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Performance of the Visual, Minicolumn and TLC Methods in Detecting Aflatoxin in 20 Contaminated Lots of Farmers Stock Peanuts

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ABSTRACT

Standard grade samples (16) from each of 20 selected minilots were used to evaluate three methods for detecting minilots of farmers stock peanuts with unacceptable concentrations of aflatoxin. A visual, a minicolumn and a modified thin layer chromatography (TLC) method were used to compare analytical results, variation, and probability of acceptance for minilots having mean aflatoxin concentrations ranging from 8 - 255 ppb. Mean values obtained by each of the three methods increased linearly with mean aflatoxin concentrations of the minilots and variation for each method as determined by the variance and coefficient of variation (CV) was very large. The CV for all three methods decreased as aflatoxin concentration increased. Overall performances of the three methods were similar in accepting and rejecting these minilots on the basis of the 1.8 kg grade samples. The greatest difference in the three methods occurred at the zero acceptance level where the modified TLC, minicolumn and visual methods rejected 97, 98 and 88%, respectively, of the minilots with more than 60 ppb aflatoxin. At this acceptance level the TLC, minicolumn and visual methods also rejected 55, 50 and 30%, respectively, of the minilots with less than 30 ppb aflatoxin.

Key Words: Aflatoxin, aflatoxin detection, *A. flavus*, TLC, minicolumn, visual, sampling, variance, analytical, coefficient of variation.

Aflatoxin produced by *Aspergillus flavus* in agricultural commodities is of great concern to the agricultural industry and the consumer. The U. S. peanut industry has been a leader in the prevention, detection and removal of aflatoxin (12). A provision of the USDA Peanut Marketing Agreement (9) requires that all of the kernels from each official grade sample of farmers stock peanuts be examined by the Dickens method (3,4,5) for visible growth of the aflatoxin-producing mold, *Aspergillus flavus*. Lots found to contain kernels with visible *A. flavus* growth are not allowed to enter the edible channels, but such lots are segregated (Segregation 3) for restricted oil processing. Economic losses associated with Segregation 3 peanuts and continuing efforts to reduce aflatoxin in peanuts have emphasized the need to develop improved methods for detecting lots of farmers stock peanuts with unacceptable concentrations of aflatoxin.

The visual method (3, 4, 5) for farmers stock peanuts has been used in conjunction with a comprehensive aflatoxin testing program (5) for the shelled peanuts to provide a means for preventing the edible use of raw skin peanuts that have an aflatoxin concentration greater than 25 ppb. The BF (8) method has been adopted as official first action by the AOAC and AOCS. The Agricultural Marketing Service (AMS) of the USDA (13) uses a modification of the BF method along with thin-layer chromatography (TLC) for determining the aflatoxin concentration in shelled peanuts. In this report we will refer to the AMS procedure as the "standard TLC" method. The method used to evaluate the 1.8 kg grade samples will be referred to as the "modified TLC" method.

The Holaday minicolumn method (6,7) was developed at the National Peanut Research Laboratory as a low-cost rapid chemical method for detecting aflatoxins in agricultural products. This method has been evaluated in collaborative studies (11) and found to accurately detect known amounts of aflatoxin in ground, blended samples of corn and peanuts. A modified version of this method, the Holaday-Velasco method, has been approved by the Association of Official Analytical Chemists (AOAC) (8) and the American Association of Cereal Chemists (AACC) (10) for determining aflatoxin in ground, blended samples.

The objective of this paper is to compare the performance of the visual, TLC and minicolumn methods relative to detecting lots of farmers stock peanuts with un-

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acceptable concentrations of aflatoxin. The comparisons in this manuscript are based upon the current official grade sample size (1800 g) and 20 Segregation 3 minilots (8-255 ppb) of Crop Year (CY) 1980 Florunner peanuts. Estimates of aflatoxin concentration in the minilot portions are presented as well as the *A. flavus* and aflatoxin data for 16 grade samples from each minilot. The mean and variation of measurements of *A. flavus* kernels or aflatoxin for the grade samples are then correlated with the respective mean aflatoxin content of the minilot. Finally, comparisons were made of the average performance of the three methods in detecting minilots with unacceptable levels of aflatoxin.

