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Reduction of Fumonisin B_1 in Corn Grits by Single-Screw Extrusion

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This study was designed to determine the efficacy of extrusion in reducing fumonisin B_1 in corn flaking grits in the presence and absence of glucose. In addition, degradation products of fumonisin B_1 during extrusion were identified and quantitated with a mass balance approach. Uncontaminated clean corn grits, grits spiked with 30 μg/g fumonisin B_1, and grits fermented with *Fusarium verticillioides* M-2552 (40–50 μg/g fumonisin B_1) were extruded in the presence and absence of glucose (10%, w/w) using a single-screw extruder. Extrusion decreased fumonisin B_1 by 21–37%, whereas the same process with added glucose further decreased fumonisin B_1 by 77–87%. LC−fluorescence and LC-MS showed that most fumonisin in the extruded samples without added glucose was the fumonisin B_1 form, whereas the main degradation product in grits extruded with glucose was *N*-(deoxy-D-fructos-1-yl)fumonisin B_1. The formation of hydrolyzed fumonisin B_1 was not significant during extrusion. Results suggest that extrusion in the presence of glucose may reduce fumonisin B_1 in corn grits significantly.

KEYWORDS: Fumonisin; reduction; *N*-(deoxy-D-fructos-1-yl)fumonisin; extrusion

INTRODUCTION

Fumonisin B_1 (FB_1)has been found to be the most commonly occurring mycotoxin in corn (maize), and the main microorganisms involved in the production of fumonisin B_1 in North America are *Fusarium verticillioides* (formerly *F. moniliforme*) and *F. proliferatum* (*1*). Surveys have shown that fumonisin B_1 occurs not only in corn grain but also in finished corn-based processed food products worldwide (*2–5*). The occurrence of fumonisin B_1 raises a significant food safety concern because it has been linked to human diseases including esophageal cancer in addition to the known toxicities in animals (*6–9*). More recently, fumonisin B_1 has been identified as a risk factor for neural tube defect in humans (*10–12*).

Extrusion cooking of cereal products is commonly used to produce breakfast cereals, snack foods, and pet foods. Extrusion processes, with single- or twin-screw configuration, employ high temperatures, high pressures, and severe shear forces that cause chemical changes and molecular transformations of food components and contaminants (*13*). Whereas fumonisin B_1 is very heat stable (*14, 15*), significant reduction of this toxin by extrusion processing has been documented, particularly when the substrate was processed with added glucose (*16, 17*). However, reaction products of fumonisin B_1 with toxic potential that may be formed during extrusion processing have not been studied. Additionally, in previous studies, reductions of FB_1 were determined by enzyme-linked immunosorbent assay (ELISA) or high-performance liquid chromatography (HPLC) based on the fluorescence derivatization reaction between the primary amine in fumonisin B_1 and *o*-phthaladehyde (OPA) (*18*). Therefore, reduced concentration of fumonisin B_1 determined by HPLC may not be directly correlated to destruction of the compound but alteration in the primary amine resulting in loss of reactivity with OPA. Murphy et al. (*19*) suggested that the loss of reactivity was due to the formation of fumonisin B_1−sugar complexes in a Maillard reaction.

When the complexity of food matrices and the availability of reducing sugars in various food processes are considered, it becomes important to understand the fate of fumonisin B_1 during extrusion cooking as an effective model system in reducing the toxin in corn-based products. The objectives of this study were to investigate the fate of fumonisin B_1 in corn grits during single-screw extrusion and to quantify degradation products using a mass balance approach.

MATERIALS AND METHODS

Preparation of Contaminated Grits. To evaluate the fate of fumonisin B_1 during single-screw extrusion of corn grits, the grits were contaminated by two different methods: direct addition of purified fumonisin B_1 (spiking) and growth of a known fumonisin-producing mold on the grits for natural production of fumonisin B_1. Number 4

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flaking grits, graciously provided by Bunge Milling, Crete, NE, were used as the substrate for both methods and screened for the presence of fumonisins.

Purified fumonisin B_1 (>95% pure) was provided by Frederick Thomas, FDA/CFSAN, College Park, MD. One batch of spiked corn was prepared by mixing fumonisin B_1 solution prepared in water with clean (uncontaminated) corn grits to achieve a final concentration of 30 μg/g. The moisture content of the grits was also adjusted to 20% [dry weight basis (dw)] and allowed to equilibrate overnight.

