Fate of Fumonisins during the Production of Fried Tortilla Chips

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The fate of fumonisin B_1 (FB_1), a mycotoxin found in corn, during the commercial manufacture of fried tortilla chips was studied. FB_1 and hydrolyzed FB_1 (HFB_1) concentrations in four lots of corn and in the masa, other intermediates, liquid and waste byproducts, and fried chips were determined by HPLC. FB_1 concentrations in the masa and chips were reduced significantly, up to 80% in the fried chips, compared to that in the raw corn. HFB_1 was also found in the masa and chips, but at low concentrations compared to FB_1. LC-MS analyses corroborated HPLC findings and further showed the presence of partially hydrolyzed FB_1 ($PHFB_1$), which, like HFB_1, was formed during the nixtamalization (cooking/steeping the corn in alkaline water to make masa) step and found predominantly in the cooking/steeping liquid and solid waste. No significant amounts of *N*-(carboxymethyl)-FB_1 or *N*-(1-deoxy-D-fructos-1-yl)-FB_1, indicative of fumonisin−sugar adduct formation, were found. Thus, FB_1 is removed from corn and diverted into liquid and waste byproducts during the commercial production of fried tortilla chips. Nixtamalization and rinsing are the critical steps, whereas grinding, sheeting, baking, and frying the masa had little effect.

Keywords: *Fumonisins; food processing; nixtamalization; corn; masa; tortilla chips*

INTRODUCTION

Fumonisins are mycotoxins produced by *Fusarium moniliforme* (=*F. verticillioides*), *F. proliferatum*, and some other *Fusarium* species (*1*−*3*). They are found worldwide in corn, sometimes at high levels (*4*). For example, up to 117 ppm of fumonisin B_1 (FB_1) was found in moldy, home-grown corn in southern Africa, while "healthy", home-grown corn contained as much as 7.9 ppm (*5*). Concentrations >1 ppm in corn meal or grits have been reported, but levels in corn-based food products, including commercially produced masa and tortillas, from industrialized countries are generally low, below 1 ppm (*5*−*15*).

The most common homologue, FB_1, causes leukoencephalomalacia in horses (*16*, *17*), pulmonary edema in swine (*18*), and hepato- and nephrotoxicity in multiple species (*1*, *19*−*21*) and is a liver and kidney carcinogen in rodents (*22*, *23*). Similar in vivo effects were elicited by exposure to fumonisins B_2 (FB_2) and B_3 or hydrolyzed FB_1 (HFB_1) (*24*−*28*). The human health risks posed by fumonisins are less well-defined but are of concern. Although there is no direct unequivocal evidence that fumonisins cause human disease, high esophageal cancer rates in parts of southern Africa and China have been correlated with the consumption of home-grown corn contaminated with *F. moniliforme* or fumonisin (*5*, *14*, *29*−*31*). Hypercholesterolemia and evidence of hepatic injury were found in nonhuman primates fed fumonisin-containing diets (*32*), leading the authors to suggest a role for fumonisins in atherosclerotic vascular disease. Others have speculated that fumonisins may contribute to liver cancer (*33*) or neural tube defects (*34*).

Given the growing food safety concerns regarding fumonisins (*2*−*4*), it is important to understand what happens to these compounds when corn is processed. Wet milling (*35*) and dry milling (*8*, *36*) reduce fumonisin levels in food products and divert fumonisins to those fractions used in animal feeds. Extrusion also reduces fumonisin concentrations in food products (*6*, *37*). The effects of cooking are unclear. It has been reported that canning, baking, and frying have little effect on fumonisin levels (*8*, *38*, *39*). In contrast, others found that frying polenta or autoclaving cornmeal significantly reduced (70−80%) fumonisin concentrations (*40*). The purpose of this study was to determine the relative amounts of fumonisins in corn, masa, and fried tortilla chips produced under standard production line conditions. This model was selected because the fate of fumonisins under large-scale, commercially relevant conditions could be studied and the effects of several processes could be directly compared. During fried chip production, corn is sequentially subjected to cooking/steeping in water under alkaline conditions (nixtamalization), rinsing, grinding, sheeting, baking, and frying.

