Fate of Fumonisin B_1 in Corn Kernel Steeping Water Containing SO_2

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Six 100 ppm fumonisin B_1 (FB_1) solutions were prepared by dissolving pure standard in six different solvents containing SO_2. Two of the solvents contained 0.2 or 0.4% SO_2 in distilled water. The other four solvents were obtained by steeping corn kernels at 60 °C in a 0.2% SO_2 aqueous solution for 6, 12, 24, or 48 h. After the addition of FB_1, all solutions were maintained at 60 °C for 7 days. Fumonisin B_1 content in each solution was determined in triplicate by HPLC. Steeping corn kernels in 0.2% solution at 60 °C for 6 h seems to be the most effective treatment to decrease the amount of FB_1.

Keywords: *Fumonisins; corn; decontamination; steeping; sodium bisulfite; HPLC*

INTRODUCTION

Fumonisins are mycotoxins produced by cereal contaminants belonging to the *Fusarium* genera, most notably *F. moniliforme* and *F. proliferatum*. These toxins are of particular concern because they have been epidemiologically and experimentally associated with several animal diseases, including leukoencephalomalacia (ELEM) in horses, pulmonary edema in pigs (PPE), and hepatotoxicity in rats. Furthermore, their presence in foodstuffs could be the reason for high incidence rates of human esophageal cancer in areas of Africa and China (Marasas et al., 1988; Chu and Li, 1994). Because these toxins have been declared a class 2B carcinogen (Vainio et al., 1993), it is necessary to screen routinely for their presence in foods and to develop procedures for reducing both animal and human exposure.

Several current management practices may reduce fumonisin concentrations. They can be classified into two categories: (1) develop genetic resistance of cereals (basically corn) to *F. moniliforme* and other *Fusarium* species and (2) prevent continued fungal development after harvest and process infected kernels to decrease fumonisin contents by physical or chemical procedures. Avoiding fungal contamination and development is problematic because selections are based on visual ratings that do not always reflect symptomless infection or fumonisin levels (Nelson et al., 1993). More satisfying results are obtained with physical and/or chemical decontamination procedures.

Dry heating of corn or corn meal reduces fumonisin B_1 (FB_1) levels, but it is difficult to conclude whether the high temperature reduces the amount of FB_1 or only results in the chemical blockage of the primary amine group (Dupuy et al., 1993). On the other hand, the rate of FB_1 hydrolysis products increases with processing temperature in aqueous model systems (Jackson et al., 1996). FB_1 levels may be substantially reduced in foods that reach >190 °C (Scott and Lawrence, 1994). During nixtamalization, calcium hydroxide causes ester hydrolysis, thus reducing detectable FB_1 (Sydenham et al., 1992). Norred et al. (1991) and Park et al. (1992) have investigated the application of ammonia treatment of fumonisin-contaminated maize at high temperatures and pressures that resulted in an 80% reduction in FB_1 content. Nonenzymatic browning in the presence of fructose at pH 7 results in the transformation of the primary amine group present in the fumonisin molecule, thus decreasing the detectable fumonisin in the samples and its toxicity (Lu et al., 1997). The treatment of maize with a combination of H_2O_2 and $NaHCO_3$ reduces the fumonisin concentration by up to 100% (Park et al., 1996).

We have observed previously (Canela et al., 1996) that after 48 h of steeping, FB_1 migrates from contaminated corn kernels to the steeping water as a result of its high polarity. We also detected very low contents of FB_1 present in the steeping water and the resulting wet corn when high temperatures and SO_2 were used to avoid biological proliferation (results not published). The aim of this research was to observe the evolution of FB_1 in the first step of the industrial wet-milling process of corn. Thus, we studied the behavior of pure FB_1 in 0.2 and 0.4% SO_2 solutions in distilled water and in four steeped corn water solutions containing 0.2% SO_2 over the course of 7 days at 60 °C.

MATERIALS AND METHODS

Caution. Fumonisins are known carcinogens. Consequently, fungal cultures and solvent extracts should be handled with extreme care.

