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The effect of thermal processing on fumonisin B_1 (FB_1) levels in maize-based foods

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Fumonisin B_1 (FB_1) is a mycotoxin from *Fusarium verticillioides* that is frequently associated with maize. Fumonisins have been implicated as the causal agents of a variety of animal diseases and are epidemiologically linked to the high incidence of human esophageal cancer in some regions of the world. Thermal treatments are used in many processes involving grain and its derivatives, but little is known about the effects of common processing methods on the fumonisin content of food. The objectives of this study were to determine the thermostability of this toxin in contaminated maize, at different time/temperature combinations, as well as to determine the effect of baking and frying on the stability of FB_1 spiked into maize-based foods. The identity of FB_1 in extracts before and after heat treatments was confirmed by high-performance liquid chromatography. For each thermal process, the fumonisin content was inversely proportional to the processing temperatures. An initial FB_1 concentration of 217 mg/g in the control, was reduced to 184 mg/g when treated at 100°C for 2 h. Oven temperature of 220°C for 30 min showed extensive reduction of FB_1 to a concentration of 1.1 mg/g as compared to 94 mg/g in the control. Baking maize muffins spiked with 1.25 µg/g (dry weight) FB_1 at 200°C for 20 min resulted in an average FB_1 loss of 70%. Frying of maize chips spiked with 5 µg/g (dry weight) FB_1 at 190 to 210°C for 5 to 10 min resulted in an average FB_1 loss of 67%. The results of this study indicate that boiling temperatures are ineffective in producing any significant reduction in FB_1 levels. Thus, there might be correlation between the under processing of fumonisin-contaminated foods and the high incidence of esophageal cancer in certain regions of South Africa.

Key words: Mycotoxins, *Fusarium verticillioides,* maize-based food products, esophageal cancer.

INTRODUCTION

Fungal growth and subsequent contamination of animal feeds and human foodstuffs by toxins (mycotoxins) constitute both an economic and a health risk. The mycotoxins of major concern for human health are produced by three main genera of fungi *(Aspergilius, Fusarium* and *Penicillium)* (Nelson et al., 1994). The

fumonisins produced by *Fusarium verticillioides* and *Fusarium proliferation,* is one family of mycotoxins, which contaminate feeds and foodstuffs, predominantly maize-based products throughout the world. This fungus has been suspected of being involved in human and animal diseases since its original description in 1904 (Sheldon,

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1904).

They can produce high amounts of fumonisins in tropical and subtropical regions (Marasas, 2001; Reddy et al., 2009; Shephard et al., 1996), whereas in colder regions, the fumonisin contamination is much lower (Logrieco et al., 2002; Miller, 2008). In addition to maize-derived products, fumonisins have also been detected in rice (Yazar and Omurtag, 2008), black tea leaves (Martins et al., 2001), asparagus (Logrieco et al., 1998) and pine nuts (Marin et al., 2007).

Maize is the staple cereal food grown and consumed by the rural farming communities of Africa, especially in the Transkei region of South Africa (Shephard et al., 2002), where the effects of fumonisins were first discovered in 1988. The Transkei region has one of the highest incidences of esophageal cancer in the world, which seems to be associated with the fumonisin intake (Makaula et al., 1996; Shephard et al., 2002). A positive correlation in the number of ecological studies between dietary fumonisins and human esophageal cancer rates has been reported in Africa and China (Sydenham et al., 1990; Chu and Li, 1994). In Southern Africa, the highest rate of human esophageal cancer occurred in the southwestern districts of Transkei where maize is the main diet, and these populations can be chronically exposed to highly contaminated food (Marasas et al., 1981). Several strains of F. verticillioides isolated from maize produced in these districts have been found to be acutely toxic to ducklings. When culture material of these isolates grown on autoclaved maize, was fed to experimental animals, the lesions induced included cirrhosis and nodular hyperplasia of the liver and intraventricular cardiac thrombosis in rats, leukoencephalomalacia (LEM) and toxic hepatosis in horses, pulmonary edema in pigs, nephrosis and hepatosis in sheep and acute congestive heart failure in baboons (Kriek et al., 1981).