MATERIALS AND METHODS

The FB_1 standard for HPLC, >98% purity, was purchased (Sigma, St. Louis, MO). FB_2 (*41*) and HFB_1 (*42*) HPLC standards were prepared using published methods and their purities (≥95%) independently determined by mass spectral

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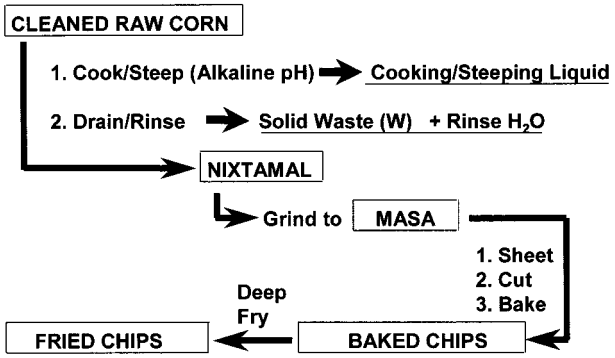

Figure 1. Flow diagram of a production line for fried tortilla chips. After a short cooking period in alkaline water, the corn is steeped in the liquid for 12 h. After steeping, the corn, now lacking the pericarp, is called nixtamal. The nixtamal is removed from the tanks, rinsed to remove the waste, drained, and ground into masa. The masa is then sheeted, cut, and baked. The baked chips are deep-fried to make the final product.

analyses (R. D. Plattner, USDA Agricultural Research Service, Peoria, IL). The HFB_1 and partially hydrolyzed FB_1 ($PHFB_1$) standards used for LC-MS were prepared as previously described (*43*). Glass-distilled, deionized water, HPLC grade solvents (Burdick & Jackson), and analytical grade (or better) reagents were used for all procedures. Samples were analyzed in triplicate unless indicated otherwise.

Corn. Four lots of raw corn were used in the experiment. Three consisted of sound corn containing low fumonisin levels (<4 ppm of FB_1) and were used for cooks 2−4 (the term "cook" designates one replicate of the process using a single lot of corn, beginning with the cooking/steeping and ending with the frying steps) (Figure 1). A fourth lot was used for cook 5. It was highly contaminated (FB_1 = 34.6 ppm by LC-MS) and consisted predominantly of damaged or broken kernels that would normally have been discarded.

Production Line and Sampling. Fried tortilla chips were prepared at the pilot production line, Frito-Lay Technology Processing Center, Duncanville, TX. Equipment, recipe, and operating conditions followed Frito-Lay's proprietary procedures for the manufacture of this product (Figure 1). The production line was stabilized beforehand using a fifth lot of corn (cook 1). To avoid cross-contamination, the highly contaminated lot (cook 5) was processed last.

Samples of the cooking/steeping liquid were collected from the tanks at 2, 6, and 12 h after the start of cooking. Masa, baked chip, and fried chip samples were taken from the production line at least 30 min after (to allow the production line to stabilize) cooking/steeping was completed. The baked and fried chips were ground using a food processor. Solid waste samples were also collected. All samples, including raw corn samples, were stored frozen.

Sample Preparation. Raw corn and fried chips (all cooks), masa, baked chips, and fried chips (cooks 2 and 5), and solid waste (cooks 4 and 5) were freeze-dried and then ground to a fine powder using a mortar and pestle. The powdered samples (5 g for raw corn; otherwise, 10 g) were extracted overnight at room temperature with gentle agitation (model G-2 laboratory rotator, New Brunswick Scientific, New Brunswick, NJ) using acetonitrile/water (1:1, pH adjusted to pH 4.5 using 6 N HCl). The final volume of pH-adjusted solvent was 5 mL/g of powdered sample. The extract was then filtered through Whatman no. 4 paper prior to cleanup.

Two cleanup methods were used. For FB_1, filtered extracts were diluted to an appropriate volume (to prevent overloading of the immunoaffinity column, dilution volumes ranged from 1:4 v/v for most samples to 1:30 for cook 5 corn) with phosphate-buffered saline (pH 7.0) and then filtered (0.45 μm nylon syringe filters, Alltech Associates, Deerfield, IL). Ten milliliters of the filtered, diluted extract (providing 67−500 mg equivalents of corn, depending on the sample dilution) were loaded on a Fumonitest immunoaffinity column (Vicam, Watertown, MA) and washed with 10 mL of phosphate-buffered saline, and the FB_1 and FB_2 were then eluted from the column with 1.5 mL of methanol. The methanol was evaporated under a gentle stream of nitrogen at 50 °C, and the dried samples stored frozen. Samples of cooking/steeping liquid were not extracted but were filtered (Whatman no. 4 paper) and then processed as described.

