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Determination of Aflatoxins in Individual Peanuts and Peanut Sections[1]

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Abstract

Subsamples of a given lot of peanuts may vary greatly in aflatoxin content due to extreme variability in the degree of contamination of individual kernels. A micro method, adapted from the aqueous acetone procedure recently proposed by Pons and Goldblatt for the determination of aflatoxins in cottonseed products, was developed to permit accurate determination of aflatoxins in individual kernels and kernel sections.

Use of this procedure permitted the topographic distribution of aflatoxins within single kernels to be mapped and indicated that the toxins are not uniformly distributed within contaminated kernels, even when the kernel contains a high level of aflatoxins. Although wrinkling or discoloration sometimes indicated that a kernel was contaminated, this type of physical damage was not found to be a reliable indication of aflatoxin content. Also it was noted that a few apparently sound and mature kernels contained high levels of aflatoxins.

Introduction

STRAINS OF THE COMMON MOLD *Aspergillus flavus* have been found to produce highly toxic metabolites recently named aflatoxins (1). These aflatoxins have been designated B_1, B_2, G_1, and G_2, the letters indicating that 2 fluoresce blue and 2 green under ultraviolet light, and the subscripts identifying their relative mobility under specific conditions of chromatography.

Although the toxins are not limited to peanuts (2,3) this commodity was the first to be implicated and is one of the commodities most seriously threatened in the United States since such a large portion is consumed as food. As numerous samples of peanuts were analyzed for aflatoxins at this laboratory, it became evident that sampling would be a major problem: it appeared that a few highly contaminated kernels unevenly distributed among a large number of uncontaminated ones caused large differences among assays of subsamples. To identify the contaminated kernels and also to determine the distribution of aflatoxin within the kernel, it was necessary to devise a method for assaying individual peanuts or parts of peanuts.

Most of the current methods involve Soxhlet extraction (4), large column (5), or phasic separations (6). The aqueous acetone method originally developed by Pons and Goldblatt for the determination of aflatoxins in cottonseed (7) is, however, particularly adaptable to micro techniques. Because it extracts only negligible amounts of oil while readily dissolving aflatoxins, this solvent is especially useful for examination of individual peanuts and peanut sections weighing as little as half a milligram. The amount of sample necessary is inversely related to the parts per billion aflatoxins. For example, on a sample weighing 400 mg it is possible to estimate aflatoxins in concentrations as low as 24 ppb; on the other hand, if the level of toxin is high enough, samples weighing as little as 0.3 mg can be assayed accurately.

Procedure and Results

Method for Assay of Aflatoxins

Whole peanuts or peanut sections are weighed and then comminuted into fine particles (0.5 to 1 mm) with a sharp razor blade without the expression of oil. The sample is allowed to soak for 30 min in 5 ml of 70% acetone in a 15 ml graduated conical tipped centrifuge tube and is stirred at intervals to ensure maximum extraction. Two ml of 20% lead acetate is then added, the mixture stirred, and 6 ml of distilled water added. The sample is centrifuged for 10 min at 4000–5000 rpm in an angle head centrifuge. The supernatant is quantitatively decanted into a 60 ml separatory funnel. The amount of 5 ml of 70% acetone is added to the residue, and the mixture is stirred and allowed to stand for 30 min. After 8 ml of distilled water is added, the mixture is recentrifuged for 5 min; the centrifugate from this extraction is added to that in the separatory funnel. This combined centrifugate is then shaken in the separatory funnel with 2 separate 20 ml portions of chloroform. After the lower chloroform layer has separated, it is filtered through about 25 g of anhydrous sodium sulfate into a 50 ml beaker. The bed of sodium sulfate can be conveniently prepared by inserting a plug of glass wool into the constriction of a drying tube (1.4 x 10 cm). After most of the

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TABLE I
Aflatoxin Content of Subsamples of Ground Peanuts and Finely Ground Peanut Meal Determined by the Micro Aqueous Acetone Method

Ground peanuts		*Peanut meal*	
Weight of subsamples	Aflatoxin B_1	Weight of subsamples	Aflatoxin B_1
g	ppb	g	ppb
0.24	2600	0.07	3100
0.13	1800	0.11	3000
0.38	1700	0.17	2800
0.22	1600	0.20	2800
0.31	1600	0.07	2700
0.41	1500	0.14	2700
0.33	1200	0.26	2500
0.25	1200	0.20	2500
0.20	1100	0.22	2500
0.25	1000	0.31	2400
0.25	500	0.24	2400
0.20	400	0.08	2300

chloroform is boiled off on a steam bath, the extract is quantitatively transferred into a small (3–5 ml) vial; evaporated to dryness, preferably under a slow stream of nitrogen; and cooled to room temperature. Chromatostrips (8) are used to obtain a preliminary estimation of the concentration of toxins. With this exception, the procedure, calculation, and equipment used for chromatography are identical to those previously reported from this laboratory (6,7).

