Effects of Time, Temperature, and pH on the Stability of Fumonisin B_1 in an Aqueous Model System

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Fumonisins, mycotoxins produced by *Fusarium moniliforme* in corn, have been implicated in several animal and human diseases. The effects of processing time and temperature on fumonisin B_1 (FB_1) stability (5 ppm) in aqueous solutions at pH 4, 7, and 10 were determined. Analysis of the thermally processed solutions by liquid chromatography/mass spectrometry indicated the predominant presence of hydrolysis products of FB_1. The rate and extent of FB_1 decomposition increased with processing temperature. After processing at ≤125 °C for 60 min, <27% of FB_1 was lost; after 60 min at 150 °C, 18−90% was lost, depending on buffer pH. Overall, FB_1 was least stable at pH 4 followed by pH 10 and 7, respectively. At ≥175 °C, >90% of FB_1 was lost after processing for 60 min, regardless of pH. FB_1 levels may be substantially reduced in foods that reach ≥150 °C during processing.

Keywords: *Fumonisin B_1; thermal processing; decomposition*

INTRODUCTION

Fumonisins are a group of mycotoxins produced by *Fusarium moniliforme* and *Fusarium proliferatum*, two of the most prevalent molds associated with corn and other grains. These compounds have been implicated as the causative agents in a variety of naturally occurring animal diseases including equine leukoencephalomalacia (Sydenham et al., 1992; Wilson et al., 1992) and porcine pulmonary edema (Harrison et al., 1990), as well as experimentally induced hepatotoxicity, nephrotoxicity, and hepatic cancer in rats (Gelderblom et al., 1991, 1992) and atherosclerosis in nonhuman primates (Fincham et al., 1992). Epidemiological studies have suggested that fumonisins may be partially responsible for the high incidence of esophageal cancer in the Transkei region of southern Africa (Sydenham et al., 1991; Rheeder et al., 1992), the Linxian region of China (Cheng et al., 1985), and the northeast region of Italy (Franceschi et al., 1990).

Data on the toxicity and carcinogenicity of the fumonisins suggest that these compounds should be evaluated as potential risks to human and animal health. Fumonisins B_1 (FB_1) and B_2 (FB_2), two of the most abundant forms of fumonisin in food, have been classified as possible human carcinogens by the International Agency for Research on Cancer (1993). Work is under way at the U.S. Food and Drug Administration (FDA) National Center for Toxicological Research (NCTR) and other government agencies and academic institutions to determine the health implications of chronic consumption of fumonisins.

Surveys have shown that fumonisins are found worldwide in virtually all types of corn products (Sydenham et al., 1991; Stack and Eppley, 1992; Murphy et al., 1993; Doko and Visconti, 1994). In a survey of corn-based foods purchased in the Washington, DC, area, Stack and Eppley (1992) reported higher concentrations of fumonisins in corn meal and grits than in thermally processed products (cereals, tortilla chips, corn chips). Similar results were reported for products purchased in Switzerland (Pittet et al., 1992) and in Italy (Doko and Visconti, 1994). Murphy et al. (1993) reported that more than half of the corn obtained from the midwestern United States during a 3-year sampling period (1988−1991) contained fumonisins. FB_1 and FB_2 concentrations ranged from 0 to 38 ppm and from 0 to 12 ppm, respectively. Corn screenings appeared to be a source of particularly high levels of fumonisins, averaging 10 times the fumonisin level in whole corn. Cagampang (1994) found that fumonisins were most concentrated in the germ and bran fractions of dry-milled corn and least concentrated in the flour and flaking grits fractions.

Currently, increased attention has been directed at establishment of residue limits for fumonisins in corn as well as methods of detoxification. Studies performed thus far on the effects of different methods for reducing the fumonisin content of corn products have shown variable results. Ammoniation of corn, a method for detoxifying aflatoxins, was shown to have little efficacy in reducing fumonisin content or toxicity (Norred et al., 1991). Conversely, Park et al. (1992) demonstrated a significant decrease in the FB_1 content of naturally contaminated corn after ammonia treatment at high pressure/ambient temperature and low pressure/high temperatures. Bothast et al. (1992) found that FB_1 was not degraded in fermentation processes and that the toxin concentrated in the spent grains rather than in the distilled alcohol. A physical method for removing "fines" from bulk shipments of corn has been an effective method for reducing the fumonisin levels of corn (Sydenham et al., 1994).

