FOOD CHEMICAL CONTAMINANTS

Variability Associated with Testing Shelled Corn for Fumonisin

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Variances associated with sampling, sample preparation, and analytical steps of a test procedure that measures fumonisin in shelled corn were estimated. The variance associated with each step of the test procedure increases with fumonisin concentration. Functional relationships between variance and fumonisin concentration were estimated by regression analysis. For each variance component, functional relationships were independent of fumonisin type (total, B1, B2, and B3 fumonisins). At 2 ppm, coefficients of variation associated with sampling (1.1 kg sample), sample preparation (Romer mill and 25 g subsample), and analysis are 16.6, 9.1, and 9.7%, respectively. The coefficient of variation associated with the total fumonisin test procedure was 45% and is about the same order of magnitude as that for measuring aflatoxin in shelled corn with a similar test procedure.

Fumonisins are mycotoxins produced by several fungi of the genus *Fusarium* (1). Fumonisin is found in various grains including shelled corn and is carcinogenic in laboratory animals such as rats (2). At present, there is no U.S. Food and Drug Administration action level for fumonisin in food or feed products produced in the United States (3).

The test procedure used to estimate the concentration of fumonisin in a bulk lot is similar to test procedures used to measure other mycotoxins, such as aflatoxin, in agricultural products. The test procedure consists of 3 steps. First, a random sample (test sample) is taken from the lot (sampling step). Second, the entire test sample is comminuted in a mill or grinder, and a random subsample is removed from the comminuted test sample. Grinding and subsampling are collectively called the sample preparation step. Third, the fumonisin in the subsample is extracted with solvent and quantitated (analytical step).

The variability associated with each of the 3 steps contributes to the total variability associated with the test procedure. The variability associated with the test procedure makes it difficult to estimate the true fumonisin concentration of a bulk lot with a high degree of confidence and consequently makes it difficult to accurately classify lots into categories such as that required by regulatory activity. If the variability of the overall fumonisin test procedure can be reduced, the lot concentration can be estimated with more confidence and lots can be classified more accurately.

Previous studies that measured the variability associated with test procedures used to measure aflatoxin in corn, peanuts, and cottonseed lead one to expect that the variability of each step of the test procedure would be different and that the cost associated with reducing the variability of each step would be different (4–6). It is important to design a fumonisin test procedure that will have the lowest variability that resources will allow. Therefore, the objectives of this study were to measure the variabilities of sampling, sample preparation, and analytical steps of the test procedure used to measure fumonisin in shelled corn and to show how to change the design of the test procedure to decrease variability and achieve more precise results.

Experimental

Sample Preparation

Twenty-four bulk lots of shelled corn harvested from 24 different fields in North Carolina were identified as having possible fumonisin contamination. A bulk sample of ca 45 kg (100 lbs) was taken from each of the 24 lots. Each bulk sample of shelled corn was riffle divided into thirty-two 1.1 kg test samples. Each test sample was comminuted in a Romer mill.

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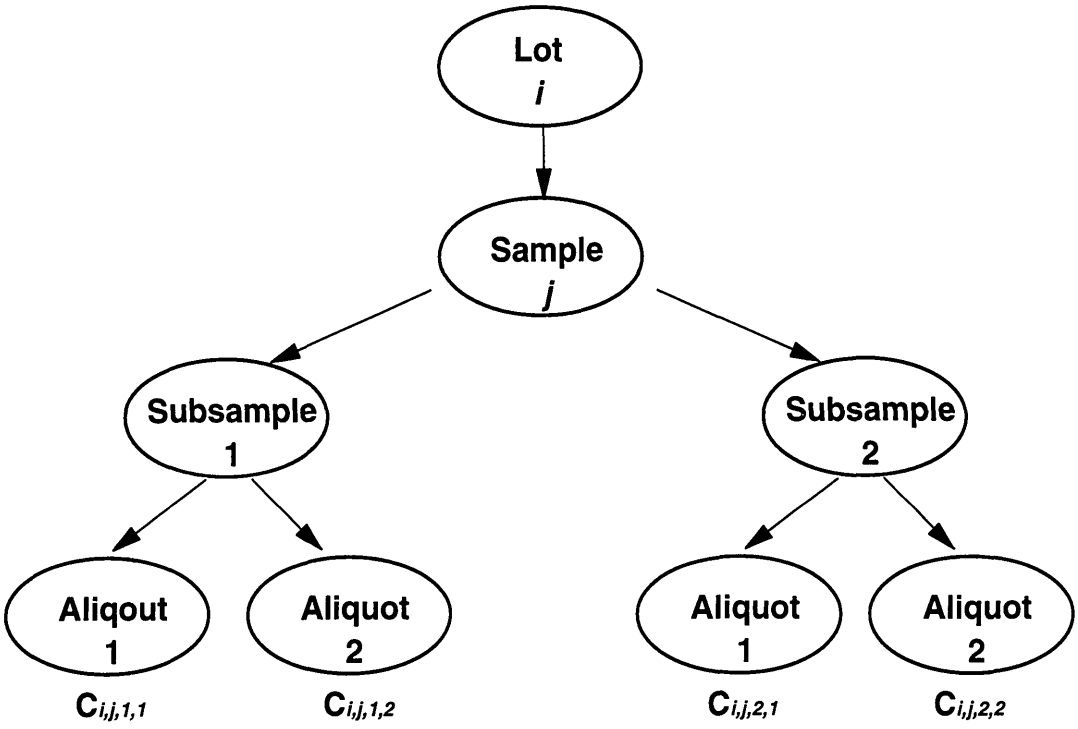