To determine the reliability of the micro procedure, 50 g stock samples of roughly ground raw peanut kernels and of finely ground peanut meal were prepared. Individual subsamples ranging from 0.07–0.4 g of each material were then analyzed for aflatoxins by application of the micro procedure. The data recorded in Table I indicate considerable variation in the individual subsamples from the ground peanuts but very little variation in those from the meal. This can easily be attributed to the nature of the sample. While the meal was ground to pass a 60-mesh screen, thus affording a fairly homogeneous sample, this could not be done with ground peanuts due to the high oil content. Consequently the values obtained on replicate micro determinations on ground peanuts were not in good agreement. However, the average of the 12 micro analyses from the ground peanut sample, 1350 ppb of aflatoxin B_1, was in essential agreement with the average of the two values, 1100 and 1400, obtained by analysis of the same stock sample by the macro aqueous acetone procedure (7). For the peanut meal stock sample the agreement among the 12 micro replicates was good and the average of these values, 2640 ppb aflatoxin B_1, was in excellent agreement with the value of 2500 obtained on duplicate analyses of the meal using the macro procedure (7). These results tend to confirm the accuracy and reliability of the micro procedure and emphasize the uneven distribution of aflatoxins in particles of raw peanuts.

Observations and Assays

Most samples of sound mature peanuts do not contain aflatoxin. Results obtained on samples examined

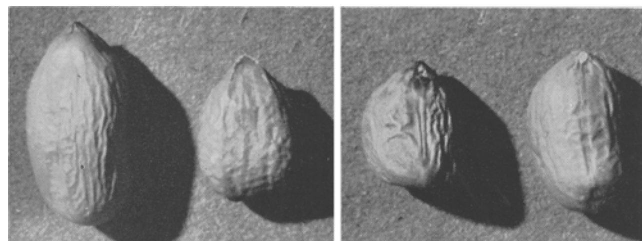

FIG. 1. Photograph of kernels No. 15, 16, 19 and 20 from Table II.

TABLE II
"Wrinkled" Peanuts Segregated from a Sample of Sound Mature Kernels

Peanut No.	Dimension of kernel	*Microscopic* examination for mold	Aflatoxins	
			B_1	G_1
	mm		ppb	ppb
1	10 x 8	N.D.[a]	400	300
2	12 x 7	+[b]	100	50
3	11 x 9	++[c]	150	150
4	15 x 9	+	100	250
5	12 x 8	+	N.D.	N.D.
6	11 x 9	+	N.D.	N.D.
7	13 x 8	++	150	250
8	12 x 8	++	Trace	Trace
9	15 x 9	N.D.	25	20
10	13 x 10	++	Trace	Trace
11	12 x 9	+	N.D.	N.D.
12	15 x 9	++	Trace	50
13	13 x 9	++	N.D.	Trace
14	13 x 9	+	N.D.	N.D.
15	17 x 9	N.D.	N.D.	N.D.
16	12 x 9	+	6,300	13,500
17	14 x 9	++	N.D.	40
18	14 x 9	+	N.D.	N.D.
19	11 x 9	+	N.D.	N.D.
20	12 x 10	+	N.D.	N.D.

[a] None detected.
[b] + = Hyphae in one spot, either in skin or on surface.
[c] ++ = Many hyphae on skin or cut surface.

at this laboratory indicate that those which do contain aflatoxins can be roughly divided into two groups. In the first, there is a higher prevalence of contaminated kernels than in the second, but the level of aflatoxins is lower. In the second type, contamination is highly selective; a few kernels carry very large amounts of aflatoxins, whereas the vast majority contain no detectable amounts. In both types the proportion of contaminated kernels to noncontaminated ones is quite low.

