

## Characterization of Fumonisin B<sub>1</sub>–Glucose Reaction Kinetics and Products

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The reaction of fumonisin B<sub>1</sub> with the reducing sugar D-glucose can block the primary amine group of fumonisin B<sub>1</sub> and may detoxify this mycotoxin. A method to separate hundred milligram quantities of fumonisin B<sub>1</sub>–glucose reaction products from the excess D-glucose with a reversed-phase C<sub>18</sub> cartridge was developed. Mass spectrometry revealed that there were four primary products in this chain reaction when fumonisin B<sub>1</sub> was heated with D-glucose at 65 °C for 48 h: *N*-methyl-fumonisin B<sub>1</sub>, *N*-carboxymethyl-fumonisin B<sub>1</sub>, *N*-(3-hydroxyacetyl)-fumonisin B<sub>1</sub>, and *N*-(2-hydroxy, 2-carboxyethyl)-fumonisin B<sub>1</sub>. The *N*-(1-deoxy-D-fructos-1-yl) fumonisin B<sub>1</sub> (fumonisin B<sub>1</sub>–glucose Schiff's base) was detected by mass spectrometry when fumonisin B<sub>1</sub> was heated with D-glucose at 60 °C. The nonenzymatic browning reaction of fumonisin B<sub>1</sub> with excess D-glucose followed apparent first-order kinetics. The activation energy, *E*<sub>a</sub>, was 105.7 kJ/mol. Fumonisin B<sub>1</sub> in contaminated corn could precipitate the nonenzymatic browning reaction with 0.1 M D-glucose at 60 and 80 °C.

**KEYWORDS:** Fumonisin B<sub>1</sub>; fumonisin B<sub>1</sub>–glucose; detoxification; *N*-(1-deoxy-D-fructos-1-yl) fumonisin B<sub>1</sub>

### INTRODUCTION

Fumonisin B<sub>1</sub> is a family of mycotoxins produced mainly by the corn fungi *Fusarium verticillioides* (synonym *Fusarium moniliforme*) and *Fusarium proliferatum* (1, 2). Fumonisin B<sub>1</sub> is the most abundant natural contaminant in corn-based foods and feeds among the several structurally related homologues. Fumonisin B<sub>1</sub> has been found in corn and corn products in Europe (3–6), Asia (7, 8), North America (3, 9–11), and Africa (3, 12–14), and has, therefore, become an important food safety concern due to its toxicity and human and animal exposure.

Fumonisin B<sub>1</sub> can cause a variety of diseases in animals. Equine leukoencephalomalacia (15, 16) and porcine pulmonary edema (17, 18) can be caused by fumonisin B<sub>1</sub>. Fumonisin B<sub>1</sub> is hepatocarcinogenic, hepatotoxic, and nephrotoxic in rats (19, 20). Rat liver cancer can be promoted and initiated by fumonisin B<sub>1</sub> (21, 22). Fumonisin B<sub>1</sub> is cytotoxic to various mammalian cell lines (23–26). The high incidence of human esophageal cancer in South Africa and China has been associated with

fumonisin B<sub>1</sub> (7, 12). Fumonisin B<sub>1</sub> has been declared a class 2B carcinogen, which is a possible human carcinogen (27).

Fumonisin B<sub>1</sub> is very thermostable (28) and is apparently not detoxified by heating. Nixtamalization, the calcium hydroxide traditional processing technique used to produce masa (tortilla flour) from corn, can convert fumonisin B<sub>1</sub> to hydrolyzed fumonisin B<sub>1</sub>. However, this process does not reduce the toxicity of the contaminated corn (29, 30). Gelderblom et al. (24) suggested that the primary amine group of fumonisin B<sub>1</sub> was critical to the toxicity of fumonisin B<sub>1</sub> because naturally occurring *N*-acetyl-fumonisin B<sub>1</sub> was not considered toxic. Reacting fumonisin B<sub>1</sub> with reducing sugars, such as fructose or glucose, may block the primary amine group and detoxify fumonisin B<sub>1</sub> (31).

Lu et al. (32) reported that a fumonisin B<sub>1</sub>–fructose reaction mixture fed to diethylnitrosamine-initiated Fischer344/N rats resulted in significantly less hepatic cancer promotion than that observed in rats fed fumonisin B<sub>1</sub>. However, <sup>14</sup>C-hydrolyzed fumonisin B<sub>1</sub> and <sup>14</sup>C-fumonisin B<sub>1</sub>–fructose were better absorbed than <sup>14</sup>C-fumonisin B<sub>1</sub> in Fischer344/N rats (33). The reduced carcinogenic promotional activity of fumonisin B<sub>1</sub>–fructose cannot be due to reduced absorption.

The reaction of primary amines, such as fumonisin B<sub>1</sub>, with fructose is more complicated than the reaction of primary amines with glucose (34, 35). Clifford (36) investigated the Maillard nonenzymatic browning reaction of fumonisin B<sub>1</sub> and glucose.

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However, the reaction products and the reaction pathway were not characterized. The objectives of the current study were to develop a method to separate the fumonisin B<sub>1</sub>-glucose reaction products from the excess glucose, to characterize the reaction products, and evaluate the reaction kinetics of glucose with fumonisin B<sub>1</sub> in a model system and in fumonisin B<sub>1</sub>-contaminated corn.

## MATERIALS AND METHODS

**Safety Precautions.** Fumonisin B<sub>1</sub> is a class 2B carcinogen and was handled accordingly.

**Chemicals.** The fumonisin B<sub>1</sub>, used as a standard for high-performance liquid chromatography (HPLC) analysis and to produce fumonisin B<sub>1</sub>-glucose, was obtained by the liquid fermentation method of Dantzer et al. (37) and purified to >95% purity as described by Dantzer et al. (38).

