Use of Loose Shelled Kernels to Estimate Aflatoxin in Farmers Stock Peanut Lots[1]

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ABSTRACT

Loose shelled kernels (LSK) are a defined grade component of farmers stock peanuts and represented, on the average, 33.3% of the total aflatoxin mass and 7.7% of the kernel mass among the 120 farmers stock peanut lots studied. The functional relationship between aflatoxin in LSK taken from 2-kg test samples and the aflatoxin in farmers stock peanut lots was determined to be linear with zero intercept and a slope of 0.297. The correlation between aflatoxin in LSK and aflatoxin in the lot was 0.844 which suggests that LSK taken from large test samples can be used to estimate the aflatoxin concentration in a farmer's lot. Using only LSK allows large test samples to be used to estimate the lot concentration since LSK can be easily screened from a large test sample. If LSK accounts for 7.7% of the lot kernel mass, a 50-kg sample will yield about 3.9 kg of LSK which can be easily prepared for aflatoxin analysis. Increasing the test sample size from 2 to 50 kg reduced the coefficient of variation associated with estimating a lot with 100 parts per billion (ppb) aflatoxin from 114 to 23%, respectively. As an example, a farmers stock aflatoxin sampling plan with dual tolerances (10 and 100 ppb) that classified lots into three categories was evaluated for two test sample sizes (2 and 50 kg). The effect of increasing test sample size from 2 to 50 kg on the number of lots classified into each of the three categories was demonstrated when measuring aflatoxin only in LSK.

Key Words: Grade, LSK, sampling, risk components.

The current aflatoxin control program for farmers stock peanuts marketed in the U.S. requires that peanut kernels in a grade sample be visually inspected for the aflatoxin-producing mold *Aspergillus flavus* Links Fr. (5). If one or more kernels are found with the mold then the lot is diverted from the edible market because of the risk that the lot may be contaminated (3). Several studies have been conducted to determine if the method that visually identifies the mold (called the VAF method) can be replaced by measuring aflatoxin directly in test samples taken from a farmer's lot (1,10). However, no official USDA/peanut industry sampling program has been developed to date. As a result, some shellers on an ad-hoc basis measure aflatoxin in farmers stock peanut lots and

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segregate the lots based on the estimated aflatoxin in the lot. Shellers estimate the lot aflatoxin concentration by measuring aflatoxin in the combined damaged (DAM) and loose shelled kernel (LSK) grade components taken from an official 2-kg grade sample.

Shellers use LSK and DAM kernels because aflatoxin studies with farmers stock peanuts (2,6,13) have shown that aflatoxin is more likely to be found in the low quality peanuts such as damaged kernels, loose shelled kernels, and small or other kernels (OK) than in the high quality peanuts such as sound mature kernels and sound splits (SMKSS). Studies (13) where aflatoxin was measured in each of the above four farmers stock grade components from each of 120 farmers stock lots showed that, on the average, aflatoxin in DAM, LSK, OK, and SMKSS represented 51.9, 33.3, 7.9, and 6.9% of the total aflatoxin mass among 600 peanut test samples, respectively. Cumulatively, the three low quality grade components (DAM, LSK, and OK) accounted for 93.1% of the total aflatoxin, but only 18.4% of the total kernel mass. The DAM, LSK, and OK grade components are often referred to as the high risk grade components from an aflatoxin standpoint.

Studies (13) showed that the aflatoxin concentration in the combined LSK and DAM (A_{ld}) grade components taken from a 2-kg test sample was linearly related to the aflatoxin concentration in the lot (A_{lot}) by the following equation:

$$A_{lot} = 0.139 * A_{ld} \quad \text{[Eq. 1]}$$

where concentration is nanograms of total aflatoxin per gram of peanuts (ng/g) or parts per billion (ppb). The correlation coefficient for the functional relationship in Eq. 1 was 0.88 and the standard error associated with the regression coefficient (0.139) is 0.0025.

