

Decontamination of Aflatoxin B1

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Abstract

Aflatoxins are a class of highly toxic carcinogenic mycotoxins by food contaminant *Aspergillus* fungi: *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are classified into four compounds: aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2), while AFB1 is the most potent carcinogenic agent associated with hepatocellular carcinoma (HCC). *Aspergillus* fungi is ubiquitously found in the soil and contaminates the crops such as maize, groundnuts, rice, and so on. Prevention of aflatoxin contamination, detection and degradation of *Aspergillus* fungi contamination, and the concentration of AFB1 in the foodstuffs are of the primary task to prevent health problems from aflatoxin. Here, the different ways are summarized to degrade or decontaminate the aflatoxins available with the foods. Traditional decontamination of aflatoxin includes physical (heat and irradiation), biological, and chemical treatments. However, these traditional aflatoxin decontamination technologies are not enough to remove the aflatoxin from the foods. Recently, some novel processing approaches have been explored to achieve full degradation of the aflatoxin available with the foodstuffs, like microwave heating, gamma and electron beam irradiation, pulsed light, electrolyzed water, cold plasma, and so on. Decontamination mechanism, degradation efficiency, advantages, and limitations of these new technologies shall be discussed herein.

Keywords: aflatoxin B1, detoxification, toxicity, decontamination, biological degradation

1. Introduction

Mycotoxins are low-molecular-weight nature products as secondary metabolites by filamentous fungi or mold, which display overlapping toxicities to invertebrates, plants, and microorganism. The term mycotoxin was coined in 1962 in the aftermath of unusual veterinary crisis close to London, England, where nearly 100,000 turkey poultry died. It came out that was linked to peanut meal contaminated by aflatoxins. Now, more than 300 mycotoxins are found; however, only very few mycotoxins caught scientists' eyeball, which have been proven to be carcinogenic and toxic.

Human food can be contaminated with mycotoxins during storage. One of the principal classes of highly toxic carcinogenic mycotoxins is the metabolite of *Aspergillus flavus* and *Aspergillus parasiticus* aflatoxins [1]. Aflatoxins are classified into such four compounds as aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) based on their fluorescence under UV light (blue or green), and in the milk it was aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) which are the metabolites of AFB1 and AFB2, respectively [2–4]. The toxic effect

upon the living organisms of aflatoxin decreases in an order B1, G1, B2, and G2 [5]. AFB1 is the most potent carcinogenic agent associated with hepatocellular carcinoma (HCC), one of the most lethal and common cancers in the world, especially in Asia and Africa. The mycotoxin aflatoxin B1 (AFB1), the most notorious liver carcinogen, has been proven to be genotoxic. Epidemiological studies have shown that chronic exposure to aflatoxin B1 in the diet is one of the important factors in the etiology of liver cancer in experimental animal models, including rats and rainbow trouts [6].

Discovery of practical and economical procedure to prevent aflatoxin contamination, detect and degrade *Aspergillus* fungi contamination, and lower the concentration of AFB1 in the foodstuffs is of the primary task to prevent or eliminate the aflatoxin risk.

Aspergillus fungi is ubiquitously found in the soil and contaminates the crops in the field and during storage, such as maize, groundnuts, rice, and so on. Mycotoxins are the low-molecular weight nature products as the secondary metabolites of molds that make the industrial factories lose money resulting from condemnation of contaminated crops. They have been detected in various commodities such as maize, wheat, barley, oilseed, peanut, and beverages made from contaminated grains and other foods. Thus direct exposure to aflatoxin-contaminated commodities may impose a great risk to the consumers.

