Detection of Fumonisins B_1, B_2, and B_3 and Hydrolyzed Fumonisin B_1 in Corn-Containing Foods

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Selected corn-containing foods were analyzed for the presence of fumonisins B_1, B_2, and B_3 (FB_1, FB_2, and FB_3) and hydrolyzed fumonisin B_1 (HFB_1). Samples were extracted with H_2O/CH_3CN (1:1). Following a cleanup procedure using a C_{18} SPE cartridge, analytical reversed-phase HPLC and fluorometric detection of the *o*-phthaldialdehyde derivatives were performed. Detection limits were 25 and 50 ng/mL for the FB_1 and FB_2 standards, respectively. FB_1 recovery for three tested levels, ranging from 250 to 1000 ng/g, was 115%. FB_1 and FB_2 contents ranged from 17 to 1410 ppb and from 0 to 414 ppb in foods, respectively. FB_3 was detected in 10 of 13 foods. HFB_1 was detected in tortilla chips, masa, and canned yellow corn.

INTRODUCTION

Fumonisins B_1, B_2, and B_3 (FB_1, FB_2, and FB_3, respectively) are the main members of a recently discovered family of mycotoxins produced by *Fusarium moniliforme* (Cawood et al., 1991). They were first isolated by Gelderblom et al. (1988), and their structure was elucidated by Bezuidenhout et al. (1988). Subsequent research has shown that the fumonisins are responsible for many of the toxic properties of corn culture material of *F. moniliforme* MRC 826 or corn and corn screenings naturally contaminated with this fungus. Marasas et al. (1988) induced equine leukoencephalomalacia (ELEM) in a horse by intravenously injecting FB_1 in purified form. Kellerman et al. (1990) reported induction of ELEM in two horses by oral dosing of FB_1. Porcine pulmonary edema (PPE) has been shown to be caused by FB_1 by Harrison et al. (1990). The fumonisins have been associated with high human esophageal cancer risk in the Transkei, South Africa (Sydenham et al., 1990). FB_1 was shown to have a cancer-promoting effect in rats (Gelderblom et al., 1988). These researchers later concluded that FB_1 was responsible for the hepatocarcinogenic and hepatotoxic activities but not for all other toxic effects (lesions in esophagus, heart, and forestomach) caused by culture material of *F. moniliforme* MRC 826 observed in rats (Gelderblom et al., 1991). Both Gelderblom et al. (1992) and Norred et al. (1992) found that the FBs lacked genotoxicity. Previously, Gelderblom and Snyman (1991) had shown that neither of the FBs was mutagenic in the *Salmonella* mutagenicity test. Kraus et al. (1992) reported that a synthesized FB_1 analogue containing only the tricarboxylic acid side-chain units was not cytotoxic to a rhesus monkey kidney cell line. An analogue containing both tricarboxylic acid side-chain units and the amine group, but not the hydroxyl groups, was equally as toxic as FB_1, while two analogues containing all three functional groups were found to be more cytotoxic than FB_1. Wang et al. (1991) have reported on the disruption of sphingolipid biosynthesis by the FBs and suggested that this may be a critical event in the diseases associated with these mycotoxins.

Hydrolyzed FB_1 (HFB_1) has been produced when corn was nixtamalized and appears to be more toxic to rats than FB_1. A feeding study in which rats were fed corn containing 50 ppm of FB_1 or 10 ppm of HFB_1 resulted in roughly equal toxicity signs in both groups, indicating that HFB_1 could be more toxic than FB_1 itself (Hendrich et al., 1993).

The presence of fumonisins in corn-containing foods could have implications for human health. The results of analysis of selected corn-containing foods, pet foods, and rat chow are reported here. This paper is the first report of the presence of FB_3 and HFB_1 in human foods.

EXPERIMENTAL PROCEDURES

Analytical Standards. FB_1 was provided by P. G. Thiel from the Research Institute for Nutritional Diseases, South African Medical Research Council, South Africa. FB_2 was purchased from Sigma Chemical Co. FB_3 was purified from corn culture material of *Fusarium proliferatum* 6104, an overproducer of FB_3 (P. F. Ross, personal communication; confirmed by R. D. Plattner). HFB_1 was produced from FB_1 according to the method of Wilson et al. (1990). The FBs are suspected carcinogens and should be handled as such.

Corn-Containing Foods. Food products (yellow and white cornmeal, canned corn, tortilla chips, masa, and dog and cat foods) from two crop years (1989 and 1990) were purchased locally. A rat chow sample was provided by the experimental animal housing facilities of the department. Samples were stored at room temperature prior to analysis.

