Reduction of Aflatoxin Levels in Cottonseed and Peanut Meals by Ozonization

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Abstract

Cottonseed and peanut meals were treated with ozone to destroy or eliminate aflatoxins. High meal moistures (cottonseed 22%, peanut 30%), high temperature (100C), and longer treatment times favored inactivation as measured by thin-layer chromatography. Aflatoxins B_1 and G_1 were readily destroyed by the ozone processes whereas aflatoxin B_2 appeared relatively resistant. In cottonseed meal, 91% of the total aflatoxins was destroyed in 2 hr, a decrease from 214 to 20 ppb; in peanut meal, 78% was destroyed in 1 hr, a decrease from 82 to 18 ppb. In both meals, aflatoxin B_1 was totally inactivated within the times specified.

Introduction

DURING THE PAST FEW YEARS the presence of mycotoxins in food and feeds has become a matter of considerable public interest. Some agricultural commodities may contain toxic metabolites emanating from several strains of *Aspergillus flavus* (1) and certain other molds. These metabolites, termed aflatoxins, have been characterized chemically (2,3), and feeding experiments have indicated that, if fed at sufficiently high levels, they induce adverse physiological responses in some laboratory and farm animals (4–7). It has been reported that the ingestion of aflatoxins by certain lactating animals results in the secretion of similar toxins in the milk (8,9).

Aflatoxins may contaminate oilseeds and their corresponding products (10,11). In some cases, such as peanuts, conventional processing removes the toxins to yield an edible oil (12), but the residual meal may retain a sufficiently high aflatoxin level to preclude its use in animal feeds. It has therefore been recommended that this material be diverted to use as fertilizer (13). Such diversion however results in loss of valuable protein supplementation for animals, and the intrinsic economic value of the meal is greatly reduced.

It is therefore important to investigate practical methods of treatment to inactivate chemically or to eliminate aflatoxins from contaminated oilseed meals. Previous publications have indicated that heat treatments alone, either dry or wet, do not effectively eliminate aflatoxins from contaminated cottonseed or peanut meals (14–17). Autoclaving of wet toxic peanut meals has been reported to reduce aflatoxin content, but the nutritive value of the end-product seems questionable (18). More recently, hydrogen peroxide treatment of aqueous peanut meal slurries has been reported to effect aflatoxin detoxification (19).

The present research was undertaken to determine the effect of ozone gas in reducing aflatoxin concentration in contaminated cottonseed and peanut meals under varying conditions of moisture content, temperature, and time.

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Experimental Section

Materials

The cottonseed meal was a selected, prepressed, solvent-extracted sample specially chosen because of its aflatoxin content. It contained 144 ppb aflatoxin B_1 and 70 ppb aflatoxin B_2. No aflatoxins G could be detected. Meal moisture was 6.6% as received. Other analytical data on the meal include (moisture-free basis): nitrogen, 7.06%; crude fiber, 15.2%; lipids, 0.85%; free gossypol, 0.03%; and total gossypol 0.94%. The available lysine content was 3.42 g/16 g nitrogen (23).

The peanut meal was a selected, prepressed, solvent-extracted sample specially chosen because of its aflatoxin content. It contained 54 ppb aflatoxin B_1, 18 ppb aflatoxin B_2, and 10 ppb aflatoxin G. No aflatoxin G_2 could be detected. Meal moisture was 7.2% as received, nitrogen, 9.82%; crude fiber, 5.0%; lipids, 0.75%. The available lysine content was 2.78 g/16 g nitrogen.

Equipment

Ozone gas was generated by a Welsbach Model T-23 laboratory ozonator, supplied with a flow of air which was dried by passage through a silica gel column.

Ozonization treatments and control experiments in which air or oxygen was substituted for ozone were carried out in a bench-scale reactor that had been designed and constructed at this laboratory (20). The reactor consists of a jacketed, 3-liter stainless steel vessel fitted with a gasketed cover and agitator which are designed to mix the meal efficiently. In the cover are a thermometer well and two ports, one to allow a reflux condenser to be inserted, and the other, to allow reaction gas to be introduced deep into the meal through a tube that extends to approximately 1.5 cm from the bottom of the vessel. Effluent gases from the reactor were vented to a hood via plastic tubing connected to the top of the reflux condenser. Constant temperature was maintained by controlling the flow of hot water or steam through the jacket.

