Determination of Aflatoxins in Peanut and Cottonseed Soapstocks[1]

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Abstract

An accurate and sensitive procedure is proposed for estimating aflatoxins in both alkaline and acidulated soapstocks. Sample suspensions in aqueous acetone are adjusted to pH 3 with hydrochloric acid, extracted in a high speed blender, treated with lead acetate and partitioned into chloroform. After silica gel cleanup, aflatoxins in purified extracts are estimated by thin layer chromatography. The use of acetone and lead acetate together apparently catalyzes the relactonization of aflatoxins B_1 nd G_1 and leads to essentially quantitative recovery of aflatoxin B_1 and somewhat lower recovery of G_1 added to alkaline or acidulated soapstock.

Introduction

Under unfavorable climatic and storage conditions, oilseeds, in common with many agricultural products, are subject to invasion by toxigenic strains of *Aspergillus flavus* and *parasiticus*, with the subsequent elaboration of fluorescent toxic metabolites known as aflatoxins. Processing of such mold damaged oilseeds results in the removal of a portion of the aflatoxins along with the crude oil (1–4). However, the conventional alkali refining and bleaching techniques remove the toxins and the finished oils are free of detectable aflatoxins (1,3). The lactone rings of aflatoxins B_1, B_2, G_1, and G_2 are readily opened in alkaline media, and the sodium salts of the hydroxy acids are water soluble. But under mild acidification conditions relactonization occurs and the aflatoxins can be regenerated (1,4). Thus, it is reasonable to assume that aflatoxins are concentrated in alkaline soapstocks from contaminated oils, and may be regenerated following acidification.

Soapstocks or vegetable oil foots containing some 290 million pounds of anhydrous fatty material are produced annually as a by-product in the refining of domestic vegetable oils (5). A considerable portion of this amount is utilized as a source of fat in various types of mixed feeds. Therefore, there is a need for an accurate and sensitive procedure for estimating aflatoxins in both alkaline and acidulated soapstocks. Present analytical procedures for the estimation of aflatoxins in peanuts, cottonseed and other agricultural products (6–8) were found to be subject to interferences when applied to acidulated soapstocks, and inapplicable to alkaline soapstocks. The method described here was developed in conjunction with research on the fate of aflatoxins during the acidulation of commercial peanut and cottonseed soapstocks.

Analytical Procedure

Reagents

Reagents used were: (a) solvents, ACS, acetone, chloroform; (b) 3N HCl (dilute 265 ml concentrated hydrochloric acid to 1 liter with distilled water); (c) lead acetate solution (dissolve 200 g neutral lead acetate trihydrate in water with warming, add 3 ml glacial acetic acid, dilute to 1 liter); and (d) celite-analytical filter aid, Johns Mansville.

Sample Extraction

Alkaline Soapstock. Weigh a 20 g sample into a 250 ml beaker, add 60 ml of distilled water, and warm on a steam bath with stirring to disperse the sample. Add 60 ml of acetone, cool to room temperature and while stirring with a magnetic or suitable blade stirrer, add 3 N HCl to pH 3, using a glass electrode to measure pH.

Acidulated Soapstock. Weigh a 10 g sample into a 250 ml beaker, add 60 ml of acetone, and stir to disperse the sample. Add 60 ml of water and while stirring adjust to pH 3 with 3 N hydrochloric acid. Some acidulated soapstocks may not require pH adjustment.

Transfer the pH adjusted samples above to a Waring blender jar and blend at high speed for 3 min. Transfer quantitatively to a ₮ 250 ml graduated cylinder, washing the blender jar and sample beaker with a fine stream of water. Dilute to 175 ml with water, add 25 ml of lead acetate solution, 4–5 g of Celite filter aid, stopper, and mix well. Filter through a folded 15 cm circle of Whatman No. 4, or equivalent, paper.

Chloroform Partition. Measure 100 ml of filtrate above into a 250 ml separatory funnel, add 50 ml of chloroform, and shake ca. 1 min. Drain the chloroform phase through a small amount of anhydrous sodium sulfate in a Butt or similar tube, collecting the filtrate in a clean 250 ml beaker. Repeat the extraction with a second 50 ml portion of chloroform. Evaporate the extract to near dryness on a steam bath.