Unquestionably, prevention is the best method to control mycotoxin contamination. As the result of the high prevalence of AFB1 in the foods, many strategies are being developed to prevent or remove AFB1 contamination. In general, two ways are available to prevent AFB1 from contamination: pre- and postharvest treatment. Pre-harvest techniques are the first barrier to prevent mycotoxin contamination in all kinds of grains or feed. Pre-harvest techniques include the use of genetically altered crops that are resistant to *Aspergillus* infection and environmental stressors, pesticide usage, crop rotation, and timing of planting. Postharvest strategies include physical methods (proper drying, packaging, storage, preservative/pesticide usage). These strategies play the roles as the preventative measures to reduce the chance and the amount of contaminations that are introduced to the crops. However, these strategies fail to prevent the contaminations fully and effectively; thus some postharvest techniques are being developed to degrade or reduce AFB1 contamination. In this review, we aimed to investigate AFB1 decontamination methods, including several traditional strategies, and update some new methods.

2. Physical treatment of aflatoxin removal and detoxification

2.1 Cleaning and segregation of aflatoxins

The first option to reduce aflatoxin is to physically separate the mold-contaminated grains or feed (kernel, seeds, and nuts) from the intact and apparently uncontaminated product. The physical procedure is the safe way not to alter the products significantly, including cleaning, sorting, and handpicking [7]. In the developing countries or economically underdeveloped areas, the people have little or no access to do mycotoxin testing of their foods. Hand sorting is still the primary method to remove AFB1. As compared with other methods including flotation and dehulling of the grains, hand sorting of maize grains boasts <6% of AFB1 and <5% of fumonisin B1. Thus hand sorting of maize grains is being recommended as a last line of defense against mycotoxin exposure among subsistence consumers [8].

2.2 Heating treatment

Aflatoxins are well known to be stable at high temperature. In modern food/feed manufacturing technology, heating treatment is always used to degrade mycotoxins to a certain extent during the processing. Recent studies have shown that AFB₁ could be significantly removed at high humidity [9–12]. However, several possible factors are associated with the prediction of the extent of mycotoxin reduction, such as initial mycotoxin concentration, the extent of binding between mycotoxin and food or feed products, heat penetration, moisture content, and processing conditions. Nonetheless, heat treatment to partially reduce the mycotoxin concentration in the food/feed stuffs is still the feasible physiological method because heating technique can be carried out easily at low cost. Extrusion cooking is broadly used in the field of food industry, which is an efficient process in food/feed process. High temperature with short-time extrusion is commonly used in the industry [5].

2.3 Microwave heat treatment

Perez-Flores et al. [13] found that aflatoxin content could be significantly reduced by microwave thermal-alkaline treatment in the traditional Mexico food tortillas. Using extract acidification methods to mimic human stomach digestion procedure to quantify AFB₁ concentration, the results indicated that the aflatoxin reduction was almost permanent. However, this thermal-alkaline treatment for tortilla-making could only remove most of AFB; thus some AFB would be left in the food.

2.4 Irradiation treatment

Another most commonly reported physical decontamination technology is γ radiation. The use of γ radiation has been reported on some kinds of food substrates including groundnuts, grains, palm juice, soybean, and animal feed. Irradiating the food products with a γ -ray source is moderately effective with an average percent reduction of 65% at high irradiation dose [14–20]. Gamma irradiation is a promising method to improve the safety and economy of moderately fungi-damaged feedstuffs.

2.5 Electrolyzed water (EOW) treatment

Electrolyzed water treatment is a sort of newly developing skill to treat AFB₁-contaminated foods or feeds. The AFB₁ was markedly reduced when treated with EOW, particularly with neutral electrolyzed oxidizing water (NEW). The levels of OH that exist in EOW could be the important reason that leads to significant fungicidal efficiencies against *A. flavus*. After 15 min treatment with EOW, AFB₁ was mostly degraded [21, 22]. Another study performed by Fan et al. [23] showed that alkaline electrolyzed water (AIEW) could remove AFB₁, and its best working condition was at pH 12.2. When 10 ml AIEW with pH 12.2 was added into 5.0 g peanut oil or olive oil, followed by oscillation for 5 min at 20°C, AFB₁ removal could reach nearly 100%.

