Characterization of Fumonisin B₁–Glucose Reaction Kinetics and Products

YUN LU,† LAURA CLIFFORD,‡ CATHERINE C. HAUCK,† SUZANNE HENDRICH,† GARY OSWEILER,§ AND PATRICIA A. MURPHY*,†

Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50011; Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824; and Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, Iowa 50011

The reaction of fumonisin B₁ with the reducing sugar D-glucose can block the primary amine group of fumonisin B₁, and may detoxify this mycotoxin. A method to separate hundred milligram quantities of fumonisin B₁–glucose reaction products from the excess D-glucose with a reversed-phase C₁₈ cartridge was developed. Mass spectrometry revealed that there were four primary products in this chain reaction when fumonisin B₁ was heated with D-glucose at 65 °C for 48 h: N-methyl-fumonisin B₁, N-carboxymethyl-fumonisin B₁, N-(3-hydroxyacetonyl)-fumonisin B₁, and N-(2-hydroxy, 2-carboxyethyl)-fumonisin B₁. The N-(1-deoxy-o-fructos-1-yl) fumonisin B₁ (fumonisin B₁–glucose Schiff’s base) was detected by mass spectrometry when fumonisin B₁ was heated with D-glucose at 60 °C. The nonenzymatic browning reaction of fumonisin B₁ with excess D-glucose followed apparent first-order kinetics. The activation energy, $E_a$, was 105.7 kJ/mol. Fumonisin B₁ in contaminated corn could precipitate the nonenzymatic browning reaction with 0.1 M D-glucose at 60 and 80 °C.

KEYWORDS: Fumonisin B₁; fumonisin B₁–glucose; detoxification; N-(1-deoxy-o-fructos-1-yl) fumonisin B₁

INTRODUCTION

Fumonisins are a family of mycotoxins produced mainly by the corn fungi Fusarium verticilloides (synonym Fusarium moniliforme) and Fusarium proliferatum (1, 2). Fumonisin B₁ is the most abundant natural contaminant in corn-based foods and feeds among the several structurally related homologues. Fumonisin B₁ 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 B₁ can cause a variety of diseases in animals. Equine leukoencephalomalacia (15, 16) and porcine pulmonary edema (17, 18) can be caused by fumonisin B₁. Fumonisin B₁ is hepatocarcinogenic, hepatotoxic, and nephrotoxic in rats (19, 20). Rat liver cancer can be promoted and initiated by fumonisin B₁ (21, 22). Fumonisin B₁ 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 B₁ (7, 12). Fumonisin B₁ has been declared a class 2B carcinogen, which is a possible human carcinogen (27).

Fumonisin B₁ 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 B₁ to hydrolyzed fumonisin B₁. 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 B₁ was critical to the toxicity of fumonisin B₁ because naturally occurring N-acetyl-fumonisin B₁ was not considered toxic. Reacting fumonisin B₁ with reducing sugars, such as fructose or glucose, may block the primary amine group and detoxify fumonisin B₁ (31).

Lu et al. (32) reported that a fumonisin B₁–fructose reaction mixture fed to diethylnitrosamine-initiated Fischer344/N rats resulted in significantly less hepatic cancer promotion than that observed in rats fed fumonisin B₁. However, ¹⁴C-hydrolyzed fumonisin B₁ and ¹⁴C-fumonisin B₁–fructose were better absorbed than ¹⁴C-fumonisin B₁ in Fischer344/N rats (33). The reduced carcinogenic promotional activity of fumonisin B₁–fructose cannot be due to reduced absorption.

The reaction of primary amines, such as fumonisin B₁, 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 B₁ and glucose.

* To whom correspondence should be addressed. Tel: (515) 294-1970. Fax: (515) 294-8181. E-mail: pmurphy@iastate.edu.
† Department of Food Science and Human Nutrition, Iowa State University.
‡ Department of Food Science and Human Nutrition, Michigan State University.
§ Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University.
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 B₁-glucose reaction products from the excess glucose, to characterize the reaction products, and evaluate the reaction kinetics of glucose with fumonisin B₁ in a model system and in fumonisin B₁-contaminated corn.

MATERIALS AND METHODS

Safety Precautions. Fumonisin B₁ is a class 2B carcinogen and was handled accordingly.