The aflatoxin in LSK and DAM (A_{ld}) will vary among test samples taken from the same lot. The variability, as measured by the variance, associated with estimating the lot aflatoxin concentration (V_{lot}) was determined from 2-kg test samples to be:

$$V_{lot} = (2/ns) * 0.392 * A_{ld}^{1.53} \quad \text{[Eq. 2]}$$

where ns is the test sample size in kg from which the DAM and LSK are taken. For example, if the aflatoxin in LSK and DAM grade components (A_{ld}) taken from a 2-kg test sample (ns = 2) is 500 ppb, then the aflatoxin concentration in the lot (A_{lot}) is estimated to be 69.5 ppb (Eq. 1). From Eq. 2, the variance associated with the estimated lot concentration of 69.5 ppb is 5281 and the coefficient of variation (CV) is equal to $(100 \times (5281)^{0.5})/69.5$ or 104.6%. Ninety-five percent confidence limits associated with the estimated lot concentration are 69.5 +/- 142.4 (assuming normal distribution, 142.4 = 1.96* square root of 5281) or 0 to 211.9 ppb. The large variation (CV = 104.6%) is due primarily to the small 2-kg test sample from which the LSK and DAM kernels are taken.

Because of the large variability associated with measuring aflatoxin in LSK and DAM peanuts taken from a small 2-kg test grade sample, some farmers stock lots will be misclassified based on aflatoxin estimates from test samples taken from the lot (12). Variability and misclassifications can be reduced by increasing the test sample size (or number of sampling units) associated with the aflatoxin test procedure. However, it will be difficult to pick damaged kernels from a large test sample because damaged kernels are identified visually and removed manually from the test sample. On the other hand, LSK can be readily removed from large test samples using screens to separate LSK from pods. One possible approach to increasing the test sample size (and reducing misclassification) is to measure aflatoxin only in LSK removed from a test sample. The objectives of this study were to (a) determine the relationship between the aflatoxin in LSK and aflatoxin in the lot, (b) determine the variability associated with estimating aflatoxin in the lot by measuring aflatoxin in LSK, and (c) determine the effect of increasing test sample size on reducing variability and reducing the misclassification of farmers stock lots.

Materials and Methods

One hundred twenty segregation 3, runner-type, farmers stock lots were identified by the Federal State Inspection Service (FSIS) using the VAF method. An 11-kg bulk sample was removed from each farmers stock lot during the grading process at the buying point. Foreign material was removed (and discarded) from each bulk sample and the remaining bulk sample of pods and LSK (about 10 kg) were riffle divided into five 2-kg test samples. The LSK were removed from each 2-kg test sample before all pods in each test sample were shelled. The hulls were discarded after weighing. Using standard FSIS practices, the shelled kernels were divided into SMKSS, OK, and DAM grade components and each grade component, along with the LSK, were placed into separate sample bags. The kernel mass, type grade component (SMKSS, OK, LSK, and DAM), along with the sample and lot identification were recorded. A total of 2400 component samples (four component samples per test sample × five test samples per lot × 120 lots = 2400 component samples) were analyzed for aflatoxin.

The aflatoxin concentration of each component sample was estimated by first grinding each component sample. An AMS mill (4) was used to grind the larger SMKSS component samples while a coffee grinder was used to grind each of the smaller OK, LSK, and DAM component samples. A 75-g subsample (or less) of comminuted peanuts was used in the extraction process. When the component sample weighed less than 75 g, the aflatoxin in the entire comminuted component sample was extracted. Acetonitrile/water (84/16) and a solvent/peanut ratio of 4/1 was used to extract aflatoxin from the comminuted subsample. The aflatoxin in the solvent was quantified by HPLC analysis (8,9). All four aflatoxins (B1+B2+G1+G2) were measured and total aflatoxin results (sum of B1+B2+G1+G2) were recorded in concentration units, ng of aflatoxin/g of peanuts or ppb.