Type I. Because the appearance of kernels comprising the first group did not suggest mold damage, an attempt was made to categorize the kernels by differences in physical appearance. A sample of segregation 1 (peanuts with 2% or less damaged kernels) Spanish peanuts was divided into the following categories: *1*) well-shaped, sound kernels (70% of the sample by weight); *2*) kernels having red dappled skins (15%); *3*) green-veined kernels (2%); *4*) splits (5%); and *5*) dark and wrinkled kernels (8%). One hundred grams of each category were assayed by the modified Broadbent procedure (4). Although no aflatoxins could be detected in the first four categories, 2,250 ppb aflatoxin B_1 was found in category 5, the dark and wrinkled kernels. A second experiment in which the dark kernels were analyzed separately from the wrinkled ones showed that the wrinkled kernels contained about 70 times as much toxin as did the dark and comprised about 60% of this category.

This concentration of aflatoxins in the wrinkled kernels suggested that it would be informative to look at individual wrinkled peanuts. Before the chemical assay, 20 peanuts in this category were examined visually, but no gross evidence of mold was detected. They were then observed with the aid of a low power microscope at magnifications of 10 X and 40 X. Notations were made of their appearances and of any mold structures such as hyphae or conidiphores. After the skin and whole kernels were observed, the cotyledons were split apart with a razor blade to permit the inner surface and the heart to be observed. Parts of questionable portions, especially the skin of 7 of the 20 kernels, were mounted in lactophenol cotton blue to make the mold structures clearer and then observed at magnifications of 100 X and 450 X.

Chemical assay showed that 12 of the 20 peanuts had detectable levels of aflatoxins. Results of the microscopic examination and the assays tabulated in

TABLE III

Peanuts Segregated from a 2 kg Sample of Sound Mature Kernels

No.	Weight of half peanut	Visual examination		Aflatoxin B_1
	g	Outside	Inside	ppb
1	0.24	Normal	Brown	3,200
2	0.19	Broken skin	Hyphae & spores	1,100,000
3	0.08	Shrivelled	Sl. brown	5,400
4	0.19	Normal	Hyphae	N.D.
5	0.19	Normal	Orange spot & hyphae	800,000
6	0.26	Normal	Sl. brown	2,900
7	0.13	Normal	Sl. brown	N.D.
8	0.10	Dark brown	Black	N.D.
9	0.14	Normal	Brown	N.D.
10	0.14	Normal	Sclerotia	5,300
11	0.18	Normal	Brown	N.D.
12	0.28	Broken skin	Hyphae & spores	250,000
13	0.12	No skin	Sl. brown	N.D.
14	0.21	Normal	Hyphae & spores	45,000
15	0.29	Normal	½ Brown & ½ white	300
16	0.20	Normal	Hyphae & spores	100,000
17	0.21	Sl. wrinkled	Normal	1,200
18	0.21	Brown	Hyphae	N.D.
19	0.20	No skin	Sprouting	N.D.
20	0.17	No skin	Brown & white	134,000

Table II show that even observable mold structure is not necessarily correlated with aflatoxin content. The assay on No. 1, which had no visible mold, gave positive results (400 ppb B_1 and 300 ppb G_1). Peanut No. 16, which showed hyphae in only one spot (+), assayed 6,300 ppb B_1 and 13,500 ppb G_1. Of the 7 peanuts showing visible mold structure (++), only 4 had measurable amounts of toxin. Photographs of 4 of these wrinkled peanuts are shown in Figure 1. One had a large amount of aflatoxin (No. 16) and the others were negative (Nos. 15, 19, 20). It is also interesting to note that although several individual wrinkled kernels contained aflatoxin G_1, it was not detectable in the portion of wrinkled peanuts assayed as a composite sample.

Type II. The second type of contamination—lower incidence but at higher levels—occurred in a sample of sound mature kernels reserved for seed stock. Two separate 2 kg subsamples taken at random were ground, mixed, and each 2 kg sample riffled separately to two 50 g aliquots, which were assayed by the macro aqueous acetone method (7). Analyses of the replicates from the same 2 kg sample were in good agreement while the values between the 2 kg samples varied from 30 to 400 ppb aflatoxin B_1. Again, contamination in a few unevenly distributed kernels could have caused this variability between subsamples. In the original seed stock, peanuts containing aflatoxin could *not* be identified by any *one* physical characteristic, such as discoloration or wrinkling. As an example, individuel assays of 50 slightly wrinkled peanuts from the seed stock showed that one peanut contained 1,200 ppb aflatoxin B_1 and 4 had only trace amounts and the rest were negative.