Figure 1. Schematic diagram of nested experimental design showing how the fumonisin test result $C_{i,j,k,l}$ was obtained. The identification for lot is *i*, where *i* = 1 to 10; for sample is *j*, where *j* = 1 to 10; for subsample is *k*, where *k* = 1 to 2; and for analysis is *l*, where *l* = 1 to 2.

The comminuted test samples were placed in plastic bags, each plastic bag was identified by lot number and sample number, and the bags were stored at 5°C until needed for several planned fumonisin studies.

Experimental Design

A nested design was used to determine the variability associated with each step of the fumonisin test procedure (Figure 1). Ten lots, with an expected wide range in fumonisin concentration, were chosen from the 24 lots by using fumonisin estimates for each of the 24 lots obtained from a previous study. For each of the 10 lots, 10 comminuted test samples were selected from the 32 test samples by arbitrarily taking every third sample. By using a riffle divider, two 25 g subsamples were taken from each of the 10 comminuted test samples. The fumonisin content of the 25 g subsamples was measured by using AOAC Official Method **995.15** (7). As specified by the analytical method, each 25 g subsample was blended with 50 mL methanol–water (3 + 1, v/v) for 3 min. Two portions were removed from the blended extract. The concentrations of B1, B2, and B3 fumonisins, in parts per million (ppm), were quantitated. Total fumonisin (sum of B1, B2, and B3) was also calculated for each portion. The nested design resulted in 400 analyses (10 lots × [10 samples/lot] × [2 subsamples/sample] × [2 aliquots/subsample]).

Variability Estimates

The error structure or sources of variability associated with the fumonisin test procedure are also illustrated in Figure 1. The total variance among fumonisin test results is composed of at least 3 variance components: sampling, sample preparation, and analysis (5). The variances in this study were estimated with a model in which an observed fumonisin test result, *C*, may be represented as follows:

$$C = \mu + S + SP + A \quad (1)$$

where μ is the true fumonisin concentration in the lot being tested, *S* is the random deviation of sample concentrations about the lot concentration with expected value 0 and variance δ^2_s, *SP* is the random deviation of subsample concentrations about the sample concentration with expected value 0 and variance δ^2_{sp}, and *A* is the random deviation of analytical assay results about the subsample concentration with expected value 0 and variance δ^2_a. By assuming independence among the random deviations in equation 1, the following variance relationship is obtained:

$$\delta^2_t = \delta^2_s + \delta^2_{sp} + \delta^2_a \quad (2)$$

where δ^2_t is the total variance associated with the fumonisin test procedure. With a Statistical Analysis System (SAS) nested analysis of variance procedure (8), each of the variance components in equation 2 was determined for each lot. Estimates of the true variance components and the true fumonisin concentration by experimental values are denoted by s^2 and *C*, respectively. Variances were determined for total, B1, B2, and B3 fumonisins.

