

Reduction of Fumonisin B₁ in Corn Grits by Twin-Screw Extrusion

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Abstract: This study was designed to investigate the fate of fumonisins in flaking corn grits during twin-screw extrusion by measuring fumonisin B₁ (FB₁) and its analogs with a mass balance approach. Food grade corn grits and 2 batches of grits contaminated with FB₁ at 10 and 50 μg/g by *Fusarium verticillioides* M-2552 were processed with or without glucose supplementation (10%, w/w) with a twin-screw extruder. Extrusion reduced FB₁ in contaminated grits by 64% to 72% without glucose and 89% to 94% with added glucose. In addition, extrusion alone resulted in 26% to 73% reduction in the levels of fumonisin B₂ and fumonisin B₃, while levels of both mycotoxins were reduced by >89% in extruded corn grits containing 10% glucose. Mass balance analysis showed that 38% to 46% of the FB₁ species detected in corn extruded with glucose was *N*-(deoxy-D-fructos-1-yl)-FB₁, while 23% to 37% of FB₁ species detected in extruded corn grits with and without added glucose was bound to the matrix. It was also found that the hydrolyzed form of FB₁ was a minor species in extruded corn grits with or without added glucose, representing <15% of the total FB₁ species present. Less than 46% of FB₁ originally present in corn grits could be detected in the fumonisin analogues measured in this study. Research is needed to identify the reaction products resulting from extrusion processing of fumonisin-contaminated corn products.

Keywords: fumonisin, *N*-(deoxy-D-fructos-1-yl) fumonisin, reduction, twin-screw extrusion

Practical Application: Twin-screw extrusion is widely used in food industry for its versatility. This technology may reduce the level of fumonisins in corn particularly with added glucose.

Introduction

Fusarium verticillioides and *F. proliferatum* commonly infect corn (maize) in all corn-growing regions in the world (Marasas 2001). These ubiquitous fungi are known to produce fumonisins, among which fumonisin B₁ (FB₁) is of the most concern due to its prevalence and toxicity. FB₁ has been found in many processed corn-based products, such as corn grits, corn flakes, fried tortilla chips, and baked products (Castelo and others 2001; Voss and others 2001; Brera and others 2004; Humpf and Voss 2004). FB₁ has been associated with various diseases in animals and correlated with human esophageal cancer rates in certain regions of the world where corn is consumed as a staple food (Rheeder and others 1992; Chu and Li, 1994; Haschek and others 2001). In addition, epidemiological and experimental evidence suggest that fumonisins are a possible risk factor for neural tube defects, and their teratogenic potential was elucidated with laboratory animals

(Gelineau-van Waes and others 2005; Missmer and others 2006).

While FB₁ is very heat stable and survives conventional heat processes, such as baking and frying (Jackson and others 1997), previous studies suggest that extrusion may reduce the toxin in finished food products (Katta and others 1999; Pineiro and others 1999). Bullerman and others (2008) studied the effect of single-screw extrusion on the reduction of FB₁ in corn grits in the presence and absence of glucose. The results indicated that the extrusion process reduced the amount of FB₁ by 21% to 37% in the absence of sugar, while the addition of glucose further decreased the toxin by 77% to 87%. It was also reported that 35% to 43% of FB₁ in extruded grits, and 65% to 78% of FB₁ in extruded grits with glucose could not be detected in the form of known fumonisin degradation products such as hydrolyzed FB₁ (HFB₁), *N*-(deoxy-D-fructos-1-yl)-FB₁ (NDF-FB₁), *N*-(carboxymethyl)-FB₁ (NCM-FB₁) using conventional analytical methods.

During thermal processes, such as extrusion, fumonisins are believed to react with reducing sugars to form sugar adducts such as NDF-FB₁, NCM-FB₁, and other *N*-substituted FB₁ compounds that can be detected by LC-mass spectrometry (LC-MS) (Murphy and others 1996; Lu and others 2002; Poling and others 2002; Bullerman and others 2008). Thermal processing may also result in binding of FB₁ to proteins and starches in the food matrix to cause reduction in recovery (Kim and others 2003; Seefelder and others 2003). Several methods using detergents and alkaline hydrolysis, have been developed to improve recovery of these "hidden" or "masked" fumonisins in thermally processed food (Kim and others

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2003; Park and others 2004).

