Atmospheric Pressure–Ambient Temperature Reduction of Aflatoxin B_1 in Ammoniated Cottonseed

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Naturally contaminated whole cottonseed containing an average of 800 μg/kg total aflatoxin was treated with ammonium hydroxide under varying ammonia concentrations (0, 1.0, 1.5, and 2.0%), moisture contents (0, 7.5, 10.0, and 12.5% added), and temperatures (21, 32, and 43 °C) and stored for either 5, 10, or 15 days at atmospheric pressure. Results indicate that ammonia degradation of AFB_1 was successfully accomplished under all treatment conditions of temperature, moisture, and ammonia concentration. Decreases of 98.8 and 99.9% were found by using conditions of (a) 2.0% ammonia, 12.5% added moisture, and 43 °C for 15 days and (b) 2.0% ammonia, no added moisture, and 43 °C for 15 days. These conditions effectively reduced the total aflatoxin content from 800 μg/kg to less than the FDA action level of 20 μg/kg.

Aflatoxins, a group of naturally occurring toxins produced by the molds *Aspergillus flavus* and *Aspergillus parasiticus*, contaminate a variety of agricultural food and feed products. Since 1960, when aflatoxin (AF) was first isolated and discovered to be a potential health hazard, extensive research has been conducted to determine a method of preventing or controlling AF contamination (Council for Agricultural Science and Technology, 1979). Corn, peanuts, and cottonseed are the major crops that are the most often contaminated by AF (Bagley, 1979). Because AF contamination can occur naturally prior to harvesting (a field problem) or after harvesting (a storage problem), contamination may be difficult to prevent or control (Goldblatt and Dollear, 1977). At present, detoxification of agricultural crops appears to be the most successful method for controlling AF contamination (Ciegler, 1978). Each commodity presents specific problems in reducing or eliminating AF; a process developed for one commodity cannot necessarily be used for another (Bagley, 1979). The detoxification process not only must degrade the AF but also must cause minimal nutritional impairment and must not produce residues which are hazardous to the animals consuming the treated feeds. Detoxification with ammonia appears to be the most successful process with oilseeds and corn (Council for Agricultural Science and Technology, 1979). A study conducted by Vesonder et al. (1975) using ammonium hydroxide (17 N) and crystalline AFB_1, at room temperature for 21 days, gave a brown product which upon acetone extraction yielded a mixture of *o*-coumaric acid, AFB_1, and an insoluble brown residue. This study showed the brown residue to be nontoxic to chicken embryos at 0.31 μg/egg. Studies conducted using high-pressure, high-temperature ammoniation (Lee et al., 1974; Mann et al., 1971) resulted in the formation of other less potent compounds; the most prevalent compound was decarboxylated AF, termed AFD_1.

Aflatoxin contamination of cottonseed in Arizona seems to be the result of cultural practices and uncontrollable environmental conditions (Price, 1980). During the late summer and fall of 1978, the State of Arizona, by executive order, dumped thousands of gallons of AF-contaminated milk (Council for Agricultural Science and Technology, 1979). An investigation revealed that the contaminated milk was the result of feeding highly contaminated whole cottonseed to dairy cows. The contaminated milk and cottonseed resulted in an estimated loss of greater than $9 million to the cotton and dairy industries of Arizona. Because cotton and diary are major industries in Arizona, the University of Arizona has developed a field process for detoxification that is carried out at atmospheric pressure and ambient temperature (APAT) (Lough, 1980). The conditions used for this study were based on results from a study conducted by Bagley (1979) for ambient pressure detoxification of corn. The APAT process involves the use of bags 10 ft in diameter and 100 ft long, a bagging machine, and an ammonia applicator originally designed for addition of ammonia to silage. A preliminary study was conducted by mixing contaminated cottonseed with 1.5% aqueous ammonia and 10% added moisture in plastic bags and storing them at ambient temperature for 21 days (Lough, 1980). The results of this preliminary study showed a 90.0% decrease in the AF level after treatment as compared to the pretreatment level (Lough, 1980). Since ~1% of AFB_1 consumed by the animal is converted to AFM_1 in milk (McKinney et al., 1973; Polan et al., 1974; Moreau, 1979), control of AF in cottonseed is imperative; if the AF level in the cottonseed is decreased, the levels of AFM_1 in the milk should also decrease (McKinney et al., 1973).

