Aflatoxin Inactivation of Undelinted Cottonseed by Ammoniation

Stanley P. Koltun
Southern Regional Research Center, Agricultural Research Service, USDA, P.O. Box 19687, New Orleans, LA 70179

The inactivation of aflatoxin in cottonseed products has been restricted principally to ammoniation of cottonseed meal. More recently, attention has been focused on the feasibility of ammoniating whole cottonseed as a feed for ruminants. Preliminary work is presented on treatment with gaseous ammonia of undelinted (fuzzy) cottonseed containing an average of 1,500 μg/kg total aflatoxins. These seed were treated with 4% anhydrous ammonia (w/w) for 30 min at temperatures ranging from 66 C to 82 C. The data obtained in this study indicate that inactivation of aflatoxins in undelinted whole cottonseed may be accomplished using processing conditions comparable to those proposed for cottonseed meal.

Undelinted (fuzzy) whole cottonseed is an excellent protein supplement for dairy cows (1). However, periodic invasion of cottonseed in southern California and Arizona by the fungus *Aspergillus flavus* and subsequent contamination of the seed by its metabolite, aflatoxin, have caused much concern to dairymen using this seed in rations for lactating dairy cows (1,2). The inactivation of aflatoxin in contaminated cottonseed meal and other oilseed products with ammonia has been reported (3–6). Generally, prepress solvent extracted cottonseed meal and peanut meal were the oilseed products investigated. The meals usually were hydrated to 15% and treated with anhydrous ammonia for approximately 30 min at about 120 C and 3.5 kg/cm² pressure.

Long term feeding studies with rats fed ammoniated meal indicate that the protein value of the meal is reduced somewhat by ammoniation but that no histopathological abnormalities or other adverse effects were present (Booth, A. N., Western Regional Research Center, USDA, personal communication, 1972).

Cavanagh and Ensminger (7) found that ammoniated cottonseed meal is equivalent to regular cottonseed meal on an iso-nitrogenous basis for ruminants, when evaluated for rate and efficiency of meat and milk produced. When cattle were fed up to six times normal levels of ammoniated cottonseed meals, no cases of toxicity developed.

Chemical and biological data from feeding trials presented by McKinney et al. (8) demonstrate that ammoniation is an effective, practical method for the elimination of aflatoxin in contaminated cottonseed and cottonseed meal that will allow their utilization in the ration of lactating cows.

Fowler (2) reported the results of a full-scale test conducted with 90 dairy cows, for which whole cottonseed, adjusted to 20% moisture content and containing as much as 879 μg/kg aflatoxins, was ammoniated (1.5%) NH_3) at ambient temperature and atmospheric pressure for 10 days. The treated seed, containing 30 to 80 μg/kg total aflatoxins, was fed to cows for 7 days, during which time aflatoxin M_1 in the milk samples ranged from 0.11 to 0.16 μg/l, well under the FDA action level of 0.5 μg/l.

Price and coworkers (9) reported on the analysis of milk for aflatoxin M_1 of individual lactating dairy cows fed ammonia-treated cottonseed (17% moisture content, 1.2% NH_3 at 18 to 23 C max. during the day for 21 days) and non-ammonia-treated seed. Before treatment, the levels of total aflatoxins in this cottonseed were 650 μg/kg; after treatment, total aflatoxin content of the seed varied from 15 to 80 μg/kg. Aflatoxin M_1 in milk from cows fed the untreated seed reached almost 2.0 μg/l in 7 days; the average aflatoxin M_1 content in milk from cows fed the treated seed rose very slightly but was less than 0.2 μg/l.

The present study was undertaken to determine if the aflatoxin in naturally contaminated, undelinted whole cottonseed could be more completely inactivated in less time by the ammoniation processing conditions reported by Koltun et al. (10) than by treatment under ambient temperature and atmospheric pressure (9,11).

EXPERIMENTAL PROCEDURE

Twenty-three kg of undelinted whole cottonseed with an initial moisture content of 8.7% and total aflatoxin content of 1,500 μg/kg were used in the inactivation study. Moisture was adjusted to approximately 15% by spraying calculated quantities of water on the seed and equilibrating in a polyethylene bag for three days. Ammoniation of the hydrated seed was carried out in a jacketed pressure reactor (10) with 4% anhydrous liquid ammonia (w/w). Ammonia was kept in an unstoppered polyethylene bottle inserted upright in the cottonseed. As the reactor rotated, liquid ammonia spilled onto the warmer cottonseed, volatilizing instantly and pressurizing the reactor. Desired temperatures were attained in approximately 65 min by adjusting the amount of steam introduced into the reactor jacket; these temperatures were maintained for an additional 30 min. Reactor pressure reached 1.4 kg/cm² in about 3 min.

