JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Extraction of Fumonisins B_1 and B_2 from White Rice Flour and Their Stability in White Rice Flour, Cornstarch, Cornmeal, and Glucose

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To extract fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) from Thai white rice flour, different solvent mixtures, temperatures, pH values, and addition of enzymes or ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) were examined. Three extractions with 0.1 M Na_2EDTA achieved the highest recoveries. Initial recoveries of fumonisins added to white rice flour, cornstarch, cornmeal, and glucose varied with commodity. Fumonisins disappeared in Thai white rice flour after 12 h, but 55% remained in another white rice flour. With cornstarch 20−30% fumonisins remained after 24 h; only 43% of ^{14}C-labeled FB_1 materials extracted from cornstarch was eluted with methanol from an immunoaffinity column. Fumonisins were stable in cornmeal for 24 h but only ~50% remained after 30 days. With glucose, 25% of FB_1 and FB_2 remained 24 h after addition; *N*-(1-deoxy-D-fructos-1-yl)FB_1 and *N*-(carboxymethyl)FB_1 were detected in lower amounts than residual FB_1 after 3 months.

KEYWORDS: Fumonisins; extraction; stability; white rice flour; cornstarch; cornmeal; glucose

INTRODUCTION

Fumonisins are a group of structurally related mycotoxins produced mainly by *Fusarium verticillioides* (*F. moniliforme*) and *Fusarium proliferatum*, both of which frequently infect corn crops worldwide (*1*−*3*). Fumonisin B_1 (FB_1) is 2*S*-amino-12*S*,-16*R*-dimethyl-3*S*,5*R*,10*R*,14*S*,15*R*-pentahydroxyeicosane with the C-14 and C-15 hydroxy groups esterified by a terminal carboxyl group of propane-1,2,3-tricarboxylic acid (tricarballylic acid), and fumonisin B_2 (FB_2) is 10-deoxy-FB_1 (**Figure 1**). FB_1 is the most abundant fumonisin and is known to cause equine leukoencephalomalacia and porcine pulmonary edema. It is also a non-genotoxic kidney or liver carcinogen in rats and mice (*4*, *5*). Epidemiological studies suggest that FB_1 may be associated with human esophageal cancer (*2*). Methods of analysis for fumonisins have been reviewed by Shephard (*6*).

Fumonisins are mainly found as natural contaminants of corn and corn-based foods (*7*, *8*). However, there is evidence that they can occur in other crops and derived foods: sorghum (*9*) and sorghum syrup (*10*); white beans, adzuki beans, and mung beans (*11*−*13*); wheat, barley, and soybean (*14*); black tea and medicinal plants (*15*); rough rice in the southern United States (*16*); rough rice, polished rice, and rice cakes in Korea (*17*, *18*); rice in China (*19*); and unpolished rice imported into Germany from various countries (*20*). Previous studies in this laboratory on the extraction of fumonisins from rice, corn-based foods, and beans found that FB_1 and FB_2 could not be recovered when added to a sample of Thai white rice flour and extracted with methanol/acetonitrile/water (25:25:50, v/v/v) (*21*). There were also indications that the fumonisins were unstable in naturally contaminated rough rice and cornstarch over long time periods. In view of these observations, we proposed developing a method of analysis for FB_1 and FB_2 in Thai white rice flour by optimizing the extraction procedure and then studying their stability at room temperature in white rice flour (two types), cornstarch, cornmeal (as control), and glucose. Because starch is the major component (~90%) of milled rice (*22*, *23*), a comparison of stability in the first two foods appeared to be warranted. Resch and Shier (*24*) recently indicated that heating ^{14}C-labeled FB_1 in a cornstarch matrix resulted in binding to the starch, whereas alkaline hydrolysis released hydrolyzed FB_1 and related compounds. Although a wet-milling study by Bennett et al. (*25*) on corn naturally contaminated with fumonisins indicated the absence of fumonisins in the starch fraction, contrary reports indicated the natural occurrence of fumonisins in cornstarch (*26*−*28*).

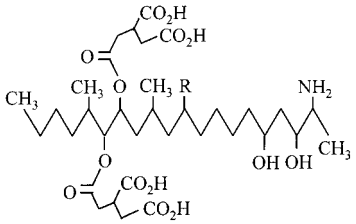

Figure 1. Structures of FB_1 (R = OH) and FB_2 (R = H).

Approaches reported in the literature for improving the extraction of fumonisins from corn and certain corn-based foods include addition of acid to the extraction solvent (*29*−*32*), raising the extraction temperature (*33*), addition of enzymes (also

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10.1021/jf020058s CCC: $22.00 Published 2002 by the American Chemical Society
Published on Web 05/10/2002

used to improve recovery of fumonisins from rice) (*34*), and addition of ethylenediaminetetraacetic acid (EDTA) (*35*−*37*). We applied these approaches to the extraction of spiked Thai white rice flour in an attempt to improve recoveries using methanol/acetonitrile/water (25:25:50, v/v/v), a solvent mixture that has recently come into favor for extraction of rice and corn foods (*21*, *37*−*40*).

Glucose is known to react with FB_1 on heating. The product is not toxic in rats (*41*). *N*-(1-Deoxy-D-fructos-1-yl)FB_1, the Amadori rearrangement product of an FB_1−glucose Schiff base (*42*), and *N*-(carboxymethyl)FB_1, formed by its oxidation (*43*, *44*), have been chemically characterized as products of this reaction. Adding glucose during thermal processing of foods can in fact result in a large reduction in the concentration of FB_1 (*45*). We chose to examine the stability of FB_1 added to solid glucose at room temperature as a possible model experiment for the stability studies with white rice flour and cornstarch.

MATERIALS AND METHODS

Chemicals. Methanol (HPLC grade) and acetonitrile (HPLC grade) were obtained from EM Science (Gibbstown, NJ). All reagents were of analytical grade. EDTA disodium salt (Na_2EDTA) dihydrate, α-amylase, *β*-glucosidase, and *o*-phthaldialdehyde (OPA) were purchased from Sigma (St. Louis, MO). Deionized water (HPLC grade) was obtained using a Millipore Milli-Q water system (Millipore, Bedford, MA). D-Glucose (minimum assay = 99.5%) was from Sigma (St. Louis, MO).

