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AFLATOXIN CONTAMINATION IN PEANUT BUTTERS AND SPICES ISTANBUL

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SUMMARY

Strains of *Aspergillus flavus* and *A. parasiticus* that produce aflatoxins are ubiquitous in nature. Aflatoxins have been frequently detected in grains, oil seeds, beverages made from grains, milk and many agricultural commodities.

In this study, aflatoxins (AFLs) were determined in 62 samples of food including peanut butter, nut butter, spices such as dried red pepper, cumin, black pepper, allspice and feedstuffs including corn. Samples were provided from markets, street bazaars, spice-sellers and some of them were homemade. AFL analysis in the samples was performed using high performance liquid chromatography (HPLC) with fluorescence detector, after extraction and sample clean-up procedure. Of 19 samples of peanut butter examined, all samples have contained AFLs. Of 24 dried red pepper examined, 14 were contaminated with AFLs. No AFL was detected in homemade dried red pepper. As AFLs belong to the most carcinogenic substances, the legal limits are very low for their occurrence in food. In Turkey these limits are 10 ppb for the sum of AFLs B_1, B_2, G_1, G_2 and 5 ppb for B_1 as a single component and total AFL level is 50 ppb for feedstuffs. AFL B_1 levels were higher than the limit in the 47.3% of peanut butter and 35.7% of AFL B_1 –contaminated dried red pepper samples. Total AFL (B_1+B_2+G_1+G_2) levels were higher than the limit in the 36.8% of peanut butter and in the 21.4% AFLs –contamina-

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ted dried red pepper samples. The highest levels of AFL B_1 were 24.70 ppb and 29.90 ppb respectively, in the peanut butter and dried red pepper samples.

ÖZET

Aflatoksin üreten *Aspergillus flavus* ve *A. parasiticus* doğada her yerde üreyebilir. Aflatoksinler hububatlarda, yağlı tohumlarda, hububatlardan yapılan fermente içkilerde, sütte ve birçok tarım ürünlerinde sıklıkla bulunabilir.

Bu çalışmada fıstık ezmesi, fındık ezmesi, kırmızı biber, karabiber, kimyon, yenibahar gibi baharatlar ve mısır örneklerinden oluşan 62 adet gıda maddesi örneği analiz edildi. Örnekler değişik market, pazar, baharatçılardan ve bunun yanında ev imalatı olanlardan sağlandı. Örneklerde aflatoksin analizleri ekstraksiyon ve temizleme işleminden sonra fluoresans detektörlü yüksek basınçlı sıvı kromatografisi ile yapıldı. Analizi yapılan 19 fıstık ezmesi örneğinin tümü aflatoksin içeriyordu. 24 Kırmızı biber örneğinin 14 ünde aflatoksin tesbit edildi ve ev imalatı olan kırmızı biber örneklerinde aflatoksin bulunmadı. Bu toksik bileşiklerin gıdalarda bulunmasına müsaade edilen miktarları için çok düşük değerler bulunmaktadır. Memleketimizde bu limitler toplam aflatoksin B_1, B_2, G_1 ve G_2 için 10 ppb, sadece aflatoksin B_1 için 5 ppb ve yemlerde 50 ppb olarak kabul edilmiştir. Fıstık ezmelerinin %47.3 ünde ve aflatoksin içeren kırmızı biber örneklerinin %35.7 inde memleketimizde kabul edilebilir en yüksek değer olan 5 ppb nin üzerinde $AFLB_1$ tesbit edildi. Fıstık ezmelerinin %36.8 inde ve aflatoksin içeren kırmızı biber örneklerinin %21.4 ünde tüm gıda maddelerinde kabul edilebilir en yüksek değer olan 10 ppb nin üzerinde toplam AFL (B_1+B_2+G_1+G_2) bulunmuştur. En yüksek AFL B1 fıstık ezmesi örneğinde 24.70 ppb, kırmızı biber örneğinde 29.90 ppb idi.