*o*-Phthaldialdehyde, 2-mercaptoethanol, and  $\alpha$ -amylase (from porcine pancreas) were from Sigma Chemical Company (St. Louis, MO). All other reagents were from Fisher Scientific Company (Chicago, IL). Water was purified with the MilliQ system (Waters, Milford, MA) and used throughout.

**Quantitative Analysis of Fumonisin B<sub>1</sub> and Fumonisin B<sub>1</sub>-Glucose.** *Reversed-Phase HPLC with Fluorescence Detection of o-Phthaldialdehyde Derivative.* Fumonisin B<sub>1</sub> was detected as a derivative with *o*-phthaldialdehyde following reversed-phase HPLC separation by fluorescence detection (9). A 100- $\mu$ L aliquot of sample was mixed with 100  $\mu$ L of 50 mM potassium phosphate buffer, pH 8.3, and 100  $\mu$ L of *o*-phthaldialdehyde solution (5 mg of *o*-phthaldialdehyde and 10  $\mu$ L of 2-mercaptoethanol in 5 mL of acetonitrile) at room temperature. After 10 min, the mixture was quenched with 100  $\mu$ L of water and the mixture was injected manually into the HPLC system using a 20- $\mu$ L loop. The *o*-phthaldialdehyde solution was stored in dark at 5 °C and made fresh weekly.

The HPLC system included a 250  $\times$  4.6 mm, 5  $\mu$ m reversed-phase C<sub>18</sub> analytical column (Alltech Associates, Deerfield, IL) and a HPLC fluorescence detector (Waters model 470, Milford, MA) with the excitation wavelength at 335 nm and the emission wavelength at 440 nm (39). A gradient mobile phase system of 40 to 60% acetonitrile in 1% aqueous acetic acid using a flow rate of 1.0 mL/min was used. The column was flushed with 100% acetonitrile using a flow rate of 2.0 mL/min before recycling to initial gradient conditions. All samples were analyzed in duplicate, and the results were averaged.

*Enzyme-Linked Immunosorbent Assay (ELISA).* Total FB<sub>1</sub> and FB<sub>1</sub>-glucose reaction products concentrations were quantified using the quantitative Veratox ELISA test kit (Neogen, Lansing, MI). The absorbance of the solution was read at 650 nm with a Biotek ELx808U microplate reader (Winooski, VT). Purified fumonisin B<sub>1</sub>, purified hydrolyzed fumonisin B<sub>1</sub>, and fumonisin B<sub>1</sub>-glucose reaction mixture were tested with the ELISA test kit and the results were compared with those of the HPLC *o*-phthaldialdehyde derivatization method.

**Preparation and Cleanup of Fumonisin B<sub>1</sub>-Glucose Reaction Products for Mass Characterization.** For fumonisin B<sub>1</sub>-glucose products characterization, 50 mL of 1.39 mM fumonisin B<sub>1</sub> with 100 mM D-glucose in 50 mM potassium phosphate buffer, pH 7.0, were heated at 65 and 80 °C for 48 h or at 60 °C for 24, 48, 72, or 96 h and were further purified. To ensure the reaction was stopped at the appropriate time points between 0 and 96 h, 12 N hydrochloric acid was used to adjust the reaction mixture pH to 2.7.

A 10-g, 60-mL, reversed-phase C<sub>18</sub> SPE cartridge (Supelco, Bellefonte, PA) was attached to a vacuum multiport (Supelco). The cartridge was preconditioned with 50 mL of 100% methanol with an apparent pH 2.7, followed by 100 mL of water adjusted to a pH of 2.7 with hydrochloric acid. A 50-mL aliquot of the 1.39 mM fumonisin B<sub>1</sub> and 100 mM D-glucose reaction mixture was loaded on the cartridge. The cartridge was attached to the vacuum multiport and the solvent flow rate was adjusted to 1–2 mL/min. The cartridge was then washed with 100 mL of water and 100 mL of 30% methanol in water with an apparent pH 2.7. The cartridge was eluted with 50-mL aliquots of 40, 50, 60, 70, and 100% methanol with apparent pH of 2.7, and the eluants

were collected. Fumonisin B<sub>1</sub> and fumonisin B<sub>1</sub>-glucose products were evaluated by the HPLC *o*-phthaldialdehyde method and by ELISA. The used cartridge was rinsed with 100 mL of water with a pH 2.7 and was ready to be reused for fumonisin B<sub>1</sub>-glucose reaction product purification.

**Characterization of Fumonisin B<sub>1</sub>-Glucose Reaction Products by Mass Spectrometry.** The 60% methanol eluant obtained from the reversed-phase C<sub>18</sub> SPE cartridge was evaporated to dryness with a rotary evaporator at 35 °C. The residue was completely redissolved in methanol, and mass spectrometry was performed by the Analytical Service Laboratory in the Department of Chemistry at Iowa State University (Ames, IA). The FB<sub>1</sub> products were analyzed by positive electrospray ionization (ESI) mass spectrometry on a Finnigan TSQ 700 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA). The ESI conditions were as follows: the spray voltage was 4.5 kV; the capillary temperature was 200 °C; the scan rate was 2 s/scan; and the auxiliary gas pressure was 40 psi.

