Effects of Thermal Processing on the Stability of Fumonisin B_2 in an Aqueous System

Keywords: *Fumonisin B_2; thermal processing; stability; decomposition*

INTRODUCTION

Fusarium moniliforme, a prevalent fungal contaminant of corn, has been implicated in several animal diseases including equine leukoencephalomalacia (ELEM) (Thiel et al., 1991), porcine pulmonary edema (PPE) (Harrison et al., 1990), liver toxicity and liver cancer in rats (Voss et al., 1993), and esophageal cancer in humans (Sydenham et al., 1991; Rheeder et al., 1992). The fumonisins, which are secondary metabolites of *F. moniliforme*, are believed to be responsible for many of the toxicological effects in animals and humans (Marasas et al., 1988; Wilson et al., 1992; Colvin et al., 1993, Gelderblom et al., 1991; Sydenham et al., 1991).

Toxicological studies have shown that purified fumonisin B_1 (FB_1) causes ELEM (Marasas et al., 1988; Wilson et al., 1992), PPE (Colvin et al., 1993), and liver tumors (Gelderblom et al., 1991) in rats. FB_2 has been shown to cause ELEM in ponies (Ross et al., 1994) and cytotoxicity in mammalian cell lines (Gelderblom et al., 1993). FB_1 and FB_2 have been found to inhibit sphingolipid biosynthesis by blocking the conversion of sphinganine to ceramide (Wang et al., 1991; Norred et al., 1992).

Fumonisins are diesters of propane-1,2,3-tricarboxylic acid and a pentahydroxyicosane containing a primary amino group. To date, seven different fumonisin analogues have been identified and characterized (Bezuidenhout et al., 1988; Branham and Plattner, 1993; Cawood et al., 1991; Gelderblom et al., 1992; Plattner et al., 1992). Of the seven, FB_1 and FB_2 are the major toxins in contaminated corn. In corn contaminated with *Fusarium proliferatum*, the ratio of FB_1 to FB_2 is approximately 3 to 1 (Ross et al., 1992). Structurally, FB_2 differs from FB_1 in its lack of a hydroxyl group on the C-10 position of the 22-carbon backbone.

Several surveys have shown that thermally processed corn products (e.g., tortillas, ready-to-eat cereal, and muffins) generally contain lower concentrations of fumonisins than unprocessed products (e.g., cornmeal and grits) (Stack and Eppley, 1992; Pittet et al., 1992). Few studies, however, have focused on the effects of thermal processing on the fumonisin content of food. Alberts et al. (1990) reported that boiling culture material of *F. moniliforme* in water for 60 min resulted in no loss of FB_1. In contrast, baking (190 and 220 °C) muffins from contaminated cornmeal resulted in a partial apparent loss of FB_1 (Scott and Lawrence, 1994). Dupuy et al. (1993) and Jackson et al. (1996) reported that the loss of FB_1 in dry corn and in an aqueous model system, respectively, followed pseudo-first-order kinetics. Studies by Bordson et al. (1993) and Scott and Lawrence (1994) suggest that the observed losses of fumonisin in thermally processed food may be due to matrix-related difficulties of recovery and detection, rather than actual fumonisin decomposition. Murphy et al. (1996) reported that the primary amine group of fumonisins can be chemically blocked when foods are heated. This results in loss of the availability of the amine group to react with derivitizing agents that are used to analyze fumonisins.

To date, little information is available concerning the effects of time, temperature, and pH on the stability of FB_2. The objective of this study was to determine the thermal stability of FB_2 in an aqueous matrix-free environment at acidic, neutral, and basic pH levels.

MATERIALS AND METHODS

FB_2 and *o*-phthaldialdehyde (OPA) were purchased from Sigma Chemical Co. (St. Louis, MO). Fully and partially hydrolyzed FB_2 qualitative standards were prepared by incubating pure FB_2 with 1 N KOH (Rice and Ross, 1994). All reagents were of analytical grade, and solvents were of high-performance liquid chromatography (HPLC) grade.