Over the past 40 y, the use of twin-screw extruders has increased due to an enhanced desire for innovative food products, particularly breakfast cereals and snack foods. In comparison with single-screw extrusion, the twin-screw configuration has greater ability and flexibility to control process and product parameters (Riaz 2001). Although there have been published studies on the fate of fumonisins in foods processed with single-screw extruders, little information exists on the stability of fumonisins during twin-screw extrusion, particularly about the levels of matrix-bound fumonisins in extruded corn grits. The main objective of this study was to use a mass balance approach to evaluate the fate of FB₁ during twin-screw extrusion of corn grits with and without glucose supplementation. Methods to measure the levels of matrix-bound FB₁ in addition to HFB₁ and a *N*-substituted FB₁ analogue were used in an attempt to account for loss of the toxin during extrusion processing.

Materials and Methods

Preparation of contaminated grits

Food grade corn grits (nr. 4 flaking grits) were provided by Bunge Milling (Crete, Nebr., U.S.A.) and were screened for the presence of fumonisins prior to inoculation with fungi. To produce contaminated corn grits, 1 kg of grits was weighed into 1-gallon size glass jars. Two sheets of filter paper (Whatman nr. 1) were placed on the mouth of each jar. Each jar was clapped with a lid that had a 7.6-cm dia hole to facilitate air flow for fungal growth, and then autoclaved for 1 h at 121 °C. Following equilibration overnight, the moisture content was adjusted to 30% (w/w; dry weight basis, db) by adding sterile distilled water in 2 steps (half and half) within a 24-h period to ensure even distribution of moisture.

For inoculation on corn grits, *F. verticillioides* M-2552 was grown on carnation leaf agar (CLA) in Petri plates for 7 to 10 d and one-half of the agar with spores was added to each jar. The jars were shaken daily to prevent clumping and provide good air exchange in the mass of grits. The production of fumonisin was monitored with Veratox[®] enzyme-linked immunosorbent assay (ELISA) kits (Neogen Corp., Lansing, Mich., U.S.A.). When batches of grits reached the desired fumonisin concentration, they were pooled and mixed. Two batches of grits containing 10 and 50 µg/g FB₁ were produced by mixing clean grits with contaminated grits using a paddle mixer. Uncontaminated, clean grits were designated as negative (unextruded) and positive (extruded) controls.

Extrusion of corn grits

Uncontaminated clean grits and contaminated grits were extruded with or without addition of food grade glucose (ADM Corn Processing, Decatur, Ill., U.S.A.). The level of added glucose and the moisture content of all treatments were set to 10% (w/w) and 20% (w/w; db), respectively, which showed greatest effect in reducing fumonisin concentration during extrusion in previous studies (Katta and others 1999; Castelo and others 2001). Extrusion was carried out using a model CTSE-V twin-screw extruder (C.W. Brabender, South Hackensack, N.J., U.S.A.) with a 3-mm nozzle. The screw speed was 40 rpm and the temperatures of the barrel were set at 50, 140, and 160 °C for the feed zone, metering zone, and compression zone, respectively.

During the extrusion process, parameters including torque, temperature, pressure, and output were recorded. An equal amount of unextruded grits was kept for each sample for comparison. The extruded and unextruded samples were ground using a Romer

Sampling Mill (Model 2A, Romer Labs, Union, Mo., U.S.A.) and separated into portions for LC-fluorescence and LC-MS analyses.

Analysis of fumonisins

Solvents used for solid phase extraction and for high-performance liquid chromatography (HPLC) mobile phases were of HPLC grade from Fisher Scientific (Pittsburgh, Pa., U.S.A.) or Acros Organics (Morris Plains, N.J., U.S.A.). Formic acid (Puriss grade) was from Fluka (St. Louis, Mo., U.S.A.), while hydrochloric acid and potassium chloride (certified ACS grade) were purchased from Fisher Scientific. Orthophthaldialdehyde (OPA), 2-mercaptoethanol, and sodium tetraborate decahydrate (borax) were from Sigma Chemical (St. Louis, Mo., U.S.A.). Deionized water was used to prepare solvent for extraction.