Analytical Standards. FB_1 (batch D/93) and FB_2 (batch M/93) were gifts from Agriculture and Agri-Food Canada (M. E. Savard and J. D. Miller). *N*-(Carboxymethyl)FB_1 was supplied by P. C. Howard (U.S. Food and Drug Administration, Jefferson, AR), and *N*-(1-deoxy-D-fructos-1-yl)FB_1 was from S. M. Poling (U.S. Department of Agriculture, Peoria, IL). ^{14}C-labeled FB_1 (specific activity = 650 *μ*Ci/mmol; *46*) was from M. E. Savard (Agriculture and Agri-Food Canada, Ottawa, ON). Each standard solution was prepared by dissolving 1 mg of the FB_1, FB_2, and [^{14}C]FB_1 in 1 mL of acetonitrile/water (3:1) and then kept at 4 °C for further dilution. *N*-(Carboxymethyl)FB_1 and *N*-(1-deoxy-D-fructos-1-yl)FB_1 were dissolved in water just before analysis.

Reagents. Phosphate-buffered saline (PBS) was a solution of 0.2 g of potassium chloride, 0.2 g of potassium dihydrogen phosphate, 1.16 g of disodium hydrogen phosphate, 8 g of sodium chloride, and 1 g of sodium azide in 1 L of distilled water, adjusted to pH 7.0 with concentrated hydrochloric acid. Phosphate buffer (0.1 M; pH 6.0) was prepared by mixing 87.7 mL of 0.2 M sodium dihydrogen phosphate with 12.3 mL of 0.2 M disodium hydrogen phosphate and diluting with water to 200 mL. Sodium citrate buffer (0.1 M; pH 3.3) was prepared by mixing 46.5 mL of 0.1 M citric acid, 3.5 mL of 0.1 M sodium citrate, and 50 mL of water. OPA reagent was a solution of 40 mg of OPA in 1 mL of methanol diluted with 5 mL of 0.1 M sodium tetraborate plus 50 *μ*L of 2-mercaptoethanol; it could be stored in the dark for up to 1 week.

Foods. All food samples used in this study were purchased locally and stored at 4 °C.

Extraction of FB_1 and FB_2 from Thai White Rice Flour. To optimize the extraction of FB_1 and FB_2 from Thai white rice flour, various temperatures and apparent pH values of extraction solvents were studied. Different solvent mixtures, including Na_2EDTA mixed with organic solvents, and the addition of α-amylase and *β*-glucosidase enzymes were also investigated to increase extraction efficiency. Ten grams of Thai white rice flour was weighed into a 250 mL Erlenmeyer flask and spiked with 500 *μ*L of standard solution (10 *μ*g each/mL) to give a final spiking level of 500 ng of FB_1 and 500 ng of FB_2/g. The sample was shaken briefly by hand, and 50 mL of the extraction solvent was added immediately. The general extraction solvent chosen was methanol/acetonitrile/water (25:25:50) as previously reported (*21*). To study the effect of the extraction temperature of the solvent mixture (20, 40, 50, and 60 °C), a 500 mL Erlenmeyer flask with a glass stopper was used in a horizontal shaking water bath (TBVS Hetomix, Hetto-Holten, Allerød, Denmark) for 1 h of extraction. To study the effect of pH, the apparent pH of the extraction solvent was adjusted with orthophosphoric acid to pH 2, 3, 4, 5, and 6, and samples were extracted for 1 h at room temperature (20 °C) in a horizontal shaking machine (Arthur H. Thomas, Philadelphia, PA). To study the effects of α-amylase and *β*-glucosidase, 25 mL of 0.1 M phosphate buffer (pH 6.0) containing 50 mg of α-amylase (1950 units, 39 units/mg from *Aspergillus oryzae*) or 40 mg of *β*-glucosidase (1140 units, 2.85 units/mg from almonds) was added to the sample. The mixtures were incubated at 40 °C for 1 h in a shaking water bath before the extraction solvent was added. The effect of including 0.1 M Na_2EDTA dihydrate (adjusted to pH 6.0 with 0.1 M sodium hydroxide) in the extraction solvent mixture was studied at room temperature and 50 °C, both for 1 h. The room temperature experiment with methanol/acetonitrile/0.1 M Na_2EDTA (25:25:50) was also carried out with two consecutive extractions, and the extracts were combined. Extractions with 0.1 M Na_2EDTA (pH 6.0)/methanol (50:50) and with 0.1 M aqueous Na_2EDTA (three times for 30 min at room temperature) were also carried out. All extracts were centrifuged for 15 min at 3000 rpm. Extracts or, when applicable, combined extracts were cleaned up on FumoniTest columns (Vicam, Watertown, MA) fitted with 15 mL reservoirs; 10 mL of extract was mixed with 10 mL of PBS, and 10 mL of this mixture was added to the column with gravity elution. The column was washed with 5 mL of PBS, which was discarded. FB_1 and FB_2 were eluted with 2 mL of HPLC grade methanol into 4 mL vials with Teflon-lined screw-caps. Samples were evaporated under a gentle stream of nitrogen at ∼55 °C in a heating block, and the residue was dissolved in 200 *μ*L of 0.1 M sodium borate. The samples were derivatized with OPA reagent and analyzed by LC fluorescence detection. All analyses for method development on Thai white rice flour were performed in duplicate.

Studies on the Stability of FB_1 and FB_2 Added to White Rice Flour, Cornstarch, Cornmeal, and Glucose. Ten grams of each sample was spiked with 500 ng each of FB_1 and FB_2/g in a manner similar to the extraction studies. All stability experiments were single analyses. After spiking, each sample was kept at room temperature for up to 60 days before analysis. Thai white rice flour was extracted three times with 50 mL of 0.1 M Na_2EDTA (pH 6.0). The U.K. white rice flour, cornstarch, and cornmeal were extracted with 50 mL of methanol/acetonitrile/water (25:25:50) by shaking for 1 h. For glucose, 0.1 M Na_2EDTA (pH 6.0) was used. Immunoaffinity column cleanup was performed as above. For ^{14}C-labeled FB_1 in cornstarch, 10 g of cornstarch was spiked at the same concentration as FB_1 and extracted (after 1 day) with 50 mL of methanol/acetonitrile/water (25:25:50) followed by centrifuging at 3000 rpm for 15 min. The radioactivity of the crude extract and of each fraction from the immunoaffinity column was determined. The sample-containing tubes were analyzed in duplicate—total count and background activity were counted. Radioactivity for ^{14}C was quantified as counts per minute (cpm) using a Beckman 7800 liquid scintillation analyzer (Beckman Instruments, Inc., Fullerton, CA).