Table I. List of Treatment Conditions

% ammonia	% moisture	time, days	temp, °F
0	7.5	5	70
1.0	15.0	10	90
1.5	17.5	15	110
2.0	20.0		

This study was designed to determine, experimentally, the optimum processing conditions for APAT ammoniation of cottonseed. It has been shown in previous studies that higher ammonia concentrations (3, 4, and 5%) and higher temperatures (160–180 °F), under pressure (20–50 psig), will readily degrade AF (Koltun et al., 1979). The specific intent of this study was to determine the optimum ammoniating conditions using a milder treatment to achieve comparable degradation. The results indicate the APAT processing conditions that proved to be the most effective in reducing the AF levels in whole cottonsed.

EXPERIMENTAL SECTION

Ammoniation Procedure. Aflatoxin-contaminated cottonseed was obtained from a commercial dairy operation in the Gilbert, AZ, area. It was treated without further delinting. The cottonseed was divided into 432 samples, each weighing 200 g, and was placed in 13 × 18 cm plastic packages (75-Maraflex, American Canning Co.). These

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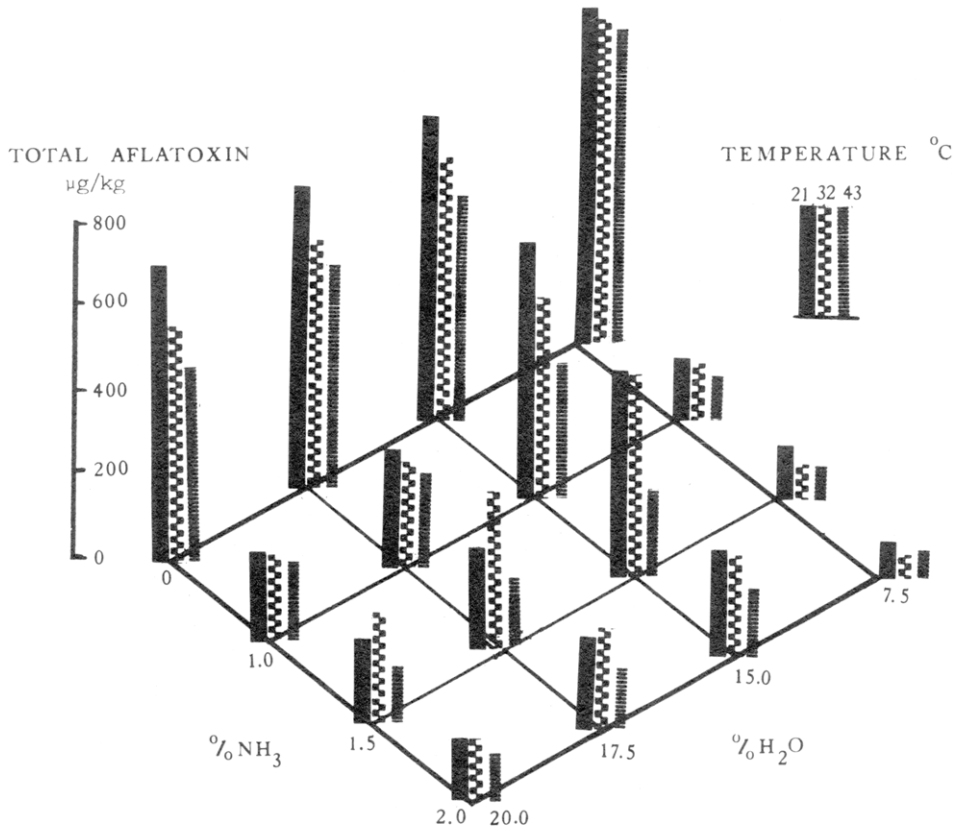

