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Effect of Extrusion Processing on Fumonisin B_1 and Hydrolyzed Fumonisin B_1 in Contaminated Alkali-Cooked Corn

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Fumonisins, toxins produced by *Fusarium verticillioides (=Fusarium moniliforme Sheld.)* Nirenberg, *F. proliferatum*, and other *Fusarium* species, have been detected worldwide in food and feed materials containing corn. Fumonisins produce a wide range of biological effects in animals. In humans, have been associated with high rates of esophageal cancer in areas of the world where corn is the main food source (Sydenham et al 1990, Yang 1980). Fumonisin B_1 (FB_1)is relatively heat stable (Dupuy et al 1993) and water soluble. Alkali-cooking or nixtamalization, which is used in the processing industry to produce snack and tortilla products from corn, reduces the original FB_1 level in contaminated corn (Sydenham et al 1995). However, it produces a hydrolyzed form of fumonisin B_1 (HFB_1), an aminopentol moiety (AP_1), which is more toxic to primary rat hepatocytes than the original FB_1 (Gelderblom et al 1993). Hendrich et al (1993) reported that treatment of corn culture material of *F. proliferatum* with $Ca(OH)_2$ reduced levels from 50 μg/g FB_1 to 7 and 10 μg/g AP_1. Rat feeding experiments in the study showed that the hydrolysis product or products produced during the treatment of corn with $Ca(OH)_2$ were more toxic. Voss et al (1996) reported that nixtamalized *F moniliforme*-culture material with 58 ppm HFB_1 but no FB_1 was hepatotoxic and nephrotoxic.

The occurrence of HFB_1 in tortilla chips and masa has been reported (Hopmans and Murphy 1993). Additionally, Stack (1998) found average FB_1 and HFB_1 levels of 187 and 82 ng/g in tortillas and 262 and 64 ng/g in masa, respectively, collected from the Texas-Mexico border. This research was part of an epidemiologic study by the Texas Department of Health to investigate an increased incidence of neural tube defects since FB_1 interferes with the sphingolipid-containing folate receptor in vitro. Meredith et al (1999) reported FB_1 and AP_1 levels of 0.8 and 26.1μg/g, respectively, in tortillas and nixtamalized corn from Guatemala. Extrusion is commonly used in the food industry to produce snacks and tortilla products. It has been reported that extrusion reduce the level of FB_1 in artificially contaminated corn grits (Castelo et al 1998, Katta et al 1999). No studies have investigated extrusion processing of alkali-cooked corn. Our objective was to evaluate the effects of moisture content and die configuration during extrusion processing on FB_1 and HFB_1 contaminated alkali-cooked corn.

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MATERIALS AND METHODS

Corn cultured with *F. verticillioides* M5991 and containing 4,461 ppm of FB_1 was obtained from the *Fusarium* Research Center at Pennsylvania State University. Sound yellow corn grain (US #2) was mixed with the contaminated corn 10 min in a 50 lb capacity Wenger horizontal ribbon mixer. The final FB_1 concentration was 250 ppm.

Two thousand grams of corn and 1.2% (w/w) solution of lime [$Ca(OH)_2$] were cooked in a stockpot for 55 min at 95-100°C (Ramirez-Wong et al 1994). The cooked corn was steeped for 14 hr. A fraction of the steep water was collected and refrigerated until analyzed. The cooked grain (nixtamal) was washed with approximately 3 volumes of tap water. The nixtamal was ground coarsely with a manual corn grinder and then dried in a forced air oven at 45°C for 16-18 hours. The dried nixtamal was ground to flour using a Fitz mill with a 0.85 mm screen. The flour was placed in sealed plastic bags and stored in a freezer until analyzed or extrusion-processed. Samples of fumonisin-contaminated raw corn, sound raw corn, alkali-cooked fumonisin-contaminated ground corn (nixtamalized), and steep water were collected for analyses of FB_1 and HFB_1 using HPLC.