A different cleanup procedure was needed for measuring HFB_1. Extracts were filtered, diluted with water (1:4), and applied to a preconditioned (2 mL of water, 2 mL of acetonitrile, 2 mL of water) C18 Sep-Pak Cartridge (part WAT051910, Waters Associates, Watertown, MA). The C18 column was eluted with (in sequence) 2 mL each of water, 15% acetonitrile/water, and 70% acetonitrile/water. The 15 and 70% acetonitrile/water eluates were dried at 50 °C under a gentle stream of nitrogen and stored frozen.

HPLC Determination of Fumonisins. All samples were redissolved in 200 μL of water/acetonitrile (1:1). Aliquots of each redissolved sample (injection volume = 10 μL) were analyzed using a model 1090 HPLC (Hewlett-Packard, Atlanta, GA) equipped with a C18 reversed phase column, particle size = 5 μm (Rainen Instrument Co., Woburn, MA). Sample derivatization [with *o*-phthalaldehyde (OPA), Pierce, Rockford, IL] chromatographic conditions, and fluorescence detection parameters have been previously described (*41*). For those samples prepared using C18 Sep-Pak cleanup, both the 15 and 70% acetonitrile/water eluates were analyzed and the results added together.

HPLC Recovery Studies. Corn, masa, baked chip, and fried chip samples (cook 4) were spiked (to a nominal level of 0.89 ppm) with 1 mL of an aqueous FB_1 standard solution. The spiked samples were air-dried and gently mixed with a spatula. Thereafter, the samples were handled (immunoaffinity column cleanup) as described above, and recovery was calculated. Recovery of HFB_1 from masa (cook 5) was similarly (C18 Sep-Pak cleanup) determined.

LC-MS Analysis of Fumonisins. Samples for LC-MS were extracted as described above with the exceptions that the raw corn and masa were not freeze-dried, the pH of the solvent was not adjusted, and the extracts were evaluated without further cleanup. A SpectraSYSTEM P4000 pump was coupled to an LCQ mass spectrometer via an electrospray interface (ESI) (Finnigan-MAT, San Jose, CA). An Inertsil ODS-3 15 cm × 3.0 mm i.d. column (0396-150 × 030, MetaChem Technologies, Torrance, CA) was used. For the first 5 min, the HPLC eluate was diverted to waste, and then the entire HPLC eluate was introduced into the detector. A 10 min linear gradient from 50:35:15 to 5:35:60 water/1% acetic acid in methanol/methanol was used and the final composition held for 20 min. The flow rate was 0.3 mL/min. Mass spectra were obtained by scanning from *m/z* 300 to 950. The ESI spray voltage was 5.0 kV, and the capillary temperature was 220 °C. The auxiliary gas flow was 65 (arb). Quantitation was performed in the SIM mode with maximum ion time set at 50 ms and 3 microscans. The following time program was used: 0−10.5 min, *m/z* 406.3 (HFB_1) and 564.3 ($PHFB_1$);10.5−11.5 min, *m/z* 564.3 and 722.3 (FB_1); 11.5−15 min, 722.3 and 706.3 (FB_2 and FB_3); and finally, 15−30 min, 722.3 and 780.3 [*N*-(carboxymethyl)-FB_1]. Stock solutions containing 1 mg/mL HFB_1, $PHFB_1$, or FB_1 in 50:50 acetonitrile/water were prepared and used to make the combined standard that contained 100 ng/μL of each component. The combined standard was diluted with 10:90 acetonitrile/water to give the two working standards that contained 100 or 10 pg/μL of each component. Various amounts of the two dilute standard solutions were injected to give 20, 40, 100, 200, 400, 1000, 2000, or 3000 pg of HFB_1, $PHFB_1$, or FB_1. Using the areas of the ion chromatograms, the response was linear between 40 and 3000 pg for each component. The standards and samples were diluted with 10:90 acetonitrile/water to ensure that HFB_1, which elutes first, gave reproducible retention times and peak areas. The coefficients of determination (r^2) of all three components were 0.99. The total area of the peaks for the two forms of $PHFB_1$ was used for quantification. The samples, extracts of freeze-

