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REVIEW OF PREVENTION, ELIMINATION, AND DETOXIFICATION OF AFLATOXINS

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Abstract - The best approach to contain aflatoxin contamination is prevention. Good farm management practice is essential. This includes use of sound, fungus-free, viable seed, proper fertilization, control of insects and diseases, prevention of lodging, and harvesting practices that avoid damaging the crop and picking up excessive leaves, trash, and dirt. Special attention should be given to diverting aflatoxin-containing lots from food and feed channels as early as possible in the marketing chain. Clean, dry, adequately cooled and ventilated storage and good sanitation are essential to minimize mold contamination. Genetic approaches and use of mold inhibitors may be helpful. When prevention fails, hand or electronic sorting can remove contaminated seeds. Aflatoxin may be removed during processing by alkali refining of crude oils or by extraction of contaminated oilseed meals with polar organic solvents. Some aflatoxin is destroyed or degraded during normal preparation of some foods. Many chemicals, especially oxidizing and reducing agents and acids and bases, have been screened as reagents for destroying aflatoxin in contaminated feeds. Several processes have been patented. In the USA aflatoxin in copra has been destroyed on a commercial scale by hot aqueous calcium hydroxide and in cottonseed meal by treatment with ammonia under pressure.

INTRODUCTION

When it was first recognized that effects associated with Turkey-X disease were due to what we now call aflatoxins and that the aflatoxins are metabolites of the common mold Aspergillus flavus, it was considered that the contamination of commodities with aflatoxins was a storage problem. We now know that although development of aflatoxin can be a storage problem it is also a field problem. We know that aflatoxin can frequently be found in peanuts in the ground, in corn growing in the field, and in some tree nuts while they are still on the tree. In the case of cottonseed it appears that the problem in the United States is largely confined to a few locations, and there it is primarily a field problem. These findings, exactly the reverse of what was commonly believed only a few years ago, prompted greater interest and research in methods of prevention.

PREVENTION: AGRICULTURAL PRACTICES

The U.S. Department of Agriculture has issued a bulletin, ARS 20-16, "Preventing Mycotoxins in Farm Commodities" (Ref. 1). This bulletin emphasized the importance of good farm management practices. It included specific as well as general recommendations such as using sound, fungus-free, viable seed; controlling insects and diseases; harvesting promptly at maturity; and properly adjusting and operating harvesting equipment to avoid damaging the crop and picking up excessive amounts of leaves and dirt. The farmer is told it is his responsibility to take proper measures to ensure that commodities are neither damaged by mold in the field nor harvested and stored in a condition favorable to molding before they reach marketing channels.

The Department has issued several other relevant bulletins, and various other public and private organizations have also written publications in simple, easily understood language to provide practical suggestions and guidance. In short, at least for peanuts in the U.S.A., the farmers and all others involved in the chain from grower to handler have ready access to appropriate information to guide them in their efforts to prevent formation of mycotoxins. It appears that the major causes of preharvest contamination in peanuts are drought-induced plant stress and faulty agronomic practice. A doctoral thesis (Ref. 2) which appears not to have received the attention it merits, reported on the influence of kernel water content on invasion by Aspergillus flavus and aflatoxin production in peanuts. The impressive data obtained by the author in laboratory and field experiments led him to conclude that A. flavus

can invade peanut cotyledons only when the water content is within the critical range of 9% to about 25%. Below 9%, or once the seed has germinated, the fungus does not invade the turgid tissues of the cotyledon. When mature kernels with more than 25% water were incubated with A. flavus, they were rarely invaded by the fungus and contained only traces of aflatoxin. The National Peanut Council (USA) has been active in developing the highest standards of peanut quality and good practices and has developed, disseminated, and promoted separate Voluntary Codes of Good Practices for Peanut Shellers, Warehousemen, Cold Storage Plants, and Manufacturers of Peanut Products. Members are urged to see that all appropriate people in their organizations read and understand the codes. These codes emphasize the importance of general good housekeeping, sanitation, insect and rodent control, close periodic checking on the condition of the peanuts, and testing for aflatoxin content at different stages. However, they also recommend specific operating or processing conditions.

DIVERSION

Bulletin ARS 20-16 (Ref. 1) emphasizes that special attention should be given to detecting lots that contain aflatoxin as early as possible in the marketing process. Early detection and diversion of small consignments of contaminated material may prevent contamination of much larger supplies. However, to achieve this, rapid screening methods of detection are required. These are becoming available. It was noted several years ago (Ref. 3) that there is a high correlation between aflatoxin content and the presence of A. flavus mold that is readily observable in damaged peanut kernels when viewed under low magnification. The commercial grading system for peanuts in the United States, which provides the basis for the price to be paid, calls for examination of the damaged kernels in the sample that is graded. Another step was introduced into the inspection system--examination of the damaged kernels for A. flavus mold. Each inspector is given a folder with a pair of photographs, about 20 cm by 30 cm, that shows him what to look for and what not to look for. These photographs have been published in color (Ref. 4). This additional operation is said to require less than a minute for each sample, but it has been extremely valuable to the peanut industry in the USA. A bright, greenish-yellow (BGY) UV-induced fluorescence has been associated with corn naturally contaminated with aflatoxin; the characteristic color of this fluorescence is shown by Fennel et al. (5).

