Effects of Baking and Frying on the Fumonisin B_1 Content of Corn-Based Foods

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Fumonisins are mycotoxins produced primarily by *Fusarium moniliforme* and *Fusarium proliferatum* in corn. Fumonisins have been implicated as the causal agents in a variety of animal diseases and are epidemiologically linked to the high incidence of human esophageal cancer in some regions of the world. Little is known about the effects of common processing methods on the fumonisin content of food. The objective of this study was to determine the effects of baking and frying on the stability of fumonisin B_1 (FB_1) spiked into corn-based foods. Baking corn muffins spiked with 5 μg/g (dry weight basis) FB_1 at 175 and 200 °C for 20 min resulted in 83.7 ± 3.5% and 72.4 ± 5.9% retention of FB_1, respectively. At both temperatures, losses of FB_1 were significantly ($p < 0.05$) greater at the surface than at the core of the muffins. No significant losses of FB_1 were found when spiked corn masa was fried at 140−170 °C for 0−6 min. FB_1 began to degrade at frying temperatures ≥180 °C and times ≥8 min. Frying chips for 15 min at 190 °C resulted in 67% loss of FB_1. These processing studies suggest that fumonisins are heat stable compounds that survive under most conditions used during baking or frying.

Keywords: *Fumonisin B_1; processing; frying; baking; heating; decomposition*

INTRODUCTION

Fumonisins are reported to contaminate corn and corn-based foods throughout the world (Sydenham et al., 1991; Fazekas and Hajdu, 1996). These mycotoxins are produced primarily by *Fusarium moniliforme* and *Fusarium proliferatum*, two common fungal contaminants of corn and other grains. To date, at least eight different fumonisin analogues and structurally related compounds have been identified and characterized (Bezuidenhout et al., 1988; Branham and Plattner, 1993; Cawood et al., 1991; Gelderblom et al., 1992; Musser et al., 1995, 1996; Plattner et al., 1992). Fumonisins B_1 (FB_1) and B_2 (FB_2) are the major forms in contaminated corn. Laboratory studies have shown that fumonisins can cause equine leukoencephalomalacia (Marasas et al., 1988; Wilson et al., 1992), porcine pulmonary edema (Harrison et al., 1990; Colvin et al., 1993), and liver cancer and toxicity in rats (Gelderblom et al., 1991). In humans, consumption of food containing *F. moniliforme* has been linked epidemiologically to the high incidence of esophageal cancer in some areas of the world (Sydenham et al., 1991; Rheeder et al., 1992). Because of their toxicity and widespread natural occurrence, the fumonisins are of public health concern.

Surveys of corn-based foods for fumonisins have shown that tortillas, ready-to-eat cereals, muffins, and other processed foods generally contain lower fumonisin levels than unprocessed foods such as corn meal, corn flour, and grits (Sydenham et al., 1991; Stack and Eppley, 1992; Doko and Visconti, 1994). Chemical processing such as ammoniation (Norred et al., 1991; Park et al., 1993) and alkaline treatment (Hendrich et al., 1993; Sydenham et al., 1995; Voss et al., 1996) had varying success at detoxifying fumonisins in corn. Preliminary work by Canela et al. (1996) has shown that steeping in water or solutions of sodium bisulfite may reduce the fumonisin content of naturally contaminated corn. Physical treatments such as sieving "fines" from bulk shipments of corn have reduced fumonisin levels by 26−69% (Sydenham et al., 1994). Dry milling of fumonisin-contaminated corn tended to concentrate the fumonisins in the bran and germ fractions and produced grits relatively free of contamination (Katta et al., 1997).

Jackson et al. (1996a,b) and Alberts et al. (1990) found minor losses when aqueous solutions of FB_1 and FB_2 were heated at temperatures <150 °C. Similarly, Dupuy et al. (1993) found minimal losses of FB_1 in naturally contaminated dry corn meal heated at temperatures <125 °C. Maragos and Richard (1994) found that heating whole milk spiked with FB_1 and FB_2 for 30 min at 62 °C did not reduce levels of these toxins. Jackson et al. (1996a,b) found that temperatures ≥150 °C were required to observe decomposition of fumonisins in aqueous solutions. Scott and Lawrence (1994) found that heating moist corn meal at 190 °C for 60 min resulted in ≈80% reduction in FB_1 and FB_2 levels. Heating dry corn meal spiked with FB_1 and FB_2 at 190 and 220 °C resulted in 60% and 100% loss of both toxins after 60 and 25 min, respectively (Scott and Lawrence, 1994).

