Occurrence of Aflatoxins in selected Colombian Foods

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Abstract

A survey of aflatoxin contamination in selected Colombian foods was conducted over a 12-month period on a total of 248 samples. Samples were collected in supermarkets, retail stores and stock centres and were grouped into five categories: (1) corn and corn products, (2) cereal grains, (3) rice and rice products, (4) legume seeds; and (5) snacks and breakfast cereals. Aflatoxins were identified and quantitated using a liquid chromatographic technique with a limit of detection of 1 ng/g for each aflatoxin. Aflatoxins were detected in 14 of 109 samples of corn and corn products, 4 of 40 samples of rice and rice products, 2 of 30 samples of legume seeds, and 2 of 11 samples of snacks and breakfast cereals. None of the cereal grains samples analysed contained detectable levels of aflatoxins. Twelve of the total of 22 positive samples exceeded the maximum tolerable level of aflatoxin B_1 adopted in most countries (5 ng/g); 10 of these 12 samples corresponded to corn and corn products. The results of the present study indicate that aflatoxin B_1 contamination in certain foods in Colombia is a major public health concern. Continuous monitoring of aflatoxin B_1 levels in Colombian foods is advised.

Introduction

Aflatoxins are a closely related group of heterocyclic metabolites synthesized predominantly by the fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. The chemistry and toxicology of aflatoxins were reviewed by Leeson *et al.* (1). Aflatoxins were first identified as the causative agent of the severe outbreak of "Turkey X" disease, a toxicosis that killed over 100,000 turkey poults in England in 1960 (2). Aflatoxins are a major concern as human hepatocarcinogens and are considered to play an important role in the high incidence of human hepatocellular carcinoma in certain areas of the world (3). Animal exposure to aflatoxins, particularly to aflatoxin B_1, may result in hepatotoxicosis, mutagenesis, immunosuppression, teratogenesis, or carcinogenesis (1). The incidence and level of aflatoxin contamination in food and feeds is continuously monitored worldwide (4, 5). However, while many surveys of natural occurrence of mycotoxins in several commodities are conducted every year in the industrialized countries, very little information is generated in developing countries. The lack of adequate laboratories and human and economic resources may be one of the reasons for the lack of information on mycotoxin occurrence in developing countries.

Moulds which can produce aflatoxins are considered to be more common in warm and humid tropical climates; however, little information concerning aflatoxin contamination in Colombian foods has been gathered to date. The aim of the present study was to investigate the incidence and level of contamination with aflatoxins in selected Colombian foods.

Experimental

Samples

A total of 248 samples of different grains, cereals, legume seeds and processed products for human consumption were collected and analysed over a period of 12 months. Sampling was conducted in supermarkets, retail stores, and stock centres, using the sampling methodology described by Piñeiro *et al.* (6). Samples were grouped into 5 categories: corn and corn products, cereal grains (wheat, barley, and oats), rice and rice products, legume seeds, and snacks and breakfast cereals. The samples were a random selection of the most popular local brands taken from store shelves. A subsample of 1 kg of each was ground and subsampled using a Romer Mill Series 2A (Romer Labs Inc., Union, MO, USA). The collected samples were stored in paper bags in a cool dry place until they were analysed (within one week of collection).

Analysis

Aflatoxins were analysed using the method reported by Trucksess *et al.* (7) and modified by Céspedes and Diaz (8), as follows: a 50 g of ground analytical sample was extracted with 100 mL acetonitrile-water (84:16, v/v) for 1 hour using a wrist-action shaker at high speed (a 25 g sample was taken in the case of hygroscopic