On the basis of the toxicological evidence, the International Agency for Research on Cancer has declared F. verticillioides toxins as potentially carcinogenic to humans as class 2B carcinogens (Shephard et al., 1996). In view of these toxic effects and the ubiquitous nature of F. verticillioides contamination of maize, control measures and potential decontamination procedures are required to reduce the health risks posed by contaminated maize consumption in various areas of the world. Also, South Africa cannot afford to dump imported grain or export grain if it is contaminated. Thus, contamination of maize by F. verticillioides and fumonisins is a growing concern that may threaten animal and human health and is detrimental to the marketability of maize throughout the world.

Studies on the occurrence of fumonisins in maize and maize-based foods have shown that the highest levels of the toxin are in the whole grain and those maize products that undergo the least/mildest forms of processing such as maize meal, maize flour and grits. Those maize products that are more highly processed such as corn flakes and cereals tend to have either no detectable, or very low levels of fumonisins (Stack and Eppley. 1992). Industrial processes such as milling, extrusion, baking and frying are used by the food industry to convert raw cereal grains into a multitude of consumer products. Little is known about the effects of thermal processing on fumonisin levels in maize-based foods. Thus, the purpose of this study was to determine the thermal stability of FB_1 in maize-based foods.

MATERIALS AND METHODS

Growth and maintenance of *F. verticillioides*

A toxigenic strain of *F. verticillioides* (MRC 826, Pretoria, South Africa) was used throughout this study. The strain was subcultured on malt extract agar (Oxoid) and Potato Dextrose Agar (Oxoid), for 7 to 12 days at 25°C until profuse sporulation was evident. Mycelial plugs from a sporulating region were excised from the cultures using a scalpel blade and used for inoculation of maize. Stock cultures were maintained by subculturing onto malt extract agar and Sabouraud dextrose agar slants fortnightly.

Inoculation of maize patty cultures

Two 250-ml Erlenmeyer flasks were filled with 100 g of dry maize meal (Nyala, Premier Milling, Newtown), plus 100 ml distilled water and autoclaved at 121°C for 15 min. The flasks were inoculated with mycelial plugs from a 15 day old culture of *F. verticillioides* and incubated at 25°C for 4 weeks. The flasks were then dehydrated in a vacuum oven for 48 h at 45°C. Dried maize cultures were ground (Warring blender) to the consistency of flour. The FB_1 concentration in this contaminated maize was determined by high performance liquid chromatography analysis and was considered the initial concentration.

Processing treatments

Boiling

The inoculated maize cultures as previously prepared were subsequently used to determine the effects of thermal treatments. Two procedures were used for treatment. Firstly, boiling water, a moist heat source was used. The maize cultures were processed by the method of Dupuy et al. (1993) with slight modifications. A 500 mg sample of contaminated maize was placed into stoppered test tubes and immersed in boiling water at 100°C for 30 min, 1 and 2 h. *F. verticillioides* inoculated maize cultures which were not thermally treated were used as the controls to determine the FB_1 concentration prior to processing. Temperature was measured using calibrated thermocouples (Omega Engineering). Secondly, an oven was used to provide a dry heat source. A 500 mg sample of contaminated maize was placed into stoppered test tubes and processed at 100°C for 30 min, 1 and 2 h in an oven. This was achieved by placing the test tubes in beakers filled with sand. All experiments were performed and analyzed in triplicate.

Baking

The baking and frying studies were modified from the method described by Jackson et al. (1997) with a few changes as follows. A commercial maize muffin mix (Golden Cloud, Tiger Milling and

Baking, Germiston) was used for baking studies. The muffin mix was spiked with 1.25 µg/g FB_1 (w/w). Fresh milk, eggs and FB_1 standard solution were blended together and mixed with the dry muffin mix. Muffin batter (50 g) was placed onto each of the six cups in a nonstick muffin pan and baked in a laboratory convection oven at 220°C for 20 min. Calibrated thermocouples (Omega Engineering) were used to monitor the oven temperature. Inoculated maize cultures were processed at temperatures of 240 to 300°C for 20 min in a muffle furnace. The controls were treated the same except that they were not baked. All experiments were performed and analyzed in triplicate.