Reaction of Solid Glucose with FB_1. In an attempt to characterize the reaction of solid glucose with FB_1, glucose was spiked at a higher level of 50 *μ*g/g and analyzed after 1 and 8 days and 3 months. Ten gram samples were extracted by shaking for 1 h with 50 mL of 0.1 M Na_2EDTA (pH 6.0) (glucose dissolved thoroughly in the 0.1 M Na_2EDTA), and 20 mL was applied to a Bond Elut HF C_{18} cleanup column (500 mg, Varian, Harbor City, CA), preconditioned with 5 mL of methanol and then 5 mL of water. The cartridges were washed with 5 mL of water, followed by 5 mL of methanol/water (1:3). The FB_1 and glucose−FB_1 reaction products were eluted with 10 mL of methanol. The eluates were dried under a gentle stream of nitrogen gas at ∼55 °C and dissolved in water. The samples were analyzed by LC evaporative light scattering detector (ELSD) and LC electrospray ionization (ESI) mass spectrometry (MS).

Determination of OPA Derivatives by LC Fluorescence. LC was carried out on the *o*-phthaldialdehyde/mercaptoethanol derivatives of FB_1 and FB_2 with fluorescence detection. A Shimadzu HPLC equipped with a model LC-6A pump, an SCL-6B system controller, an SIL-6B autoinjector, a Shimadzu CLASS-VP (version 4.2) chromatography data system, and an RF-551 fluorescence detector with excitation at 335

nm and emission cutoff at 440 nm (Shimadzu, Kyoto, Japan) was used. The autoinjector was programmed to mix 80 μL of sample extract with the same amount of OPA reagent. Ten microliters of the mixture was injected after 2 min of incubation. Separation was carried out on a MetaChem Inertsil ODS-2, 5 μm, 250 × 3.0 mm i.d., column (MetaChem, Torrance, CA) with a 2 cm Supelguard LC-18 guard column (Supelco, Bellefonte, PA). Mobile phase solvents for LC fluorescence were (A) methanol/0.05 M sodium dihydrogen phosphate (55:45), acidified to apparent pH 3.3 with orthophosphoric acid, and (B) acetonitrile/water (8:2), acidified to apparent pH 3.3 with orthophosphoric acid. The following gradient profile was employed: 100% A from 0 to 5 min, linear gradient from 0 to 95% B from 5 to 9 min, 95% B from 9 to 15 min, and 95 to 0% B from 15 to 17 min; flow rate was 0.5 mL/min. Measurements were made by peak area. The detection limit (signal/noise = 3) obtained by this procedure was 1 ng each of FB_1 and FB_2.

Determination of Underivatized Fumonisins by LC-ELSD. *N*-(1-Deoxy-D-fructos-1-yl)FB_1 and *N*-(carboxymethyl)FB_1 were determined without derivatization using LC-ELSD, a technique previously used for fumonisins lacking a free NH_2 group (*47*, *48*). The column was a TSK-GEL ODS-80T_M, 5 μm, 250 × 4.6 mm i.d. (TosoHaas, Montgomeryville, PA), and mobile phases were (A) acetonitrile/water/acetic acid (20:80:1) and (B) acetonitrile/water/acetic acid (80:20:1) with a gradient of 100% A from 0 to 3 min, 0 to 100% B from 3 to 30 min, and 100% B from 30 to 40 min. The flow rate was 1 mL/min. The detector was an ELSD 2000 (Alltech, Deerfield, IL). Impactor-off mode was used; the tube temperature was 115 °C, and the flow rate of nitrogen gas was maintained at 2.0 L/min.

Determination of FB_1, *N*-(1-Deoxy-D-fructos-1-yl)FB_1, and *N*-(Carboxymethyl)FB_1 by LC-ESI MS. The LC column was a Luna C_{18}, 3 μm, 150 × 2.0 mm i.d. (Phenomenex, Torrance, CA), and mobile phases were (A) 0.1% aqueous acetic acid or aqueous formic acid and (B) acetonitrile/methanol (1:1), with a gradient of 35% B from 0 to 1 min, linear gradient to 60% B from 1 to 15 min, and 60% B from 15 to 32 min. The mobile phase was returned to 35% B from 32 to 34 min. The flow rate was 0.2 mL/min. An HP 1100 system (with degasser, binary pump, and autosampler) was interfaced to a Quatro II tandem mass spectrometer (Micromass, Manchester, U.K.) through an ESI interface. Mass spectra were obtained by scanning from *m*/*z* 300 to 1000. The capillary voltage was 3.5 kV, the cone voltage was 50 V, and the source temperature was 130 °C.

RESULTS AND DISCUSSION

Method Development for Thai White Rice Flour. Recoveries of FB_1 and FB_2 added to a sample of Thai white rice flour each at 500 ng/g were <5% using methanol/acetonitrile/water (25:25:50) as extraction solvent. This result is in agreement with that obtained previously by Scott et al. (*21*). Even three consecutive extractions yielded only 13% recoveries of each fumonisin (single analysis). Recoveries of FB_1 and FB_2 added to a blank extract at the same equivalent level were 104 and 96%, respectively, indicating the losses did not occur on the immunoaffinity column. Several experiments were tried to improve recoveries from the Thai white rice flour by modifying the extraction solvent and conditions. Increasing the temperature of the extraction solvent to 40−50 °C improved recoveries to 20−25%, although recovery at 60 °C was lower (**Figure 2a**). Adjusting the apparent pH of the extraction solvent to pH 3 (with orthophosphoric acid) also gave fumonisin recoveries of 20%, although recoveries reverted to 5% at pH 4−6 (**Figure 2b**). Combining a temperature of 50 °C with methanol/acetonitrile/0.1 M sodium citrate buffer (pH 3.3) (25:25:50) again gave only 20−21% recoveries. Addition of the enzymes α-amylase or β-glucosidase in 0.1 M phosphate buffer (pH 6.0) to Thai white rice flour for 1 h at 40 °C, followed by extraction with methanol/acetonitrile/water (25:25:50) at 40 °C, gave low recoveries (0−16%), indicating the pretreatment could be detrimental. Incorporation of Na_2EDTA into the extraction solvent mixture resulted in an improvement in recoveries of FB_1 and FB_2. The best recoveries achieved were 50% for FB_1 after three extractions with 0.1 M Na_2EDTA (no organic solvents) (**Figure 2c**). Agreement between duplicate analyses was excellent; the highest variation was noted for the series of extractions with methanol/acetonitrile/water (25:25:50) at four temperatures, where the analyses differed from the means by an average of 10 and 11% for FB_1 and FB_2, respectively.

