurry method, a sample of peanuts was ground briefly in a vertical cutter mixer. Five subsamples of the ground peanuts were spiked with aflatoxins at each of two concentration levels: 10 ng/g B_1 and G_1, 3 ng/g B_2 and G_2; and 100 ng/g B_1 and G_1, 30 ng/g B_2 and G_2. The slurry method, including the withdrawal of a portion of the slurry for moisture determination, was used to analyze all samples.

RESULTS AND DISCUSSION

Moisture. Preliminary experiments with the slurry method demonstrated that three hours in the oven was sufficient to dry the slurries to a constant weight.

Results of the two experiments to compare the slurry method with an official method for determining the %KMC of peanuts are shown in Table 1. In the first test, the only significant difference ($P \leq 0.05$) between the two methods was seen in sample 5, which was at low moisture. The variation among the replications within a sample (as shown by the CV) was similar for the two methods, except for sample 5 of the slurry method (10.2%).

In the second test, significant differences ($P \leq 0.05$) between the two methods were found for samples 3 and 5. The KMC for sample 3 as determined by the official method averaged 22.22% compared with 21.65% for the slurry method. For sample 5, the averages were 12.30% and 11.93% for the official and slurry methods, respectively. Although these differences are statistically significant, they are considered acceptable in the context of our application of this methodology, which is the determination of %KMC of peanuts from individual plants with an accuracy of ±1%. The CV for the two methods was also similar in the second test, but, as in the first test, the lowest moisture sample showed a slightly higher CV. More testing would need to be done to determine if the slurry method produces more variation consistently in samples of low moisture content (<10%). Our application of this method measures moisture and aflatoxin in "green" peanuts that typically contain >20% KMC; therefore, the

TABLE 2
Recovery of Aflatoxins from 50 g of Spiked Peanut Meal

	Aflatoxins							
Statistic	B_1		B_2		G_1		G_2	
Added (ng/g)	10.0	100.0	3.0	30.0	10.0	100.0	3.0	30.0
Recovered[a]	9.7	96.8	2.8	27.8	9.8	101.0	3.7	35.0
Std. Dev. (ng/g)	0.3	4.2	0.2	0.9	0.3	4.1	0.1	1.7
CV (%)	2.8	4.4	7.0	3.4	2.9	4.0	1.9	4.7
% Recovered	97.4	96.8	94.7	92.8	98.2	101.0	121.7	116.7

[a]Mean of five determinations. Aflatoxin was not detected in the unspiked peanut meal. See Table 1 for abbreviation.

variation associated with low-moisture samples was not a major concern.

Aflatoxin. Results of the aflatoxin recovery study are presented in Table 2. Recovery of the four toxins ranged from 92.8 to 121.7%. Importantly for this study, the CV were quite good (range 1.9–7.0%), and they compared favorably with those reported earlier (13). Withdrawing 10 g of the slurry for the moisture determination could introduce an error and produce more variability in the aflatoxin results if the aflatoxin were not homogeneously distributed throughout the slurry. However, these results indicate that good homogeneity was achieved in the slurries and that confidence in the aflatoxin analysis was not compromised.

This method has provided a way to determine %KMC and aflatoxin concentrations in discrete samples of peanuts. It can be used for samples that range in size from 10 g to several hundred grams by using an appropriately sized blender. The application of this method in our laboratory has been primarily for determining %KMC and aflatoxin content of peanuts from single plants. In determining the KMC of green peanuts, it is necessary to shell and weigh the kernels immediately after digging. The other important factor in achieving good results is that accurate weights, preferably to the nearest 0.01 g, should be taken at every step.

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