**Fumonisin B<sub>1</sub>-Glucose Nonenzymatic Browning Model System Kinetics.** Fumonisin B<sub>1</sub> and glucose in 50 mM potassium phosphate buffer (pH = 7.0) were prepared in disposable glass test tubes, then the tubes were covered with aluminum foil and heated in a convection oven. The residual free FB<sub>1</sub> was evaluated by the HPLC *o*-phthaldialdehyde derivatization method at different reaction time points. The volume of the solutions was brought back to 1.0 mL before HPLC analysis. Different initial FB<sub>1</sub> concentrations of 69, 139, 347, or 693  $\mu$ M fumonisin B<sub>1</sub> in 100 mM glucose at 80 °C were used to evaluate the effect of fumonisin B<sub>1</sub> concentration on the reaction rate. Different initial D-glucose concentrations of 10, 50, 100, 500, or 1000 mM D-glucose with 139  $\mu$ M fumonisin B<sub>1</sub> at 80 °C were used to evaluate the effect of D-glucose concentration on the reaction rate. The effects of different reducing sugars, D-glucose, D-fructose, maltose, lactose, and mannose, were investigated at 100 mM with 139  $\mu$ M fumonisin B<sub>1</sub> at 80 °C over 48 h. To determine the energy of activation, the reaction rates of 139  $\mu$ M fumonisin B<sub>1</sub> with 100 mM D-glucose were examined at 40, 50, 60, and 80 °C between 0 and 48 h. To ensure the reaction was stopped at the appropriate time points, 12 N hydrochloric acid was used to adjust the reaction mixture pH to 2.7.

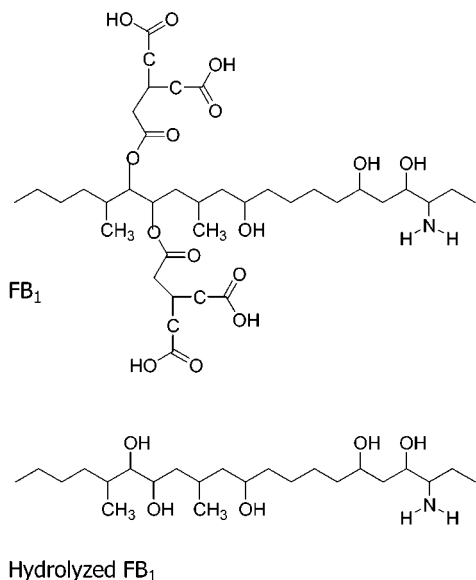
**Nonenzymatic Browning Corn Model System.** Corn was fermented with *Fusarium proliferatum* strain M5991 to produce fumonisin B<sub>1</sub>-contaminated corn using the method described by Hendrich et al. (29). The fumonisin B<sub>1</sub>-contaminated corn (moisture = 64.6%) was ground with a coffee grinder. The ground, fermented fumonisin B<sub>1</sub>-corn was mixed with ground fumonisin B<sub>1</sub>-free corn to attain a final concentration of 10.1 nmol fumonisin B<sub>1</sub>/g corn (dry basis). Triplicate 2.0-g samples of the ground fumonisin B<sub>1</sub>-corn were heated at 80 °C with 100 mM D-glucose in 25 mL of 50 mM potassium phosphate pH 7.0. After 1.5 h, 100 mg of dry bovine pancreas  $\alpha$ -amylase was added to the suspension to digest the corn starch. The reaction was stopped by adjusting the pH of the reaction mixture to 2.7 with concentrated hydrochloric acid at different time points. Twenty-five mL of acetonitrile was added to the reaction mixture. The samples were extracted and cleaned-up using the method described by Murphy et al. (10). Free fumonisin B<sub>1</sub> was measured with HPLC *o*-phthaldialdehyde derivatization method. Fumonisin B<sub>1</sub> and fumonisin B<sub>1</sub>-glucose reaction products total concentrations were determined by ELISA.

Triplicate 2.0-g samples of the ground 10.1 nmol fumonisin B<sub>1</sub>/g corn were heated at 40, 50, or 60 °C in 25 mL of 100 mM D-glucose in 50 mM potassium phosphate, pH 7.0, without  $\alpha$ -amylase. Fumonisin B<sub>1</sub> and fumonisin B<sub>1</sub>-glucose analyses were the same as at 80 °C.

**Statistical Methods.** Linear regressions, correlation coefficients, and analysis of variance were calculated using SAS program version 7.0 (SAS Institute Inc., Cary, NC, 1998). A 95% confidence interval was used to determine statistical significance. Comparison of 95% confidence intervals on regression slopes was used to determine the difference between reaction rate constants under different conditions.

## RESULTS AND DISCUSSION

The HPLC *o*-phthaldialdehyde derivatization method could detect fluorescent derivatives of fumonisin B<sub>1</sub> and hydrolyzed fumonisin B<sub>1</sub>, but not of fumonisin B<sub>1</sub>-glucose. Because

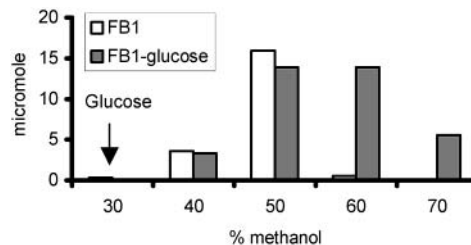


**Figure 1.** Structures of fumonisin B<sub>1</sub> and hydrolyzed fumonisin B<sub>1</sub>.

*o*-phthalaldehyde reacts only with primary amines, the HPLC data suggested that fumonisin B<sub>1</sub>-glucose has no primary amine group. These results supported the previous findings of Clifford (36) that fumonisin B<sub>1</sub>-glucose could not be detected by the HPLC *o*-phthalaldehyde derivatization method and the hypothesis of Murphy et al. (31) that reacting fumonisin B<sub>1</sub> with a reducing sugar could block its primary amine group. Fumonisin B<sub>1</sub> and hydrolyzed fumonisin B<sub>1</sub> gave linear standard curves.