substrates such as rice and rice products). The extract was filtered using qualitative high speed filter paper and approximately 5 mL of the filtered extract were transferred into a 10 mL culture tube. A multifunctional cleanup column (Romer Mycosep™ 224, Romer Labs Inc., Union, MO, USA) was used for the purification of the extract. The column was slowly pushed into the culture tube until approximately 0.5 mL of purified extract appeared in the column reservoir, then 200 µL of purified extract were quantitatively transferred to a derivatization vial with teflon-lined screw cap. To the extract was then added 700 µL derivatizing solution (trifluoroacetic acid-glacial acetic acid-water, 2:1:7, v/v/v) in order to transform aflatoxins B_1 and G_1 into aflatoxins B_{2a} and G_{2a}, respectively. The vial was tightly closed, shaken in vortex for 30 seconds, and heated in a 65°C water bath for 10 minutes. After cooling the vial to room temperature the contents were filtered and injected into the liquid chromatograph. Chromatographic conditions were as follows, mobile phase: isocratic water-methanol (60:40, v/v) at a flow rate of 0.6 mL/min (LC-9A Liquid Chromatograph, Shimadzu, Kyoto, Japan); analytical column: reversed phase ODS, 12.5 cm x 4.0 mm I.D. (Lichrospher 100 RP-18, Merck, Darmstadt, Germany); column temperature: 50°C (Column oven CTO-6A, Shimadzu, Kyoto, Japan); fluorescence detector: excitation wavelength 350 nm; emission wavelength 450 nm (Fluorescence detector Model RF-535, Shimadzu, Kyoto, Japan). The limit of detection was 1.0 ng/g for individual aflatoxins (B_1, B_2, G_1, and G_2). Figure 1 shows chromatograms of a naturally contaminated corn meal sample and a standard. The quality control used in this study included confirmation of 10 positive samples (taken at random) by TLC. Two mL of the purified extract (obtained from the multifunctional cleanup column) were taken to dryness and dissolved with 100 µL of benzene-acetonitrile (98:2, v/v), then 20 µL of this solution were spotted on silicagel TLC plates and developed with chloroform-acetone (90:10, v/v), along with appropriate standards. Aflatoxin spots were visualized under long-wave UV light. The identity of aflatoxin B_1 was also confirmed by injecting into the chromatograph the extract of positive samples without previous derivatization (the peak of AFB_1 disappears). Aflatoxin standards were obtained from Sigma Chemical Co. (St. Louis, MO). Authenticity of the standards was made by TLC, and quantification by absorption of individual aflatoxins in the UV range, using a recording spectrophotometer (Shimadzu Model 160A UV-Visible spectrophotometer).

Results and Discussion

Measurable amounts of aflatoxins were found in all categories of foods analysed, except in cereal grains (Table 1). As expected, aflatoxin B_1 was the major contaminant in all the positive samples. The overall incidence of aflatoxin B_1 was 8.9% (22 out of 248 samples), and the overall levels of aflatoxin B_1 ranged from 1.0 to 103.3 ng/g with a mean value of 12.6 ng/g. The incidence and levels of aflatoxin B_2 were much lower than those found for aflatoxin B_1. Only 3 samples (1.2 %) had aflatoxin B_2 and the levels ranged from 1.4 to 6.7 ng/g, with a mean value of 4.2 ng/g. Aflatoxins G_1 and G_2 were found in only one sample (0.4 %), and were present in a sample of corn kernel that also contained aflatoxins B_1 and B_2.