Figure 1. Effect of temperature on APAT degradation of AFB_1: 5 days.

samples were grouped and labeled according to the specific treatments. Specific treatments were dictated by the multifactorial design (Table I). There were three replicates for each treatment. Measured amounts of distilled water were added to the cottonseed in the bags to increase the moisture content, and concentrated ammonium hydroxide (28.7%, Mallinckrodt) was added to bring the ammonia concentration to the proper level. After the bags were heat sealed, the contents were mixed, and they were allowed to react at 21, 32, and 43 °C for 5, 10, or 15 days. After the specified time, all samples were stored at 4 °C until analysis could be completed.

Cottonseed Analysis Procedure. All samples were masked and randomly analyzed. The contents of each package were uniformly ground (mesh size 20) by using a hammermill grinder. Twenty-five-gram samples were neutralized with 40 mL of 0.1 N HCL according to Pons and Franz (1977) and analyzed by using a modification for thin-layer chromatography (TLC) of the same method for high-performance liquid chromatography (LC). The modifications were as follows: (a) the filtrate was washed twice with 50 mL hexane to remove lipids prior to $CHCl_3$ extraction; (b) the $CHCl_3$ extract was not evaporated prior to application to a silica gel column (Bio-Rad Econo column, 1.0 cm i.d. × 30 cm length, Merck 7734 silica gel, and anhydrous Na_2SO_4) and the AF was eluted from the column with 100 mL of $CHCl_3$–acetone (4:1). The eluant was collected and evaporated at 50 °C to near dryness by using a rotary evaporator; it was transferred with two washings of 2 mL of $CHCl_3$ to a 2-dram vial and dried in a steam bath under a stream of N_2 gas. The extract was then reconstituted with 0.1 mL of benzene–acetonitrile (98:2).

All samples were analyzed according to the modified procedure and visually quantitated by using thin-layer chromatography according to the Association of Official Analytical Chemists rapid method for AFB_1 in cottonseed products 26.A15–26.A16 (1975). The sample was spotted by using 5- and 10-µL aliquots of the AF standard (Supelco, Inc., 4-6300). Since visual quantitation was done by comparing a known amount of AF standard to the amount extracted from the cottonseed, all samples containing greater than 50 µg/kg were diluted and respotted. Aflatoxin verification was done using trifluoroacetic acid (Trucksess, 1976).

RESULTS AND DISCUSSION

Preliminary results have indicated that AF levels could be reduced in whole cottonseed by treatment with aqueous ammonia at atmospheric pressure and ambient temperatures. Under the parameters of this study, there was a significant difference between the amounts of AF remaining and the specific conditions used.

During the initial phase of this experiment, several cottonseed analysis procedures were tested for percent of AF recovery. Because the modification of the Pons and Franz (1977) high-performance method for LC had the largest recovery, it was the method of analysis chosen.

Aflatoxin mean values for all treatments are shown in Figures 1, 2, and 3 which correspond to 5, 10, and 15 days. All treatments resulted in a reduction of AF in whole cottonseed, even those in which ammonia was not added.

These results show that increasing the temperature for all treatment conditions reduced the amount of AF. A similar study with corn under atmospheric pressure, using temperatures ranging from 0 to 140 °F, found that the higher temperatures resulted in larger decreases in AF content (Bagley, 1979). Since the temperatures chosen, 21, 32, and 43 °C, are common to Arizona, these results provide the necessary information for this process to be carried out at various times during the year.

The ammonia concentrations chosen for the APAT process (0, 1.0, 1.5, and 2.0%) were based on the low-pressure ammoniation study conducted on corn. These concentrations ranged from 0.5 to 1.5% (Bagley, 1979).