2.6 Pulsed light technology to remove AFB₁

Pulsed light has been demonstrated to be an effective decontamination technique capable of destroying bacteria, viruses, fungi, and spores at the surface of food and material [24]. The work of Moreau et al. [25] provides the first

demonstration of a nonthermal technology allowing mycotoxin destruction and inactivation of their mutagenic activity. They evaluated that the effectiveness of the pulsed light technology for the degradation of mycotoxins. AFB₁ was destroyed around 98% by eight flashes of pulsed light.

3. Chemical treatment to degradation of aflatoxins

The use of chemical additives upon the contaminated foods has been one popular method, especially the additives themselves would be used in the foods.

3.1 Ammonia decontamination treatment

Ammonization of maize, rice, barley, peanuts, and cottonseeds to alter the toxic and carcinogenic effects of aflatoxin contamination has been intensely researched by the scientists from government agencies and universities in the world. Several studies have shown that aflatoxin B₁ levels were reduced effectively and permanently by 1 hour ammonia treatment. Treatment with either NH₄OH at high temperature or gaseous NH₃ can effectively reduce aflatoxin B₁ content sometimes reaching above 99%. But at lower temperature, for example, at 25°C, AFB₁ level could not be reduced very well. Their study revealed that the moisture level of the product and holding temperature were the crucial factors to have influence upon the efficacy of aflatoxin decontamination [26–29]. The degradation of AFB₁ is ammonization of aflatoxin (AFD₁), which has been shown to be far less mutagenic than AFB₁.

3.2 Hydrochloric acid (HCl) treatment

Aly and Hathout [30] investigated the effect of hydrochloric acid on AFB₁ degradation in contaminated corn gluten under different HCl concentrations. The effect of AFB₁ degradation by HCl is in a temperature-, HCl concentration-, and time-dependent manner. During the wet milling process, treating with 1 mol/L HCl at 100°C resulted in degradation of AFB₁ by 27.6% after 4 hours and reached to 42.5% after 8 hours. When concentration of HCl increased, the degradation of AFB₁ increased, and it will completely degrade AFB in the presence of 5 mol/L HCl after 4 hour at 110°C.

3.3 Lactic acid and citric acid treatment

Previous studies have shown that some organic acids have detoxification ability in treatment of aflatoxin-contaminated foods [31]. Mendez-Albores et al. showed that citric acid and lactic acid have efficiency upon aflatoxin degradation. When the acid concentration increased, the amount of B-aflatoxins decreased, and citric acid has more notable effect upon AFB degradation. Lee et al. also found the reduction rates of AFB₁ in 1.0 N citric acid and lactic acid treatment for 18 hour could reach 94.1 and 92.7%, respectively [11].

3.4 Ozonation treatment

Ozonation is another commonly used chemical control method. Ozonolysis at a concentration 6–90 mg/L is effective to degrade AFB₁ in short-time treatment. As short as 15 min, all molds were inactivated, and *Aspergillus parasiticus* and *Aspergillus flavus* were isolated from dried figs, while AFB₁ was degraded in

time-dependent manner in dried figs [32]. Aflatoxins in the peanuts at moisture content of 5% (w/w) were sensitive to ozone and easily degraded when treated with 6.0 mg/l of ozone for half hour at room temperature. The detoxification rates of the total aflatoxins and aflatoxin B₁ (AFB₁) were 65.8% and 65.9%, respectively [33]. Another study also showed that 89.4% AFB₁ in the peanuts was decomposed by ozone with a concentration at 50 mg/L, flow rate 5 L/min for 60 hours [34].

4. Biological treatment to degrade aflatoxins

Using microorganisms or enzymes for biodegradation of aflatoxins is one of the well-known strategies to decrease the level of aflatoxins in the foods or feed products. The methods of biologically based interventions are being actively studied because they are efficient, specific, and environmentally friendly as compared with other non-biological degradation methods.