The ELISA assay could detect fumonisin B<sub>1</sub> and fumonisin B<sub>1</sub>-glucose but not hydrolyzed fumonisin B<sub>1</sub>. The ELISA assay produced a nonlinear standard curve. A solution of 666 μmol fumonisin B<sub>1</sub> was heated with 100 mM D-glucose in 50 mM potassium phosphate buffer, pH 7.0, at 80 °C for 48 h. Before heating, the ELISA and the HPLC *o*-phthalaldehyde methods gave fumonisin B<sub>1</sub> masses of 612 ± 21 and 666 ± 32 μmol, respectively. After heating, the ELISA test and the HPLC *o*-phthalaldehyde method detected 597 ± 26 and 282 ± 3 μmol fumonisin B<sub>1</sub>, respectively. The data suggest that ELISA assay determines fumonisin B<sub>1</sub> and fumonisin B<sub>1</sub>-glucose total concentration. The structural difference between fumonisin B<sub>1</sub> and hydrolyzed fumonisin B<sub>1</sub> is the lack of two tricarballic acid groups in hydrolyzed fumonisin B<sub>1</sub> (Figure 1). The results suggested that fumonisin B<sub>1</sub>-glucose should have the aminopentol chain with two of the side chain hydroxyls esterified with tricarballic acid.

The fumonisin B<sub>1</sub>-glucose mixture was fractionated using the reversed-phase C<sub>18</sub> SPE cartridge, and the fractions were evaluated by *o*-phthalaldehyde-HPLC and ELISA. A representative histogram from a SPE elution is shown in Figure 2. The yellow color of the eluant produced with 30% methanol apparently was the excess glucose in the model system reaction mixture. Using acidified 40% methanol in the next gradient step eluted a small amount of fumonisin B<sub>1</sub> and fumonisin B<sub>1</sub>-glucose. A 50% methanol step eluted most of the fumonisin B<sub>1</sub> and about 40% of fumonisin B<sub>1</sub>-glucose. The 60 and 70% methanol eluants contained principally the fumonisin B<sub>1</sub>-glucose and a small amount of fumonisin B<sub>1</sub>. The 60% eluants were used for mass spectrometric characterization of fumonisin B<sub>1</sub>-glucose reaction products. After the fumonisin B<sub>1</sub>-glucose model system fractions were eluted, the cartridges were washed with water acidified to pH 2.7, and could be reused. In general, the 10-g reversed-phase C<sub>18</sub> SPE cartridge provided a relatively

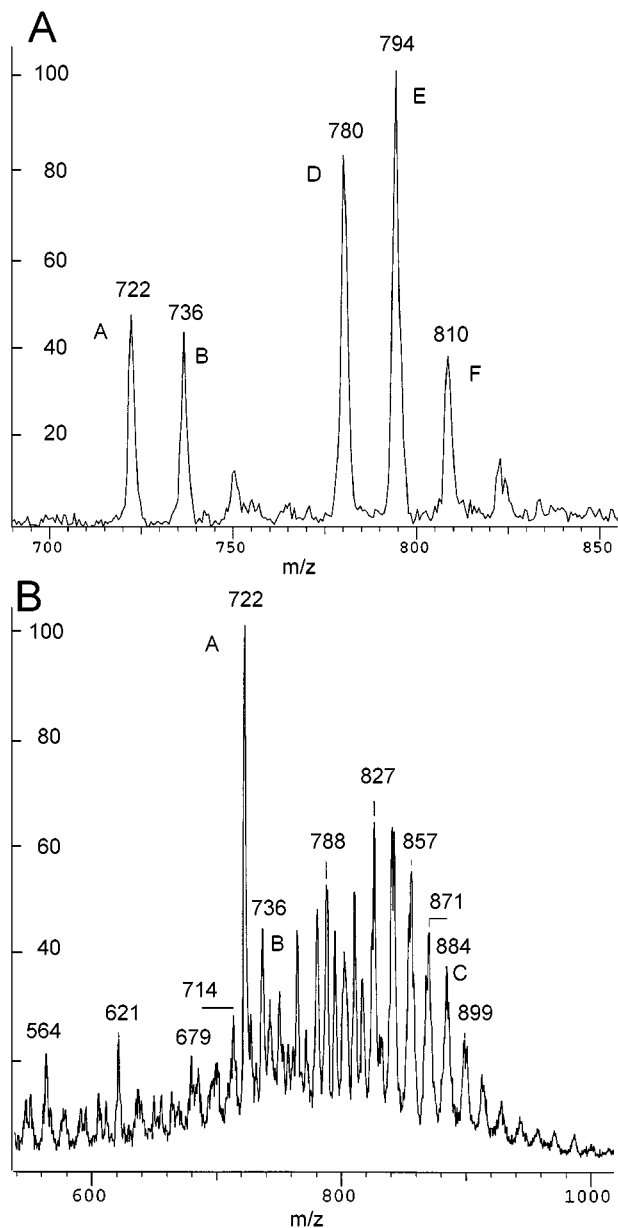


**Figure 2.** Reversed-phase C<sub>18</sub> SPE cartridge separation of fumonisin B<sub>1</sub> and fumonisin B<sub>1</sub>-glucose in a 1.39 mM fumonisin B<sub>1</sub> and 100 mM D-glucose reaction mixture.

fast and economical method to purify hundred-mg quantities of fumonisin B<sub>1</sub>-glucose products for characterization.