Table 1. FB_1 in Raw Corn, Masa, and Baked and Fried Tortilla Chips from Three Lots of Cleaned Corn Having Commercially Relevant FB_1 Concentrations (Cooks 2−4) and a High FB_1 Lot Serving as a Model (Cook 5), As Measured by HPLC Analyses of Extracts Cleaned up Using Immunoaffinity Columns[a]

	cook 2		cook 3		cook 4		cook 5	
fraction	ppm	% raw corn	ppm	% raw corn	ppm	% raw corn	ppm	% raw corn
raw corn	1.48 (±0.54)	100	0.22 (±0.06)	100	1.92 (±0.04)	100	46.5 (±6.00)	100
masa	0.09 (±0.02)	6.1	NA[b]	NA	NA	NA	5.48 (±0.82)	11.8
baked chips	0.14 (±0.09)	9.5	NA	NA	NA	NA	11.3 (±0.76)	24.3
fried chips	0.33 (±0.10)	22.3	0.14 (±0.03)	63.6	0.97 (±0.06)	50.5	10.6 (±3.91)	22.8

[a] Values indicate the mean (± standard deviation), $n = 3$. [b] NA, not analyzed

dried material, or the samples of the cooking/steeping liquid were filtered through 0.5 μm filters and analyzed without further cleanup. Qualitative analysis with full scan spectra was obtained by injecting the filtered samples. For quantitative analysis, 100 μL of the filtered sample was diluted with 900 μL of 10:90 acetonitrile/water and then 5 or 10 μL injected.

RESULTS AND DISCUSSION

Fumonisin concentrations of the raw corn had no noticeable effect on the results when cooks 2 and 5 were compared. FB_1 concentrations in masa and baked chips were significantly reduced and FB_1 concentrations in the fried tortilla chip product were reduced almost 80% compared to their respective raw corn samples (Table 1). In each case, reduction was achieved during the conversion of the corn to masa, which involves cooking/steeping, rinsing, and grinding the nixtamal (the corn after cooking/steeping in an alkaline solution). Sheeting, cutting, baking, and frying had little effect on fumonisins in the masa, a finding that is in agreement with the reported heat stability of these compounds (*8*, *44*). That the concentration of FB_1 in the fried chips was slightly higher than that of masa is attributable to the loss of water that occurred when masa was baked and fried. The relative amounts of FB_2 measured in the raw corn, masa, and chips followed the same pattern. Measured FB_2 concentrations (not corrected for recovery) of masa and fried chips averaged 6−8% of those found in cook 5 raw corn and 6−11% of those found in cook 2 raw corn, whereas the corresponding FB_1 concentrations (before correction for recovery) in masa and fried chips relative to FB_1 in raw corn were 10−11% for cook 5 and 6−10% for cook 2. Because the patterns of reduction appeared to be similar for FB_1 and FB_2, we focused on FB_1 (results corrected for recovery) and its derivatives during the subsequent experiments.

Fumonisins are converted to their hydrolyzed forms during nixtamalization (*8*, *45*). Cook 5 samples were therefore analyzed a second time to quantify HFB_1. HFB_1 measurements were not done for cooks 2−4 because its concentration in the intermediates and fried chips was too low to be confidently quantified by HPLC. Because HFB_1, unlike FB_1, does not bind to the immunoaffinity columns and our attempts to quantify the HFB_1 in the immunoaffinity column eluates were unsuccessful, C18 Sep-Pak columns were used for sample cleanup during the HFB_1 analyses. After C18 cleanup, FB_1 showed the same distribution pattern in the raw corn, intermediates, and fried chips as found after immunoaffinity column cleanup (data not shown).