Table 1. Fumonisin concentration and sampling, sample preparation, and analytical variances associated with measuring fumonisins in shelled corn

Fumonisin type	Lot ID	Fumonisin concentration (μg/g)	Total	Variance[a]		
				Sample	Sample preparation	Analytical
B1	5	0.6	0.0319	0.0150	0.0080	0.0089
B1	18	3.0	0.6806	0.5874	0.0572	0.0360
B1	16	4.3	0.9774	0.7102	0.2229	0.0443
B1	15	5.5	1.0022	0.7571	0.0572	0.1879
B1	23	6.5	2.4380	1.6142	0.6259	0.1979
B1	19	8.6	1.8009	1.1260	0.2752	0.3997
B1	2	11.6	3.0845	1.9999	0.6331	0.4515
B1	22	14.5	4.3885	2.4088	0.5325	1.4472
B1	14	14.7	2.5686	—[b]	2.1026	0.4661
B1	3	16.0	3.7095	2.1837	0.4854	1.0404
B2	5	0.2	0.0134	0.0014	0.0022	0.0098
B2	18	1.1	0.1003	0.0830	0.0098	0.0076
B2	16	1.3	0.0843	0.0358	0.0379	0.0106
B2	15	1.9	0.1382	0.0807	0.0210	0.0365
B2	23	2.0	0.3734	0.3017	0.0412	0.0305
B2	19	4.0	0.5881	0.4274	—	0.1607
B2	14	5.1	0.4742	0.2216	0.1661	0.0866
B2	2	5.4	1.2444	0.9021	0.2187	0.1236
B2	22	6.7	1.5876	1.0461	0.1651	0.3764
B2	3	7.6	1.2589	1.0141	0.0676	0.1772
B3	5	0.0	—	0.0001	—	—
B3	18	0.3	0.0062	0.0045	0.0002	0.0015
B3	16	0.5	0.0258	0.0052	0.0039	0.0167
B3	15	0.6	0.0126	0.0078	0.0015	0.0034
B3	23	0.7	0.0481	0.0330	0.0110	0.0042
B3	19	1.0	0.1137	0.0762	0.0240	0.0135
B3	2	1.4	0.0615	0.0367	0.0095	0.0153
B3	14	2.0	0.1290	0.0178	0.0804	0.0307
B3	22	2.1	0.1329	0.0618	0.0224	0.0487
B3	3	2.2	0.0892	0.0644	0.0111	0.0138
Total[c]	5	0.8	0.0892	0.0280	0.0265	0.0347
Total	18	4.4	1.3421	1.1658	0.1009	0.0754
Total	16	6.2	1.8246	1.1685	0.5330	0.1231
Total	15	8.0	0.1057	1.5223	0.1695	0.4139
Total	23	9.3	5.3131	3.8800	1.0168	0.4163
Total	19	13.7	4.3813	2.7495	0.3825	1.2494
Total	2	18.5	9.1663	6.4071	1.5971	1.1621
Total	14	21.8	5.6314	—	4.4938	1.1376
Total	22	23.3	12.9603	7.4069	1.5820	3.9714
Total	3	25.7	10.1210	6.7057	1.0758	2.3395

[a] Sample variance reflects 1.1 kg sample. Sample preparation variance reflects Romer mill and 25 g subsample. Analytical variance reflects AOAC Method **995.15** and LC.
[b] —, missing value.
[c] Total fumonisin = B1 + B2 + B3.

Results

Table 1 shows the total, sampling, sample preparation, and analytical variance estimates for each type of fumonisin (B1, B2, B3, and total) for each of the 10 lots. Because of experimental error, some variance estimates were calculated to be negative (not physically possible values) and were treated as missing values in Table 1 (negative values ignored and not used in any subsequent analysis). For each type of fumonisin, variance results are ordered by the fumonisin concentration, which varied from less than 1 to about 26 ppm. In general, each variance component increases with fumonisin concentration regardless of fumonisin type as has also been observed in aflatoxin studies for corn, peanuts, and cottonseed (4–6).

