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A STUDY ON AFLATOXINS IN VARIOUS FOODS, SPICES AND FEEDSTUFFS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

103 Samples from various food, feedstuff, spices and some other materials were analyzed for aflatoxin levels during 1991-1995. Aflatoxin was found in 5 samples, one of them (peanut butter) was over the accepted limits.

ÖZET

1991-1995 Yıllarında çeşitli gıda, yem, baharat ve diğer gıda maddelerinden toplam 103 örnekte aflatoksin analizi yapıldı. Bunlardan 5 örnekte aflatoksine rastlandı ve bir örnekte (fıstık ezmesi) limitlerin üzerinde bulundu.

Key words: Aflatoxin, high performance liquid chromatography

INTRODUCTION

In 1960 in the United Kingdom, over one hundred thousand turkeys and ducks died in the course of a few months. Because the causative agent was unknown and the affected animals exhibited similar symptoms, the disease was initially called, 'Turkey "X" Disease ' (1, 2). In 1961 a toxic compound was isolated from moldy agricultural commodities (3) used as ingredients in turkey feed. The molds responsible for the production of aflatoxins are primarily *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. Since it was produced

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by *Aspergillus flavus* Link and exhibited a characteristic blue fluorescence on TLC plates under longwave UV ligh, it was named aflatoxin B_1 (3).

Toxicologic studies confirmed that aflatoxin B_1 was extremely hepatotoxic, hepatocarcinogenic, mutagenic and teratogenic (l, 4, 5, 6).

In 1967, this problem was first encountered in Turkey, when hazelnuts that had been exported to Canada were returned back (7). Confrontation with the same problem occured in the summer of 1971 again (8).

In this report, the results of analysis of 103 various food samples including hazelnuts, hazelnut butter, peanuts, peanut butter, potato chips, sunflower meal, spices, red pepper, etc. were given. These samples were collected between 1991 and 1995 during 5 years.

RESULTS and DISCUSSION

Table 2. shows the results of aflatoxin levels determined between 1991 and 1995. The highest value of aflatoxin has been found in peanut butter (6.3 ppb).

The accepted maximal aflatoxin levels by Ministery of Agriculture (Official Journal 2nd May 1990 /Nr. 20506) were as following: foods and agricultural products (aflatoxin B_1) 5ppb, total aflatoxins B_1, B_2,G_1 and G_2 20 ppb, feedstuff

Table 1. Number of samples investigated for aflatoxin content between the years 1991-1995.

Sample	1991	1992	1993	1994	1995
Hazelnut butter	3	2			
Hazelnuts	3	5	5	1	
Peanut butter	2				
Peanuts	1	1	3	3	
Potato chips	23	2			
Sunflower meal (feedstuff)	1				
Red pepper		2	5		11
Spices (Cumin,black pepper)			5		
Others (Coffee, cocoa, fig, walnut, etc.)	15	4	5	1	
The overall sum	48	16	23	5	11

Table 2. The amount of aflatoxin found in food and feedstuff samples (ppb)

Sample	Date	Aflatoxin B_1	B_2	G_1	G_2
Peanut butter	25.2.1991	6.3 *	–	–	–
Peanut butter	11.7.1991	3.44	3.16	–	–
Sunflower meal	4.2.1991	11.1	3.56	–	–
Red pepper	8.2.1995	1.048	–	–	–
Red pepper	9.2.1995	1.273	–	–	–

* Exceeds the limits of Ministery of Agriculture

50 ppb. The toxic limit for total aflatoxins given by WHO is above 30 ppb (9). But in some countries (Japan, U.S.A., Switzerland, United Kingdom) most of the regulation levels have been set between 10 to 20 ppb (1) for human foods except milk and dairy products.

Occasionally very heavy contamination was found in maize, cottonseeds, peanuts and peanut products, tree nuts, many other foods and feedstuffs. Some of these commodities are very important economic crops, therefore aflatoxin contamination does not only concern public health but also stands to be an economic problem.

EXPERIMENTAL

Apparatus: High Performance Liquid Chromatograph (HPLC,Waters Corporation, U.S.A.)

The attachments: Pump M 501 solvent delivery system, Detection: M 420-AC Fluorescence Detector at 365 nm excitation, 425 nm emission with aflatoxin lamp, injector: U6 K Universal injector, Data Station: M 746 integrator, Column: 5 µm resolve C_{18} Radial-Pak cartridge+RCSS C_{18} quard pak insert (Waters).