Several studies have shown that established analytical methods tend to underestimate the fumonisin content of thermally processed foods. The losses of fumonisin observed during heating (Dupuy et al., 1993; Scott and Lawrence, 1994) may be due to matrix-related difficulties of recovery and detection, rather than actual

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S0021-8561(97)00405-6 CCC: $14.00

fumonisin decomposition. Bordson et al. (1995) reported that drying corn at temperatures above 50 °C, but lower than temperatures required to decompose fumonisins, resulted in decreased fumonisin recovery during analysis. Scott and Lawrence (1994) reported poor recoveries when the method of Shephard et al. (1990) was used to analyze FB_1 and FB_2 spiked into corn bran flour. Poor recoveries of FB_1 were obtained when the methods of Shephard et al. (1990) and Ross et al. (1991) were used to measure the FB_1 content of canned sweet corn (Trucksess et al., 1995). Jackson (1997) found that a commercially available ELISA kit and the Shephard et al. (1990) method greatly underestimated the fumonisin levels in corn muffins and extruded corn grits prepared from spiked corn. Using 1:1 acetonitrile/H_2O (pH 1.0) to extract fumonisins and the method of Ross et al. (1991) to purify and quantify the compounds gave excellent (>90%) recoveries of fumonisins spiked into several processed foods such as tortillas, extruded corn grits, and corn muffins (Jackson et al., unpublished data).

More work is needed to gather accurate data on the effects of processing on the fumonisin content of food. This study determined the effect of different processing temperatures used during baking and frying on the FB_1 content of corn muffins and corn chips, respectively.

MATERIALS AND METHODS

Fumonisin B_1 (98% pure) and fully hydrolyzed FB_1 (HFB_1) for standards and processing studies were donated by Drs. Robert Eppley and Steven Musser (FDA, Washington). Standards were prepared in 1:1 acetonitrile/H_2O. *O*-Phthaldialdehyde (OPA) was purchased from Sigma Chemical Co. (St. Louis, MO). All reagents were of analytical grade, and solvents were of HPLC grade. FB_1 is a suspected carcinogen and should be handled with care.

Baking Study. A commercial corn muffin mix (Jiffy Mix, Chelsea Milling Co., Chelsea, MI), containing <0.1 μg/g FB_1, was used for baking studies. These studies involved spiking the corn muffin mix with FB_1 standard at a level of ≈5 μg/g (dry weight basis). Skim milk, eggs, and FB_1 standard solution were blended together and then mixed with the dry muffin mix as directed by the instructions on the box. Muffin batter (70 g) was placed into each of the six cups in a nonstick muffin pan and baked in a laboratory convection oven (Precision Instruments, Chicago, IL) at 175 and 200 °C for 20 min. Calibrated thermocouples, made from type T thermocouple wire (Omega Engineering, Stamford, CT), were used to monitor the temperature in the center and close to one surface of three muffins/pan. A data acquisition system (Labtech Control, Laboratory Technologies, Wilmington, MA) installed on a Hewlett-Packard HP 7500 Series B computer recorded muffin temperature during baking.

For one baking trial at 175 and 200 °C, an attempt was made to determine if there were differences in FB_1 levels at the surface and in the center of muffins. The surface of the muffins, defined as the outer 3 mm, was removed with a razor blade. The core or center of the muffin was defined as the remainder of the muffin after removal of the surface. The core and surface of muffins were ground and frozen until analyzed. Moisture contents of muffins (whole and portions) were determined after drying at 60 °C under vacuum for 24 h.

Frying Study. Corn masa (Quaker, Barrington, IL), containing no detectable FB_1, was spiked at 10 μg/g FB_1 on a dry weight basis. This was accomplished by mixing masa with an equal weight of 10 μg/g FB_1 solution. The resulting dough was formed into circles 12.1 cm in diameter and 1.2 mm in thickness with a tortilla press. The circles were cut into four equal pieces and fried (Frymaster Model J1C, Shreveport, LA) in partially hydrogenated soybean oil (Crisco, Proctor and Gamble, Cincinnati, OH) at 140−190 °C for 1−15 min. The corn chips were dried for 16 h at 60 °C and then ground in a food processor. The ground chips and masa dough (before frying) were frozen until they were analyzed for FB_1 content.

