FOOD CHEMICAL CONTAMINANTS

Estimating Aflatoxin in Farmers' Stock Peanut Lots by Measuring Aflatoxin in Various Peanut-Grade Components[1]

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Five, 2 kg test samples were taken from each of 120 farmers' stock peanut lots contaminated with aflatoxin. Kernels from each 2 kg sample were divided into the following grade components: sound mature kernels plus sound splits (SMKSS), other kernels (OK), loose shelled kernels (LSK), and damaged kernels (DAM). Kernel mass, aflatoxin mass, and aflatoxin concentration were measured for each of the 2400 component samples. For 120 lots tested, average aflatoxin concentrations in SMKSS, OK, LSK, and DAM components were 235, 2543, 11 775, and 69 775 ng/g, respectively. Aflatoxins in SMKSS, OK, LSK, and DAM components represented 6.9, 7.9, 33.3, and 51.9% of the total aflatoxin mass, respectively. Cumulatively, 3 aflatoxin risk components—OK, LSK, and DAM—accounted for 93.1% of total aflatoxin, but only 18.4% percent of test sample mass. Correlation analysis suggests that the most accurate predictor of aflatoxin concentration in the lot is the cumulative aflatoxin mass in the high 3 risk components OK + LSK + DAM (correlation coefficient, r = 0.996). If the aflatoxin in the combined OK + LSK + DAM components is expressed in concentration units, r decreases to 0.939. Linear regression equations relating aflatoxin in OK + LSK + DAM to aflatoxin concentration in the lot were developed. The cumulative aflatoxin in the OK + LSK + DAM components was not an accurate predictor (r = 0.539) of aflatoxin in the SMKSS component. Statistical analyses of 3 other data sets published previously yielded similar results.

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Farmers' stock (FS) peanuts are graded by the Federal State Inspection Service (FSIS) of the Agricultural Marketing Service of the U.S. Department of Agriculture (USDA) to determine support price (1). A grade sample is removed from the farmer's lot, and the percentage by mass of the following grade components are determined: foreign material, loose shelled kernels (LSK), small or other kernels (OK), damaged kernels (DAM), and sound mature kernels plus sound splits (SMKSS). In addition, peanut kernels in the grade sample are inspected visually for the aflatoxin-producing fungus *Aspergillus flavus* by the visual *A. flavus* (VAF) method, which is used as an indication that the lot may contain aflatoxin (2). If the fungus is found, the lot is diverted from the edible market because of the risk that the lot is contaminated with aflatoxin. At present, USDA does not inspect FS peanuts for aflatoxin by directly measuring aflatoxin in a sample taken from the lot.

The U.S. peanut industry conducted 2 major studies to determine if the VAF method can be replaced by measuring aflatoxin directly in samples taken from FS lots at the point of purchase: (*1*) a time and motion or feasibility study (3) to determine if sampling, sample preparation, and analytical steps associated with an aflatoxin test would fit into the grading operation at the point of purchase and (*2*) a variability and distribution study to determine effects of sample size on the efficiency of detecting and classifying contaminated FS lots into aflatoxin categories (4–7). These 2 studies assumed that the entire peanut sample would be comminuted in a mill, aflatoxin would be extracted from a 100 g subsample of comminuted peanuts, and aflatoxin would be quantitated by immunochemical methods.

Even with information from these 2 studies, no official USDA/peanut industry aflatoxin-sampling program has been developed for FS lots to date. However, buyers (shellers) of FS peanuts have developed aflatoxin-sampling programs on an ad hoc basis. Most shellers testing FS lots for aflatoxin use a method whereby aflatoxin is measured only in the combined LSK and DAM components. FS lots are then segregated by the sheller on the basis of aflatoxins measured in the LSK and DAM components. However, the relationship between aflatoxin concentration in the combined LSK and DAM components and aflatoxin concentration in the lot is presently unknown.

Shellers measure aflatoxin in the LSK and DAM grade components because previous studies (8, 9) have demonstrated that DAM kernels contain the greatest percentage of total aflatoxin mass in the sample, followed by LSK, OK, and SMKSS. Three components—DAM, LSK, and OK—are considered to be the aflatoxin risk components in an FS lot. In addition, DAM and LSK generally constitute a small percentage, by mass, of the sample. By excluding SMKSS from aflatoxin analysis, smaller quantities of peanuts are used in sample preparation, and the volume of extraction solvent required is minimized.

