Effect of Nixtamalization (Alkaline Cooking) on Fumonisin-Contaminated Corn for Production of Masa and Tortillas

Mary A. Dombrink-Kurtzman,*,† Timothy J. Dvorak,† Marc E. Barron,‡ and Lloyd W. Rooney‡

Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604-3902, and Cereal Quality Laboratory, Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas 77843-2474

Studies were undertaken to determine the fate of the mycotoxins, fumonisins, during the process of alkaline cooking (nixtamalization), using normal-appearing corn that was naturally contaminated with fumonisin B_1 (FB_1) at 8.79 ppm. Corn was processed into tortillas, starting with raw corn that was cooked with lime and allowed to steep overnight; the steeped corn (nixtamal) was washed and ground into masa, which was used to make tortillas. Calculations to determine how much of the original fumonisin remained in the finished products took into consideration that FB_1 will be converted to hydrolyzed fumonisin B_1 (HFB_1) by the process of alkaline cooking. All fractions, including steeping and washing water, were weighed, and percent moisture and fumonisin content were determined. Tortillas contained approximately 0.50 ppm of FB_1, plus 0.36 ppm of HFB_1, which represented 18.5% of the initial FB_1 concentration. Three-fourths of the original amount of fumonisin was present in the liquid fractions, primarily as HFB_1. Nixtamalization significantly reduced the amount of fumonisin in maize.

Keywords: *Alkaline cooking; corn; fumonisin B_1; hydrolyzed fumonisin B_1; nixtamalization*

INTRODUCTION

Corn (maize) is the staple food for millions of people throughout the world. In Mexico, Central America, and certain parts of Africa and China, corn is the major source of energy for a large part of the general population (Rooney and Serna-Saldivar, 1987). It has been estimated that in Mexico the annual consumption of corn is 410 pounds per person (Watson, 1988). Prior to consumption, corn is processed by various technologies. In both Mexico and Central America, as well as in the United States, the major way of processing corn to make masa and tortillas is nixtamalization, the ancient, traditional process of alkaline cooking, using lime as the alkaline component in the process (Serna-Saldivar et al., 1990). Major benefits of nixtamalization are improved protein quality and availability of niacin (Rooney and Suhendro, 1999), an increased calcium content in the processed corn (Bressani et al., 1958), and the increased availability of lysine (Trejo-Gonzalez et al., 1982).

Corn products destined for human consumption can contain quite low levels of fumonisin, although under certain environmental conditions, such as drought stress, corn can contain higher levels of fumonisin. People who consume high amounts of corn may be exposed to increased amounts of fumonisin if growing conditions are favorable for production of the mycotoxin.

Previously, when we analyzed commercially produced masa and tortillas from Mexico and the United States for fumonisin content, the amount of fumonisin B_1 (FB_1) present in Mexican samples was 1.0−1.8 ppm (Dombrink-Kurtzman and Dvorak, 1999). Alkaline hydrolysis of FB_1 removes the tricarballylic side chains at C-14 and C-15 to form hydrolyzed FB_1 (HFB_1) (Figure 1). Fumonisins (FB_1 and HFB_1) have been detected in alkaline-processed food produced in the United States and Canada, but the levels were generally <1 ppm (Hopmans and Murphy, 1993; Maragos et al., 1997; Murphy et al., 1996; Scott and Lawrence, 1996; Stack, 1998). Sydenham et al. (1996) reported that with alkaline hydrolysis treatment of intact corn kernels, only 5.1% of the original FB_1 remained if the outer pericarp had been removed from the kernels.

Fusarium verticilliodes (Sacc.) Niremberg (synonym *Fusarium moniliforme* Sheldon) and *Fusarium proliferatum*, fungi capable of producing fumonisins, are frequently found in corn worldwide due to the endophytic nature of these fungi (Nelson et al., 1993; Bacon and Hinton, 1996). Fumonisins have been associated with various animal diseases, including equine leukoencephalomalacia (ELEM) (Marasas et al., 1988; Thiel et al., 1991), porcine pulmonary edema (Harrison et al., 1990), and hepatotoxicity and nephrotoxicity in rats (Voss et al., 1993). A higher incidence of human esophageal squamous cell carcinoma has been linked epidemiologically with consumption of fumonisin-contaminated corn in both the Transkei area, South Africa (Rheeder et al., 1992), and Linxian County, Henan providence, China (Chu and Li, 1994). Recently, intestinal disturbances have been reported in humans who had consumed moldy sorghum and corn that contained FB_1 (Bhat et al., 1997). Toxicity problems related to FB_1 are probably less likely with sorghum than with corn because many of the *F. moniliforme* strains recovered

* Author to whom correspondence should be addressed [telephone (309) 681-6254; fax (309) 681-6686; e-mail dombrink@mail.ncaur.usda.gov].

† U.S. Department of Agriculture.

‡ Texas A&M University.