Because it was not possible to consign the contaminated kernels to one category, a third 2 kg sample was carefully examined for *any* suspect kernels. To ensure segregation of all possible contaminated kernels, each peanut was cut in half longitudinally so that each part consisted of half of each cotyledon. In this way, any hidden damage was easily detected. Approximately five thousand peanuts were cut and examined visually. Of these only 20 were slightly discolored or shrivelled or contained visible mold. The rest of the 2 kg sample, i.e., the kernels not considered "suspect," were ground dry and the two 50 g aliquots assayed (7) were negative.

One half of each of the 20 "suspect" kernels was assayed individually. Results of visual examination and the assays are recorded in Table III. Twelve of the 20 kernels contained aflatoxins. These values

TABLE IV

Aflatoxin B_1 Content of Constituent Parts of Selected Type II Peanuts

Part	Peanut No. 2 [a]	Peanut No. 5 [a]	Peanut No. 16 [a]	Peanut No. 20 [a]
	ppb	ppb	ppb	ppb
Skin	570,000	110,000	....	....
Heart	4,000,000	N.D.	27,000	1,100,000
Abaxial surface	3,000,000	130,000	98,000	210,000
Layer 1	2,300,000	110,000	....	....
Layer 2	760,000	....	....	....
Mesophyll	650,000	18,000	130,000	20,000
Adaxial surface	60,000	44,000	340,000	152,000
Layer 1	....	24,000		76,000
Mold	N.D.	....	10,000	....
Brown area next to heart	....	....	....	700,000
Orange spot	....	2,000,000	....	....

[a] Represents the other half of the peanut kernel listed in Table III.

ranged from 1,100,000 ppb of aflatoxin B_1 in kernel No. 2 to 300 ppb in kernel No. 15. The second half of kernel No. 1 was assayed and contained 6,000 ppb B_1, an indication that even though the kernels were cut longitudinally so that each part consisted of half of each cotyledon, these two parts are not necessarily true duplicates. Two assays of the other half of kernel No. 12 showed that the proximal portion contained 126,000 ppb B_1, while the distal portion contained only 84,000. This difference indicated that the toxin is not uniformly distributed even within a small area.

Milligram quantities of selected portions of the other half of kernels Nos. 2, 5, 16 and 20 of Table III were assayed separately. Thin layers were carefully sliced from the curved surface and other selected portions were completely excised with a razor blade, which was more convenient than a microtome because of the contour of the peanut kernel. Results reported in Table IV do not show a consistent pattern of contamination. In kernel No. 2 (Figure 2) the aflatoxin content was higher in the heart and outer surface and gradually decreased in slices closer to the center of the kernel, whereas in kernel No. 16 there was more toxin in the inner surface and mesophyll and less in the outer surface. The assay of the hearts of kernels Nos. 2 and 20 indicated a high level of toxin; the heart of No. 5 had no detectable amount. In this kernel, the greatest concentration of toxin was in an orange-brown spot imbedded in the center of the peanut.

Of the 12 contaminated kernels, those containing over 100,000 ppb of aflatoxins seemed to contain only B_1 and traces of B_2, an indication that the *A. flavus* strains contaminating these kernels produced the B toxins exclusively. Cultures of molds positively identified as *A. flavus* were made from kernels No. 2, 5, and 16. All of these strains produced the B aflatoxins exclusively on peanuts and on shredded wheat.

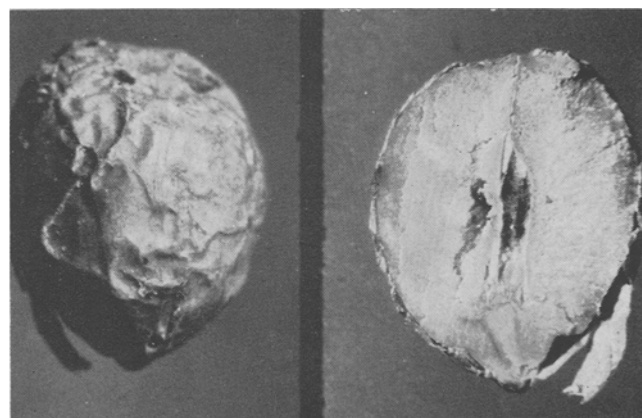

FIG. 2. Photograph of the other half of kernel No. 2 from Table III showing abaxial surface (a) and adaxial surface (b).