FB_2 solutions (5 ppm) were prepared in Teorell and Stenhagen's citrate−phosphate−borate buffer (CRC, 1968) adjusted to pH 4, 7, or 10. This buffer was chosen since it has a broad pH range (2−12). The solutions (500 mL) were placed in a 1-L stainless steel pressure vessel (Parr Instrument Co., Moline, IL) and heated to processing temperatures of 100−200 °C with an electric heating mantle (Jackson et al., 1996). Use of the pressurized vessel enabled solution temperatures of >100 °C to be reached. A Parr Model 4841 proportional controller was used to maintain each reaction mixture at the desired temperature while it was agitated at a constant speed. The come-up times, i.e. the lengths of time necessary for FB_2 solutions to reach the desired processing temperatures, were 18, 29, 32, 40, and 44 min for temperatures of 100, 125, 150, 175, and 200 °C, respectively. Once the desired processing temperature was attained, aliquots of the reaction mixture were removed at 10-min intervals for 60 min and analyzed for FB_2 levels as previously described by Jackson et al. (1996).

HPLC Determination of FB_2. Losses of FB_2 in the processed solutions were measured according to the method of Shephard et al. (1990) with modifications (Jackson et al., 1996). Because FB_2 was processed in aqueous buffer, steps normally used to extract and purify fumonisin from corn were omitted. Consequently, the FB_2 solutions required minimal preparation for analysis by HPLC, and recovery correction was not necessary. A 10-μL aliquot of the FB_2/OPA mixture was used for HPLC determination. A Waters (Milford, MA) HPLC equipped with a Model 600 pump, a Rheodyne (Cotati, CA) injector, and a Model 740 fluorescence detector (335-nm excitation wavelength and 440-nm emission wavelength) and Millenium 2010 software (Waters) was used to identify and quantify FB_2 in the solutions. Separations were carried out at 23 °C on a Supelco (Bellefonte, PA) ODS-80 column (4.6 mm × 25 cm) with an LC-18-DB (Supelco) precolumn. The mobile phase was methanol/1 M sodium dihydrogen phosphate (80:20) adjusted to pH 3.3 with concentrated phosphoric acid at a flow rate of 1.0 mL/min.

Kinetic Calculations. Kinetic constants were calculated according to the procedure of Jackson et al. (1996).

Statistical Analysis. All processing runs were performed in duplicate. Processed solutions were analyzed for FB_2 in duplicate. Means and standard deviations were calculated with Minitab (State College, PA) statistical software. Linear regression analyses, used to determine reaction constants, half-lives of FB_2, and correlation coefficients, were performed by using Psiplot graphics software (Poly Software International, Salt Lake City, UT). Minitab statistical software was used to verify significant differences between rate constants and half-lives by one-way analysis of variance (ANOVA) followed by least significance difference (lsd) tests at 95% confidence. A three-way analysis of variance (ANOVA) was used to determine if processing variables (time, temperature, and pH) significantly affected loss of FB_2.

Safety Precaution. FB_2 is a suspected carcinogen and should be handled with care.

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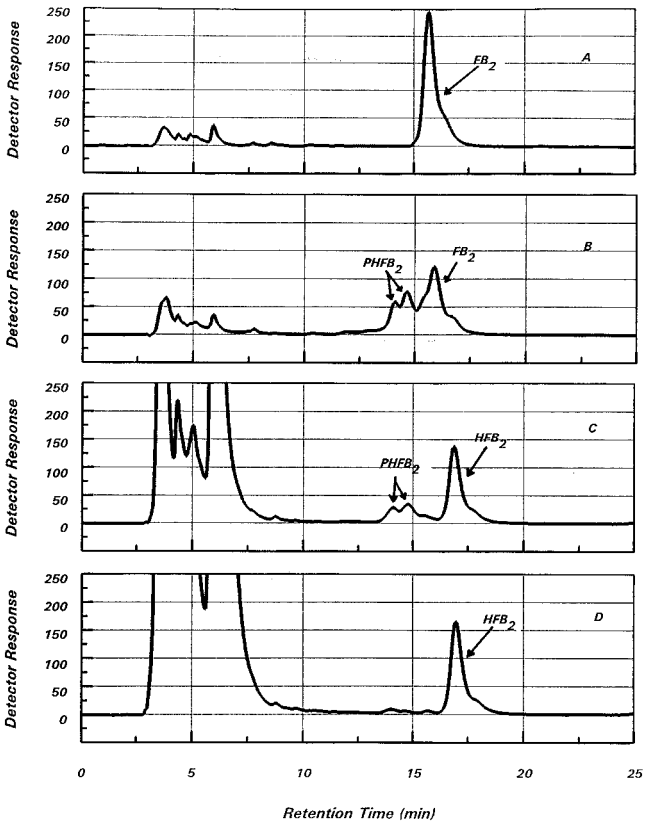

