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Review

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Tools for Defusing a Major Global Food and Feed Safety Risk: Nonbiological Postharvest Procedures To Decontaminate Mycotoxins in Foods and Feeds

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ABSTRACT: Mycotoxin contamination of foods and animal feeds is a worldwide problem for human and animal health. Controlling mycotoxin contamination has drawn the attention of scientists and other food and feed stakeholders all over the world. Despite best efforts targeting field and storage preventive measures, environmental conditions can still lead to mycotoxin contamination. This raises a need for developing decontamination methods to inactivate or remove the toxins from contaminated products. At present, decontamination methods applied include an array of both biological and nonbiological methods. The targeted use of nonbiological methods spans from the latter half of last century, when ammoniation and ozonation were first used to inactivate mycotoxins in animal feeds, to the novel techniques being developed today such as photosensitization. Effectiveness and drawbacks of different nonbiological methods have been reported in the literature, and this review examines the utility of these methods in addressing food safety. Particular consideration is given to the application of such methods in the developing world, where mycotoxin contamination is a serious food safety issue in staple crops such as maize and rice.

KEYWORDS: *decontamination, feeds, foods, fungi, mycotoxins*

INTRODUCTION

Mycotoxins are secondary metabolites produced by some filamentous fungi that are poisonous to humans and animals.[1] The problem of mycotoxin contamination in foods and animal feeds is global, depending greatly on the climate and agronomic practices in a given region.[2−4] Whereas previously the Food and Agriculture Organization of the United Nations (FAO) estimated that about 25% of the food crops in the world are contaminated with mycotoxins,[5] studies in tropical African countries indicate higher rates of contamination in common staple crops.[6] Despite implementation of preventative strategies, mycotoxin contamination in foods and feeds still occurs. This creates a demand for additional practical and economical detoxification methods when preventive measures are inadequate.[7−9] Countries and international communities have set maximum standard levels of different mycotoxins in foods and feeds that are tolerable in market systems.[10] Considerable losses of foods and feeds occur each year due to discarding contaminated products that do not meet such standards.[11] Not all contaminated food is wasted, especially with alternative nonfood use of grains gaining popularity.[12,13] However, where food is scarce, food safety and food security considerations may cause conflict.[14]

Mycotoxins are toxic at both acute and chronic exposure levels and interfere with nutrition processes and, hence, are associated with serious health effects including retarded growth in humans and livestock.[15,16] This raises the importance of finding ways to make contaminated foods and livestock feeds useful without harming the health of the consumers, especially when discarding food is not an easy option. Studies have highlighted the potential of rendering mycotoxin-contaminated foods and feeds useable without significantly affecting their nutritive value through processes of dilution, separation, or decontamination.[15] Dilution methods are perhaps the simplest of these and involve mixing highly contaminated products with less contaminated ones to achieve final products with reduced contamination levels. Where permitted, this can help reduce exposure to the toxins. Although dilution is not a permissible treatment in the European Union,[17] elsewhere the practice of mixing highly contaminated products such as peanut cake with other less contaminated ingredients in making livestock feeds is considered acceptable.[18] Separation involves sorting and