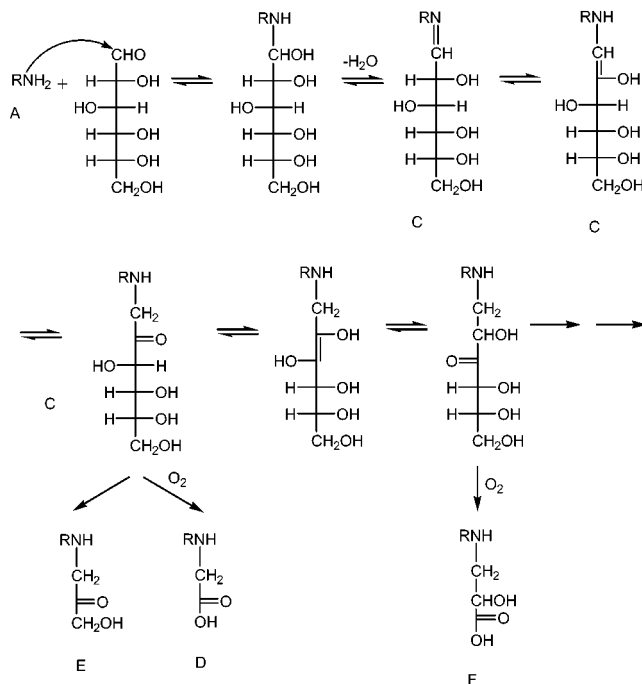
The positive electrospray ionization (ESI) mass spectrum analysis of the 60% methanol eluant of fumonisin B<sub>1</sub>-glucose model system produced after 48 h at 65 °C is shown in Figure 3A. ESI is a soft ionization technique so intact molecular ion adducts are often observed with minimal decompositions. The mass spectrum suggested that there were four primary products: (B) *N*-methyl-fumonisin B<sub>1</sub> (MW = 735.87), (D) *N*-carboxymethyl-fumonisin B<sub>1</sub> (MW = 779.88), (E) *N*-(3-hydroxyacetyl)-fumonisin B<sub>1</sub> (MW = 793.90), and (F) *N*-(2-hydroxy, 2-carboxyethyl)-fumonisin B<sub>1</sub> (MW = 809.90) in fumonisin B<sub>1</sub>-glucose reaction mixture in addition to the residual fumonisin B<sub>1</sub> (A). A proposed mechanism of fumonisin B<sub>1</sub> and D-glucose nonenzymatic browning reaction is presented in Figure 4 based on the nonenzymatic browning reaction scheme of Yaylayan and Huyghues-Despointes (40), the report of Poling et al. (41), and the mass spectrum presented in Figure 3A. When fumonisin B<sub>1</sub> was heated with D-glucose at 80 °C for 48 h, there were more reaction products (> 10) found in the mass spectrometric analysis (Figure 3B). The results supported the well-recognized chain reaction complexity in the Maillard reaction by yielding a variety of products. The mass spectra of fumonisin B<sub>1</sub> heated with D-glucose at 60 °C for 24, 72, and 96 h are shown in Figure 5. The rate of nonenzymatic browning reaction was temperature dependent, with higher reaction rate and complexity of the nonenzymatic browning reaction products increasing with temperature. These spectra of products produced at lower temperatures indicated the fumonisin B<sub>1</sub>-glucose Schiff's base (MW = 883.98) had formed. Poling et al. (41) have identified this first product of fumonisin B<sub>1</sub>-glucose condensation as *N*-(deoxy-D-fructosyl-1-yl) fumonisin B<sub>1</sub>, the Amadori rearrangement product expected from glucose, with the same MW reported here. Howard et al. (42) claimed that there was only one primary product, *N*-carboxymethyl-fumonisin B<sub>1</sub>, with a molecular weight of 779 when fumonisin B<sub>1</sub> reacted with D-glucose at 78 °C for about 13 h. Their reaction products were separated with reversed-phase HPLC, and the HPLC column eluant was delivered to the mass spectrometer. Only one product was observed from this reaction probably because the HPLC separated it from other potential nonenzymatic browning reaction products. The shorter reaction time (42) than that used here may have resulted in fewer reaction products in their reaction mixture. Poling et al. (41) reported *N*-carboxymethyl-fumonisin B<sub>1</sub> as a secondary product from this reaction. Voss et al. (43) reported minor amounts of *N*-(deoxy-D-fructosyl-1-yl) fumonisin B<sub>1</sub>, *N*-carboxymethyl-fumonisin B<sub>1</sub>, and their hydrolyzed fumonisin B<sub>1</sub> analogues formed upon frying of tortilla chips. However, because corn meal and masa meal contain virtually no reducing sugars, the minor concentrations of these adducts is not unexpected.

The effect of different fumonisin B<sub>1</sub> initial concentrations on fumonisin B<sub>1</sub> loss rate is shown in Figure 6. The reaction



**Figure 3.** (A) ESI positive ion mass spectrum of a mixture of fumonisin B<sub>1</sub> and D-glucose nonenzymatic browning deduced reaction products formed at 65 °C for 48 h: A, [fumonisin B<sub>1</sub> + H]<sup>+</sup>; B, [N-methyl-fumonisin B<sub>1</sub> + H]<sup>+</sup>; D, [N-(carboxymethyl)-fumonisin B<sub>1</sub>]<sup>+</sup>; E, [N-(3-hydroxyacetyl)-fumonisin B<sub>1</sub> + H]<sup>+</sup>; F, [N-(2-hydroxy, 2-carboxyethyl)-fumonisin B<sub>1</sub> - H]<sup>+</sup>. (B) ESI positive ion mass spectrum of fumonisin B<sub>1</sub> and D-glucose nonenzymatic browning deduced reaction products formed at 80 °C for 48 h: A, [fumonisin B<sub>1</sub> + H]<sup>+</sup>. The peaks with mass-charge ratio higher than 722.9 are probably nonenzymatic browning reaction products.