Figure 1. HPLC chromatograms using fluorescence detection (335-nm excitation wavelength and 440-nm emission wavelength) for FB_2 dissolved in an aqueous buffer at pH 10. Chromatograms A, B, C, and D refer to the FB_2 solution before processing, the solution after reaching 200 °C, the solution after 30 min at 200 °C, and the solution after 60 min at 200 °C, respectively. FB_2, partially hydrolyzed FB_2 ($PHFB_2$), and fully hydrolyzed FB_2 (HFB_2) are indicated by arrows. Peaks with retention times of less than 10 min have not been identified.

RESULTS AND DISCUSSION

Thermal Decomposition Products of FB_2. HPLC chromatograms for FB_2 processed at 200 °C (pH 10) are shown in Figure 1. The chromatograms indicate that the concentration of FB_2 (retention time of approximately 15.7 min) decreased during processing, while the levels of three apparent decomposition products (retention times of 14.1, 14.7, and 16.9 min) generally increased. Because the decomposition products had similar retention times as partially (14.1 and 14.7 min) and fully hydrolyzed (16.9 min) FB_2 standards, they were tentatively identified as $PHFB_2$ and HFB_2 in Figure 1.

Several researchers (Bezuidenhout et al., 1988; Jackson and Bennett, 1990; Sydenham et al., 1990a,b) have reported that fumonisins hydrolyze to the C_{22} aminopolyol backbone and tricarballylic acid in the presence of heat and strong acid or base. For example, fully hydrolyzed FB_1 can be found in tortillas prepared from corn treated with calcium hydroxide and heat (Hendrich et al., 1993). Hopmans and Murphy (1993) detected HFB_1 in tortilla chips, masa, and canned corn. However, little is known about the levels of hydrolyzed FB_2 in these and other thermally processed corn-based foods.

The data presented here (Figure 1) indicate that the thermal processing of FB_2 in the presence of water results primarily in the formation of hydrolysis products. The pH had an effect on the types of hydrolysis products detected in the processed solutions. At pH 10, the major species throughout the process was HFB_2, while at pH 4 and 7, $PHFB_2$ was also present.

Table 1. Statistical Analysis of Variance of Time, Temperature, and pH on the Loss of FB_2 during Processing

source	DF	sum of squares	mean squares	*F* value	Pr > *F*
I. Dependent Variable: Percent FB_2 Remaining (Model with Interaction)					
pH	2	11971	5985	96.60	0.0001
temp	4	266335	66584	1074.58	0.0001
time	6	33505	5584	90.12	0.0001
pH × temp	8	8067	1008	16.27	0.0001
pH × time	12	276	23	0.37	0.9710
temp × time	24	29296	1220	19.70	0.0001
pH × temp × time	48	9289	194	3.12	0.0001
error	105	6506	62		
total	209	365243			
II. Dependent Variable: Percent FB_2 Remaining (Model with Main Effect Only)					
pH	2	11971	5985	22.07	0.0001
temp	4	266335	66584	245.49	0.0001
time	6	33505	5584	20.59	0.0001
error	197	53432	271		
total	209	365243			