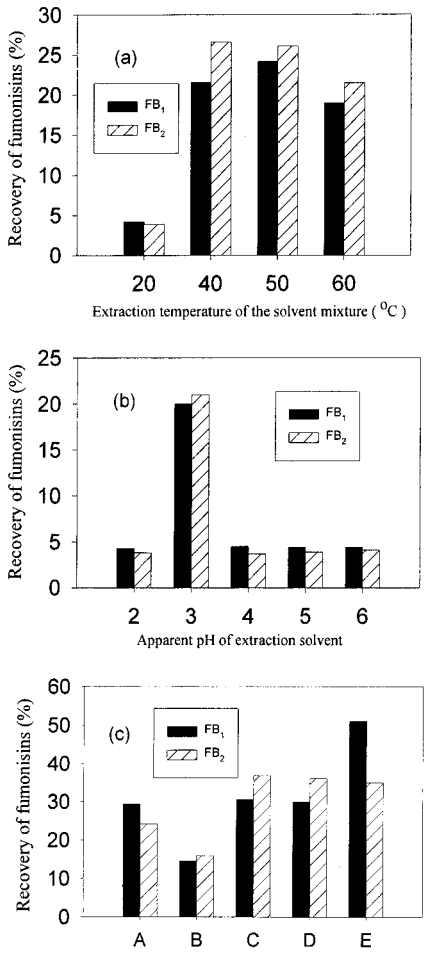

Figure 2. Effects of changing (a) the temperature and (b) the apparent pH of the extraction solvent methanol/acetonitrile/water (25:25:50) on the recovery of fumonisins added to Thai white rice flour. (c) Effect of Na_2EDTA on the recovery of fumonisins added to Thai white rice flour extracted with (A) methanol/acetonitrile/0.1 M Na_2EDTA (pH 6.0, adjusted with 0.1 M sodium hydroxide) (25:25:50) for 1 h at room temperature; (B) methanol/acetonitrile/0.1 M Na_2EDTA (pH 6.0) (25:25:50) for 1 h at 50 °C; (C) methanol/acetonitrile/0.1 M Na_2EDTA (pH 6.0) (25:25:50) for 1 h at room temperature, extracted two times; (D) methanol/0.1 M Na_2EDTA (50:50) extracted at room temperature; and (E) 0.1 M Na_2EDTA, extracted three times for 30 min at room temperature.

Stability Studies on Fumonisins Added to White Rice Flour, Cornstarch, and Cornmeal. With a useable method involving EDTA developed, it was possible to show that FB_1 and FB_2 were in fact unstable when added to the Thai white rice flour and that they disappeared completely after 10 h at room temperature (**Figure 3a**). Another type of white rice flour (produced in the United Kingdom) gave somewhat different results. Initial recoveries of fumonisins from this sample were 90−110% using methanol/acetonitrile/water (25:25:50), and the instability was much less, so that even after 60 days ∼10% remained (**Figure 3b**). A possible explanation could be the coarser nature of the U.K. rice flour compared to the Thai rice

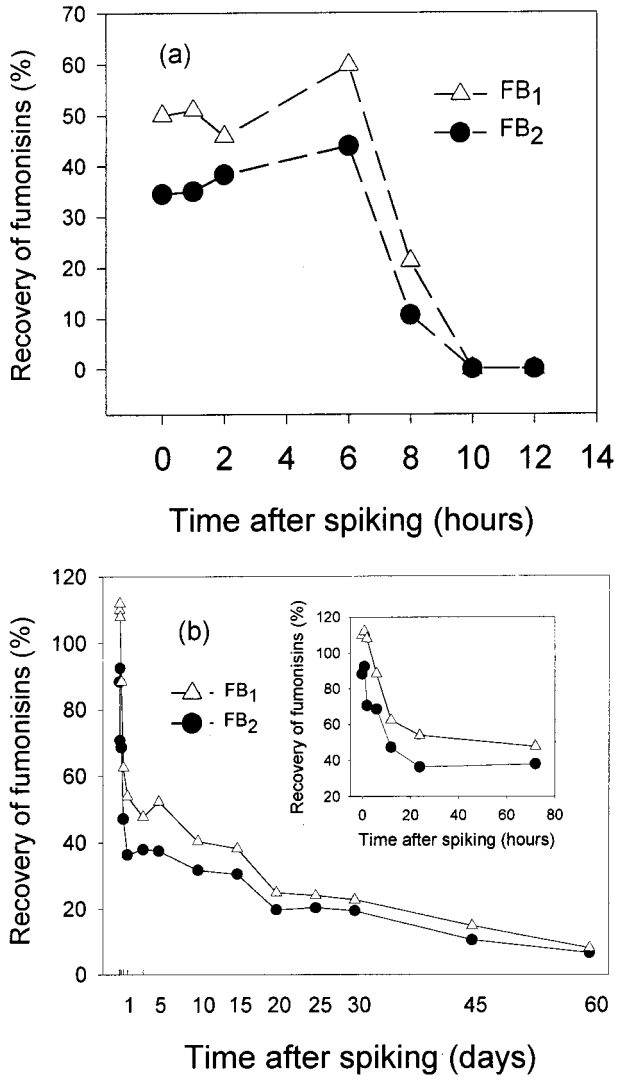

Figure 3. Stability of FB_1 and FB_2 (each 500 ng/g) in (a) Thai white rice flour, extracted three times with 50 mL of 0.1 M Na_2EDTA (pH 6.0), and (b) white rice flour from the United Kingdom, extracted with 50 mL of methanol/acetonitrile/water (25:25:50).

flour, which was a fine powder. Another explanation could be the difference in water contents. The package label for Thai rice flour stated that water was added. We also suspected metal ions might cause the difference in fumonisins' stability and checked for aluminum and iron in both rice flours, but found no difference in the amounts (results not shown).

The rates of disappearance of FB_1 and FB_2 in cornstarch were similar (**Figure 4a**). These results are in agreement with previous observations (*21*) that fumonisins were unstable in cornstarch over a 2-day period and had completely disappeared after 5.5 months.

