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# Effects of Thermal Processing on the Stability of Fumonisin B<sub>2</sub> in an Aqueous System

Lauren S. Jackson

*Food and Drug Administration, National Center for Food Safety and Technology, Summit-Argo, Illinois*

Jason J. Hlywka

*University of Nebraska - Lincoln*

Kannaki R. Senthil

*Illinois Institute of Technology, National Center for Food Safety and Technology, Summit-Argo, Illinois*

Lloyd B. Bullerman

*University of Nebraska-Lincoln, lbullerman1@unl.edu*

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# Effects of Thermal Processing on the Stability of Fumonisin B<sub>2</sub> in an Aqueous System

**Keywords:** *Fumonisin B<sub>2</sub>*; 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<sub>1</sub> (FB<sub>1</sub>) 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<sub>2</sub> has been shown to cause ELEM in ponies (Ross et al., 1994) and cytotoxicity in mammalian cell lines (Gelderblom et al., 1993). FB<sub>1</sub> and FB<sub>2</sub> have been found to inhibit sphingolipid biosynthesis by blocking the conversion of sphinganine to ceramide (Wang et al., 1991; Norred et al., 1992).

Fumonisin is a diester of propane-1,2,3-tricarboxylic acid and a pentahydroxycosane 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<sub>1</sub> and FB<sub>2</sub> are the major toxins in contaminated corn. In corn contaminated with *Fusarium proliferatum*, the ratio of FB<sub>1</sub> to FB<sub>2</sub> is approximately 3 to 1 (Ross et al., 1992). Structurally, FB<sub>2</sub> differs from FB<sub>1</sub> 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<sub>1</sub>. In contrast, baking (190 and 220 °C) muffins from contaminated cornmeal resulted in a partial apparent loss of FB<sub>1</sub> (Scott and Lawrence, 1994). Dupuy et al. (1993) and Jackson et al. (1996) reported that the loss of FB<sub>1</sub> 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<sub>2</sub>. The objective of this study was to determine the thermal stability of FB<sub>2</sub> in an aqueous matrix-free environment at acidic, neutral, and basic pH levels.

## MATERIALS AND METHODS

FB<sub>2</sub> and *o*-phthaldialdehyde (OPA) were purchased from Sigma Chemical Co. (St. Louis, MO). Fully and partially hydrolyzed FB<sub>2</sub> qualitative standards were prepared by incubating pure FB<sub>2</sub> with 1 N KOH (Rice and Ross, 1994). All reagents were of analytical grade, and solvents were of high-performance liquid chromatography (HPLC) grade.

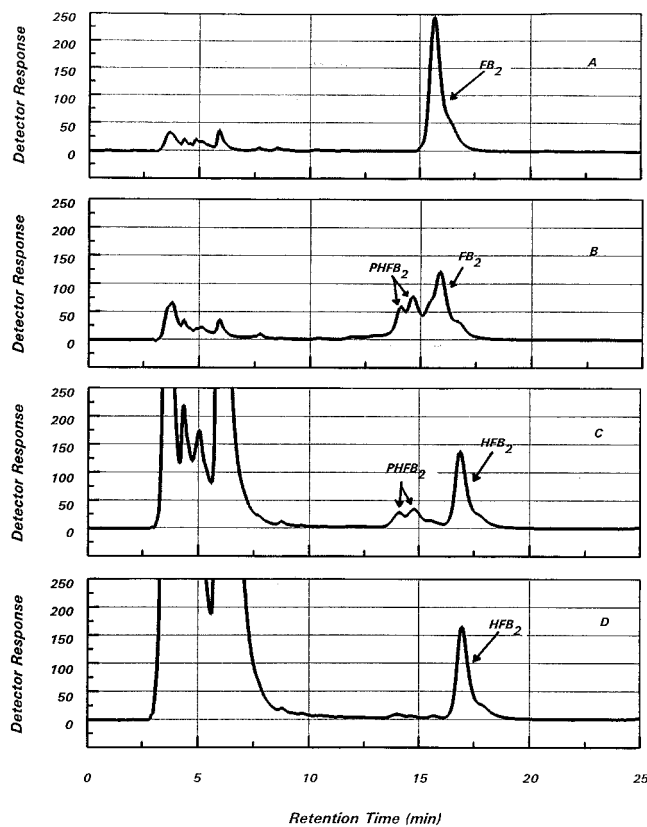
FB<sub>2</sub> solutions (5 ppm) were prepared in Teorell and Stenhagen's citrate-phosphate-borate buffer (CRC, 1968) adjusted to pH 4, 7, or 10. This buffer was chosen since it has a broad pH range (2–12). The solutions (500 mL) were placed in a 1-L stainless steel pressure vessel (Parr Instrument Co., 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<sub>2</sub> 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<sub>2</sub> levels as previously described by Jackson et al. (1996).