Corn Samples. Corn seed samples (*Zea mays* sp. Juanita) were obtained from a local farm in Menàrguens (Lleida, Spain). Seed was stored after harvesting under ambient conditions for 6−8 months.

Analytical Standards. Analytical standards of FB_1 were isolated and purified from solid corn cultures of *F. moniliforme* and *F. proliferatum* according to the method of Cawood et al. (1991). An additional HPLC purification step was used to achieve >95%. Purity of our standards was determined by

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10.1021/jf9805045 CCC: $18.00
Published on Web 12/23/1998

Table 1. Degradation of 100 ppm of FB_1 in Different Solvents with SO_2 at 60 °C[a]

solution	0 days	1 day	2 days	7 days
control	ND[b]	ND	ND	ND
0.2% SO_2	83.56 ± 2.36^{ac}	92.46 ± 3.55^{a}	93.87 ± 4.00^{a}	77.74 ± 4.27^{a}
0.4% SO_2	44.01 ± 5.53^{b}	67.92 ± 11.81^{b}	83.43 ± 7.51^{ab}	86.37 ± 9.13^{a}
steep water				
6 h	76.80 ± 17.34^{c}	29.28 ± 23.32^{c}	8.48 ± 5.07^{d}	ND
12 h	53.70 ± 18.03^{b}	31.60 ± 25.42^{c}	38.36 ± 29.70^{c}	1.80 ± 0.58^{b}
24 h	92.90 ± 7.00^{a}	75.59 ± 10.80^{ab}	67.92 ± 15.22^{b}	1.01 ± 0.13^{b}
48 h	88.27 ± 7.14^{ac}	91.48 ± 9.53^{a}	69.77 ± 12.28^{b}	1.50 ± 0.20^{b}

[a] Each value represents the mean of three replicates and the standard error of the mean. Within a column, the same letters indicate no significant differences ($P < 0.5$, Duncan test). [b] ND, not detected.

comparison with FB_1 purchased from the Division of Food Science and Technology (CSIR, Pretoria, South Africa). An independent confirmation of purity was carried out using electrospray mass spectrometry (Dantzer et al., 1996).

FB_1 polyalcohol (2-amino-12,16-dimethyl-3,5,10,14,15-eicosanpentol), a degradation product of FB_1, was synthesized by heating FB_1 in 1 M KOH to 60 °C for 12 h (Thakur and Smith, 1996). FB_1 polyalcohol was used as a chromatographic standard for the hydrolysis product of fumonisin B_1.

SO_2 Solutions. SO_2 solutions (0.2 and 0.4%) were prepared by dissolving 13.9 and 27.8 g, respectively, of $K_2S_2O_5$ in 1000 mL of distilled water. The four corn steeping water solutions were prepared by steeping 200 g of corn seed in 250 mL of 0.2% SO_2 aqueous solution. The mixture was maintained at 60 °C in a sand bath using a reflux system to avoid concentration. Fifteen milliliter aliquots of steeped corn water were removed at 6, 12, 24, and 48 h.

Sample Preparation. Six 5 mL vials for each SO_2 solution were prepared. Three contained 2 mL of solution with 100 ppm of FB_1; the other three vials contained only 2 mL of solution as a control. Each vial was vortex mixed for 3 min and maintained for 0.5 min in an ultrasonic bath. Levels of FB_1 were quantified when the solution was made and after 1, 2, and 7 days at 60 °C in a sand bath.

Chromatographic Analysis. *Sample Purification.* Two hundred microliters of each solution was obtained and adjusted to pH 6 with 7% aqueous $NaHCO_3$. Samples were applied to previously conditioned 1 mL SAX columns (Superclean LC-SAX, Supelco) (Scott and Lawrence, 1996). The cartridges were washed with 1.5 mL of methanol/water (3:1), and fumonisins were eluted with 1.5 mL of methanol/acetic acid (99:1). Eluants were evaporated to dryness under a nitrogen stream until no acetic acid was detected by smell. The residues were redissolved in 1 mL aliquots of methanol prior to HPLC analysis. Methanol/water SAX column washes from all steeping water solutions collected on day 7 were also analyzed by HPLC to determine the possible presence of the FB_1 alcohol as a fumonisin degradation product. HPLC analysis of FB_1 in nonsteeping solutions was carried out without sample purification.