Methods

To obtain the specific meal moistures described, 700 g of the meal was blended in a Model C-10 Hobart mixer, equipped with a stainless steel bowl and beater; the calculated quantity of distilled water was gradually added, and the blending continued for an additional 10 min. The maximum moisture level used for cottonseed meal was 22%, since it tended to form a tough plastic mass at higher levels. Peanut meal, however, was processed without difficulty at levels as high as 30%.

The hydrated meal then was transferred to the bench-scale reactor to be mixed and heated. When the desired temperature was attained, approximately 25 mg of ozone per minute was introduced, for a total gas effluent rate of 850 ml per minute. This output was determined by wet test flowmeter readings and iodometric chemical titrations.

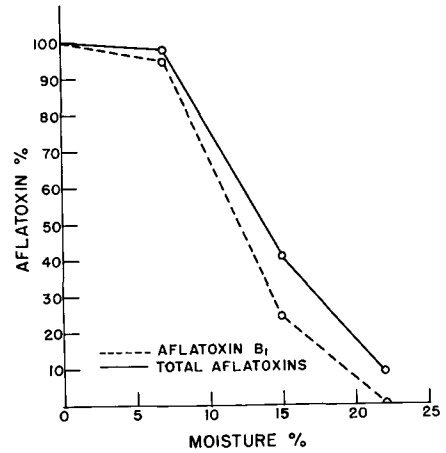

FIG. 1. Effect of moisture on inactivation of aflatoxin in cottonseed meal: ------ aflatoxin B_1, ——— total aflatoxins.

After reaction conditions had been maintained for the designated time, the meal was removed from the reactor, spread in a glass tray, and allowed to air-dry at ambient temperature for at least 24 hrs before it was assayed for aflatoxin.

Meal samples were assayed for aflatoxin content by the method of Pons et al. (21,22). Briefly, the procedure involved extraction of the aflatoxins from the meals with 70% (v/v) aqueous acetone, purification by precipitation with lead acetate, partitioning of aflatoxins into chloroform, purification of the extract on a silica gel column, separation of aflatoxins on TLC plates coated with silica gel GHR, and visual evaluation of the intensity of fluorescence of test spots viewed under ultraviolet light.

Results and Discussion

Treatment with Ozone

Effect of Moisture. Increased moisture in cottonseed and peanut meals greatly enhanced the effectiveness of ozone treatments in reducing or eliminating the aflatoxin contaminants. This relationship for cottonseed meal which was treated for 2 hr at 100C with varying meal moistures is shown in Fig. 1. At 7% moisture aflatoxin B_1 was reduced only 5% whereas at 22% moisture it was totally inactivated. Similarly, ozone treatment of the peanut meal for 1 hr at 100C resulted in increased inactivation as the meal moisture was increased (Fig. 2).

Effects of Temperature and Time. The effects of temperature and time on the inactivation of aflatoxin

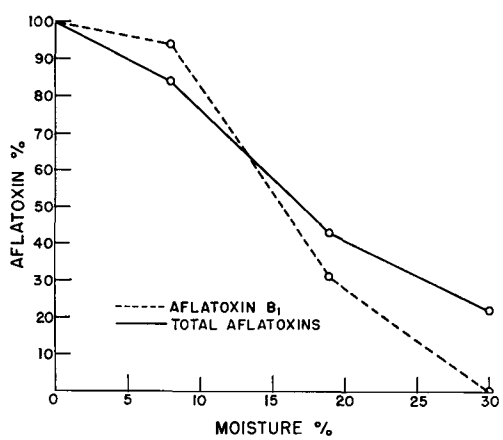

FIG. 2. Effect of moisture on inactivation of aflatoxin in peanut meal: ------ aflatoxin B_1, ——— total aflatoxins.