Two batches of naturally contaminated grits were obtained by fermentation of clean corn grits with *F. verticillioides* M-2552 until fumonisin B_1 levels in the grits reached 40–50 μg/g. For the fermentation, 1 kg of corn grits was weighed into gallon glass jars, autoclaved for 1 h at 121 °C, and then allowed to equilibrate overnight. The moisture content was then adjusted to 30% (dw) with the addition of sterile distilled water, which was done in two steps (half and half) with a 24 h period between additions to ensure even distribution of moisture. The corn grits were then inoculated with *F. verticillioides* M-2552 spores that were obtained by growing the mold on carnation leaf agar (CLA) in Petri plates for 7–10 days. Each agar plate was then divided in two, and half of the agar with spores was added to each jar. The openings of the jars were covered with two sheets of filter papers and closed with lids, with a 7.6 cm diameter hole to facilitate air flow. The jars were shaken every day to prevent clumping and provide good air exchange in the mass of grits. The moisture content was checked once a week and readjusted to 30% by the addition of sterile distilled water as needed. The production of fumonisin was monitored by Veratox enzyme-linked immunosorbent assay (ELISA) kits (Neogen Corp., Lansing, MI), and fermentation was allowed to proceed until the desired fumonisin concentration was achieved. Fermented grits were frozen until used for extrusion. Uncontaminated corn grits were used in the extrusion process as a control.

Extrusion of Corn Grits. Contaminated grits and uncontaminated clean corn grits (control) were extruded in the presence and absence of 10% (w/w) food grade glucose (ADM Corn Processing, Decatur, IL) in a model 2003 GR-8 single-screw extruder (C.W. Brabender, South Hackensack, NJ) with a nozzle diameter of 3 mm. The screw speed used was 60 rpm, and the temperature of the first (feed) zone was set to 100 °C, whereas the second (metering) and the third (compression) zones were set at 160 °C. The moisture content of all treatments was adjusted to 20% (dw) as it was determined to be the condition for maximum reduction of fumonisin B_1 (*16, 17*).

For each sample, ca. 2.5 kg of prepared grits was extruded, and parameters including torque, temperature, pressure, and output were recorded during the process. The same amount of unextruded grits for each sample was kept for comparison.The extruded and unextruded samples were ground using an S-500 disk mill (Glen Mills, Marywoods, NJ). The ground materials were then separated into portions for ELISA, HPLC, and HPLC−mass Spectrometry (MS) analyses.

Analyses of Fumonisins and Degradation Products. Acetonitrile, acetic acid, methanol, and water were used for solid-phase extraction (SPE), and the HPLC mobile phases were of HPLC grade from Fisher Scientific (Pittsburgh, PA) or Acros Organics (Morris Plains, NJ). Formic acid was of Puriss grade from Fluka (St. Louis, MO). Hydrochloric acid and potassium chloride were of certified ACS grade from Fisher. OPA, 2-mercaptoethanol, and sodium tetraborate decahydrate (borax) were from Sigma Chemical (St. Louis, MO). Deionized water was used to prepare solvent and to extract corn samples.

Fumonisin B_1 standard from Sigma (98% pure; molecular weight = 721.8) was used as received. Fumonisin B_2 (molecular weight = 705.8), fumonisin B_3 (molecular weight = 705.8), and hydrolyzed fumonisin B_1 (molecular weight = 405.6) (all >95% pure) were kindly provided by Frederick Thomas of FDA/CFSAN, College Park, MD, and used as received. *N*-(Deoxy-D-fructos-1-yl)fumonisin B_1 (>95% pure; molecular weight = 883.9) was prepared according to the method reported earlier (*20*) and kindly provided by Steven Poling of USDA/NCAUR (Peoria, IL). Individual 1.0 mg/mL stock standards were prepared in acetonitrile/water (1:1, v/v). Mixed standards containing fumonisin B_1, fumonisin B_2, fumonisin B_3, hydrolyzed fumonisin B_1, and *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 were prepared by serial dilution in the same solvent in the range of 0.01−10.00 μg/mL. Standards were stored in amber vials at 5 °C. These standards were used to spike control samples for recovery studies and for instrument calibration.