Because little information is known about the relationship between aflatoxins in the risk components OK, LSK, and DAM and the aflatoxin concentration in the lot, the risk component or combination of risk components that best correlates with the aflatoxin concentration in the lot needs to be determined. If a strong correlation exists, then relationships need to be developed that predict aflatoxin concentration in the lot based on measurements of aflatoxin in one or more risk components. Once the risk component(s) is defined, the variability associated with measuring aflatoxin in that component must be determined so that the performance of aflatoxin-sampling plans can be predicted and efficient sampling plans can be designed.

In this paper, we focus on determining the correlation between aflatoxin concentration in the lot and aflatoxin in risk components and developing regression equations to predict aflatoxin concentration in the lot from measurement(s) of aflatoxin in optimum risk component(s) chosen on the basis of correlation analysis. Variability among appropriate risk component samples will be investigated in a separate study.

Experimental

FSIS inspectors identified 120 runner-type FS lots by the VAF method. An 11 kg bulk sample was removed from each FS lot during grading at the point of purchase. Foreign material was removed from each bulk sample and discarded, remaining pods and LSK (ca 10 kg) were riffle-divided into five 2 kg test samples. LSK was removed from each test sample before pods were shelled, and hulls were discarded after weighing. According to standard FSIS practices, shelled kernels were divided into SMKSS, OK, and DAM grade components and, along with LSK, placed in separate sample bags. Component kernel mass, type grade component (SMKSS, OK, LSK, and DAM), and sample and lot identification were recorded. A total of 2400 component samples (4 components per test sample × 5 test samples per lot × 120 lots) were analyzed for aflatoxin.

The aflatoxin concentration of each component sample was estimated. Each component sample was ground; an AMS mill (10) was used for larger SMKSS component samples, and a coffee grinder was used for smaller OK, LSK, and DAM component samples. A subsample (75 g or less) of comminuted peanuts was extracted. When the sample weighed less than 75 g, the entire sample was extracted with acetonitrile–water (84 + 16) at a solvent/peanut ratio of 4/1. Aflatoxin was quantitated by liquid chromatography (11, 12). All 4 aflatoxins (B1 + B2 + G1 + G2) were measured, and results were recorded in concentration units or nanogram of aflatoxin per gram of peanuts (ng/g).

The aflatoxin mass in each component was calculated by multiplying component mass (g) by aflatoxin concentration in the component (ng/g). The aflatoxin concentration in each test sample was calculated by adding the aflatoxin masses in the 4 components and dividing by the total mass of the 4 component samples (equal to mass of the test sample). The aflatoxin concentration in each test sample (SMKSS + OK + LSK + DAM) was used to estimate aflatoxin concentration in the lot.

Regression and correlation analyses were conducted on all 600 component sample aflatoxin values by using the SAS program (13).

Results

Aflatoxin concentrations in the 120 lots varied from 4 to 24 301 ng/g. Average aflatoxin concentrations in SMKSS, OK, LSK, and DAM component samples

Table 1. Component sample masses and aflatoxin concentrations component samples[a]

Component[b]	Component sample mass,[c] g	Aflatoxin concentration, ng/g peanuts
SMKSS	1274	235
OK	134	2543
LSK	122	11775
DAM	32	69775
Test sample	1562	2762

[a] $n = 600$ for each component; 5 test samples were taken from each of 120 FS lots.

[b] SMKSS = sound mature plus sound split kernels, OK = other kernels (pass a 16/64 inch slotted screen), LSK = loose shelled kernels, DAM = damaged kernels, Test sample = SMKSS + OK + LSK + DAM.

[c] Foreign material and peanut hull mass excluded.