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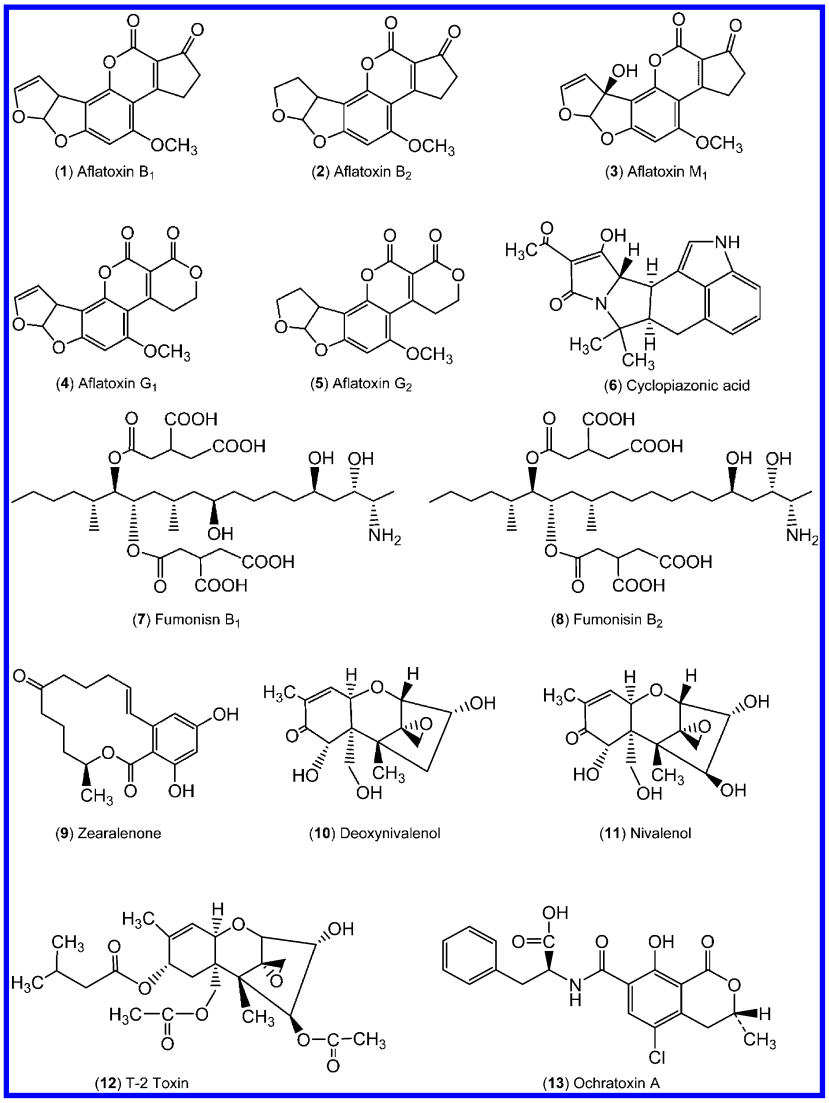

Figure 1. Chemical structures of the mycotoxins discussed.

removal of excessively contaminated portions of the product, with such processes more suited to some particular products such as peanuts.[19] Despite the added costs for rendering food products less contaminated or mycotoxin free, it is established that when well-informed, consumers are ready to pay a premium for such food.[20]

Methods proposed for mycotoxin decontamination and suppression in foods and feeds should meet the criteria recommended by FAO and (1) inactivate, destroy, or remove the toxin; (2) not produce or leave toxic residues in the food or feed; (3) retain nutritive value and food/feed acceptability of the product; (4) not alter significantly the technological properties of the product; and (5) if possible, destroy fungal spores.[9] Such methods can be grouped into biological and nonbiological. Biological decontamination and suppression methods involve the use of living organisms or enzymatic extracts to facilitate biodegradation of the toxins or reduce their absorption and/or bioavailability when ingested.[21−23] Nonbiological methods include the use of chemical or physical entities to degrade or bind the toxins in foods and feeds and render them nontoxic, less toxic or biologically unavailable when consumed.[19] It is, however, understood that some treatments of mycotoxin-contaminated products may result in less characterized but yet potentially toxic modified forms of the toxins.[24,25]

This review focuses on nonbiological methods of mycotoxin decontamination as biological methods have been the subject of several recent reviews.[21−23] We analyze different advances in these nonbiological methods, highlighting their potential and applications, identifying gaps, and proposing possible ways of filling them. The review encompasses chemical treatments (including ozonation, ammoniation, nixtamalization, sulfites, and other food additives) physical degradation, processing and binding of a range of important mycotoxins particularly aflatoxins (**1**−**5**), cyclopiazonic acid (**6**), fumonisins (**7**, **8**), zearalenone (**9**), trichothecenes (**10**−**12**), and ochratoxin A (**13**) (Figure 1). When possible, we highlight the proposed mode of action, the fate of the resulting products, and how useful and successful the methods have been. So-called "masked" mycotoxins are an important emerging issue, and the chemical treatments discussed in this review could actually result in the release of the mycotoxins,[24] but subsequent chemical degradation could then occur paralleling the degradation of the free mycotoxins.