Key words: Aflatoxin; HPLC; Peanut; Red pepper; Spices.

INTRODUCTION

Aflatoxins are highly potent carcinogenic compounds produced by *Aspergillus flavus* Link and *A. parasiticus* Speare (1). Spices are often produced in countries with tropical climates which have extreme ranges of rainfall, temperature and humidity and it is believed that the sun drying process presents the greatest potential for contamination. It is also possible for pre-harvest contamination to occur in tropical environments (2). To reduce the risk many countries regulated the maximum permissible levels of AFLs in foods; examples are as follows: 20 ppb in the United States, 15 ppb in Canada, 10 ppb in

France, 10 ppb in the United Kingdom, 5 ppb in Australia, and 10 ppb in Japan (3). The accepted maximal AFL levels by Ministery of Agriculture in Turkey (Official Gazette 16 November 1997/Nr. 23172) (4) are as follows: spices, foods and agricultural products (AFL B_1) 5 ppb, milk and dairy products 0.5 ppb, total AFLs B_1, B_2, G_1 and G2 10 ppb, feedstuff 50 ppb. Epidemiological studies have shown a correlation between AFL exposure and primary hepatocellular carcinoma incidence in United Kingdom and several Third World Countries (5). Toxicologically, AFLs may be regarded as a quadruple threat: it can function as a potent toxin, a carcinogen, a teratogen, and a mutagen (1). $AFLB_1$ is acutely toxic in all species studied with an LD_{50} ranging from 0.5 mg/kg for the duckling to 60 mg/kg for the mouse (6). There is, of course, no established toxic dose for humans; but strong circumstantial evidence from Southeast Asia, India, and Africa, plus a suspect case in Germany, indicates that AFLs have been involved in human deaths, particularly among children. The AFL problem was first encountered in Turkey in June 1967, when hazelnuts exported to Canada were returned to Turkey with a report from their Department of Health and Welfare asserting that they contained AFLs. Turkey was again confronted with the same problem in the summer of 1971 (7, 8) and also with the red peppers in 1995 (9).

Many different methods have been used for the estimation of AFLs based on high performance liquid chromatography (HPLC) (10, 11, 12), gas chromatography (GC) (13), thin layer chromatography (TLC) (3, 14), (enzyme-linked immunosorbent assays (ELISA) (15, 16), immunoaffinity column (16), mini column chromatography (15). In this study, AFLs were determined in samples using HPLC. Confirmation of AFLs was by TLC.

RESULTS AND DISCUSSION

Typically spices are laid out on the ground to dry in the open air where the climatic conditions of high temperature and humidity are ideal for growth of *aspergilli* and production of AFLs (2).

AFLs B_1 , B_2 ,G_1 and G_2 can be readily separated and detected using either normal- or reversed-phase TLC or HPLC techniques. The samples were initially screened semi-quantitatively by TLC; this provides a rapid means of analysing a large number of samples. It was easy, fast and inexpensive however, HPLC using fluorescence detection is now the method of choice for determining AFLs and is also growing in popularity for their identification. Further devolepment of monoclonal antibodies in preliminary clean-up will help in HPLC methods for many commodities that contain many interfering substances (17).

In a study, of the spices analysed, chilli powder and ground ginger were the most likely to be contaminated; some samples contained over 20 ppb total AFLs. More than 50 % of the spice samples were found to be contaminated at levels of greater than 1 ppb. (10). In an another study, of 157 retail samples in UK which included curry powders, pepper, cayenne pepper, chilli, paprika, ginger, cinnamon and coriander, nearly 95 % of samples contained below 10 ppb total AFLs and only nine samples had higher levels. The highest concentration in a retail sample was 48 ppb in a chilli powder (2). Tabata *et al.* (3) found nearly 7.4% (mean 1.4 ppb) of samples of AFL-positive peanut products and nearly 20% (the highest 9.1 ppb for AFL B_1) of red pepper samples. Our previous studies on AFLs in various foods, spices and feedstuffs by HPLC in Turkey in 1995, the amounts of $AFLB_1$ two red pepper samples 1.048, 1.273 ppb and two peanut butter samples 6.3, 3.44 ppb (12) have been found and the highest $AFLB_1$ level was found as 109.7 ppb in an unmarked dried red pepper samples collected from a spice-sellers in Istanbul in 1998 (18).