Table 2. Correlation between aflatoxin concentration in the lot and aflatoxin in various components

Component[a]	Correlation coefficient: Aflatoxin concentration, ng/g	Correlation coefficient: Aflatoxin mass, ng
SMKSS	0.585	0.561
OK	0.413	0.473
LSK	0.775	0.859
DAM	0.868	0.939
OK + LSK	0.813	0.883
OK + DAM	0.905	0.952
LSK + DAM	0.881	0.989
OK + LSK + DAM	0.931	0.996
SMKSS + OK + LSK + DAM[b]	1.000	1.000

[a] Components defined in footnote to Table 1.

[b] Sum of all components or estimate of aflatoxin concentration in lot.

(average of 600 component samples per grade component) were 235, 2543, 11 775, and 69 775 ng/g, respectively (Table 1). Aflatoxin in SMKSS, OK, LSK, and DAM component samples represented 6.9, 7.9, 33.3, and 51.9%, respectively, of the total aflatoxin mass in the test sample.

Masses of SMKSS, OK, LSK, and DAM component samples averaged 1274, 134, 122, and 32 g, respectively. Test sample masses (without hulls) averaged 1562 g (Table 1). SMKSS, OK, LSK, and DAM component samples represented 81.6, 8.6, 7.7, and 2.1%, of the test sample mass on a kernel basis (hulls not included).

SMKSS had the highest component mass and the lowest component aflatoxin concentration, while DAM had the lowest component mass and the highest aflatoxin concentration (Table 1). Cumulatively, the 3 risk components DAM, LSK, and OK accounted for 93.1% of the aflatoxin contamination but only 18.4% of the test sample mass. Thus, a small percentage of the peanuts in an FS lot contributes to a large percentage of the aflatoxin contamination.

Correlations between aflatoxin concentration in the lot and aflatoxin in various component samples were calculated (Table 2). Correlations were higher with aflatoxin masses than with aflatoxin concentrations, as expected because aflatoxin mass takes the sample component mass into account. When samples are uniform in size, concentration gives a relative indication of the quantity of aflatoxin among the 4 component samples within a lot. However, when samples differ in size, as among the 4 component samples, aflatoxin mass is a better indicator of aflatoxin concentration in the lot.

Aflatoxin mass in OK samples gave the lowest correlation (0.473) and aflatoxin mass in DAM samples gave the highest correlation (0.939) with aflatoxin concentration in the lot. When aflatoxin in all possible combinations (excluding SMKSS) of 2 risk component samples were combined, aflatoxin in LSK + DAM samples gave the highest correlation (0.989) with aflatoxin concentration in the lot. The combined aflatoxins in the OK + LSK + DAM components had the highest correlations with lot concentration, whether expressed in mass (0.996) or concentration (0.931).

Table 2 suggests that if the peanut industry wants to exclude SMKSS peanuts from the aflatoxin test, the best predictor of aflatoxin concentration in the lot is the cumulative aflatoxin mass in the OK + LSK + DAM components. Relationships between aflatoxin in the 3 risk components and aflatoxin concentration in the lot are shown in Figures 1 and 2. Each plot contains 600 observations. The scatter among aflatoxin concentrations (Figure 2) is more than among aflatoxin masses (Figure 1). Multiplying concentrations by the cumulative mass of the 3 risk components samples

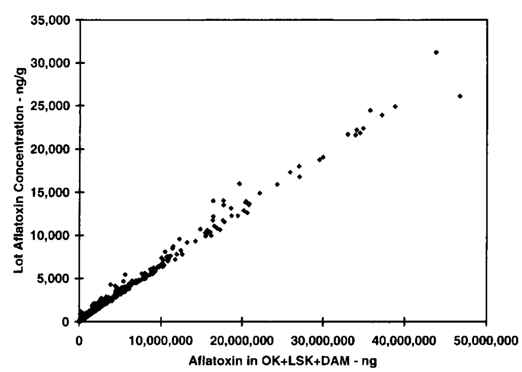

Figure 1. Aflatoxin concentration (ng/g) in FS peanut lots versus cumulative mass (ng) of aflatoxin in other kernels (OK), loose shelled kernels (LSK), and damaged kernels (DAM).