In 1955, before the discovery of aflatoxin, it was reported that a previously undescribed boll rot of cotton characterized by a BGY UV-induced fluorescence in the fibers is caused by Aspergillus flavus attack on the fiber. With the advent of aflatoxin, attempts were made to relate this BGY fluorescence to contamination of cottonseed with aflatoxin. The same BGY fluorescence that occurs in the cotton fibers may also be found in the seed fuzz. Analysis of an extensive series of samples indicated that fuzzy seed samples that fluoresce under ultraviolet light tend to have aflatoxin in them (Ref. 6). On the other hand, analysis of individual fluorescent cottonseeds indicated that there was no detectable aflatoxin in nearly half of them (Ref. 7). It is now recognized that the fluorescence in cotton fiber and cottonseed fuzz is due not to aflatoxin but to a derivative of kojic acid, a well known metabolite of A. flavus. The fluorescent derivative of kojic acid is soluble in water and the test is not applicable to weathered cottonseed. Because of the apparent inapplicability of the BGY fluorescence test for aflatoxin in cottonseed, a simple and rapid chemical test was devised (Ref. 8). This millicolumn test is sensitive to about 5-10μg/kg and can be completed in 15 minutes or even less. It has been very useful in detecting lots of contaminated cottonseed before they are unloaded so they could be diverted for separate processing. The millicolumn procedure has also been used with various other agricultural commodities, and numerous modifications have been reported.

ANTIFUNGAL AGENTS

The use of antifungal agents to control fungal infestation and development of aflatoxin has been the subject of much study, but until rather recently there has not appeared to be any large scale application. With peanuts, spray treatment with fungicides has not effectively prevented A. flavus growth in farmers' stock (in shell) peanuts. Better results have been obtained with corn. Certain volatile fatty acids such as propionic preserve high moisture corn without reducing its value as an animal feed, and this method is now widely used. Both ammonia (2%) and propionic acid (1%) significantly reduced mold growth and subsequent aflatoxin formation (Ref. 9). It was concluded that both should have practical application for preventing the formation of mycotoxins in stored corn. Dichlorvos has been reported to strongly inhibit aflatoxin biosynthesis by a potent strain of A. parasiticus without affecting fungal growth and to be much more potent than other organophosphorus insecticides (Ref. 10).

GENETIC APPROACHES

Development of commercially acceptable varieties that would resist toxin-producing molds or

completely inhibit production of toxin would be an ideal solution, and genetic approaches that may result in resistance to elaboration of aflatoxin are being investigated. Impermeable seed coat cottonseed (so-called "hard seed") was reported to have less tendency to allow A. flavus to grow and produce aflatoxins than seed without this "hard coat" trait (Ref. 11). This indicates that genetic control of mold invasion, and hence production of aflatoxin in cottonseed is possible. Laboratory and field studies have presented evidence for broad varietal differences in resistance of corn to A. flavus and its production of aflatoxin (Ref. 12). Research to identify peanut lines with resistance to toxin-producing molds is under way, and at least three lines with seeds resistant, to some degree, to A. flavus invasion have been identified. Yield and other characteristics make these of no commercial value, but the Agricultural Research Service of the USDA has released two of the genotypes with tolerance to toxin producing strains of A. flavus, for use in peanut breeding programs.

ELIMINATION: REMOVAL BY PHYSICAL SEPARATION

The vast majority of the aflatoxin in contaminated commodities generally resides in a relatively small number of seeds or kernels. This affords an exceptional opportunity for effectively yet economically reducing the aflatoxin content by mechanical removal of those few that are contaminated. Physical separation is used successfully in the peanut industry. Culling is typically accomplished by screening at shelling plants, by removing discolored kernels by hand sorting on picking tables, and by various mechanical and electronic sorters. Electronic sorting is even more effective after blanching. Almonds may be sorted in commercial practice by machines equipped with ultraviolet illumination. Such electronic sorters have been reported to effectively remove cottonseed exhibiting BGY fluorescence, but this may not be economic. Bockelee-Morvan and Gillier (13) reported that overall aflatoxin contamination of unshelled peanuts can be significantly reduced by removing defective pods by hand or by pneumatic sorting, and that hand sorting for unshelled, edible products is now employed in Senegal. Contamination of Brazil nuts can be significantly reduced by pneumatic sorting. Results of two tests to separate aflatoxin-contaminated cottonseed by mechanical projection from a moving belt were inconclusive. In one test, 63% of the aflatoxin was concentrated in 6% of the seed, but in the other little or no segregation was achieved (Ref. 14).