Materials and Methods

During the normal marketing of CY 1980 peanuts, 40 farmers stock lots were selected from lots identified as Segregation 3 peanuts by the Dickens method (3,4,5). These lots came from 40 different farms in Terrell County, Georgia. To minimize sampling errors (14), a large minilot (approximately 61 kg) was removed from each of these lots with the Federal State Inspection Service pneumatic sampler (2). As diagrammed in Figure 1, each minilot was divided in half (Half A and Half B) by using the Federal State Inspection Service farmers stock divider. Half A of each lot was cleaned (foreign material was removed) and the loose shelled kernels (LSK) and inshell peanuts were separated to permit independent aflatoxin (standard TLC method) analyses of the LSK and shelled portions by the AMS Laboratory in Albany, Georgia. The aflatoxin determinations of Half A were used to select 20 of the 40 minilots that had estimated aflatoxin levels within the 5 to 300 ppb range. As diagrammed in Figure 1, the other half (Half B) of the minilot from each of the 20 selected lots was divided into 16 official grade samples by using the Federal State Inspection Service farmers stock divider. The number of kernels or kernel pieces with visible *A. flavus* growth in the grade sample were counted, the aflatoxin concentration in each grade sample was determined by the modified TLC and minicolumn methods. The residue from Half B was evaluated for aflatoxin by the standard TLC method. The weighted mean aflatoxin concentration of each minilot was calculated using the standard TLC values for Half A and residue of Half B as well as the modified TLC values for the grade samples.

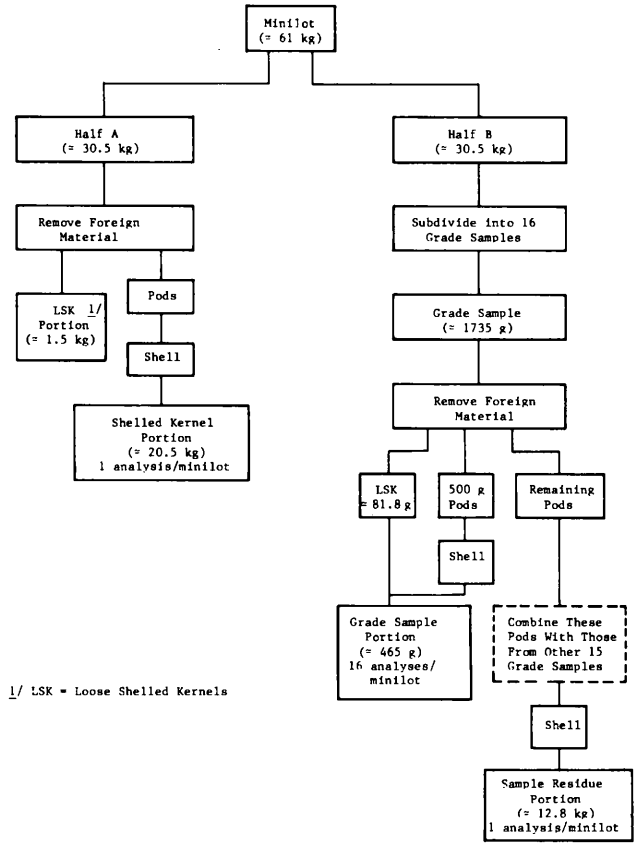

Fig. 1. The procedure used in obtaining the various portions for each of the 20 selected minilots.

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Initial extractions for minicolumn and modified TLC analyses were performed by blending the approximately 500 g samples (2:1, w/v) with methanol:water (4:1, v/v) for 1 min in 2-L blending jars. The extract was vacuum filtered through a Reeve Angel[1] glass fiber filter paper (934AH) to obtain approximately 250 mL. Of this extract, 125 mL was placed in plastic bottles to be delivered for modified TLC analysis.

The modified TLC method consisted of adding 60 mL of water to 100 mL of the minicolumn filtrate and blending 30 sec. Hexane (70 mL) was added to the aqueous filtrate and blended for an additional 30 sec. The entire 230 mL was then centrifuged and 50 mL of methanol:water extract was analyzed as with the standard TLC method.