Frying

Maize meal spiked at 5 µg/g FB_1 (w/w) was used for frying experiments. This was achieved by mixing a 100 g maize meal with an equal weight of water containing the FB_1 solution. The resulting dough was formed into circles of approximately 10 cm in diameter and 1.5 mm in thickness with a rolling pin. The circles were cut into four equal pieces and fried in pure sunflower oil at 190 to 210°C for 5 to 10 min. In this study, the control used to determine the FB_1 concentration prior to processing was FB_1 spiked maize chips that were not fried. Calibrated thermometers were used to monitor the oil temperatures during frying. For all the frying runs, oil temperatures dropped by 10 to 20°C after the chips were added to the oil. However, the required temperatures were reached within 2 min of starting the frying run. All experiments were performed and analyzed in triplicate.

Extraction

Fumonisin B_1 (98% pure) for standards and processing studies were obtained from Sigma Chemical Co; Midrand South Africa. Standards were prepared in 1:1 acetonitrile - water. The standards allowed qualitative and quantitative validation, by TLC and HPLC respectively. δ-Phthaldialdehyde (OPA) was purchased from Sigma Chemical Co. All reagents were of analytical grade and solvents were of HPLC grade.

Loss of FB_1 in maize cultures, muffins and chips was measured according to the method of Sydenham et al. (1992). Contaminated processed and unprocessed samples were extracted with methanol-water (3:1) (v/v), in a 1:2 (v/v) ratio and placed onto an orbital shaker for 1 h. The resulting slurries were centrifuged (500 xg, 10 min at 4°C). The supernatants were then filtered through Whatman No. 4 paper. The filtered extracts before and after processing were purified using the Bond Elut SAX cartridges (Varian, Harbor City, USA). Cartridges were fitted onto the Vac-Elut manifold (Varian) and were conditioned by washing first with 5 ml methanol followed by 5 ml of methanol-water (3:1) (v/v). Cartridges were not allowed to run dry. A 5 ml aliquot of the filtered extract was applied to the cartridge, thereafter the cartridge was washed with 5 ml methanol-water (3:1) followed by 3 ml methanol to remove contaminants. A flow rate of 2 ml/min through the cartridge was maintained. Fumonisins were eluted and collected with 10 ml of 0.5% acetic acid in methanol at a flow rate not higher than 1 ml/min in a suitable vial. The eluate was evaporated to dryness under a stream of nitrogen at 50°C. Dried samples were stored at 4°C until HPLC analysis.

Thin layer chromatography

Detection of FB_1 in extracts before and after processing were done by the method of Dupuy et al. (1993) with modifications, dried extracts and standards were reconstituted in 20 µl of acetonitrile-

water (1:1), by vortexing for 5 min. 5 µl of the corn extracts and standards was spotted on Silica Gel 60 plates (10 x 10 cm, Merck). The plate was developed in 1-butanol-acetic acid-water (20:10:10) (v/v/v). After solvent evaporation, the plate was sprayed with 0.5% ρ-anisaldehyde and heated at 100°C for 10 min. For each plate, extracts and standards were spotted in duplicate.

High-performance liquid chromatography

Loss of FB_1 in inoculated maize cultures, muffins and chips were measured according to the method of Sydenham et al. (1992) with modifications. Briefly, residues were dissolved in 200 ul of acetonitrile-water (1:1). A 50 µl volume of the purified extracts or standards were transferred to the bottom of a small test tube and 450 µl of -phthalaldehyde reagent was added to each test tube. The solution was vortexed for 20 s, and 20 µl was injected into the HPLC system within 2 min of adding the OPA reagent. The HPLC column used was a Lichrosorb 5-µm C_{18} reversed phase column (Merck). A liquid chromatography M45 pump and injector with a fluorescence detector (335 nm excitation wavelength and 440 nm emission wavelength) was used to determine the identity and concentrations of FB_1 in the sample extracts. The mobile phase was methanol : 0.1 M sodium dihydrogen phosphate (68:32) adjusted to pH 3.35 with ortho-phosphoric acid. The flow rate was 1.0 ml/min. The fumonisin content of the maize sample was calculated from the chromatographic peak areas according to Shepard (2000).