Extraction/Purification of Fumonisins for HPLC and HPLC-MS. Preparation of samples was based on the method of Jackson et al. (*15*) with minor modifications. Briefly, 5.0 g of extruded corn samples was weighed into 50 mL polypropylene tubes, and 25 mL of acetonitrile/0.1 N HCl (1:1, v/v) was added. The tubes were placed on a wrist action shaker at maximum speed for 60 min. The samples were then centrifuged at 10000 rpm for 15 min. The supernatant fluid (15 mL) was filtered with a 0.45 μm Maxi-Spin PVDF filter (Alltech Chromatography, Deerfield, IL) at 3000 rpm for 2 min. Two milliliters of the filtrate was transferred to a 15 mL screw-cap centrifuge tube, and then 8 mL of 1% KCl solution was added. The filtrate was centrifuged at 4000 rpm for 10 min, and the supernatant fluid was decanted into a clean tube.

A C_{18} Sep-Pak cartridge (Waters Chromatography, Milford, MA) was conditioned with methanol and then water using a vacuum manifold (Supelco, Bellefonte, PA). The sample extract was applied to the cartridge, and the vacuum was applied until the liquid level was just above the bed of the cartridge. The cartridge was washed with 1 mL of acetonitrile/water (15:85, v/v). The fumonisins were eluted from the cartridge with 3.0 mL of acetonitrile/water (70:30, v/v) into a 5 mL graduated centrifuge tubes. Sample extracts were analyzed for fumonisin levels without further concentration. Dilutions of the initial 3 mL sample were made with acetonitrile/water (50:50, v/v).

HPLC Analysis of Fumonisins. Quantitative determination of fumonisins B_1, B_2, B_3, and hydrolyzed fumonisin B_1 was based on the method of Thakur and Smith (*21*) with OPA reagent for fluorescence derivatization prepared as described by Shephard et al. (*22*). A Waters HPLC system equipped with an Alliance 2695 solvent module, a model 2475 fluorescence detector (excitation wavelength = 335 nm; emission wavelength = 440 nm; gain = 10), and Empower software were used. The autoinjector was programmed to mix 10 μL of sample extracts with 20 μL of OPA reagent, incubate the mixture for 2 min, and then inject 10 μL of the derivatized sample. Separations were carried out at 35 °C on a 150 mm × 3 mm i.d. Supelcosil LC-18-DB column (Supelco). The mobile phases were acetonitrile/water/acetic acid (30:69:1, v/v/v) (solvent A) and acetonitrile/water/acetic acid (60:39:1, v/v/v) (solvent B). The flow rate was 0.5 mL/min, and the column temperature was 35 °C. The initial mobile phase was 60:40 solvent A/solvent B. This solvent mixture was kept constant for 5 min, and then levels of solvent B were increased to 50% over a period of 5 min. After 5 min, levels of solvent B were increased to 80% over a 10 min period. After 5 min, the column was equilibrated for 5 min at the initial mobile phase.

Calibration standards for fumonisins were injected in the range of 0.01−1 μg/mL. Empower software was used to construct linear calibration curves from the standard responses. Concentrations of analytes in samples were calculated with the instrument software.

LC-MS Analysis of Degradation Products. A Waters HPLC equipped with an Alliance 2695 solvent module, a ZQ mass detector, and Empower software was used to identify and quantify *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 (molecular weight = 780.0) in sample extracts and to estimate levels of *N*-(carboxymethyl)fumonisin B_1. The MS was operated in positive ESI mode with scan time of 0.5 s, a dwell time of 0.5 s, a capillary voltage of 3000 V, and a cone voltage of 30 V. The instrument was operated in single ion mode (SIM) for *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 (M + H; *m/z* 884).

N-(Deoxy-D-fructos-1-yl)fumonisin B_1 calibration standards in acetonitrile/water (50:50, v/v) were injected in the range of 0.05−2 ppm. Two partially resolved peaks, isomers of NDF-FB_1, were observed in the *m/z* 884 SIM chromatogram. The ratio of the peak areas was the same in standards and corn samples. A peak group function was used to sum the peak areas for quantitation.