For the minicolumn method, a 15 mL aliquot was analyzed similar to published procedures (6,7) by adding 15 mL of salt solution (600 g sodium chloride, 600 g zinc acetate and 15 mL glacial acetic acid in 4L distilled water) to the aliquot in a test tube. The tube was closed and shaken vigorously for ca. 10 sec and the mixture was filtered (Reeve Angel 934AH) to obtain 15 mL. To this 15 mL aliquot, 3 mL of toluene was added with gentle mixing. After the layers separated, 1 mL of the toluene layer was placed on a minicolumn attached to a vacuum source. The toluene was followed with 2 mL of methylene chloride:acetone (90:10, v/v). A blue band at the interface of the Florisil and alumina in the minicolumn indicated the presence of at least 1 ppb of aflatoxin. For positive samples, 1 mL of the toluene was diluted to 10 mL and 0.12 mL was added to a minicolumn. A blue band at the interface indicated at least 100 ppb; however, if no band was present, aliquots of 0.04, 0.08, 0.24, 0.32 and 1.6 mL, each followed by 2 mL of methylene chloride:acetone (9:10, v/v), were added sequentially to the same minicolumn until a blue band was present. Presence of a blue band after addition of these aliquots was indicative of at least 75, 50, 25, 15, and 5 ppb, respectively. If the sample contained at least 100 ppb, a second 1:10 dilution (i.e. 1:100) was made and the indicated aflatoxin concentrations increased by a factor of 10.

Results

Aflatoxin Results for Minilots and Minilot Portions

The aflatoxin data for each of the 20 selected minilots and their portions are presented in Table 1. As expected (4), the LSK portion usually had much higher concentrations of aflatoxin than the shelled portion. Since the residue and shelled portions contained no LSK, these portions generally had lower aflatoxin values than for the mean aflatoxin of the 16 grade samples. Comparison of total aflatoxin values for Half A with Half B and comparison of aflatoxin in the shelled kernels (Half A) with that in the residue (Half B) indicate that there were large sampling errors even though extensive mixing and blending procedures were used. Nine of the minilots had a mean aflatoxin concentration less than 50 ppb. Thirteen of the minilots had a mean aflatoxin concentration less than 150 ppb. The remaining 7 minilots had a mean aflatoxin concentration ranging from 157 to 255 ppb.

Table 1. Aflatoxin concentrations (ppb) in twenty 61-kg minilots and their protions.

Minilot No.	Half A[1] LSK Portion	Half A[1] Shelled Kernel Portion	Half A[1] Total[2]	Half B (Mean of 16 Grade Samples) Residue Portion[1]	Half B (Mean of 16 Grade Samples) Sample Portions[3]	Half B (Mean of 16 Grade Samples) Total[4]	Minilot[5]
1	78	3	7	3	16	8	8
2	64	0	6	11	21	15	10
3	64	3	10	11	33	19	14
4	0	16	15	32	22	28	21
5	43	3	6	48	40	44	25
6	46	46	46	0	20	7	27
7	419	23	37	2	92	32	34
8	414	22	42	2	113	41	42
9	465	6	36	7	119	47	42
10	892	27	67	29	97	51	59
11	458	32	56	27	154	72	64
12	1105	106	159	34	119	62	111
13	1338	113	158	48	182	93	128
14	535	182	208	55	173	101	157
15	1795	56	183	28	365	148	166
16	425	128	166	163	172	167	166
17	2348	52	193	43	383	164	179
18	1268	71	182	266	170	220	198
19	1595	194	271	230	175	210	242
20	376	291	300	184	245	203	255

1/ Aflatoxin concentrations were determined by the standard TLC method.
2/ Value is weighted mean of LSK and shelled kernel portions.
3/ Aflatoxin concentrations were determined by the modified TLC method.
4/ Value is weighted mean of sample and residue portion.
5/ Value is weighted mean of the total values.

A flavus and Aflatoxin Results for the 320 Grade Samples

The visible *A. flavus* and aflatoxin data for each of the grade samples are presented in Table 2. There were many more zero values for either of the three methods for minilots 1-9 (low aflatoxin levels) than for minilots 14-20 (high aflatoxin levels). Using the minicolumn and TLC methods aflatoxin was found in 85.1% and 97.8% of the same samples for minilots 1-9 and minilots 10-20 respectively. Using the visual method, there was also good agreement (87%) as to the presence of *A. flavus* kernels and aflatoxin (modified TLC) in the samples from minilots 10-20, but poor agreement (60%) in results from minilots 1-9. Generally, the number of *A. flavus* kernels or aflatoxin concentration of a single grade sample did not provide a reliable estimate of the aflatoxin concentration of the minilot. Even though the same extract was evaluated by the minicolumn and modified TLC methods, occasionally there were large differences in the aflatoxin concentration determined by these two methods.