Calibrated type T thermocouples (Omega Engineering) were used to monitor oil temperature during frying. During all frying runs, oil temperature dropped by 10−20 °C after masa dough was dropped into the oil. However, the desired frying temperatures were reached within 2 min of starting frying runs. At the desired frying temperatures, oil temperature fluctuated by ±5 °C.

HPLC Determination of FB_1. Loss of FB_1 in muffins and corn chips was measured according to the method of Ross et al. (1991) with modifications. Briefly, samples were blended with 1:1 acetonitrile/H_2O (adjusted to pH 1.0 with HCl) for 30 s and then shaken with a wrist action mixer for 60 min. To estimate the percent FB_1 recovered during the extraction and purification procedure, samples were spiked with FB_1 standard and extracted with acetonitrile/H_2O as mentioned above. The resulting slurry was filtered through Whatman No. 2 paper. Two milliliters of filtrate was mixed with 6.0 mL of 1% (w/v) KCl and then loaded on a preconditioned (2 mL of methanol followed by 2 mL of 1% KCl) C_{18} SepPak (Waters, Milford, MA) column. The columns were washed with 2 mL of 1% KCl and then with 1 mL of 15:85 acetonitrile/water. FB_1 was eluted from the columns into vials with 3 mL of 70:30 acetonitrile/water. Extracts were dried using a SpeedVac concentrator (Savant Instruments, Farmingdale, NY), and the residues were dissolved in 200 μL of 1:1 acetonitrile/water. The presence of HFB_1 in samples was determined according to the method of Hopmans and Murphy (1993).

A Waters HPLC equipped with a Model 600 pump, a WISP 716 autoinjector, and a Model 740 fluorescence detector (335 nm excitation wavelength and 440 nm emission wavelength) and Millenium 2010 software were used to identify and quantify FB_1 in the sample extracts. The autoinjector was programmed to mix 20 μL of sample extracts with 125 μL of OPA reagent and then to inject 10 μL of the mixture after 5 min of incubation. Separations were carried out at 23 °C on a Supelco ODS-80 column (4.6 mm × 25 cm) with a 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. The flow rate was 1.0 mL/min.

Kinetic Calculations. Kinetic constants were calculated according to the method of Jackson et al. (1996a). Briefly, processing time was plotted with respect to C_A, $\ln(C_A)$, and $1/C_A$, where C_0 and C_A refer to the initial FB_1 concentration and the remaining FB_1 concentration (micrograms per gram) after time t (minutes), respectively. Reaction order was obtained by determining whether zero-, first-, or second-order plots 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 frying and baking trials were performed in duplicate and triplicate, respectively. Triplicate FB_1 analyses were performed on all samples. Means and standard deviations (SD) were calculated with Minitab (State College, PA) statistical software. Minitab was used to verify significant differences between treatments by one-way analysis of variance (ANOVA) followed by a Ryan−Einot−Gabriel−Welch multiple-range test at 95% confidence. Linear regression analyses, used to determine reaction constants and half-lives of FB_1, were performed by using Psiplot graphics software (Poly Software International, Salt Lake City, UT).

RESULTS AND DISCUSSION

Corn samples (before and after frying and baking) were spiked with FB_1 to estimate the percentage recovered during the HPLC extraction/purification procedure. Percent recoveries of FB_1 spiked (3 μg/g) into masa dough, fried corn chips, muffin batter, and baked corn muffins were 92.3 ± 5.9%, 96.7 ± 4.6%, 79.4 ± 7.8%, and 93.1 ± 7.3%, respectively. The detection limit for the method was 0.05 μg/g.

Table 1. Effect of Baking on the FB_1 Content of Corn Muffins[a]

baking temp (°C)	trial	% FB_1 remaining after baking		
		whole muffin	surface	core
175	A	79.8 ± 5.3	81.5 ± 3.7 a	86.6 ± 5.0 a
	B	86.7 ± 3.4		
	C	84.6 ± 3.2		
		x = 83.7 ± 3.5 a		
200	A	66.6 ± 9.4	57.3 ± 2.6 b	88.5 ± 1.3 a
	B	78.4 ± 4.2		
	C	72.4 ± 3.2		
		x = 72.4 ± 5.9 b		

[a] Means in the same column having different letters are significantly different at $p < 0.05$.