Column Cleanup. Dissolve the dry sample extract in ca. 5 ml of chloroform and apply to a silica gel cleanup column as previously described (6,8).

Thin Layer Chromatography. Spot and develop plates as previously described (6,8) with the following exception. For development, place ca. 150 ml of chloroform-acetone (85:15 v/v) in an unlined and unequilibrated tank, and place three 30 ml beakers half filled with distilled water near the front of the tank. Introduce the plate behind the beakers, and develop for ca. 12 cm of solvent travel. The presence

TABLE I

Effects of pH on Extraction of Aflatoxins From Aqueous Solutions

pH of Aqueous solution[a]	Aflatoxin recovery, %[b]	
	B_1	G_1
1	95	67
2	86	75
3	67	55
4	63	43
5	52	29
6	43	21
7	41	22
12	0	0

[a] Adjusted with hydrochloric acid, except pH 12.
[b] One hundred milliliters aqueous pH 12 solution containing 10 μg each of B_1 and G_1, acidified to pH indicated and extracted twice with 50 ml chloroform.

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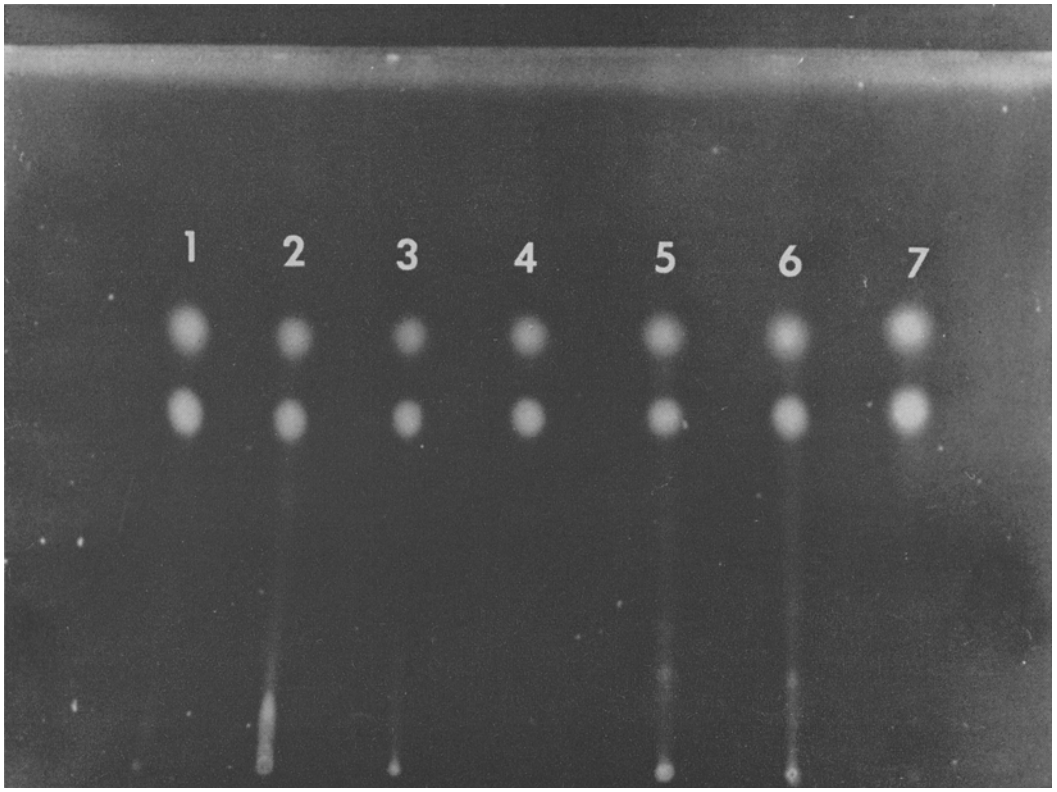

FIG. 1. Thin layer chromatograms, aflatoxins in soapstocks: 1,4,7 standard containing 0.01 μg each B_1 and G_1; 2, alkaline cottonseed; 3, acidulated cottonseed; 5, alkaline peanut; 6, acidulated peanut soapstock spiked with B_1 and G_1. Spots contain ca. 0.01 μg each of B_1 and G_1.

of water vapor in the atmosphere during development gives improved separation of aflatoxins. This is an adaptation of the chloroform-acetone-water development solvent suggested by Stubblefield et al. (9).