Because a standard for *N*-(carboxymethyl)fumonisin B_1 was not available, concentrations of this fumonisin B_1 species in sample extracts were estimated on the basis of the area of the ion chromatogram of the protonated molecule (M + H; *m/z* 780). This required an assumption that the response factor for *N*-(carboxymethyl)fumonisin B_1 was identical to the factor for *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 and

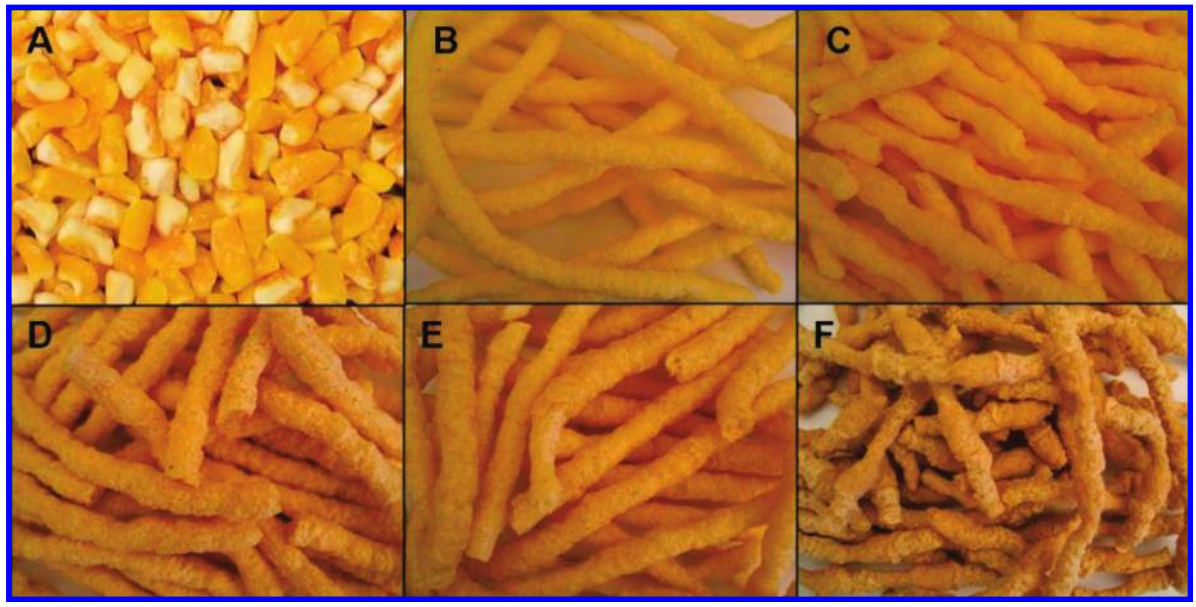

Figure 1. Appearance of clean grits and the extruded products: (**A**) unextruded clean corn flaking grits; (**B**−**F**) extruded products, (**B**) clean grits, (**C**) spiked grits without glucose, (**D**) spiked grits with glucose, (**E**) fermented grits without glucose, and (**F**) fermented grits with glucose.

Table 1. Parameters Measured during and After Extrusion in the Presence and Absence of Glucose

sample[a]	torque (Nm)	temperature (°C)	pressure (psi)	output[b] (g/min)	diameter (mm)	expansion ratio[c]
CG-E	68.4 ± 8.7	156.4 ± 2.8	400.8 ± 44.2	43.9	9.14 ± 0.55	3.05 ± 0.18 ab
SG-E	57.1 ± 21.4	156.8 ± 3.7	370.0 ± 34.6	52.0	9.44 ± 1.36	3.15 ± 0.45 a
SG-EG	35.1 ± 2.8	156.0 ± 0.9	315.7 ± 19.6	50.3	8.74 ± 0.72	2.91 ± 0.24 b
FG1-E	48.6 ± 6.2	157.3 ± 0.9	413.8 ± 43.7	50.0	NA[d]	NA
FG1-EG	52.7 ± 3.6	157.5 ± 0.5	293.4 ± 17.5	52.7	NA	NA
FG2-E	71.1 ± 6.3	157.0 ± 0.8	402.6 ± 33.2	47.8	11.17 ± 1.53	3.72 ± 0.51 c
FG2-EG	49.0 ± 2.8	156.4 ± 1.0	288.0 ± 12.9	47.0	7.32 ± 1.50	2.44 ± 0.50 d

[a] CG-E, clean grits extruded; SG-E, spiked grits extruded; SG-EG, spiked grits extruded with glucose; FG1-E, fermented grits batch 1 extruded; FG1-EG, fermented grits batch 1 extruded with glucose; FG2-E, fermented grits batch 2 extruded; FG2-EG, fermented grits batch 2 extruded with glucose. [b] Only one measurement was taken for each of the samples. [c] Groups not sharing letters are significantly different ($p < 0.05$). [d] NA, not measured.

the use of the *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 calibration curve to estimate concentration.