The interaction of fumonisins with cornstarch at room temperature was investigated further using ^{14}C-labeled FB_1 (500 ng/g) and immunoaffinity column chromatography. Of the [^{14}C]-FB_1 material extracted (actual mean recovery determined by radioactivity counting was 61 ± 3.7%, $n = 4$), only 43% (relative recoveries of 42 and 44% in two experiments) of the radioactivity was eluted from the column with 2 mL of methanol, which would normally be expected to elute all of the fumonisin from a FumoniTest column (*31*, *38*). The FumoniTest immunoaffinity column was used in this laboratory with extracts of U.K. white rice flour and cornmeal, and 2 mL

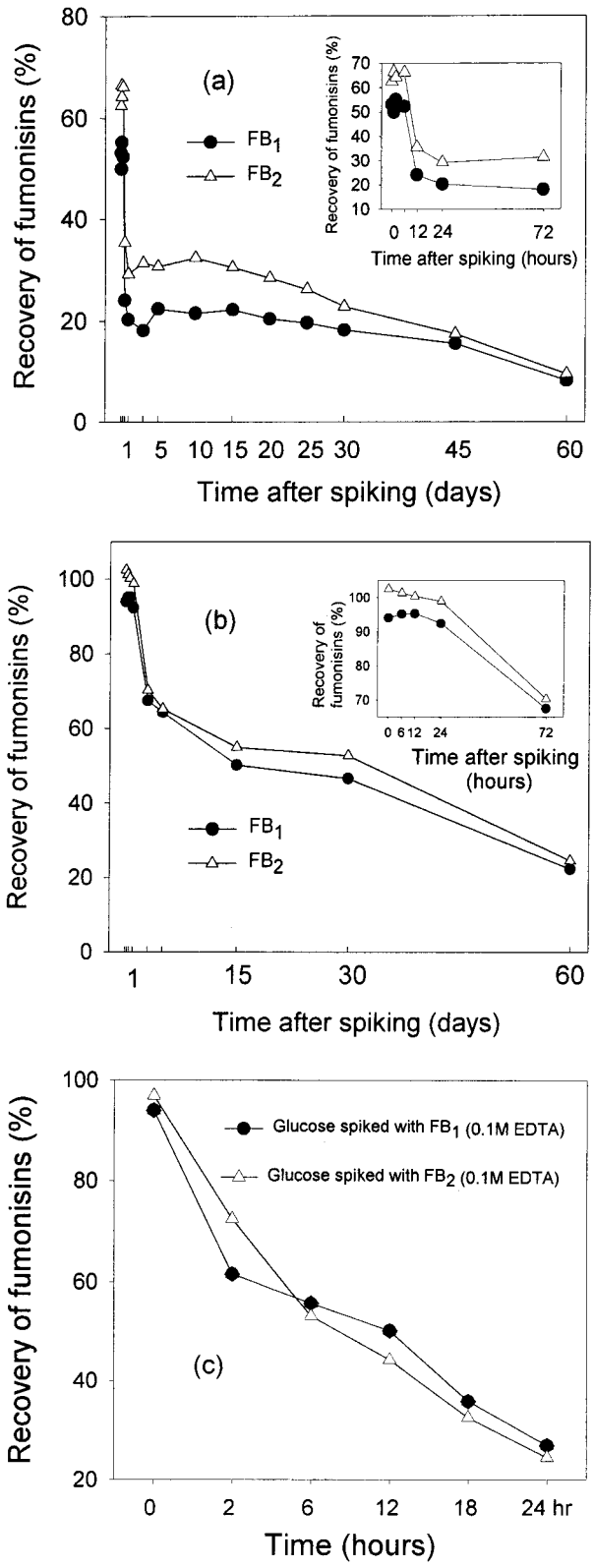

Figure 4. Stability of FB_1 and FB_2 (each 500 ng/g) in (a) cornstarch and (b) cornmeal, each extracted with 50 mL of methanol/acetonitrile/water (25:25:50), and (c) glucose, extracted with 0.1 M Na_2EDTA (pH 6.0).

of methanol did elute most of the fumonisins (see overall recoveries at zero time in **Figures 3b** and **4b**). The 24 h recovery of FB_1 from starch measured in the 2 mL of methanol eluate by LC (20%, **Figure 4a**) compares well with the overall radioactivity recovery (61 × 43 = 26%). Most of the remainder of the radioactivity in the starch extract could be eluted from

Table 1. ^{14}C-Labeled FB_1 Added to Cornstarch (500 ng/g) and Analyzed after 24 h Using Immunoaffinity Column Cleanup

	^{14}C-FB_1 equivalent recovered (ng)	recovery[a] (%)
crude extract	331	
loading (20 mL): extract + PBS	0	0
washing with PBS (5 mL)	6.44	1.9
elution with methanol (2 mL)	141	42.3
elution with methanol/water (2 mL) (8:2, v/v, 1% trifluoroacetic acid)	180	53.6
elution with dimethyl sulfoxide (2 mL)	4.03	1.2
total relative recovery[a]		99.0

[a] Relative recovery from extract.

the immunoaffinity column with methanol/water (80:20, containing 1% trifluoroacetic acid) (**Table 1**) or with methanol/dimethyl sulfoxide mixtures, followed by dimethyl sulfoxide (data not shown). In a separate experiment, essentially all of the radioactivity could be eluted with 2 mL of methanol/water (80:20) alone (no trifluoroacetic acid) instead of methanol. The same experiment as that shown in **Table 1** was carried out with nonlabeled FB_1 added to cornstarch followed by immunoaffinity chromatography. The methanol eluate contained only FB_1 as determined by LC-ESI MS total ion current and positive ion scan at *m*/*z* 722.6. The methanol/water (80:20, plus 1% trifluoroacetic acid) fraction contained mainly FB_1 (retention time = 14.7 min); there was a small amount of a monomethyl ester (14.9 min, *m*/*z* 736.6), an artifact from acid-catalyzed methylation of FB_1 (*49*). It is known that starch forms inclusion complexes with compounds such as flavors and lipids, which are bound to the amylose and to amylopectin segments (*50*−*52*). It could be supposed that fumonisins might form such complexes. If extractable, they would probably not behave like free fumonisin during immunoaffinity chromatography. Nonextractable bound fumonisins would not be detected at all by the present methods, and future investigation of such complexes is needed.

The stability of FB_1 and FB_2 added to cornmeal was investigated as a control experiment. Although initial recoveries were 95−100%, slow disappearance of the fumonisins was observed over a period of 2 months so that only ~25% remained at that time (**Figure 4b**). Previously, a 56% loss of FB_1 in naturally contaminated cornmeal (from 111 to 49 ng/g) over 5.5 months had been observed, whereas there were no observed losses in a naturally contaminated white corn flour (*21*). The present findings have serious implications for interlaboratory studies of analytical methods, for which samples of cornmeal spiked with fumonisins would be sent to collaborating laboratories and may be stored for days (or even weeks) before analysis.