In this study, the detection limits of HPLC were 0.075 ng for AFLs B_1 and G_1 and 0.06 ng for B_2 and G_2 . The detection limits of TLC were 1 ng for AFLs. AFLs recoveries were 91% for B_1 , 71% for B_2 , 97% for G1 and 67% for G_2 . Figure 1. shows HPLC chromatograms of AFLs standards, a peanut butter and dried red pepper samples and also Table 1 and 2 show results of HPLC method for AFLs in samples. AFLs were determined in 62 samples of food or feedstuffs (2 samples), including peanuts (3 samples), peanut butter (19 samples), nut butter (6 samples), spices such as dried red pepper (24 samples), cumin (1 sample), black pepper (1 sample), allspice (1 sample) and corn (5 samples). Measurable levels of AFL were found in 19 peanut butters and in14 of 24 dried red pepper specimens.

No AFL was detected in the other samples. Dried red pepper samples were provided from markets, street bazaars, spice-sellers and some of them were homemade. The

Table 1: Incidence and level of aflatoxins in peanut butters

	Range (mean of positives) (µg/kg); mean µg/kg±SD			
	AFL B1	AFL B2	AFL G1	AFL G2
No. positives samples/ No. of samples	0.85-24.70; 6.9±6.7 19/19	0.42-3.97; 2.1±1.2 11/19	2.47-3.05; 2.76±0.4 2/19	ND 0/19

ND: Not detected

Table 2 : Incidence and level of aflatoxins in dried red peppes (µg/kg)

Known origin	Bought from	$AFLB_1$	$AFLB_2$	$AFLG_1$	$AFLG_2$	AFL (B1+B2+ G1+G2)
Antakya	Spice-seller, Unmarked	1.10	-	-	-	1.10
Antakya		2.70	-	-	-	2.70
Antakya		-	-	-	-	
Gaziantep		21.90	1.63	-	-	23.53
India		8.12	-	-	-	8.12
Şanlıurfa		3.21	-	-	-	3.21
Şanlıurfa		-	-	-	-	
Şanlıurfa		-	-	-	-	
Şanlıurfa		-	-	-	-	
Kahramanmaraş		-	-	-	-	
Kahramanmaraş		15.71	1.32	-	-	17.03
UO		6.77	0.48	0.62	-	7.87
UO		1.81	0.87	-	-	2.68
UO		1.68	-	-	-	1.68
UO		-	-	-	-	
UO		2.36	7.04	-	-	9.40
Kilis	Street-bazaar, Unmarked	3.13	-	-	-	3.13
UO		2.43	-	-	-	2.43
UO		-	-	-	-	
UO	Markets, Marked	29.90	8.82	-	-	38.72
UO		0.64	-	-	-	0.64
Gaziantep	Home-made	-	-	-	-	-
Gaziantep		-	-	-	-	-
Gaziantep		-	-	-	-	-

UO: Unknown origin

highest AFLB1 level was found as 24.70 ppb in a peanut sample and 29.90 ppb in a dried red pepper sample collected from a market. In 47.3% of peanut butter samples AFLB1 levels were higher than 5 ppb and total AFL levels were higher than 10 ppb in about 36.8% of samples. In 35.7% of AFL-contaminated dried red pepper samples (5 samples), $AFLB_1$ levels were higher than 5 ppb and total AFL levels were higher than 10 ppb in about 21.4% of samples (3 samples). In Turkey, the use of dried red pepper in some special foods and the snack food business has increased. Hence exposure to AFLs from these sources will also be increasing, putting some additional health risk to the con-