Means, Variances and Correlations with Aflatoxin Concentration of Minilots

The means and variances of the *A. flavus* and aflatoxin determinations for the 16 grade samples are presented in Table 3. As expected, the mean number of *A. flavus* kernels and the mean aflatoxin concentrations in the 16 grade samples as determined by the three methods increased with aflatoxin concentration of the minilots. Both Student's t test and the nonparametric sign test indicated that the mean aflatoxin concentrations of the minilots as determined by the minicolumn method were not significantly different from those respective means determined by the modified TLC method.

Table 3. Mean and variance for each minilot.

Minilot No.	Minilot aflatoxin concentration (ppb)	Mean number of A. flavus kernels in 16 grade samples	Aflatoxin concentration in 16 grade samples TLC[1] (ppb)	Aflatoxin concentration in 16 grade samples Minicolumn (ppb)	Variance[2] Visual	Variance[2] TLC	Variance[2] Minicolumn
1	8	0.2	16	96	0.46	1374	97213
2	10	0.5	21	22	0.82	3947	2766
3	14	1.2	33	29	2.56	2135	970
4	21	2.9	22	67	5.59	977	23703
5	25	0.3	40	22	0.73	4420	1245
6	27	0.2	20	19	0.57	3981	2445
7	34	1.4	92	66	1.30	16572	3404
8	42	2.0	113	134	3.82	31344	41688
9	42	0.7	119	32	1.14	92420	9340
10	59	2.0	97	116	2.51	13163	8981
11	64	2.2	154	130	8.45	64599	47309
12	111	2.4	119	113	4.98	24675	9104
13	128	2.8	182	190	6.18	44591	100352
14	157	4.4	173	217	3.95	23437	39300
15	166	8.6	365	355	9.78	148473	135852
16	166	3.4	172	356	5.08	29549	125764
17	179	7.8	383	451	13.85	90870	148665
18	198	5.6	170	287	5.82	20804	45290
19	242	3.1	175	335	2.29	32613	72484
20	255	5.5	245	294	3.05	67170	91916

1/TLC values were determined by the modified TLC method.
2/Variance consisted of sampling plus analytical variances. Variance for the visual method was assumed to be equal to its sampling variance (i.e. analytical variance = 0).

Linear regressions of the mean data are presented in Figure 2.

Total variances were very large (Table 3). Procedures to partition out the analytical and sampling variance for each minilot were unreliable since they often resulted in negative variance estimates.

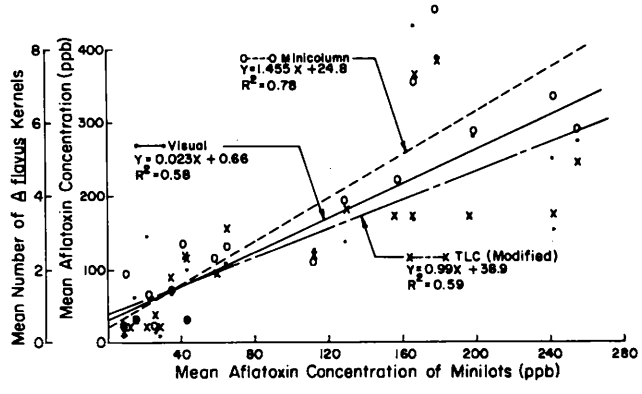

Fig. 2. Linear regression of mean number of *A. flavus* kernels and mean aflatoxin concentration of 16 grade samples on the mean aflatoxin concentration of the minilot.

Quadratic regressions of the coefficients of variation for the three methods on the mean aflatoxin concentrations of the minilots are shown in Fig. 3. The regression

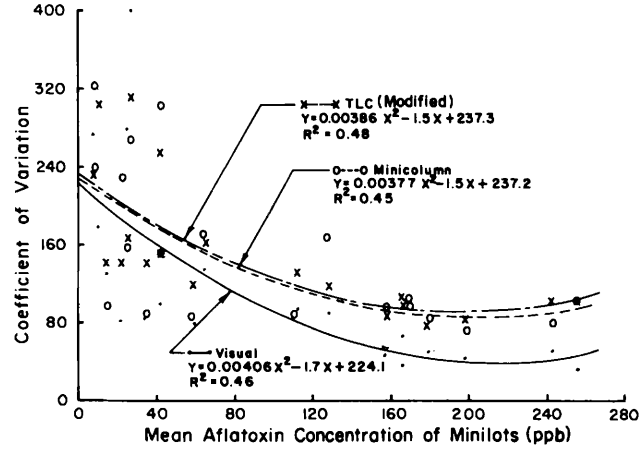

Fig. 3. Quadratic regression of coefficient of variation on the mean aflatoxin concentration of the minilot.