FB₁ used to prepare calibration standards and for recovery experiments was purchased from Sigma (98% pure), while fumonisin B₂ (FB₂), fumonisin B₃ (FB₃), and hydrolyzed fumonisin B₁ (HFB₁) (all >95% pure) were kindly provided by the Mycotoxin Team of FDA/CFSAN, College Park, Md., U.S.A. NDF-FB₁ (>95% pure) was supplied by Steven Poling of USDA/NCAUR (Peoria, Ill., U.S.A.). Stock solutions containing 1.0 mg/mL of the individual fumonisin standards were prepared in acetonitrile:water (1:1, v/v). Mixed standards containing all 5 forms of fumonisin were prepared by serial dilution in the same solvent in the range of 0.01 to 10.00 µg/mL. Standards, stored in amber vials at 5 °C, were used to spike control samples for recovery studies and for instrument calibration.

Extraction/purification of fumonisins for LC-fluorescence and LC-MS

Unbound forms of fumonisins (FB₁, FB₂, FB₃, HFB₁, NDF-FB₁) were extracted from samples and purified using the method described previously by Bullerman and others (2008). The method of Park and others (2004) with modifications was used to extract and purify bound FB₁ in samples. Briefly, fumonisin-contaminated corn grits (1.0 g) were extracted with 50 mL of acetonitrile:methanol:water (25:25:50, v/v) in 50-mL polypropylene tubes to remove unbound fumonisins. Contents of the tubes were filtered through Whatman nr. 541 filter paper. The solid material remaining on the filter paper was re-extracted with 50 mL of acetonitrile:methanol:water (25:25:50, v/v). The filtrates were discarded, while the solid material obtained after extraction of unbound fumonisins was treated with KOH to hydrolyze bound FB₁ to HFB₁, which was then extracted and quantified by LC-fluorescence. First, the solid corn residue remaining after extraction was transferred to a 250-mL Erlenmeyer flask, and 25-mL of 2 N KOH was added. The flask was held in a shaking (140 rpm) water bath at 60 °C for 1 h. The hydrolysate was diluted with 50 mL of 25:25:50 methanol:acetonitrile:water, and then placed in a shaker (room temperature) set at 140 rpm for 30 min. The hydrolysate was centrifuged at 10000 × *g* for 10 min, and then 10 mL of the supernatant fluid was applied to a preconditioned OASIS HLB (Waters; Milford, Mass., U.S.A.) SPE column, and vacuum was applied until the liquid level was just above the bed of the cartridge. The cartridge was washed with 3 mL of methanol:water (20:80, v/v), 3 mL of methanol:water (50:50, v/v), followed by 3-mL methanol:water (67:33, v/v). HFB₁ was eluted from the column with 1-mL methanol:water (75:25, v/v), followed by 3-mL methanol:8% acetic acid (75:25, v/v). Eluates were combined and then evaporated to dryness under nitrogen using a Turbovap LV system (Biotage; Charlotte, N.C., U.S.A.)

set at 40 °C. The dry residues were redissolved in 1-mL acetonitrile:water (70:30, v/v) and analyzed by LC-fluorescence to determine concentrations of HFB₁. Bound FB₁ was estimated from HFB₁ by multiplying the concentration of HFB₁ by the mass ratio of FB₁:HFB₁ (721.8:405.6). It was assumed that HFB₁ released during the alkaline hydrolysis step was due to matrix-bound FB₁ rather than matrix-bound HFB₁, since TCA side chains believed to be responsible for binding to the food matrix are not present in HFB₁.

LC-fluorescence and LC-MS analysis of fumonisins

Quantitative determination of FB₁, FB₂, FB₃, and HFB₁ in sample extracts was based on the method of Thakur and Smith (1996) using OPA derivatization as described by Shephard and others (1990). Analyses were carried out with a Waters HPLC system equipped with an Alliance 2695 solvent module and a model 2475 fluorescence detector ($\lambda_{EX} = 335$ nm; $\lambda_{EM} = 440$ nm; gain = 10). The autosampler was programmed to mix 10 μ L of sample extracts with 20 μ L of OPA reagent, incubate the mixture for 2 min, and then inject 10 μ L of the derivatized samples. Separations were carried out at 35 °C on a 150 \times 3 mm² i.d. Supelcosil LC-18-DB column (Supelco; Bellefonte, Pa., U.S.A.). 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) and the flow rate was 0.6 mL/min. 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 3 min at the initial mobile phase. Calibration standards for fumonisins were injected in the range of 0.05 to 2.5 μ g/mL.