Stability of Fumonisins in Glucose. The stability experiments described above were carried out at room temperature, and the reaction of FB_1 with D-glucose had only previously been studied under heating conditions. Therefore, we spiked solid glucose with FB_1 and FB_2 (each 500 ng/g) and extracted the mixture with 0.1 M Na_2EDTA. Initial recovery was good (>90%), but 24 h after spiking, only ~20% remained as extractable fumonisins (**Figure 4c**). We do not have an explanation for what happens to most of the fumonisins. When D-glucose was spiked with a higher concentration of FB_1 (50 μg/g) and kept for 3 months at room temperature, only a small amount of *N*-(1-deoxy-D-fructos-1-yl)FB_1 (*42*) was seen in

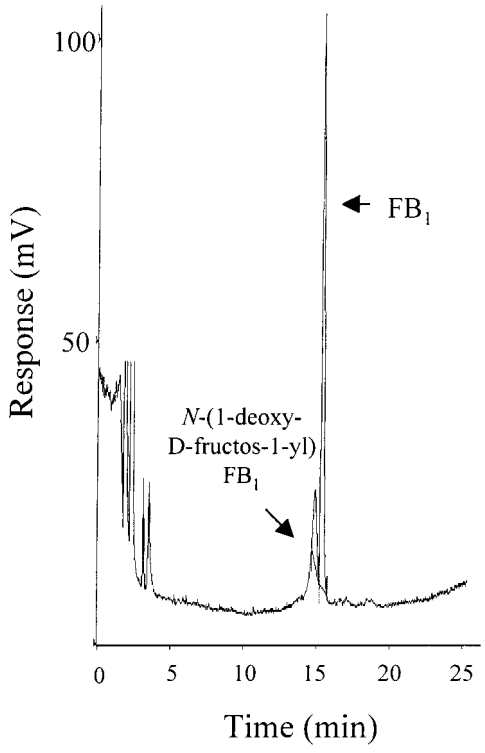

Figure 5. LC-ELSD of glucose spiked with 50 μg of FB_1/g after 3 months at room temperature and extracted with 0.1 M Na_2EDTA (pH 6.0).

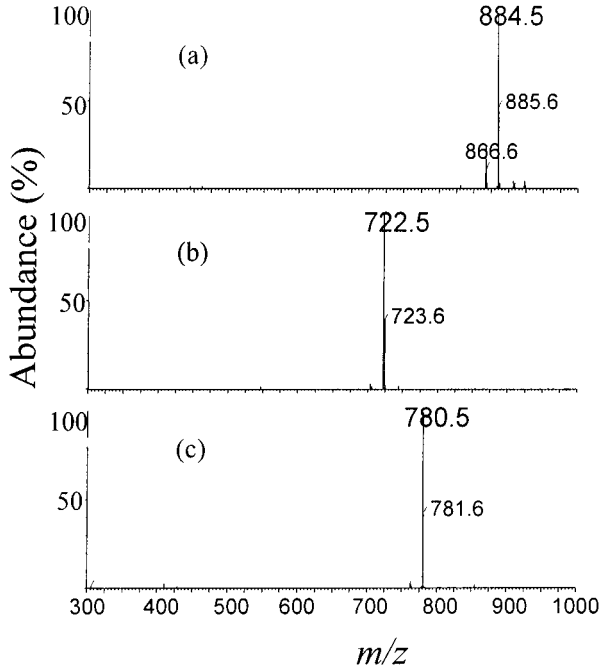

Figure 6. Mass spectra recorded by LC-ESI MS of (a) *N*-(1-deoxy-D-fructos-1-yl)FB_1 ($[M + H]^+ = 884$, identified by comparison with a standard from the USDA), (b) FB_1 ($[M + H]^+ = 722$), and (c) *N*-(carboxymethyl)-FB_1 ($[M + H]^+ = 780$) in the same extract as in **Figure 5**.

comparison to residual FB_1 by LC-ELSD and no *N*-(carboxymethyl)FB_1 (*43*, *44*) was detected (**Figure 5**); both compounds could be detected by LC-ESI MS (mass spectra are shown in **Figure 6**). Minor concentrations of these two products could also be detected by LC-MS after 8 days of reaction, but after 1 day, no *N*-(carboxymethyl)FB_1 was observed. Clearly our results show that something else is happening to FB_1 in the presence of excess D-glucose at room temperature. *N*-(1-Deoxy-D-fructos-

1-yl)FB_1 exists in several isomeric forms (*42*). In the standard provided by Dr. Poling we were able to observe by LC-ELSD a minor peak, which could be either an impurity or an isomer, on the front shoulder (14.6 min compared to 14.9 min for the major peak).

Conclusions. Our findings on the instability of fumonisins in white rice flour, cornstarch, cornmeal, and glucose indicate that we need more knowledge about "hidden fumonisins" bound to foodstuffs at room temperature. Hidden fumonisins in heat-processed foods also need further study in addition to research already published. Such studies raise important implications related to human intake of fumonisins. In addition, our experiments have indicated the need for analysts to be aware of the instability of fumonisins in stored analytical samples, particularly spiked samples used in interlaboratory (collaborative) method studies.

SAFETY

Fumonisin B_1 is carcinogenic in rodents.

ABBREVIATIONS USED

FB_1, fumonisin B_1; FB_2, fumonisin B_2; EDTA, ethylenediaminetetraacetic acid.; OPA, *o*-phthaldialdehyde; PBS, phosphate-buffered saline.

ACKNOWLEDGMENT

We are grateful to M. E. Savard and J. D. Miller, Agriculture and Agri-Food Canada, Ottawa, for standards of FB_1 and FB_2; P. C. Howard, U.S. Food and Drug Administration, Jefferson, AR, for a standard of *N*-(carboxymethyl)FB_1; S. M. Poling, U.S. Department of Agriculture, Peoria, IL, for *N*-(1-deoxy-D-fructos-1-yl)FB_1; and M. E. Savard, Agriculture and Agri-Food Canada, Ottawa, for ^{14}C-labeled FB_1 and providing facilities for its use. We thank R. W. Dabeka and A. D. MacKenzie, Food Research Division, Health Canada, for analyses of white rice flours for metals.

LITERATURE CITED

(1) Jackson, L. S., DeVries, J. W., Bullerman, L. B., Eds. *Fumonisins in Food. Advances in Experimental Medicine and Biology*; Plenum Press: New York, 1996; Vol. 392.

(2) Marasas, W. F. O. Discovery and occurrence of the fumonisins: a historical perspective. *Environ. Health Perspect.* **2001**, *109* (Suppl. 2), 239−243.

(3) Marasas, W. F. O.; Miller, J. D.; Riley, R. T.; Visconti, A. *Environmental Health Criteria 219. Fumonisin B_1*; International Programme on Chemical Safety, World Health Organization: Geneva, Switzerland, 2000.