10.1021/jf000529f CCC: $19.00
Published on Web 10/12/2000

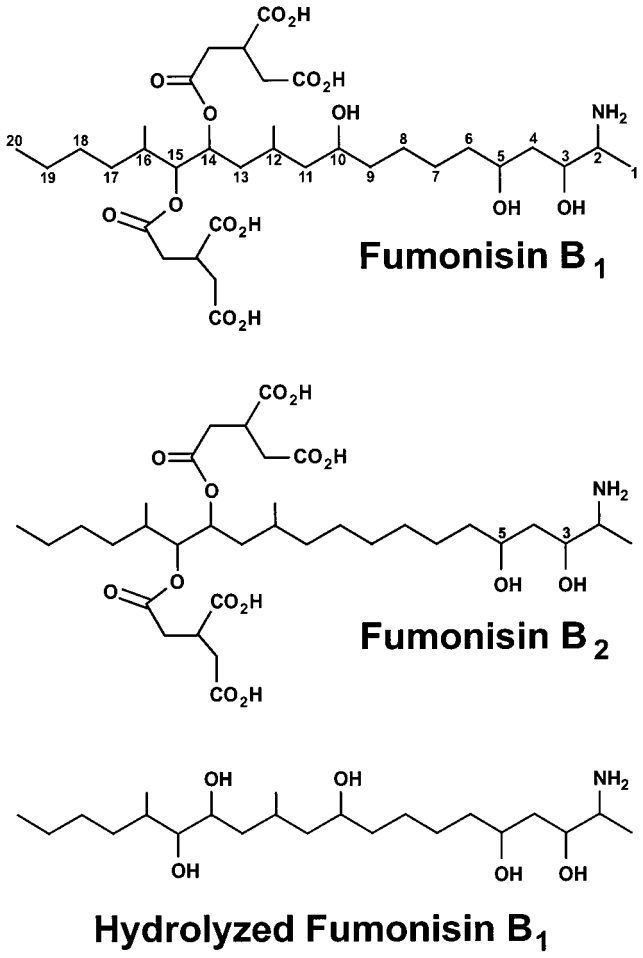

Figure 1. Chemical structures of fumonisin B_1, fumonisin B_2, and hydrolyzed fumonisin B_1.

from sorghum produced no more than a trace of FB_1 (Nelson et al., 1991).

Fumonisins represent a family of structurally related compounds containing a hydrocarbon backbone that resembles the sphingoid bases, sphinganine and sphingosine. Fumonisins are able to inhibit sphingolipid biosynthesis by interfering with the enzyme sphinganine (sphingosine) *N*-acyltransferase (ceramide synthase), resulting in sphinganine accumulation and blocked biosynthesis of ceramides and complex sphingolipids (Wang et al., 1991). A common occurrence noted following in vivo and in vitro exposure to fumonisins is the elevation of sphinganine levels (Merrill et al., 1996). In vitro exposure to FB_1 is also capable of inhibiting folate receptor-mediated transport of 5-methyltetrahydrofolate in Caco-2 cells (Stevens and Tang, 1997) and inducing cell cycle arrest in the G_1 phase and apoptosis in normal-type African green monkey kidney cells (CV-1) (Wang et al., 1996).

The present research was undertaken to determine the fate and distribution of fumonisin in corn products in the various fractions produced during the process of nixtamalization. Normal-appearing corn was processed in pilot plant trials that simulated commercial production, using naturally contaminated corn containing a moderate amount (8.79 ppm) of FB_1. All solid and liquid fractions were analyzed to quantitate both the parent molecules, FB_1, fumonisin B_2 (FB_2), and HFB_1.

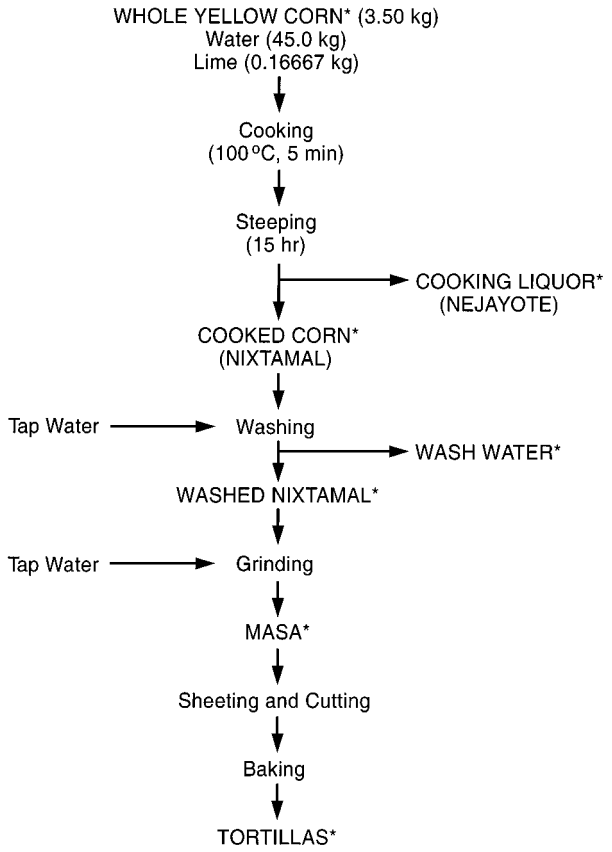

Figure 2. Production of tortillas. Asterisks indicate samples taken for moisture content and fumonisin analysis.

