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A Research Note

ELISA Survey of Retail Grain-Based Food Products for Zearalenone and Aflatoxin B_1

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ABSTRACT

Seventy-nine grain-based food products were purchased from mid-Michigan retail grocery outlets in 1985 and analyzed for the mycotoxins zearalenone and aflatoxin B_1 by enzyme-linked immunosorbent assay. Twenty-two percent of these samples contained detectable zearalenone (limit $\geq$2.5 μg/kg). Zearalenone was found in breakfast cereal, snack foods, popcorn, corn meal, and cake-muffin mixes representing 10, 11, 57, 78, and 20% of these samples, respectively. The average level of this toxin among the positive samples was 20 μg/kg with maximum levels of 120 and 130 μg/kg being found in samples of corn meal and popcorn, respectively. Zearalenone was not found in any of the wheat flour or baby foods samples. Detectable aflatoxin B_1 (limit $\geq$5.0 μg/kg) was not found in any of the 79 samples tested.

Zearalenone and aflatoxin B_1 (AFB_1) are two mycotoxins that commonly contaminate cereal grains in the U.S. and throughout the world. Zearalenone is produced by toxigenic *Fusarium* sp. in corn, wheat, and barley both in the field and during storage *(7)*. The compound is estrogenic and has been associated with field cases of hyperestrogenism and reproductive problems in domestic animals *(1,7)*. AFB_1 is produced by toxigenic strains of *Aspergillus flavus* and *A. parasiticus* in a variety of agricultural staples including corn, peanuts and cottonseed *(8)*. AFB_1 is an extremely potent liver carcinogen in experimental animals and a correlation has been drawn between degree of AFB_1 exposure and primary hepatocellular carcinoma in developing countries *(1)*.

To make appropriate risk assessments on the effects of mycotoxins on humans, it is essential that reliable data be available concerning the frequency and levels of mycotoxins in the U.S. food supply. Efforts in collecting such data have been largely hindered by the lack of suitable rapid, sensitive chemical methods for mycotoxin detection. Several enzyme linked immunosorbent assays (ELISAs) have been developed for zearalenone *(2,6,14,16)* and AFB_1 *(3,5,10,12,13)* which can circumvent these problems. The purpose of this study was to survey retail grain-based products for the presence of zearalenone and AFB_1 by ELISA.

MATERIALS AND METHODS

Reagents

All inorganic chemicals and organic solvents were reagent grade or better. Chicken egg albumin (ovalbumin, grade II), 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS), hydrogen peroxide, horseradish peroxidase, and Tween 20 were purchased from Sigma Chemical Co. (St. Louis, MO). Zearalenone and AFB_1 antisera and horseradish peroxidase conjugates were prepared as described previously *(12,16)*.

Samples

Seventy-nine grain-based samples representing different lots of breakfast cereals, snack foods, popcorn, wheat flour, corn meal, cake/muffin mixes, and baby foods were purchased from mid-Michigan retail grocery outlets during summer, 1985.

Sample extraction for ELISA

Triplicate samples (5 g) were blended in 25 ml of methanol-water-dimethylformamide (70+29+1) extracting solvent for 5 min and filtered through Whatman No. 4 filter paper. The filtrate was used directly for zearalenone and AFB_1 ELISAs.

ELISA

Procedures used for ELISA were identical to those reported in detail previously for zearalenone *(16)* and AFB_1 *(12)*. Briefly, wells of polystyrene microtiter plates (Immunolon Removawells, Dynatech Laboratories, Alexandria, VA) were coated with specific mycotoxin antisera. Mycotoxin standards (in extracting solvent) or extracts were mixed with equal volumes of mycotoxin-horseradish peroxidase conjugate and 50 μl of this mixture was incubated over the antibody solid phase for 1 h at 37°C. The plates were washed and bound mycotoxin-horseradish peroxidase was determined *(9)*. Mycotoxin content was calculated from standard competition curves comparing log mycotoxin concentration vs. absorbance. Detection limits for zearalenone and AFB_1 were 2.5 and 5.0 μg/kg, respectively.