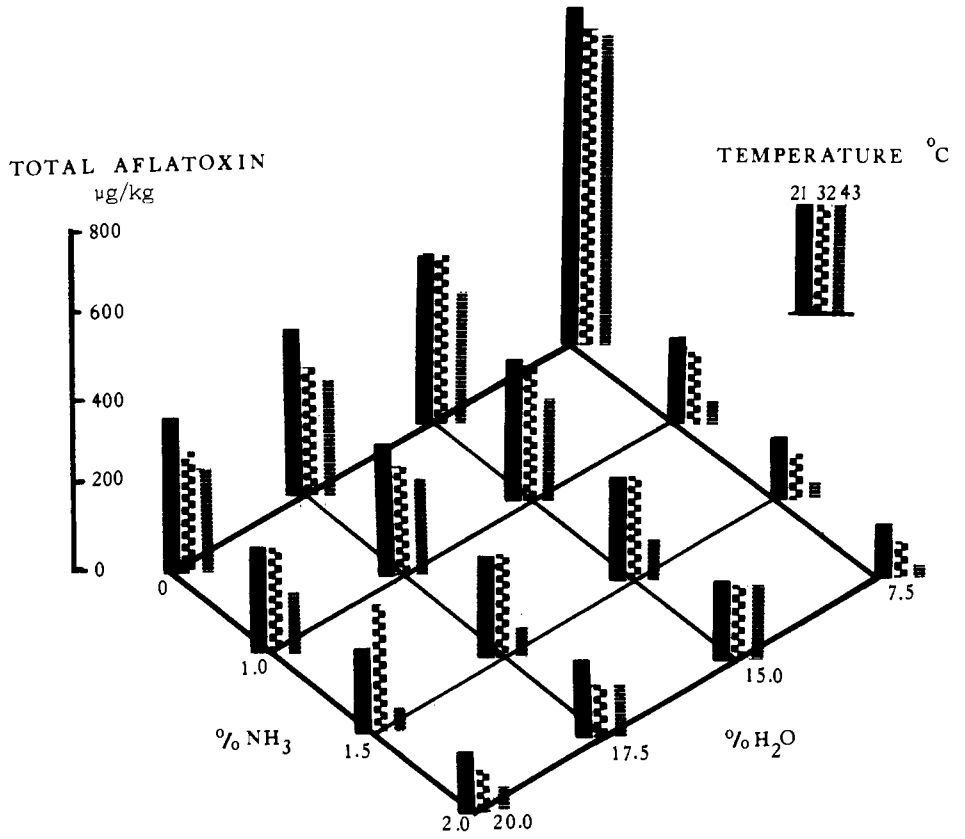

Figure 2. Effect of temperature on APAT degradation of AFB_1: 10 days.

