Stability of Fumonisins in Thermally Processed Corn Products

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ABSTRACT

Little is known about the stability of fumonisins in corn-based foods during heating. This study investigated the effects of canning, baking, and roasting (dry heating) processes on the stability of fumonisins in artificially contaminated and naturally contaminated corn-based foods. All samples were analyzed for fumonisin levels by both a commercial enzyme-linked immunoabsorbent assay (ELISA) and a high-performance liquid chromatographic (HPLC) method. Canned whole-kernel corn showed a significant (P ≤ 0.05) decrease in fumonisins by both ELISA (15%) and HPLC (11%) analyses. Canned cream-style corn and baked corn bread showed significant (P ≤ 0.05) decreases in fumonisin levels at an average rate of 9% and 48%, respectively, as analyzed by ELISA. Corn-muffin mix artificially contaminated with 5 µg of fumonisin B1 (FB1) per g and naturally contaminated corn-muffin mix showed no significant (P ≤ 0.05) losses of fumonisins upon baking. Roasting cornmeal samples artificially contaminated with 5 µg of FB1 per g and naturally contaminated cornmeal samples at 218°C for 15 min resulted in almost complete loss of fumonisins.

Fumonisins are secondary metabolites produced by several species of the fungus Fusarium. Among the most common and highest-producing fumonisin species are Fusarium moniliforme and Fusarium proliferatum, both of which are frequently found on corn (17). Fumonisin B1 (FB1) is the most common naturally occurring compound in this group of mycotoxins. Fumonisins have been associated with several diseases in animals, including leukoencephalomalacia in horses (12), pulmonary edema in pigs (8), and hepatocarcinoma in rats (7). Epidemiological evidence also indicates a possible correlation between the fumonisins and the high incidence of esophageal cancer in South Africa (19), China (4), and northern Italy (5).

Fumonisins have been detected in animal feeds and human foods. Pestka et al. (15) found fumonisin levels ranging from 400 to 6,300 ng/g in cornmeal, 0 to 1,200 ng/g in corn-muffin mix, 200 ng/g in corn tortilla mix, and none in cornflakes cereals. Sydenham et al. (20) reported ranges of 0 to 2,790 ng of FB1 per g and 0 to 920 ng of FB2 per g in cornmeal samples collected from the United States. They found low levels or nondetectable levels of fumonisins in cornflakes and corn tortillas. Doko and Visconti (5) reported that in corn-based foods in Italy the highest fumonisin levels, ranging from 500 ng/g to 4,700 ng/g, were found in cornmeal, corn grits, and polenta.

A number of studies have reported on the stability of fumonisins in various food processes. Bothal et al. (3) studied the fate of fumonisin B1 during ethanol fermentation. They found that FB1 was not degraded during the fermentation of contaminated corn. Ethanol distilled from the whole stillage did not contain any FB1; however, all the other products produced from this fermentation contained FB1. The traditional process to produce masa, called nixtamalization, was evaluated as a method to detoxify fumonisins (9). It was found that treating corn with lime water and heat hydrolyzed FB1 to the aminopentol backbone and tricarboxylic acid. However, when the corn was fed to rats, it was found that the toxicity was not reduced by the treatment. Norred et al. (13) reported that at atmospheric pressure and ambient temperature, ammoniation reduced the fumonisin content of F. moniliforme culture material but did not reduce the toxicity of the material when fed to rats. Park et al. (14) studied the effects of ammoniation on fumonisins as a means to detoxify fumonisin-contaminated corn and found a 79% reduction of fumonisin levels in corn after high-pressure and ambient temperature ammoniation followed by a low-pressure and high-temperature treatment. However, they did not measure the toxicity of the treated corn. Scott and Lawrence (18) reported 60% loss of fumonisins in dry cornmeal heated at 190°C for 60 min, 70 to 80% loss of fumonisins in moist cornmeal heated at 190°C for 60 min, and complete loss in dry cornmeal heated at 220°C for 25 min. They also found that fumonisin content was partially reduced in cornmeal muffins baked at 220°C for 25 min. These authors likewise did not measure the toxicity of the treated products.