Table 2. Visible *A. flavus* and aflatoxin data obtained from 320 grade samples.

Minilot No.	Mean aflatoxin concentration of minilot (ppb)	Method	Number of visible A. flavus kernels (visual) and aflatoxin concentration in ppb (minicolumn[1] TLC[2]) for respective grade samples															
			Grade Sample															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		Visual	0	0	0	0	0	0	0	0	2	0	0	0	0	2	0	0
1	8	TLC	12	0	12	0	11	0	3	0	0	50	21	0	0	146	0	0
		Minicolumn	50	0	0	0	15	0	0	0	0	150	5	0	0	1000	0	0
		Visual	0	0	2	0	0	0	0	0	0	0	0	0	2	2	2	0
2	10	TLC	0	0	12	0	0	0	6	0	0	35	0	0	27	0	253	0
		Minicolumn	5	0	5	0	0	0	5	0	0	75	0	0	25	0	150	0
		Visual	4	2	0	1	1	1	0	0	4	0	0	0	3	4	0	0
3	14	TLC	0	0	22	125	12	6	37	12	12	0	6	47	6	-	150	58
		Minicolumn	5	0	15	25	25	15	5	25	15	0	5	25	25	0	100	50
		Visual	7	0	7	2	0	3	2	2	3	3	6	0	4	0	5	3
4	21	TLC	110	0	22	21	0	0	25	31	0	11	50	0	7	0	70	0
		Minicolumn	500	0	25	15	0	0	75	75	0	15	50	0	5	0	100	0
		Visual	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0	0
5	25	TLC	102	0	62	256	37	0	6	6	12	12	0	-	7	50	8	41
		Minicolumn	100	0	0	25	50	0	0	5	0	0	0	5	0	25	5	50
		Visual	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	27	TLC	0	0	0	0	0	253	6	46	11	0	5	4	0	0	0	0
		Minicolumn	0	0	5	5	0	150	15	0	25	5	0	5	0	0	0	0
		Visual	2	2	0	2	3	2	0	2	2	2	0	0	3	0	2	0
7	34	TLC	0	12	0	108	248	0	79	360	394	110	57	44	7	6	17	22
		Minicolumn	0	15	0	75	150	0	100	100	15	100	75	75	5	50	50	25
		Visual	7	3	0	1	0	2	2	2	0	3	5	0	2	3	2	0
8	42	TLC	41	353	36	0	0	0	19	0	6	300	5	50	369	560	76	0
		Minicolumn	100	100	25	0	5	0	25	0	5	500	15	100	150	500	150	0
		Visual	0	0	0	0	3	0	0	1	0	0	1	0	2	0	1	3
9	42	TLC	0	5	0	0	662	6	5	1075	0	3	0	0	7	0	3	132
		Minicolumn	0	0	0	0	250	0	0	0	0	0	0	0	0	0	5	100
		Visual	4	3	1	2	1	1	2	2	3	2	0	6	0	2	3	0
10	59	TLC	6	380	79	0	6	44	124	84	50	76	200	36	66	66	340	0
		Minicolumn	50	100	50	5	5	150	150	75	100	50	100	150	100	75	250	0
		Visual	0	2	2	1	3	3	4	0	12	2	0	2	2	2	0	0
11	64	TLC	87	83	17	119	44	46	54	12	149	22	177	54	100	994	508	0
		Minicolumn	100	75	25	75	75	0	75	15	100	5	15	75	50	750	250	0
		Visual	0	7	4	3	1	3	2	1	2	2	3	7	0	0	4	0
12	111	TLC	6	39	227	99	0	11	-	108	62	82	113	79	66	645	157	86
		Minicolumn	5	50	100	75	0	15	25	75	75	150	100	250	100	150	150	50
		Visual	0	6	6	2	3	7	4	0	6	3	1	3	0	0	3	0
13	128	TLC	57	24	323	161	54	180	43	50	12	66	288	634	12	276	677	63
		Minicolumn	75	25	100	100	50	100	25	25	25	75	150	1000	15	100	500	50
		Visual	10	4	2	4	5	5	5	6	4	5	2	5	2	2	5	4
14	157	TLC	100	38	157	54	360	62	253	175	265	475	32	22	157	44	480	93
		Minicolumn	100	5	100	15	250	75	250	250	100	500	100	75	100	50	500	150
		Visual	10	7	14	15	6	8	10	10	7	10	10	10	3	6	5	7
15	166	TLC	27	161	392	653	323	129	630	221	177	180	513	677	50	19	145	1541
		Minicolumn	75	75	100	250	100	100	500	150	150	250	500	1000	25	50	100	1000
		Visual	4	5	0	5	0	6	2	4	2	4	8	3	2	4	0	5
16	166	TLC	25	276	44	127	315	132	38	215	6	127	125	0	50	466	207	604
		Minicolumn	75	250	25	150	500	100	75	500	750	100	250	15	50	1000	150	500
		Visual	20	10	7	9	6	11	8	7	4	4	8	6	5	8	5	6
17	179	TLC	215	490	108	365	79	63	160	187	699	19	991	495	677	778	171	633
		Minicolumn	500	250	100	250	100	50	100	500	500	5	1000	500	150	1000	150	500
		Visual	9	5	5	6	3	7	4	4	5	4	4	1	9	9	6	9
18	198	TLC	190	-	153	538	37	63	41	50	346	219	250	177	25	6	238	221
		Minicolumn	250	248	100	500	25	75	75	75	250	250	250	250	100	25	500	500
		Visual	3	3	0	3	4	4	0	3	5	2	3	2	4	5	5	3
19	242	TLC	145	415	22	41	353	79	0	0	551	88	165	37	127	490	54	229
		Minicolumn	250	250	15	50	250	100	0	500	750	100	250	250	150	750	150	250
		Visual	8	6	8	5	3	4	3	7	4	7	6	7	4	7	6	3
20	255	TLC	129	-	453	253	66	44	165	265	196	1060	48	256	430	163	95	57
		Minicolumn	250	100	250	75	75	25	100	500	250	1000	100	250	250	100	100	100