HFB_1 was not detected in cook 5 raw corn. HFB_1 concentration in the cook 5 masa was 0.64 ppm, a relatively low amount, ∼12%, compared to the FB_1 concentration in this intermediate. HFB_1 was also detected in baked and fried chips but at levels that were even lower than those in masa and not readily quantifiable by HPLC. These findings agreed with others (*7*, *8*, *10*, *44*−*46*) who have reported that HFB_1 concentrations in tortilla chips or tortillas were considerably lower than FB_1 levels. For example, Dombrink-Kurtzman and Dvorak (*7*) found that HFB_1 concentrations in Mexican tortillas averaged ≤5% of the FB_1 concentrations therein. In contrast, FB_1 was completely converted to HFB_1 when corn culture materials (surface-sterilized corn fermented with *F. moniliforme* under laboratory conditions) were nixtamalized (*26*, *47*). The culture materials were ground beforehand, whereas (as per commercial practice) whole kernel corn was nixtamalized in the present study. Thus, it appears that the pericarp interferes with penetration of the alkali into the intact corn kernel and, as previously suggested, that removal of the pericarp is important for reducing fumonisin concentration in food products (*46*−*49*). It is of interest that there is good agreement between our HPLC results and those of Dombrink-Kurtzmann et al. (*46*), who measured fumonisins in masa and tortillas (different from fried tortilla chips) that they prepared in the laboratory from contaminated (8.79 ppm of FB_1) corn. They found 0.50 ppm of FB_1 and 0.36 ppm of HFB_1 in the tortillas, values representing ∼18.5% of the FB_1 originally present in the corn. FB_1 (0.75 ppm) and HFB_1 (0.39 ppm) concentrations in the masa were likewise reduced.

The fate of fumonisins during food processing has important toxicological implications. Steeping and rinsing ground *F. moniliforme* culture material with water significantly reduced both its fumonisin concentration, by almost 90%, and its toxicity to rats (*47*). On the other hand, water washing was ineffective for lowering fumonisin concentrations of whole corn (*8*). In this study, 2.4 and 1.6 μg/mL of FB_1 and HFB_1, respectively, were found (HPLC) in the 2 h cooking/steeping liquid from cook 5. After 6 h, FB_1 and HFB_1 concentrations were 1.1 and 1.3 μg/mL, respectively, whereas 1.5 μg/mL FB_1 and 2.2 μg/mL HFB_1 were found in the cooking/steeping liquid after 12 h. The ratio of FB_1/HFB_1 (μg/mL) in the liquid decreased from 1.5 at 2 h to ∼0.7 after 12 h. Thus, a significant amount of FB_1 was extracted into the cooking/steeping liquid. These findings are again consistent with those of Dombrink-Kurtzmann et al. (*46*), who found about three-fourths of the FB_1 in the liquid fractions after nixtamalizing corn for tortillas and, as in our study, that the majority of the FB_1 in the liquid was hydrolyzed. Our data further show that most of the fumonisins were extracted during the first 2 h of steeping and then, with time, were increasingly hydrolyzed.

Extractability of fumonisins from various corn-based products can vary significantly and is influenced by factors such as the chemical composition of the matrix, solvent, solvent pH, and other experimental conditions (*8 10*, *44*, *50*, *51*). Recoveries of FB_1 from spiked corn

Table 2. Concentrations of FB_1, $PHFB_1$, and HFB_1 in Raw Corn, Masa, Solid Waste, and Cooking/Steeping Liquid from Cooks 4 and 5, As Determined by LC-MS/SIMS [Values Are Mean (± Standard Deviation)]

	cook 4			cook 5		
	FB_1	$PHFB_1$	HFB_1	FB_1	$PHFB_1$	HFB_1
raw corn[a] (ppm)	3.79 (± 0.13) [100][c]	ND[b]	ND	34.6 (±1.07) [100]	1.34 (±0.04)	0.95 (±0.09)
masa[a] (ppm)	0.42 (±0.10) [11.8]	0.06 (±0.04)	0.28 (±0.02)	4.72 (±0.10) [13.6]	0.90 (±0.09)	0.90 (±0.04)
solid waste[d] (ppm)	0.05 (±0.02)	0.07 (±0.02)	0.47 (±0.01)	0.20 (±0.01)	0.96 (±0.03)	1.72 (±0.03)
cooking/steeping liquid[e]						
2 h (μg/mL)	0.12 (±0.07)	ND	0.36 (±0.01)	0.30 (±0.03)	1.02 (±0.01)	2.82 (±0.26)
6 h (μg/mL)	0.10 (±0.04)	ND	0.52 (±0.04)	0.19 (±0.01)	0.68 (±0.05)	3.30 (±0.15)
12 h (μg/mL)	0.07 (±0.02)	ND	0.56 (±0.02)	0.12 (±0.02)	0.48 (±0.01)	3.14 (±0.15)