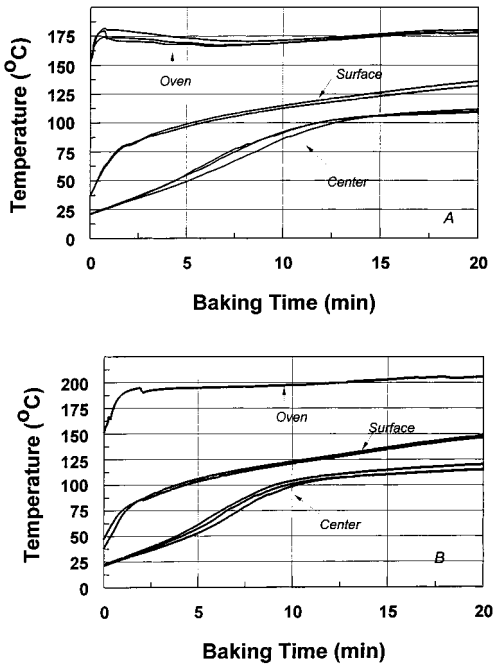

Figure 1. Temperature profiles in corn muffins baked in a convection oven at 175 (A) and 200 °C (B) for 20 min. Temperature was recorded in the oven and in the center and on of the surfaces of the muffins.

Baking Study. Baking at 175 and 200 °C resulted in small, but statistically significant ($p < 0.05$), losses of FB_1 in corn muffins (Table 1). The percent of FB_1 retained in muffins baked at 175 °C (83.7 ± 3.5%) was slightly greater than that in muffins baked at 200 °C (72.4 ± 5.9%). Compared to the data reported here, Scott and Lawrence (1994) observed less retention of FB_1 (28%) and FB_2 (36%) in corn muffins that were baked at 220 °C for 25 min. The lower retentions reported by Scott and Lawrence (1994) may be due to the higher baking temperatures, longer baking times, and/or analytical problems that prevented accurate quantitation of fumonisins.

The temperature profile in the muffins had a significant effect on losses of FB_1. When baked at 175 °C, the surface and core temperatures of the muffins reached 135 and 107 °C, respectively, while muffins baked at 200 °C had maximum surface and core temperatures of 150 and 115 °C, respectively (Figure 1). At both baking temperatures, losses of FB_1 at the surface of the muffins were significantly greater ($p < 0.05$) than those at the core (Table 1). At 200 °C, FB_1 levels in the surface were two-thirds those in the core of the muffins.

The results shown here are generally in agreement with those of previous studies on the thermal stability of FB_1 in corn. Dupuy et al. (1993) found similar reductions in FB_1 levels when dry corn was heated at temperatures <150 °C; however, the authors did not measure the temperature in the corn during heating. The data presented here appear to contradict those found by Jackson et al. (1996a,b). They did not observe losses of FB_1 and FB_2 in solutions heated at temperatures <150 °C for 30 min. The mechanism for fumonisin loss, however, may be different in a simple aqueous medium. Jackson et al. (1996a,b) showed that when solutions reached temperatures ≥150 °C, partial and fully hydrolyzed fumonisins were the major decomposition products. The loss of FB_1 at temperatures <150 °C and the lack of hydrolysis products in HPLC chromatograms (not shown) suggest that hydrolysis may not be the major mechanism for decomposition in the baked corn muffins studied here. However, another possible explanation is that HFB_1 formed during heating reacted with other components of the muffin mix.

The loss of FB_1 during baking may be due to the nonenzymatic browning reaction described by Murphy et al. (1996). The reaction occurs when the primary amine group of FB_1 reacts with free aldehyde or ketone groups in reducing sugars such as glucose or fructose. Murphy et al. (1996) found that when 100 mM fructose or glucose was heated with 5 μM FB_1 at 80 °C, the reaction followed apparent first-order kinetics. The presence of reducing sugars in the muffin mix and added milk (lactose) suggests that losses of FB_1 observed in the baked muffins may have been due to nonenzymatic browning. The rate and extent of nonenzymatic browning increase in food with increasing heating time and temperature (Mottram, 1994). The darker brown color of muffins baked at 200 vs 175 °C suggests that browning was more extensive at the higher temperature. This would explain the greater losses of FB_1 in muffins and portions of muffins (i.e. surfaces) that received greater heat treatments.

Frying Study. HPLC chromatograms for extracts of corn chips fried at 190 °C for 2−15 min are shown in Figure 2. The chromatograms show the progressive loss of FB_1 as frying time increased. No hydrolysis products of FB_1 were detected in the chromatograms. This suggests that hydrolysis was not the mechanism of loss of FB_1 in corn chips during frying or that the hydrolysis products may have reacted with other components of the masa.