Quantitation. Estimate aflatoxins on the developed plates as previously described (6,8), using either visual or preferably fluorodensitometric estimation. In calculations the final purified extract from alkaline soapstocks represents 10 g of original sample, and that from acidulated soapstock, 5 g.

Results and Discussion

Final purified extracts are clean and free of interfering materials as shown by a photograph of a developed plate taken under long wave ultraviolet light (Fig. 1). The chromatograms represent aliquots of spiked peanut and cottonseed soapstocks, and an aflatoxin standard, each containing ca. 0.01 μg of B_1 and G_1.

Recovery of Aflatoxins From Acidified Solutions. Aflatoxins are readily soluble in aqueous alkaline media, but the transfer into chloroform following acidification is dependent on the pH, as shown in Table I. In these experiments, 100 μg each of B_1 and G_1 were deposited as a film in a 2 liter beaker, and dissolved in 1 liter of water adjusted to pH 12. One hundred milliliter aliquots representing 10 μg of B_1 and G_1 were adjusted to the pH levels shown in Table I, and extracted by shaking with two 50 ml portions of chloroform. After evaporation of the solvent, each extract was dissolved in 10 ml of chloroform, and the recovery determined by thin layer chromatography and fluorodensitometric measurements. The recovery of B_1 was essentially complete at pH 1 and progressively decreased from pH 2–7. Recovery of G_1 was lower than B_1 at all pH levels. No aflatoxins were recovered by chloroform extraction of the pH 12 aqueous solution.

For analytical purposes, acidity levels in the range of pH 1 are undesirable, since at this pH level there is a fairly rapid conversion of B_1 and G_1 to low R_f derivatives, B_{2a} and G_{2a} (10). At the milder acidulation level of pH 3 selected for use in the method, aflatoxins are stable at moderate temperatures, and the presence of both acetone and lead acetate, as employed in the analytical procedure, apparently catalyzes the relactonization of both B_1 and G_1. This is illustrated by the data recorded in Table II. In these experiments, aliquots of an alkaline solution containing aflatoxins B_1 and G_1 were adjusted to pH 3 and acetone only, lead acetate solution only, and acetone plus lead acetate solution were added prior to the chloroform extraction. The amounts added were in conformity with the analytical procedure. It can be noted (Table II) that the addition of acetone and lead acetate together results in almost

TABLE II
Effect of Lead Acetate and Acetone on Recovery of Aflatoxins

Acidification conditions[a]		Treatment after acidification[b]	Aflatoxin recovery, %[c]	
Acid	pH		B_1	G_1
None	13	.....	0	0
HCl	3	None	75	38
HCl	3	60 ml acetone	33	26
HCl	3	25 ml lead acetate soln.	86	48
HCl	3	Lead acetate plus acetone	96	62

[a] One hundred milliliters of aqueous pH 12 solutions containing 10 μg each of B_1 and G_1.
[b] Treatment given acidified, pH 3 solution before chloroform extraction.
[c] One hundred milliliters of treated solutions extracted twice with 50 ml of chloroform.

TABLE III
Recovery of Aflatoxins Added to Soapstocks

Soapstock	Sample weight, g	Aflatoxin recovery, %[a]			
		10 μg each B_1 and G_1 added		1 μg each B_1 and G_1 added	
		B_1	G_1	B_1	G_1
Alkaline, peanut	20	....	....	100	80
Alkaline, peanut	20	90	71	100	64
Alkaline, peanut	20	93	94	98	92
Alkaline, cottonseed	20	90	89	83	89
Acidulated, peanut	10	91	93	96	95
Acidulated, cottonseed	10	78	81	67	71

[a] Fluorodensitometric measurement of TLC plates.

quantitative recovery of B_1, and the highest recovery of G_1.