Extrusion processing was performed in the Dept. of Grain Science & Industry at Kansas State University using a Leitzstritz 18 mm twin screw, co-rotating, intermeshing extruder. The extruder barrel was 53 cm in length and 1.9 cm in diameter and had a feed block and six temperature control zones. To obtain a processing temperature of 171°C, the following temperature settings were used: zone 1 (75 ± 21°C), zone 2 (97 ± 6°C), zone 3 (115 ± 7°C), zone 4 (152 ± 4°C), zone 5 (161 ± 7°C), and zone 6 (152 ± 9°C). The water was pumped directly into the extrusion barrel and regulated by setting the stroke length to 0.8, 1.0, 1.25, or 1.5 strokes, which provided either 6, 8, 10, or 12 mL/min to give target moistures of 24, 27, 30, and 33%, respectively, in the material being extruded. The screw speed was held constant at 122 rpm. Two dies with 10 mm land length were used: a tapered-angular die with a 3 mm opening and a tapered-circular die with 5 mm opening. The feed rate of the material to be extruded was fixed at 30 g/min. The extruder was brought to a steady state by running a sample at the target moisture level until uniform flow was achieved. After 10 min of uniform flow, 300 g of each extruded sample were collected. Extruded samples were dried overnight at 45°C in a forced-air oven, ground, and analyzed for FB_1 and HFB_1.

A 1000 µg/ml FB_1 standard stock solution was prepared by dissolving 1 mg FB_1 standard (Sigma Chemical Co., St. Louis, MO) in 1 ml acetonitrile-water (1:1, v/v). Serial dilutions of 100, 50, 25, 10, 5, and 1µg/ml were made. HFB_1 stock solution was prepared using base hydrolysis. Complete hydrolysis was achieved by mixing 1 mg of FB_1 with 2 ml of 2*N* potassium hydroxide and allowing it to react at room temperature overnight.

Fumonisin B_1 and HFB_1 were extracted from the samples based on the procedure described by Sydenham et al (1992). Twenty-five grams of ground sample were

extracted with 50 mL of methanol:water (3:1) in a blender at high speed for 2 min. Centrifuged at 500 G for 10 min, and filtered through Whatman No.4 filter paper. The pH of the eluate was adjusted with 0.1*M* KOH to 5.8-6.5, if necessary.
Ten milliliters of the filtered extract were applied to a C_{18} cleanup cartridge (Sep-Pak Waters, 3 mL capacity containing 500 mg of sorbent) previously conditioned by successive washing, first with 5 mL of methanol and then with 5 mL of methanol:water (3:1). A 10 mL of aliquot of filtered sample extract was applied to the cartridge as the flow rate was maintained at ≤2 mL/min. The cartridge was washed with 8 mL of MeOH:H_20 (3:1) followed by 3 mL of MeOH. The fumonisins were eluted with 10 mL of 1% methanolic acetic acid at a flow rate ≤1 mL/min and collected in a clean test tube. The eluate was evaporated to dryness at 60°C. Collection tubes were rinsed with 1 mL MeOH and evaporated to dryness. Dried eluates were stored at 4°C for less than 24 hr prior to HPLC analysis. For cleanup of extract from the raw corn samples, a Varian Bond Elut SAX column (10 cc, 500 mg of sorbent) was used. For analysis of the steep water, 10 mL aliquots were adjusted to pH 2.5 by the addition of 0.1*M* HCl (Sydenham et al 1995). Then, they were passed through the C_{18} column previously conditioned as described above. FB_1 and HFB_1 were eluted by the addition of 10 mL of 1% methanolic acetic acid, and the eluate was evaporated to dryness and retained for analysis.

A standard curve was constructed by injection of known concentrations of FB_1 and HFB_1 from their serial dilutions. Retention times were 10 and 7 min for FB_1 and HFB_1, respectively. The detection limit of this method was 0.02 ppm. Fumonisin B_1 and HFB_1-OPA (*o*-phthaldialdehyde) derivatives were prepared according to Sydenham et al (1992). Evaporated sample eluates were redissolved in 200 µL of methanol, and 50 µL was transferred into a test tube with 100 µL of OPA reagent and mixed. Within 40 sec of adding the OPA reagent, 100 µL of the derivatized solution was injected into the HPLC system.

The sample was chromatographed on a stainless steel analytical column (350 x 4.6 mm id) packed with Hypersil C_8 5 µm reverse-phase packing material. Isocratic conditions were used. The mobile phase consisted of methanol and 0.1*M* sodium dihydrogen phosphate (15.6 g $NaH_2PO_4.2H_2O$ in 1 L distilled water, 64:36) adjusted to pH 3.3 with o-phosphoric acid. The flow rate was 1.0 mL/min. The fluorescent derivative was detected using an excitation wavelength of 334 nm and an emission wavelength of 440 nm and recorded on an integrator with a chart speed of 0.5 in/min. The equation of Sydenham et al (1996) was used to calculate the fumonisin content of each aliquot injected. The HPLC confirmation analyses of samples were based on the procedure by Thakur and Smith (1996).