**HPLC Determination of FB<sub>2</sub>.** Losses of FB<sub>2</sub> in the processed solutions were measured according to the method of Shephard et al. (1990) with modifications (Jackson et al., 1996). Because FB<sub>2</sub> was processed in aqueous buffer, steps normally used to extract and purify fumonisin from corn were omitted. Consequently, the FB<sub>2</sub> solutions required minimal preparation for analysis by HPLC, and recovery correction was not necessary. A 10- $\mu$ L aliquot of the FB<sub>2</sub>/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 Millennium 2010 software (Waters) was used to identify and quantify FB<sub>2</sub> in the solutions. Separations were carried out at 23 °C on a Supelco (Bellefonte, PA) ODS-80 column (4.6 mm  $\times$  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<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub>.

**Safety Precaution.** FB<sub>2</sub> is a suspected carcinogen and should be handled with care.



**Figure 1.** HPLC chromatograms using fluorescence detection (335-nm excitation wavelength and 440-nm emission wavelength) for  $\text{FB}_2$  dissolved in an aqueous buffer at pH 10. Chromatograms A, B, C, and D refer to the  $\text{FB}_2$  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.  $\text{FB}_2$ , partially hydrolyzed  $\text{FB}_2$  (PH $\text{FB}_2$ ), and fully hydrolyzed  $\text{FB}_2$  (H $\text{FB}_2$ ) are indicated by arrows. Peaks with retention times of less than 10 min have not been identified.

## RESULTS AND DISCUSSION

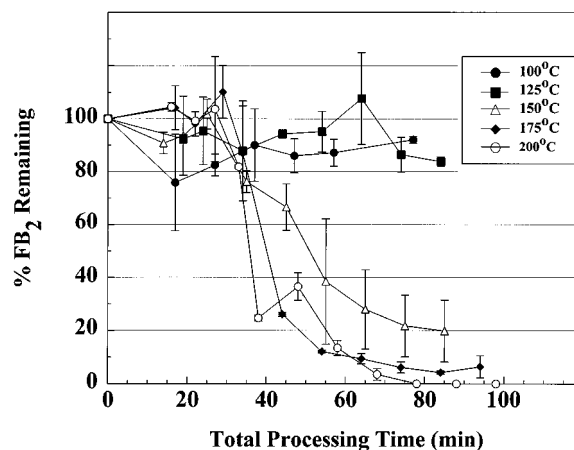
**Thermal Decomposition Products of  $\text{FB}_2$ .** HPLC chromatograms for  $\text{FB}_2$  processed at 200 °C (pH 10) are shown in Figure 1. The chromatograms indicate that the concentration of  $\text{FB}_2$  (retention time of approximately 15.7 min) decreased during processing, while the levels 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)  $\text{FB}_2$  standards, they were tentatively identified as PH $\text{FB}_2$  and H $\text{FB}_2$  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  $\text{C}_{22}$  aminopolyol backbone and tricarballic acid in the presence of heat and strong acid or base. For example, fully hydrolyzed  $\text{FB}_1$  can be found in tortillas prepared from corn treated with calcium hydroxide and heat (Hendrich et al., 1993). Hopmans and Murphy (1993) detected H $\text{FB}_1$  in tortilla chips, masa, and canned corn. However, little is known about the levels of hydrolyzed  $\text{FB}_2$  in these and other thermally processed corn-based foods.