The aflatoxin concentration in each 2-kg test sample (SMKSS+DAM+LSK+OK) was used as an estimate of the aflatoxin concentration in the lot. The relationship between the aflatoxin in the LSK and aflatoxin in a lot was estimated by averaging the aflatoxin in the five LSK components samples and averaging the aflatoxin in the five test samples (all components) for each lot. This provided 120 estimates (one for each lot) of aflatoxin in the LSK and aflatoxin in the lot. The variance associated with measuring aflatoxin in the LSK component was determined from the five LSK component samples for each lot. This provided 120 estimates of

variance (one for each lot). All statistical analyses were conducted using the SAS system (7). Operating characteristic (OC) curves were used as a measure of misclassifications and were calculated using the negative binomial distribution where distributional parameters were calculated from measured variances (11).

Results

A plot of aflatoxin concentration in the LSK component samples, A_l, and the aflatoxin concentration in the lot A_{lot} in Fig. 1 suggests the relationship between A_{lot} and A_l is linear. A linear regression analysis performed on the 120 values gave the following expression:

$$A_{lot} = 0.297 * A_l \quad [Eq. 3]$$

with a correlation coefficient of 0.844. Regression of Eq. 3 was forced through the zero intercept because the intercept (negative 46) and was not significantly different from zero (standard error of 266). The standard error associated with the regression coefficient 0.297 in Eq. 3 was 0.017. The predicted curve (Eq. 3) also is shown in Fig. 1 along with the observed aflatoxin values. The correlation coefficient of 0.844 reflects the initial regression with an intercept. The correlation coefficient associated with a regression forced through the intercept is usually higher.

The mean and variance among the five LSK component aflatoxin values are shown in Fig. 2 for each of the 120 lots. As with past studies (10), it appears that variance is a function of aflatoxin concentration and is approximately linear in a full-log plot. As a result, a function of the form shown in Eq. 4 was used to describe the relationship between variance (V) and aflatoxin concentration (A):

$$V = a * A^b \quad [Eq. 4]$$

where a and b are constants. From a regression analysis, the following regression equation was obtained:

$$V_l = 10.498 * A_l^{1.642} \quad [Eq. 5]$$

with a correlation coefficient of 0.95 in the log scale. The predicted variance, V_l, among LSK component samples also is shown in Fig. 2 along with the observed variance values and is specific for LSK being taken from a 2-kg test sample.

The variance among LSK aflatoxin estimates (Eq. 5) can be used to determine the variance associated with estimating the lot concentration V_{lot} since the relationship between the aflatoxin in the LSK risk components and the aflatoxin in the lot (Eq. 3) is known. If the aflatoxin in the lot A_{lot} is linearly related to the aflatoxin in the LSK component A_l,

$$A_{lot} = c * A_l \quad [Eq. 6]$$

then the variance associated with predicting the aflatoxin in the lot A_{lot} from LSK can be computed if the variance associated with measuring aflatoxin in the LSK component A_l is known.

$$V_{lot} = c^2 * V_l \quad [Eq. 7]$$

where c is assumed to be a constant equal to the regression coefficient (0.297) in Eq. 3 and V_l is given by Eq. 5. Therefore, the variance associated with estimating aflatoxin concentration in the lot, V_{lot}, by measuring aflatoxin concentration in LSK taken from a 2-kg test sample is given in Eq. 8 as:

$$V_{lot} = (0.297)^2 * 10.498 * A_l^{1.642} \quad [Eq. 8]$$

The variation associated with estimating lot concentration when using the LSK risk components can be reduced by increasing the size of the test sample. Statistical law states that, if the sample size is doubled, the variance is reduced by half. Since the variance Eq. 8 is specific for a 2-kg test sample of farmers stock peanuts, Eq. 8 can be modified to predict the variance for any test sample size ns in kg.

$$V_{lot} = (2/ns) * (0.297)^2 * 10.498 * A_l^{1.642} \quad [Eq. 9]$$

An example of how Eqs. 3 and 9 would be used to predict the lot concentration and the variation associated with estimating the lot concentration is shown below. A 2-kg test sample of farmers stock peanuts is removed from a lot and screened to separate LSK from the pods. The LSK are comminuted in a mill and the aflatoxin is measured in a subsample. Assuming the aflatoxin concentration in the LSK component A_l is 337 ppb, then the estimated lot concentration A_{lot}, from Eq. 3 is 100 ppb. Using Eq. 9, the variance associated with the estimated lot concentration of 100 ppb (LSK = 337 ppb) is 13,062. The standard deviation is 114 and the CV is 114%. The standard deviation of 114 is large relative to the true lot concentration (100 ppb) indicating that aflatoxin estimates will be rather unreliable. In fact, the 95% confidence interval (for a normal distribution) for estimates of the true lot concentration is from 0 to 328 ppb.