TABLE 1. *Zearalenone content of retail grain-based foods.*

Item	No. of samples analyzed	No. of positive samples[a,b]	Zearalenone content of positive samples (μg/kg)		
			Maximum	Minimum	Average
Breakfast cereals	39	4 (10)	8.6	2.6	4.6
Snack foods	9	1 (11)	2.9	2.9	2.9
Popcorn	7	4 (57)	130	2.5	38
Wheat flour	5	0 (0)	---	---	---
Corn meal	9	7 (78)	120	3.2	23
Cake/muffin mixes	5	1 (20)	3.1	3.1	3.1
Baby food	5	0 (0)	---	---	---
TOTAL	79	17 (22)	130	2.5	20

[a]Minimal detection limit for zearalenone was 2.5 μg/kg.
[b]Number in parentheses indicates % positive samples.

RESULTS AND DISCUSSION

AFB_1 was not detected in any of the 79 survey samples tested. The results of the retail product survey for zearalenone are summarized in Table 1. Detectable zearalenone was found in breakfast cereals, snack foods, popcorn, corn meal, cake/muffin mix but not wheat flour or baby food. Of the 17 zearalenone-positive samples, 4 were wheat products (3 breakfast cereals and 1 snack food) and the remaining 13 were corn products. Samples with detectable zearalenone comprised 22% of the total items surveyed and contained an average of 20 μg of zearalenone/kg.

ELISAs have been demonstrated to be specific, rapid, and sensitive alternatives to conventional chemical methods currently available for mycotoxin analysis *(2,3,5,6,9-13,16)*. The direct competitive ELISA employed here is based on competition between free mycotoxin in the sample extract and mycotoxin enzyme conjugate for binding to mycotoxin-specific solid phase antibody. Recently, our laboratory has demonstrated that direct competitive ELISAs for zearalenone *(2,16)* and AFB_1 *(12)* can readily be conducted on methanol-water-dimethylformamide extracts of spiked and naturally contaminated food samples, thereby simplifying the overall procedure and further reducing analysis time. This approach is thus highly compatible with screening large numbers of samples for mycotoxins and was therefore used in this survey.

Eppley et al. *(4)* found that 17% of 223 samples of market corn contained zearalenone at concentrations ranging between 100 and 5000 μg/kg. Scott et al. *(14)* previously detected between 13-20 μg of zearalenone/kg in a sample of corn flakes. In a recent retail product survey, deoxynivalenol (vomitoxin), a *Fusarium* mycotoxin that is often a cocontaminant with zearalenone, was found in 60% of 60 breakfast cereals analyzed *(15)*. It was therefore not surprising to find in our survey that zearalenone was also a frequent contaminant in grain-based retail products. Fifteen of the 17 positive samples contained zearalanone at levels less than 20 μg/kg. However, the observation that samples of corn meal and popcorn contained 120 and 130 μ/kg, respectively, may be cause for some concern. Currently, action levels do not exist for zearalenone in human foods in the U.S. Mirocha et al. *(7)* reported that feed samples containing as little as 100 μg/kg have been associated with swine hyperestrogenism. However, much higher levels of zearalenone are required to cause reproductive effects experimentally. The significance of our finding to human health might be further debated by the fact that a zearalenone-contaminated food (e.g. corn meal or popcorn) would represent only a small fraction of a consumer's overall diet. Nevertheless, it would be most prudent for commercial processors to screen raw ingredients for zearalenone before processing to minimize human exposure. The ELISA described here provides a simple approach for such screening.

Our inability to detect AFB_1 can be explained for several reasons. AFB_1 is an infrequent contaminant of wheat. The most likely source of AFB_1 in a grain-based food would be via corn produced in the southeastern U.S. This represents only a small portion of total U.S. corn production that may not be reflected in our sample survey. Since, the U.S. Food and Drug Administration has established a 20 μg/kg action level for AFB_1 in foods, producers and processors are cognizant of the need to screen incoming market corn for AFB_1 thus further decreasing the possibility of this toxin entering a finished food product. In related work, we have recently surveyed 63 peanut butter samples by ELISA and found that only 3 contained detectable AFB_1—all of which were below the 20 μg/kg action level *(13)*.

It must be noted that interpretation of this survey is subject to several limitations. Mycotoxin frequency and levels are largely determined by weather conditions and thus vary on a yearly and regional basis. Our samples were collected over a short time period in a small distribution area. No attempt was made to identify the origin of grain used in these finished products. Thus our data may not be truly indicative of actual average and maximum mycotoxin levels in U.S. foods. Such information could only be obtained by collecting samples from a large number of regional distribution points over the period of several years. Using ELISAs, such as those de-

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scribed here, a single technician could analyze hundreds of samples for mycotoxins in a short time period, making such extended surveys mechanistically feasible.

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