A Waters HPLC equipped with an Alliance 2695 solvent module, ZQ mass detector, and Empower software was used to identify and quantify NDF-FB₁ in sample extracts. 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 NDF-FB₁ (M+H; $m/z = 884.4$). Separations were carried out at 35 °C on a 150 \times 3 mm² i.d. Supelcosil LC-18-DB column with 10- μ L 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 linearly 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

Extrusion parameters for each treatment (batch of grits) were analyzed for statistical differences using 1-way ANOVA followed by Tukey's multiple comparison test with a family-wise error rate of 0.05. All analyses for fumonisin analogues were done in triplicate. Means and standard deviations were calculated with Minitab[®] (State College, Pa., U.S.A.) statistical software for the LC-fluorescence and LC-MS analysis. Minitab was also used to verify statistical differences between treatments in the same batch of grits by 1-way ANOVA followed by Tukey's multiple comparison test at a 0.05 error rate.

Results and Discussion

Extrusion of corn grits

Conditions used during the extrusion process that may affect fumonisin reduction are summarized in Table 1. The moisture content and temperature are regarded as the main factors affecting expansion ratio while other factors including protein content may play an important role (Bhattacharya and others 1986; Scudamore and others 2008). The variations in expansion ratio may be attributed to relative protein contents since the moisture content and temperature of samples were kept constant. The pressure in the extruder barrel was also influenced by the torque ($R^2 = 0.7301$) suggesting the effect of matrix, that is, relative protein content, and presence of glucose. The relative protein content in contaminated grits tends to be greater than that of clean grits since carbohydrate is preferred source of nutrient for the growth of mold. In addition, the expansion ratios had a high degree of correlation with the torque ($R^2 = 0.9291$, Figure 1).

In general, differences in values of observed parameters may be attributed to the composition of the samples and presence of glucose since the screw speed, moisture content, and temperature were controlled in this study. For example, extrusion with glucose supplementation led to reduced torque and pressure, and resulted in lower expansion ratio in comparison with the samples extruded without glucose. The torque is mainly dependent on the viscosity of the matrix in the barrel when the screw speed is constant. Considering the melting point of glucose (146 °C), added glucose may act as a plasticizer to reduce the viscosity and torque at the temperature employed during extrusion (160 °C). Nonetheless, the reductions in torque and pressure were 34% and 24% in Batch 1 and 54% and 29% in Batch 2, respectively, suggesting that the addition of glucose (10% w/w) was not the only contributing factor.

Analyses of fumonisin and degradation products by LC-fluorescence and LC-MS

Extruded and unextruded clean grits were spiked at levels of 1.0, 5.0, and 10.0 μ g/g with each of the fumonisin standards to estimate the amount of each unbound fumonisin analogue recovered during the analytical procedure. Mean recoveries (\pm RSD) for FB₁, FB₂, FB₃, HFB₁, and NDF-FB₁ were 102 \pm 17%, 92 \pm 20%, 100 \pm 12%, 72 \pm 29%, and 97 \pm 22% for 6 analyses with an LOQ of 0.2 μ g/g for all species.

For the samples without added glucose (batch 1; batch 2), levels of unbound FB₁ decreased by 63% to 71% after extrusion (Table 2). Extrusion of the corn grits after addition of glucose resulted in substantial (88% to 94%) reduction in FB₁ levels. Similarly, extrusion alone resulted in 26% to 73% reduction in FB₂ and FB₃ levels, while levels of both toxins were reduced by >89% in extruded corn-containing glucose, suggesting that they likely also interacted with glucose to form N-fructosyl derivatives.

HFB₁ and NDF-FB₁ were quantified as the major reaction products for FB₁ in extruded corn-containing glucose. Of these forms in extruded contaminated corn with added glucose, NDF-FB₁ was the dominant species, while HFB₁ was a minor reaction product. There were no attempts to measure levels of NCM-FB₁ in the extruded corn samples since an analytical standard for NCM-FB₁ was not available, and previous studies (Seefelder and others 2001; Bullerman and others 2008) indicated that this fumonisin analogue is a minor reaction product in thermally processed corn products containing added sugars.