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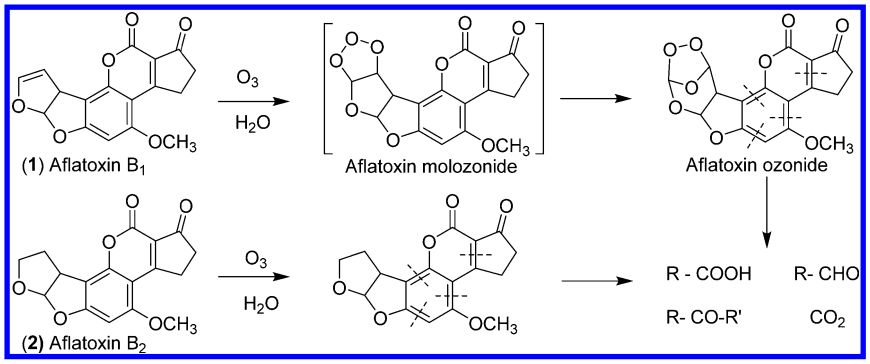

Figure 2. Chemical reaction pathway for degradation of aflatoxins B_1 and B_2 by ozonation.

CHEMICAL PROCEDURES FOR MYCOTOXIN DETOXIFICATION

Ozonation. Ozonation is an oxidation process that has been investigated extensively and found to be efficient in mycotoxin degradation. This decontamination treatment uses ozone to react with, and degrade, different types of mycotoxins. The process is used widely in sanitation, and, being a strong oxidizing agent, ozone destroys organic entities through direct or indirect oxidation.[26−31] In this review, we focus on the potential of ozonation in degrading mycotoxins, the proposed mechanism of action, and resulting breakdown products.

Several studies have been conducted on the potential of ozone to decontaminate mycotoxins in both foods and feeds, but reservations about the safety of degradation products have limited the application to animal feeds rather than human foods. In different studies, the process has been demonstrated to detoxify a range of mycotoxins including aflatoxin, zearalenone, cyclopiazonic acid, ochratoxin A, patulin, secalonic acid D, and trichothecenes.[27,30,32] The effectiveness of ozonation in degrading mycotoxins in pure preparations has been reported,[27] and studies have gone further to investigate their detoxification in foodstuffs, including fruits, grains, vegetables, spices, processed products, pet foods, and livestock feeds.[33−37]

Ozonation involves pumping in dry or moistened ozone gas or dipping the material being treated in ozonized water. Different rates and extents of detoxification are observed with each different method of ozonation, and the effectiveness also depends on other factors such as the pH, temperature, and physical state of the material on which detoxification is carried out. With moist ozone, a 90% reduction of deoxynivalenol (DON) in corn containing 1000 mg/kg of the toxin was observed as compared to a 70% reduction when dry ozone gas was used.[38] Treating dry figs with dry ozone gas had higher efficiency in degrading aflatoxin B_1 than dipping them in ozonized water.[39] Another factor that influences detoxification efficacy of ozone to mycotoxins is pH level, with higher efficiency in degradation of trichothecenes observed at lower pH than higher.[30] At pH 4−6, trichothecene mycotoxins were readily degraded by aqueous ozone, with notably reduced reactivity at more neutral pH, and at pH 9 there was negligible detoxification.[30] The efficacy of ozone in degrading mycotoxins is also influenced by the reaction temperature as reported in a study to assess the detoxification of aflatoxins B_1 and G_1 in peanuts using ozone.[40] In this study, ozonation efficiency was improved by increasing temperatures (from 25 to 75 °C) and treatment time (from 5 to 15 min) and in contaminated peanut whole kernels achieved maximal detoxifications of 77% for aflatoxin B_1 and 80% for aflatoxin G_1.

The physical status of the substrate also affects the effectiveness of ozonation with lower efficacies achieved with peanut flour (up to 56% degradation for aflatoxin B_1 and 61% for aflatoxin G_1) as compared to intact kernels in which up to 76 and 81% degradations of the two respective aflatoxins were achieved under comparable conditions.[40] Similarly, total aflatoxins and aflatoxin B_1 were reduced by 24 and 23%, respectively, by treating pistachio kernels with 9 mg/kg ozone concentration for 7 h, whereas with ground kernels only 5% reduction for both aflatoxin B_1 and total aflatoxins could be achieved.[41] This might be due to poor penetration of the gas into ground pistachios as studies suggest mycotoxins localize more on kernel surfaces.[42]