The effect of increasing test sample size (from which LSK is taken) from 2 to 50-kg on the precision associated

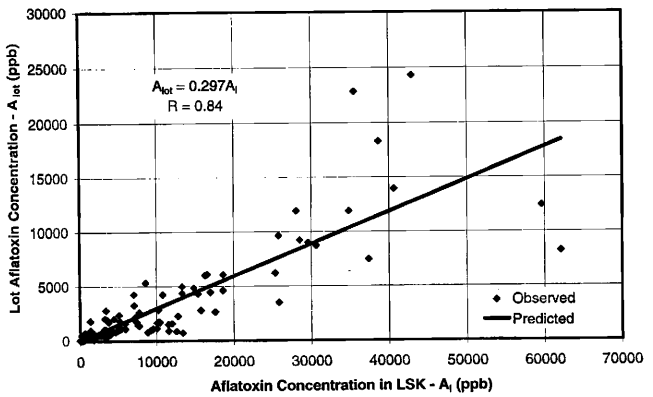

Fig. 1. Aflatoxin concentration in farmers stock peanut lots (A_{lot}) versus aflatoxin concentration in loose shelled kernels (A_l).

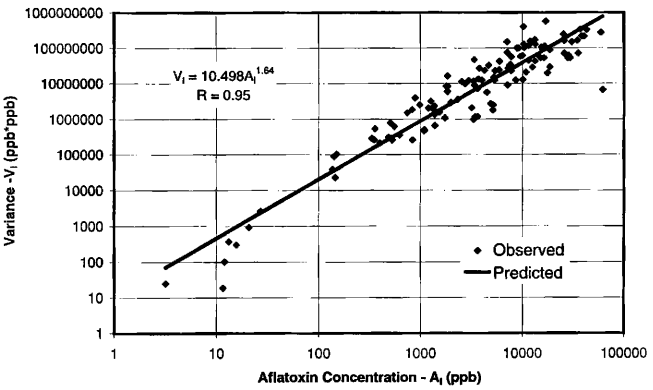

Fig. 2. Full log plots of variance (V_l) versus aflatoxin when measuring aflatoxin (A_l) among loose shelled kernel samples.

with estimating aflatoxin concentration in a farmer's lot with a true concentration of 100 ppb is shown in Table 1. As the sample size increases, the variance and CV associated with measuring the true lot concentration of 100 ppb decreases. The CV associated with a 50-kg test sample is 23% compared to 114% for a 2-kg test sample.

Table 1. Effect of sample size on the precision associated with estimating a farmers stock lot at 100 ppb by measuring aflatoxin in loose shelled kernels taken from samples of various sizes.

Sample size	Variance	Coefficient of variation	95% Confidence limits	
			Low	High
kg	ppb*ppb	%	------- ppb -------	
2	13062.4	114.3	0.0	328.6
5	5225.0	72.3	0.0	244.6
10	2612.5	51.1	0.0	202.2
15	1741.7	41.7	16.5	183.5
20	1306.2	36.1	27.7	172.3
25	1045.0	32.3	35.3	164.7
30	870.8	29.5	41.0	159.0
40	653.1	25.6	48.9	151.1
50	522.5	22.9	54.3	145.7

To show the effect of aflatoxin testing variability on the misclassification of lots, a sample design with one or more aflatoxin tolerances needs to be defined. While the peanut industry has not defined a sampling plan (sample size or aflatoxin tolerances) on an industry-wide basis, the industry has indicated an interest in classifying lots into three or more categories based on aflatoxin estimates. Some shellers have indicated that classifying lots based on low, medium, and high aflatoxin levels helps them to manage more effectively their shelling facilities to reduce aflatoxin in processed lots.