MATERIALS AND METHODS

Analytical Standards. Pure standards of FB_1 and FB_2 were purchased from Sigma Chemical Co. (St. Louis, MO). HFB_1 was prepared by mild hydrolysis of FB_1, provided by R. D. Plattner, USDA, ARS, NCAUR, Peoria, IL, and was >99.8% pure by mass spectrometric analysis.

Corn Sample. Naturally contaminated corn containing 8.79 ppm of FB_1 was used in this study. The kernel characteristics were as follows: density, 1.306 g/cm^3; thousand kernel weight (TKW), 355.4 g; bulk density, 59 lb/bu; and floaters, 80%. The grade 2 yellow dent corn was free of cracked kernels and had no significant mold or insect damage.

Physical and Chemical Analysis. Moisture content of the samples taken throughout the process was measured by drying to constant weight in a forced-air oven at 105 °C for 24 h (AACC, 1986). The calcium content of the fractions (solid and liquid) was measured, in triplicate, using a Perkin-Elmer Plasma 400 ICP emission spectrometer (Norwalk, CT).

Production of Tortillas: Pilot Scale Nixtamalization of Fumonisin-Contaminated Corn Kernels. The procedure was a modification of the methods described by Serna-Saldivar et al. (1990) (Figure 2). The procedure was replicated twice. Contaminated corn (3.5 kg) was placed in a perforated nylon bag and cooked for 5 min at a temperature of 100 °C in a steam kettle (model TDC/2-20, Groen Div., Dover Corp., Elk Grove Village, IL) containing lime solution (166.67 g of CaO/45 kg of water). The steam was turned off, and the corn was steeped overnight (15 h). After the corn had been steeped, it was removed from the kettle.

Excess water was allowed to drain back into the kettle, and the bag was placed in a bucket. Samples were taken from the cooking liquor (nejayote) after the corn was removed. The liquor was stirred before each sample was taken; random samples were taken from five different locations in the kettle. The steeping water was weighed after it was removed from the kettle. Steeped corn (nixtamal) was weighed before washing. Samples were taken for moisture and fumonisin analyses

from both liquid and solid samples (Figure 2). The nixtamal was washed with preweighed water; the washing water (water used to wash the nixtamal) was collected and weighed.

The washed nixtamal was ground into masa using a stone grinder equipped with a 12 in. diameter lava stone (model CG, Casa Herrera Inc., Los Angeles, CA). During grinding, 326 g (rep I) of water were added to the nixtamal. All of the masa was collected from the stone grinder and weighed. The masa was divided into two parts (for rep I only) to make table tortillas and thin tortillas for frying; only table tortillas were made for rep II. The masa was continuously sheeted (model CH4-STM, Superior Food Machinery Inc., Pico Rivera, CA), formed into unbaked tortilla disks, and baked into tortillas in a gas-fired oven with three moving tiers (model C0440, Superior Food Machinery Inc.). The thickness of the tortillas was controlled by adjusting the rollers to produce optimum thickness for table tortillas (30 g per tortilla before baking). To produce tortilla chips, the thickness of the tortillas was reduced (18−20 g per tortilla before frying). The tortillas were fried in partially hydrogenated soy oil at 190 °C for 60 s.

Mass Balance Calculations. A mass balance was calculated on each batch to quantify the movement of moisture and corn dry matter in the corn−water−lime system. Data included the initial weight of each component, corn moisture content, and wastewater solids content at each stage.

Determination of FB_1, HFB_1, and FB_2 in Solid Fractions. The procedure used for the isolation of fumonisins was basically as described by Dombrink-Kurtzman and Dvorak (1999). Samples were dried at room temperature, noting the initial and dried weights, before being ground for analysis. Briefly, 25 g of ground corn, masa, or ground tortillas was extracted with 25 mL of methanol plus 25 mL of EDTA(aq). The concentration of EDTA was adjusted so that it exceeded that of calcium by a factor of 1.36. The aqueous phase was separated by filtration, and 10 mL of the extract (adjusted to pH 2−3 with 1 N HCl) was applied to a Sep-Pak Vac C_{18} SPE cartridge (containing 500 mg of sorbent; Waters Corp., Milford, MA) that had been preconditioned with 5 mL of methanol and 5 mL of water. The cartridges were washed with 3 mL of water, followed by 5 mL of methanol/water (1:3). The fumonisins were eluted with 15 mL of methanol. The flow rate of the columns was <1 mL/min. For comparison, some of the samples were also processed using a Bond Elut C_{18} solid-phase extraction (SPE) cartridge (containing 500 mg of sorbent; Varian, Harbor City, CA).