A nonenzymatic browning reaction involving FB_1 and reducing sugars could explain the increasing loss of FB_1 detected in these samples as frying time increased. Chips became progressively browner in color as frying times and temperatures increased. Chips fried for 1−2 min (all temperatures) were barely cooked, while those fried for 4−6 min were light brown in color and resembled commercially available corn chips in appearance. Chips fried at higher temperatures (180−190 °C) and longer times (8−15 min) were dark brown in color.

Figure 3 indicates that the loss of FB_1 is highly dependent on temperature and that, in general, the extent of loss increased with frying temperature and time. No significant losses of FB_1 were found when chips were fried at frying temperatures <180 °C. This finding conflicts with the results of the baking study for which losses of FB_1 were found in muffins baked at 175 °C. Differences in the results may be reflected by compositional differences between the muffin batter and

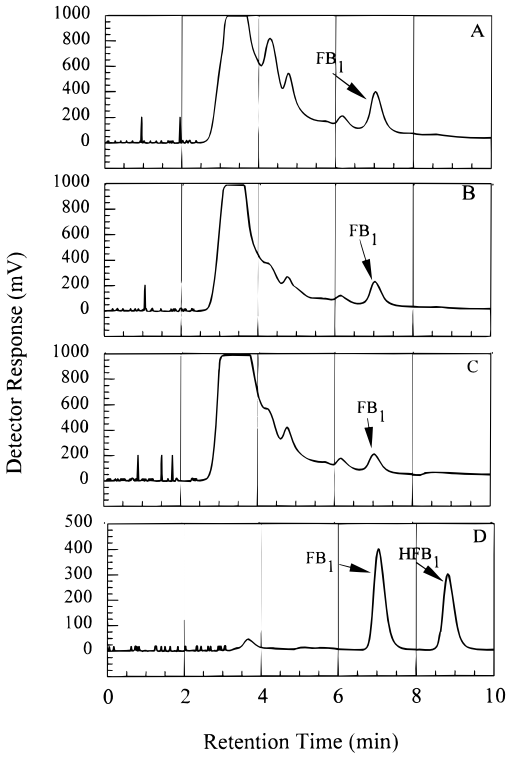

Figure 2. HPLC chromatograms using fluorescence detection (335 nm excitation wavelength and 440 nm emission wavelength) for extracts of corn chips fried at 190 °C. Chromatograms A, B, and C refer to the extracts fried at 190 °C for 2, 10, and 15 min, respectively. Chromatogram D shows peaks for standards of FB_1 and HFB_1. Peaks with retention times of <7 min have not been identified.

the masa dough and the fact that the masa dough was heated for shorter lengths of time (≤15 min) than the muffin batter (30 min).

At 180 and 190 °C, losses increased with frying time and began when chips were fried for ≥8 min. At both temperatures, the loss of FB_1 in the corn chips followed an apparent first-order reaction. Half-lives were 14.83 ± 0.32 and 6.42 ± 0.72 min at 180 and 190 °C, respectively, while the pseudo-first-order reaction constants were 0.0467 ± 0.0042/min and 0.1078 ± 0.0121/min, respectively. Dupuy et al. (1993) and Jackson et al. (1996a) studied the kinetics of FB_1 decomposition in dry corn and aqueous solutions, respectively, during thermal processing. Similar to the results reported here, they found that the decomposition of FB_1 during heating followed first-order kinetics. The half-lives and rate constants reported here are within the ranges calculated by Jackson et al. (1996a) for aqueous solutions of FB_1 processed at 175−200 °C.

Conclusions. Processing is used by the food industry to convert raw cereal grains and other agricultural products into a multitude of consumer products. Examples of these processes are milling to produce flour, extrusion to produce ready-to-eat cereals and snacks, baking to produce cakes and muffins, and frying to produce snack foods. Little is known about the effects of these processing operations on the levels of fumonisins in food. This study indicates that under normal commercial frying and baking conditions, FB_1 losses are small. More substantial losses of FB_1 may occur when foods are processed at more extreme (i.e. higher temperatures/longer times) conditions. More work is needed to determine if processes that subject food to high temperatures and pressures (i.e. extrusion) affect fumonisin levels.