Several studies have focused on the effect of thermal processing on the fumonisin content of food. Alberts et

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al. (1990) reported that FB_1 was heat-stable; no toxin was lost when culture material of *F. moniliforme* was boiled for 30 min. When raw milk spiked with FB_1 and FB_2 was pasteurized (heated at 62 °C for 30 min), there were no losses of either toxin (Maragos and Richard, 1994). In contrast, Scott and Lawrence (1994) observed losses of FB_1 and FB_2 exceeding 70% in corn meal heated to 190 °C for 60 min and to 220 °C for 25 min. Similarly, fumonisin content was partially reduced in corn meal muffins baked at 220 °C for 25 min (Scott and Lawrence, 1994). Dupuy et al. (1993) reported that the decomposition of fumonisin in dry corn heated at 100−150 °C followed first-order kinetics but at a rate indicative of high thermal stability. Studies by Bordson et al. (1993) and Scott and Lawrence (1994) have indicated that the observed decreases in the fumonisin content of processed foods may be due to matrix-related difficulties of recovery and detection, rather than to actual fumonisin decomposition.

The objective of this study was to determine the thermal stability of FB_1 in an aqueous environment at acidic, neutral, and basic pH levels. Buffered solutions of 5 ppm of FB_1 at pH 4, 7, and 10 were heated at temperatures of 100−235 °C for 60 min. Interfering matrix effects, as such, were nonexistent, and analytical samples required minimal preparation for high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Materials. FB_1 for the processing studies was kindly supplied by Dr. Robert Eppley (FDA, Washington, DC). FB_1 standard and *o*-phthaldialdehyde (OPA) were purchased from Sigma Chemical Co. (St. Louis, MO). Fully hydrolyzed FB_1 standard was prepared by incubating pure FB_1 with 1 N KOH. Partially hydrolyzed FB_1 standard was purified from culture material by HPLC. All reagents were of analytical grade, and solvents were of HPLC grade.

Processing of Model System. FB_1 solutions (5 ppm) were prepared in Teorell and Stenhagen's citrate−phosphate−borate buffer (CRC, 1968) adjusted to pH 4, 7, or 10, and 500-mL aliquots were placed in a 1-L stainless steel reaction vessel (Parr Instrument Co., Moline, IL) and heated to processing temperatures of 100−235 °C for 1 h with an electric heating mantle. A Parr Model 4841 proportional controller was used to maintain the reaction mixture at the desired temperature while it was agitated at a constant speed. During heating, aliquots of the reaction mixture were removed from the vessel through a sampling valve and analyzed for FB_1 content by HPLC. 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_1 levels. Processed aliquots were immediately placed in an ice bath and then frozen to prevent further reaction.

HPLC Determination of FB_1. Loss of FB_1 in the processed solutions was measured according to the method of Shephard et al. (1990). A 50-μL aliquot of the processed FB_1 solution was mixed with 200 μL of an OPA reagent prepared by dissolving 40 mg of OPA in 1 mL of methanol and adding 5 mL of 0.1 M sodium borate buffer and 50 μL of 2-mercaptoethanol. A 10-μL aliquot of the FB_1/OPA mixture was used for HPLC determination. An HPLC system consisting of a Waters (Milford, MA) Model 510 pump, a Model U6K injector, a Model 740 fluorescence detector (335-nm excitation wavelength and 440-nm emission wavelength), and a Model 1020 integrator (Perkin-Elmer, Norwalk, CT) was used to separate and quantify FB_1 in the solutions. Separations were carried out on a Supelco (Bellefonte, PA) ODS-80 column (4.6 mm × 25 cm) with a Supelco LC-18-DB precolumn at 23 °C. The mobile phase was methanol/1 M sodium dihydrogen phosphate (80:20) adjusted to pH 3.3 with phosphoric acid, and the flow rate was 0.8 mL/min.