(4) Howard, P. C.; Eppley, R. M.; Stack, M. E.; Warbritton, A.; Voss, K. A.; Lorentzen, R. J.; Kovach, R. M.; Bucci, T. J. Fumonisin B_1 carcinogenicity in a two-year feeding study using F344 rats and B6C3F_1 mice. *Environ. Health Perspect.* **2001**, *109* (Suppl. 2), 277−282.

(5) Gelderblom, W. C. A.; Kriek, N. P. J.; Marasas, W. F. O.; Thiel, P. G. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B_1, in rats. *Carcinogenesis* **1991**, *12*, 1247−1251.

(6) Shephard, G. S. Chromatographic determination of the fumonisin mycotoxins. *J. Chromatogr. A* **1998**, *815*, 31−39.

(7) Shephard, G. S.; Thiel, P. G.; Stockenström, S.; Sydenham, E. W. Worldwide survey of fumonisin contamination of corn and corn-based products. *J. AOAC Int.* **1996**, *79*, 671−687.

(8) Weidenbörner, M. Foods and fumonisins. *Eur. Food Res. Technol.* **2001**, *212*, 262−273.

(9) da Silva, J. B.; Pozzi, C. R.; Mallozzi, M. A. B.; Ortega, E. M.; Corrêa, B. Mycoflora and occurrence of aflatoxin B_1 and fumonisin B_1 during storage of Brazilian sorghum. *J. Agric. Food Chem.* **2000**, *48*, 4352−4356.

(10) Trucksess, M. W.; Cho, T.-H.; Ready, D. E. Liquid chromatographic method for fumonisin B_1 in sorghum syrup and corn-based breakfast cereals. *Food Addit. Contam.* **2000**, *17*, 161−166.

(11) Tseng, T. C.; Tu, J. C.; Tzean, S. S. Mycoflora and mycotoxins in dry bean (*Phaseolus vulgaris*) produced in Taiwan and in Ontario, Canada. *Bot. Bull. Acad. Sin.* **1995**, *36*, 229−234.

(12) Tseng, T. C.; Tu, J. C.; Soo, L. C. Natural occurrence of mycotoxins in *Fusarium* infected beans. *Microbios* **1995**, *84*, 21−28.

(13) Tseng, T. C.; Tu, J. C. Mycoflora and mycotoxins in adzuki and mung beans produced in Ontario, Canada. *Microbios* **1997**, *90*, 87−95.

(14) Castellá, G.; Bragulat, M. R.; Cabañes, F. J. Surveillance of fumonisins in maize-based feeds and cereals from Spain. *J. Agric. Food Chem.* **1999**, *47*, 4707−4710.

(15) Martins, M. L.; Martins, H. M.; Bernardo, F. Fumonisins B_1 and B_2 in black tea and medicinal plants. *J. Food Prot.* **2001**, *64*, 1268−1270.

(16) Abbas, H. K.; Cartwright, R. D.; Shier, W. T.; Abouzied, M. M.; Bird, C. B.; Rice, L. G.; Ross, P. F.; Sciumbato, G. L.; Meredith, F. I. Natural occurrence of fumonisins in rice with *Fusarium* sheath rot disease. *Plant Dis.* **1998**, *82*, 22−25.

(17) Chung, S. H.; Kim, Y. B. Natural occurrence of fumonisin B_1 in Korean corn and rough rice. *Foods Biotechnol.* **1995**, *4*, 212−216.

(18) Kim, E.-K.; Kim, Y.-B.; Shon, D.-H.; Ryu, D.; Chung, S.-H. Natural occurrence of fumonisin B_1 in Korean rice and its processed foods by enzyme-linked immunosorbent assay. *Food Sci. Biotechnol.* **1998**, *7*, 221−224.

(19) Trucksess, M. W. (Chair). Joint Mycotoxin Committee. *J. AOAC Int.* **2000**, *83*, 536−541.

(20) Usleber, E.; Schneider, E.; Märtlbauer, E. Fumonisins in rice from the German market. *Abstracts, AOAC International Annual Meeting*, Sept 7−11, 1997, San Diego, CA; AOAC: Gaithersburg, MD, 1997; p 54.

(21) Scott, P. M.; Lawrence, G. A.; Lombaert, G. A. Studies on extraction of fumonisins from rice, corn-based foods and beans. *Mycotoxin Res.* **1999**, *15*, 50−60.

(22) Juliano, B. O. *Rice in Human Nutrition*; U.N. Food and Agriculture Organization: Rome, Italy, 1993.

(23) Juliano, B. O. Rice: recent progress in chemistry and nutrition. In *Cereals for Food and Beverages. Recent Progress in Cereal Chemistry and Technology*; Inglett, G. E., Munck, L., Eds.; Academic Press: New York, 1980; pp 409−428.

(24) Resch, P.; Shier, W. T. The fate of fumonisin during thermal food processing. *Lebensmittelchemie* **2000**, *54*, 31−44.

(25) Bennett, G. A.; Richard, J. L.; Eckhoff, S. R. Distribution of fumonisins in food and feed products prepared from contaminated corn. In *Fumonisins in Food. Advances in Experimental Medicine and Biology*; Jackson, L. S., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Press: New York, 1996; Vol. 392, pp 317−322.

(26) Maragos, C. M.; Bennett, G. A.; Richard, J. L. Affinity column clean-up for the analysis of fumonisins and their hydrolysis products in corn. *Food Agric. Immunol.* **1997**, *9*, 3−12.

(27) Omurtag, G. Z. Determination of fumonisins B_1 and B_2 in corn and corn-based products in Turkey by high-performance liquid chromatography. *J. Food Prot.* **2001**, *64*, 1072−1075.

(28) Kim, E.-K.; Shon, D.-H.; Chung, S.-H.; Kim, Y.-B. Survey for fumonisin B_1 in Korean corn-based foods. *Food Addit. Contam.* **2002**, *19*, in press.

(29) Alberts, J. F.; Gelderblom, W. C. A.; Marasas, W. F. O. Evaluation of the extraction and purification procedures of the maleyl derivatization HPLC technique for the quantification of the fumonisin B mycotoxins in corn cultures. *Mycotoxin Res.* **1993**, *9*, 2−12.

(30) Zoller, O.; Sager, F.; Zimmerli, B. Vorkommen von Fumonisinen in Lebensmitteln. *Mitt. Gebiete Lebensm. Hyg*. **1994**, *85*, 81−99.