[1] Minicolumn values represent the lowest estimate of the aflatoxin band (B) as follows:

0 = B <1, 5 = 5 <B <15, 15 = 15 <B <25, 25 = 25 <B <50, 50 = 50 <B <75, 75 = 75 <B <100, 100 = 100 <B <150,

150 = 150 <B <250, 250 = 250 <B <500, 500 = 500 <B <750, 750 = 750 <B <1000, 1000 = 1000 <B

[2] TLC values were determined by the modified TLC method.

equations were similar. The coefficients of variation decreased substantially as the mean aflatoxin of the minilots increased from 8 to 100 ppb. Based upon Whitaker's work on sampling of shelled peanuts (15), Figure 3 indicates that sampling errors were much larger than the analytical errors. The visual method had the lowest coefficient of variation. The coefficient of variation for the modified TLC and minicolumn methods

were practically the same.

Detection of Minilots with Unacceptable Levels of Aflatoxin

The percent of samples indicating rejection (R) from each of the 20 minilots when either of 5 different acceptance levels were used for each of the three methods are given in Table 4. The percent of samples from these minilots indicating rejection decreased as acceptance levels increased. For all three methods the percent of samples rejected at levels 3 and 4 with aflatoxin concentration as high as 260 ppb was generally 80% or less. At the 0 acceptance level (maximum sensitivity), the percent of samples rejected with the modified TLC and minicolumn methods was higher than the visual method. For example, the modified TLC and minicolumn rejected 97 and 98%, respectively, of the samples from minilots having aflatoxin concentrations above 60 ppb aflatoxin and the visual method rejected 88% of the samples from the same minilots. On the other hand, the modified TLC and minicolumn methods rejected about 55 and 50%, respectively, of the samples from minilots having aflatoxin concentrations below 30 ppb and the visual method rejected about 30% of the samples from these same minilots. The percent rejection by the visual method at the 0 level appeared to be in the range of that predicted by Dickens and Satterwhite (4). The percent of samples indicating acceptance (P) is the complement of the percent of samples indicating rejection (R) (i.e. P = 100 - R).