The data presented here (Figure 1) indicate that the thermal processing of  $\text{FB}_2$  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,

**Table 1. Statistical Analysis of Variance of Time, Temperature, and pH on the Loss of  $\text{FB}_2$  during Processing**

source	DF	sum of squares	mean squares	F value	Pr > F
I. Dependent Variable: Percent $\text{FB}_2$ 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 $\text{FB}_2$ 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			



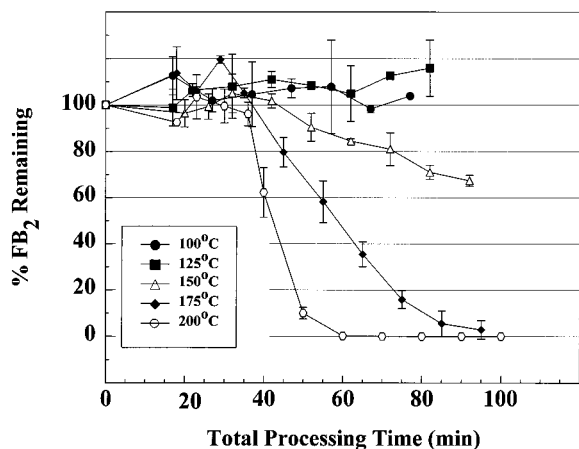
**Figure 2.** Effects of processing temperature and time on the decomposition of  $\text{FB}_2$  in an aqueous buffer at pH 4. Each point represents the average of two replicates, and error bars indicate one standard deviation of the mean.

the major species throughout the process was H $\text{FB}_2$ , while at pH 4 and 7, PH $\text{FB}_2$  was also present.

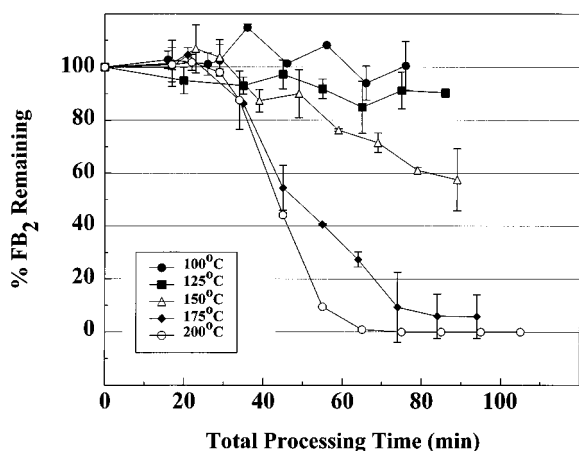
**Effect of pH, Time, and Temperature on the Stability of  $\text{FB}_2$ .** Statistical analysis of the processing data was performed to determine if independent variables (time, temperature, and pH) were related to the dependent variable (percent  $\text{FB}_2$  remaining after processing). Results of a three-way ANOVA indicate highly significant effects ( $p < 0.01$ ) of pH, time, and temperature on loss of  $\text{FB}_2$  (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  $\text{FB}_2$  during thermal processing depended on the pH of the solution. Overall,  $\text{FB}_2$  was least stable at pH 4 (Figure 2) and most stable at pH 7 (Figure 3). At processing temperatures <200 °C, the decomposition of  $\text{FB}_2$  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  $\text{FB}_2$ . After 60 min of processing at 200 °C, all  $\text{FB}_2$  was decomposed at each pH level.

Figures 2–4 and Table 1 indicate that the rate of decomposition of  $\text{FB}_2$  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

**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 ( $R^2$ )<sup>a</sup>

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

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

reaction constants for the decomposition of  $FB_2$  (Table 2) were in general agreement with those reported by Jackson et al. (1996) for  $FB_1$ .

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 nixtamalization 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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**Lauren S. Jackson,\*† Jason J. Hlywka,‡  
Kannaki R. Senthil,§ and Lloyd B. Bullerman‡**  
*Food and Drug Administration, National Center for Food Safety and Technology, Summit-Argo, Illinois 60501, Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska 68583, and Illinois Institute of Technology, National Center for Food Safety and Technology, Summit-Argo, Illinois 60501*

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\* Author to whom correspondence should be addressed.

† Food and Drug Administration.

‡ University of Nebraska.

§ Illinois Institute of Technology.