Estimation of Aflatoxin in Peanut Butter

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SUMMARY

Food retailed in South Africa must by law be free of the toxic and carcinogenic mycotoxin, aflatoxin. Peanut butter is particularly liable to contamination, yet no nationally accepted analytical method suitable for the product exists. Collaborative studies with various laboratories were organised to test the efficiency of various methods, but the results revealed a disturbing frequency of underestimation. The 'best food' method in conjunction with a personnel training programme is recommended.

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Mycotoxicoses, particularly ergotism, have been known to man for much of his recorded history. In 1960 a renaissance of mycotoxin research developed as a direct result of outbreaks of aflatoxicosis in poultry and fish.

A number of outbreaks of disease, including fatalities, have occurred in which circumstantial evidence suggests the possibility of aflatoxin involvement in acute toxicosis in man. For instance, Thai children dying from an acute syndrome of unknown aetiology had substantial quantities of aflatoxin in their tissue and body fluids.[1] Pathological changes were very similar to those induced in monkeys by aflatoxin.[2] Several epidemiological studies in Africa have related the aflatoxin intake of populations to the incidence of liver cancer.[3] In the laboratory aflatoxin has been shown to be the most potent chemical carcinogen known. A variety of tumours, particularly hepatocellular carcinoma, may be produced. As little as 0,015 parts-per-million in the feed will induce tumours in 100% of test animals.

Aflatoxins are produced by the fungus *Aspergillus flavus*. Conditions of high humidity conducive to mould growth are inherent in some methods of harvesting and drying groundnuts, and are considered a major factor in mould contamination of this commodity. Even in dry seasons termites damage the husks and may introduce fungal spores in their search for moisture. Because some mouldy kernels have a normal external appearance, the possibility exists that aflatoxin-containing kernels may escape detection and be processed into peanut butter and peanut meal. The aflatoxin B_1 present in contaminated peanut meal fed to cows, is metabolised by the cow and excreted in the milk as aflatoxin M_1—also a potent liver toxin.[4]

In certain seasons a disturbingly high proportion of the groundnut crop may be contaminated with aflatoxin.[5] Experience has shown that control of the problem by improved harvesting and storage is not completely effective in preventing contamination of the final processed product. Legislation has made chemical control by industry in South Africa imperative, yet no nationally acceptable chemical method exists. The collaborative studies described here were designed to investigate and to identify the most suitable assay method. Originally ten laboratories consented to collaborate, although only six completed the study.

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MATERIALS AND METHODS

Preparation of Samples

In view of reports that the level of aflatoxin in artificially contaminated peanut butter decreases with time, naturally contaminated peanut butter was prepared. Mouldy groundnuts, which were known to contain the four aflatoxins (B_1 B_2, G_1 and G_2), were rolled into butter. Desired levels of aflatoxin-containing peanut butter were obtained by thorough mixing of this naturally contaminated product with commercial peanut butter. Samples of 100 g, labelled at random, were dispatched to the collaborators.

Analytical Methods

Two analytical methods were used for the estimation of the aflatoxin B_1. The first, referred to as the best food (BF) method,[6] involves the solvent extraction of the aflatoxin from 50 g of peanut butter, using 250 ml methanol : water (55 : 45) and hexane (100 ml). After blending for 2 min the suspension is centrifuged for 5 min at 2 000 rpm. The lipids dissolve in the hexane and are discarded, while the aflatoxins quantitatively enter the methanol : water phase from which they are removed by extraction with chloroform (125 ml). Evaporation of the dried chloroform extract gives a mixture of aflatoxin B_1, B_2, G_1 and G_2, which are separated by thin-layer chromatography (TLC). The amount of aflatoxin B_1 in the spot from the extract is visually estimated by comparison with a spot of standard aflatoxin B_1 solution. The TLC plate is viewed under UV-light. The smallest amount of aflatoxin B_1 that can accurately be estimated on TLC is 0,005 μg.

The second method, called the rapid method,[7] involves the solvent extraction of the aflatoxins in the peanut butter (50 μg) with 110 ml methanol : water (10 : 1). The lipids are essentially insoluable in this solvent and do not interfere with the TLC separation. This method takes less time than the BF method—an important consideration in an industrial laboratory and the reason for its inclusion in this study. The BF method was chosen

because it has been examined extensively in laboratories overseas and adopted as the method of choice in the USA.

The collaborating laboratories were also sent standard solutions of aflatoxin B_1, plastic sheets precoated with silica gel for TLC prepared by Machery-Nagel and Co., Düren, and weighed amounts of Camag D-5 silica gel for the preparation of TLC plates.

Study Programme

The collaborative study was conducted in three parts. In the first part, collaborators were sent 6 identical peanut samples and instructions to analyse 2 samples by each of the methods described and 2 by the method currently in use in their laboratory. In the second part, collaborators received solutions of aflatoxin and were asked to estimate the concentrations of aflatoxin after separation by TLC. Finally, a second set of peanut butter samples was to be analysed by the 3 methods used in the first part.