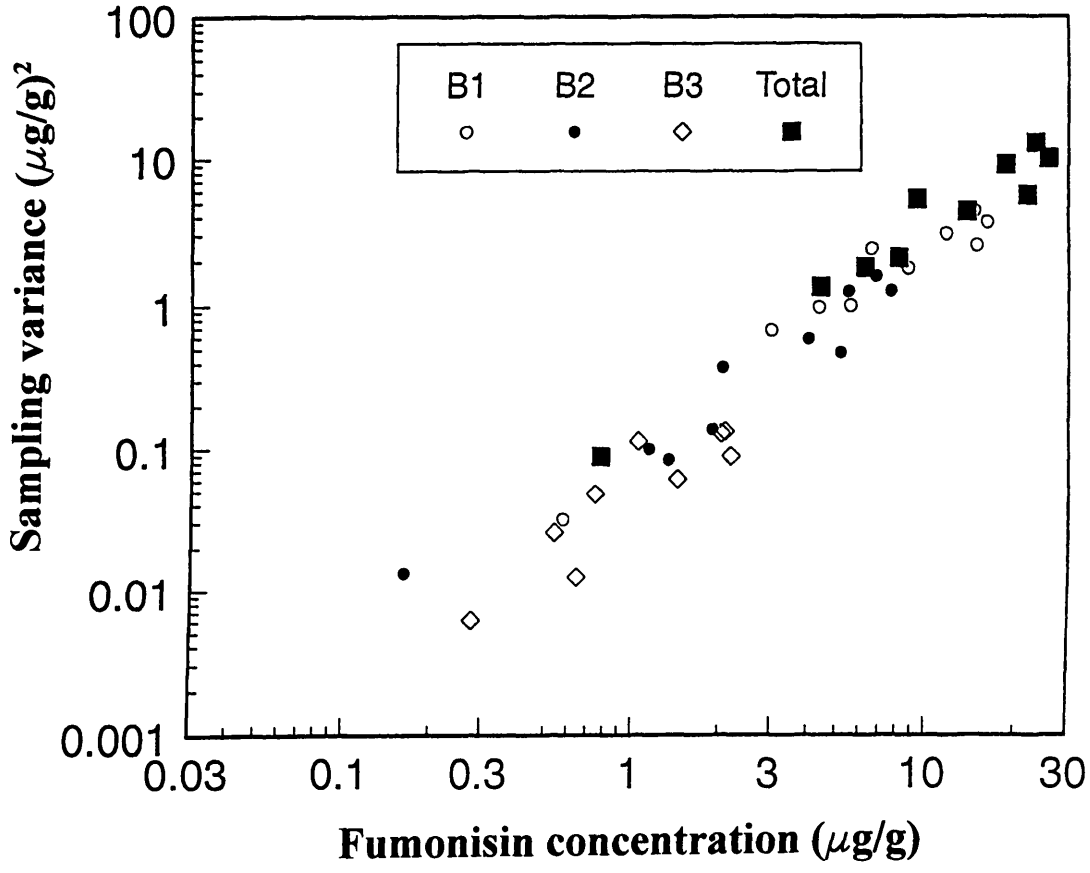

Figure 2. Sampling variance versus fumonisin concentration for 1.1 kg sample of shelled corn.

