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Comparison of the Amounts of Aflatoxin Extracted From Raw Peanuts Using AOAC Methods I and II[1]

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ABSTRACT

Four lots of raw shelled peanuts, naturally contaminated with aflatoxin, were each ground into a paste. Sixty-four 50-g samples were removed from three of the lots and forty 50-g samples were removed from the fourth lot. For each lot, aflatoxin was extracted from half of the samples by the AOAC Method I (CB) and from the remaining half by the AOAC Method II (BF). The 4 lots averaged 57.8, 127.6, 238.5, and 447.1 parts per billion (ppb) total aflatoxin when measured by the CB method. On the average across the four lots, the BF method extracted 26, 25, 22, and 18% less aflatoxin B1, B2, G1, and G2, respectively, than the CB method.

Key Words: Aflatoxin, peanuts, extraction, efficiency, AOAC Methods I and II

Method I (CB) and Method II (BF) are AOAC approved quantitative procedures to analyse aflatoxin in raw peanuts and peanut products (1). The BF method is considered to be faster and more economical, but the CB method is reported to provide a better cleanup (2). The BF and other similar methods (3, 4) are generally used by aflatoxin laboratories in industry and government to test peanuts and peanut products for aflatoxin (5).

The results of 21 tests which compared the BF and CB methods are summarized in Table 1. Fifteen of the 21 tests made in the studies were on peanuts or peanut products. The BF method is not AOAC approved for analysis of wheat flour and corn meal used in 4 of the tests. For approved products, the average of analyses by the CB method was higher than the average by the BF method 76% of the time for aflatoxin B1, 92% of the time for aflatoxin B2, 58% of the time for aflatoxin G1, 82% of the time for aflatoxin G2 and 62% of the time for total aflatoxin. When statistical analysis was used to differentiate between sample means for approved products the mean of analysis by the CB method was shown to be significantly higher than for the BF method 27, 33, 33, 11, and 33% of the time for B1, B2, G1, G2 and total aflatoxin, respectively. The BF method was never shown to be significantly higher than the CB method.

Seventeen of the 21 tests were based on studies which involved 7 or more laboratories. Variability among laboratories might have prevented the detection of statistically significant differences between the BF and the CB method in some of these 17 tests. A statistical analysis was not made to differentiate between sample means by the 2 methods for 4 tests based on analyses within the same laboratory (tests 14 through 17).

The purpose of this study was to compare the BF and CB methods for raw peanuts when a large number of replicated analyses were made on samples from homogenous, naturally contaminated peanut material.

Procedure

Three 15 kg lots of raw shelled peanuts, each naturally contaminated with a different concentration of aflatoxin, were ground into a paste with a Morehouse stone mill. A fourth lot of raw peanut paste, naturally contaminated with aflatoxin, was obtained from the Food and Drug Administration, Washington, DC. Each lot of paste was thoroughly blended and kept in cold storage during the testing period. Sixty-four 50-g samples were removed from 3 of the lots and forty 50-g samples were removed from the FDA lot. For each of the 4 lots, half of the samples were analysed for aflatoxin by the CB method and half by the BF method. Samples from only 1 lot were analysed at a time. Because of slow filtration and concern over excessive chloroform evaporation, centrifugation was substituted for filtration in the extraction step of the CB method. Sample extract from each analytical method was spotted on thin layer chromatography (TLC) plates in pairs (one extract from the BF method and one extract by the CB method). The pair of sample extracts was spotted on 2 TLC plates (2 replications) for two of the lots and on 4 TLC plates (4 replications) for the third and fourth lots (16). The spots were quantified densitometrically (17). Computation of ppb values associated with the CB method incorporated a correction factor, described in 26.031 of the AOAC Official Methods of Analysis, that accounts for the high fat content of peanuts.

Results and Discussion

The average concentration and average coefficient of variation (CV) associated with analysis of B1, B2, G1, G2, and total aflatoxin by the CB and BF methods for the four lots of raw peanut paste are shown in Table 2. The total aflatoxin in lots 1, 2, 3, and 4 (determined by the CB method) averaged 57.8, 238.5, 447.1 and 127.6 parts per billion (ppb), respectively. Each aflatoxin value in the table is the average of 64 observations (2 plates per

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Table 1. A summary of studies comparing aflatoxin analyses by the CB and BF methods.