RESULTS AND DISCUSSION

Boiling water

Migration rates of crude extracts of inoculated maize patty cultures processed at a 100°C in boiling water for 30 min, 1 and 2h can be seen in Figure 1A. Trailing and co-elution were present in the sample extracts, indicating that the fumonisins and the dirt were bound, thus affecting the migration rates (Rf) values of the sample extracts. Positive identification of fumonisins was achieved after SAX cleanup in the processed and unprocessed samples (Figure 1A). Purification of extracts resulted in no dirt beneath the sample extracts and thus equivalent (Rf values) between samples and FB_1 standards were obtained. Positive identification was achieved after SAX clean up in processed and unprocessed *F. verticillioides* inoculated maize cultures by TLC. In Figure 1B, sample extracts migration rates (Rf values) are equivalent to those of the standards. Unprocessed inoculated maize patty cultures used for the boiling water experiments contained an initial concentration of 217.11 mg/g. Thermal treatments resulted in small decreases of fumonisin levels. Inoculated maize cultures processed at 100°C for 30 min contained 205.95 mg/g of FB_1. Thermal treatments at 100°C for 1 h contained a FB_1 concentration of 194.80 mg/g. Processing at 100°C for 2 h resulted in a FB_1 concentration of 183.65 mg/g (Table 1). Unprocessed inoculated maize cultures used for the oven experiments contained 1706.80 µg/g FB_1. Thermal treatment in an
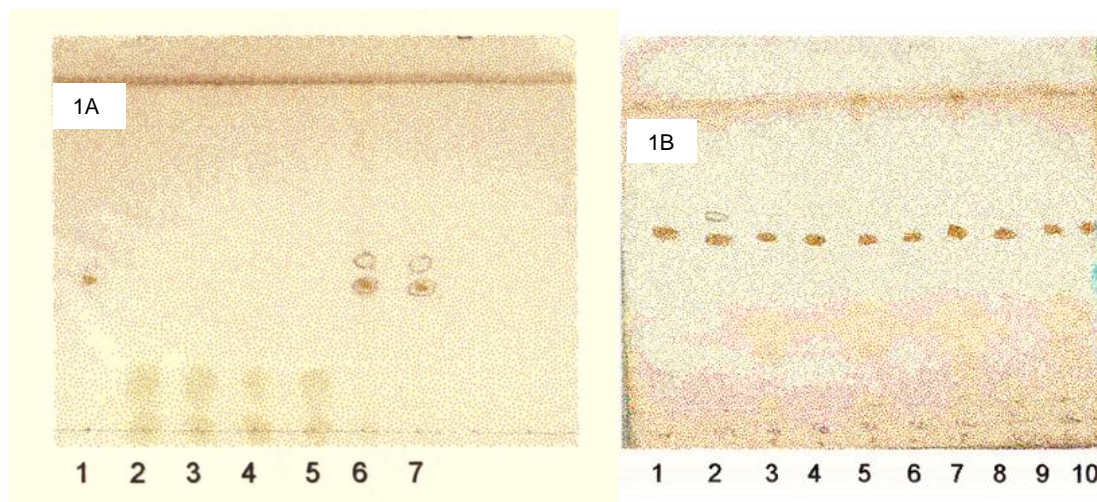
Figure 1. A: TLC of crude processed and unprocessed *F. moniliforme* inoculated maize cultures. Lane 1, 6, 7– FB$_1$ standards, lane 2 represents the control, lane 3- samples processed at 100°C for 30 min, lanes 4- samples processed at 100°C for 1 h and lanes 5- samples processed at 100°C for 2 h. B: TLC of thermally processed purified extracts in boiling water (100°C). Lanes 1 and 2– FB$_1$ standards, lanes 3 and 4- control, lanes 5 and 6- sample extracts treated at 100°C for 30 min, lanes 7 and 8- sample extracts treated at 100°C for 1 h and lanes 9 and 10- sample extracts treated at 100°C for 2 h.