For comparison, FB_1 solutions from several processing runs were analyzed according to the OPA derivatization method of Rice and Ross (1994). A 100-μL aliquot of the solution was derivatized at room temperature through a 10-min reaction with 100 μL of 0.1 M sodium borate (pH 8.2), 100 μL of OPA reagent (2 mg of OPA and 20 μL of 2-mercaptoethanol in 10 mL of acetonitrile), and 100 μL of water. The components in a 5-μL aliquot of the derivatized solution were separated on a Perkin-Elmer 3-μm C_{18} high-speed column, 3 cm × 4.6 mm, with a Waters Model 510 HPLC pump equipped with a Waters Model U6K injector. FB_1 was identified with a Spectroflow 980 programmable fluorescence detector (Kratos Analytical, Ramsey, NJ), with an excitation wavelength of 335 nm and an emission wavelength of 418 nm, and quantitated with an HP3395 integrator (Hewlett-Packard, Avondale, PA). The HPLC conditions used to separate FB_1 and its decomposition products were as follows: an isocratic mobile phase of 40% acetonitrile and 60% 0.1 M potassium dihydrogen phosphate (adjusted to pH 3.3 with phosphoric acid) at a flow rate of 1.0 mL/min and a temperature of 22 °C.

Mass Spectrometry. Mass spectrometry was used to identify decomposition products resulting from some of the thermal processing runs. To enable detection of the products by the liquid chromatography/mass spectrometry (LC/MS) system, concentrated FB_1 solutions (approximately 130 ppm) were thermally processed according to the procedures described above. Fifty microliters of the processed FB_1 solution was mixed with 50 μL of 40 mM formic acid buffer, and 20 μL of the mixture was used for the LC/MS determinations. A Hewlett-Packard Model 1050 quaternary gradient HPLC pump was used to supply a 200 μL/min flow and solvent gradient to a J-sphere ODS-L80 column, 2.0 × 250 mm (YMC Inc., Wilmington, NC). An acetonitrile gradient beginning at 25% B for 5 min and then changing linearly to 40% B produced the best separations (solvent A = 99% H_2O/1% acetonitrile, 0.1% formic acid; solvent B = 10% H_2O/90% acetonitrile, 0.1% formic acid). The entire 200 μL/min effluent from the column was directed into the electrospray ion source of a Model TSQ-7000 triple quadrupole mass spectrometer (Finnigan, San Jose, CA). The ion source was operated with a needle voltage of 4.5 kV, N_2 gas pressure of 70 psi, and capillary temperature of 275 °C. The instrument was operated in the positive ion mode and scanned over the range of 350−800 amu at 1 s/scan. Peaks were identified by comparing retention times and $(M + H)^+$ ions with those of standards.

Kinetic Calculations. The rate of decomposition of FB_1 (R_A) was expressed by the equation

$$R_A = -\mathrm{d}(C_A)/\mathrm{d}t = kC_A^{\,n} \quad (1)$$

where C_A is the remaining FB_1 concentration (ppm) at time t (min), k is the reaction rate constant (min^{-1}), and n is the reaction order. Several equations that could describe the individual reaction order are obtained by integration

$$\text{zero order} \qquad C_0 - C_A = kt \quad (2)$$

$$\text{first order} \qquad \ln(C_A) - \ln(C_0) = -kt \quad (3)$$

$$\text{second order} \qquad 1/C_A - 1/C_0 = kt \quad (4)$$

where C_0 and C_A refer to the initial FB_1 concentration and the remaining FB_1 concentration (ppm) after time t (min), respectively. C_0 was taken to be the concentration of FB_1 when the desired processing temperature was reached. Processing time was plotted with respect to C_A, $\ln(C_A)$, and $1/C_A$. Reaction order was obtained by determining which plot had the best linear fit. The reaction rate constant was calculated from the slope of the linearized rate law equation. The half-life was calculated from the rate law equation by allowing C_A to equal $0.5C_0$.

Statistical Analysis. All processing runs were performed in triplicate. Means and standard deviations were calculated with Minitab statistical software. Linear regression analyses, used to determine reaction constants, half-lives of FB_1, and

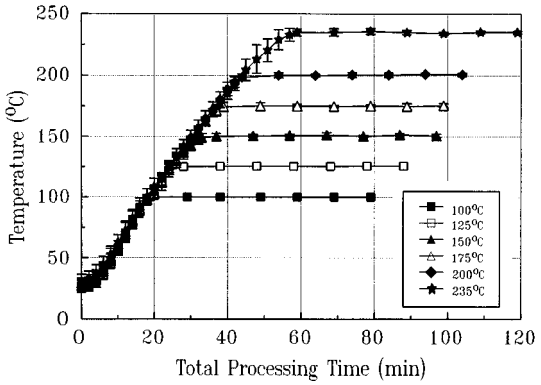

Figure 1. Temperature recorded in aqueous solutions of FB_1 (5 ppm) during thermal processing. The solutions were processed for 1 h at temperatures of 100−235 °C in a 1-L Parr pressure reactor. Points indicate the average for nine replicates. Error bars indicate one standard deviation of the mean.

correlation coefficients, were performed by using Psiplot graphics software (Poly Software International, Salt Lake City, UT).