The reaction pathway of ozone with mycotoxins depends on the chemical structure of the given mycotoxin. The mechanism of reaction between ozone and the alkene double bond to form ozonides has been proposed by Criegee,[43] whereby the triatomic ozone molecule undergoes 1,3-dipolar cycloaddition with a double bond forming 1,2,4-trioxolane (ozonides) from alkenes with aldehyde or ketone oxides formed as intermediates. Ozonides undergo oxidative disintegration into carbonyl compounds, carboxylic acids, or ketones, and six aflatoxin B_1 ozonation degradation products have been identified.[44,45] The existence of double bonds between C8 and C9 of aflatoxins B_1 (**1**) and G_1 (**4**) explains their greater susceptibility to ozonation as compared to aflatoxins B_2 (**2**) and G_2 (**5**), where this double bond is absent (Figure 2).[27] The primary mechanism for degradation by ozonation is oxidation of the C9−C10 double bond in trichothecenes[30] and oxidation the aromatic ring of zearalenone.[46] It is expected that similar ozone-induced oxidation of alkene/aromatic structures occurs in cyclopiazonic acid, ochratoxin A, patulin, and secalonic acid D.

Under certain circumstances, ozonation results in undesirable nutritional and physical changes of the treated commodity.[31,47−49] Ozone may also react with some mycotoxins (such as fumonisin) to form compounds toxic to humans and/or animals.[27] Studies have indicated that in some crops, ozonation causes changes in organoleptic properties of the food, affecting mainly the color, taste, smell, rancidity, texture, and overall palatability. Inan et al.[50] reported slight changes in all Hunter color parameters (*L*, *a*, and *b*) in ozone-treated as compared to untreated red pepper samples, although the changes did not significantly affect the color and the pepper samples were still acceptable. In a study by Akbas and Ozdemir,[41] ozonation of whole kernels affected only the rancidity of pistachio nuts, but

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when ground nuts were used, additional effects were observed in sweetness, flavor, appearance, and palatability. Wang et al.[51] reported a reduction in whole protein levels in maize kernels treated with ozone gas (10−12%) for 96 h, and extensibility of the aleurone layer of wheat was reduced by ozonation.[52] Extending the ozone exposure time from 9 to 45 min and elevating the ozone levels from 200 to 1000 mg/kg on flour were reported to deteriorate the quality of bread.[53]

Other studies have reported a lack of serious side effects in ozone-treated products and, in some cases, positive outcomes in addition to mycotoxin degradation. For instance, baking properties of wheat flour as well as biochemical composition are not affected by ozonation and subsequent milling.[48,54] Furthermore, treatment with 50 mg/kg gaseous ozone for 30 days had no effect on the popping volume of popcorn.[48] This study also found no effects on the fatty acid and amino acid composition of soybean, wheat, and maize or the milling characteristics of wheat and maize as well as the baking characteristics of wheat and the stickiness of rice. Elsewhere, ozone treatment of moistened wheat eased dehulling; the required total energy for milling after ozonation of food grains was about 80−90% of that required for untreated grains.[52]

Ammoniation. Treatment of contaminated food products and animal feeds with ammonia to reduce the toxicity of mycotoxins has been an area of intense research by scientists worldwide. The efficiency and safety of ammoniation have received remarkable support as a practical method for mycotoxin detoxification in foodstuffs and animal feeds. Ammoniation involves treating a food or feed material with either gaseous ammonia or aqueous ammonium hydroxide. Early studies investigating ammonia detoxification of aflatoxin-contaminated animal feeds showed considerable success,[55,56] and it was reported in several studies that treating feeds, especially peanut and corn meal with ammonia, reduces aflatoxicosis in animals.[57−60] Ammoniation was thereafter shown to degrade ochratoxin A, citrinin, and penicillic acid and, to a lesser extent, zearalenone in corn, wheat, and barley.[61] In later studies, the absorption, distribution, and excretion of ammoniation products have been reported.[62]

The use of ammoniation in selected agricultural commodities in various locales has been approved.[56] Ammoniation reduced aflatoxin B_1 levels from 121 μg/kg to nondetectable levels in peanut meal, whereas in cottonseed meal the level was reduced from 350 to 4 μg/kg.[63] Generally, it is widely agreed that the efficiency of ammoniation in detoxification of aflatoxins is positively correlated with the amount of ammonia applied, the duration of the reaction, and the temperature and pressure levels.[60,64] Studies on the reaction of aflatoxin B_1 (**1**) with ammonia gas have identified decomposition products and demonstrated that the first step in ammoniation is the opening of the lactone ring to form the hydroxy amide (Figure 3).[56] This reaction is reversible, and if carried out under mild temperature and pressure, the product will revert to aflatoxin B_1. The relative proportion of reaction products depends on the temperature and pressure and whether ammonium hydroxide or ammonia gas is employed. The tricyclic product, which lacks the cyclopentenone ring, is formed in higher quantity under high temperature and pressure conditions and is not necessarily a decomposition product of the other major product, aflatoxin D_1 (**14**).