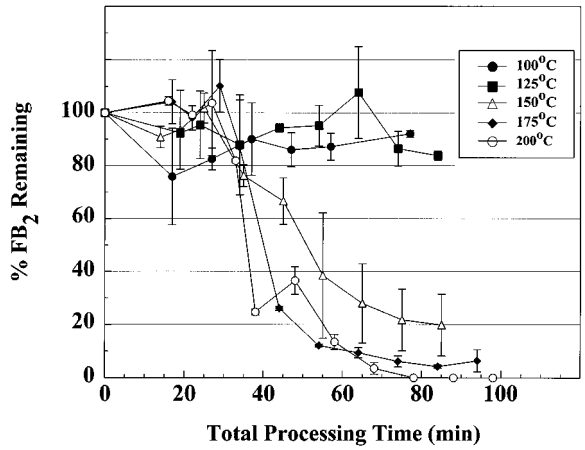

Figure 2. Effects of processing temperature and time on the decomposition of FB_2 in an aqueous buffer at pH 4. Each point represents the average of two replicates, and error bars indicate one standard deviation of the mean.

Effect of pH, Time, and Temperature on the Stability of FB_2. Statistical analysis of the processing data was performed to determine if independent variables (time, temperature, and pH) were related to the dependent variable (percent FB_2 remaining after processing). Results of a three-way ANOVA indicate highly significant effects ($p < 0.01$) of pH, time, and temperature on loss of FB_2 (Table 1). In addition, Table 1 indicates that there was a highly significant three-way interaction ($p < 0.01$) between these independent variables.

Figures 2−4 and Table 1 indicate that decomposition of FB_2 during thermal processing depended on the pH of the solution. Overall, FB_2 was least stable at pH 4 (Figure 2) and most stable at pH 7 (Figure 3). At processing temperatures <200 °C, the decomposition of FB_2 was most rapid and extensive at pH 4, followed by pH 10 (Figure 4) and 7, respectively. At 200 °C, pH had little effect on the rate of loss of FB_2. After 60 min of processing at 200 °C, all FB_2 was decomposed at each pH level.

Figures 2−4 and Table 1 indicate that the rate of decomposition of FB_2 was highly temperature dependent

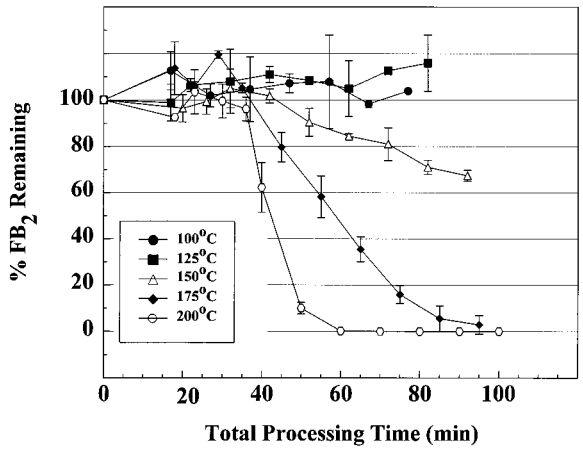

Figure 3. Effects of processing temperature and time on the decomposition of FB_2 in an aqueous buffer at pH 7. Each point represents the average of two replicates, and error bars indicate one standard deviation of the mean.

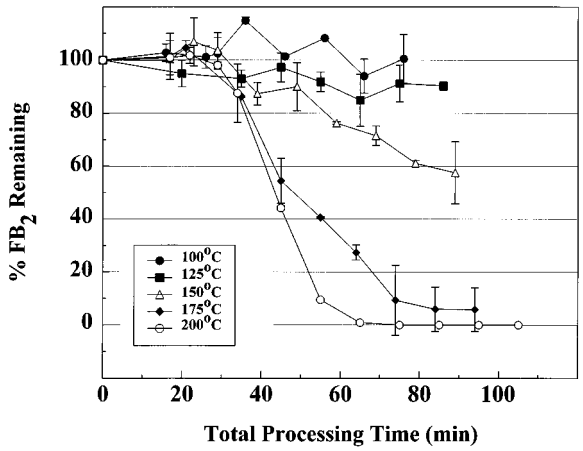

Figure 4. Effects of processing temperature and time on the decomposition of FB_2 in an aqueous buffer at pH 10. Each point represents the average of two replicates, and error bars indicate one standard deviation of the mean.

and, in general, the extent of decomposition increased with processing temperature and time. At all three pH levels, no significant losses in FB_2 occurred during processing at 100 and 125 °C. These results are parallel to those of Jackson et al. (1996), who reported no significant losses of FB_1 at 100 °C and minor losses (<27%) at 125 °C. Similarly, Alberts et al. (1990) found that boiling culture material of *F. moniliforme* for 30 min did not reduce FB_1 concentration. Dupuy et al. (1993) also found minimal losses of FB_1 in naturally contaminated dry corn meal heated at 100 °C for 45 min.