Bullerman and others (2008) reported that single-screw extrusion decreased FB₁ levels in corn grits by 21% to 37%, and 77% to 87% in grits supplemented with glucose, while Castelo and others (2001) showed apparent losses of FB₁ with and without added glucose were 35% to 46% and 55% to 68%, respectively. The improved efficacy of twin- in contrast to single-screw extrusion in reducing fumonisin levels may be a result of more complete mixing of sample in the barrel of a twin-screw extruder (Connelly and Kokini 2007). Other studies have demonstrated that the stability of fumonisins during extrusion is influenced by several factors, including the screw configuration, screw speed, extrusion temperature, and moisture content of the food matrix (Katta and others 1999; Scudmore and others 2008).

It was hypothesized that the apparent loss of fumonisins in extruded samples may have been due, at least in part, to binding of FB₁ to polysaccharides and proteins in corn during heating. To test this hypothesis, the method developed by Park and others (2004) was used to measure bound FB₁ in unextruded and extruded samples. Initial experiments estimated recovery of bound FB₁ during the hydrolysis/extraction procedure by spiking clean grits and clean, extruded grits with 1.0 and 10.0 µg/g FB₁ after the steps used to extract unbound fumonisins from the samples. Mean (±RSD) recovery of FB₁, as determined as HFB₁ after al-

kaline hydrolysis of the samples, was 83.2 ± 9.9% (*n* = 7). No significant difference was found in the recoveries of FB₁ spiked into unextruded and extruded grit samples.

Table 3 shows the levels of bound FB₁ in contaminated grits before and after extrusion. As expected, no bound fumonisin was found in the grits before extrusion. However, extruded grits contained considerable amounts of bound FB₁, with levels roughly equal to those of unbound FB₁. Similar to the results presented here, Kim and others (2003) and Park and others (2004) found higher levels of bound FB₁ in corn-based breakfast cereals, tortilla chips, and corn chips compared with unbound FB₁. In contrast with our results, Dall'Asta and others (2009) reported the presence of bound fumonisins in corn flour and other corn products that did not receive a thermal treatment.

Fumonisin undergo reactions in foods during processing that alter their bioavailability, toxicity, and/or ability to be detected (Murphy and others 1996; Hopmans and others 1997; Humpf and Voss 2004). Several toxicological studies using swine and rats established reduced toxicity of reaction products of FB₁ and reducing sugars compared to the unsubstituted analogue (Howard and others 2002; Fernandez-Surumay and others 2005; Voss and others 2008). It is hypothesized that the reactive amine group imparts toxicity to FB₁ since studies have shown that chemically blocking the group reduces toxicity in monkey kidney cells (Meca and