4.1 Soil bacteria

Many bacteria in the soil are able to degrade aflatoxins. *Flavobacterium aurantiacum* NRRL B-184, a kind of bacteria from the soils and water, showed that it can detoxify aflatoxins in high efficiency. The study from Ciegler et al. [35] showed that *F. aurantiacum* NRRL B-184 removed aflatoxin irreversibly from contaminated milk, oil, peanut butter, peanuts, and corns and partially removed from soybeans. The aflatoxins are not only removed away by *F. aurantiacum* NRRL B-184 but also failed to form any new toxic products. The bacteria was also reported that AFM₁ could be removed by it from milk [35]. During monitoring the roles of such metal ions as Cu²⁺, Mn²⁺, Zn²⁺, and other chemical materials on AFB₁ degradation by the bacteria, they could increase AFB₁ degradation by 10–15% [36–38], suggesting enzymatic system was involved in aflatoxin B₁ degradation by *F. aurantiacum*. Except *F. aurantiacum*, other microorganisms, for example, *Nocardia asteroides* and *Corynebacterium rubrum*, are able to detoxify aflatoxin [5, 39]. *Mycobacterium fluoranthenivorans* sp. nov. DSM44556, as a single carbon source from soil of a former coal gas plant, could reduce AFB₁ concentration to amounts of 70–80% of the initial concentration within 36–48 hours, and no AFB₁ could be detected in 72 hours [40, 41], while the cell-free extracts of *M. fluoranthenivorans* sp. nov. DSM44556 degraded AFB₁ more than 90% the initial amount of AFB₁ at high temperature within 4 hours and fully degraded in 8 hours [41]. Teniola et al. showed that *N. corynebacterioides* DSM20 151 could degrade more than 90% of AF₁B after 24 hours treated in cell-free extracts. Alberts et al. [42] examined that AFB₁ was biodegraded by *Rhodococcus erythropolis* in liquid cultures. AFB₁ was dramatically degraded to 32% of initial concentration by extracellular extracts from *R. erythropolis* liquid cultures. Thus *F. aurantiacum*, *M. fluoranthenivorans*, and *N. corynebacterioides* could be a potential and promising application because of their potent efficient degradation of AFB₁ in the food and feed process.

4.2 Fungi

Fungi can not only produce aflatoxins but also degrade aflatoxin. Such four fungal strains *Aspergillus niger*, *Eurotium herbariorum*, a *Rhizopus* sp., and non-aflatoxin-producing *A. flavus* were able to convert AFB₁ to aflatoxicol-A (AFL-A); then AFL-A was converted to aflatoxicol-B (AFL-B) by the actions of medium components or organic acids produced from the fungi. Fungi *Penicillium raistrickii* NRRL 2038 could transform AFB₁ to a new compound which is similar to AFB₂.

Kusumaningtyas et al. found *Rhizopus oligosporus* was able to inhibit synthesis or to degrade AFB₁ when cultured together with AFB₁-producing fungi *A. flavus* [43].

4.3 Yeasts and lactic acid bacteria

The mechanism of degradation AFB by yeasts and lactic acid bacteria is due to their adhesion to cell wall components. However the role of yeasts and lactic acid bacteria on AFB is controversial. A few study showed there was no effect of yeasts and lactic bacteria upon aflatoxin [44]. The results showed that high levels of aflatoxins in raw maize would not be degraded during the fermentations in the processing of the west African traditional food “kenkey.” Other studies reported very efficient aflatoxin reductions after fermentation. Chu et al. [45] reported that AFB₁ concentration dramatically decrease during brewing process, which suggested that *S. cerevisiae* yeasts sorb mycotoxin. AFB₁ was detoxified into a nontoxic new fluorescing compound corresponding to AFB₂a during yogurt-making and dairy product fermentation [46, 47]. Drinking water with *S. cerevisiae* strain showed a positive protection effect on the relative weight of the liver and histopathological and biochemical parameters when giving the diets contaminated with AFB₁ [48].