[a] $n = 3$, except where indicated otherwise. [b] ND, not detected. [c] Numbers in brackets indicate relative amounts of FB_1 in raw corn and masa, normalized to raw corn. [d] $n = 2$ for cook 4 and $n = 3$ for cook 5; values indicate ppm in freeze-dried samples that were not included in mass balance calculations. [e] $n = 2$.

and masa in this study were similar, averaging 48 and 44%, respectively, using the immunoaffiinitiy cleanup procedure (taking into account both extraction efficiency and irreversible adherence of some FB_1 to the column). The reason these recoveries were lower than those reported by others (*7*, *8*, *10*, *46*, *51*) was not determined. We did observe, however, that only 70−75% of the [^{14}C]FB_1, which was spiked into an acetonitrile/water extract of fumonisin-contaminated corn just before immunoaffinity cleanup, was recovered in the 1.5 mL of methanol eluate. This suggests that retention of fumonisins by the column contributed significantly to the low recoveries. That recoveries of FB_1 from spiked baked and fried chips, 21−22%, were even lower than from masa and raw corn offers further evidence that the chemical matrix influences extractability and suggests that additional FB_1 binding sites may have been created in the matrix upon heating.

LC-MS analyses were undertaken for several reasons. First, HPLC analysis of OPA-derivatized fumonisins did not allow detection of any fumonisins lacking a primary amino function, whereas OPA-nonreactive compounds such as *N*-(carboxymethyl)-FB_1 and *N*-(1-deoxy-D-fructos-1-yl)-FB_1, indicative of fumonisin−sugar interactions, could be measured by LC-MS. Detection of fumonisin−sugar or other fumonisin-bound products is important, not only for understanding the fate of fumonisins during fried chip production but also because of the toxicological implications. It is likely that ceramide synthase inhibition, a key event in toxigenesis (*52*), requires the primary amino function of the molecule (*50*, *52*, *53*). Interestingly, Lu et al. (*50*) reported that an FB_1−sugar reaction product or products, in which the primary amine group was modified so that it could no longer react with OPA but which was not otherwise identified (*55*), was not toxic to rats, a finding which implies that fumonisins are detoxified when converted to fumonisin−sugar compounds. Second, LC-MS also eliminated the need for extract cleanup and therefore allowed simultaneous quantification of FB_1, HFB_1, and $PHFB_1$ using a single aliquot of extract. Third, the HPLC chromatograms obtained after C18 cleanup were not optimal for quantifying low concentrations of HFB_1. They contained a number of peaks with HFB_1 appearing as a small peak or shoulder on the leading edge of a larger peak. It was therefore deemed prudent to corroborate the HPLC results using a second method. Finally, that relatively high amounts of HFB_1 were found in the cooking/steeping liquid by HPLC suggested that significant amounts of $PHFB_1$ might also have formed. $PHFB_1$, which we had not included in the HPLC measurements, was therefore also considered during LC-MS analyses.

N-(Carboxymethyl)- and *N*-(1-deoxy-D-fructos-1-yl)-FB_1 were used as markers for the formation of fumonisin−sugar adducts. Howard et al. (*56*) showed that, under mildly alkaline conditions, FB_1 reacts with glucose to give *N*-(carboxymethyl)-FB_1. A small amount of a compound with *m*/*z* 884 was also observed when their experiment was repeated. This compound, which can be isolated as a stable product, was identified as *N*-(1-deoxy-D-fructos-1-yl)-FB_1 (Poling, unpublished observations), the Amadori rearrangement product of the Schiff base formed by the reaction of FB_1 with glucose. *N*-(1-Deoxy-D-fructos-1-yl)-FB_1 is formed under milder conditions than used by Murphy et al. (*8*) to form their fumonisin−sugar adducts. Upon alkaline hydrolysis, it yields a mixture of FB_1, *N*-(carboxymethyl)-FB_1, and the partially hydrolyzed and hydrolyzed forms of FB_1 (HFB_1 and $PHFB_1$), *N*-(1-deoxy-D-fructos-1-yl)-FB_1, and *N*-(carboxymethyl)-FB_1. Three of the six possible forms of *N*-(1-deoxy-D-fructos-1-yl)-FB_1 and *N*-(carboxymethyl)-FB_1 and their hydrolyzed or partially hydrolyzed forms were found at low levels in corn, masa, waste, and fried chips or in the cooking/steeping liquid from cook 5. The following results are estimates and are based on the relative areas of the ion chromatograms of the protonated molecules. They were not corrected for response, as response factors are not known for all of the compounds. Under our conditions, the response factor for *N*-(1-deoxy-D-fructos-1-yl)-FB_1 compared to FB_1 is 0.55 on a weight basis and the response factors are between 0.5 and 1 for *N*-(carboxymethyl)-FB_1 and hydrolyzed *N*-(carboxymethyl)-FB_1. *N*-(1-Deoxy-D-fructos-1-yl)-FB_1 was found only in cook 5 raw corn, at an amount equaling ∼1.3% of the area of the FB_1 peak. *N*-(Carboxymethyl)-FB_1 was detected in the raw corn, masa, and fried tortilla chips from cook 5 at levels ranging from 0.3 to 0.6% of the area of the FB_1 peak found in these samples. Thus, although there was a significant reduction in the amount of FB_1 in masa compared to the raw corn (Table 2), the ratio of *N*-(carboxymethyl)-FB_1 to FB_1 in the corn and masa remained constant.