Determination of FB_1, HFB_1, and FB_2 in Aqueous Fractions. For the washing water and steeping water samples, 25 mL of sample was mixed with 25 mL of methanol; dry EDTA was added so that the final concentration of EDTA exceeded that of Ca by a factor 1.36, as described for the solid samples. The aqueous fractions were then processed in the manner described above for the solid samples. To determine optimal conditions for processing aqueous fractions, four different extraction methods were compared in duplicate using the steeping water or the washing water sample: water alone; water plus EDTA; water with an equal volume of methanol; and water with an equal volume of methanol plus EDTA (as described above for analysis of the solid fractions).

Analysis of Fumonisins by High-Performance Liquid Chromatography (HPLC). Samples were derivatized as described previously (Dombrink-Kurtzman and Dvorak, 1999). Briefly, the eluates were evaporated to dryness (under nitrogen), dissolved in 1 mL of methanol, and derivatized with naphthalene dicarboxaldehyde (NDA) (Bennett and Richard, 1994), with the following modification: 7 mL of acetonitrile/water/acetic acid (60:40:1) was used in place of acetonitrile/phosphate buffer (60:40). A reversed-phase (RP) C_{18} column, SGE (250GL4) column (250 mm × 4 mm) packed with 5 mm of ODS2-I-10/5 (or ODS2-I-15/5) material (SGE, Austin, TX) was used in the HPLC method for analysis of fumonisins. The mobile phase of 65% acetonitrile/acetic acid (99:1) plus 35% water/acetic acid (99:1) was pumped at a flow rate of 0.7 mL/min. An excitation wavelength of 280 nm and an emission wavelength of 470 nm were used for fluorescence detection.

Table 1. Effect of Alkaline Cooking on Moisture and Weight of Nixtamal, Masa, Tortillas, Tortilla Chips, Steeping Water, and Washing Water

	rep I	rep II
total wt of nixtamal (after steeping)	7.36 kg	7.34 kg
moisture content	60.49%	58.81%
total wt of steeping water (after steeping)	37.04 kg	37.19 kg
moisture content	99.16%	99.17%
total wt of nixtamal (before washing)	6.90 kg	6.918 kg
total wt of nixtamal (after washing)	6.18 kg	6.04 kg
moisture content	54.46%	54.24%
total wt of washing water (after washing)	27.56 kg	23.29 kg
moisture content	99.47%	99.47%
total wt of nixtamal (before grinding)	5.69 kg	5.30 kg
wt of water added (during grinding)	0.326 kg	0.319 kg
total wt of masa (after grinding)	3.90 kg	3.94 kg
moisture content	54.44%	55.73%
total wt of masa processed into tortillas	1.805 kg	2.0 kg
total wt of tortilla	0.966 kg	1.40 kg
moisture content	43.31% (table) 34.25% (thin)	43.99%

RESULTS

Production of Tortillas: Pilot Scale Nixtamalization of Fumonisin-Contaminated Corn Kernels. The weight of each component and their respective moisture content were determined for all fractions, both solid and liquid (Table 1). Samples were removed at each step of the processing for moisture (AACC, 1986) and fumonisin determinations (Figure 2). The samples were air-dried prior to determination of fumonisins because treatment at 105 °C for 24 h (AACC, 1986) would have resulted in decreased fumonisin recoveries (Bordson et al., 1995). There was close correlation between the moisture content determined by the AACC (1986) method and the estimate of moisture comparing the wet and dry weights of the solid fractions. To determine the dry matter loss (DML), the amount of solids in the two aqueous fractions was calculated. The DML for reps I and II of 14.9 and 14.1%, respectively, compared favorably with losses of 13% for tortillas made by the traditional method (Khan et al., 1982) and 8.5−12.5% reported in commercial corn masa production (Pflugfelder et al., 1988a). Similar yields (wet basis) of nixtamal, masa, and tortillas were obtained for reps I and II: nixtamal/grain, 1.77 and 1.73; tortilla/grain, 1.42 and 1.41; and tortilla/masa, 0.80 and 0.82, respectively.

Calcium Content. The amount of calcium present was determined on a dry weight basis by triplicate analyses. For raw corn, the calcium concentration was 40 mg/kg. Calcium concentrations for each of the solid samples decreased with successive processing with the calcium content of steeped corn and washed corn higher than that of masa and tortillas. For nixtamal, washed nixtamal, masa, tortillas, tortilla chips, steeping water, and washing water for rep I, calcium concentrations were 3790, 2670, 2090, 2130, 1980, 780, and 280 mg/kg, respectively. The calcium concentrations in the masa (2090 mg/kg), tortillas (2130 mg/kg), and tortilla chips (1980 mg/kg) were comparable to the content in masa harina de maiz (1980 mg/kg), as reported previously (Dombrink-Kurtzman and Dvorak, 1999). For rep II, calcium concentrations for nixtamal, washed nixtamal, masa, tortillas, steeping water, and washing water were 10,960, 6950, 5810, 6060, 970, and 350 mg/kg, respectively. The amount of calcium present in the solid fractions from rep II was 2−3-fold higher than the amount present from rep I, but the calcium concentrations of the specific aqueous fractions from reps I and II were fairly similar, with the values for rep II 25% higher than those of rep I.