Recovery of Aflatoxins from Soapstock. The recovery of both B_1 and G_1 added at two levels to alkaline and acidulated peanut and cottonseed soapstock is outlined in Table III. Aflatoxins were added to the dispersed samples prior to pH adjustment, and carried through the entire procedure. Fluorodensitometry was used to estimate aflatoxins on the thin layer plates. The average recovery of added B_1 was 93% and that of G_1 85% except for the acidulated cottonseed soapstock for which the recovery averaged 70% and 76% respectively. It can also be noted that the recoveries of G_1 were higher than those shown in Tables I and II, where soapstock was absent.

Application of Method. Application of the procedure to a number of commercial soapstocks is shown in Table IV. Peanut soapstocks from oil mills in the United States (2–6,10–11,13), South America (1), and Europe (12,14) and six domestic cottonseed soapstocks were assayed. Aflatoxin B_1 ranging from 259 to 12 μg/kg was detected in 10 of 11 peanut soapstocks, and at 2 μg/kg in one of six cottonseed soapstocks. Aflatoxin B_2 at a level of 2 μg/kg was found in sample 3, but was not detected in any of the other samples. Only 3 of the 17 commercial soapstocks assayed had any detectable aflatoxin G_1.

The fact that aflatoxins were detected in most of the peanut soapstocks is not surprising inasmuch as only low quality peanuts, unsuitable for edible use, are processed for oil and meal.

TABLE IV
Aflatoxin Content of Commercial Soapstocks

Sample	Moisture content, %[a]	pH[b]	Aflatoxins μg/kg, moisture-free basis[c]	
			B_1	G_1
Alkaline, peanut				
1	22.5	11.5	12	ND[d]
2	81.5	12.5	92	ND
3	48.5	9.5	243	ND
4	52.5	11.0	259	ND
5	47.5	9.8	110	ND
6	50.0	9.4	46	ND
Alkaline, cottonseed				
7	31.0	13.0	ND	ND
8	35.0	14.0	ND	ND
9	48.0	14.0	ND	ND
Acidulated, peanut				
10	5.0	7.0	ND	ND
11	5.9	7.0	20	ND
12	2.0	5.2	23	12
13	1.5	5.3	89	68
14	3.0	5.1	24	15
Acidulated, cottonseed				
15	2.5	3.0	2	ND
16	6.0	6.5	ND	ND
17	8.0	6.0	ND	ND

[a] By toluene distillation.
[b] pH of sample dispersion prior to pH adjustment in procedure.
[c] By fluorodensitometric measurement of TLC plates.
[d] None detected at sensitivity of procedure, ca. 1 μg/kg.

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REFERENCES

1. Parker, W. A., and D. Melnick, JAOCS *43*, 635–638 (1966).
2. Vorster, L. J., Rev. Frans. Corps Gras *13*, 7–12 (1966).
3. Chang, Y. H., and C. G. Beng, Med. J. Malaya *20*, 49–50 (1965).
4. Feuell, A. J., Trop. Sci. *8*, 61–70 (1966).
5. U.S. Department of Commerce, "Current Industrial Reports," Ser. M-20K(66)-13, 5, Bureau of Census, December 1967.
6. Pons, Jr., W. A., A. F. Cucullu, A. O. Franz, Jr. and L. A. Goldblatt, JAOCS *45*, 694–699 (1968).
7. Association of Official Analytic Chemists, J. Ass. Offic. Anal. Chem. *51*, 485–489 (1968).
8. Ibid. *52*, 405–409 (1969).
9. Stubblefield, R. D., G. M. Shannon and O. L. Shotwell, Ibid. *52*, 669–672 (1969).
10. Pons, Jr., W. A., A. F. Cucullu, L. S. Lee, H. J. Janssen and L. A. Goldblatt, AOCS Meeting, Minneapolis, October 1969, Abstr. No. 101.

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