A 2x4 factorial experiment was carried out to determine the effect of the die configuration (tapered-angular with 3 mm opening and tapered-circular with 5 mm opening) and processing moisture contents of 24, 27, 30, and 33% on the recovery of FB_1 and HFB_1. The experiment was performed in triplicate in a completely randomized design. Data were analyzed using SAS software (SAS Institute, 1987).

RESULTS AND DISCUSSION

The amount of recoverable FB_1 was reduced during the nixtamalization step (alkali-cooking), because FB_1 was transformed into its hydrolysis product, HFB_1. A similar result was reported by Sydenham et al (1995). In this study, the steep water contained 39% of the original FB_1 content, but the HFB_1 was the predominant form (Table 1). The control corn contained 1μg/g FB_1, none was detected in the steep water and the nixtamal.

Table 1. Fumonisin B_1 and hydrolyzed fumonisin B_1 in contaminated and non-contaminated alkali-cooked corn.

	Fumonisin-Contaminated Corn				Non-Contaminated Corn
Sample	FB_1 (μg/g)	HFB_1 (μg/g)	HFB_1 Equiv. to FB_1(μg/g)	FB_1 (μg/g)	FB_1 (μg/g)
Raw Corn	239[a]	nd[b]	-	239	1
Steep water	6	50	88	94	Nd
Alkali-cooked corn	41	59	104	145	Nd

[a] Data are averages of 3 replications, 4 HPLC injections per replication.
[b] = Not detected

The removal of about one-third of the initial fumonisin contamination by the steep water probably was facilitated by the high solubility of fumonisins in water. This could be considered a preliminary decontamination step, increasing the safety of the product by reducing the fumonisin contamination. However, the steep water usually is discarded during commercial processing and discharged into wastewater. This water effluent represents a potential source of contaminants of surface water and groundwater. Conventional water treatments will be ineffective in removing fumonisins because of their high solubility. Water treatment plants usually monitor the amounts of insecticides, herbicides and other organic compounds, but they do not monitor for mycotoxins.

The nixtamal contained 61% of the original FB_1 (FB_1 + HFB_1), but again the major fumonisin compound present was HFB_1 at 72% (Table 1). Only 17% of the FB_1 remained unchanged during the alkali-cooking of fumonisin-contaminated grain. Material balance calculations based on the initial FB_1 level and the liquid and solid fractions obtained from nixtamalization showed that 96% of the FB_1 amount in the raw corn was recovered. In this study, care was taken not to discard the pericarp during the washing of the nixtamal. Thus, the remaining percentages of FB_1 and HFB_1 are higher than those reported by Sydenham et al (1995). Furthermore, those authors reported that corn kernels with their pericarps partially removed retained 31% of the original FB_1 concentration, predominantly as the intact toxin and a smaller amount of the AP_1. Those kernels from which the

pericarps were removed fully retained only 5.1% of the original FB_1, with the majority being present as AP_1.

The recoverable FB_1 and HFB_1 concentrations of alkali-cooked corn flour were affected significantly ($P \leq 0.05$) by the die configuration during extrusion processing. The tapered-angular die (Table 2) reduced the FB_1 and HFB_1 levels more than the tapered-circular die (Table 3).

Table 2. Fumonisin B_1 and hydrolyzed fumonisin B_1 in alkali-cooked corn before and after extrusion with the tapered-angular die.

Sample	HFB1	HFB1(μg/g) equiv. FB1	FB1 (μg/g)	FB1 total in sample (μg/g)	% Reduction	
					HFB1	FB1
Before extrusion	59	104	41	145	-	-
After extrusion						
[1]Control	nd	nd	nd	nd	nd	nd
[2]33%	37	65a	15a	80	38a	63a
[2]30%	40	70a	4b	74	33a	90b
[3]27%	24	42b	1.0b	43	60b	98b
[3]24%	19	34b	0.4b	34.4	67b	99b

nd= Not detected.
Values followed by the same letter in a column are not significantly different at 5% level
[1]Control uncontaminated corn flour.
[2]Mean of three replicates, 4 HPLC repetitions per replicate.
[3]Single measurement, 4 HPLC repetitions.