Lactic acid bacteria have been previously reported to possess antimycotoxigenic activities both in vitro and in vivo. The specific strains of lactic acid bacteria will bind selected dietary aflatoxin contaminants. The ability of 12 *Lactobacillus*, 5 *Bifidobacterium*, and 3 *Lactococcus* bacteria strains to bind AFB₁ was investigated by Peltonen et al. [49]. Two *Lactobacillus amylovorus* strains and one *Lactobacillus rhamnosus* strain removed more than 50% AFB₁ rapidly after a 72-hour incubation period. Another two lactic acid bacteria *Lactobacillus rhamnosus* strain GG (LBGG) and *L. rhamnosus* strain LC-705 (LC705) can significantly and very quickly remove approximately 80% AFB₁ from culture media in both temperature- and bacteria concentration-dependent manner [50]. Kankaanpaa et al. [51] found that the binding of AFB₁ to *L. rhamnosus* GG decreased its subsequent adhesion capability to Caco-2 cells, thereupon which the bacteria may reduce the accumulation of aflatoxins in the intestine via increasing aflatoxin-bacteria complex excretion.

4.4 Aflatoxin degradation by enzymes

Some specific enzymes to degrade aflatoxins have been purified from microbial systems. Using enzyme to degrade aflatoxins have some merits, such as avoiding to change flavor or impairing the nutritional value. Motomura et al. [52] investigated the ability of degrading AFB₁ in cultured supernatants from 19 fungi and purified 1 enzyme with aflatoxin degradation activity from *P. ostreatus* supernatant. The enzyme showed that AFB could make the best degradation of activity at 25°C with a pH of 4.0–5.0. The novel enzyme could cleave the lactone ring of aflatoxin. Another study showed that an intracellular enzyme, named aflatoxin-detoxifizyme, exhibited detoxification activity on aflatoxin B₁ and the optimum activity for the enzyme was at 35°C with a pH of 6.8 [53]. Shcherbakova et al. [54] also proved AFB₁ degradation by *Phoma glomerata* PG41 strain was stable and reproducible.

4.5 Cold plasma technology to remove AFB₁

In the past cold plasma is used for sterilization of sensitive materials. Lately, much attention has been paid to cold plasma as a new microbial decontamination technology in the food industry. It has the advantages of high efficiency and short treatment time, no residue, and low impact on the quality of treated food products [55, 56]. Recently the degradation of mycotoxins by cold plasma was studied.

It was reported that AFB₁ could be successfully removed by 5 s of treatment with microwave-induced argon plasma [57]. Nitrogen gas plasma could efficiently be degraded to 10% of initial concentration within a 15-min treatment [58]. Low-temperature radio-frequency plasma degraded 88% of AFB₁ within 10 min [59]. High-voltage atmospheric cold plasma (HVACP) is a novel nonthermal decontamination technology that has the potential to be used in the food industry. HVACP treatment of aflatoxin has been shown to degrade 70% of the total aflatoxin in 12 min [60, 61].

5. Sorbent additives for degradation of AFB₁

There is one approach to solve AFB₁ contamination is the addition of sorbents in the foods. This process is not the same as the degradation process, because it does not involve destroying or reducing the amount of AFB₁ in the foods or feeds. They act as binding agents to prevent AFB₁ absorbed from intestinal tract after ingestion. Chlorophyllin added to the contaminated feeds could reduce AFB₁-DNA adduct by 37% in rainbow trout which led to a 77% reduction of tumor incidence [62]. Another study observed that chlorophyllin exhibited the reduction of AFB₁-DNA adducts, boasting the reduction of AFB₁-album adducts by 65% and urinary AFM₁ by 90% in rats; chlorophyll also reduces AFB₁-DNA adducts, AFB₁-album adducts, and urinary AFM₁ levels by 55, 51, and 92%, respectively [63].