Chemicals. The fumonisin B₁, used as a standard for high-performance liquid chromatography (HPLC) analysis and to produce fumonisin B₁-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).

α-Phthalaldialdehyde, 2-mercaptoethanol, and α-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 B₁ and Fumonisin B₁-Glucose. Reversed-Phase HPLC with Fluorescence Detection of α-Phthalaldialdehyde Derivative. Fumonisin B₁ was detected as a derivative with α-phthalaldialdehyde following reversed-phase HPLC separation by fluorescence detection (9). A 100-μL aliquot of sample was mixed with 100 μL of 50 mM potassium phosphate buffer, pH 8.3, and 100 μL of α-phthalaldialdehyde solution (5 mg of α-phthalaldialdehyde and 10 μL of 2-mercaptoethanol in 5 mL of acetonitrile) at room temperature. After 10 min, the mixture was quenched with 100 μL of water and the mixture was injected manually into the HPLC system using a 20-μL loop. The α-phthalaldialdehyde solution was stored in dark at 5 °C and made fresh weekly.

The HPLC system included a 250 × 4.6 mm, 5 μm reversed-phase C₁₈ analytical column (Altech 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 FB₁ and FB₂-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 B₁, purified hydrolyzed fumonisin B₁, and fumonisin B₁-glucose reaction mixture were tested with the ELISA test kit and the results were compared with those of the HPLC α-phthalaldialdehyde derivatization method.

Preparation and Cleanup of Fumonisin B₁-Glucose Reaction Products for Mass Characterization. For fumonisin B₁-glucose products characterization, 50 mL of 1.39 mM fumonisin B₁ 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 C₁₈ 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 B₁ 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 B₁ and fumonisin B₁-glucose products were evaluated by the HPLC α-phthalaldialdehyde 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 B₁-glucose reaction product purification.

Characterization of Fumonisin B₁-Glucose Reaction Products by Mass Spectrometry. The 60% methanol eluant obtained from the reversed-phase C₁₈ 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 FB₁ 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 B₁-Glucose Nonenzymatic Browning Model System Kinetics. Fumonisin B₁ 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 FB₁ was evaluated by the HPLC α-phthalaldialdehyde derivatization method at different reaction time points. The volume of the solutions was brought back to 1.0 mL before HPLC analysis. Different initial FB₁ concentrations of 69, 139, 347, or 693 μM fumonisin B₁ in 100 mM glucose at 80 °C were used to evaluate the effect of fumonisin B₁ concentration on the reaction rate. Different initial D-glucose concentrations of 10, 50, 100, 500, or 1000 mM D-glucose with 139 μM fumonisin B₁ 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 μM fumonisin B₁ at 80 °C over 48 h. To determine the energy of activation, the reaction rates of 139 μM fumonisin B₁ 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 MS5991 to produce fumonisin B₁-contaminated corn using the method described by Hendrich et al. (29). The fumonisin B₁-contaminated corn (moisture = 64.6%) was ground with a coffee grinder. The ground, fermented fumonisin B₁-corn was mixed with ground fumonisin B₁-free corn to attain a final concentration of 10.1 nmol fumonisin B₁/g corn (dry basis). Triplicate 2.0-g samples of the ground fumonisin B₁-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 α-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 B₁ was measured with HPLC α-phthalaldialdehyde derivatization method.

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 α-phthalaldialdehyde derivatization method could detect fluorescent derivatives of fumonisin B₁ and hydrolyzed fumonisin B₁, but not of fumonisin B₁-glucose. Because
Figure 1. Structures of fumonisin B₁ and hydrolyzed fumonisin B₁.

*o*-phthalaldehyde reacts only with primary amines, the HPLC data suggested that fumonisin B₁-glucose has no primary amine group. These results supported the previous findings of Clifford (36) that fumonisin B₁-glucose could not be detected by the HPLC *o*-phthalaldehyde derivatization method and the hypothesis of Murphy et al. (31) that reacting fumonisin B₁ with a reducing sugar could block its primary amine group. Fumonisin B₁ and hydrolyzed fumonisin B₁ gave linear standard curves.