Table 2. Fumonisin Content in Solid Fractions Produced by Nixtamalization of Fumonisin-Contaminated Corn

	FB_1 (ng/g)		HFB_1 (ng/g)		FB_2 (ng/g)	
sample	dry	wet	dry	wet	dry	wet
rep I						
steeped nixtamal	280	111	579	229	54	21
washed nixtamal	377	172	433	197	165	75
masa	706	322	667	304	110	50
tortillas	717	406	497	282	177	100
tortilla chips	498	327	415	273	189	124
rep II						
steeped nixtamal	2106	867	1399	576	1347	555
washed nixtamal	2349	1075	1266	579	1548	708
masa	1129	500	826	366	436	193
tortillas	1075	602	795	445	562	315

Determination of FB_1, HFB_1, and FB_2 in Solid Fractions. Following nixtamalization of fumonisin-contaminated corn (in duplicate) for production of masa and tortillas, the solid fractions were analyzed for FB_1, HFB_1, and FB_2 (Table 2). Tortilla chips were prepared only in rep I. The values for rep II represent the means of two to four analyses.

Recoveries of FB_1, HFB_1, and FB_2 from the solid fractions (duplicate analyses) were 93.4, 76.0, and 93.5% for tortillas and 81.2, 74.9, and 91.8% for tortilla chips, respectively, at spiking levels of 500 ng/mL FB_1, 500 ng/mL HFB_1, and 250 ng/mL FB_2.

The pH of the extracts of the different fractions was highest for steeped corn (pH 7 and 10 for reps I and II, respectively). For all of the other processing fractions, the pH of the extracts was between pH 5 and 7 for rep I, although it was pH 7 for rep II. The pH of the corn extract was 5.

Determination of FB_1, HFB_1, and FB_2 in Aqueous Fractions. Of the four different extraction methods compared in duplicate to extract FB_1, HFB_1, and FB_2 from the two aqueous fractions (steeping water and washing water), the highest levels of FB_1, HFB_1, and FB_2 were detected using the method that incorporated dry EDTA in the liquid fraction with an equal volume of methanol (data not shown). This extraction solution was analogous to the manner in which the solid fractions were extracted. The aqueous fractions produced during the process of nixtamalization were analyzed for FB_1 and HFB_1 (Table 3) and FB_2. Although no FB_1 was detected in aqueous fractions from rep II, more HFB_1 was present in aqueous fractions from rep II, compared to rep I. No FB_2 was detected in either the steeping or washing water from reps I and II.

Recoveries of FB_1, HFB_1, and FB_2 from the aqueous fractions were 91.6, 84.8, and 96.1% for the steeping water and 90.7, 79.8, and 91.3% for the washing water, respectively, based on triplicate analyses at spiking levels of 500 ng/mL FB_1, 500 ng/mL HFB_1, and 250 ng/mL FB_2.

The pH of the extracts of the steeping water was 9−10 for rep I and 11−12 for rep II. For the washing water, the pH of the extracts was between 6 and 7 for both reps I and II.

The major amount of FB_1 that had been present in the raw corn was recovered in the steeping water as HFB_1 (Table 3). The total amount of FB_1 plus HFB_1 present in the steeping water was 9−18-fold greater than the amount present in the washing water. Thus, the combined aqueous fractions (steeping water and washing water) contained as FB_1 plus HFB_1 an average of 76.1% of the initial amount of FB_1 present in the raw corn.

Table 3. Fumonisin Content in Aqueous Fractions Produced by Nixtamalization of Fumonisin-Contaminated Corn

sample	FB_1 (ng/g)	% of original	HFB_1 (ng/g)	FB_1 equiv (ng/g)	% of original	total % of original
			Steeping Water			
rep I						
1	285	3.2	3125	5563	63.3	66.5
2	220	2.5	2938	5230	59.5	62.0
3	297	3.4	3061	5449	62.0	65.4
mean	267	3.0	3041	5414	61.6	64.6
			Washing Water			
rep I						
1	370	4.2	38	68	0.8	5.0
2	343	3.9	185	329	3.7	7.6
3	366	4.2	235	418	4.8	9.0
mean	360	4.1	153	272	3.1	7.2
			Steeping Water			
rep II						
1	ND[a]	ND	3092	5504	62.6	62.6
2	ND	ND	4453	7926	90.2	90.2
3	ND	ND	3715	6612	75.2	75.2
mean	ND	ND	3753	6681	76.0	76.0
			Washing Water			
rep II						
1	ND	ND	224	399	4.5	4.5
2	ND	ND	211	376	4.3	4.3
3	ND	ND	197	351	4.0	4.0
mean	ND	ND	211	375	4.3	4.3

[a] ND, not detected (<10 ng/g).