Table 1. Thermal treatments of inoculated maize cultures at 100°C in boiling water and in oven.

Sample (n=3)	Boiling water (100°C)		Oven (100°C)	
	Concentration (mg/g)	Loss (%)	Concentration (µg/g)	Loss (%)
Control	217.11	0 ± 2	1706.80	0 ± 2
100°/30min	205.95	5 ± 1	1085.51	36 ± 1
100°/1h	194.80	10 ± 2	709.65	58 ± 1
100°/2h	183.65	15 ± 2.5	272.50	84 ± 5

Results are the mean and standard deviation of three determinations.

oven resulted in significantly higher FB$_1$ percentage losses as compared to boiling water. Processed inoculated maize cultures at 100°C for 30 min contained 1085.51 µg/g of FB$_1$. Processed maize cultures at 100°C for 1 h contained 709.65 µg/g of FB$_1$ and treatment at 100°C for 2 h contained a FB$_1$ content of 272.50 µg/g (Table 1).

Oven treatments

A convection oven was used to process *F. verticillioides* maize patty cultures at a higher range of temperatures from 140 to 220°C for 30 min. None of the above temperatures appeared to affect fumonisin levels at this stage. Equivalent migration rates (Rf values) between sample extracts and the FB$_1$ standards achieved positive identification. The control refers to an unprocessed inoculated maize patty culture sample extract which contained an initial concentration of 93.73 mg/g. Thermal treatments showed increase in FB$_1$ reduction as

temperatures increased. Inoculated maize cultures processed at 140°C for 30 min contained 75.11 mg/g of FB$_1$. Processing at 160°C resulted in a FB$_1$ content of 48.59 mg/g, treatment at 180°C resulted in 34.75 mg/g FB$_1$. While processing at 200 and 220°C for 30 min resulted in FB$_1$ concentrations of 22.54 and 1.14 mg/g, respectively (Table 2).
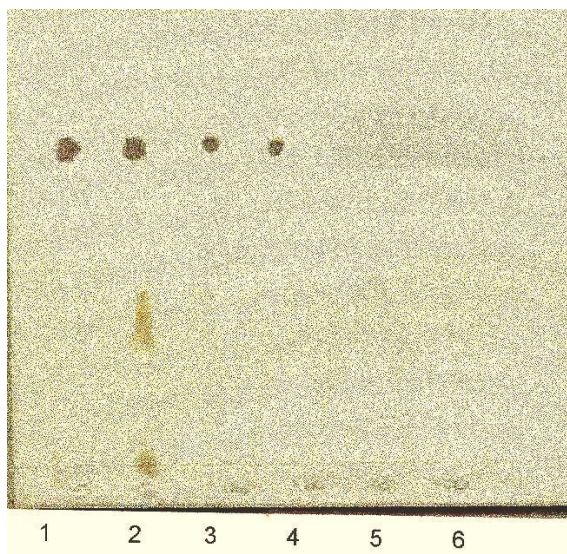
These results support the data obtained by Alberts et al. (1990), who reported that boiling culture material of *F. verticillioides* for 30 min had no effect on the FB$_1$ concentration. It highlights the fact that FB$_1$ might be found in foods cooked at 100°C for a few minutes. Dupuy et al. (1993) found similar reductions in FB$_1$ levels when dry maize was heated at temperatures <150°C.

Muffle furnace

In an effort to determine the temperature at which fumonisins are eliminated, inoculated maize cultures were processed at temperatures of 240 to 300°C for 20

Table 2. Effects of thermal treatments on *F. moniliforme* inoculated maize cultures processed for 30 min in an oven.

Sample (n=3) (°C)	Concentration (mg/g)	Loss (%)
Control	93.73	0
140	75.11	20
160	48.59	49
180	34.75	63
200	22.54	76
220	1.14	99

Table 3. Effects of baking on fumonisin levels in maize muffins.