Loss of fumonisins may result from a nonenzymatic browning reaction involving the primary amine in fumonisins and reducing sugars in the food. More work is needed to identify the sugar−fumonisin browning product(s) and quantify these products in processed

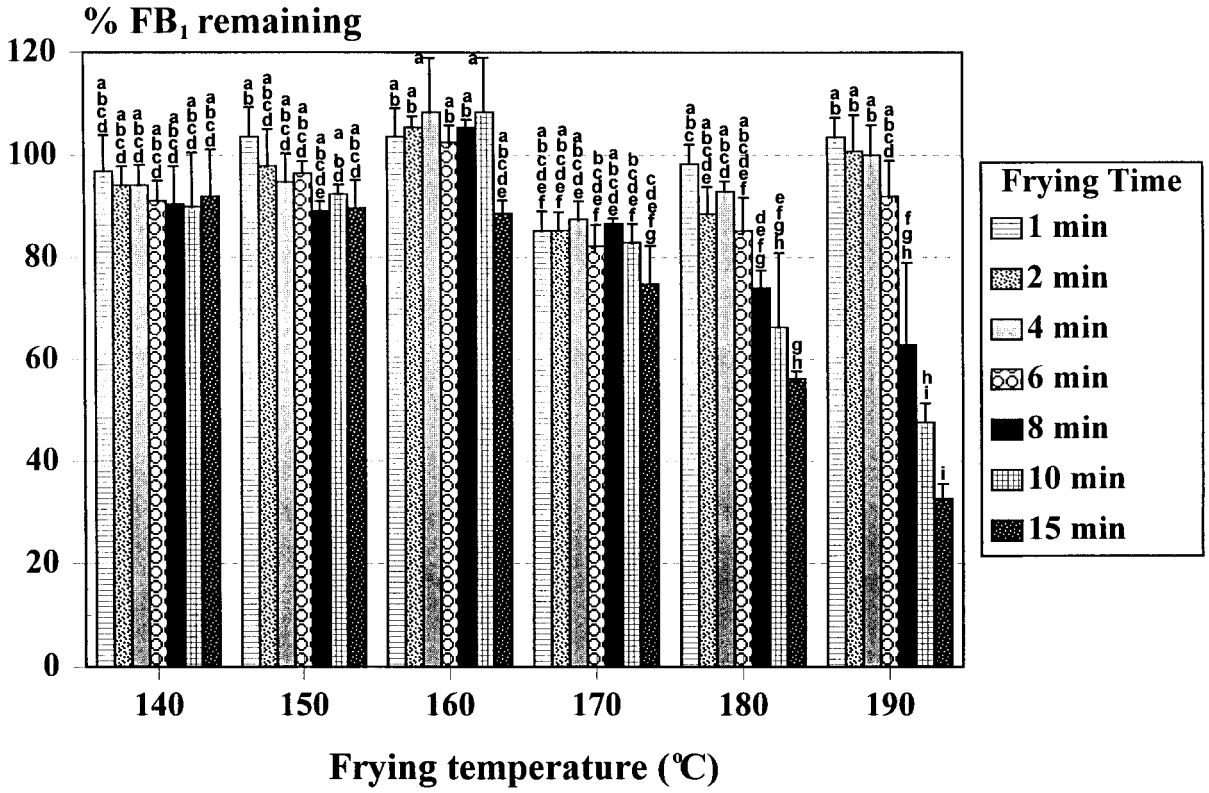

Figure 3. Effects of temperature and time on the loss of FB_1 in corn chips fried in hydrogenated soybean oil. Each bar represents the average of two replicate runs, and error bars indicate one SD of the mean. Bars with different letters are significantly different ($p < 0.05$).

food. Further studies are also needed to determine if adding reducing sugars to food before processing results in loss of fumonisin.

ACKNOWLEDGMENT

We thank Stuart J. Chirtel (FDA, CFSAN, Division of Mathematics) for assisting with the statistical analysis of the data.

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Received for review May 16, 1997. Accepted September 23, 1997.[⊗] This work was partially supported by Cooperative Agreement FD-000431 from the U.S. Food and Drug Administration and the National Center for Food Safety and Technology. Its contents are solely the opinions of the authors and do not necessarily represent official views of the U.S. Food and Drug Administration. This material is based upon work supported in part by the Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture, under Agreement 95-37201-2343. Financial support for this project was also provided in part by a grant from the Anderson Research Fund (of NC 213), Maumee, OH. Published as Paper 11951 Journal Series, Agricultural Research Division, University of Nebraska, Lincoln, NE. Research was conducted under Projects 16042 and 16056 (NC213).

JF970405B

[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1997.