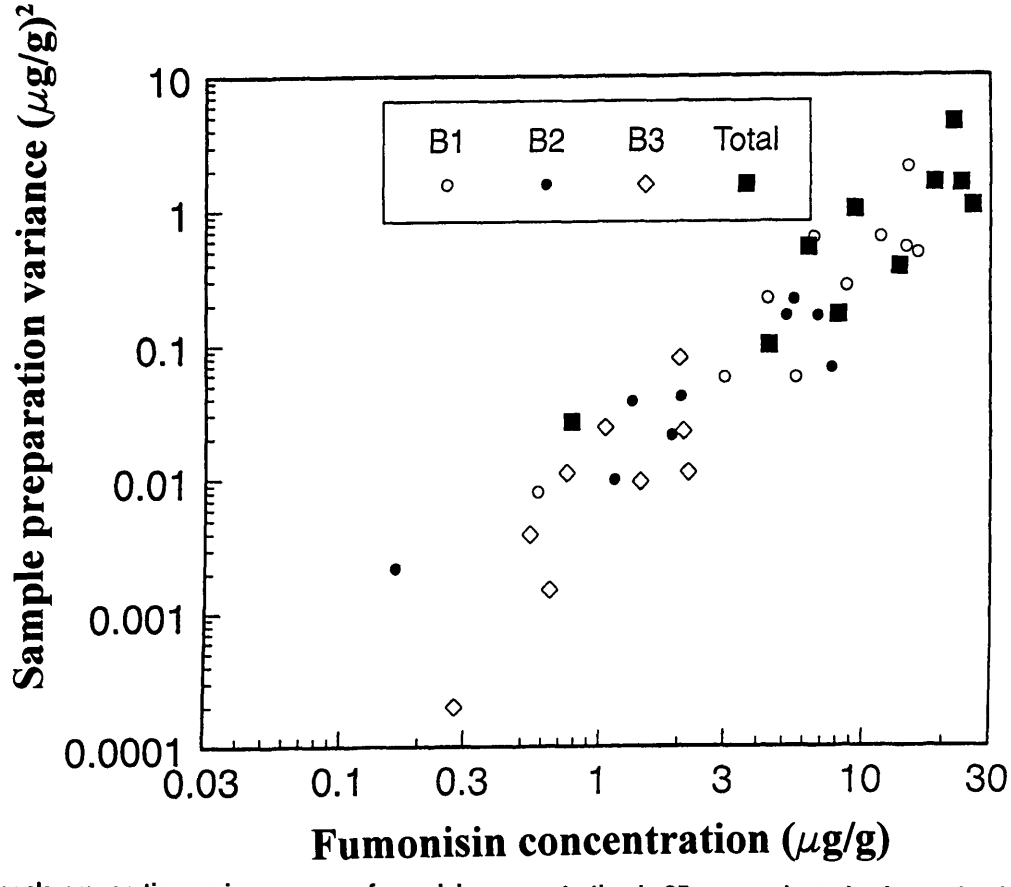

Figure 3. Sample preparation variance versus fumonisin concentration in 25 g comminuted subsample of shelled corn.

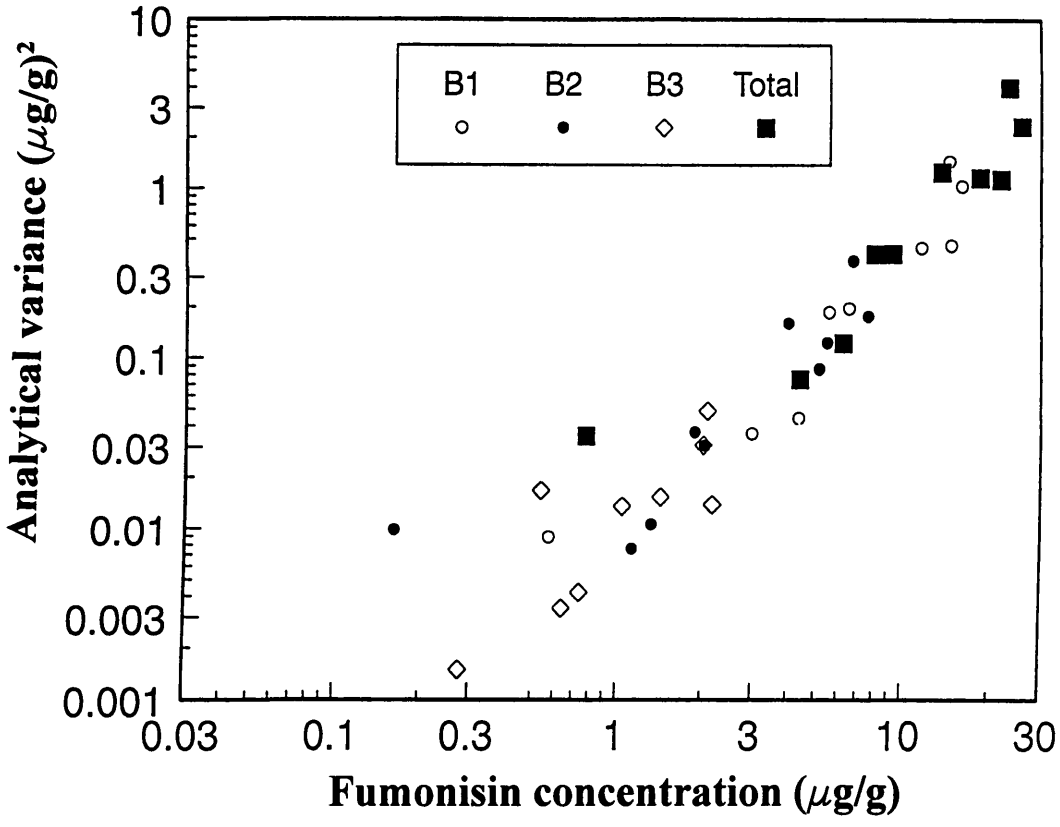

Figure 4. Analytical variance versus fumonisin concentration for shelled corn in 1 portion of extract analyzed by AOAC Official Method 995.15.