Clay works similarly to chlorophyll and chlorophyllin. By addition of the clay into the animal feeds, AFM₁ level in milk is reduced accordingly with the decrease of AFB₁ absorption rate [64]. And no overt toxicities were observed after SD rats were fed with NovaSil clay (NS) for more than half year [65]. For human beings, NS was performed for clinical study, and the side effect were reported in 99.5% of the persons as compared to the control group. After 3 months, the level of AFB₁-albumin adduct was significantly decreased in both low-dose group and high-dose group. The level of AFM₁ in urine samples decreased 58% in the high-dose group in 3 months. And there was no liver and kidney function or hematological parameter change reported [66, 67]. From these studies, NS diet can be regarded as a safe and effective method to reduce AFB₁ toxicity in the foods.

In addition, different types of mineral clays have been tested for their capabilities to bind AF in animal feeds. These absorbents, such as activated carbon (charcoal), zeolite, and saponite-rich bentonite, reduced AFB₁ absorption in the gastrointestinal tract [68].

6. Other methods to degrade of AFB₁

Recently, some inexpensive, new promising methods on top of conventional methods for decontamination of food and raw materials have been developed. In the beer or wine factories, some fermentation residues were observed to have the ability of degradation of AFB. A group in Italy have shown that biosorption of mycotoxins onto grape pomace may be a reasonably low-cost decontamination method. The theoretical maximum adsorption capacities (mmol/kg dried pomace) were calculated at pH 7 and 37°C; around 1 hour of contact, that pomace could adsorb almost half of initial AFB₁ concentration, but it seems the adsorption rate was kept stable within pH ranges [69]. Similarly, Bovo et al. [70] also found AFB adsorption by beer fermentation residue (BFR) ranged from 45.5 to 69.4% at pH 3.0 and from 24.0 to 63.8% at pH 6.0.

7. Conclusion

Among all mycotoxins, the group of aflatoxins has received much attention due to their severe impact on human and animal health. AFB₁ is the most potent carcinogenic agent associated with hepatocellular carcinoma. And AFB₁ can negatively affect nutrition absorption, growth and development, and immune system function. AFB₁ contamination in the food/feed supplies was found in various countries, particularly in Asia and Africa. A lot of methods to remove or prevent AFB₁ contamination, degrade AFB₁, or inhibit AFB₁ absorption have been developed in the last several decades. The efficiency of aflatoxin decontamination is subject to such factors as food conditions (food constituents, moisture content, pH conditions) and decontamination technologies and conditions. The traditional physical methods for separation or dehulling of the contaminated grains are the simple and safe ways without expensive costs to reduce the dietary exposure to aflatoxins; they can be chosen by anyone or in any area, which makes them the best methods for poor or undeveloped area. Decontamination of aflatoxin is one of the significant challenges for the food industry. The treated food should keep their nutrition values or other important desired qualities, and no residues be left or new contaminants be produced. Either most of the physical and chemical approaches for aflatoxin detoxification might affect the nutritional properties of the foods or be unsafe for human consumption; however, gamma radiation, ozone applications, microwave heating, electron beam, pulsed light, electrolyzed water, and cold plasma showed great potentials for future applications. Recently some inexpensive methods showed good perspectives for reducing aflatoxins in beer and wine factory, which are good choice to be adopted in commercial factory. Biological approaches based on removal or degradation of aflatoxins by bacteria and yeasts are of the promising perspectives, although these practices cannot be currently adopted for foods commercially. While applying these new technologies to make decontamination of aflatoxin, it shall be vital to make clear of the mechanisms of aflatoxin detoxification to determine the practical applications of these approaches in food products, especially concerning their impacts upon the food constituents. Furthermore, combination with traditional and novel technologies shall be also considered to improve the efficiency of decontamination and break through the limitations for specific technologies.

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