The objective of this work was to extend the studies of Scott and Lawrence (18) and to determine the effects of canning, baking, and roasting on the stability of fumonisins in spiked and naturally contaminated corn-based foods by using two analytical methods, enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC).
MATERIALS AND METHODS

Samples. Canned products, including cream-style corn (main ingredients: corn, water, sugar, modified cornstarch, and salt), whole-kernel corn (main ingredients: corn, water, salt), creamed corn for infants (main ingredients: corn, water, nonfat milk, and rice flour) and dog food (main ingredients: corn, water, soybean meal, chicken, poultry by-products, beef, mineral and vitamin supplements) were purchased at a local market. The FB₁ used to artificially contaminate the samples was obtained from Dr. Robert Eppley (U. S. Food and Drug Administration, Washington, D.C.). FB₁ was dissolved in a 70:30 solution of acetonitrile (ACN) and water to a final concentration of 5 µg/ml. Canned products were removed from their original cans and 500 g of each were mixed separately in a 1-liter beaker with a sufficient amount of FB₁ solution to obtain approximately 5 µg of FB₁ per g of food. These were then mixed with a spatula for 10 min and refrigerated overnight. The samples were then mixed again for 10 min prior to recanning. For canning, 100 g of each sample was placed into 113-g (ca. 4-oz) glass canning jars. The jars were retorted (canned) in a laboratory autoclave at 121°C. While internal temperatures of the containers were not measured, industry conditions for processing times were followed as closely as possible. Using these guidelines, cream-style corn, creamed corn for infants, and dog food were heated to initial temperatures of 60°C prior to processing. The cream-style corn and creamed corn for infants were processed at 121°C for 65 min, the whole-kernel corn for 22 min, and the dog food for 87 min. After processing, the jars were cooled at room temperature.

For the baking experiment, naturally contaminated corn-muffin mix and corn-bread samples were prepared following package instructions. In addition, a fumonisin-free corn-muffin mix was prepared following package instructions and then artificially contaminated to obtain approximately 5 µg of FB₁ per g with the required volume of a stock solution of 5 µg of FB₁ per µl of ACN and water (70:30) and mixed with a spatula for 10 min. Fifty grams of each corn-muffin mix and corn-bread samples were then placed in paper cupcake cups (76 mm in diameter by 38 mm high). Corn-muffin mix samples were baked in an oven (Partlow, National MFG Co., Lincoln, Neb.) at 204°C for 20 min. The corn-bread sample was baked at 232°C for 20 min.

Three naturally contaminated cornmeal samples and one artificially contaminated cornmeal sample were roasted (dry heated) at 218°C for 15 min in the same oven used for baking. FB₁ was added to the artificially contaminated cornmeal sample to obtain an approximate concentration of 5 µg/g. First, the cornmeal (200 g) was dried at 105°C for 24 h and then distilled water containing the FB₁ was added to restore the moisture and artificially contaminate the sample with FB₁. The artificially contaminated sample was mixed for 10 min, left at room temperature overnight, and then roasted along with the naturally contaminated samples as described above. Fifty grams of each sample were evenly distributed on the surface of foil pans (EZ foil, 203 by 133 by 38 mm) and roasted.

Forty grams of each sample were reserved as controls prior to each of the three processing treatments and analyzed by the methods described below to determine the actual amount of FB₁ in the samples. For the baking and roasting experiments, a thermocouple (model 650/660 microprocessor-controlled temperature indicator, Omega Engineering, Inc., Stamford, Conn.) was used to measure the temperature inside the oven and in the center of the samples. Canning, baking, and roasting treatments were done in triplicate.

Analyses. All samples were analyzed by HPLC for FB₁ as described by Rice et al. (16) with a slight modification in the cleanup of the extract. Solid-phase extraction (SPE) columns (C₁₈ Waters Sep-Pak cartridge) were conditioned with 2 ml of acetonitrile (ACN) followed by 2 ml of 1% potassium chloride (KCl) solution. The diluted samples were then loaded onto the columns and allowed to flow at a rate of 2 ml/min. The columns were washed with 2 ml of 1% KCl followed by 1 ml of a 15:85 solution of ACN and water. The rinses were discarded and air was forced through the columns to expel all of the rinse solutions. Fumonisins were eluted with 3 ml of a 70:30 solution of ACN and water. Then 100 µl of the eluate from the SPE column was derivatized with 100 µl of borate buffer solution (pH 8.3 to 8.5), 100 µl of o-phthaldehyde (OPA) solution (1.5 mg of o-phthaldehyde per ml of ACN in 0.2% mercaptoethanol), and 100 µl of water, and allowed to react for 10 min at room temperature.

SAX columns (Supelclean LC-SAX, SPE tubes, 1 ml; Supelco, Inc., Bellefonte, Pa.) were also used for cleaning of the dog food and corn-bread samples in accordance with the method of Thiel et al. (21) with some modifications. Briefly, the samples were extracted with 50 ml of a 50:50 solution of ACN and water and 2 ml of the extract was evaporated under a stream of nitrogen at 60°C. The samples were redisolved in 10 ml of a 70:30 solution of methanol (MeOH) and water and loaded on SAX columns preconditioned with 5 ml of MeOH and 5 ml of a 70:30 solution of MeOH and water. The columns were washed with 8 ml of a 70:30 solution of MeOH and water followed by 3 ml of MeOH. FB₁ was eluted with 10 ml of 1% acetic acid in MeOH. The eluate was evaporated under nitrogen at 60°C and then redisolved in 3 ml of a 70:30 solution of ACN and water. The samples were analyzed as described by Rice et al. (16).

