A Fluorometric Rapid Screen Method for Aflatoxin in Peanuts

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ABSTRACT

Peanuts were screened for aflatoxin using a rapid, inexpensive fluorometric method. Peanuts were ground and extracted with methanol, and the extract was treated with acidified zinc-acetate-sodium chloride solution, filtered, and diluted with water. Fluorescence of the extracts was compared with that from aflatoxin-free control peanuts. Test samples (160) of several varieties and grades of peanuts were obtained from storage and from several commercial sources and were screened for the presence of aflatoxin. One hundred thirty-five samples (84%) were identified by this method as aflatoxin positive (15 ppb+) or aflatoxin negative (<15 ppb). Although 22 samples (13.6%) were incorrectly labeled as aflatoxin positive, most of these showed evidence of the presence of mold metabolites other than aflatoxin. Three samples (1.8%) were incorrectly labeled as aflatoxin negative when they actually contained 20, 33, and 34 ppb aflatoxin.

INTRODUCTION

A new approach for the quantitative and rapid measurement of aflatoxin in corn and for rapid screening of corn for aflatoxin was recently reported (1,2). These methods were based on the treatment of aqueous solutions of aflatoxin with iodine and a comparison of the fluorescence of spiked and nonspiked extracts of control corn with test sample extracts. These methods were not suitable for peanuts. However, a new procedure was developed for peanuts. This procedure, called the FLAP method (fluorometric analysis of peanuts), is similar to that reported for corn (2), but does not include the enhancement of aflatoxin fluorescence by treatment with iodine, an approach which was found to be unsatisfactory for screening peanuts for aflatoxin due to the presence of interfering substances in the aqueous extracts. In this manuscript we report the results of screening 160 samples of peanuts using the FLAP method.

MATERIALS AND METHODS

Standards

Controls were prepared from segregation No. 1 Florunner peanuts, which analyses showed to be aflatoxin-free (3). These controls were prepared daily by processing the peanuts by the same procedure as the samples under test.

Reagents

The extraction solvent was technical grade methanol. The zinc salt solution consisted of 150 g of zinc acetate, 150 g of sodium chloride, and 3.75 ml of glacial acetic acid per liter of water (4).

Equipment

Fluorometric analysis of extracts was made with a Coleman Model 12-C Electronic Photofluorometer and filter combination as previously reported (1).

Method

The 160 samples of peanuts were randomly selected from several hundred samples of segregation No. 1-3 peanuts obtained from several commercial and noncommercial sources (see Acknowledgments). Peanuts were supplied in 300-g to 4.5-kg lots. Each lot was ground (as received) in a modified Dickens-Satterwhite Mill with 1/8 in. screen (Federal-State Inspection Service, Albany, GA). The modification was the addition at one of the two subsample ports of a riffle system, which in the larger lots further divided the subsample into portions of ca. 75 g each. One portion was selected at random and used for both a 50-g analytical sample and a 10-g rapid screen sample. Ten-g samples were used for convenience and economy. In practice it would be prudent to extract 50-g samples and process one-fifth of the extract. The 50-g sample was analyzed for aflatoxin using the Pons aqueous acetone procedure (3). Rapid screening was by the FLAP method described herein.

A 10-g sample was stirred intermittently for 3 min with 20 ml of methanol and then filtered through Whatman No. 4 rapid filter paper. Zinc acetate solution (25 ml) was added to the filtrate, and the mixture was left undisturbed for 5 min. Distilled water (55 ml) was added, and the mixture was filtered through a Gelman No. 61631 glass filter pad. Fluorescence of the clear filtrate was then compared with that of an extract of an aflatoxin-free peanut control prepared as described above. Three control peanut samples were prepared daily. These three extracts were mixed just prior to fluorometric analysis to obtain a 3-sample average.

Fluorometric analysis was conducted as follows: (a) shutter fully open; (b) standard control knob maximum sensitivity (fully clockwise); (c) blank control used to set meter to zero using distilled water. Fluorescence of control extracts (zero aflatoxin) was determined. Controls read 10 or below or were discarded. It was essential that all controls and test samples be processed immediately after grinding. The background fluorescence of test samples and controls gradually increased following grinding, an increase which after a few hours invalidated the screening procedure. Where necessary, ground test samples or controls were frozen and kept for several days and were analyzed immediately on thawing. After verification of the acceptability of the controls (meter reading 10 or less), the blank control was used to set the meter reading for the control samples to zero. Fluorescence of test sample extracts was then determined. Meter readings of >12 were interpreted as presumptive evidence of aflatoxin (15 ppb+) and a meter reading of 12 or less as aflatoxin negative (0-14 ppb).