If P_i is the percent of grade samples accepted in the i-th minilot, (with aflatoxin concentration A_i), the average percent samples accepted is

$$P = (P_1 + P_2 + \ldots + P_{20})/20.$$

Assume a population of N lots, with the same aflatoxin distribution as the 20 minilots in the experiment. Thus, NP_1 lots with aflatoxin concentration A_1, NP_2 lots with aflatoxin concentration A_2, etc. will be accepted, so that the average aflatoxin concentration of the accepted lots will be

$$A = (NP_1A_1 + NP_2A_2 + \ldots + NP_{20}A_{20})/(NP_1 + NP_2 + \ldots + NP_{20})$$
$$= (P_1A_1 + P_2A_2 + \ldots + P_{20}A_{20})/(P_1 + P_2 + \ldots + P_{20}).$$

The values of P and A were calculated for each method and each acceptance level. These values are plotted in Figure 4. Even though these points do not represent continuous functions, regression equations were developed. The regression equations of P on A were very similar for all 3 methods. The correlation coefficients were very high. Regression analyses indicated the lines were parallel and that fitting all the calculated data to

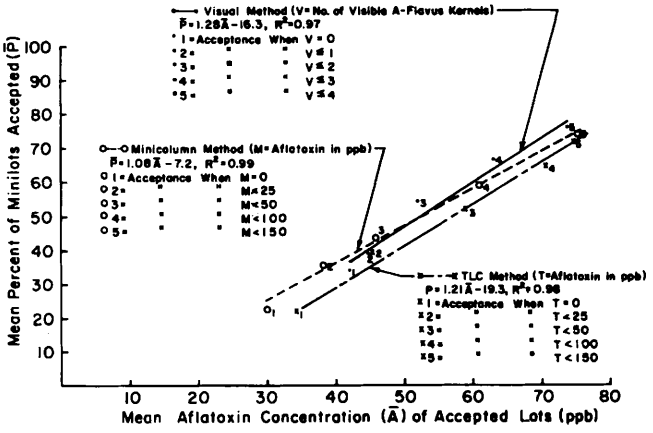

Fig. 4. Average percent of minilots accepted and average aflatoxin of accepted minilots ($8 \leq A \leq 255$) at 5 sensitivity levels.

Table 4. Percent of samples from each of the 20 minilots indicating rejection when using various acceptance levels[1].

Minilot No.	Aflatoxin concentration of minilot (ppb)	Visual method acceptance levels (no. kernels)					TLC method[2] acceptance levels (ppb)					Minicolumn method acceptance levels (ppb)				
		0(0)	1(1)	2(2)	3(3)	4(4)	0(0)	1(25)	2(50)	3(100)	4(150)	0(0)	1(25)	2(50)	3(100)	4(150)
1	8	12	12	0	0	0	44	12	6	6	0	31	19	19	12	12
2	10	25	25	0	0	0	31	19	6	6	6	38	19	12	6	6
3	14	50	31	25	19	0	80	33	20	13	0	81	44	12	6	0
4	21	75	75	56	31	25	56	25	12	6	0	56	38	31	12	6
5	25	12	12	6	0	0	80	40	20	13	7	50	31	19	6	0
6	27	6	6	6	0	0	38	12	6	6	6	44	12	6	6	6
7	34	62	62	12	0	0	81	50	44	31	19	81	62	56	25	6
8	42	69	62	31	12	12	69	50	31	25	25	75	56	44	44	25
9	42	38	19	12	0	0	56	19	19	19	12	19	12	12	12	6
10	59	81	62	31	12	6	88	75	56	25	19	94	81	81	50	25
11	64	69	62	25	12	6	94	75	62	31	19	88	69	62	25	12
12	111	75	62	44	25	12	94	80	73	33	20	94	81	75	44	25
13	128	69	62	56	31	25	100	81	69	44	44	100	94	69	44	19
14	157	100	100	75	75	50	100	94	75	50	50	100	88	88	69	38
15	166	100	100	100	94	94	100	94	81	81	69	100	100	94	75	50
16	166	81	81	62	56	31	94	81	62	62	38	100	94	88	69	56
17	179	100	100	100	100	88	100	94	94	81	75	100	94	94	88	69
18	198	100	94	94	88	62	100	87	67	60	60	100	100	88	69	56
19	242	88	88	75	38	19	88	81	69	50	38	94	88	88	81	69
20	255	100	100	100	81	62	100	100	87	67	60	100	100	94	81	44

1/ The acceptance level as indicated by the numbers in parentheses is either the maximum number of peanut kernels (or pieces) with visible A. flavus growth or the maximum concentration of aflatoxin allowed in the sample before rejection of the lot.