After 60 min at 150 °C, loss of FB_2 ranged from 30 to 80%, with the greatest decomposition occurring at pH 4 and the least at pH 7. At temperatures of 175 and 200 °C, over 90% of FB_2 was degraded after 60 min of processing time, regardless of pH. The results shown here are in agreement with previous results that measured the thermal stability of FB_1 in an aqueous system (Jackson et al., 1996) and in corn. Dupuy et al. (1993) observed losses of FB_1 of 87% in dry corn heated to 150 °C for 40 min. FB_1 and FB_2 levels were reduced by 70−80% in moist corn meal heated for 60 min at 190 °C (Scott and Lawrence, 1994).

The decomposition of FB_2 in pH 4, 7, and 10 buffers heated at 150, 175, and 200 °C followed an apparent first-order reaction similar to that of FB_1 (Jackson et al., 1996). In general, half-lives and pseudo-first-order reaction constants for the decomposition of FB_2 (Table 2) were in general agreement with those reported by Jackson et al. (1996) for FB_1.

Table 2. Reaction Rate Constants (k) and Half-Lives ($t_{1/2}$) for the Decomposition of FB_2 in Teorell and Stenhagen's Phosphate−Citrate−Borate Buffer at pH 4, 7, and 10 and Linear Relationships between Processing Time and Fraction of Remaining FB_2 As Indicated by Correlation Coefficients (R^2)[a]

temp, °C	pH	k, min^{-1}	$t_{1/2}$, min	R^2
150	4	0.0296 ± 0.0046^a	23.9 ± 2.8^a	0.972
175	4	0.0564 ± 0.0058^b	12.3 ± 1.3^b	0.923
200	4	0.2846 ± 0.0569^c	2.4 ± 0.5^c	0.962
150	7	0.0077 ± 0.0005^d	88.9 ± 3.9^d	0.983
175	7	0.0625 ± 0.0095^b	11.1 ± 2.6^b	0.953
200	7	0.0975 ± 0.0212^e	7.2 ± 1.6^e	0.986
150	10	0.0096 ± 0.0011^f	70.3 ± 8.1^f	0.962
175	10	0.0909 ± 0.0354^e	17.9 ± 3.9^e	0.943
200	10	0.1941 ± 0.0233^g	3.6 ± 0.4^g	0.977

[a] Kinetic constants were calculated according to the method of Jackson et al. (1996); those having the same superscripts (a−g) are not significantly different ($p < 0.05$).

The purpose of this study was to determine the thermal stability of FB_2 in an aqueous matrix-free environment under conditions that may be encountered when foods are processed. Processing temperatures of 100 and 125 °C were chosen since they are used when foods are boiled and retorted, respectively. The other temperatures studied here (150−200 °C) are within the range used to bake, extrude, and fry corn-based foods. The pH values used in the thermal processing study were used to mirror those found in corn-based foods. Batters used to make corn muffins/breads are typically at neutral pH. Buffer at pH 10 was used to simulate the high pH values (>10) seen during the process of nixtamilization or alkaline cooking and steeping of corn. Buffer at pH 4.0 was used to mirror the low pH values (3.5−4.0) encountered in the wet milling operation.

The results reported here indicate that, similar to FB_1, FB_2 is a fairly heat stable compound in an aqueous environment. Thermal processes such as boiling or retorting, which occur at temperatures <125 °C, would be expected to have little effect on fumonisin content. However, processing at temperatures >150 °C (frying, extrusion, baking) may result in the destruction of FB_2 and lead to a decrease in the overall fumonisin content.

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