DISCUSSION

Nixtamalization significantly reduced the amount of fumonisin in corn. Nixtamalization had the effect of dramatically lowering the amount of FB_1 (8.79 ppm) present in the raw corn to an average of 0.50 ppm of FB_1, plus 0.36 ppm HFB_1, detected in prepared tortillas (wet basis) (Table 2), representing 18.5% of the initial FB_1 concentration. The initial concentration of FB_2 (1.97 ppm) was reduced to an average of 0.21 ppm of FB_2 in tortillas. The amount of fumonisin (FB_1 plus HFB_1) remaining in the tortillas represented between 6.6 and 9.6% (mean = 8.1%) as FB_1 and between 8.1 and 12.7% (mean = 10.4%) as HFB_1. To calculate the percentage of the initial FB_1 represented by HFB_1, a correction factor of 1.78 was used to account for the difference in molecular weights for FB_1 (721) and HFB_1 (405). A similar reduction of aflatoxin content (<17% remained in the masa) has been observed when corn spiked with radioactive aflatoxin underwent the process of nixtamalization (Guzman de Pena et al., 1995).

Higher levels of fumonisins were detected at intermediate processing steps because treatment may be aiding in the extraction of fumonisins. Additionally, baking associated with tortilla production may result in nonenzymatic browning (Maillard reaction) with monosaccharides, such as glucose, becoming conjugated to the primary amino group of FB_1, making it undetectable by the HPLC method used here. Nonenzymatic browning reactions may have been responsible for the reduction in recoverable FB_1 that was observed following extrusion cooking of corn grits (Castelo et al., 1998).

An average of 72.5% of FB_1 was converted to HFB_1 and remained in the steeping and washing water, which is discarded. An additional 3.5% of FB_1 remained unchanged in the aqueous fractions. Thus, the waste liquid fractions contained 76% of the initial FB_1.

Studies to determine the effects of temperature and pH on the stability of FB_1 and FB_2 in an aqueous system

have indicated that no significant losses occur at neutral pH during processing at 100−125 °C; however, at pH 10, HFB_1 and HFB_2 were the major decomposition species detected (Jackson et al., 1996a,b). Drying samples at 30 and 50 °C did not affect the recoveries of FB_1 and FB_2, but drying at 110 °C for 24 h reduced recoveries by >80% (Bordson et al., 1995). For this reason, solid fractions were air-dried prior to fumonisin analysis.

Research to understand the role of lime, $Ca(OH)_2$, which is incorporated during alkaline cooking, has indicated that, in addition to aiding in pericarp removal, lime incorporation is responsible for the cross-linking of starch molecules via formation of a calcium bridge with negatively charged amylose molecules (Rodriguez et al., 1996). The high pH of the alkaline processing that promotes ionization of starch hydroxyl groups (Bryant and Hamaker, 1997) is also responsible for hydrolysis of the parent FB_1 to HFB_1. The calcium content of masa is influenced by various factors: concentration of lime used for the processing; cooking/steeping temperatures; and specific characteristics of the corn (Pflugfelder et al., 1988b).

Different factors may have contributed to the greater amount of calcium detected in fractions from rep II. Significant time had elapsed between the processing of reps I and II, which occurred in early spring and mid summer, respectively. Slightly different processing conditions (cooking temperatures) may have been present, related to the time of the year when the processing was performed. There may have been variation between the batches of lime used; the lime used for rep I may have aged over time, affecting its activity in terms of removing pericarp components effectively, and a new batch of lime may have been used for rep II.

Although nixtamalization involves cooking at an alkaline pH, the only extracts that had a pH between 10 and 12 were those of the steeped corn (before it was rinsed) and the steeping water. After washing, extracts of all subsequent fractions had pH values between 5 and 7. Interestingly, the calcium content and pH of rep II extracts were higher than those of rep I extracts, and the amount of fumonisin detected was also higher.

The ease of pericarp removal during alkaline cooking is closely linked to the genotype of the corn, with some contribution due to environmental factors. Thorough washing and rubbing of nixtamal after steeping will remove greater amounts of pericarp and germ (Bedolla et al., 1983). Major components of dry matter in wastewater include pericarp, starch, protein, and germ solubles, with loss/removal of pericarp representing the major factor contributing to DML in alkaline cooking.