Despite the reported potential of ammoniation for detoxification of mycotoxins in feeds, the method has shown to be less than perfect and hence needs to be used in

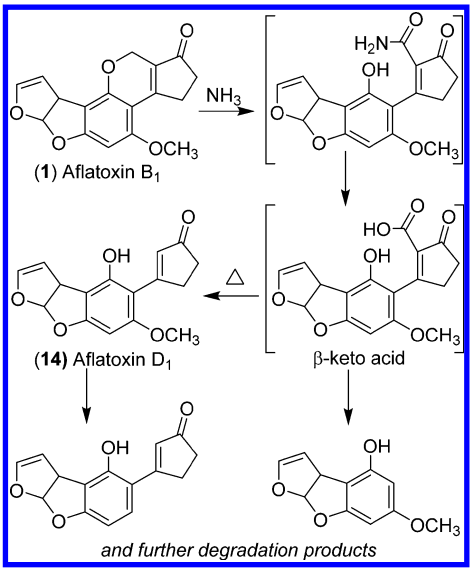

Figure 3. Chemical reaction pathway for ammoniation of aflatoxin B_1.

combination with other techniques. For instance, ammoniation of dairy cow feeds results in considerable reduction of aflatoxin B_1, but that did not deliver the same reduction of aflatoxin M_1 levels in milk.[65] The ammoniation process results in products that, under acidic conditions (as in the gastric fluids), revert back to aflatoxin B_1 through recyclization of the aflatoxin lactone.[58,66] The outcome is that an animal may, after gastric digestion, absorb a greater quantity of toxin than that analyzed in the feed, hence, more aflatoxin M_1 in milk. However, the major aflatoxin−ammonia products are not easily extractable with highly reduced bioavailability,[62,67] and ammoniation remains a useful method to reduce aflatoxin exposure through animal feeds.

The effectiveness of ammoniation for one mycotoxin does not necessarily imply efficacy against other mycotoxins. For example, an ammoniation process that lowered levels of aflatoxins in corn had no significant decontamination effect on zearalenone.[68] Similarly, another study on ammoniation reported a significant reduction in the level and toxicity of aflatoxins, but similar treatment of fumonisin-contaminated material decreased the level of the toxins but not the toxicity of the material.[55] Ammonia treatment has been reported to substantially reduce fumonisin B_1 contamination,[55,56] but did not reduce toxicity in rats fed the ammoniated product.[55] It is suggested that ammoniation of fumonisin produces amino-polyols, which still exert hepatotoxic and nephrotoxic effects.[69]

In addition to degradation of mycotoxins, the ammoniation process has been applied to increase proteaceous nitrogen while reducing levels of nonreducing sugars, lysine, and sulfur-containing amino acids in corn and cottonseed meal.[56,70,71] However, studies indicated reduced nutritive values of corn[71] and lowered acceptance by pigs of aflatoxin-contaminated maize meal treated with ammonia when the ammonia concentration was increased. This side effect was avoided when the concentration of ammonia was reduced to ≤100 mg/kg in the maize dry matter, but less detoxification was achieved.[72]

Nixtamalization. The addition of alkaline media during food processing is a common practice in many developing countries, especially for flavor enhancement and tenderization. In Mexico, an ancient and traditional process used to make

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corncake (tortillas) using corn exposed to a lime and heat treatment is referred to as "nixtamalización". It is from this the word nixtamalization has been derived, which basically means the process of preparing maize or other grain by soaking in water, cooking in alkaline solution, and removing the pericarp.

