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Modification of the Method 1 AOAC (CB-Method) for the Detection of Aflatoxins

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Many methods are described in the literature for the detection of mycotoxins. Some methods rely on traditional analytical techniques involving thin layer chromatography (TLC), while others require advanced technology that is unavailable in many developing countries. Thus, most developing countries continue to use traditional analytical techniques to detect aflatoxins.

Preliminary studies have been performed to compare various methods of aflatoxin analysis currently in use and to determine the most appropiate methodology with the aim of modifying it to meet the limitations in resources prevailing in most developing countries (Guzman de peña et al. 1988). Method number 1 of the Official Methods of the Association of Official Analytical Chemists (CB-method), Shannon method (modified rapid screening method), and the Eppley method were compared. Results of such study indicated that the CB-method (AOAC 1984) was more efficient than Shannon (Shannon 1973) and Eppley (Eppley et al. 1975) methods. Thus, the CB-method was selected as a convenient starting point for modifications to obtain a method more suitable for the use in developing countries. Furthermore, diverse collaborative studies have found that method number 1 (CB-method) is the most efficient procedure due to the use of chloroform as a solvent for extraction (Wilson 1984). Also, the CB method has been the method of choice for aflatoxin analysis in 60 countries (Friesen 1989). However, the CB- method, as described in the Official Methods of the AOAC, presents two major disadvantages: (1) each analysis requires a time-consuming process and (2) the expense incurred due to the enormous volume of reagents is substantial.–

This communication describes modifications made to the CB-method for the detection of aflatoxin that address the problems and conditions existing in Mexico and other developing countries today. These countries require a method for aflatoxin determination based on simple analytical techniques that are inexpensive, time saving, efficient,

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sensitive, and, most importantly, recognized as valid for international transactions.

MATERIAL AND METHODS

Reagents and apparatus used in this study were as outlined in section 26 of the Official Methods of the Association of Official Analytical Chemists, (AOAC 1984).
Extraction was performed according to section 26.029 (AOAC 1984). Modification of the procedure started when all the extract (220 mL) was precipitated with 50 mL of ammonium sulfate solution (70 g/100 mL water) for 3 min and filtrated; 100 mL of the bottom layer was collected and evaporated to 5 mL for separation under column chromatography.

Column chromatography was performed on a 5-cm plastic syringe cartridge used as a chromatographic column with only 0.5 g of anhydrous sodium sulfate, 1.0 g of 60-200 mesh silica gel and 1.5 g of anhydrous sodium sulfate, that is, 10% of the reagents called for in sec. 26.030, (AOAC 1984). Only 3.3 % of the original solvents was needed to clean the sample (5 mL of hexane and 5ml of diethyl ether) and 5 mL of chloroform : methanol (98:2) to elute aflatoxins from the column. Quantitative TLC was performed as outlined in sec. 26 (AOAC 1984) using 10-cm chromatographic plates.

Two types of experiments were performed to evaluate the modified method. First, the recovery of aflatoxin B_1 in fortified corn was determined. Three different amounts of aflatoxin B_1 were added to non-contaminated corn flour. Pure aflatoxin B_1 was added using microsyringes to 50 g of corn flour contained in 500-mL Erlenmeyer flasks. The flour was mixed with a glass rod 30 min before extraction. The amounts of B_1 added to each 50-g sample were equivalent to 32, 64, and 96 μg/kg with five replicates of each level. The original CB was also evaluated using the same protocol. Second, to compare how both methods perform, analysis of corn with the modified method and the original CB method was performed on 24, 5-kg samples. The samples were collected from three different storage locations in the Northeast of Mexico.

RESULTS AND DISCUSSION

Modifications of the CB-method proposed by our laboratory are : (1) precipitation of the chloroform extract of aflatoxin with ammonium sulfate to reduce sample contaminants, (2) reduction of the extracting volume from 100 mL to 5 mL to permit the use of a 5-cm chromatographic column, (3) reduction to 10 % of the original volume of column reagents and to 3% of the original volume of solvents required for analysis. In addition to the above modifications of the

The amounts of aflatoxin B_1 recovered from the fortified corn using the modified CB method were 21, 44, and 84 μg/kg, and with the original CB method the amounts were 28, 45, and 57 μg/kg, respectively.These amounts of aflatoxin B1 represent recoveries of 65, 68, and 87% with the modified CB, versus 87, 71, and 60% with the original CB considering that the fortified corn originally contained 32, 64, and 96 μg/kg (Table 1). Thus, the performance of detection was similar for both methods since the mean recovery was around 74%. Therefore, the modifications made to the CB-method do not diminish the efficiency of recovery of the original CB-method and the modified-CB could be used in place of the original CB.

Table 1 Recovery of Aflatoxin B_1 from Fortified Corn Samples.

Aflatoxin B_1 Added	Recovery of Aflatoxin B_1			
	modified CB		original CB	
μg/kg	μg/kg*	%	μg/k*	%
32	21+ 0	65	28+ 0	87
64	44+ 4	68	45+ 8	71
96	84+ 0	87	57+ 15	60

* mean of 5 replicates

\+ standard deviation

Table 2 gives the results obtained using the modified and original methods for the analysis of aflatoxin B_1 in naturally contaminated white corn samples. Of the 24 samples analyzed with both methods, 14 samples yielded the same aflatoxin values. Two samples (4 and 23) were negative with the modified CB, but positive with the original CB with values of only 11 μg/kg. This discrepancy is considered acceptable to meet Mexican government regulations because of the low value of the positive measurements. Six samples analyzed by the original procedure yielded values of aflatoxin contamination that were more than two fold those determined by the modified CB. Although consistently lower, the values obtained with the modified CB were positive for such samples .These different values could be interpreted as a consequence of the irregular distribution of aflatoxin within a given lot of grain and to the subsampling error.

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Table 2 Determination of Aflatoxin B_1 in Naturally Contaminated White Corn by the Modified CB (short-CB) and Original-CB (#1 AOAC)

Sample number	Storage location	Aflatoxin B_1 Found µg/ kg	
		modified-CB	original CB
1	Puertecitos	15	66
2	Puertecitos	23	23
3	Puertecitos	132	371
4	Porvenir	<1	11
5	Puertecitos	66	132
6	Puertecitos	15	15
7	Puertecitos	11	11
8	Puertecitos	11	11
9	Puertecitos	11	11
10	Puertecitos	11	23
11	Puertecitos	15	15
12	Rio Bravo	<1	<1
13	Rio Bravo	11	11
14	Puertecitos	<1	<1
15	Puertecitos	92	66
16	Puertecitos	11	66
17	Valle Hermoso	< 1	< 1
18	Puertecitos	< 1	< 1
19	Valle Hermoso	11	11
20	Puertecitos	11	11
21	Rio Bravo	< 1	< 1
22	Puertecitos	11	66
23	Rio Bravo	< 1	11
24	Puertecitos	46	66

These results show that the modified CB method produced an acceptable performance of aflatoxin recovery in fortified corn. Also, the results in naturally contaminated corn are acceptable since only 14 % of the results were different.

The cost of the modified CB method is around 75% cheaper than the original CB. The reasons of this cost reductions are: the small amount of reagents used due to the small size of the column and the reduction of the analysis time. Further reduction in cost is obtained when a rotary evaporator is used to recover chloroform and hexane which can be redistilled and recycled for extraction.

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