A three-way classification sampling plan with two tolerances based upon aflatoxin in LSK was evaluated. Aflatoxin tolerances based upon aflatoxin in LSK of 33.7 and 337 ppb or 10 and 100 ppb for lot concentrations were chosen for evaluation. Use of two tolerances classifies farmers stock lots into three categories. Lots where LSK tests less than or equal to 33.7 ppb (lots with 10 ppb or less) are classified Category 1, lots where LSK tests greater than 33.7 ppb (10 ppb for lots) but less than or equal to 337 ppb (100 ppb for lots) are classified Category 2, and lots where LSK tests greater than 337 ppb (100 ppb for lots) are classified Category 3. To show the effect of test sample size on reducing misclassifications, two test sample sizes of 2 and 50 kg were evaluated. The tolerances and test sample sizes were chosen only as examples and not because they are specifically under industry consideration. Tolerances will be referred to as 10 and 100 ppb in the lot scale.

The effect of measuring aflatoxin in LSK taken from a 2-kg test sample along with the two tolerances 10 and 100 ppb on the classification of farmers stock lots into three categories is shown in Fig. 3 for a wide range of lot concentrations. Each curve indicates the fraction of lots at various lot concentrations that are classified into each

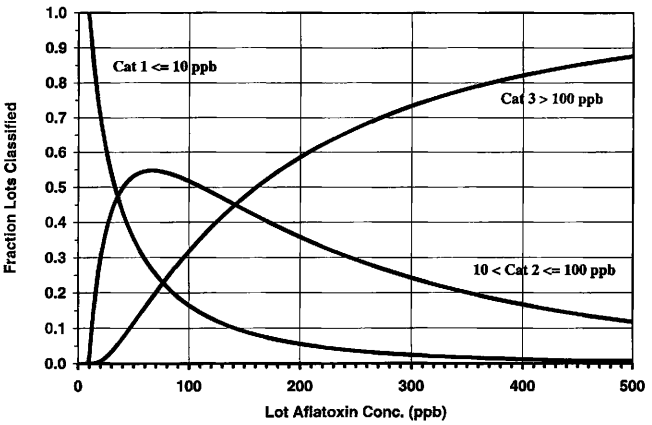

Fig. 3. Fraction of lots classified into three categories when measuring loose shelled kernels taken from a 2-kg farmers stock sample and using dual tolerances of 10 and 100 ppb.

category. For a given lot concentration, the fraction of lots classified Categories 1, 2, and 3 should add to 1 (decimal basis) or 100%. For example, about 37, 52, and 11% of the lots at 50 ppb will be classified into Categories 1, 2, and 3, respectively, using a 2-kg test sample (Fig. 3). With a perfect sampling plan (no testing variability), 100% of all lots at 50 ppb should be classified into Category 2 by the sampling plan. But 48% of the lots at 50 ppb are misclassified and 52% are correctly classified. Similar observations can be made for lots at other concentrations.

Fig. 4 shows how lots at various concentrations are classified when using a 50-kg test sample. Comparing curves in Fig. 3 and Fig. 4 show that more lots are correctly classified with a 50-kg test sample than with a 2-kg test sample. For example, 0, 100, and 0% of the lots at 50 ppb are classified into Categories 1, 2, and 3, respectively. Increasing the test sample size from 2 to 50 kg eliminated all misclassification of lots at 50 ppb.