In general, the snack foods industry prefers the removal of pericarp during cooking (Rooney and Suhendro, 1999). Loss of pericarp during alkaline cooking is expected and, considering it is the fraction containing the higher amounts of fumonisins (Sydenham et al., 1995), it is desirable. Sydenham et al. (1995) reported that following alkaline hydrolysis (at room temperature) of fumonisin-contaminated corn, only 5.1% of the original FB_1 concentration was detected in kernels devoid of pericarp, with the majority present as HFB_1. Sydenham et al. (1995) also noted variability between replicates of treated ground corn; two solid corn fractions had no detectable FB_1, but one had 755 ng/g FB_1.

Recently, Howard et al. (1998) described the formation of *N*-(carboxymethyl)fumonisin B_1 when FB_1 was incubated with D-glucose overnight at 78 °C under alkaline conditions. Because conditions for forming *N*-(carboxymethyl)fumonisin B_1 are similar to parameters involved in nixtamalization, studies are currently underway in our laboratory to determine if this compound is generated during nixtamalization.

LITERATURE CITED

AACC. *Approved Methods of the American Association of Cereal Chemists*; St. Paul, MN, 1986.

Bacon, C. W.; Hinton, D. M. Fusaric acid and pathogenic interactions of corn and non-corn isolates of *Fusarium moniliforme*, a nonobligate pathogen of corn. In *Fumonisins in Food*; Jackson, L., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Press: New York, 1996; pp 175−191.

Bedolla, S.; de Palacios, M. G.; Rooney, L. W.; Diehl, K. C.; Khan, M. N. Cooking characteristics of sorghum and corn for tortilla preparation by several cooking methods. *Cereal Chem*. **1983**, *60*, 263−268.

Bennett, G. A.; Richard, J. L. Liquid chromatographic method for analysis of the naphthalene dicarboxaldehyde derivative of fumonisins. *J. AOAC Int.* **1994**, *77*, 501−506.

Bhat, R. V.; Shetty, P. H.; Amruth, R. P.; Sudershan, R. V. A foodborne disease outbreak due to the consumption of moldy sorghum and maize containing fumonisin mycotoxins. *Clin. Toxicol.* **1997**, *35*, 249−255.

Bordson, G. O.; Meerdink, G. L.; Bauer, K. J.; Tumbleson, M. E. Effects of drying temperature on fumonisin recovery from feeds. *J. AOAC Int.* **1995**, *78*, 1183−1188.

Bressani, R.; Paz y Paz, R.; Scrimshaw, N. S. Chemical changes in corn during preparation of tortillas. *J. Agric. Food Chem*. **1958**, *6*, 770−774.

Bryant, C. M.; Hamaker, B. R. Effect of lime on gelatinization of corn flour and starch. *Cereal Chem*., **1997**, *74*, 171−175.

Castelo, M. M.; Katta, S. K.; Sumner, S. S.; Hanna, M. A.; Bullerman, L. B. Extrusion cooking reduces recoverability of fumonisin B_1 from extruded corn grits. *J. Food Sci*. **1998**, *63*, 696−698.

Chu, F. S.; Li, G. Y. Simultaneous occurrence of fumonisin B_1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. *Appl. Environ. Microbiol.* **1994**, *60*, 847−852.

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

Guzman de Pena, D.; Trudel, L.; Wogan, G. N. Corn "nixtamalizacion" and the fate of radiolabelled aflatoxin B_1 in the tortilla making process. *Bull. Environ. Contam. Toxicol.* **1995**, *55*, 858−864.

Harrison, L. R.; Colvin, B. M.; Greene, J. T.; Newman, L. E.; Cole, R. J. Pulmonary edema and hydrothorax in swine produced by fumonisin B_1, a toxic metabolite of *Fusarium moniliforme*. *J. Vet. Diagn. Invest.* **1990**, *2*, 217−221.

Hopmans, E. C.; Murphy, P. A. Detection of fumonisin B_1, B_2, and B_3 and hydrolyzed fumonisin B_1 in corn-containing foods. *J. Agric. Food Chem*. **1993**, *41*, 1655−1658.

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.

Jackson, L. S.; Hlywka, J. J.; Senthil, K. R.; Bullerman, L. B.; Musser, S. M. Effects of time, temperature, and pH on the stability of fumonisin B_1 in an aqueous model system. *J. Agric. Food Chem*. **1996a**, *44*, 906−912.

Jackson, L. S.; Hlywka, J. J.; Senthil, K. R.; Bullerman, L. B. Effects of thermal processing on the stability of fumonisin B_2 in an aqueous system. *J. Agric. Food Chem*. **1996b**, *44*, 1984−1987.

Khan, M. N.; Des Rosiers, M. C.; Rooney, L. W.; Morgan, R. G.; Sweat, V. E. Corn tortillas: Evaluation of corn cooking procedures. *Cereal Chem*. **1982**, *59*, 279−284.

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.

Marasas, W. F. O.; Kellerman, T. S.; Gelderblom, W. C. A.; Coetzer, J. A. W.; Thiel, P. G.; van der Lugt, J. J. Leukoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. *Onderstepoort J. Vet. Res.* **1988**, *55*, 197−203.