2/ The modified TLC method was used.

one line provided a good fit ($P = 1.15A - 11.4$, $R^2 = 0.95$). The average percent of lots accepted ranged from 34.4 to 75.3, 21.9 to 71.9, and 22.8 to 73.4 for the visual, modified TLC and minicolumn methods, respectively. The visual and minicolumn method tended to provide slightly lower (5 ppb) average aflatoxin in the accepted lots for the same average percentage of accepted lots. The overall performance of the visual and minicolumn methods were similar except at the 0 acceptance level (maximum sensitivity). At this acceptance level the minicolumn accepted approximately 23% of the minilots and the accepted lots had an average aflatoxin concentration of 30 ppb. The visual method accepted about 34% of the lots and the accepted lots had an average aflatoxin concentration of 42 ppb.

Discussion

The large sampling and analytical errors greatly affected the performance of these three methods. Sampling errors were much larger than the analytical errors. The coefficient of variation was less for the visual method than for the minicolumn and modified TLC methods. The smaller coefficient of variation may have resulted from lower variability of the Poisson distribution, a lower sensitivity, integer type measurements and/or because the visual method had no analytical error. The coefficients of variation and statistical analyses indicated that the modified TLC and minicolumn methods provided similar analytical results. The distribution of the modified TLC and minicolumn data is best described by the negative binomial distribution.

Linear regressions of the mean number of *A. flavus* kernels and mean aflatoxin measurements (by modified TLC and minicolumn) on the mean aflatoxin of the minilots were significant, but the coefficients of determination ranged from only 0.58 to 0.78. Based upon the regression and correlation coefficients, the minicolumn had the best response to the mean aflatoxin of the minilot. It was quite evident from this study that neither the number of *A. flavus* kernels nor the aflatoxin concentration in an 1800 gram official grade sample provided a reliable estimate of the aflatoxin concentration in a lot of farmers stock peanuts. However, it was quite evident that the presence of *A. flavus* kernels or aflatoxin in the official grade sample would mean that some aflatoxin was present in the load of farmers stock peanuts, and segregating these loads (zero acceptance level) would prevent the acceptance of many lots with very high aflatoxin concentrations. At the zero acceptance level, the visual method rejected most of the minilots with mean aflatoxin concentration above 150 ppb while the modified TLC and minicolumn rejected most minilots with aflatoxin concentration above 60 ppb. Thus, the modified TLC and minicolumn showed potential at the zero acceptance level in reducing the risk of accepting lots with high concentrations of aflatoxin. However, at this acceptance level the modified TLC and minicolumn will reject more lots and the economic losses to the farmer because of Seg. 3 peanuts would be greater. If an above zero acceptance level is allowed in farmers stock peanuts, the evaluation of a second larger sample from the rejected lots would reduce these economic losses. Since aflatoxin concentrations are generally much higher in LSK, damaged kernels, small kernels, and kernels with low specific gravity than for other peanut fractions (1), it appears that sampling plans and marketing procedures can be improved.

The economic losses to the farmer and risk of rejecting lots with low levels of aflatoxin concentration could also be reduced by using higher acceptance levels. However, the risk of accepting highly contaminated lots would also become much greater. At the higher acceptance levels, the overall performance of the three methods were very similar. It appeared that the visual and minicolumn methods accepted lots with slightly lower levels of aflatoxin than did the modified TLC method.

Using the current sample size, the only apparent benefits of using the minicolumn and modified TLC methods to detect lots of farmers stock peanuts with unacceptable aflatoxin concentration is at the zero acceptance level to provide a lower risk of accepting highly contaminated lots. However, with the current sampling plan, this procedure would increase the chances of rejecting lots with low concentrations of aflatoxin. Use of larger sample sizes and improved sampling methods would benefit all three methods. However, the intended use of the peanuts and the additional cost must be considered along with the benefits to determine the feasibility of such changes.

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