The ELISA assay could detect fumonisin B₁ and fumonisin B₁-glucose but not hydrolyzed fumonisin B₁. The ELISA assay produced a nonlinear standard curve. A solution of 666 μmol fumonisin B₁ 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 B₁ 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 B₁, respectively. The data suggest that ELISA assay determines fumonisin B₁ and fumonisin B₁-glucose total concentration. The structural difference between fumonisin B₁ and hydrolyzed fumonisin B₁ is the lack of two tricarballylic acid groups in hydrolyzed fumonisin B₁ (Figure 1). The results suggested that fumonisin B₁-glucose should have the aminopentyl chain with two of the side chain hydroxyls esterified with tricarballylic acid.

The fumonisin B₁-glucose mixture was fractionated using the reversed-phase C₁₈ SPE cartridge, and the fractions were evaluated by *o*-phthalaldehyde-HPLC and ELISA. A representative histogram for an 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 B₁ and fumonisin B₁-glucose. A 50% methanol step eluted most of the fumonisin B₁ and about 40% of fumonisin B₁-glucose. The 60 and 70% methanol eluants contained principally the fumonisin B₁-glucose and a small amount of fumonisin B₁. The 60% eluants were used for mass spectrometric characterization of fumonisin B₁-glucose reaction products. After the fumonisin B₁-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 C₁₈ SPE cartridge provided a relatively fast and economical method to purify hundred-mg quantities of fumonisin B₁-glucose products for characterization.

The positive electrospray ionization (ESI) mass spectrum analysis of the 60% methanol eluant of fumonisin B₁-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 B₁ (MW = 735.87), (D) N-carboxymethyl-fumonisin B₁ (MW = 779.88), (E) N-(3-hydroxyacetonyl)-fumonisin B₁ (MW = 793.90), and (F) N-(2-hydroxy, 2-carboxyethyl)-fumonisin B₁ (MW = 809.90) in fumonisin B₁-glucose reaction mixture in addition to the residual fumonisin B₁ (A). A proposed mechanism of fumonisin B₁ and D-glucose nonenzymatic browning reaction is presented in Figure 4 based on the nonenzymatic browning reaction scheme of Yaylayan and Huýghues-Despointes (40), the report of Poling et al. (41), and the mass spectrum presented in Figure 3A. When fumonisin B₁ 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 B₁ 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 B₁-glucose Schiff’s base (MW = 883.98) had formed. Poling et al. (41) have identified this first product of fumonisin B₁-glucose condensation as N-(deoxy-D-fructosyl-1-yl) fumonisin B₁, 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 B₁, with a molecular weight of 779 when fumonisin B₁ 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 B₁ as a secondary product from this reaction. Voss et al. (43) reported minor amounts of N-(deoxy-D-fructosyl-1-yl) fumonisin B₁, N-carboxymethyl-fumonisin B₁, and their hydrolyzed fumonisin B₁ 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 B₁ initial concentrations on fumonisin B₁ loss rate is shown in Figure 6. The reaction
appears to be an apparent first order with respect to fumonisin B₁ because log₁₀ of fumonisin B₁ concentration had a linear relationship with respect to time. The fumonisin B₁ loss rate constants k₁, ₁₃, and ₃₄ for ₆₉, ₁₃₉, and ₃₄₇ μM fumonisin B₁ 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 B₁ concentration is in agreement with apparent first-order kinetics. The fumonisin B₁ loss rate constant k for ₆₉₃ μM fumonisin B₁ heated with 100 mM D-glucose at 80 °C was 0.043/h, which was significantly smaller compared with the k₁ values at lower fumonisin B₁ concentrations. These data suggest that the reaction does not follow first-order kinetics at high fumonisin B₁ concentrations.

A comparison of initial fumonisin B₁ 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 B₁ loss rates for 10, 50, 100, and 500 mM D-glucose heated with 139 μM fumonisin B₁ 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 B₁ 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 B₁ 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 sugars. The percentage open chain is greater for reducing monosaccharides than for reducing disaccharides (45).