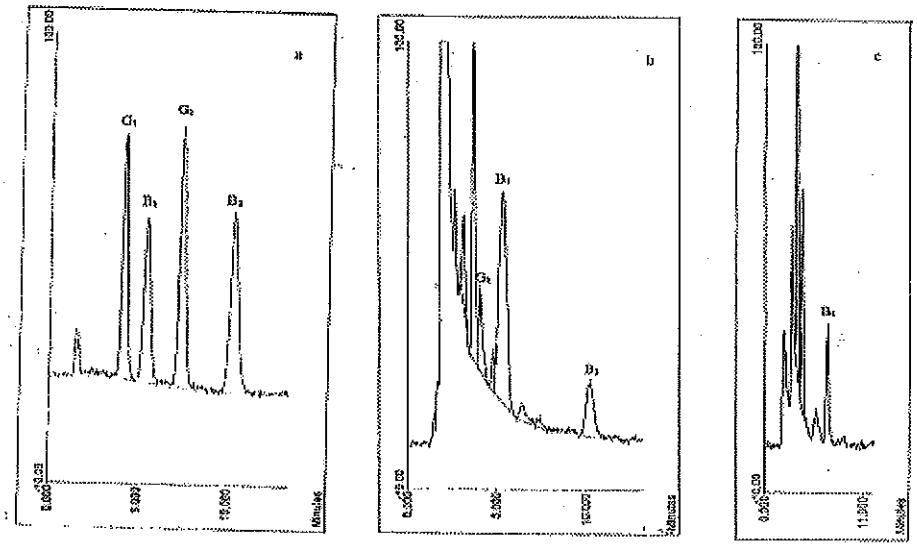

Fig.1 : HPLC chromatograms:a) Aflatoxin standards (AFL G1 B1, G2, B2 ; 400 pg), b) Naturally contaminated peanut butter extract (AFL B1 20.06 µg/kg, B2 3.97µg/kg, G1 3.05µg/kg), c) Naturally contaminated dried red pepper extract (.AFL B1 2.70 µg/kg).

sumer. As a result of our study we believe that AFL analysis has to be done and declared to the consumer for food products such as peanut butter and dried red pepper samples that can have a high risk of AFL contamination in Turkey.

EXPERIMENTAL

Samples

Analyses were carried out on peanut butter, nut butter, spices such as dried red pepper, cumin, black pepper, allspice and corn samples provided from commercially available markets, street bazaars and spice-sellers collected in Istanbul during the years 1997-1999. The home-made dried red pepper samples were from Gaziantep.

Aflatoxin determination

AFLs were determined by HPLC method reported earlier (12), as follows: 340-1000 g samples were blended, AFLs were extracted with chloroform- water from 25 g ground sample, purified by Sep-Pak silica column chromatograhy (WAT020520), and examinated by HPLC. Confirmation was performed by TLC, according to the method prescribed by Omurtag *et al.* 1998 (18).

Chemicals: $AFLB_1$ (Sigma A-6636), $AFLB_2$ (Sigma A-9887), $AFLG_1$ (Sigma A-0138), $AFLG_2$ (Sigma A-0263) were used as standards. AFLs B_1, B_2, G_1 and G_2 were dissolved in $MeOH/CHCl_3$ (1:1, v/v) so as to contain 20 ng of AFL/ml and other chemicals were HPLC grade Merck products. High purity water obtained through a Millique Q-RG water purification system (Millipore, Bedford, MA, U.S.A.) was used in all procedures.

Apparatus: HPLC (Waters Corp., U.S.A.).The attachments: Pump M 510 solvent delivery system, Detection: M 420-AC Fluorescence Detector at 365 nm excitation, 425 nm emission, Rheodyne 7725 sample injector(100 µl accessory), Data Station: Unicam 4880 Chromatography Data Handling System, Column: µBondapak C_{18} 125 A° 10µ 3.9x300 mm (Part No. 027324, Waters).

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