Separations were carried out at 35 °C on a 150 mm × 3 mm i.d. Supelcosil LC-18-DB column with a 10 μL injection volume. The mobile phases were acetonitrile/40 mM formic acid (10:90, v/v) (solvent A) and acetonitrile/40 mM formic acid (90:10, v/v) (solvent B). The flow rate was 0.5 mL/min, and the column temperature was 35 °C. The initial mobile phase was solvent A/solvent B (75:25, v/v). Levels of solvent B were increased to 75% over 10 min, and this mixture was held for 2 min. The column was equilibrated at the initial conditions at a flow rate of 1.0 mL/min for 3 min.

Statistical Analysis. All fumonisin analyses were done in triplicate. Means and standard deviations were calculated with Minitab (State College, PA) statistical software for the HPLC and HPLC-MS analysis. Minitab was used to verify significant differences between treatments in the same group (i.e., spiked corn, fermented corn batch 1, and fermented corn batch 2) by one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test. Treatments in each group were compared to the negative control and the extrusion control.

The ELISA determinations were repeated three times, and the means and standard deviations were calculated with SAS (SAS Institute, Cary, NC) statistical software. SAS was also used to verify significant differences between treatments in the same group (i.e., spiked corn, fermented corn batch 1, and fermented corn batch 2) by ANOVA for the difference in the least-squares means. Comparisons between the analysis methods (ELISA and HPLC) were also done by ANOVA using SAS.

RESULTS AND DISCUSSION

Extrusion of Corn Grits. During the extrusion of the samples, when the dough leaves the extruder, it expands because of a change in pressure and normal forces, which reduces the amount of moisture in the cooked product (*23*). The appearance of the final product varies depending upon the ratio of expansion and browning reactions that may occur during extrusion. The extruded products obtained were visually different from one another, depending upon the presence or absence of glucose (**Figure 1**). Such differences could also be attributed to the relative amount of protein in the corn grits.

Averaged torque, temperature at the nozzle, and pressure inside the barrel, collected every 10 s during the extrusion process, are shown in **Table 1**, along with the output of extruded sample. For all samples the feeding rate was maintained constant at about 64 g of grits/min. The expansion ratio, which is defined as the ratio of the cross-sectional area of the extruded material to that of the extruder nozzle (*24*), was calculated for each sample on the basis of 50 measurements of the diameter of the extruded material (**Table 1**).

Fermented grits showed the highest expansion ratio when compared to the other extruded materials. According to Bhattacharya and Hanna (*24*) the factors that affect the expansion ratio the most are moisture content and temperature, whereas the composition of the material may also play a role. However,

Table 2. Concentration of Fumonisin Species Determined by HPLC and LC-MS[a]

sample[b]	FB_1	HFB_1	NDF-FB_1	FB_2	FB_3	total fumonisins[c]
Controls						
CG	nd[d]	nd	nd	nd	nd	nd
CG-E	nd	nd	nd	nd	nd	nd
Spiked Corn Grits						
SG	31.0 ± 4.1 b	0.6 ± 0.1 b	0.3 ± 0.1 b	nd	nd	31.9 ± 4.1 b
SG-E	28.0 ± 3.3 b	0.7 ± 0.1 b	0.9 ± 0.1 b	nd	nd	29.2 ± 3.1 b
SG-EG	7.9 ± 0.6 c	0.2 ± 0.1 c	17.0 ± 1.5 c	nd	nd	25.0 ± 3.5 b
Fermented Corn Grits (Batch 1)						
FG1	32.7 ± 9.0 b	0.5 ± 0.2 b	0.4 ± 0.1 a	13.4 ± 3.3 b	4.9 ± 1.1 b	51.9 ± 13.6 b
FG1-E	23.4 ± 1.1 b	0.5 ± 0.1 b	0.3 ± 0.1 a	8.2 ± 0.1 c	2.8 ± 0.1 b	35.2 ± 1.3 b
FG1-EG	4.8 ± 0.4 a	0.2 ± 0.1 a	9.0 ± 0.9 b	1.7 ± 0.5 a	0.6 ± 0.1 a	16.3 ± 1.4 c
Fermented Corn Grits (Batch 2)						
FG2	47.9 ± 1.0 b	0.8 ± 0.2 b	0.5 ± 0.1 a	18.1 ± 0.9 b	7.0 ± 0.4 b	74.2 ± 2.2 b
FG2-E	41.0 ± 0.7 c	1.0 ± 0.1 c	0.7 ± 0.1 a	12.9 ± 0.3 c	4.7 ± 0.1 c	60.3 ± 0.7 c
FG2-EG	9.5 ± 0.2 d	0.4 ± 0.1 d	15.3 ± 0.8 b	2.9 ± 0.3 d	1.1 ± 0.1 d	29.2 ± 1.4 d