The apparent reaction rate constants k for fumonisin B₁ 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 Eₐ of 105.7 kJ/mol or 25.28 kcal/mol. This magnitude of Eₐ 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 B₁ and D-glucose was of practical significance, the reaction in fumonisin B₁-corn was investigated. The rates of fumonisin B₁ loss and fumonisin B₁-D-glucose formation in the nonenzymatic browning corn model system at 60 and 80 °C are shown in Figure 8. The free fumonisin B₁ was determined by the HPLC o-phthalaldehyde derivatization method, and the total concentrations of fumonisin B₁ and fumonisin B₁-D-glucose reaction products were quantified with the ELISA assay.

Figure 3. (A) ESI positive ion mass spectrum of a mixture of fumonisin B₁ and D-glucose nonenzymatic browning deduced reaction products formed at 65 °C for 48 h: A, [fumonisin B₁ + H]+; B, [N-methyl-fumonisin B₁ + H]+; C, [N-(carboxymethyl)-fumonisin B₁ + H]+; D, [N-(3-hydroxyacetyl)-fumonisin B₁ + H]+; E, [N-(2-hydroxy, 2-carboxyethyl)-fumonisin B₁ - H]+. (B) ESI positive ion mass spectrum of fumonisin B₁ and D-glucose nonenzymatic browning deduced reaction products formed at 80 °C for 48 h: A, [fumonisin B₁ + H]+. The peaks with mass-charge ratio higher than 722.9 are probably nonenzymatic browning reaction products.

Figure 4. Proposed nonenzymatic browning reaction scheme of fumonisin B₁ 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). RNH₂, fumonisin B₁. Letters next to fumonisin–carbohydrate adduct correspond to Figures 3 and 5.
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 B₁ after 48 h as measured by o-phthaldialdehyde-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 B₁-glucose reaction from proceeding. 

R-Amylase can hydrolyze amylose in corn. It was hypothesized that by adding R-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 R-amylase was added to the fumonisin B₁-corn-glucose model system, no starch gelatinization was observed. R-Amylase was expected to be denatured at 80 °C, but it was apparently active long enough to prevent the inhibition of the fumonisin B₁-glucose reaction. The free fumonisin B₁ concentrations, determined by o-phthaldialdehyde-HPLC measurements, decreased with reaction time while the total fumonisin B₁, determined by ELISA, remained constant (Figure 8B). These results showed that there was loss of the fumonisin B₁ primary amine while concentration of total fumonisin B₁ plus fumonisin B₁-glucose reaction products remained the same. The data confirmed that the reaction of fumonisin B₁ with glucose occurred at 80 °C. At 40, 50, and measured by o-phthaldialdehyde-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 B₁-glucose reaction from proceeding. 

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 B₁ after 48 h as
60 °C, there was no need to add α-amylase because corn starch does not gelatinize at these temperatures (48). There was no apparent fumonisin B₁ reaction with glucose in corn when heated with α-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. Fumonisins were probably produced by the fungi at 40 °C which may explain why we observed an increase of fumonisin B₁ and fumonisin B₁-glucose total concentration at this temperature (data not shown). At 60 and 80 °C, total fumonisin B₁ plus fumonisin B₁-glucose concentration remained constant, while free fumonisin B₁ concentration decreased (Figure 8). These results showed that nonenzymatic browning reaction occurred at 60 and 80 °C, and fumonisin B₁ had been converted into fumonisin B₁-glucose products in corn. Fumonisin B₁ has caused economic losses to farmers because of its toxicity to livestock. This reaction may be used as a method to detoxify fumonisin B₁ in corn. If the reaction was carried out at 60 °C, 8 days were needed to decrease the fumonisin B₁ 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 B₁ to half, but the usage of α-amylase would increase the cost. The optimum reaction time and temperature would depend on initial fumonisin B₁ concentration in contaminated corn and the desired residual fumonisin B₁ in the treated food or animal feed.

Castelo et al. (49) recently reported 45–70% reductions in detectable fumonisin B₁ in corn muffins with added glucose when baked at 200 °C, and 90% reduction of fumonisin B₁ upon extrusion of 560 μmol glucose/g corn (26% moisture) at 160 °C at an extruder speed of 40 rpm. Less than 1% of fumonisin B₁ was detected as N-carboxymethyl-fumonisin B₁ in the muffins. As the internal temperature of the baked muffin would never exceed 100 °C, these results are not unexpected. Unfortunately, the extruded corn was not examined for N-carboxymethyl-fumonisin B₁ content.

LITERATURE CITED