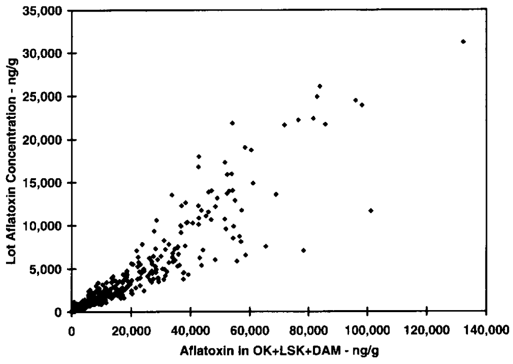

Figure 2. Aflatoxin concentration (ng/g) in FS peanut lots versus cumulative aflatoxin concentration (ng/g) in other kernels (OK), loose shelled kernels (LSK), and damaged kernels (DAM).

stabilizes the results, as indicated by Figure 1 and correlation coefficients (r), in Table 2.

Figures 1 and 2 suggest linear relationships between the parameters plotted. Regression analysis yielded the following relationships:

$$\text{Aflatoxin in lot, ng/g} = 0.000662 \times \text{aflatoxin mass in OK + LSK + DAM (ng)} \quad (1)$$

$$\text{Aflatoxin in lot, ng/g} = 0.223 \times \text{aflatoxin concentration in OK + LSK + DAM (ng/g)} \quad (2)$$

The standard errors of the estimated linear regression coefficients in equations 1 and 2 are 0.00000222 and 0.003, respectively. Regression equations 1 and 2 were forced through the intercept (0,0) under the assumption that if all 3 risk components have zero aflatoxin, SMKSS probably has zero aflatoxin, and the concentration of aflatoxin in the lot is also zero.

The ratio of the linear coefficients in equations 1 and 2 (0.223/0.000662) is 336 and should approximately equal the average of the sum of the masses of OK + LSK + DAM, which is 288 g (Table 1). The regression coefficient for aflatoxin concentration, 0.223 (equation 2), should hold for any test sample size. However, the regression coefficient for aflatoxin mass, 0.000662 (equation 1), should depend on the cumulative mass of OK + LSK + DAM peanuts.

Three additional data sets developed by other researchers (8, 9) were analyzed similarly to determine if analogous results could be obtained that would further support results of this study. Table 3 shows the characteristics of the 3 data sets and the data set described in this study. The 4 data sets vary widely in number of lots tested, number of test samples analyzed per lot, and size of test samples. Test sample sizes, on a kernel mass basis, vary from a low of 287.3 g (data set JWD1) to 9292.9 g (data set FED1).

Table 3 also shows regression equations determined for each data set. Results indicate that linear coefficients for aflatoxin concentration in the 3 risk components (OK + LSK + DAM) are stable, varying from 0.176 to 0.246. However, linear coefficients for aflatoxin mass in the 3 risk components (OK + LSK + DAM) vary inversely with sample size. For each data set, the ratio of the linear coefficients is approximately equal to the average combined mass of the OK + LSK + DAM component samples. The percentages of the OK + LSK + DAM mass to test sample mass are about the same for all 4 data sets.

When aflatoxin is expressed as a concentration, the average of the 4 linear coefficients (C1 in Table 3) weighted by the number of test samples in each data set is calculated with the following equation:

$$\text{Aflatoxin in lot, ng/g} = 0.216 \times \text{aflatoxin concentration in OK + LSK + DAM (ng/g)} \quad (3)$$

Table 3. Regression analysis results for 4 data sets for the correlation between aflatoxin concentration in lot and aflatoxin in the risk components OK + LSK + DAM

Data set[a]	Number of lots	Number of test samples per lot	Test sample mass,[b] g	Risk component mass,[b,c] g	Risk mass/test sample mass ratio	C1[d]	C2[e]	C1/C2
JWD1	151	1	287	50	0.174	0.176	0.00378	47
TBW1	120	5	1561	288	0.184	0.223	0.00067	342
JWD2	26	2	7808	1270	0.163	0.160	0.00013	1194
FED1	17	10	9293	1919	0.206	0.246	0.00011	2239

[a] Data sets JWD1 and JWD2 are from reference 9; FED1 is from reference 8; TBW1 is from the current study.
[b] All masses are expressed on a kernel basis.
[c] Risk components = OK + LSK + DAM.
[d] Correlation coefficients for the equation: Aflatoxin concentration in lot, ng/g = C1 × aflatoxin concentration in OK + LSK + DAM (ng/g).
[e] Correlation coefficients for the equation: Aflatoxin concentration in lot, ng/g = C2 × aflatoxin mass in OK + LSK + DAM (ng).