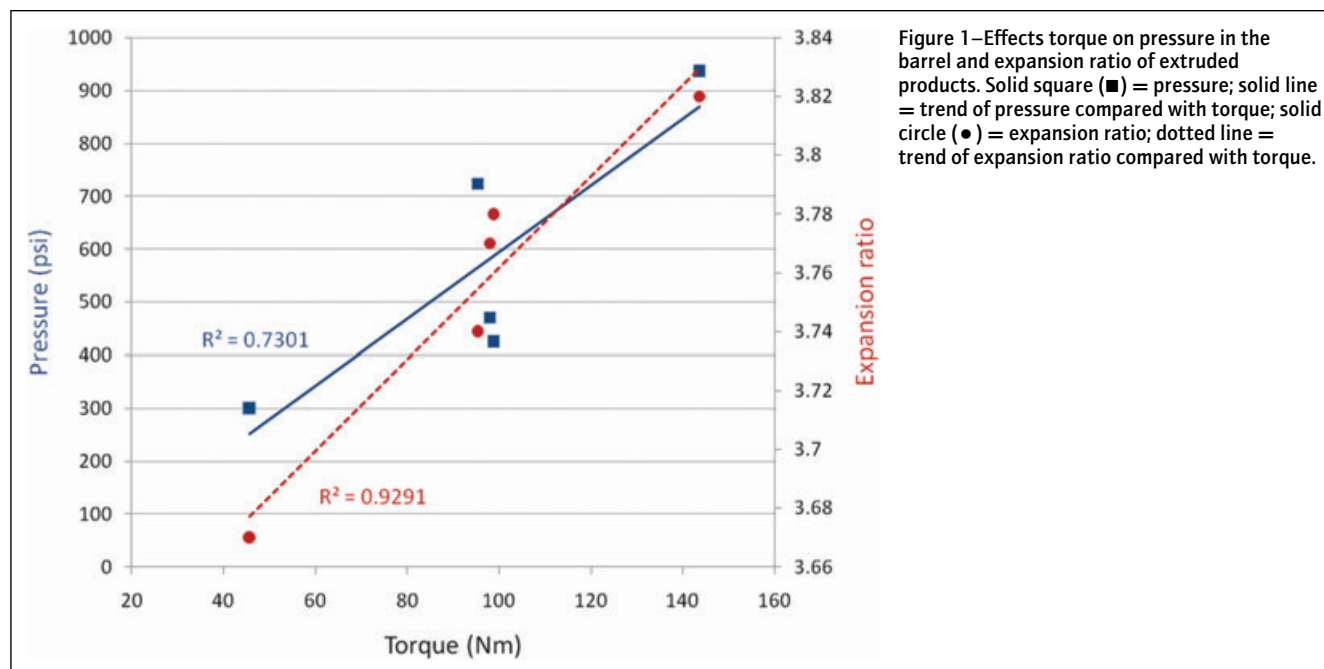
Table 1—Parameters measured during and after twin-screw extrusion in the presence and absence of glucose.

Sample ¹	Torque (Nm)	Temperature (°C)	Pressure (psi)	Output (g/min)	Diameter (mm)	Expansion ratio ²
CG-E	97.99 ± 8.67 ^a	160.2 ± 4.7 ^a	470.10 ± 54.54 ^a	98.9 ± 4.9 ^a	5.84 ± 0.03 ^a	3.77 ± 0.04 ^a
Batch 1-E	143.66 ± 12.8 ^b	159.9 ± 4.7 ^a	937.06 ± 105.47 ^b	94.7 ± 3.0 ^a	5.87 ± 0.02 ^b	3.82 ± 0.02 ^b
Batch 1-EG	95.39 ± 13.16 ^a	161.6 ± 2.4 ^a	723.41 ± 73.23 ^c	94.9 ± 9.2 ^a	5.80 ± 0.01 ^c	3.74 ± 0.01 ^c
Batch 2-E	98.84 ± 7.81 ^a	159.5 ± 4.5 ^a	425.27 ± 50.95 ^a	99.5 ± 1.9 ^a	5.83 ± 0.01 ^a	3.78 ± 0.02 ^a
Batch 2-EG	45.57 ± 8.85 ^c	160.9 ± 5.3 ^a	300.77 ± 68.14 ^d	84.0 ± 4.2 ^b	5.75 ± 0.01 ^d	3.67 ± 0.01 ^d

¹Legends for the samples in column—CG-E = clean grits extruded; Batch 1-E = grits contaminated with 10 ppm FB₁ extruded; Batch 1-EG = Batch 1 extruded with 10% glucose; Batch 2-E = grits contaminated with 50 ppm FB₁ extruded; Batch 2-EG = Batch 2 grits extruded with 10% glucose.

²The expansion ratio was calculated as the ratio of the cross-sectional area of the extruded material to that of the nozzle based on 50 measurements per sample.

^{a10d}Data in columns not sharing superscripts are significantly different (*P* < 0.05).



others 2010) and primary rat hepatocytes (Gelderblom and others 1993). Unlike *N*-substituted adducts that are formed through covalent reaction between the free amine group of FB₁ and food components, the toxin has been shown to bind covalently to protein and starch during food processing via its 2 tricarballic acid side chains (Seefelder and others 2001; Kim and others 2003). These "hidden fumonisins" may be of health concern if they are released into the gastrointestinal tract and during digestion (Humpf and Voss 2004; Dall'Asta and others 2009).