(31) Meister, U. Effect of extraction and extract purification on the measurable fumonisin content of maize and maize products. Tests on the efficiency of acid extraction and use of immunoaffinity columns. *Mycotoxin Res*. **1998**, *15*, 13−23.

(32) Murphy, P. A.; Hendrich, S.; Hopmans, E. C.; Hauck, C. C.; Lu, Z.; Buseman, G.; Munkvold, G. Effect of processing on fumonisin content of corn. In *Fumonisins in Food. Advances in Experimental Medicine and Biology*; Jackson, L. S., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Press: New York, 1996; Vol. 392, pp 323−334.

(33) Lawrence, J. F.; Niedzwiadek, B.; Scott, P. M. Effect of temperature and solvent composition on extraction of fumonisins B_1 and B_2 from corn products. *J. AOAC Int*. **2000**, *83*, 604−611.

(34) Akiyama, H.; Miyahara, M.; Toyoda, M.; Saito, Y. Determination of fumonisin B_1 and fumonisin B_2 in grain and corn processed products by HPLC. *Shokuhin Eiseigaku Zasshi* **1996**, *37*, 54−58.

(35) Sydenham, E. W.; Stockenström, S.; Thiel, P. G.; Shephard, G. S.; Koch, K. R.; Marasas, W. F. O. Potential of alkaline hydrolysis for the removal of fumonisins from contaminated corn. *J. Agric. Food Chem*. **1995**, *43*, 1198−1201.

(36) Dombrink-Kurtzman, M. A.; Dvorak, T. J. Fumonisin content in masa and tortillas from Mexico. *J. Agric. Food Chem*. **1999**, *47*, 622−627.

(37) Scott, P. M.; Lawrence, G. A. Determination of hydrolysed fumonisin B_1 in alkali-processed corn foods. *Food Addit. Contam*. **1996**, *13*, 823−832.

(38) Solfrizzo, M.; De Girolamo, A.; Visconti, A. Determination of fumonisins B_1 and B_2 in cornflakes by high performance liquid chromatography and immunoaffinity clean-up. *Food Addit. Contam*. **2001**, *18*, 227−235.

(39) Visconti, A.; Solfrizzo, M.; De Girolamo, A. Determination of fumonisins B_1 and B_2 in corn and corn flakes by liquid chromatography with immunoaffinity column cleanup: collaborative study. *J. AOAC Int*. **2001**, *84*, 1828−1837.

(40) Hartl, M.; Humpf, H.-U. Simultaneous determination of fumonisin B_1 and hydrolyzed fumonisin B_1 in corn products by liquid chromatography/electrospray ionization mass spectrometry. *J. Agric. Food Chem*. **1999**, *47*, 5078−5083.

(41) Liu, H.; Lu, Y.; Haynes, J. S.; Cunnick, J. E.; Murphy, P.; Hendrick, S. Reaction of fumonisin with glucose prevents promotion of hepatocarcinogenesis in female F344/N rats while maintaining normal hepatic sphinganine/sphingosine ratios. *J. Agric. Food Chem*. **2001**, *49*, 4113−4121.

(42) Poling, S. M.; Plattner, R. D.; Weisleder, D. N. *N*-(1-deoxy-D-fructos-1-yl) fumonisin B_1, the initial reaction product of fumonisin B_1 and D-glucose. *J. Agric. Food Chem*. **2002**, *50*, 1318−1324.

(43) Howard, P. C.; Churchwell, M. I.; Couch, L. H.; Marques, M. M.; Doerge, D. R. Formation of *N*-(carboxymethyl)fumonisin B_1, following the reaction of fumonisin B_1 with reducing sugars. *J. Agric. Food Chem*. **1998**, *46*, 3546−3557.

(44) Seefelder, W.; Hartl, M.; Humpf, H.-U. Determination of *N*-(carboxymethyl)fumonisin B_1 in corn products by liquid chromatography/electrospray ionization-mass spectrometry. *J. Agric. Food Chem*. **2001**, *49*, 2146−2151.

(45) Castelo, M. M.; Jackson, L. S.; Hanna, M. A.; Reynolds, B. H.; Bullerman, L. B. Loss of fumonisin B_1 in extruded and baked corn-based foods with sugars. *J. Food Sci*. **2001**, *66*, 416−421.

(46) Blackwell, B. A.; Miller, J. D.; Savard, M. E. Production of carbon ^{14}C-labeled fumonisin in liquid culture. *J. AOAC Int*. **1994**, *77*, 506−511.

(47) Wilkes, J. G.; Sutherland, J. B.; Churchwell, M. I.; Williams, A. J. Determination of fumonisins B_1, B_2, B_3 and B_4 by high performance liquid chromatography with evaporative light scattering detection. *J. Chromatogr. A* **1995**, *695*, 319−323.

(48) Wilkes, J. G.; Churchwell, M. I.; Billedeau, S. M.; Vollmer, D. L.; Vollmer, D. A.; Thompson, H. C., Jr.; Lay, J. O., Jr. Determination of underivatized fumonisin B_1 and related compounds by HPLC. In *Fumonisins in Food. Advances in Experimental Medicine and Biology*; Jackson, L. S., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Press: New York, 1996; Vol. 392, pp 93−103.

(49) Josephs, J. L. Detection and characterization of fumonisin mycotoxins by liquid chromatography/electrospray ionization using ion trap and triple quadrupole mass spectrometry. *Rapid Commun. Mass Spectrom*. **1996**, *10*, 1333−1344.

(50) Jackson, D. S. Starch. Functional properties. In *Encyclopaedia of Food Science, Food Science and Nutrition. Vol. 7. Spectroscopy−Z*; Macrae, R., Robinson, R. K., Sadler, M. J., Eds.; Academic Press: London, U.K., 1993; pp 4381−4384.

(51) Hahn, D. E.; Hood, L. F. Factors influencing corn starch−lipid complexing. *Cereal Chem.* **1987**, *64*, 81−85.

(52) Osman-Ismail, F.; Solms, J. The formation of inclusion compounds of starches with flavor substances. *Lebensm.-Wiss. Technol*. **1973**, *6*, 147−50.

Received for review January 18, 2002. Revised manuscript received March 19, 2002. Accepted March 21, 2002. This work was presented at the 115th AOAC International Annual Meeting, Sept 9−13, 2001, Kansas City, MO. Financial support for this project was provided in part by the Korean Science and Engineering Foundation (KOSEF) and the Natural Sciences and Engineering Research Council of Canada (NSERC), as a Visiting Fellowship to E.-K.K.

JF020058S