Table 3. Fumonisin B_1 and hydrolyzed fumonisin B_1 in alkali-cooked corn before and after extrusion with the tapered-circular die.

Sample	HFB_1	HFB1(μg/g) equiv. FB_1	FB_1 (μg/g)	FB_1 total in sample (μg/g)	% Reduction	
					HFB_1	FB_1
Before extrusion	59	104	41	145	-	-
After extrusion						
[1]Control	nd	nd	nd	nd	nd	nd
33%	28	50b	4c	54	52a	90a
30%	54	96a	40ab	136	8c	2c
27%	37	65ab	23bc	88	38b	44b
24%	32	57ab	59a	116	45ab	-

nd = Not detected.
Values followed by the same letter in a column are not significantly different at 5% level
[1]Control uncontaminated corn flour.
Mean of three replicates, 4 HPLC repetitions per replicate.

With the tapered angular-die, the amount of moisture during processing also significantly ($P<0.05$) affected the FB_1 and HFB_1 levels in the extruded product. The greatest reductions in FB_1 (90-99%) were obtained by extruding the alkali-cooked flour at moisture contents of 24, 27 and 30% (Table 2). The HFB_1 also was reduced but by a lesser amount. The greatest reductions were 67 and 60% at 24 and 27% moistures, respectively.

Alkali-cooked, contaminated, corn flour extruded with the tapered-circular (5 mm) die exhibited a different fumonisin-reduction pattern (Table 3). The greatest reductions in recoverable FB_1 and HFB_1 occurred in the product extruded at 33% moisture content, but the relationship between the rate of water injection and the reduction in recoverable fumonisin did not appear to be consistent. At 24% moisture content FB_1 was not detected. Because the water was pumped directly into the extrusion barrel, differences in between the initial moisture content and the extruded product ranged from +1 to -10% depending on the die pressure and temperature during processing (Table 4). These differences are attributed to the differences between pressure inside the extruder barrel and the atmosphere, which cause moisture loss when the product exited the extruder. The higher the pressure difference, the higher the moisture loss. So, based on the moisture content of the extruded product, the level of FB_1 and HFB_1 reductions were low at moisture contents ≤22% (Tables 3 and 4).

Table 4. Mean die pressure, die temperature and moisture content of extruded product after extrusion processing at four moisture content using two die configurations.

Initial Moisture	Tapered-Angular Die			Tapered-Circular Die		
	Pressure (lbs/in^2)	Temp. (°C)	Extruded Product Moisture (%)	Pressure (lbs/in^2)	Temp. (°C)	Extruded Product Moisture (%)
24	270	141	26	957	141	14
27	400	144	28	560	144	18
30	187	151	24	497	147	22
33	150	147	27	363	148	28

Data are means of three replications

Our data showed that extrusion processing reduced the recoverable FB_1 and HFB_1 from alkali-cooked contaminated corn flour. The low percentage recovery may indicate that the FB_1 and FB_1 were destroyed, transformed into other products, or rendered less extractable. Perhaps the decomposition products from extrusion did not react with the OPA reagent and, thus, were not detected by chromatography. Such low recoveries may be due to binding and matrix related problems. Murphy

et al (1996) stated that the heating process may chemically block the FB amine thus losing reactivity during the derivatization step for HPLC detection.

Castelo et al (1998) reported that extrusion processing with mixing screws caused more reduction of FB_1 in corn grits spiked at 5 mg/g. In a study on the effect of temperature and screw speed, Katta et al (1999) reported that the FB_1 recovered, decreased with an increase in temperature and a decrease in screw speed. They stated further that about 46-76% of the spiked FB_1 was lost when grits were extruded at temperatures and screw speeds that resulted in acceptable product expansion and color. Extrusion processing may be effective in reducing the FB_1 and HFB_1 levels of alkali-cooked corn. However, further studies are needed to evaluate the effects of moisture content, temperature, pressure, and die configuration during extrusion processing. Toxicity studies also are needed to evaluate whether the remaining FB_1 and HFB_1 in extruded products are bio-available and toxic. Information on the reduction of FB_1 and HFB_1during processing will be important to regulators, corn producers, processors, and consumers.

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