The HPLC system consisted of a model 510 HPLC pump and a U6K loop injector (Waters, Milford, Mass.), a high-speed reverse-phase column (C₁₈, 3 mm, 33 by 4.6 mm; Perkin-Elmer Corp., Norwalk, Conn.), a model 474 scanning fluorescence detector (Waters) with wavelength set at 335 nm excitation and 440 nm emission, and an HP 3395 recorder-integrator (Hewlett-Packard Co., Wilmington, Del.).

Fumonisin levels were also quantitated with ELISA test kits (quantitative kit for fumonisins, Veratox, Neogen Corp., Lansing, Mich.) used according to kit instructions.

RESULTS AND DISCUSSION

Canning. Statistical analysis of fumonisin concentrations determined by ELISA and HPLC in processed canned creamed corn for infants and canned dog food showed no significant (P ≤ 0.05) differences from concentrations in

<table>
<thead>
<tr>
<th>Product</th>
<th>Control (unprocessed)</th>
<th>Processed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cream-style corn</td>
<td>ELISA: 6.9 ± 0.43</td>
<td>HPLC: 5.1 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>ELISA: 6.3 ± 0.15</td>
<td>HPLC: 4.6 ± 0.29</td>
</tr>
<tr>
<td>Whole-kernel corn</td>
<td>ELISA: 7.6 ± 0.5</td>
<td>HPLC: 5.3 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>HPLC: 6.5 ± 0.28</td>
<td>HPLC: 4.7 ± 0.32</td>
</tr>
<tr>
<td>Creamed corn for infants</td>
<td>ELISA: 6.0 ± 0.36</td>
<td>HPLC: 4.6 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>HPLC: 6.6 ± 0.28</td>
<td>HPLC: 4.8 ± 0.16</td>
</tr>
<tr>
<td>Dog food</td>
<td>ELISA: 7.7 ± 0.36</td>
<td>HPLC: 4.1 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>HPLC: 5.3 ± 1.18</td>
<td>HPLC: 4.1 ± 0.1</td>
</tr>
</tbody>
</table>

*Total fumonisins by ELISA; FB₁ by HPLC.

b Significantly (P ≤ 0.05) different from control.
unprocessed controls (Table 1). A significant ($P \leq 0.05$) decrease in fumonisin levels (9%) was observed in cream-style corn analyzed by ELISA (Table 1). Canning whole-kernel corn resulted in a significant ($P \leq 0.05$) decrease in fumonisin levels measured by both ELISA and HPLC at average rates of 15% and 11%, respectively (Table 1).

**Baking and roasting.** Baking artificially contaminated (5 μg of FB$_1$ per g) and naturally contaminated corn-muffin mixes at 204°C for 20 min resulted in no significant ($P \leq 0.05$) loss of fumonisins measured by either the ELISA or HPLC method (Table 2). Only baking corn bread at 232°C for 20 min showed a significant ($P \leq 0.05$) decrease in fumonisin levels (48%) by ELISA analyses, but not by HPLC (Table 2). Roasting (dry heating) artificially contaminated (5 μg/g) and naturally contaminated cornmeal samples at 218°C for 15 min resulted in almost complete reduction of fumonisins (Table 2).

Previous studies have demonstrated that fumonisins are quite heat stable compounds. Jackson et al. (10, 11) studied the effects of thermal processing on FB$_1$ and FB$_2$ in an aqueous buffer. They found that the rate and extent of fumonisin decomposition increased with processing temperature. Small reductions of FB$_1$ (<27%) and FB$_2$ (<20%) were observed when the processing temperature was less than or equal to 125°C for 60 min. More than 80% of FB$_1$ and FB$_2$ were lost at temperatures ≥175°C for 60 min. Dupuy et al. (6) reported that the decomposition of FB$_1$ in dry cornmeal heated at 100 to 150°C followed first-order kinetics. They also found that substantial losses of FB$_1$ occurred only after dry corn was heated at 150°C for 50 min. Therefore, it appears that the internal temperature of the processed corn products reached during thermal processing plays an important role in the stability of fumonisins. In baked samples, the maximum internal temperature was 102°C, whereas in the roasted cornmeal samples the maximum internal temperature was 192°C (Table 2), and no loss of fumonisins was observed with baked products, whereas almost complete loss of fumonisins was observed with the roasted products, which reached a much higher temperature.