Chemicals: Aflatoxin B_1 (Sigma A-6636), aflatoxin B_2 (Sigma A-9887), aflatoxin G_1 (Sigma A-0138), aflatoxin G_2 (Sigma A-0263) were used as standards. Aflatoxin B_1, B_2, G_1 and G_2 were dissolved in MeOH so as to contain 20 ng of aflatoxin/ml. Sep-Pak silica cartridge (Waters Part.No.51900) and other

chemicals were HPLC grade Merck products. Water was obtained daily from Milli-Q (Millipore) system.

Procedure: The method used was described previously by Waters (10): 25 g of the sample was mixed with 1 g NaCl in a blender. 12.5 ml distilled water and 125 ml $CHCl_3$ were added. After blending for 10 seconds, 12.5 g of celite was added and this solution was blended again for 1 minute. Then it was filtered through a bed of celite and the filtrate was collected (Buchner funnels with Whatman Nr. 4 paper and 0.5 cm of celite were used). 12.5 ml of the $CHCl_3$ filtrate was passed through a silica Sep-Pak cartridge. After the cartridge was washed with 10 ml of each hexane and then diethyl ether, the aflatoxins were eluted with 10 ml of $CHCl_3/CH_3OH$ (95: 5). The eluates were collected into a 10-15 ml screw cap vial and evaporated to dryness under a stream of nitrogen. After adding 0.2 ml of hexane into the vial, the residue was dissolved by vortexing approximately for 10 seconds and 0.2 ml 80 % trifluoracetic acid was added, then the mixture was vortexed for 30 seconds. After 1 minute 2.3 ml of CH_3CN/2.5 % acetic acid (10:90) was added and mixed well then the lower (aqueous) layer was filtered through a Millex HV (0.45 µm.).

HPLC quantitation: The samples were injected into HPLC system under the same conditions used for preparing calibration graphs. Injection: 25 µl, flow rate: 2.5 ml/min., chart speed: 0.25 cm/min., attenuation: 16, mobile phase: CH_3OH/CH_3CN/5.0 % acetic acid (14:14:72). The mobile phase was filtered through a Millipore HV (0.45 µm) membrane filter and degassed by immersion in an ultrasonic bath (Fig. 1.)

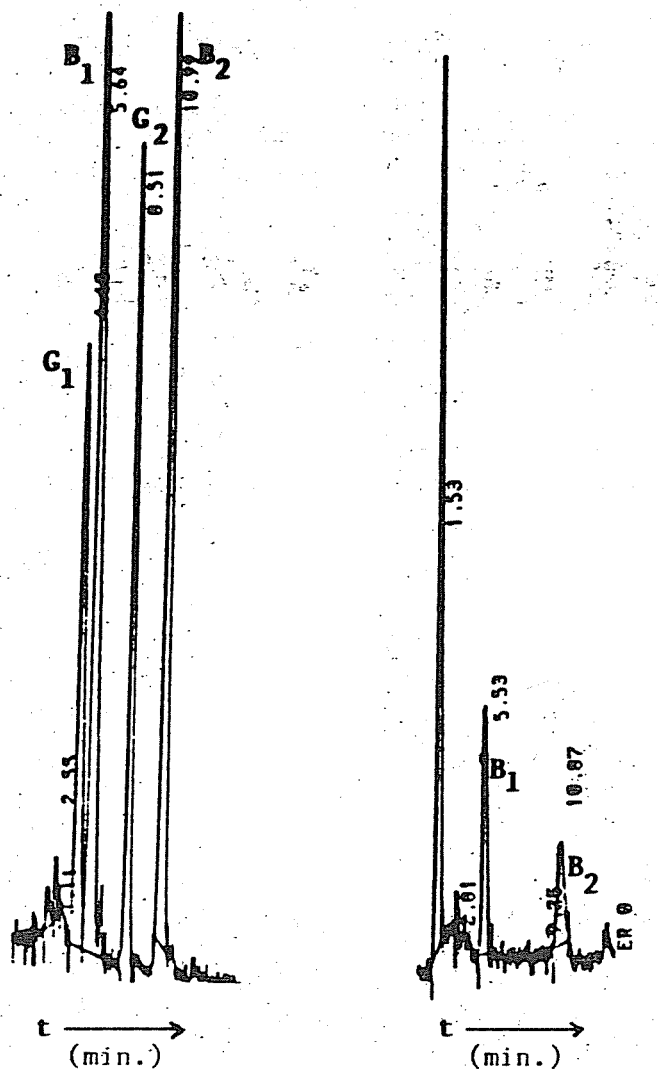

Fig. 1. Chromatogram A: aflatoxin standards
Chromatogram B: peanut butter extract.
For conditions of analysis, see text.

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