[a] All values are concentration (μg/g of dry weight), the average ± standard deviation of three determinations. Groups not sharing letters are significantly different ($p < 0.05$). [b] CG-E, clean grits (negative control); CG-E, clean grits extruded (extrusion control); SG, spiked grits; SG-E, spiked grits extruded ; SG-GE, spiked grits extruded with glucose; FG1, fermented grits batch 1 unextruded; FG1-E, fermented grits batch 1 extruded; FG1-EG, fermented grits batch 1 extruded with glucose; FG2, fermented grits batch 2 unextruded; FG2-E, fermented grits batch 2 extruded; FG2-EG, fermented grits batch 2 extruded with glucose. [c] Total fumonisins = fumonisin B_1 (FB_1) + hydrolyzed fumonisin B_1 (HFB_1) + *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 (NDF-FB_1) + fumonisin B_2 (FB_2) + fumonisin B_3 (FB_3). [d] Not detected; less than the method LOQ.

the effect of moisture content and temperature on expansion ratio would not be significant because both parameters were controlled in this study.

In relation to the effect of plant protein blends in extrusion, it was suggested that the expansion ratio increases with shear rate, where an increase in shear rate causes protein molecules to be stretched farther apart, weakening bonds and resulting in a puffier product (*23*). During fermentation of the grits, the mold assimilates starch and other nutrients, resulting in increase of the relative amount of protein in the fermented grits when compared to clean grits. This change in composition could be responsible for the protein effect observed in the expansion ratio, where the protein content in the fermented product would increase the shear rate of the dough, leading to a puffier product. Corroborating this, the torque applied to the dough was highest for the fermented grits batch 2, which indicates a more viscous dough with higher shear forces. Therefore, a greater expansion ratio could be attributed to the greater difference in pressure between barrel and atmosphere as a result of a higher protein content of the fermented materials.

The extruded materials that showed the lowest expansion ratios were those in which glucose was added before extrusion. For the fermented corn with added glucose the difference was significant, whereas for the spiked corn with added glucose the difference approached significance ($p = 0.09$). The addition of glucose reduced the relative amount of the other components of the dough compared to extrusion in the absence of glucose. Relative reductions in the amount of starch and protein, mainly responsible for the puffiness of the final product, had a direct impact in the expansion ratio. At the temperature of the extrusion process, the glucose, which has a melting point of 146 °C, contributes to the dough in a liquid form, decreasing its viscosity and leading to a reduction in the shear forces and pressure applied to the dough. Clearly, from **Table 1** spiked corn and fermented corn (second batch) extruded with glucose showed the lowest torques measured (35.1 ± 2.8 and 49.0 ± 2.8, respectively), which denotes the lowest shear forces being applied to the dough during extrusion. Once again the pressure values could also be used to help explain the reduced expansion ratio, because in the presence of glucose the pressure inside the extruder was lower when compared to the same materials in the absence of glucose, and a low pressure differential results in a less puffed product as previously suggested (*23*).