appears to be an apparent first order with respect to fumonisin B<sub>1</sub> because log<sub>10</sub> of fumonisin B<sub>1</sub> concentration had a linear relationship with respect to time. The fumonisin B<sub>1</sub> loss rate constants *k* for 69, 139, and 347 μM fumonisin B<sub>1</sub> heated with 100 mM D-glucose at 80 °C were not significantly different, with the mean of 0.051 ± 0.002/h. The observation that the rate constant *k* is independent of initial fumonisin B<sub>1</sub> concentration is in agreement with apparent first-order kinetics. The fumonisin B<sub>1</sub> loss rate constant *k* for 693 μM fumonisin B<sub>1</sub> heated with 100 mM D-glucose at 80 °C was 0.043/h, which was significantly smaller compared with the *k* values at lower fumonisin B<sub>1</sub> concentrations. These data suggest that the reaction does not follow first-order kinetics at high fumonisin B<sub>1</sub>



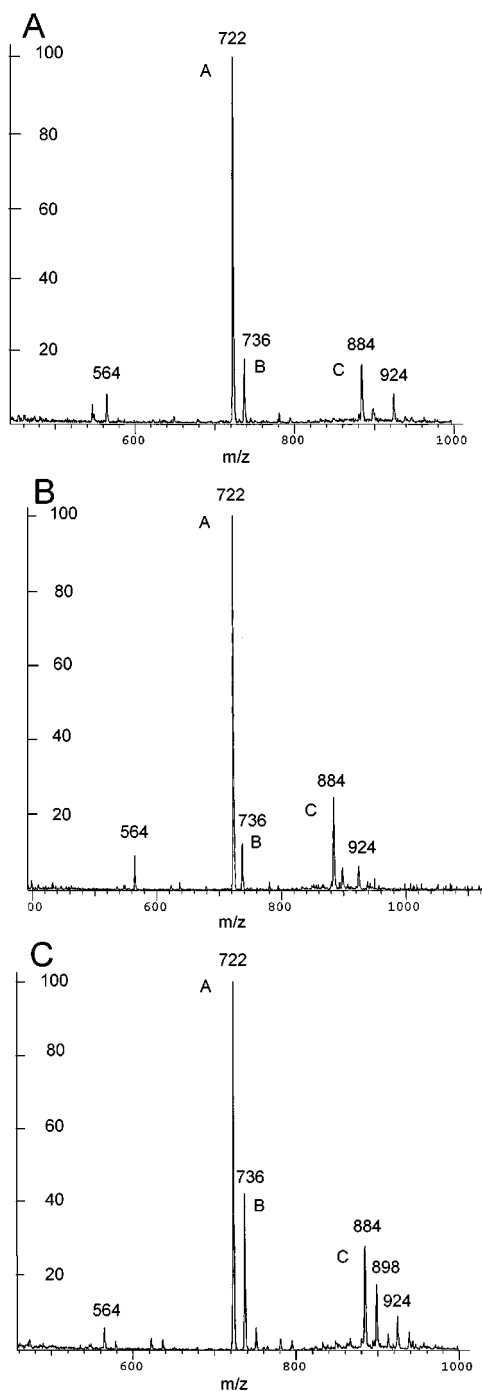
**Figure 4.** Proposed nonenzymatic browning reaction scheme of fumonisin B<sub>1</sub> and D-glucose based on the ESI positive ion mass spectra and the lysine and D-glucose nonenzymatic browning reaction scheme of Yaylayan and Huyghues-Despointes, (40). RNH<sub>2</sub>, fumonisin B<sub>1</sub>. Letters next to fumonisin-carbohydrate adduct correspond to Figures 3 and 5.

concentrations. A comparison of initial fumonisin B<sub>1</sub> loss rates at different D-glucose concentrations is shown in Figure 7. The initial reaction rate versus initial D-glucose concentration was a hyperbolic curve. The initial fumonisin B<sub>1</sub> loss rates for 10, 50, 100, and 500 mM D-glucose heated with 139 μM fumonisin B<sub>1</sub> at 80 °C were 2.91, 5.07, 6.45, and 7.81 μM/h, respectively. We observed that the higher the D-glucose initial concentration, the faster the reaction rate. The lower initial fumonisin B<sub>1</sub> loss rate with 10 and 50 mM D-glucose may be due to insufficient open chain D-glucose to support the reaction. The similar reaction rate constant *k* for 100 and 500 mM D-glucose may be due to excess open chain D-glucose in these glucose concentrations at equilibrium (44).

The effects of different reducing sugars on fumonisin B<sub>1</sub> loss rates is shown in Table 1. The different reaction of the reducing sugars may be due to their different abilities to form open-chain sugar. The percentage open chain is greater for reducing monosaccharides than for reducing disaccharides (45).

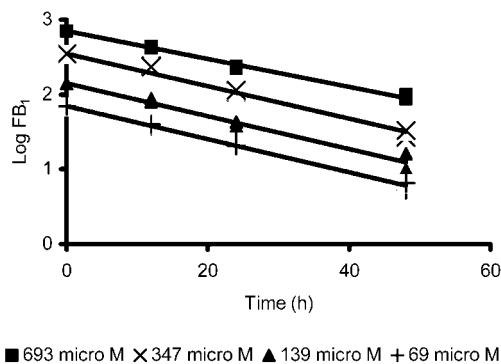
The apparent reaction rate constants *k* for fumonisin B<sub>1</sub> loss in the presence of 100 mM glucose model systems at 40, 50, 60, and 80 °C were 0.000471, 0.00243, 0.00754, and 0.0505/h, respectively. The Arrhenius plot yields an *E<sub>a</sub>* of 105.7 kJ/mol or 25.28 kcal/mol. This magnitude of *E<sub>a</sub>* suggests that temperature has a major effect on the reaction rate and heating is necessary for the reaction to be useful within the feasible processing limits of corn foods and feeds (46).