Merrill, A. H., Jr.; Liotta, D. C.; Riley, R. T. Fumonisins: fungal toxins that shed light on sphingolipid function. *Trends Cell Biol.* **1996**, *6*, 218−223.

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*; Jackson, L., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Press: New York, 1996; pp 323−334.

Nelson, P. E.; Plattner, R. D.; Shackelford, D. D.; Desjardins, A. E. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. *Appl. Environ. Microbiol.* **1991**, *57*, 2410−2412.

Nelson, P. E.; Desjardins, A. E.; Plattner, R. D. Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry and significance. *Annu. Rev. Phytopathol.* **1993**, *31*, 233−252.

Pflugfelder, R. L.; Rooney, L. W.; Waniska, R. D. Dry matter losses in commercial corn masa production. *Cereal Chem.* **1988a**, *65*, 127−132.

Pflugfelder, R. L.; Rooney, L. W.; Waniska, R. D. Fractionation and composition of commercial corn meal. *Cereal Chem.* **1988b**, *65*, 262−266.

Rheeder, J. P.; Marasas, W. F. O.; Thiel, P. G.; Sydenham, E. W.; Shephard, G. S.; van Schalkwyk, D. J. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology* **1992**, *82*, 353−357.

Rodriguez, M. E.; Yanez-Limon, M.; Alvarado-Gil, J. J.; Vargas, H.; Sanchez-Sinencio, F.; Figueroa, J. D. C.; Martinez-Bustos, F.; Martinez-Montes, J. L.; Gonzalez-Hernandez, J.; Silva, M. D.; Miranda L. C. M. Influence of the structural changes during alkaline cooking on the thermal, rheological, and dielectric properties of corn tortillas. *Cereal Chem.* **1996**, *73*, 593−600.

Rooney, L. W.; Serna-Saldivar, S. O. Food uses of whole corn and dry-milled fractions. In *Corn: Chemistry and Technology*; Watson, S. A., Ramstad, P. E., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1987; pp 399−429.

Rooney, L. W.; Suhendro, E. L. Perspectives on nixtamalization (alkaline cooking) of maize for tortillas and snacks. *Cereal Foods World* **1999**, *44*, 466−470.

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

Serna-Saldivar, S. O.; Gomez, M. H.; Rooney, L. W. Technology, chemistry and nutritional value of alkaline-cooked corn products. In *Advances in Cereal Science and Technology*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1990; Vol. 10, pp 243−307.

Stack, M. E. Analysis of fumonisin B_1 and its hydrolysis product in tortillas. *J. AOAC Int.* **1998**, *81*, 737−740.

Stevens, V. L.; Tang, J. Fumonisin B_1-induced sphingolipid depletion inhibits vitamin uptake via the glycosylphosphatidyl-inositol-anchored folate receptor. *J. Biol. Chem.* **1997**, *272*, 18020−18025.

Sydenham, E. W.; Stockenstrom, 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.

Thiel, P. G.; Shephard, G. S.; Sydenham, E. W.; Marasas, W. F. O.; Nelson, P. E.; Wilson, T. M. Levels of fumonisins B_1 and B_2 in feeds associated with confirmed cases of equine leukoencephalomalacia. *J. Agric. Food Chem.* **1991**, *39*, 109−111.

Trejo-Gonzalez, A.; Feria-Morales, A.; Wild-Altamirano, C. The role of lime in the alkaline treatment of corn for tortilla preparation. In *Modifications of Proteins*; Feeney, R. E., Whitaker, J. R., Eds.; Advances in Chemistry Series 198; American Chemical Society: Washington, DC, 1982; pp 245−263.

Voss, K. A.; Chamberlain, W. J.; Bacon, C. W.; Norred, W. P. A preliminary investigation on renal and hepatic toxicity in rats fed purified fumonisins B_1. *Nat. Toxins* **1993**, *1*, 222−228.

Wang, E.; Norred, W. P.; Bacon, C. W.; Riley, R. T.; Merrill, A. H., Jr. Inhibition of sphingolipid biosynthesis by fumonisins. *J. Biol. Chem.* **1991**, *266*, 14486−14490.

Wang, W.; Jones, C.; Ciacci-Zanella, J.; Holt, T.; Gilchrist, D. G.; Dickman, M. B. Fumonisins and *Alternaria alternata lycopersici* toxins: Sphinganine analog mycotoxins induce apoptosis in monkey kidney cells. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3461−3465.

Watson, S. A. Corn marketing, processing, and utilization. In *Corn and Corn Improvement*, 3rd ed.; Sprague, G. F., Dudley, J. W., Eds.; Agronomy Monograph 18; ASA-CSSA-SSSA: Madison, WI, 1988; pp 881−940.

Received for review April 27, 2000. Revised manuscript received August 16, 2000. Accepted August 16, 2000. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the products, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

JF000529F