Aflatoxin analyses were conducted by the rapid method for aflatoxins in cottonseed products, AOAC 26.A09-26.A16, modified by Pons and Franz (12).

RESULTS AND DISCUSSION

During the ammonia detoxification studies with aflatoxin-contaminated cottonseed meal (10), it was discovered that the particle size of contaminated meal was not a significant factor in either the rate or degree of inactivation. Meal particles measuring 1.2 mm or greater were detoxified as readily as fine powderlike meal under identical treatment conditions.

It thus seemed reasonable that similar treatment with ammonia gas might also prove effective for detoxifying aflatoxins in undelinted, whole cottonseed, because the gas readily penetrates the chalazal end of the seed and makes direct contact with the contaminated meat.

An experiment was conducted according to the techniques described using a 23-kg charge of aflatoxin-contaminated cottonseed containing 1,500 μg/kg total aflatoxins. Table 1 shows reaction conditions used and results obtained. Reduction in aflatoxin level was achieved, with total aflatoxin content in the treated seed reduced to 4 μg/kg using a reaction temperature of 82 C.

TABLE 1

Effect of Reaction Conditions on Aflatoxin Content of Undelinted Whole Cottonseed[a]

Ammoniation conditions[b]		
Peak ammonia pressure kg/cm²	Peak temperature °C	Total aflatoxins[c] μg/kg
1.4	65	110
1.4	71	56
1.4	74	59
1.4-1.8	77	41
1.4-1.8	82	4

[a]4% Ammonia concentration; 30 min reaction time at peak conditions.

[b]15% Moisture content.

[c]Values reported are means of 4 replicated runs.

Experience with detoxification of aflatoxin-contaminated cottonseed meals indicates that the presence of moisture promotes destruction of aflatoxin with ammonia gas. The influence of higher moisture levels, with a limit of approximately 15%, probably will enhance the destruction of aflatoxins in undelinted whole cottonseed.

This study supports the feasibility of ammoniating undelinted whole cottonseed as an effective means of reclaiming contaminated cottonseed for use as a feed for ruminant animals.

ACKNOWLEDGMENTS

A. O. Franz Jr. provided technical assistance.

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[Received November 7, 1985]

Chemical and Microscopic Studies of the Matrix Substance in Pigment Glands of Cotton (*Gossypium hirsutum* L.) Seeds

Lawrence Yatsu[a,*], Thomas J. Jacks[a], Henry W. Kircher[b,1] and Mary An Godshall[c]

[a]Southern Regional Research Center, ARS, USDA, P.O. Box 19687, New Orleans, LA 70179; [b]Department of Agricultural Chemistry, University of Arizona, Tucson, AZ, and [c]Sugar Processing Research Inc., New Orleans, LA

Cottonseeds contain gossypol, a toxic substance, that renders the otherwise nutritious seeds inedible. However, because the gossypol is concentrated in small, intercellular glands, it is possible to separate gossypol from other seed constituents by pulverizing the seeds and removing the glands. This procedure is practicable because gossypol remains with the glands even during seed pulverization and manipulation in hexane. Many believe that the gossypol remains within the glands because the glands are virtually indestructible, protected by tough, resilient "plates." However, we show that most of the isolated glands are broken after comminution. The gossypol is held in a water-soluble matrix within the lumen of the glands. Analysis of aqueous extracts of isolated glands showed that the bulk of the extract is a non-dialyzable arabinogalactan. We suggest that the matrix substance is an arabinogalactan.

Over 33 million tons of cottonseed was produced worldwide last year, an increase of 50% over the last decade (1). The seed, a by-product of fiber production, contains an excellent vegetable oil and high grade proteins (2). However, the protein and sometimes even the oil, are rendered unfit for human consumption because of the presence of gossypol (a toxic substance) in the seeds (3-5).

Because virtually all of the gossypol in cottonseed is concentrated in glands (6,7), it is possible to separate the gossypol from the rest of the seed constituents by pulverizing the seeds and removing the pigment glands (8-10). This procedure is practicable because gossypol remains with the glands during comminution and suspension in hexane. Many believed, and still believe, that the glands retain the gossypol because they are virtually indestructible, protected by tough, resilient "plates" (6,8,10,11).

We showed, however, that many (perhaps most) of the isolated glands were broken in pulverized cottonseed meal and concluded that intact glands really were not necessary for procedures such as the Liquid Cyclone Process (LCP) to work (7). In this communication, we show that the gossypol is enmeshed in a water-soluble matrix within the lumen of the glands. We show that

[1]Deceased.

*To whom correspondence should be addressed.