To determine whether the nonenzymatic browning reaction of fumonisin B<sub>1</sub> and D-glucose was of practical significance, the reaction in fumonisin B<sub>1</sub>-corn was investigated. The rates of fumonisin B<sub>1</sub> loss and fumonisin B<sub>1</sub>-glucose formation in the nonenzymatic browning corn model system at 60 and 80 °C are shown in Figure 8. The free fumonisin B<sub>1</sub> was determined by the HPLC *o*-phthalaldehyde derivatization method, and the total concentrations of fumonisin B<sub>1</sub> and fumonisin B<sub>1</sub>-glucose reaction products were quantified with the ELISA assay.

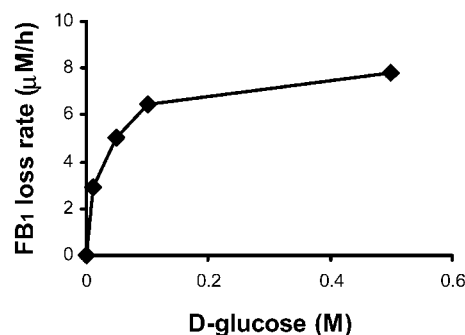


**Figure 5.** (A) ESI positive ion mass spectrum of fumonisin B<sub>1</sub> and D-glucose nonenzymatic browning reaction products formed at 60 °C for 24 h: A, [fumonisin B<sub>1</sub> + H]<sup>+</sup>; B, [N-methyl-fumonisin B<sub>1</sub> + H]<sup>+</sup>; C, [fumonisin B<sub>1</sub>-glucose Schiff's base + H]<sup>+</sup> or [N-(deoxy-D-fructos-1-yl) fumonisin B<sub>1</sub> + H]<sup>+</sup>. (B) ESI positive ion mass spectrum of fumonisin B<sub>1</sub> and D-glucose nonenzymatic browning reaction products formed at 60 °C for 72 h: A, [fumonisin B<sub>1</sub> + H]<sup>+</sup>; B, [N-methyl-FB<sub>1</sub> + H]<sup>+</sup>; C, [fumonisin B<sub>1</sub>-glucose Schiff's base + H]<sup>+</sup> or [N-(deoxy-D-fructos-1-yl) fumonisin B<sub>1</sub> + H]<sup>+</sup>. (C) ESI positive ion mass spectrum of fumonisin B<sub>1</sub> and D-glucose nonenzymatic browning reaction deduced products formed at 60 °C for 96 h: A, [fumonisin B<sub>1</sub> + H]<sup>+</sup>; B, [N-methyl-fumonisin B<sub>1</sub> + H]<sup>+</sup>; C, [fumonisin B<sub>1</sub>-glucose Schiff's base + H]<sup>+</sup> or [N-(deoxy-D-fructos-1-yl) fumonisin B<sub>1</sub> + H]<sup>+</sup>.

When the corn was heated in 50 mM phosphate, pH 7.0, and 100 mM glucose solution at 80 °C, starch began to gelatinize and there was no apparent loss of fumonisin B<sub>1</sub> after 48 h as



**Figure 6.** Effect of different initial fumonisin B<sub>1</sub> concentrations on rate of fumonisin B<sub>1</sub> loss. The fumonisin B<sub>1</sub> solutions were heated with 100 mM D-glucose at 80 °C. The mean reaction rate constant *k* for 69, 139, and 347 μM fumonisin B<sub>1</sub> is 0.0505 ± 0.002/h, and the rate constant *k* for 693 μM fumonisin B<sub>1</sub> is 0.0428/h (*n* = 2).



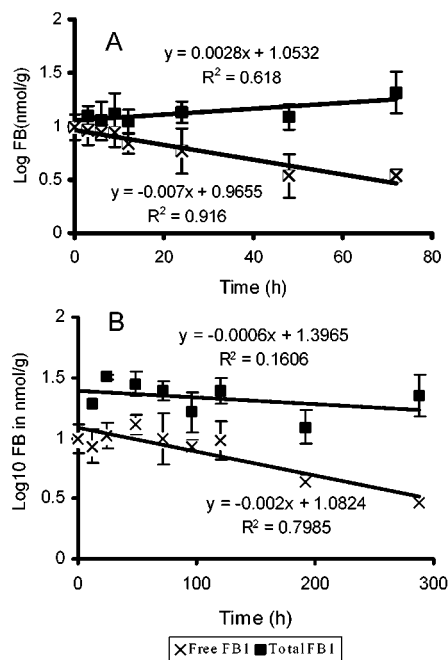
**Figure 7.** Rate of fumonisin B<sub>1</sub> loss with different D-glucose concentrations. The D-glucose solutions with 139 μM fumonisin B<sub>1</sub> were held at 80 °C (*n* = 2).