Previous studies on the degradation of fumonisins have shown that real losses of fumonisins may be difficult to measure (2). This problem may be due to possible strong binding of fumonisins to the food matrix or, in the case of HPLC analysis, modification of the structure of FB$_1$ in such a way that the amino group involved in the derivatization of FB$_1$ is removed or blocked. In this study, two analytical methods, ELISA and HPLC, were used in an attempt to more closely determine real losses of fumonisins in the processed corn products. In the samples that were roasted, both ELISA and HPLC analysis showed almost complete loss of fumonisins, whereas there were greater differences in the amounts detected by the two methods in products subjected to lower heat treatments.

Mean recoveries for the canned, baked, and roasted samples using the ELISA method were 133%, 110%, and 110%, respectively, whereas for the HPLC procedure of Rice et al. (16), which involves cleanup of the extract using C$_{18}$ columns, were 95, 98, and 108%, respectively. However, in this study it was observed that the cleanup of canned dog food and baked corn bread extracts with C$_{18}$ columns resulted in peaks that co-eluted with FBI during HPLC separation (Figure 1a and 1c). Use of SAX columns rather than C$_{18}$ columns for the cleanup of canned dog food and baked corn bread extracts resulted in HPLC chromatograms free of these interferences (Figure 1b and 1d) with mean recoveries of 82 and 76%, respectively.

In most of the analyses, higher levels of fumonisins were found by ELISA than by HPLC before and after thermal processing. This result may have been due to the presence of structurally related metabolites reacting in the ELISA test that were not determined by HPLC, fumonisin decomposition products that reacted in the ELISA test but not in HPLC, and/or possible binding of fumonisins to the food matrix, making them nondetectable by HPLC but still able to react with the ELISA antibodies (1, 15).

In the present study, toxicity testing of the processed samples was not possible because of a lack of suitable in vitro and in vivo test systems and small sample sizes. However, analysis of samples by two different analytical methods, ELISA and HPLC, was used as a quality control measure (2). This problem may be due to possible strong binding of fumonisins to the food matrix or, in the case of HPLC analysis, modification of the structure of FB$_1$ in such a way that the amino group involved in the derivatization of FB$_1$ is removed or blocked. In this study, two analytical methods, ELISA and HPLC, were used in an attempt to more closely determine real losses of fumonisins in the processed corn products. In the samples that were roasted, both ELISA and HPLC analysis showed almost complete loss of fumonisins, whereas there were greater differences in the amounts detected by the two methods in products subjected to lower heat treatments.

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**TABLE 2. Concentration of fumonisins remaining in the samples after baking (corn muffin and corn bread) and roasting (cornmeal)**

<table>
<thead>
<tr>
<th>Product</th>
<th>Oven temp. (°C)</th>
<th>Maximum internal temp. of sample (°C)</th>
<th>Time (min)</th>
<th>Control (unprocessed)</th>
<th>Processed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ELISA</td>
<td>HPLC</td>
</tr>
<tr>
<td>Corn muffin</td>
<td>204</td>
<td>102</td>
<td>20</td>
<td>5.7 ± 0.35</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>Corn muffin</td>
<td>204</td>
<td>100</td>
<td>20</td>
<td>0.4 ± 0.14</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Corn bread</td>
<td>232</td>
<td>102</td>
<td>20</td>
<td>1.2 ± 0.19</td>
<td>0.6 ± 0.08</td>
</tr>
<tr>
<td>Corn meal</td>
<td>218</td>
<td>182</td>
<td>15</td>
<td>5.6 ± 0.17</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>Corn meal</td>
<td>218</td>
<td>192</td>
<td>15</td>
<td>4.2 ± 0.21</td>
<td>2.6 ± 0.07</td>
</tr>
<tr>
<td>Corn meal</td>
<td>218</td>
<td>192</td>
<td>15</td>
<td>2.5 ± 0.28</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>Corn meal</td>
<td>218</td>
<td>186</td>
<td>15</td>
<td>8.6 ± 0.28</td>
<td>4.7 ± 0.07</td>
</tr>
</tbody>
</table>

a Total fumonisins by ELISA; FB$_1$ by HPLC.
b Spiked with 5 μg of FB$_1$ per g of product.
c Significantly ($P \leq 0.05$) different from control.
d ND, none detected (<50 ng/g).
e Naturally contaminated.
methods provided additional data not found in similar studies.

Overall, it was found in this study that fumononisins added to cornmeal and present in naturally contaminated cornmeal were unstable under roasting conditions, but remained fairly stable during the canning and baking of corn-based foods, probably because the canned and baked products reached lower internal temperatures than the roasted products.

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