Sample Preparation. Samples were prepared as described by Ross et al. (1991) with some modifications. Briefly, ground sample (25 g) was extracted with 50 mL of acetonitrile/water (1:1). Ten milliliters of the filtered extract was diluted with 30 mL of water and centrifuged for 15 min at 15 000 rpm. The supernatant was applied to a C_{18} cleanup cartridge (Sep-Pak, Waters, Division of Millipore), and the cartridge was washed with 5 mL of acetonitrile/water (1:4). The FBs were eluted with 2 mL of acetonitrile/water, either 1:1 or 7:3. FB_2 and FB_3 do not completely elute from the Sep-Pak with acetonitrile/water (1:1) but do with a 7:3 acetonitrile/water ratio (Ross et al., 1991). Initially, sample cleanup was performed using acetonitrile/water (1:1). The cleanup procedure was repeated with replicate samples using acetonitrile/water (7:3) as the eluting solvent. The FB concentrations estimated from the initial cleanup procedure were confirmed with the 7:3 acetonitrile/water cleanup, and the data were combined.

FB Analysis. The *o*-phthaldialdehyde (OPA) derivative was prepared as follows: 100 μL of the cleaned-up extract, 100 μL of borate buffer (0.05 M, pH 8.3), and 100 μL of OPA solution [1 mg of OPA (Sigma)/mL in acetonitrile; 20 μL of 2-mercaptoethanol/10 mL) were mixed. After 10 min, 100 μL of water was added and 20 μL of was chromatographed on an analytical C_{18}

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Table I. Amounts[a] of FB_1, FB_2, FB_3, and HFB_1 in Corn-Containing Foods

food	year	ppb FB_1	ppb FB_2	ppb FB_3	ppb HFB_1
white cornmeal	1989	210 ± 120	33 ± 10	+[c]	nd[d]
white cornmeal	1990	360 ± 220	58 ± 34	++	nd
yellow cornmeal	1989	790 ± 80	120 ± 21	+	nd
yellow cornmeal 1	1990	560 ± 140	266 ± 158	+	nd
yellow cornmeal 2	1990	840 ± 300	144 ± 23	++	nd
yellow cornmeal 3	1990	680 ± 170	414 ± 285	+++	nd
canned yellow corn[b]	1990	26	nd	nd	+
tortilla chips	1989	320 ± 130	nd	nd	+
tortilla chips	1990	310 ± 110	nd	nd	+
masa	1989	17 ± 3	nd	nd	+++
dry dog food	1989	1410 ± 140	144 ± 27	+++	nd
dry dog food	1990	820 ± 320	102[b]	++	nd
dry cat food	1989	987 ± 156	140[b]	++	nd
dry cat food	1990	220 ± 130	125 ± 44	++	nd
rat chow[b]	1990	219	<20	+	nd

[a] Amounts reported were not corrected for recovery. [b] Average of two measurements; all other values reported are averages of triplicate measurements. [c] +, ++, and +++ are indications of amount present. [d] Not detected.

column (3 cm × 4.6 mm, Perkin-Elmer) using isocratic conditions (61% potassium phosphate buffer, 0.05 M, pH 3.3; 39% acetonitrile; 1 mL/min for 9 min followed by an increase to 2 mL/min in 1 min, total analysis time was 30 min). The fluorescent derivative was detected using a Turner fluorometer equipped with a Corning 7-60 as the primary filter and a Wratten 2a as the secondary filter or a Waters 470 scanning fluorometer (excitation, 335 nm; emission, 440 nm).

A standard curve for FB_1 was prepared daily to account for day-to-day fluorescence variation (25–500 ng/mL using the Waters fluorometer, 100–2000 ng/mL using the Turner fluorometer). Replicate analyses of FB_1 standards (25 and 100 ng/mL for the Waters fluorometer, 1000 ng/mL for the Turner fluorometer) over a 30-day period and calculated back, using one standard curve, resulted in average FB_1 contents of 24 ± 3, 105 ± 18, and 1035 ± 116 ng/mL. FB standards were analyzed daily to verify retention times of 6.1, 18.2, and 19.4 min for FB_1, FB_2, and FB_3, respectively. A standard curve (50–500 ng/mL) for FB_2 (Sigma) was prepared when it became available, and FB_2 content was determined in retrospect. The presence of FB_3 was confirmed by addition of purified FB_3 to selected prepared food extracts. The FB_3 content was estimated by comparison of the peak height to FB_2. The presence of HFB_1 (retention time of 7.1 min) in suspect samples was confirmed by the National Veterinary Services Laboratory, ARS, USDA, and by addition of HFB_1 standard to selected prepared extracts.

Recoveries were determined by adding 250, 500, and 1000 ng/g FB_1 to yellow cornmeal (1990 crop year). Sample preparation and analysis were done as described. Determination of FB content in foods and recoveries of FB_1 were performed in triplicate.