**Table 1.** Effect of 100 mM Reducing Sugars on the Rate of 139 μM Fumonisin B<sub>1</sub> Loss at 80 °C<sup>a</sup>

	lactose	maltose	fructose	mannose	glucose
<i>k</i> (h <sup>-1</sup> )	0.0290 <sup>b</sup>	0.0318 <sup>b</sup>	0.0373 <sup>ab</sup>	0.0553 <sup>a</sup>	0.0495 <sup>a</sup>

<sup>a</sup> Reaction rates followed by different letters are significantly different at  $\alpha = 0.05$ .

measured by *o*-phthalaldehyde-HPLC detection. During the gelatinization of corn starch, the starch granules absorbed most of the water and formed a highly viscous starch paste (47). The gelatinization of starch resulted in decreased water activity, a critical component for the nonenzymatic browning reaction (45), and prevented the fumonisin B<sub>1</sub>-glucose reaction from proceeding.  $\alpha$ -Amylase can hydrolyze amylose in corn. It was hypothesized that by adding  $\alpha$ -amylase to the corn reaction mixture, the increased viscosity and water absorption by the starch could be minimized, thus allowing the nonenzymatic reaction to proceed. When  $\alpha$ -amylase was added to the fumonisin B<sub>1</sub>-corn-glucose model system, no starch gelatinization was observed.  $\alpha$ -Amylase was expected to be denatured at 80 °C, but it was apparently active long enough to prevent the inhibition of the fumonisin B<sub>1</sub>-glucose reaction. The free fumonisin B<sub>1</sub> concentrations, determined by *o*-phthalaldehyde-HPLC measurements, decreased with reaction time while the total fumonisin B<sub>1</sub>, determined by ELISA, remained constant (**Figure 8B**). These results showed that there was loss of the fumonisin B<sub>1</sub> primary amine while concentration of total fumonisin B<sub>1</sub> plus fumonisin B<sub>1</sub>-glucose reaction products remained the same. The data confirmed that the reaction of fumonisin B<sub>1</sub> with glucose occurred at 80 °C. At 40, 50, and



**Figure 8.** Rates of change of total fumonisin (fumonisin B<sub>1</sub> plus fumonisin B<sub>1</sub>-glucose) and free fumonisin B<sub>1</sub> in (A) fumonisin B<sub>1</sub>-corn heated at 80 °C and (B) fumonisin B<sub>1</sub>-corn heated at 60 °C, both with 100 mM glucose at pH 7. Error bars represent standard error,  $n = 3$ . Slopes of total fumonisin B<sub>1</sub> rate of change were not significantly different from 0,  $\alpha = 0.05$ .

60 °C, there was no need to add  $\alpha$ -amylase because corn starch does not gelatinize at these temperatures (48). There was no apparent fumonisin B<sub>1</sub> reaction with glucose in corn when heated with D-glucose at 40 and 50 °C for 22 and 16 days, respectively. At 40 °C, the *F. proliferatum* fungi began to grow in some of the corn samples and formed a red color that we have observed in this culture strain. Fumonisin B<sub>1</sub> was probably produced by the fungi at 40 °C which may explain why we observed an increase of fumonisin B<sub>1</sub> and fumonisin B<sub>1</sub>-glucose total concentration at this temperature (data not shown). At 60 and 80 °C, total fumonisin B<sub>1</sub> plus fumonisin B<sub>1</sub>-glucose concentration remained constant, while free fumonisin B<sub>1</sub> concentration decreased (Figure 8). These results showed that nonenzymatic browning reaction occurred at 60 and 80 °C, and fumonisin B<sub>1</sub> had been converted into fumonisin B<sub>1</sub>-glucose products in corn. Fumonisin B<sub>1</sub> has caused economic losses to farmers because of its toxicity to livestock. This reaction may be used as a method to detoxify fumonisin B<sub>1</sub> in corn. If the reaction was carried out at 60 °C, 8 days were needed to decrease the fumonisin B<sub>1</sub> to half of the initial concentration in the presence of excess glucose. If heated at 80 °C, only 2 days were needed to decrease fumonisin B<sub>1</sub> to half, but the usage of  $\alpha$ -amylase would increase the cost. The optimum reaction time and temperature would depend on initial fumonisin B<sub>1</sub> concentration in contaminated corn and the desired residual fumonisin B<sub>1</sub> in the treated food or animal feed.

Castelo et al. (49) recently reported 45–70% reductions in detectable fumonisin B<sub>1</sub> in corn muffins with added glucose when baked at 200 °C, and 90% reduction of fumonisin B<sub>1</sub> upon extrusion of 560  $\mu$ mol glucose/g corn (26% moisture) at 160 °C at an extruder speed of 40 rpm. Less than 1% of fumonisin B<sub>1</sub> was detected as *N*-carboxymethyl-fumonisin B<sub>1</sub> in the muffins. As the internal temperature of the baked muffin would never exceed 100 °C, these results are not unexpected. Unfor-

tunately, the extruded corn was not examined for *N*-carboxymethyl-fumonisin B<sub>1</sub> content.

Lu et al. (50) examined the hepatic cancer promotional effect of fumonisin B<sub>1</sub> and fumonisin B<sub>1</sub>-fructose reaction products by feeding these reaction mixtures to diethylnitrosamine-initiated Fischer344/N rats. The fumonisin B<sub>1</sub>-fructose reaction products fed rats had significantly less promotion of hepatocarcinogenesis than the fumonisin B<sub>1</sub> fed rats. Dantzer et al. (33) demonstrated that the reduced toxicity of fumonisin B<sub>1</sub>-fructose was not due to reduced absorption compared to that of fumonisin B<sub>1</sub>. Liu et al. (50) showed that 35  $\mu$ mol fumonisin B<sub>1</sub>-glucose/kg diet could neither promote liver cancer nor was hepatotoxic in rats. When we initially dosed swine by i.p. at 11  $\mu$ mol fumonisin B<sub>1</sub>-glucose/kg body weight, 4 out of 6 pigs died of acute pulmonary edema (51). However, in a recent preliminary study, pigs dosed at 5.5  $\mu$ mol fumonisin B<sub>1</sub>-glucose/kg body weight were partially protected from fumonisin B<sub>1</sub> toxicosis compared to animals at the same dose of fumonisin B<sub>1</sub>. Taken together, these data indicate that the primary amine group of fumonisin B<sub>1</sub> is essential to its hepatocarcinogenicity in rats and its hepatotoxicity to rats and swine, and that the reaction with glucose does provide a protective effect.

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