RESULTS AND DISCUSSION

Reproducibility of Processing Runs. To measure the effects of processing time and temperature on FB_1, it was necessary to have a processing system that would give reproducible results. The time−temperature profiles for the processing runs (Figure 1) indicate that the heating process was reproducible. The come-up times, i.e., the length of time necessary for FB_1 solutions to reach the desired processing temperatures, were 19, 28, 33, 39, 44, and 53 min for temperatures of 100, 125, 150, 175, 200, and 235 °C, respectively. The variation in temperature at each processing time was ±4 °C during the come-up time and ±2 °C after the processing temperature was reached.

Identification of Thermal Decomposition Products of FB_1. The methods of Shephard et al. (1990) and Rice and Ross (1994), with modifications, were used to monitor the decomposition of FB_1 during processing. Because of the absence of matrix effects and interferences in the processed solutions, steps normally used to extract and purify fumonisin were omitted. Consequently, the FB_1 solutions required minimal preparation for HPLC, and it was not necessary to correct for recovery.

HPLC chromatograms for FB_1 processed at 175 °C are shown in Figures 2−4. These chromatograms, obtained according to the method of Shephard et al. (1990), were similar to those acquired following the method of Rice and Ross (1994). However, the pH of the FB_1 solution affected the extent of OPA derivatization and the sensitivity of the response when this method was used. With the method of Rice and Ross (1994), the detector response to FB_1 was reduced at pH 4 compared with that at pH 7 and 10. This effect was not apparent when the method of Shephard et al. (1990) was used.

The chromatograms (Figures 2−4) indicate that the concentration of FB_1 (retention time of approximately 13.2 min) decreased over time during processing, while the levels of two apparent decomposition products (retention times of 12.7 and 15.1 min) generally increased. LC/MS analysis of the thermally processed solutions indicated that at least three decomposition products existed: two partially hydrolyzed products and a fully hydrolyzed form. In the chromatograms shown, the partially hydrolyzed isomers ($PHFB_1$) coeluted at a

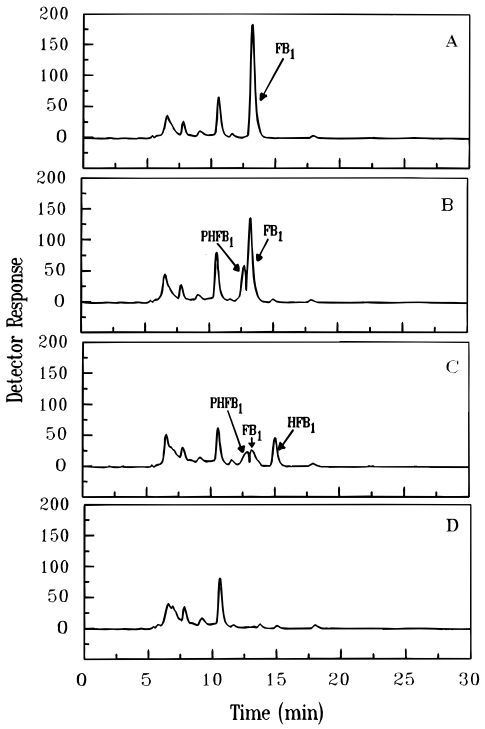

Figure 2. HPLC chromatograms using fluorescence detection (335-nm excitation wavelength and 440-nm emission wavelength) for FB_1 dissolved in an aqueous buffer at pH 4. Chromatograms A, B, C, and D refer to the FB_1 solution before processing, the solution after reaching 175 °C, the solution after 30 min at 175 °C, and the solution after 60 min at 175 °C, respectively. FB_1, partially hydrolyzed FB_1 ($PHFB_1$), and fully hydrolyzed FB_1 (HFB_1) are indicated by arrows. Unidentified peaks at retention times of 5.0−12.5 min were present in reagent blanks.

retention time of 12.7 min; the fully hydrolyzed FB_1 (HFB_1) eluted at 15.1 min.