RESULTS AND DISCUSSION

Part 1

Repeated analyses of the peanut butter in our hands gave results of 1 400 ± 100 μg aflatoxin/kg. The results from the collaborators are given in Table I.

These results show that only two laboratories were able to resolve the mixture of aflatoxins into the four components, and this probably accounts for the wide variation in the reported results. For this reason the second part of the study was confined to the standardisation of the TLC method.

Part 2

The collaborators were each sent a single solution containing the four aflatoxins (abbreviated AFB_1, AFB_2, AFG_1, and AFG_2, respectively). They were asked to separate the aflatoxins by TLC and to estimate the amount of each aflatoxin by comparing the intensity of fluorescence under UV-light of the spot of the unknown with that produced by a spot of a known amount viewed under the same UV-light. They were to use precoated TLC plates and plates prepared with Camag-D5 silica gel with the following solvent system: chloroform : trichloroethylene : isobutanol : formic acid (80 : 15 : 4 : 1). The results from six laboratories are shown in Table II.

Both the precoated and Camag-TLC plates separate the four aflatoxins in a satisfactory manner. There is no statistical significance in the difference between the results obtained on these two TLC systems.

Part 3

In the final phase of this study the newly acquired TLC skill of part 2 was applied to the original problem. Twelve peanut butter samples, contaminated at four different levels (30, 50, 100 and 120 μg/kg) with aflatoxin B_1, were numbered at random and distributed to the collaborators. Camag-D5 silica gel for TLC was used and no difficulty in separating the four aflatoxins was experienced by any of the six collaborators who returned results. As in Part 1 of the study, the rapid, the BF and their own methods were employed. Yet, in spite of correct TLC technique the results shown in Table III show an apparently disturbing frequency of gross underestimation. The possibility, however, of deterioration of the samples is not fully excluded.

TABLE I. COMPARISON OF 3 METHODS USED FOR THE DETERMINATION OF AFLATOXIN IN PEANUT BUTTER

Lab. No.	Own method (μg/kg)	No. spots on TLC	BF method (μg/kg)	No. spots on TLC	Rapid method (μg/kg)	No. spots on TLC
1	1100,2100	3	—	—	—	—
2	2332,2332	—	3180,3180	2	2915,2915	2
3	2650,2650	—	2650,2650	2	2650,2650	2
4	1893,1395	3	5300,6073	2	2968,2120	2
5	2100,1600	4	1484,1470	4	1780,1270	4
6	—	4	5300,3975	4	1060,1590	4

TABLE II. CONCENTRATION (μg/ml) OF AFLATOXINS (AFB_1, AFB_2, AFG_1 AND AFG_2) IN TEST SOLUTION

	Precoated TLC plates				Camag D-5 TLC plates			
Lab. No.	AFB_1	AFB_2	AFG_1	AFG_2	AFB_1	AFB_2	AFG_1	AFG_2
1	9,0	12,0	12,0	2,0	8,0	16,0	16,0	2,0
2	13,3	6,6	19,9	3,3	9,3	8,0	9,3	5,6
3	8,0	11,9	8,0	3,2	11,9	8,0	8,0	4,0
4	4,0	4,8	8,0	0,8	8,0	8,0	8,0	4,0
5	12,0	5,0	24,0	2,0	6,6	3,3	6,6	1,3
6	9,3	8,0	9,3	1,3	9,3	8,0	9,3	1,6
X	9,26	8,04	13,53	2,10	8,85	8,55	9,53	3,08
S	3,26	3,23	6,81	0,99	1,80	4,11	3,32	1,71

TABLE III. DETERMINATION OF AFLATOXIN IN PEANUT BUTTER USING STANDARDISED TLC TECHNIQUES

	30			50			100			120		
Lab. No.	Rapid	BF	Own	Rapid	BF	Own	Rapid	BF	Own	Rapid	BF	Own
2	**19**	**19**	**11**	**24**	**19**	**18**	**74**	**32**	**42**	**85**	**100**	**53**
3	**21**	**31**	**27**	**28**	**33**	**26**	**28**	**50**	**40**	**42**	**65**	**33**
6	**15**	**12**	**—**	**15**	**9**	**—**	**3**	**3**	**—**	**11**	**12**	**—**
7	**21**	**16**	**11**	**16**	**11**	**11**	**21**	**22**	**0**	**42**	**15**	**21**
9	**27**	**28**	**—**	**35**	**49**	**—**	**57**	**46**	**—**	**54**	**64**	**—**

1. Three naturally contaminated peanut butter samples at each of the above four levels of contamination were sent to each collaborator (a total of 12 samples).
2. The peanut butter samples were numbered at random.
3. Each collaborator was asked to analyse one peanut butter sample at each level of contamination using the following three methods: (i) rapid method; (ii) BF method; and (iii) the method currently in use in their own laboratory.

Collaborative studies overseas have shown the BF method to be the most reliable. We thus conclude that local personnel are in need of training in the use of this procedure. It is in the public interest that attention be given to the standardisation of analytical procedures for the estimation of aflatoxin in peanut butter.

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