Efficacy of ammonia in detoxification of fumonisin contaminated corn

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Fumonisin B₁ level in culture material and in naturally contaminated corn by F. moniliforme was reduced by 30 and about 40%, respectively, by ammonia treatment. Atmospheric ammoniation of corn did not appear to be an effective method for detoxification of F. moniliforme contaminated corn.

Fumonisins, an important class of mycotoxins, are originally isolated from cultures of Fusarium moniliforme. Fumonisin B₁ (FB₁) is the major fumonisin present in fungal culture and naturally contaminated corn samples. F. moniliforme is an ubiquitous fungal contaminant of corn found throughout the world. Infected corn by this fungus is known to cause leukoencephalomalacia (LEM) in horses, pulmonary edema in pigs and hepatocarcinogenic in rats and oesophageal cancer in humans. Recently, Chaurasia and Shelby have reported the production of fumonisins by F. moniliforme and F. proliferatum in Indian corn samples, which may threaten human and animal health, and is detrimental to the marketability of corn. It has been reported that ammonia prevents aflatoxicosis in animals and is also effective in decontamination of aflatoxin containing foodstuffs.

The present study has been undertaken to study the effect of ammonia treatment on FB₁ content and toxicity of F. moniliforme in culture and natural condition.

Fungal cultures and culture material—Pure strains of Fusarium moniliforme were obtained from culture collection as reported earlier. Healthy yellow corn kernels (500 g) were placed in flasks (2.8 L) containing distilled water (500 mL) and autoclaved at 15 pounds/square inch pressure at a temperature of 120°C for 30 min for 2 successive days. Fungal inoculum was prepared by growing the fungus on corn meal agar (CMA) in petri dishes for 14 days at 25°C. A water suspension of microconidia (10⁵ spores/mL) was prepared and 0.5 mL was used to inoculate each flask of corn. The inoculated corn was incubated in the dark at 25°C for 4 weeks. The flask cultures were shaken once or twice daily for 3 days for uniform distribution of the inoculum. The culture material thus prepared was freeze-dried, ground and stored at -4°C until assayed. For an experimental control, corn kernels underwent the same autoclaving and incubation procedures, except that it was not inoculated with the fungus.

Ammoniation procedure—Samples of F. moniliforme culture material and control corn kernels (1 Kg each) were placed in round-glass stoppered bottles (2L) and maintains moisture contents to 15% by addition of water. Concentrated ammonium hydroxide (29% ammonia by weight) was added to give a final ammonia concentration of 2%. The control culture material and corn kernels were treated in the same manner, except that an equal volume of water was substituted for ammonium hydroxide. The samples were incubated at 50°C for 4 days, removed from the bottles, and allowed to dry in a hood at room temperature for 4 days. Samples of corn kernels (10 g) naturally contaminated with fumonisins were similarly treated with 1, 2 and 5% ammonia.

Extraction and quantification of fumonisins—Fumonisins were assayed by reversed-phase High Performance Liquid Chromatography (HPLC) system. Naturally contaminated samples or culture material were extracted with acetonitrile-water (1:1, v/v), filtered and an aliquot was applied to a Sep-Pak C18 cartridge (Waters Associates, Milford, Massachusetts, USA). The cartridge was washed with water and then acetonitrile-water (15:85, v/v). After elution of fumonisins from the cartridge with acetonitrile-water (70:30, v/v), an aliquot was treated with o-phthalaldehyde to form fluorescent derivatives of amine-containing compounds. FB₁ was quantitated by a reversed-
phase HPLC consisting of M-45 pump and U6K injector. The analytical column (stainless steel, 250x4.6 mm) was prepacked with Ultracarb 7 ODS 30 reversed-phase material (Phenomenex, Torrance, California). The detector was a model 650 S fluorimeter from Perkin Elmer (Norwalk, Connecticut) fitted with 18 μL flow cell. The eluent was methanol and 0.1m sodium dihydrogen phosphate (80:20, v/v) adjusted to pH 3.3 with orthophosphoric acid. The flow rate was 1 mL min⁻¹. Excitation and emission wavelengths were 335 and 440 nm, respectively.

FB₁ and FB₂ standards were obtained from the Research Institute for Nutritional Diseases, Medical Research Council, Tygerberg, South Africa. The hydrolysed form of FB₁ (HFB₁) was prepared by heating a solution of FB₁ in 2N-KOH at 70°C for 1 hr. The solution was then acidified (pH 6) with 0.5 N HCl, and extracted three times with an equal volume (25 mL) of n-butanol. The upper phase was evaporated under vacuum and HFB₁ was dissolved in acetonitrile-water (1:1, v/v).

Samples of F. moniliforme culture material showed reduction (80-90%) in FB₁ content compared with non-ammoniated controls (981 ppm) within 1 day of removal from the ammoniation vessel. When the material was allowed to air-dry for 4 days, only 30% reduction had occurred. In ammoniated culture material FB₁ content was increased from 204 to 663 ppm within 1 to 4 days of air drying compared with 0-day treatment (138 ppm). Maximum reduction in FB₁ content (21 ppm) of naturally contaminated corn samples was recorded at 2% of ammonia followed by 1 (23 ppm), 3 (23 ppm) and 4% (24 ppm), and least reduction (25 ppm) was noticed at 5% of ammonia. HPLC chromatograms obtained from a standard solution of FB₁, HFB₁ and FB₂, non-ammoniated culture material, and culture material treated with 2% ammonia and assayed after 4 days of air drying (F₁) were studied. The peaks representing FB₁ and FB₂ were clearly resolved from natural background peaks. There was no sign of formation of hydrolysed FB₁ after ammoniation and no unidentified ammoniation related peaks in the chromatograms were observed.

Detoxification of aflatoxin-contaminated foodstuffs involves a series of chemical reactions of which the first step is an opening of lactone ring. The structure of FB₁ does not indicate likely sites for attack by ammonia, with the possible exception of hydrolysis of tricarballylic acid moieties as occurs upon heating (60°C) of solution of fumonisins in 1N-potassium hydroxide. The biologically active chemical form of FB₁ is not yet elucidated, and there is no report on reactions that may occur between ammonia, fumonisins, other mycotoxins and chemicals that may be present in contaminated corn. Corn may be contaminated by both Aspergillus sp. and F. moniliforme, and therefore has aflatoxins and fumonisins contaminants.

Initial experiments indicated that ammoniation was effective in reducing FB₁ level in F. moniliforme culture material by nearly 90%. However, thorough air drying after ammonia treatment, fumonisins level of ammoniated culture
material recovered to 70% or more than that of non-ammoniated material. It is unknown whether transient decrease in fumonisin level was due to interference by residual ammonia with extraction or derivatization of fumonisin, or to reversibility of a chemical or physical reaction. The mechanism of stimulation of FB₁ level in ammoniated culture material for 4 days of air drying is not known. It is possible that residual ammonia become attached to the hyphae of F. moniliforme and travel with hyphae into the tissues, altering not only the substrate but also the fungal wall-membrane structure, increasing the rate at which FB₁ metabolite can leak through the membrane. Ammoniation has been shown to destroy over 98% of aflatoxin in naturally contaminated corn. In the present study, ammonia apparently destroyed less than 50% of FB₁ in naturally contaminated corn screenings.

Ammoniation conditions used in the present investigation were similar to those previously shown to destroy aflatoxin added to corn meal. Under the same experimental conditions fumonisins was not adequately destroyed. Based on these observations, atmospheric ammoniation of corn for destruction of aflatoxin should be used after confirming that the corn is not co-contaminated with fumonisins.

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References