Complete hydrolysis of FB_1 to tricarballylic acid and the C_{22} aminopolyol backbone when FB_1 was heated in the presence of a strong acid or base was also reported by Bezuidenhout et al. (1988), Jackson and Bennett (1990), and Sydenham et al. (1990a,b). HFB_1 can be found in tortillas, i.e., corn which is treated with calcium hydroxide and heat (Hendrich et al., 1993). However, little is known about the levels of HFB_1 and $PHFB_1$ in other thermally processed corn-based foods.

The pH had no apparent effect on the types of decomposition products found in the processed solutions. However, at pH 10 the major decomposition species throughout the process was HFB_1, whereas at pH 4 and 7 $PHFB_1$ was also present. It should be noted that FB_1 and its hydrolysis products were not observed in HPLC chromatograms after 60 min at 175 °C and pH 4 (Figure 2) or by LC/MS analysis of the same solution. These results were also observed when FB_1 solutions at pH 4 were processed for 60 min at temperatures of 200 and 235 °C. It is possible that the decomposition products formed at these temperatures were not derivatizable by the OPA reagent and thus not detected by HPLC or that these decomposition products may not have been in the mass range scanned by LC/MS.

Effect of pH, Time, and Temperature on FB_1 Decomposition. Figures 5−7 illustrate that the decomposition of FB_1 during thermal processing depended on the pH of the solution. Overall, FB_1 appeared least stable at pH 4 and most stable at pH 7. At processing temperatures <200 °C, loss of FB_1 was most rapid and extensive at pH 4, followed by pH 10 and 7, respectively.

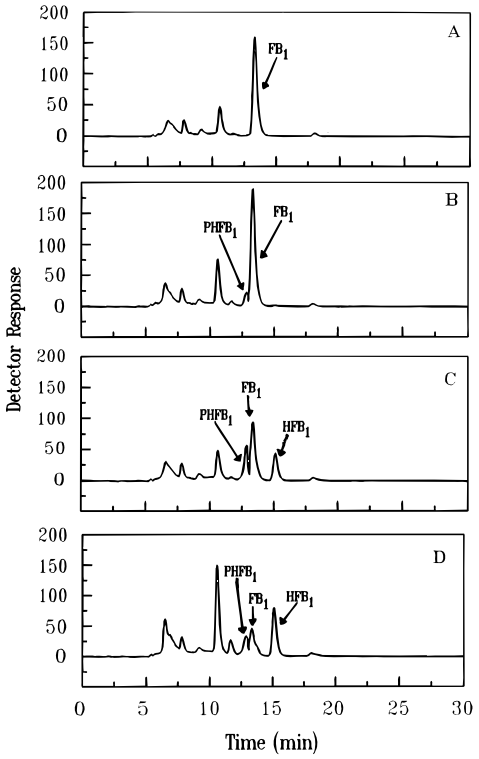

Figure 3. HPLC chromatograms using fluorescence detection (335-nm excitation wavelength and 440-nm emission wavelength) for FB_1 dissolved in an aqueous buffer at pH 7. Chromatograms A, B, C, and D refer to the FB_1 solution before processing, the solution after reaching 175 °C, the solution after 30 min at 175 °C, and the solution after 60 min at 175 °C, respectively. FB_1, partially hydrolyzed FB_1 ($PHFB_1$), and fully hydrolyzed FB_1 (HFB_1) are indicated by arrows. Unidentified peaks at retention times of 5.0−12.5 min were present in reagent blanks.