Sample (n = 3)	Concentration (μg/g)	Loss (%)
Control	0.96	0
Muffin 1	0.37	62
Muffin 2	0.28	71
Muffin 3	0.23	76

Figure 2. Thermal treatments of *F. moniliforme* inoculated maize cultures treated at 240 to 300°C in a muffle furnace. Lane 1- fumonisin standard, lane 2- control, lanes 3, 4, 5 and 6- samples processed at 240, 260, 280 and 300°C for 20 min.

min in a muffle furnace (Figure 2). Fumonisins were detected at 240 and 260°C, but not detected at 280 and 300°C.

Evaluation of processing temperatures

Baking

Evaluation of processing temperatures involved baking and frying studies. Maize muffins spiked at 1.25 μg/g FB_1 standard were baked at 220°C for 20 min in a convection oven. Fumonisins were positively identified by comparison of the migration rates (Rf values) of the sample extracts and the FB_1 standard indicating that the baking process does not sufficiently degrade fumonisins. The control for baking studies was an unbaked spiked muffin, which had an initial concentration of 0.96 μg/g.

Baking of spiked maize muffins resulted in an average FB_1 percentage loss of 70% (Table 3).

The results of these baking experiments are in accordance with those by Scott and Lawrence (1994). FB_1 and FB_2 retentions of 36 and 28% were reported for muffins baked at 220°C for 25 min. Baking of maize meal muffins at 175 and 200°C by Jackson et al. (1997) resulted in small but statistically significant (p<0.005) losses of FB_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%). As compared to the data reported here, the higher FB_1 retention obtained by Jackson (1997) could be due to the lower baking temperatures used.

Results of previous studies on the thermal stability of FB_1 in maize compares well to those obtained here. The mechanism for fumonisin loss, however, may differ in each study depending on the matrix and temperature used. Jackson et al. (1996a, b) showed that when solution 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 suggest that hydrolysis may not be the major mechanism for decomposition in the baked maize muffins studied here. However, another possible explanation is that HFB_1 formed during heating reacted with the other components of the muffin mix. The loss of FB_1 during baking may be due to the non-enzymatic browning reaction described by Murphy et al. (1996). The reaction occurs when the primary amine group of FB_1 reacts with free aldehyde 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 non-enzymatic browning. The rate and extent of nonenzymatic browning increase in food with increasing heating time and temperature (Mottram, 1994).

Frying

TLC results of FB_1 spiked fried chips, showed equivalent

Table 4. Effects of frying temperatures on fumonisin levels in maize chips.

Sample (n=3)	Concentration (µg/g)	Loss (%)
Control	1.98	
Fried 1	0.66	67
Fried 2	0.68	66
Fried 3	0.64	67

Rf values for the samples and the FB_1 standards. Thus, indicating that frying temperatures does not degrade fumonisins completely. A sample of spiked maize chips that was not fried was used as the control, which contained a FB_1 concentration of 1.98 µg/g (Table 4). Processing of maize chips at frying temperatures of 190 to 210°C for 5 to 10 min resulted in an average FB_1 percentage loss of 67%.

The results shown here for the thermal processing of fumonisin contaminated maize based-foods are generally in agreement with those of previous studies on the thermal stability of FB_1 in maize. Alberts et al. (1990) reported no loss of fumonisins when culture material of *F. verticillioides* was boiled for 30 min. When raw milk spiked with FB_1 and FB_2 was pasteurized (62°C for 30 min), there was no loss of either toxin (Maragos ad Richard, 1994). In contrast, Scott and Lawrence (1994) observed losses of FB_1 and FB_2 exceeding 70% in maize meal heated to 190°C for 60 min and about 100% in maize meal heated to 220°C for 25 min. Dupuy et al. (1993) reported that the decomposition of fumonisin in dry maize heated at 100 to 150°C followed first order kinetics but at a rate indicative of high thermal stability. Jackson et al. (1996a, b) studied the effects of heating of FB_1 and FB_2 in an aqeous buffer. They found that the rate and extent of fumonisins loss increased with increasing temperature, and that hydrolysed fumonisins were formed.

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