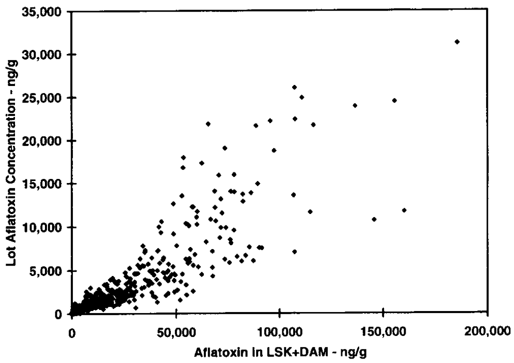

Figure 3. Aflatoxin concentration (ng/g) in FS peanut lots versus cumulative aflatoxin concentration (ng/g) in loose shelled kernels (LSK) and damaged kernels (DAM).

Equation 3 should be a better estimate than equation 2, because it incorporates results of 4 data sets, or 973 observations. The linear coefficients for aflatoxin mass (C2 in Table 3) cannot be averaged, because they depend on the combined risk component masses of OK + LSK + DAM.

The 4 data sets were not combined into one large data set because (*1*) different sample sizes were used in each study and (*2*) the TBW1 data set had aflatoxin concentrations in the lot over a much wider range than did the other data sets and would dominate any regression analysis of the combined data sets. However, Table 3 shows that all 4 data sets give very similar results.

At present, shellers testing FS peanut lots for aflatoxin are measuring cumulative aflatoxin concentrations in LSK + DAM components. The correlation between that and aflatoxin concentration in the lot is 0.881, which is the 8th highest r among the 16 listed in Table 2. A plot for the TBW1 data set (Figure 3) suggests a linear relationship between aflatoxin concentration in LSK + DAM and aflatoxin concentration in the lot. Table 4 shows regression coefficients (regressions forced through intercept) for the 4 data sets. The weighted average of the 4 linear regression coefficients (weighted on the total number of observations in each data set) provides the following relationship between aflatoxin concentration in the lot and aflatoxin concentration in LSK + DAM:

$$\text{Aflatoxin in lot, ng/g} = 0.117 \times \text{aflatoxin concentration in LSK + DAM (ng/g)} \quad (4)$$

Equation 4 indicates that 1000 ng aflatoxin/g in LSK + DAM would translate into an aflatoxin concentration of about 117 ng/g in the lot.

Table 4. Regression analysis results for 4 data sets for the correlation between aflatoxin concentration in lot and aflatoxin concentration in the risk components LSK + DAM

Data set[a]	Number of lots	Number of samples per lot	C1[b]	R^2
JWD1	151	1	0.0674	0.837
JWD2	52	2	0.0750	0.738
TBW1	120	5	0.1390	0.838
FED1	17	10	0.0972	0.987

[a] Data sets JWD1 and JWD2 are from reference 9; FED1 is from reference 8; TBW1 is from the current study.

[b] Correlation coefficients for the equation: Aflatoxin concentration in lot, ng/g = C1 × aflatoxin concentration in LSK + DAM (ng/g). Weighted average for C1 is 0.117.

Peanut shellers can better predict aflatoxin concentration in a lot simply by converting the aflatoxin concentration in LSK + DAM components to aflatoxin mass by multiplying aflatoxin concentration with component sample mass. As Table 2 shows, this conversion increases r from 0.881 to 0.989. The relation between aflatoxin mass in LSK + DAM components and aflatoxin concentration in the lot (Figure 4) is:

$$\text{Aflatoxin in lot, ng/g} = 0.0007 \times \text{aflatoxin concentration in LSK + DAM (ng)} \quad (5)$$

In shelling plants, risk components are removed from FS peanuts with screens and electronic color sorters. Lots of raw, shelled peanuts sold to manufac-