Derivatization. Two hundred microliters of *o*-phthaldialdehyde (OPA) reagent, prepared according to the method of Shephard et al. (1990), was added to 50 μL of sample solution. Twenty microliters of this solution was injected into the HPLC system between 1 and 2 min after derivatization.

Chromatographic Instruments and Conditions. The HPLC system consisted of Applied Biosystem Series (ABI Analytical Kratos Division, Ramsey, NJ), model 400 pumps, model 491 Dynamic Mixer/Injector with a 20 μL loop, model 980 fluorescence detector (excitation at 335 nm and emission at 418 nm cutoff filter), and a Hewlett-Packard 3396 Series II integrator (Hewlett-Packard, Avandole, PA). Samples were applied to a 25 × 0.4 cm Spherisorb ODS-2 (Tracer Analítica, SA) reversed-phase column preceded by a Waters (Waters Cromatografia SA, Barcelona, Spain) C_{18} guard column. The mobile phase used was methanol/0.1 M sodium dihydrogen phosphate (4:1) adjusted to pH 3.35 with orthophosphoric acid (OPA). The flow rate was 0.7 mL/min.

RESULTS AND DISCUSSION

Table 1 shows that FB_1 did not degrade significantly over 7 days at 60 °C in water solutions that contained only SO_2. Surprisingly, FB_1 contents in the 0.4% SO_2 solution are clearly lower on the first day than on day 7. This result could be explained by an interaction of SO_2 with the OPA reagent. As time goes by, this phenomenon would disappear as SO_2 is lost by evaporation. This phenomenon would be more appreciable with 0.4% SO_2 than with 0.2% SO_2. Any of the controls show FB_1 above our detection limit (500 ng of FB_1/mL, signal-to-noise ratio = 3:1).

The FB_1 concentration on day 7 is very close to our detection limit in all steeping waters, indicating that components other than SO_2 in the steeping waters induced this decrease in fumonisin levels. Comparing the four steeping waters, the longer the steeping duration, the lower the rate of FB_1 degradation. Polyalcohol of FB_1 was not detected in the steeping waters or in the methanol/water washes from the SAX cartridge, where Scott and Lawrence (1996) described its presence, or in the acidic fractions. The detection limit for this alcohol was ~5 μg/mL (signal-to-noise ratio = 3:1).

Our results suggest that a compound or compounds responsible for declining FB_1 levels in the steeping waters should be released by the corn seed to the aqueous part. The concentration of these compounds should decrease during time as binding to FB_1 proceeds. Moreover, these substances should be quite stable at 60 °C for at least 7 days. Thus, FB_1 losses could be a consequence of binding to these substances. Binding of fumonisin to some corn constituents has also been proposed by Scott and Lawrence (1994) to explain some of their results obtained after corn bran and corn meal were heated at >190 °C. In that case, adding water to the corn meal caused a decrease in the fumonisin titer.

Among the substances present in steep water (Hull et al., 1996), carbohydrates are likely candidates to react chemically with fumonisins. Thus, when FB_1 in potassium phosphate buffer at pH 7 containing 1 M fructose was heated for 48 h at 80 °C, <5% of free FB_1 remained in the solution. Fructose blocks the amine group of FB_1 in a Maillard type reaction, reducing or preventing promotion of hepatocarcinogenesis and hepatotoxicity in rats (Lu et al., 1997).

We conclude that the steep water 6 h solution was our most effective treatment for decreasing fumonisin concentration in solution. It could be considered an alternative for the decontamination process in a commercial setting, although further research is needed to characterize FB_1 degradation products to ensure consumer safety as well as to elucidate the cause of this process.

ACKNOWLEDGMENT

We are grateful to the editor and reviewers for their valuable comments and suggestions.

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Received for review May 14, 1998. Revised manuscript received November 2, 1998. Accepted November 2, 1998. This research was supported by the Spanish Government (CICYT, Grant ALI98-0509-CO4-01).

JF9805045