Of the compounds resulting from fumonisin−sugar interaction, hydrolyzed *N*-(carboxymethyl)-FB_1 was the only one found in the cooking/steeping liquid. It was present in similar amounts at 2, 6, and 12 h, averaging only ∼1.6% of the area of the HFB_1 peak. Just as most FB_1 extracted into the liquid during cooking/steeping is ultimately converted to HFB_1, *N*-(carboxymethyl)-FB_1

is similarly hydrolyzed under alkaline conditions and, therefore, *N*-(1-deoxy-D-fructos-1-yl)-FB_1 extracted from the corn or formed during cooking/steeping would probably be converted to hydrolyzed *N*-(carboxymethyl)-FB_1 or HFB_1. Because solutions of FB_1 and glucose can form small equilibrium amounts of *N*-(1-deoxy-D-fructos-1-yl)-FB_1, it is possible that some was formed after extraction. However, the pattern of the occurrence of these compounds is consistent with their being naturally present in the samples, particularly in the case of *N*-(carboxymethyl)-FB_1, which has been previously found to occur naturally in corn at low levels (*56*). That little *N*-(carboxymethyl)-FB_1 was found indicates that significant amounts of fumonisin−glucose adducts were not formed during nixtamalization and those that did form were removed from the "product stream" by extraction into the cooking/steeping liquid.

For LC-MS quantification of FB_1, HFB_1, and $PHFB_1$ in raw corn, masa, waste, and cooking/steeping liquid, the extracts (or cooking/steeping liquid) were diluted and analyzed in the SIM mode. Like HPLC, LC-MS results showed a significant (86−89%) reduction in the FB_1 concentrations of cook 4 and 5 masa compared to their respective raw corn samples (Table 2). It was further shown that only low levels of HFB_1 were present in the corn and masa, that $PHFB_1$ was distributed similarly to HFB_1 in these fractions, and that significant amounts of HFB_1 were found in the cooking/steeping liquid. By LC-MS, 6.6, 6.9, and 6.3 μg/mL total FB_1 equivalents, which includes FB_1, HFB_1, and $PHFB_1$, were found in the cooking/steeping liquid at 2, 6, and 12 h, respectively. Subtracting $PHFB_1$ from these totals, FB_1 equivalents in the liquid were 5.3 μg/mL at 2 h, 6.0 μg/mL at 6 h, and 5.7 μg/mL FB_1 at 12 h, whereas the corresponding values (FB_1 plus HFB_1) measured by HPLC were 5.3, 3.4, and 5.4 μg/mL FB_1 equivalents, respectively. The reason that less unchanged FB_1 was found in the liquid by LC-MS (0.16−0.42 μg/mL FB_1 equivalents) than by HPLC (2.52−3.32 μg/mL FB_1 equivalents) could not be determined, but the findings suggest that further hydrolysis may have occurred in the stored liquid during the time (~8 months) between HPLC and LC-MS measurements. In any event, it is the total amount of FB_1 and its derivatives in the liquid that is important and, in consideration of the total FB_1 equivalents found in each, the results are in good agreement.