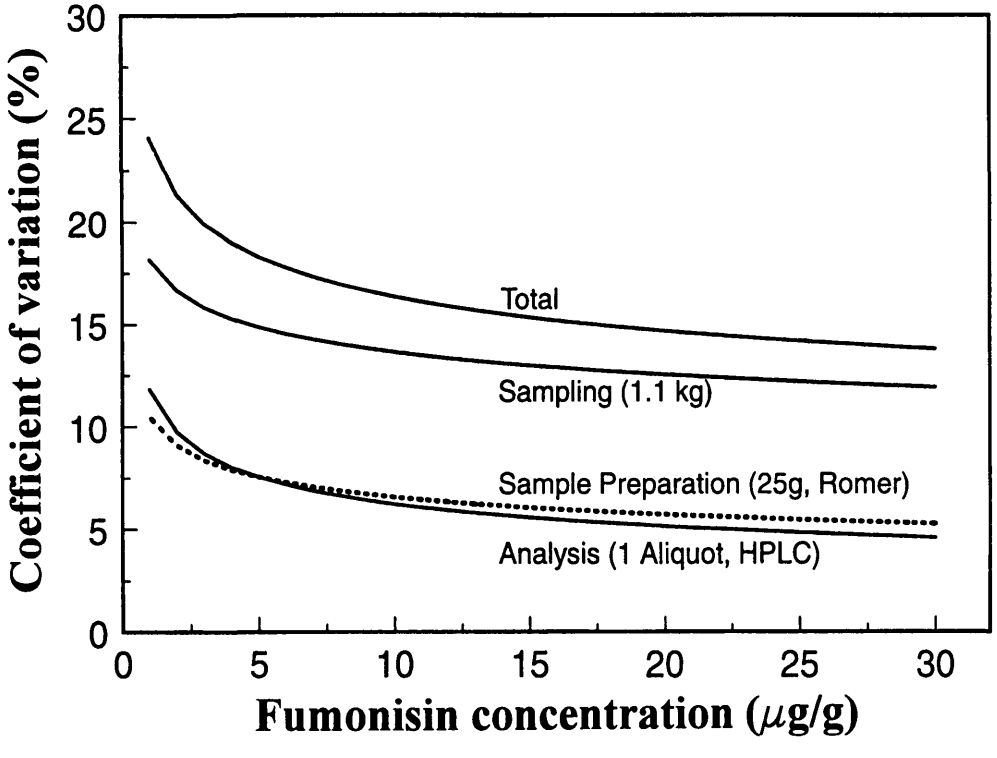

Figure 5. Coefficient of variation associated with each step of the fumonisin test procedure for shelled corn. Coefficients of variation are specific for 1.1 kg sample, Romer mill, 25 g subsample, LC, and 1 portion.

Figures 2–4 show that sampling, sample preparation, and analytical variances increase linearly with fumonisin concentration (either B1, B2, B3, and total) when plotted in full log plots. Two important observations can be made from the plots in Figures 2–4. First, the figures suggest that the relationship between variance, s^2, and fumonisin concentration, C, can be described by a power function:

$$s^2 = a \times C^b \quad (3)$$

where a and b are constants determined from regression analysis.

Second, for each variance component (Figures 2–4), the variance values, identified by type of fumonisin, appear to have about the same slope. If this is true, then the variances for each type of fumonisin can be pooled, and equation 3 for each variance component would be independent of fumonisin type. The general linear model (GLM) procedure in SAS was used to check the homogeneity of the slope values associated with fumonisin type for each variance component. The test indicated that the slopes were not significantly different at the 95% confidence level.

From regression analysis, the values of a and b in equation 3 that describe the sampling, sample preparation, and analytical variances as function of fumonisin concentration (independent of the type of fumonisin) were determined:

$$s^2_s = 0.033 \times C^{1.75} \quad (4)$$

$$s^2_{sp} = 0.011 \times C^{1.59} \quad (5)$$

$$s^2_a = 0.014 \times C^{1.44} \quad (6)$$

From the regression analysis, the coefficients of determination (R^2) for equations 4–6 are 0.946, 0.87, and 0.882, respectively. R^2, is the square of the correlation coefficient and $100R^2$ indicates the percentage of the sum of squares or variability that is accounted for by the regression equation.