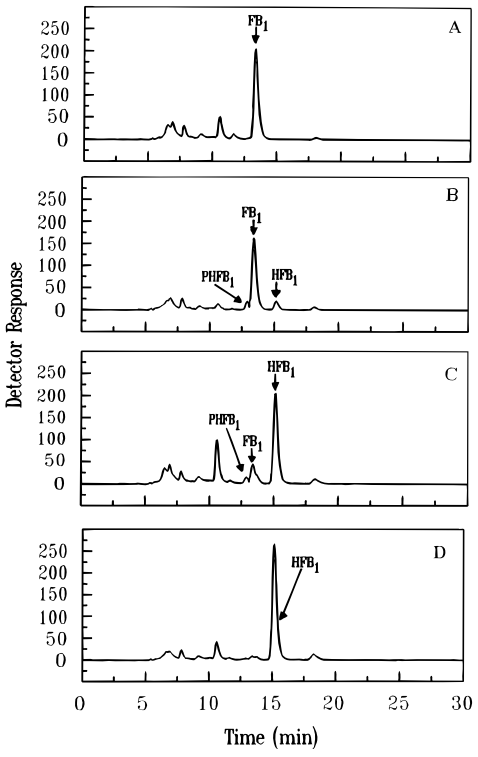

Figure 4. HPLC chromatograms using fluorescence detection (335-nm excitation wavelength and 440-nm emission wavelength) for FB_1 dissolved in an aqueous buffer at pH 10. Chromatograms A, B, C, and D refer to the FB_1 solution before processing, the solution after reaching 175 °C, the solution after 30 min at 175 °C, and the solution after 60 min at 175 °C, respectively. FB_1, partially hydrolyzed FB_1 ($PHFB_1$), and fully hydrolyzed FB_1 (HFB_1) are indicated by arrows. Unidentified peaks at retention times of 5.0−12.5 min were present in reagent blanks.

At 200 and 235 °C, pH had no effect on the rate of loss of FB_1. After 60 min of processing at temperatures >175 °C, all FB_1 was decomposed at each pH level.

Figures 5−7 indicate that the rate of decomposition of FB_1 is highly dependent on temperature and that, in general, the extent of decomposition increases with processing temperature and time. No significant losses in FB_1 occurred during processing at 100 °C, and <27% of FB_1 was lost at 125 °C. Similar results were reported by Alberts et al. (1990), who found that boiling culture material of *F. moniliforme* for 30 min did not reduce FB_1 concentration. Dupuy et al. (1993) found minimal losses of FB_1 in naturally contaminated dry corn meal heated at 100 °C for 45 min, whereas 42% was lost after 135 min. The losses reported by Dupuy et al. (1993) may be attributed to the longer heating time and possible interactions of the fumonisins with other components of the corn during heating.

At the three pH levels, FB_1 did not appear to be sufficiently degraded until the processing temperature reached 150 °C. After 60 min at 150 °C, loss of FB_1 ranged from 18 to 90%, with the greatest decomposition occurring at pH 4 and the least at pH 7. At temperatures ≥175 °C, >90% of FB_1 was lost after the 60-min processing time, regardless of pH. At 200 and 235 °C, all fumonisin was lost after 10 min of processing. The results shown here are generally in agreement with the results of previous studies on the thermal stability of FB_1 in corn. Dupuy et al. (1993) observed losses of 45 and 87% of FB_1 in dry corn heated for 40 min to 125 and 150 °C, respectively. Scott and Lawrence (1994)

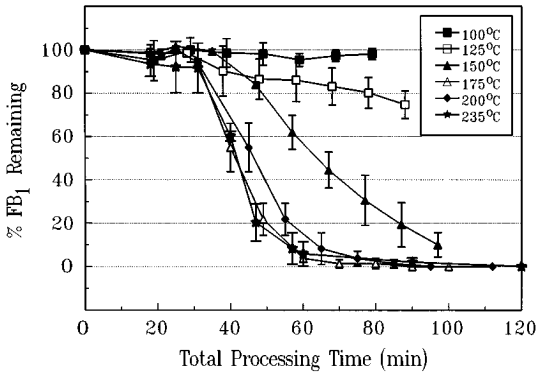

Figure 5. Effects of processing temperature and time on the decomposition of FB_1 in an aqueous buffer at pH 4. Each point represents the average of three replicates, and error bars indicate one standard deviation of the mean.

reported complete loss of FB_1 and FB_2 when spiked dry corn was heated at 220 °C for 25 min. These authors also reported that fumonisin concentrations were reduced by 70−80% in moist corn meal heated for 60 min at 190 °C.

The loss of FB_1 in pH 4, 7, and 10 buffers heated at 125, 150, 175, and 200 °C followed an apparent first-order reaction. Linear correlation coefficients (0.875−0.999) shown in Table 1 demonstrate the straight-line relationships between processing time and the natural log of the fraction of FB_1 remaining. Figure 8 demonstrates these relationships for FB_1 solutions processed at 150 °C. For processing temperatures of 175 and 200 °C, initial FB_1 concentrations (C_0) were taken as the