The detection limit for this method was 10 ppb for FB_1 and 20 ppb for FB_2. With the pet foods and rat chow, the detection limit was 200 ppb for FB_1 due to necessary dilution to prevent fluorescent interference of earlier eluting compounds. These compounds did not interfere with the detection of FB_2 or FB_3 in these samples, since the retention times of FB_2 and FB_3 are much higher.

Statistical Analysis. Statistical analysis was performed using the general linear means procedure and Scheffe's multiple-comparisons procedure using the SAS package (Cary, NC).

RESULTS AND DISCUSSION

FB_1 and FB_2 concentrations and indication of the presence of FB_3 and HFB_1 in 15 analyzed samples are given in Table I. Figure 1 shows example chromatograms of the analysis of white cornmeal (panel A) and masa (panel B). The mean concentrations of FB_1 and FB_2 of six cornmeals analyzed were 573 and 173 ppb, respectively. This includes the white cornmeals which have a significantly lower mean FB_1 concentration ($P < 0.05$) than the yellow cornmeals (285 ppb of FB_1 vs 718 ppb of FB_1, respectively). Although the mean FB_2 concentration of white cornmeal appeared to be lower than the mean FB_2 concentration of yellow cornmeal (46 FB_2 vs 236 FB_2, respectively), this difference was not statistically significant. Sydenham et al. (1991) reported mean concentrations of 1048 ppb of FB_1 and 298 ppb of FB_2 in 15 positive samples among 16 cornmeal samples from the United States. Stack and Eppley (1992) reported average levels of 910 ppb of FB_1 and 220 ppb of FB_2 in 10 cornmeals from Washington, DC. No differentiation was made between white or yellow cornmeal in these two studies.

High levels of FBs were found in three of four analyzed pet foods compared to levels found in human corn meals. Corn-based pet foods are generally produced using corn screenings (C. Hurburgh, Agricultural Engineering, Iowa State University, personal communication). Murphy et al. (1993) found that corn screenings contain about 10 times the fumonisin contents of whole corn. Stack and Eppley (1992) found higher levels of FB_1 in corn screenings compared to those in yellow field corn (1.74–196 vs 0.08–16.31 ppm of FB_1).

Our results agree with the range of FB concentrations earlier reported in corn-containing foods. Sydenham et al. (1991) reported ranges of 0–2790 ppb for FB_1 and 0–920 for FB_2. In a survey of the occurrence of FB_1 and FB_2 in corn-containing foods from Switzerland, a ranges of 0–790 ppb for FB_1 and 0–160 ppb for FB_2 were found in 120 analyzed samples (Pittet et al., 1992).

All samples analyzed in this study contained FB_1. No FBs were detected in 76 of the 120 samples analyzed by Pittet et al. (1992), while Sydenham et al. (1991) and Stack and Eppley (1992) reported several negative samples. This can partly be explained by the lower detection limits of 10 ppb for FB_1 and 20 ppb for FB_2 for the method described here, compared to detection limits of 50 ppb for both FB_1 and FB_2 reported by Sydenham et al. (1991) and Pittet et al. (1992). However, Stack and Eppley (1992) reported detection limits of 10 ppb for both fumonisins.

In this study, the average FB_1 recovery for three tested levels was 115%. The results are shown in Table II. These results compare favorably to the FB_1 recovery of 67% reported by Stack and Eppley (1992), who used the method developed by Shephard et al. (1990). Shephard et al. (1990) originally reported recoveries of 99.5% and 85.9% for FB_1 and FB_2, respectively. However, the FB recovery aliquot was added to the corn solvent extract and not to the original product, which could explain the discrepancy between these results and those reported by Stack and Eppley (1992). Others (Sydenham et al., 1991; Pittet et al., 1992) have not reported recoveries.

Analysis of corn and corn screenings showed a strong correlation among the FB_1, FB_2, and FB_3 contents (Murphy et al., 1993). The FB_1/FB_2 ratios in the analyzed foods were determined when possible. The ratios of seven samples ranged from 0.10 to 0.17 with an average of 0.14. Three samples (two cornmeals and one cat food) showed much higher ratios (0.48–0.61). These results suggest there may be a constant ratio between the FBs in human foods as observed in field corn and corn screenings. The difficulty of extracting low levels of FB_2 and FB_3 from the food samples reproducibly and the lack of a standard for FB_3 made it difficult to draw a clear conclusion for corn-containing food. However, if FB_2 or FB_3 could be detected, the apparent FB_3 contents were always less than the FB_2 content, and the FB_2 contents were always less than the FB_1 content, with one exception (commercial cat food,