A mass balance analysis was used to estimate the contribution of the various forms of FB₁ (unbound FB₁, bound FB₁, HFB₁, and NDF-FB₁) to the total FB₁ content of unextruded and extruded contaminated corn grits (Table 3). To account for differences in masses between the FB₁ species, concentrations for each species was converted to nmol/g dry weight. The mass balance indicates that HFB₁ was a minor species in extruded corn with or without added glucose, representing <15% of the total FB₁ species present. The analysis also found low levels of NDF-FB₁ (1.9% to 2.4% of total FB₁ species) in extruded corn without added glucose. In contrast, 36% to 46% of the FB₁ species detected in corn extruded with glucose was NDF-FB₁. Overall, these results agree with other published papers (Castelo and others 2001; Voss and others 2003) on the relative contributions of FB₁ species in thermally processed corn-based foods. It was found that low levels of FB₁-sugar adducts, such as NDF-FB₁, 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 (Seefelder and others 2001). These results cor-

roborate our previous study (Bullerman and others 2008) that determined that 57% to 66% and <3% of FB₁ species detected in extruded corn with and without glucose, respectively, was NDF-FB₁.

From 23% to 38% of FB₁ species detected in extruded grits with and without added sugar was bound to the food matrix, possibly through interaction of the FB₁ tricarballic acid side chains with polysaccharides and proteins in corn. Despite the use of additional methods to detect these hidden fumonisins, mass balance analysis revealed that only 37% to 46% of the FB₁ originally presented in corn grits were detected after extrusion as HFB₁. This result is not unexpected since hydrolysis of FB₁ to HFB₁ is favored at alkaline pH values (Jackson and others 1996). Interestingly, only 20% to 30% of FB₁ in grits with added glucose could be detected in the form of the fumonisin analogues measured in this study.

Conclusions

Our results suggest that the majority of fumonisins in extruded corn-based foods react via the primary amine of the toxin to food components to form a variety of products that have not been identified. Studies using radio-labeled FB₁ may enable a better understanding of the fate of fumonisins during extrusion processing. Despite the lack of information on the fate of fumonisins in extruded products, results of one study suggest that extrusion with glucose under some circumstances can reduce *in vivo* toxicity of FB₁. Specifically, Voss and others (2008) found that kidney lesions in rats fed 1 of 3 batches of fumonisin-contaminated corn grits that were (single-screw) extruded with added glucose were

Table 2—Concentration of fumonisin species in unextruded and extruded corn grits determined by LC-fluorescence and LC-MS.*

Sample ¹	FB ₁ (Unbound)	FB ₁ (Bound)	HFB ₁ (Unbound)	FB ₂ (Unbound)	FB ₃ (Unbound)	NDF-FB ₁ (Unbound)	Total fumonisins ²
Controls							
CG	0.21 ± 0.08 ^a	—	ND ^a	0.29 ± 0.11 ^a	ND ^a	ND ^a	0.50 ± 0.31 ^a
CG-E	0.20 ± 0.02 ^a	—	ND ^a	ND ^b	ND ^a	ND ^a	0.20 ± 0.02 ^a
Contaminated corn grits (Batch 1, 10 ppm FB₁)							
Batch 1	10.5 ± 1.57 ^a	ND ^a	ND ^a	5.22 ± 0.87 ^a	1.13 ± 0.14 ^a	0.72 ± 0.09 ^a	17.6 ± 2.47 ^a
Batch 1-E	3.01 ± 0.10 ^b	2.39 ± 0.25 ^b	0.54 ± 0.0 ^b	1.49 ± 0.22 ^b	0.41 ± 0.08 ^b	ND ^b	7.84 ± 0.77 ^b
Batch 1-EG	0.64 ± 0.01 ^c	0.78 ± 0.01 ^c	ND ^a	0.30 ± 0.03 ^b	ND ^c	1.01 ± 0.20 ^a	2.73 ± 0.32 ^c
Contaminated corn grits (Batch 2, 50 ppm FB₁)							
Batch 2	54.3 ± 1.62 ^a	ND ^a	0.50 ± 0.03 ^a	23.7 ± 1.40 ^a	7.46 ± 1.19 ^a	4.48 ± 1.13 ^a	90.4 ± 3.58 ^a
Batch 2-E	20.0 ± 2.39 ^b	14.4 ± 0.8 ^b	1.51 ± 0.09 ^b	17.9 ± 2.44 ^b	3.23 ± 1.07 ^b	1.14 ± 0.41 ^b	58.2 ± 4.52 ^b
Batch 2-EG	6.38 ± 0.26 ^c	4.35 ± 0.12 ^c	0.41 ± 0.02 ^a	2.71 ± 0.08 ^c	0.59 ± 0.02 ^c	8.63 ± 1.77 ^c	23.1 ± 2.48 ^c

*All values are concentration (μg/g dry weight), the average ± standard deviation of 3 determinations.