Aflatoxin may be removed by extraction with suitable solvents. The most successful application is the removal of aflatoxin from oils during normal commercial processing. Current processing of oilseeds leaves in the oil a portion of any aflatoxin present in the seed. Most of this aflatoxin ends up in the soapstock obtained when the crude oil is treated with alkali. This may be considered a special case because the aflatoxin is chemically altered in the process. In the soapstock, the aflatoxin is present as the alkali salt of the acid formed on opening the lactone ring, and the acid is readily reconverted to aflatoxin by mild acidification.

Several procedures may be used to remove aflatoxins from oilseeds and meals. These include extraction of aflatoxin with appropriate solvents, simultaneous solvent extraction of oil and aflatoxin, and selective extraction of aflatoxin and limited amounts of oil or meal components.

An aqueous solution of calcium chloride has been reported to show promise for removing aflatoxin from contaminated meals during preparation of protein isolates (Ref. 15). A solvent system of acetone, hexane, and water, originally developed and investigated on a pilot-plant scale to remove gossypol along with oil from prepared cottonseed meats, also removed aflatoxin readily and quantitatively from ground peanuts or peanut meal while removing relatively little extraneous material other than oil. Aqueous acetone may also be used as a selective solvent (Ref. 16).

Mixtures of hexane-methanol, hexane-ethanol, hexane-ethanol-water, and hexane-acetone-water were evaluated by Vorster (17). Greatest reduction in aflatoxin content was obtained with hexane-acetone-water and hexane-methanol. Good reduction in aflatoxin content of contaminated cottonseed and peanut meals has been reported for extraction with 80% aqueous isopropanol (Ref. 18) and also with 95% ethanol. Extraction of cottonseed flakes with acetone containing 25-30% water removes essentially all gossypol and aflatoxin, most of the free fatty acids, half the raffinose, and negligible quantities of neutral oil and protein. The residual, practically full-fat, product, now essentially free of any aflatoxins that may have been present, can then be processed for oil removal by any conventional means (Ref. 19). This solvent system is potentially applicable to other oilseeds.

Thus a variety of polar solvents are effective for the removal of aflatoxins. Such solvent systems have the advantage that under suitable conditions they can remove essentially all the aflatoxins with little likelihood of forming from the aflatoxins products having adverse physiological activity and without appreciable reduction of protein content or of its nutritional quality. On the other hand, there is the cost of additional processing, the need for special extraction and solvent recovery equipment, the loss of some water-soluble components of the residual meals (chiefly carbohydrates), and the problem of disposal of the extract.

DETOXIFICATION

In spite of efforts at prevention, aflatoxin contamination will sometimes be unavoidable (Ref. 20). Some aflatoxin may be destroyed in conventional processing of food products. In roasting peanuts under conditions simulating commercial oil- and dry-roasting, overall average destruction was about 66% (Ref. 21). Similar results were obtained on roasting pecans (Ref. 22). Aflatoxin destruction has also been observed in making tortillas, the overall reduction being about 70% (Ref. 23). Partial destruction of aflatoxin occurs in various stages of breadmaking, and it was proposed that this may be due to oxidation and hydrolysis phenomena (Ref. 24). Agricultural products contaminated with aflatoxin that are unsuitable for food are usually diverted to feed. The effect of heat and moisture may reduce aflatoxin levels during processing for feed (Ref. 25).

Biological approaches to aflatoxin inactivation have been investigated. Approximately 1,000 microorganisms were screened for destruction of aflatoxin B_1 and G_1 (Ref. 26) but only one, Flavobacterium aurantiacum, was effective in removing aflatoxin. Feeding animals aflatoxin contaminated grains or oilseed meals also results in biological destruction of aflatoxin. Although some aflatoxin or its metabolites may be transmitted from feed to animal products such as meat, milk, or eggs, the amount is usually a very small percentage of that fed. Thus, the animal serves as a filter and the hazard to human health is greatly reduced.

Many chemicals have been screened as reagents for the destruction of aflatoxins including acids, alkalies, aldehydes, oxidizing agents, and various gases. But the number that can destroy aflatoxins without leaving deleterious residues or excessively damaging nutrients appears to be quite small. The discussion here will be limited to treatments that have been used commercially or that seem to be most promising for practical application. As indicated earlier, refining of vegetable oils concentrates aflatoxin in the soapstock. Treatment with mineral acid at low pH values rapidly converts aflatoxin B_1 to B_{2a} (Ref. 27). Acidulation would thus reduce any toxic or carcinogenic effects and render soapstock more suitable for use in feeds.