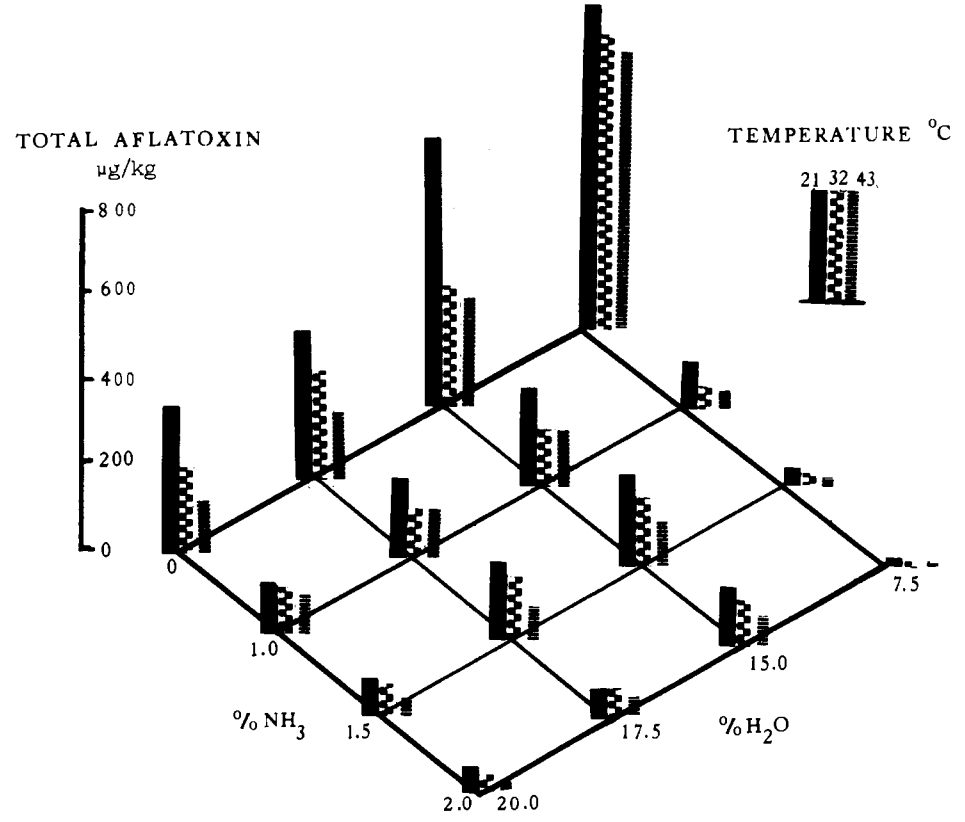

Figure 3. Effect of temperature on APAT degradation of AFB_1: 15 days.

The results from both of these studies show that increasing the ammonia concentrations decreased the total AF content and the highest concentration resulted in the largest decrease.

The effect of varying the moisture content of the cottonseed on AF reduction was the same for all time periods. As the moisture content increased from 15 to 20%, the AF content was also decreased. Since aqueous ammonia was used, moisture values were slightly higher in the more highly dosed samples. Cottonseed has a very hard seed coat and increasing the moisture content allows the seed coat to soften. It is possible that more ammonia may be

absorbed into the cottonseed. However, this hypothesis did not hold true for the samples maintained at normal moisture content (7.5%). Reduction of AF was greatest in the samples to which no water was added. The reasons for this have not yet been determined. If similar results occur in larger samples, there will be significant economic benefits for concerned industries. Moisture would not have to be added to cottonseed during treatment nor would the seed have to be dried before feeding. This would lower treatment costs and possibly produce a more desirable product. Previous studies conducted using cottonseed meal did not report the amount of AF destruction in unmoistened samples (Ciegler, 1978; Council for Agricultural Science and Technology, 1979). However, Nofsinger and Anderson (1979) showed that the total AF content of corn could be reduced at atmospheric pressure by using aqueous ammonium hydroxide and maintaining the corn at normal moisture content.

The difficulty in sampling and analysis for AF in whole cottonseed and other crops has been a subject of various studies (Schuller et al., 1976; Whitaker and Dickens, 1979). In this study the individual sample size could not exceed 200 g because of the quantity of samples tested and the limited supply of highly contaminated cottonseed. Had each sample been larger, it may have been easier to extrapolate from an experimental scale to a commercial scale operation. However, it has been the experience of this laboratory that the variability of the AF levels in the cottonseed used (Arizona 1978 crop) was somewhat less than normally expected; levels appeared to be uniformally high and the cottonseed had been mixed many times during commercial handling.

APAT treatment for 5 days was insufficient to reduce the AF levels below 20 μg/kg and was considered to be unsatisfactory. After 10 days, one set of treatment conditions (2.0% ammonia, no added moisture, and 43 °C) decreased the AF level below the 20 μg/kg. The results seen in Figure 3 indicate that 15 days was sufficient time to reduce the AF below the 20 μg/kg level under four sets of treatment conditions. The conditions were as follows: (a) 2.0% ammonia, no added moisture, and 21 °C; (b) 2.0% ammonia, no added moisture, and 32 °C; (c) 2.0% ammonia, no added moisture, and 43 °C; (d) 2.0% ammonia, 20% moisture, and 43 °C.

Since this process was conducted under atmospheric pressure rather than increased pressure, the mechanism of degradation has not yet been determined.

Although the APAT process has not yet been tested by FDA-approved protocols, it is currently being used within the State of Arizona to treat whole cottonseed being fed to dairy cattle. The results of this study give an indication of the treatments to be used under varying conditions for commercial application of this process.

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