Discussion

Samples of peanuts are currently graded as sound and mature if they contain less than 2% damaged kernels, including discolored, broken, insect-infested or mold-damaged kernels. Both of the samples described are well within these grading requirements.

In the first type of contamination where the incidence of contaminated kernels is high but the level of toxin within the kernels comparatively low, it was possible to consign the contaminated kernels to a category that represented 5% of the sample, and was characterized by a physical appearance designated as wrinkling. This wrinkling differed from the shrivelled appearance of immature peanuts that fall through the screens during sorting, and could have been associated with dampening of the kernels during storage. Even in this category, some of the wrinkled kernels were positive and some were negative, but *none* of the kernels other than the wrinkled ones contained appreciable amounts of aflatoxin.

In the second type of contamination where the incidence of kernels containing toxin was low but the level of toxin in those kernels was high, these contaminated kernels represented only 0.24% of the sample. Of the 20 "suspect" kernels only a few were dark or discolored; in fact, 11 appeared normal before cutting (Table III). These kernels were segregated as "suspect" *only* after the inside was examined.

ACKNOWLEDGMENTS

Technical assistance by A. O. Franz; advice and continued interest by W. A. Pons, Jr.

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LAS Removal Across an Institutional Trickling Filter

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Abstract

Field studies with a low-rate trickling filter have shown a paraffin-derived LAS surfactant to be removed nearly as efficiently as the rest of the organics contained in an institutional sewage. A nondomestic cracked-wax-derived LAS surfactant, which was fed over a second two-month period for reference purposes, was removed at a somewhat lower rate. These tests were conducted at the New Jersey Colony at New Lisbon during the period April to August, 1965.

Introduction

A SIX-MONTH EVALUATION of the amenability of two linear alkylate sulfonate (LAS) surfactants to biodegradation by the trickling filtration process has been completed. These tests were conducted by Union Carbide Corporation at the New Jersey Colony at New Lisbon primarily to determine the removals of paraffin-derived LAS by this somewhat inefficient process. A nondomestic cracked-wax-derived LAS was also tested to provide a point of reference to a prior study at the institution (1).

Various surfactants, shown to be degradable by laboratory testing, have established themselves as replacements for the relatively nondegradable branched-chain alkylbenzenesulfonates. As is often the case, questions have developed regarding the ability of laboratory tests to predict adequately the behavior of the new surfactants under actual treatment conditions. To this end, studies involving full-scale sewage treatment plants have established that paraffin-derived LAS removals by activated sludge average about 95% (2,3); however, ample degradability data from full-scale trickling filter studies have not been available. Added emphasis regarding trickling filter data have developed because of a recent report that a cracked-wax-derived LAS was removed quite inefficiently with this process (1).

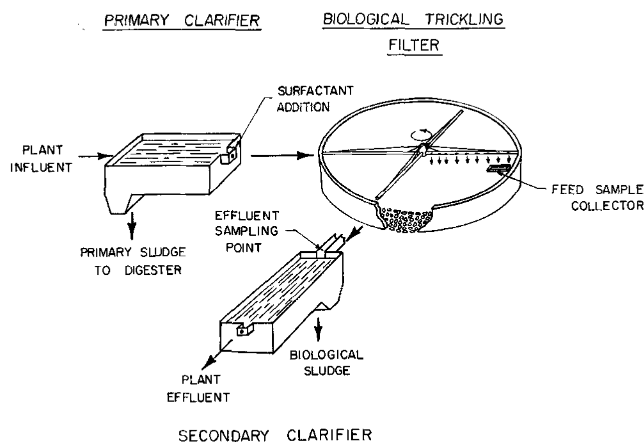

FIG. 1. State Colony at New Lisbon sewage treatment plant.

Test Site

The trickling filter facilities at the State Colony in New Lisbon, New Jersey, were selected for conducting these LAS field tests for several reasons. Close control over all process variables could be maintained. The influent had a very low background surfactant content, which made feasible the addition of surfactant at the treatment facilities. Also, data obtained during a prior study at this plant were available (1).

The State Colony at New Lisbon is an institutional home operated by the State of New Jersey for about 1250 men and boys with a daytime staff of approximately 250. The sewage treatment plant influent consists essentially of domestic waste, since all laundry is contracted out and the kitchen wastes are treated