The total variance associated with the fumonisin test procedure can be estimated by adding the sampling (equation 4), sample preparation (equation 5), and analytical (equation 6) variances, as shown in equation 2.

$$S^2_t = (0.033 \times C^{1.75}) + (0.011 \times C^{1.59}) + (0.014 \times C^{1.44}) \quad (7)$$

The total variance in equation 7 is specific for the test procedure used in this study (1.1 kg sample, Romer mill, 25 g subsample, 1 aliquot, and liquid chromatography [LC]).

As an example, the sampling, sample preparation, analytical, and total variances expected for the test procedure when fumonisin concentration is 2 ppm are 0.111, 0.033, 0.038, and 0.182, respectively. Here, sampling variance accounts for 61% of the total testing variability (s^2_s/s^2_t), sample preparation variance accounts for 18.2% of the total testing variability (s^2_{sp}/s^2_t), and analytical variance accounts for 20.8% of the total testing variability (s^2_a/s^2_t). As with other mycotoxins, sampling is the largest source of variation, especially for small sample sizes. For a normal distribution, a total variance of 0.182 indicates that repeated fumonisin test results will vary about the true lot concentration of 2 ppm by ± 0.85 ppm (2 standard deviations or 95% confidence limits).

The total testing variation can be reduced by reducing the variance associated with one or more of the steps of the testing procedure. Sampling variance can be reduced by increasing sample size, ns, or increasing the number of sampling units. Sample preparation variance can be reduced by increasing subsample size (assume use of the same mill), nss, or increasing the number of subsampling units. Analytical variance can be reduced by quantitating fumonisin in more than one portion from the blended extract, na (assume use of the same analytical method). The effects of ns, nss, and na on sampling, sample preparation, and analytical variances are shown in equations 8–10, respectively.

$$s^2_s = (1.1/ns) \times 0.033 \times C^{1.75} \quad (8)$$

$$s^2_{sp} = (25/nss) \times 0.011 \times C^{1.59} \quad (9)$$

$$s^2_a = (1/na) \times 0.014 \times C^{1.44} \quad (10)$$

where ns is in kg, nss is in g, and na is number of portions.

The total variance associated with a fumonisin test procedure for any sample size, any subsample size (Romer mill), and any number of aliquots (LC) can be determined by adding equations 8–10.

$$s^2_t = (0.0363/ns) \times C^{1.75} + (0.275/nss) \times C^{1.59} + (1/na) \times 0.014 \times C^{1.44} \quad (11)$$

Because a different cost is associated with reducing the variability of each step of the fumonisin test procedure, it is important to consider cost as well as the expected reduction in variability when designing a fumonisin test procedure.

It would be of interest to compare the variability of the test to measure fumonisin in shelled corn to the variability of the test to measure aflatoxin in shelled corn. It is difficult to compare the variabilities by using variance because aflatoxin concentration is usually reported in parts per billion (ng aflatoxin/g corn) and fumonisin is usually reported in ppm (μg fumonisin/g corn). However, the coefficient of variation (CV) can be used to compare the variabilities of the 2 mycotoxin test procedures, because CV is a relative measure of variation and is a dimensionless variable. The CV associated with each step of the fumonisin test procedure was computed from variance equations 4–6 over a range of fumonisin concentrations and is plotted as a continuous or smooth curve in Figure 5. For example, at a fumonisin concentration of 2 ppm, the CVs associated with sampling, sample preparation, and analysis are about 16.6, 9.1, and 9.7%, respectively. The CV for the fumonisin test procedure is about the same as that for testing shelled corn for aflatoxin (4, 9).

Summary and Discussion

The variability associated with the test procedure to measure fumonisin in shelled corn is similar to that associated with the test procedure to measure aflatoxin in shelled corn. For small sample sizes, sampling variance is the largest source of the total

testing variation. For example, when testing a bulk corn lot with a fumonisin concentration of 2 ppm, the CVs associated with sampling, sample preparation, and analysis are 16.6, 9.1, and 9.7%, respectively. The variances associated with each step of the test procedure increase with fumonisin concentration. Regression equations were developed to predict the variance as a function of fumonisin concentration for each step of the fumonisin test procedure. They were independent of fumonisin type (B1, B2, B3, or total).

From variance estimates, the effect of the test procedure on confidence limits with which fumonisin concentration is being estimated can be determined. Further studies are needed to determine the type of distribution (symmetrical, skewed, etc.) that will best describe the distribution of sample test results from a given lot so the performance of fumonisin sampling plans can be predicted more accurately.

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