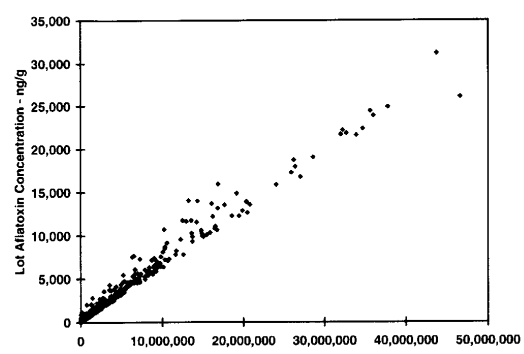

Figure 4. Aflatoxin concentration (ng/g) in FS peanut lots versus cumulative aflatoxin mass (ng) in loose shelled kernels (LSK) and damaged kernels (DAM).

Table 5. Correlation between aflatoxin concentration in SMKSS component and aflatoxin in various components

	Correlation coefficient	
Component[a]	Aflatoxin concentration, ng/g	Aflatoxin mass, ng
OK	0.307	0.326
LSK	0.469	0.409
DAM	0.499	0.495
OK + LSK	0.498	0.443
OK + DAM	0.512	0.514
LSK + DAM	0.510	0.502
OK + LSK + DAM	0.549	0.514

[a] Components defined in footnote to Table 1.

turers of consumer-ready products are produced primarily from the SMKSS component in the FS lot. As a result, the peanut industry is also interested in knowing if aflatoxin in the risk components also can be used to predict aflatoxin in the SMKSS component.

Table 5 shows correlations between aflatoxin in the SMKSS component and aflatoxin in the remaining 3 risk components (expressed as a concentration or as a mass) for the TBW1 data set. The highest r is 0.549, for aflatoxin concentration in OK + LSK + DAM components. A plot of aflatoxin concentration in the SMKSS component versus aflatoxin concentration in OK + LSK + DAM components is shown in Figure 5. Table 5 and Figure 5 indicate that aflatoxin in the SMKSS component cannot be predicted accurately by aflatoxin in OK + LSK + DAM components. If shellers want to estimate aflatoxin in the SMKSS component, direct measurement may be the better approach.

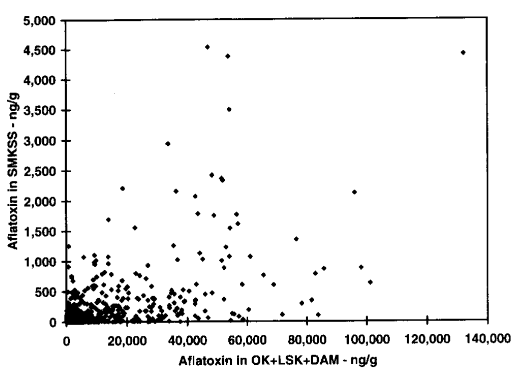

Figure 5. Aflatoxin concentration (ng/g) in sound mature kernels plus sound splits (SMKSS) versus cumulative aflatoxin concentration (ng/g) in other kernels (OK), loose shelled kernels (LSK), and damaged kernels (DAM).

Summary and Conclusions

For the 120 lots tested, SMKSS accounted for 81.6% of kernel mass and 6.9% of total aflatoxin mass. Cumulatively, the 3 risk components, OK + LSK + DAM, accounted for 18.4% of total lot mass and 93.1% of total aflatoxin mass. These findings are similar to previously reported results (8, 9).

Aflatoxin mass in OK + LSK + DAM was highly correlated (r = 0.996) with aflatoxin concentration in the FS lot. Empirical relationships were developed to predict aflatoxin concentration in the lot from aflatoxin mass and concentration in OK + LSK + DAM components.

Aflatoxin concentration in OK + LSK + DAM was not highly correlated (r = 0.549) with aflatoxin in SMKSS. Thus, precise predictions of aflatoxin concentration in SMKSS component cannot be made from measurements of aflatoxin in OK + LSK + DAM components.

Further studies will be done to measure sampling variability associated with use of aflatoxin mass in OK + LSK + DAM components so that performance (false positives, false negatives, and costs) of aflatoxin-sampling plans can be predicted and efficient sampling plans can be designed for the peanut industry.

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