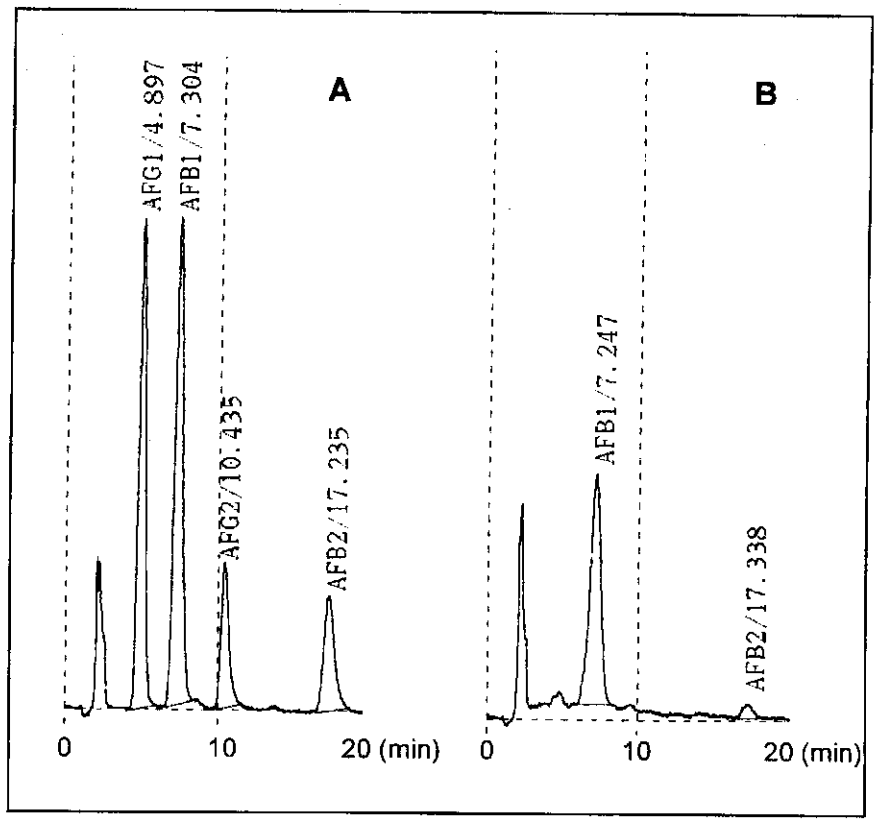

Figure 1 - Chromatograms of (A) standard aflatoxin mixture equivalent to 65 ng/g total aflatoxins (25:25:7.5:7.5, G_1, B_1, G_2, B_1), and (B) corn meal naturally contaminated with 11.2 ng/g aflatoxin B_1 and 1.4 ng/g aflatoxin B_2.

In a review of the current worldwide regulations for mycotoxins in foods, Van Egmond (9) reports that in the case aflatoxin B_1 alone, the majority of countries adopt a maximum tolerable level of 5 ng/g.

In the present study, 12 of the 22 samples found positive for aflatoxin B_1 contained levels above 5 ng/g. Further, the mean value for aflatoxin B_1 found in the 22 positive samples was 12.6 ng/g, which is more than twice the maximum tolerable level of 5 ng/g accepted in most countries. Most of the positive samples of corn and corn products (10 out of 14) had aflatoxin B_1 levels above 5 ng/g, and two of these samples had aflatoxin B_1 levels above 30 ng/g, which is the highest level allowed in the most permissible countries (10). These two samples contained 38.0 and 103.3 ng/g aflatoxin B_1, respectively. The sample that contained 103.3 ng/g aflatoxin B_1 was the only one that contained the four naturally occurring aflatoxins (B_1, B_2, G_1, and G_2, 136.7 ng/g total aflatoxins) and the sample that contained 38.0 ng/g aflatoxin B_1 also contained 4.6 ng/g aflatoxin B_2 (42.6 ng/g total aflatoxins). These high levels of contamination are obviously a major concern for public health, in light of the carcinogenic potential of aflatoxin B_1 (1). In the case of rice and rice products, two of the four positive samples had levels above 5 ng/g (9.8 and 13.6 ng/g) but these levels were much lower than those found in corn and corn products. None of the positive

samples of legume seeds or breakfast cereals and snacks had aflatoxin B_1 levels above 5 ng/g. Interestingly, none of the 58 samples of wheat, barley and oats analysed had detectable levels of aflatoxins.

Table 1 - Incidence and levels of aflatoxin B_1 in Colombian foods.

Substrate	Samples Analysed	Positiv samples	Incidence	Median (ng/g)	Mean (ng/g)	Range (ng/g)
Corn and corn products	109	14	12.8%	8.9	17.3	2.0-103.3
Cereal grains (wheat, barley,oats)	58	0	-	-	-	-
Rice and rice products	40	4	10.0%	6.8	7.1	1.0-13.6
Legume seeds	30	2	6.7%	2.3	2.3	1.0-3.6
Snacks and break-fast cereals	11	2	18.2%	1.7	1.7	1.3-2.0
TOTAL	248	22	8.9%	5.9	12.6	1.0-103.3

The results of the present survey indicate that aflatoxin occurrence is relatively low in Colombian foods (8.9 %), compared with such countries as India (11). This incidence is also much lower than the 29 % overall incidence of aflatoxin B_1 found in poultry and pig feeds and feedstuffs used in Colombia (8). Nevertheless, aflatoxin levels in some substrates, particularly corn and corn products, are a public health concern due to the high levels found in positive samples. To keep aflatoxin contamination at the lowest possible levels, surveillance of Colombian foods should continue. Continuos aflatoxin surveillance is important not only because of the high levels found in some samples in the present study but also because aflatoxin levels in corn may vary from year to year and from region to region in the same country (4, 11). Due to the lack of information, surveys on the incidence of other mycotoxins in human foods should be conducted in Colombia.

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