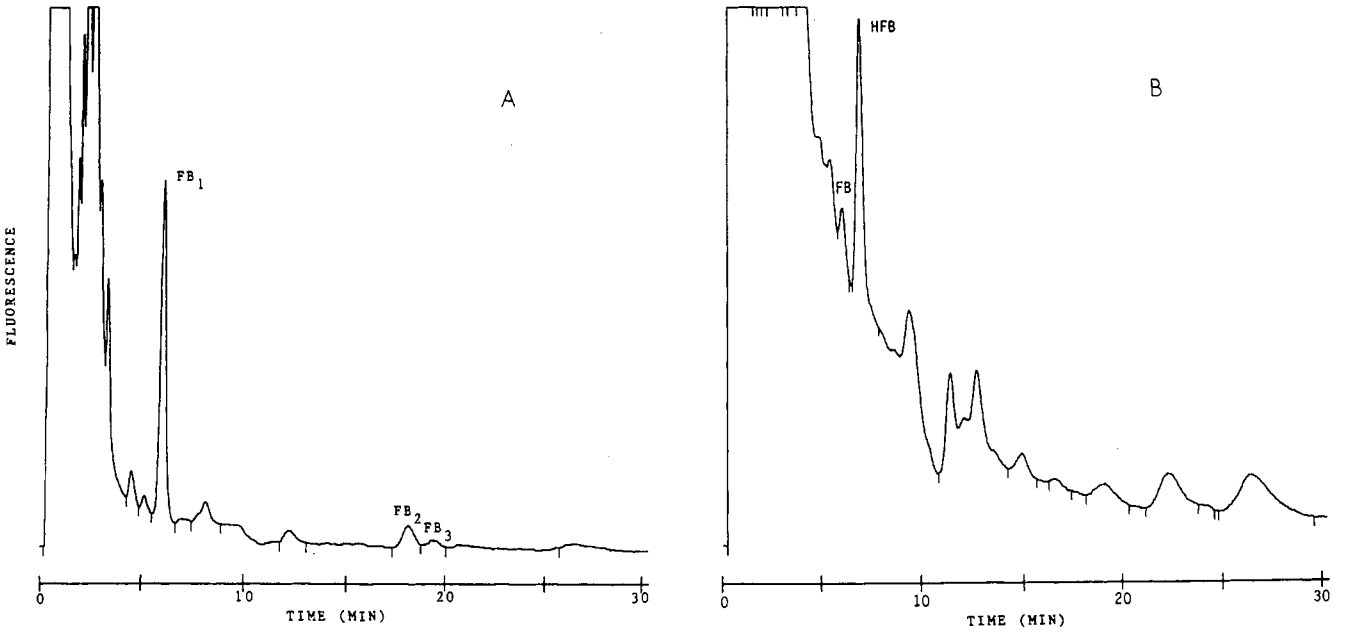

Figure 1. HPLC chromatogram of the OPA derivatives of (A) white cornmeal [crop year 1990; the FB_1 and FB_2 peaks represent 380 (60 ng/g) and 60 ng/mL (96 ng/g), respectively] and (B) masa [crop year 1989; the FB_1 peak represents 51 ng/mL (20 ng/g)].

Table II. Recovery of Fumonisin B_1 Added to Cornmeal

added, ng/g	total found, ng/g	net found, ng/g	FB_1 recovery, %
0	493 ● 6		
250	782 ● 81	289 ± 87	116 ± 35
500	1087 ● 170	594 ● 176	119 ± 35
1000	1595 ± 191	1102 ± 197	110 ± 20

1990). In the cat food, the apparent FB_3 content was slightly higher than the FB_2 content. No FB_2 or FB_3 could be detected in masa, tortilla chips, or canned yellow corn. It would be expected that FB_2 and FB_3 present in these samples were in such low concentrations that they could not be detected.

The occurrence of HFB_1 in tortilla chips, masa, and canned yellow corn was significant due to the toxicity reported by Hendrich et al. (1993). Sydenham et al. (1991) suggested that the process to prepare these foods, which involves a step with high pH and heat, would destroy FB_1. Apparently, FB_1 loses its two tricarboxylic acid groups to form HFB_1, which appears to be more toxic.

The presence of 219 ppb of FB_1 and a trace of FB_2 in corn-based commercial rat chow could provide complications in feeding studies designed to determine the activity of a carcinogenic compound. Although the FB content of the analyzed rat chow was much lower than the hepatocarcinogenic dose of 50 ppm in a rat feeding study by Gelderblom et al. (1991), sub parts per million levels could influence the multistage process of carcinogenesis. This becomes especially important in the case of a long-term study of a hepatocarcinogen, as synergistic action of the FBs present and the compound studied are a possibility.

The results of this study show that fumonisins are present in a wide range of corn-containing products for human consumption and companion animal foods. The effects of long-term exposure to low levels of FB_1 should be studied as well as the toxicological significance of FB_2, FB_3, and HFB_1.

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