¹Legends for the samples in column—CG = clean grits (negative control); CG-E = clean grits extruded (extrusion control); Batch 1 = grits contaminated with 10 ppm FB₁; Batch 1-E = Batch 1 grits extruded; Batch 1-EG = Batch 1 grits extruded with 10% glucose; Batch 2 = grits contaminated with 50 ppm FB₁; Batch 2-E = Batch 2 grits extruded; Batch 2-EG = Batch 2 grits extruded with 10% glucose.

²Total fumonisins = unbound fumonisin B₁ (FB₁) + bound fumonisin B₁ (FB₁) + hydrolyzed fumonisin B₁ (HFB₁) + fumonisin B₂ (FB₂) + fumonisin B₃ (FB₃) + *N*-(deoxy-D-fructos-1-yl) fumonisin B₁ (NDF-FB₁). ND = not detected, less than the method LOQ.

^{a,b,c} Samples within a group (batch) not sharing superscripts are significantly different (*P* < 0.05).

Table 3—Mass balance of fumonisin B₁ species in unextruded and extruded corn grits.*

Sample ¹	Unbound FB ₁	Bound FB ₁	HFB ₁	NDF-FB ₁	Total FB ₁ species ²
Contaminated corn grits (Batch 1, 10 ppm FB₁)					
Batch 1	14.5 ± 2.16 ^a	ND ^a	ND ^a	0.80 ± 0.10 ^a	15.5 ± 2.60 ^a
Batch 1-E	4.16 ± 0.13 ^b	3.32 ± 0.34 ^b	1.31 ± 0.02 ^b	ND ^b	8.97 ± 0.47 ^b
Batch 1-EG	0.89 ± 0.02 ^c	1.09 ± 0.01 ^c	ND ^a	1.14 ± 0.23 ^a	3.13 ± 2.28 ^c
Contaminated corn grits (Batch 2, 50 ppm FB₁)					
Batch 2	75.2 ± 2.27 ^a	ND ^a	1.22 ± 0.03 ^a	5.08 ± 1.28 ^a	81.4 ± 7.99 ^a
Batch 2-E	27.8 ± 1.44 ^b	19.9 ± 1.00 ^b	3.72 ± 0.09 ^b	1.30 ± 0.47 ^b	52.7 ± 3.88 ^b
Batch 2-EG	8.84 ± 0.38 ^c	6.02 ± 0.17 ^c	1.01 ± 0.04 ^a	9.76 ± 2.01 ^c	25.6 ± 2.01 ^c

*All values are concentration (nmol/g dry weight) the average ± standard deviation of 3 determinations. Legends for the samples in column—Batch 1 = grits contaminated with 10 ppm FB₁; Batch 1-E = Batch 1 grits extruded; Batch 1-EG = Batch 1 grits extruded with 10% glucose; Batch 2 = grits contaminated with 50 ppm FB₁; Batch 2-E = Batch 2 grits extruded; Batch 2-EG = Batch 2 grits extruded with 10% glucose.

²Total FB₁ species = unbound fumonisin B₁ (FB₁) + bound fumonisin B₁ + hydrolyzed fumonisin B₁ (HFB₁) + *N*-(deoxy-D-fructos-1-yl) fumonisin B₁ (NDF-FB₁) ND = not detected, less than the method LOQ.

^{a,b,c} Samples within a group not sharing superscripts are significantly different (*P* < 0.05).

significantly less severe than those found in rats fed either the unprocessed grits or the grits that were extruded without added glucose. The toxicological properties of the uncooked and extruded corn grits generated in the present study, including prevention of nephrotoxicity by extrusion with glucose supplementation of Batch 1, are reported elsewhere (Voss and others 2011).

Safety

Corn grits contaminated with fumonisin B₁, cultured with *Fusarium verticillioides* M-2552, were handled with proper caution as fumonisin B₁ is an IARC class B carcinogen.

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