The fraction of lots, shown in Fig. 3 and Fig. 4, that are classified into each category can be converted into the total number of lots classified into each category using the estimated crop distribution shown in Table 2. The crop distribution describes how many lots are tested by the sampling plan at each lot concentration. The crop

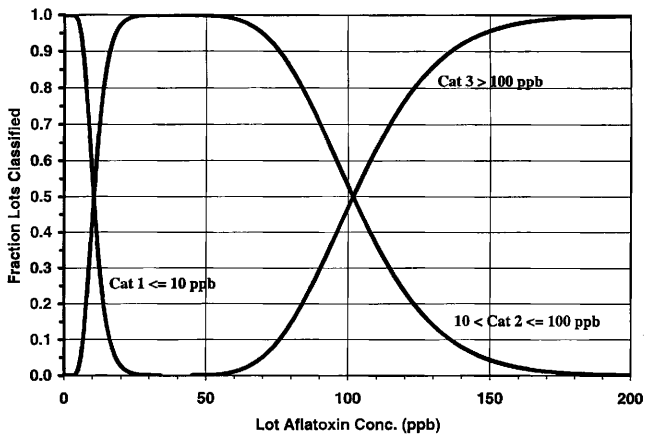

Fig. 4. Fraction of lots classified into three categories when measuring loose shelled kernels taken from a 50-kg farmers stock sample and using dual tolerances of 10 and 100 ppb.

Table 2. Cumulative distribution among farmers stock lot aflatoxin concentrations.

	Lot aflatoxin	Cumulative
	ppb	%
	0	51.81
	2	61.16
	5	66.83
	10	71.87
	15	75.05
	20	77.38
	30	80.75
	50	85.03
	75	88.36
	100	90.62
	150	93.53
	200	95.34
	300	97.40
	400	98.47
	500	99.07
	750	99.71
$\bar{X}$	33	

distribution, shown in Table 2, was developed from aflatoxin data collected in an industry wide project that measured aflatoxin in farmers stock lots marketed at five buying points located across all three growing regions (1). The average aflatoxin concentration among all lots described in Table 2 is 33 ppb. Using techniques previously described (14), the number of lots classified and the average aflatoxin concentration among lots classified into the three categories is summarized in Table 3 for the two sample sizes. Results in Table 3 are per 100 lots tested. The majority of lots (over 70%) are classified into Category 1 by both test sample sizes. As test sample size increases, fewer lots are classified into Category 1 and more lots are classified into Category 3. The average aflatoxin is much lower in Category 1 for the 50-kg test sample size. Increasing test sample size from 2 to 50 kg reduces the average amount of aflatoxin in all three categories.

Table 3. Effect of two sample sizes along with two tolerances, t, on the classification of farmers stock lots into three categories when measuring aflatoxin in loose shelled kernels screened from 2- and 50-kg samples.

Sample size	Parameter[a]	Category 1	Category 2	Category 3
kg				
2	Tolerance (ppb)	$t \leq 10$	$10 > t \leq 100$	$t > 100$
	Lots classified (%)	78.2	13.9	7.9
	Average (ppb)	5.0	69.7	245.4
50	Tolerance (ppb)	$t \leq 10$	$10 > t \leq 100$	$t > 100$
	Lots classified (%)	71.8	13.3	14.9
	Average (ppb)	1.1	26.4	192.8

[a]Average aflatoxin among crop being sampled is 33.0 ppb; tolerances of 10 and 100 ppb reflect aflatoxin in LSK of 33.7 and 337.0 ppb.

Summary and Conclusions

The functional relationship between aflatoxin in LSK and aflatoxin in farmers stock peanut lots was determined. The correlation between aflatoxin in LSK and aflatoxin in the lot (0.844) was considered high enough to suggest that LSK alone can be used to estimate the lot aflatoxin concentration especially when using large test sample sizes. Using only LSK allows for much larger test samples to be used to estimate the lot concentration since LSK can be easily screened from a large test sample. If LSK accounts for 7.7% of the lot mass, a 50-kg test sample will yield about 3.9 kg of LSK which can be easily prepared for aflatoxin analysis.

The effect of increasing test sample size on reducing the variability associated with estimating the true lot concentration also was determined. The coefficient of variation was reduced from 114 to 23% when the test sample size was increased from 2 to 50 kg, respectively. The large variability associated with measuring aflatoxin in LSK taken from small test samples shows how important it is to use large samples to estimate the aflatoxin concentration of a farmer's lot at the buying point.

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