Analyses of Fumonisin and Degradation Products. *HPLC and LC-MS Analysis of Fumonisins.* For method validation, uncontaminated corn grits (negative control) before and after extrusion were spiked with fumonisin B_1, hydrolyzed fumonisin B_1, *N*-(deoxy-D-fructos-1-yl)fumonisin B_1, fumonisin B_2, and fumonisin B_3 at levels of 0.2–1.0 μg/g to estimate the percentage of each fumonisin species recovered during the LC extraction/purification procedure as well as limits of quantitation (LOQ). Mean and RSDs of six recoveries at the 0.2 ppm level were 91 ± 12% (fumonisin B_1), 78 ± 7.3% (fumonisin B_2), 86 ± 6.0% (fumonisin B_3), and 85 ± 18% (hydrolyzed fumonisin B_1). The mean and RSD of recovery from eight analyses for *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 was 104 ± 24%. On the basis of recovery data, the method LOQ was 0.2 ppm for fumonisins B_1, B_2, and B_3 and hydrolyzed fumonisin B_1 and 0.5 ppm for*N*-(deoxy-D-fructos-1-yl)fumonisin B_1. Recovery studies were not conducted for *N*-(carboxymethyl)fumonisin B_1, because a standard of this compound was not available. The LOQ for *N*-(carboxymethyl)fumonisin B_1 was estimated from the LOQ of *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 (0.5 ppm), assuming that the response factor from LC-MS analysis was the same for both compounds.

The results of the fumonisin analyses for the extruded and unextruded spiked and fermented corn grits are summarized in **Table 2**. For all three groups of samples without added glucose (fumonisin B_1-spiked corn, fermented corn batch 1, and fermented corn batch 2), levels of fumonisin B_1 did not significantly change or decreased slightly (<19%) after extrusion. However, extrusion of the corn samples after the addition of 10% glucose resulted in substantial (75–85%) reductions in fumonisin B_1 levels. Similarly, extrusion alone resulted in <39% reduction in fumonisin B_2 and fumonisin B_3 levels, whereas levels of both toxins were reduced by >84% in extruded corn containing 10% glucose. Hydrolyzed fumonisin B_1, *N*-(deoxy-D-fructos-1-yl)fumonisin B_1, and *N*-(carboxymethyl)fumonisin B_1 were used as markers for fumonisin B_1 reaction products in extruded corn containing glucose. Of these three forms of

Table 3. Mass Balance of Fumonisin B_1 Species in Extruded and Unextruded Corn Samples[a]

sample[b]	FB_1	HFB_1	NDF-FB_1	NCM-FB_1	total FB_1 species[c]
Spiked Corn Grits					
SG	43.0 ± 5.7 a	1.4 ± 0.2 a	0.3 ± 0.1 a	<1	44.7 ± 5.6 a
SG-E	34.1 ± 4.0 a	1.5 ± 0.2 a	1.0 ± 0.1 a	<1	36.6 ± 3.8 ab
SG-EG	9.8 ± 0.7 b	0.5 ± 0.3 b	19.2 ± 1.7 b	<1	29.2 ± 2.5 b
Fermented Corn Grits (Batch 1)					
FG1	45.5 ± 12.5 a	1.3 ± 0.5 a	0.5 ± 0.1 a	<1	47.3 ± 12.3 a
FG1-E	29.0 ± 1.4 a	1.2 ± 0.2 a	0.3 ± 0.1 a	<1	30.8 ± 1.1 ab
FG1-EG	5.9 ± 0.5 b	0.6 ± 0.3 b	10.2 ± 1.0 b	<1	16.7 ± 1.8 b
Fermented Corn Grits (Batch 2)					
FG2	67.1 ± 1.4 a	1.9 ± 0.5 a	0.6 ± 0.1 a	<1	69.6 ± 2.1 a
FG2-E	50.9 ± 0.9 b	2.5 ± 0.2 b	0.8 ± 0.1 a	<1	54.3 ± 0.6 b
FG2-EG	11.7 ± 0.2 c	1.1 ± 0.3 c	17.3 ± 1.0 b	<1	30.1 ± 1.5 c

[a] All values are concentration (nmol/g of dry weight), the average ± standard deviation of three determinations. Samples within a group (spiked corn, fermented corn batch 1, fermented corn batch 2) not sharing letters are significantly different ($p < 0.05$). [b] SG, spiked grits; SG-e, spiked grits extruded; SG-EG, spiked grits extruded with glucose; FG1, fermented grits batch 1 unextruded; FG1-E, fermented grits batch 1 extruded; FG1-EG, fermented grits batch 1 extruded with glucose; FG2, fermented grits batch 2 unextruded; FG2-E, fermented grits batch 2 extruded; FG2-EG, fermented grits batch 2 extruded with glucose. [c] Total FB_1 species = fumonisin B_1 (FB_1) + hydrolyzed fumonisin B_1 (HFB_1) + *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 (NDF-FB_1) + *N*-(carboxymethyl)fumonisin B_1 (NCM-FB_1).

fumonisin B_1 in extruded spiked or fermented corn with added glucose, *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 was the dominant species, whereas hydrolyzed fumonisin B_1 and *N*-(carboxymethyl)fumonisin B_1 were minor reaction products.