Investigator	Year	Commodity[3]	Test	Number of Collaborators	Type of Aflatoxin[4] B1	B2	G1	G2	Total	Statistical[5] Analysis	Reference
Waltking	1970	Peanut Butter (S)	1	15	BF	-	BF	-	BF	Yes	15
		Peanut Butter (N)	2	15	BF	-	-	-	BF	Yes	15
Coon, et al.[1]	1972	Peanut Meal (N)	3	129	CB*	CB*	CB*	CB	CB*	Yes	2
		Deoiled Peanut Meal (N)	4	129	CB	CB	CB*	CB	CB*	Yes	2
		Raw Peanut Butter (N)	5	129	BF	BF	BF	BF	BF	Yes	2
		Roasted Peanut Butter (N)	6	129	CB	CB	CB	CB	CB	Yes	2
Coon, et al.[1]	1973	Roasted Peanut Butter (N)	7	42	CB	CB	BF	CB	BF	Yes	3
Jemmali	1973	Peanut Meal (U)	8	12	CB	-	-	-	-	No	8
		Wheat Flour (U)	9	12	CB	-	-	-	-	No	8
Stack	1974	Peanut Butter (N)	10	7	BF	CB	-	-	BF	No	13
		Peanut Butter (S)	11	7	CB	CB	-	-	CB	No	13
DiProssimo	1974	Pistachio Nuts (S)	12	13	CB	CB*	BF	CB	CB	Yes	6
		Pistachio Nuts (N)	13	13	CB*	CB	CB	CB*	CB*	Yes	6
Shotwell & Goulden	1977	Corn Meal (N)	14	1	CB	CB	CB	CB	CB	No	
		Corn Meal (S)	15	1	CB	CB	CB	CB	CB	No	
Chang, et al.	1979	Peanut Meal (N)	16	1	CB	CB	CB	CB	CB	No	1
		Peanut Butter (N)	17	1	CB	CB	CB	CB	CB	No	1
Friesen, et al.[1]	1980	Peanut Meal (N)	18	139	CB	CB	BF	BF	-	Yes	7
		Peanut Butter (N)	19	139	CB*	CB*	CB*	CB	-	Yes	7
		Corn Meal (N)	20	139	CB*	CB	CB	CB	-	Yes	7
McKinney[2]	1981	Peanut Meal (N)	21	34	CB	-	-	-	-	No	9

[1]International Check Sample Program.

[2]AOCS Smalley Check Sample Program.

[3]The BF method is not approved by AOAC for wheat flour or corn meal. "S" indicates the sample was spiked with aflatoxin, "N" indicates the sample was naturally contaminated, "U" indicates the method of contamination was not given.

[4]The method that gave the highest average result is listed. An asterick denotes that the difference between methods was shown to be statistically significant (note that statistical analysis was not used for some studies). Results were averaged when multiple sample concentrations were used in a test or when both visual and densitometric measurements were made for the same samples.

[5]This column indicates when statistical analysis was used to differentiate between sample means by the two analytical methods.

Table 2. Coefficients of variation and average aflatoxin concentrations associated with the analysis of aflatoxins in raw peanut paste by the CB and BF methods.

Lot	Aflatoxin[1]	Number of Samples	Number of Plates	BF Method AVG (ppb)	BF Method CV (%)	CB Method[2] AVG (ppb)	CB Method[2] CV (%)	Difference in AVG[3] (%)
1	B1	32	2	15.8	28	24.1	18	34*
2	B1	32	2	110.3	19	142.2	23	22*
3	B1	32	4	135.6	14	174.7	16	22*
4	B1	20	4	82.3	8	109.2	7	27*
1	B2	32	2	2.0	35	2.3	23	13*
2	B2	32	2	8.8	36	12.1	34	27*
3	B2	32	4	25.3	22	39.6	18	36*
4	B2	20	4	14.0	19	18.3	22	23*
1	G1	32	2	19.4	36	28.6	27	32*
2	G1	32	2	64.4	33	76.3	31	16*
3	G1	32	4	157.9	19	191.0	21	17*
4	G1	20	4	-	-	-	-	-
1	G2	32	2	2.6	35	2.9	26	10
2	G2	32	2	6.4	52	7.9	47	19
3	G2	32	4	31.0	20	41.7	16	24*
4	G2	20	4	-	-	-	-	-
1	Total	32	2	39.0	30	57.0	21	31*
2	Total	32	2	190.0	23	238.5	24	20*
3	Total	32	4	350.6	15	447.1	16	22*
4	Total	20	4	96.4	8	127.6	8	24*

[1]Total = B1 + B2 + G1 + G2.

[2]Centrifugation was substituted for filtration.

[3]% difference in method AVG = ((AVG(CB) - AVG (BF))/AVG (CB)) 100. The method averages significantly different ($p \leq 0.05$) for the comparison are indicated by an asterick.

sample x 32 samples) for lots 1 and 2, the average of 128 observations (4 plates per sample x 32 samples) for lot 3, and the average of 80 observations (4 plates per sample x 20 samples) for lot 4. In all cases (1 lot did not contain aflatoxin G1 or G2), the concentration of aflatoxin indicated by the CB method was greater than or equal to the concentration indicated by the BF method. In 12 cases, the differences were statistically significant at the 5% confidence level. The percent difference in aflatoxin analyses by the two methods is also shown in Table 2. The percent difference averaged across all lots for B1, B2, G1, and G2 is 26, 25, 22, and 18% respectively. On the average, the BF method indicated 24% less total aflatoxin than the CB method.

The CV values shown in the table contain both sample to sample and analytical variation. The sample to sample variation should be small due to the fine particle size associated with the peanut paste, and it should be of the same magnitude for both methods. The CV associated with each method was about the same and the averaged CV across method and lots was 20% for total aflatoxin. This CV is in good agreement with previous measurements of analytical error (18,19).

While the CB method appears to extract more aflatoxin B1, B2, G1, and G2 from raw peanuts than does the BF method, the BF method is faster and more economical. In this situation it may be appropriate to develop a correction factor for the BF method. Also future studies are required to determine what causes the difference between the two methods.

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