RESULTS AND DISCUSSION

Table I presents a comparison of the data obtained from screening 160 test samples of peanuts by the FLAP method with the Pons method (3) for quantitative analysis for aflatoxin. Results showed that 84% of the samples were correctly classified by the screening method with respect to the criteria used, i.e., meter readings of >12 = positive; 0-12 = negative; aflatoxin positive (3) = 15 ppb+; and aflatoxin negative (3) = <15 ppb. A meter reading of 12 was selected as the dividing point after statistical analysis of more than 100 analyses showed that the mean meter reading for samples containing less than 15 ppb aflatoxin

TABLE I

Screening of 160 Peanut Samples for Aflatoxin by Fluorometric Analysis

Group[a]	Samples No.	Samples %	Fluorometric analysis	Aflatoxin[b] (ppb)	Comments
I	89	55.6	positive	15-350	correctly classified
II	46	28.7	negative	0-14	correctly classified
III	22	13.7	positive	0-14	false positives
IV	3	1.8	negative	20,33,34	false negatives

[a](I) Correct positives, meter reading >12, aflatoxin content 15 ppb or more; (II) correct negatives, meter reading 12 or less, aflatoxin content less than 15 ppb.

[b]Quantitation of aflatoxin by Pons aqueous acetone method.

was 4.6 with a standard deviation of 3.9. Thus, meter readings of 0-12 included 95% of all aflatoxin negative samples tested ($\bar{x}$ + 2SD).

Twenty-two samples (13.7%) were incorrectly classified aflatoxin positive by the FLAP screen. Since such samples must be further analyzed by quantitative methods, they represent an expense but not a health hazard. However, 3 samples (1.8%) were incorrectly classified aflatoxin negative. False negatives represent a potential health hazard in that lots of peanuts from which they were derived were classified aflatoxin-free when in fact they contained 20, 33, and 34 ppb aflatoxin.

Results reported above are similar to those previously reported for the FL-IRS method for aflatoxin in corn (2), in which 88.8% of the corn samples were correctly classified, only 8.2% were false positives, and 3% were false negatives. Unlike the FL-IRS method for screening corn, the FLAP method for peanuts was not improved by iodine treatment to enhance the fluorescence of aflatoxin (1,2). This was probably due to the presence of additional mold metabolites as discussed elsewhere in this report. These results also compare favorably with those of studies evaluating the effectiveness of the Peanut Administrative Committee (PAC) aflatoxin control program for peanuts (5,6). Those studies showed that 2.7% of the lots accepted from the 1973 crop and 1.7% from the 1974 crop contained over 25 ppb aflatoxin.

The FLAP screening method required ca. 10 min per sample (excluding grinding time), and the cost (excluding capital equipment) was estimated to be 5 cents per sample. Other advantages of analysis of aqueous extracts of aflatoxin have been discussed previously with respect to corn (1,2).

Results with samples artificially contaminated with aflatoxin (unpublished data) indicated that mold metabolites other than aflatoxin were also detected. One such metabolite often observed was tentatively identified as ergosta-4,6,8(14),22-tetraen-3-one described by Porter et al. (7). They suggested that this compound might be used as a field indicator of toxic bermudagrass. They also showed that the compound was produced in culture by *Balansia epichloe*. Cooks et al. (8) previously suggested, and we concur, that this compound could be utilized as an early indicator of fungus damage in cereals. We have observed this or closely related compounds many times in extracts of moldy natural products. In research reported herein on peanuts, and previously on corn (2), the false positives are thought to result from the presence of these fluorescent steroid derivatives. Thus, although the false positives may not have contained aflatoxin, they had been contaminated by a mold metabolite. The FLAP screening test may therefore be regarded as a general test for moldiness, including *Aspergillus flavus*. When used and interpreted as described, the test was sufficiently correlated with the presence of aflatoxin so that the FLAP method can be recommended for the first or initial screening of peanuts for aflatoxin as well as other mold damage. Preferably, it should be used in conjunction with the present PAC program to increase the overall efficiency of the existing program in maintaining and improving the quality of commercial peanuts.

ACKNOWLEDGMENTS

J.W. Dickens, USDA, North Carolina State University, Raleigh, NC; W. Eaves and Texas Peanut Producers Board, Gorman, TX; D. Hartzog, Headland, AL; L. Helms, J. Alford, and Dothan Oil Milling Co., Dothan, AL; B. Smith and Toms Foods, Columbus, GA; W.P. Smith and Stevens Industries, Dawson, GA; and P. Thompson and Sessions Peanut Co., Enterprise, AL, supplied peanuts for this study.

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[Received August 6, 1979]