A mass balance analysis was used to estimate the contribution of the various fumonisin B_1 reaction products to the total fumonisin B_1 content of extruded and unextruded corn (spiked and fermented) (**Table 2**). The mass balance indicates that hydrolyzed fumonisin B_1 and *N*-(carboxymethyl)fumonisin B_1 were minor species in extruded corn with or without added glucose, representing <3% of the total fumonisin B_1 species present. The analysis also found low levels of *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 (2–5% of total fumonisin B_1 species) in extruded corn without added glucose. Overall, these results agree closely with previous results (*17, 25, 26*) on the relative contributions of fumonisin B_1 species in thermally processed corn-based foods. It was also found that low levels of fumonisin B_1−sugar adducts such as *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 and *N*-(carboxymethyl)fumonisin B_1 were present in a variety of thermally processed corn-based foods (tortilla chips, corn flakes, and nacho chips) that contain an insignificant amount of reducing sugars. It appears that *N*-(carboxymethyl)fumonisin B_1 is a minor reaction product even in corn-based foods containing reducing sugars because we found low levels of the compound in extruded corn that was supplemented with 10% glucose. Similarly, Castelo et al. (*17*) reported that 3–16% of fumonisin B_1 in muffin batter containing glucose was recovered as *N*-(carboxymethyl)fumonisin B_1.

Mass balance analysis indicates that 57–66% of the fumonisin B_1 species detected in corn (spiked and fermented) extruded with glucose was *N*-(deoxy-D-fructos-1-yl)fumonisin B_1. However, differences were found between groups of corn samples in the percent conversion of fumonisin B_1 to *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 during extrusion. In spiked extruded corn with glucose, 45% of the fumonisin B_1 originally in the corn was converted to *N*-(deoxy-D-fructos-1-yl)fumonisin B_1. In contrast, only about 25% of the fumonisin B_1 present in the fermented corn samples with glucose became converted to *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 during extrusion. These results suggest that the presence and growth of mold in the corn grits may have altered the matrix of the corn, resulting in changes in the fate of fumonisin B_1 during thermal processing. One possibility is that the fungus may have converted some of the carbohydrates and protein material in the grits to compounds that reacted with fumonisin B_1 during thermal processing. Previous papers also suggest that in addition to glucose and fructose, fumonisin B_1 reacts with polysaccharides and proteins in corn during heating via its two tricarballylic acid side chains (*27, 28*). When reacted with these food components, it is possible that fumonisin B_1 becomes bound and not detected using conventional fumonisin analyses. According to Kim et al. (*27*), heat processing, including extrusion, may lead to the binding of fumonisin B_1 to protein, which renders fumonisin less extractable. Although fumonisin B_1 in food matices may be bound to components other than protein, this may lead to underestimation of dietary exposure of fumonisin B_1 and its reaction product formed during heat processing as these toxicants may be released in the human gastrointestinal tract. Toxicological evaluations of the reaction products are underway in our laboratory to elucidate the significance of fumonisins in extruded corn product.

The kinetics of *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 formation in fumonisin B_1-contaminated corn with added glucose was reported by Lu et al. (*29*). The rate of *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 formation increased rapidly going from 40 to 80 °C in their study. The corn extruded in this study was exposed to much higher temperatures in the feed zone (100 °C) and in the metering and compression zones (160 °C). Lu et al. (*29*) added amylase to prevent starch gelatinization and water adsorption and to facilitate reaction of glucose with fumonisin B_1. In our study, no amylase was added to the corn for extrusion, and the extrusion process eliminated much of the water. The high levels of *N*-(deoxy-D-fructos-1-yl)fumonisin B_1 found in glucose-amended, extruded corn indicate that temperature is a significant factor in the formation of this reaction product. In general, the fate of fumonisin B_1 during the extrusion of naturally contaminated corn would be similar to that of fermented corn. It may be more significant in commercial applications, where the addition of sugars including glucose is commonly practiced.

SAFETY

Corn grits contaminated with fumonisin B_1, both spiked and cultured with *Fusarium verticillioides* M-2552, were handled with proper caution as fumonisin B_1 is a class B carcinogen.

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