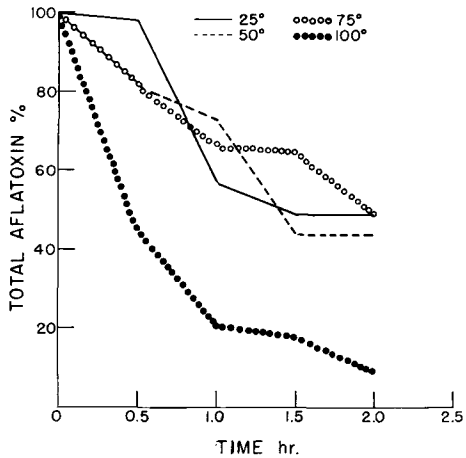

FIG. 3. Effect of temperature and time on inactivation of total aflatoxins in cottonseed meal: ——— 25C, ------ 50C, ○○○○○○○ 75C, ●●●●●●● 100C.

in the cottonseed meal containing 22% moisture are illustrated in Fig. 3. Processing at 100C for 2 hr resulted in destruction of 91% of the total aflatoxins present whereas treatments at lower temperatures were decidedly less effective. The 91% reduction achieved at 100C for 2 hr represented a 100% destruction of aflatoxin B_1. The remaining 9% toxin was aflatoxin B_2, which could not be eliminated from the meal by this treatment.

The effects of temperature and time on the destruction of aflatoxins by ozone in the peanut meal containing 30% moisture are shown in Fig. 4. Higher processing temperatures and longer periods of treatment favored the destruction of aflatoxins in the peanut meal. A maximum of 78% reduction in total aflatoxins was achieved in 1 hr at 100C, and the level remained unchanged with further treatment since this represents 100% destruction of the aflatoxins B_1 and G_1. The residual aflatoxin B_2 which was present in the peanut meal was essentially unaffected by this treatment.

The actual reduction in ppb of aflatoxin B_1 alone is shown in Fig. 5 to emphasize the fact that this aflatoxin was totally inactivated in 1 hr in peanut meal and in 2 hr in cottonseed meal.

Experiments with Air or Oxygen

In the control treatments, air or oxygen proved to be less effective than ozone in inactivating aflatoxin.

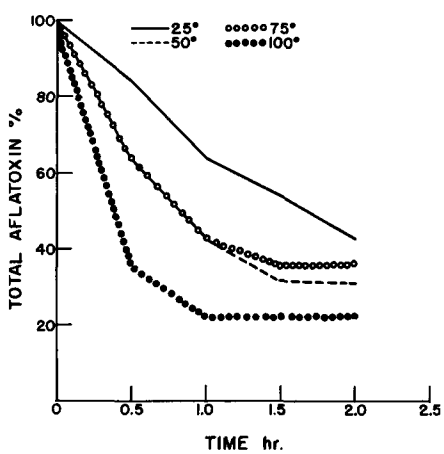

FIG. 4. Effect of temperature and time on inactivation of total aflatoxins in peanut meal: ——— 25C, ------- 50C, ○○○○○○○ 75C, ●●●●●●● 100C.

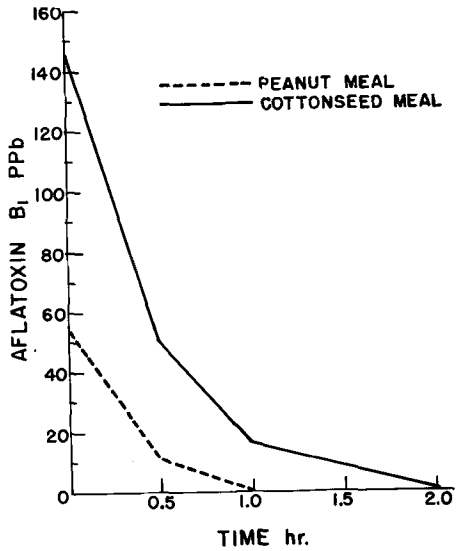

FIG. 5. Effect of time on inactivation of aflatoxin B_1 in cottonseed and peanut meals: ------- peanut meal, ——— cottonseed meal.

For example, employing air or oxygen gas at 100C for 2 hr reduced the total aflatoxin content of the cottonseed meal only 76% in comparison with the 91% achieved with the ozonized air. Treating the peanut meal with air at 100C for 2 hr reduced the total aflatoxins 67% in comparison with the 78% reduction at 100C for 1 hr with ozone.

Comparison with Standard Results

The ozonization treatments lowered the aflatoxin levels in cottonseed and peanut meals to within the value specified by a Protein Advisory Group sponsored by FAO, WHO, and UNICEF, which recently stated that "the level of aflatoxin in peanuts or other protein supplements should not exceed 0.03 mg (30 μg) per kilogram [ppb] of foodstuff" (24).

Tests in Prospect

Duckling and rat feeding tests are to be used to provide biological data on toxicity and nutritional potential of the ozonized peanut meal described in this work. Results will be reported in a subsequent publication from this laboratory.

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