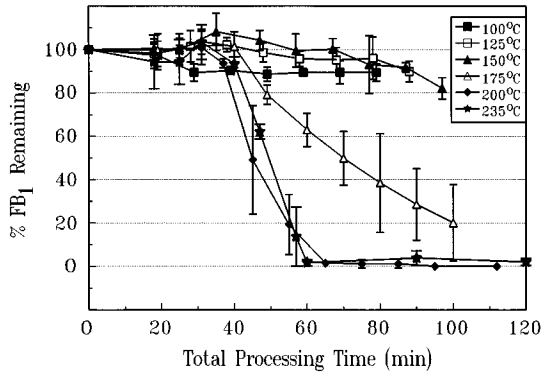

Figure 6. Effects of processing temperature and time on the decomposition of FB_1 in an aqueous buffer at pH 7. Each point represents the average of three replicates, and error bars indicate one standard deviation of the mean.

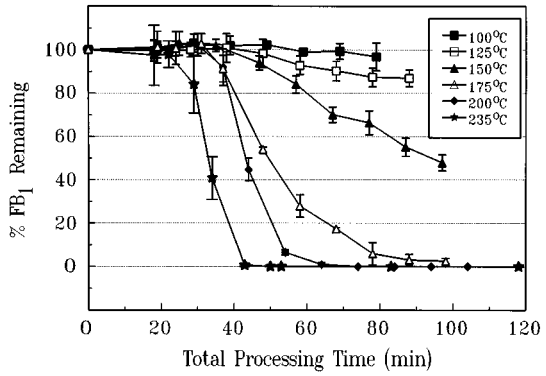

Figure 7. Effects of processing temperature and time on the decomposition of FB_1 in an aqueous buffer at pH 10. Each point represents the average of three replicates, and error bars indicate one standard deviation of the mean.

Table 1. Reaction Rate Constants (k) and Half-Lives ($t_{1/2}$) for the Decomposition of FB_1 in Teorell and Stenhagen's Phosphate−Citrate−Borate Buffer at pH 4, 7, and 10: Linear Relationships between Processing Time and Fraction of Remaining FB_1 Are Indicated by Correlation Coefficients (R^2)

temp, °C	pH	k, min^{-1}	$t_{1/2}$, min	R^2
125	4	0.00357	196	0.961
150	4	0.0375	24	0.970
175	4	0.1062	8	0.990
200	4	0.1102	6	0.994
125	7	0.0019	375	0.876
150	7	0.0044	162	0.942
175	7	0.0268	27	0.995
200	7	0.1170	6	0.986
125	10	0.0025	278	0.951
150	10	0.0127	57	0.987
175	10	0.0799	11	0.995
200	10	0.1891	3	0.999

concentrations of FB_1 present when the processing temperature was reached, i.e., after the come-up period.

Gould (1959) reported that the reaction mechanisms for acid hydrolysis of esters differ from those for base hydrolysis. Hydrolysis of esters in acidic and basic solutions is a pseudo-first-order reaction and a second-order reaction, respectively. However, base hydrolysis of esters in buffered solutions would be expected to follow pseudo-first-order kinetics since the concentration of hydroxide ion (a reactant in the hydrolysis reaction) remains constant. This is consistent with the results presented here.

Table 1 summarizes the kinetic data for the decomposition of FB_1 at temperatures of 125−200 °C. Half-lives (3.21−5.65 min) and pseudo-first-order reaction

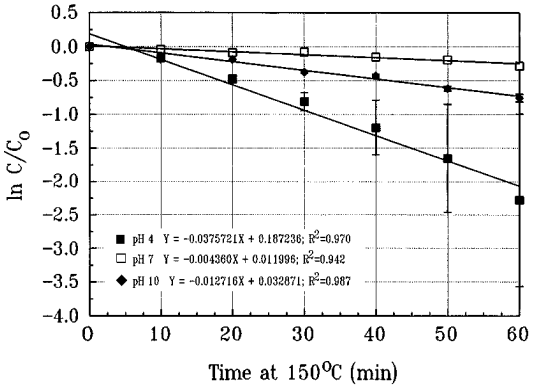

Figure 8. Pseudo-first-order rate plots for the decomposition of solutions of FB_1 at pH 4, 7, and 10 during processing at 150 °C. Error bars indicate one standard deviation of the mean.

constants (0.1102−0.1891 min^{-1}) indicate that the most rapid disappearance of FB_1 occurred at 200 °C with substantial hydrolysis occurring in the come-up period (Figures 5−7). At temperatures below 200 °C, the decomposition reaction occurred most rapidly at pH 4; at 200 °C, the reaction occurred most rapidly at pH 10.