Treatment of contaminated peanut meal with hydrogen peroxide effectively destroyed toxicity (Ref. 28). The use of sodium hypochlorite during aqueous processing of raw peanuts to produce protein isolates also destroyed aflatoxin (Ref. 29).

Treatment of aflatoxin-contaminated copra with calcium hydroxide was formerly practiced commercially in the U.S.A. and is described in U. S. Patent 3,689,275. The salt formed when aflatoxin is treated with alkali degrades on heating (Ref. 30), and this precludes reformation of the lactone on acidification following ingestion. Treatment of aflatoxin-contaminated peanut or cottonseed meals with formaldehyde and lime has been found to reduce aflatoxin to low levels, (Ref. 31). This process might be applicable for ruminant feed.

When a cottonseed meal initially containing about 500 µg/kg aflatoxin was treated with ammonia (48 pounds pressure, 118°C, for 30 minutes) the aflatoxin content was reduced to below 5 µg/kg (Ref. 32). From a 2-year feeding test in which this meal was fed to rats as 20% of the diet, it was concluded that ammoniation effectively detoxified the meal. Further feeding tests are under way to establish the safety of ammonia-detoxified cottonseed meal as feed for laying chickens and of the eggs and meat for human consumption. In the USA there is a commercial plant to ammoniate aflatoxin contaminated cottonseed for feeding to dairy cattle and two plants to ammoniate excessively contaminated cottonseed meal. The U.S. Food and Drug Administration has granted interim approval for use of ammonia-detoxified cottonseed meal for cattle feed and limited interim approval as feed for laying chickens.

Aflatoxin in peanut meal has been reduced to nondetectable levels by ammoniation for 30 minutes under 30 pounds pressure at 65°C. Ammonia detoxification of aflatoxin contaminated peanut meal has been confirmed by research on this process in France (Ref. 33). Detoxification with ammonia is covered by U.S. Patent 3,429,709 and French Patent 2,184,439. Use of alkali or organic amines is described in U.S. Patent 3,890,452 and corresponding patents in other countries. Model ammoniation experiments with aflatoxin B_1 resulted in identification of two major reaction products (Ref. 34). A scheme proposed for the reaction is shown in Fig. 1. This postulates opening the lactone ring to form the ammonium salt of a phenolic keto acid and loss of ammonia to form the free β-keto acid, followed by its decomposition to CO_2 and aflatoxin D_1 or to the furofurophenol designated by its molecular weight, 206.

A process has been proposed for detoxifying aflatoxin-contaminated corn by contact with ammonia at atmospheric pressure. A mechanism of inactivation is suggested in which aflatoxin (or its ammoniation product(s)) is bound to a macromolecule (Ref. 35). Feeding tests with ammoniated corn are under way.

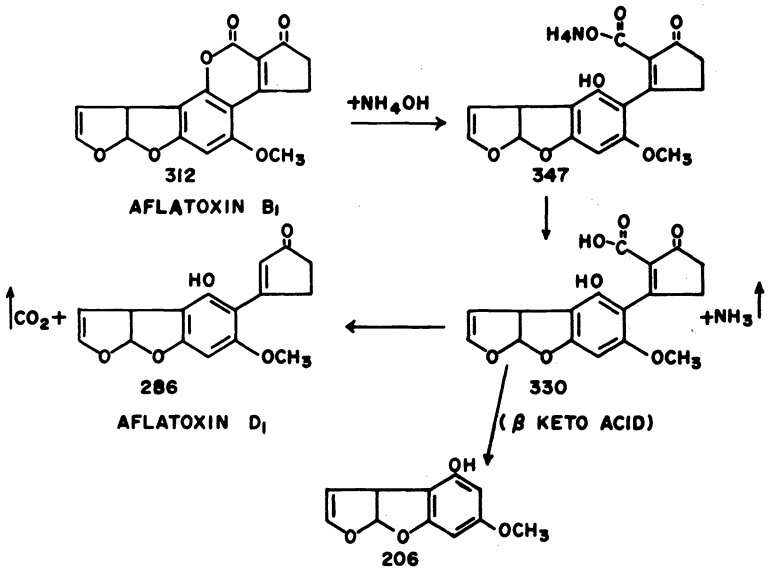

Fig. 1. A proposed scheme for formation of the major products of ammoniation of aflatoxin B_1.

To summarize -- prevention of contamination is the best approach. Appropriate preventive measures should be taken at all stages of culture, harvest, transportation, storage, and processing. If prevention fails, contaminated material may still be salvaged but at a cost, by mechanical removal of contaminated seed, by extraction with polar solvents, or by destruction of aflatoxins with appropriate chemicals.

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