To estimate the extent to which our analyses accounted for all of the FB_1 in the raw corn, the total amounts (micromoles) of FB_1, $PHFB_1$ and HFB_1 in the raw corn, masa, and 12h cooking/steeping liquid were calculated (Table 3). LC-MS results were used because the HPLC-based mass balance (not shown), although consistent with that constructed from the LC-MS data, did not account for $PHFB_1$. The mass balance estimate was constructed by taking into account the following: (a) the concentrations of FB_1, $PHFB_1$, and HFB_1 in corn and masa; (b) the molecular weights of FB_1, $PHFB_1$, and HFB_1; (c) the relative proportions of corn and water during cooking/steeping; and (d) the masa yield (w/w accounting for water uptake compared to the raw corn). It was not necessary to include the baked and fried chips, as there was no significant change in the fumonisin amounts between the masa and the chips.

Approximately 34% of the fumonisins (total micromoles) initially present in the raw corn was found in masa, whereas another 45% was found in the cooking/steeping liquid (Table 3). Thus, 79% of the fumonisins in corn was accounted for in the masa and cooking/steeping liquid. FB_1, $PHFB_1$, and HFB_1 were found (the latter was present at a relatively high concentration) in the solid waste and in the rinse liquid. They were not, however, included in the mass balance estimate because it was not possible for technical reasons to accurately determine the weight of the solid waste generated or the volume of rinse water used during cook 5. The mass balance estimate nonetheless indicates that cooking/steeping removed a significant amount of the fumonisins from the corn and diverted them from the "product stream" leading to fried chips.

Table 3. Estimated Mass Balance of Fumonisins (FB_1 + $PHFB_1$ + HFB_1) in Cook 5 Raw Corn, Masa, and Cooking/Steeping Liquid at the Conclusion of the 12 h Cooking/Steeping Step

		concentration		
	rel amt[b]	ppm[a] (corn and masa) or mg/L (liquid)	μmol/kg[d]	amt[c] (μmol)
raw corn	1.00	FB_1 = 34.6 $PHFB_1$ = 1.34 HFB_1 = 0.95	FB_1 = 48.0 $PHFB_1$ = 2.38 HFB_1 = 2.35	52.7 [100]
masa	1.74	FB_1 = 4.72 $PHFB_1$ = 0.90 HFB_1 = 0.90	FB_1 = 6.55 $PHFB_1$ = 1.60 HFB_1 = 2.22	18.0 [34]
cooking/steeping liquid	2.71	FB_1 = 0.12 $PHFB_1$ = 0.48 HFB_1 = 3.14	FB_1 = 0.17 $PHFB_1$ = 0.85 HFB_1 = 7.75	23.8 [45]
solid waste	ND[e]	FB_1 = 0.20 $PHFB_1$ = 0.96 HFB_1 = 1.72	FB_1 = 0.27 $PHFB_1$ = 1.71 HFB_1 = 4.25	ND

[a] Mean value from Table 2. [b] Relative amounts of raw corn, masa, and cooking/steeping liquid, on a per weight basis, assuming 1 L of water weighs 1 kg and accounting for water imbibed by the corn during cooking. [c] Concentration (μmol/kg) × relative amount. Number in brackets indicates percent of amount in raw corn. [d] Molecular weights of FB_1, $PHFB_1$, and HFB_1 used in the calculation were 721, 563, and 405, respectively. [e] ND, not determined. The weight of solid waste produced and the amount of rinse water used could not be determined for technical reasons. Total fumonisins in these fractions could therefore not be calculated.

In summary, the fate of fumonisins during the production of fried tortilla chips under commercial conditions was investigated. This process effectively reduced fumonisin concentrations in the masa and chips relative to that in the raw corn. Cooking and steeping the corn under mild alkaline conditions (nixtamalization) followed by rinsing with water was the critical step for achieving reduced concentrations. Sheeting, baking, and frying the masa to make the fried tortilla chips had little further effect. Additional studies are needed to characterize fumonisin−matrix interactions in the corn, intermediates, chips, and byproducts; to thereafter study in more detail the fate of fumonisins during the manufacture of various food products from sound corn containing low, commercially realistic fumonisin concentrations; and to optimize production conditions to further reduce fumonisins in corn-based food products.

SAFETY

Fumonisin B_1 is a rodent carcinogen and should be handled accordingly.

ABBREVIATIONS USED

FB_1, fumonisin B_1; HFB_1, hydrolyzed FB_1; $PHFB_1$, partially hydrolyzed FB_1.

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