Dupuy et al. (1993) studied the kinetics of FB_1 decomposition in dry corn during thermal processing. Comparable to the results reported here, they found that the decrease in the amount of FB_1 recovered after heating followed first-order kinetics. Dupuy et al. (1993) calculated FB_1 half-lives of 175 and 38 min at 125 and 150 °C, respectively. These half-life values are one-fourth to -half the respective values obtained at pH 7 in the aqueous model system (Table 1). At 125 °C, the FB_1 half-life of 175 min compares favorably with the calculated half-life of 196 min at pH 4 (Table 1). At 150 °C, the calculated FB_1 half-lives of 24 and 57 min at pH 4 and 10, respectively, were similar to the half-life of 38 min reported by Dupuy et al. (1993). FB_1 may decompose by different mechanisms in dry versus aqueous environments, with the decomposition dependent on the pH of the environment. The data presented here indicate that the thermal instability of FB_1 in the presence of water results primarily in the formation of hydrolysis products. Dupuy et al. (1993) did not identify the end products or reaction mechanism of decomposition in their study, but the lack of water in the system suggests that hydrolysis was not the primary reaction. Another explanation for the differences in the data is that Dupuy et al. (1993) studied decomposition of FB_1 at atmospheric pressure. In the system studied here, pressures were 0, 40, 70, 135, 230, and 440 psi at 100, 125, 150, 175, 200, and 235 °C, respectively. Pressure in the reaction vessel may have affected the reaction rates and mechanisms.

Bordson et al. (1993) and Scott and Lawrence (1994) proposed that the observed reductions in the fumonisin levels of heated corn may be due to binding of fumonisin to the corn matrix. Therefore, the loss of fumonisin as reported by Dupuy et al. (1990) could be due to matrix-related effects associated with recovery and detection, rather than chemical decomposition.

The results reported here, as well as those by Alberts et al. (1990), Dupuy et al. (1993), and Scott and Lawrence (1994), indicate that FB_1 is a fairly heat-stable compound. Thermal processing operations, such as boiling or retorting, at temperatures <125 °C would be expected to have little effect on fumonisin content. However, temperatures during baking or frying of food (>150 °C) may result in substantial losses of FB_1.

Although the hydrolysis of FB_1 was evident from HPLC and mass spectroscopy, detoxification of FB_1 cannot be assumed. Little is known about the toxicological effects of the fumonisin hydrolysis products reported here. Hendrich et al. (1993) found that treating FB_1-containing corn with lye and heat resulted in a product more toxic than untreated corn when fed to rats. These authors hypothesized that hydrolysis and other unidentified breakdown products of FB_1 may have played a role in the toxicity of the lye-treated corn. Wang et al. (1991) and Norred et al. (1992) investigated the effects of fumonisin and hydrolyzed fumonisin on *in vitro* sphingolipid synthesis. Their work suggests that the C_{22} aminopentol backbone of FB_1 is responsible for the alterations in sphingolipid synthesis in animals which exhibit fumonisin toxicity. They found that FB_1, FB_2, and base-hydrolyzed FB_1 inhibited microsomal sphinganine *N*-acyltransferase, a rate-limiting enzyme in sphingomyelin synthesis.

Conclusions. This is the first systematic study of the thermal stability of fumonisin in the absence of a food matrix. Consequently, the results presented here reflect true losses of FB_1 rather than binding or analytical problems with recovery. These data confirm that FB_1 is fairly heat-stable, especially at neutral pH. In general, the loss of FB_1 was more rapid and extensive in alkaline or acidic environments than at neutral pH. Physical parameters such as processing time and temperature are critical factors that affect decomposition of FB_1. These results suggest that when foods are heated at temperatures encountered in boiled or retorted foods, i.e., 100−125 °C, little change in FB_1 content would be expected. Foods that reach temperatures of >150 °C during processing (baking or frying) may have substantial losses of fumonisin. More work is needed to determine the effects of thermal processing operations on the stability of FB_1 in contaminated corn. In addition, research is needed to determine the stability of other fumonisin isomers (FB_2, FB_3, etc.) during processing.

LC/MS analysis of thermally processed FB_1 solutions identified hydrolysis products; however, little is known about the toxicity of these products. Studies are currently under way to examine the biological toxicity of thermally processed FB_1 solutions.

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