It has been demonstrated that during cooking in an alkaline solution, up to 90% reduction of aflatoxins may be achieved.[73−75] The effectiveness of the process is, however, dependent on cooking parameters with high pH required to effect modification and detoxification of aflatoxins.[74] The nixtamalization process in making tortillas is also indicated to reduce total fumonisins by 50%, with hydrolyzed fumonisins containing the aminopolyol backbone being the major product.[76,77] It has been reported that boiling maize in limewater reduced zearalenone by 59−100%, DON by 72−82%, and 15-acetyl-DON by 100%.[78] The recent introduction of the process in East Africa and prospects to be adapted highlight the potential of nixtamalization to play a role in mitigating mycotoxin exposure in wider societies especially in developing countries.[79]

The mechanism of degradation of mycotoxins by nixtamalization has not been reported in depth but has been suggested to occur through alkaline hydrolysis.[80,81] With aflatoxins, alkaline hydrolysis involves base-induced lactone ring-opening, yielding a water-soluble salt followed by decarboxylation.[82] Just like in ammoniation, re-formation of the aflatoxin may occur in acidic conditions,[83] presenting a challenge in maintaining the reliability of the nixtamalization process for alleviating mycotoxin exposure from contaminated foods. Mendez-Alborez et al.[82] reported that despite the initial 93.2% reduction of aflatoxin B_1 by nixtamalization, 34% of the original aflatoxins were re-formed in tortillas by acidification. However, modification of the process by cooking maize in a microwave oven greatly improves the outcomes with as low as 3% of the initial aflatoxins being re-formed by subsequent acidification.[84] Additionally, despite the reported degradation of fumonisins by degradation, the resulting aminopolyol products were reported to be as toxic as the parent compound,[69] a result analogous to the ammoniation process.

Food Additives. A range of food additives and preservatives from sulfites to organic acids, chlorine-containing disinfectants, and essential oils have also been demonstrated to have coincident activity in mycotoxin degradation. Sodium bisulfite ($NaHSO_3$) is a preservative commonly used in foods and beverages to hinder the growth and multiplication of microorganisms and to prevent browning by acting as an antioxidant and reducing agent.[85] Bisulfite can also effect chemical changes by the addition to double bonds including those present in mycotoxins, with reported efficacy in the detoxification of aflatoxins[80] and DON.[32] The action of sodium bisulfite on aflatoxin B_1 has been shown to yield a single major product, aflatoxin B_1S, through the addition to the double bond of the terminal furan ring.[86,87] Aflatoxin B_1S product is more hydrophilic than aflatoxin and is quite stable in highly acidic conditions.[86] The less toxic aflatoxin B_2 lacks this double bond and is not susceptible to bisulfite addition. The effectiveness of bisulfite treatment in the reduction of aflatoxin levels has been demonstrated in figs[88] and peanut meal[89] among other products. Maize treatment with aqueous bisulfite produced aflatoxin B_1S in the aqueous phase, and the authors concluded that similar aflatoxin B_1S formation would occur in the production of commercial cornstarch.[87]

DON and other trichothecenes contain an α,β-enone, which is also subject to bisulfite addition at the β-position of the 9,10 double bond forming 10-sulfonates such as DON-S and AcDON-S.[90] The effectiveness of bisulfite treatment in the reduction of DON levels has been demonstrated in corn[38] and wheat[91] with the extent of reduction dependent on concentration and contact time. However, when flour from milled bisulfite-treated wheat was baked into some products, DON levels increased to 50−75% of that present in the untreated wheat due to alkaline hydrolysis of the DON-S intermediate.[91] This implies that alkaline treatment methods such as nixtamalization should be avoided in DON-contaminated sulfite-treated products. Despite this drawback, bisulfite is still regarded as a promising reagent for reducing DON contamination. Addition of bisulfite to bread dough before baking resulted in 40% reduction in DON compared to control samples.[92] The use of sodium bisulfite has been demonstrated to affect DON degradation in noodles without reducing product quality.[93] Bisulfite treatment of animal feeds is also considered particularly promising. The performance of pigs fed bisulfite-treated DON-contaminated corn was significantly improved compared to those fed untreated contaminated corn and was similar to that of pigs fed noncontaminated corn.[94] Fumonisins do not contain double bonds that would be susceptible to bisulfite addition and are seemingly unaffected by this treatment. Soaking maize naturally contaminated with fumonisins B_1 and B_2 in a 0.3% solution of sodium bisulfite for up to 48 h did not appreciably alter the total concentration of fumonisin, although the presence of sulfite appeared to delay the extraction of the toxins into the liquid phase.[95]

The related food preservatives sodium hydrosulfite ($Na_2S_2O_4$) and sodium metabisulfite ($Na_2S_2O_5$) demonstrated mycotoxin-reducing activity similar to that of bisulfite. Aflatoxins and ochratoxin in black pepper were reduced 77−100% by treatment with 2% $Na_2S_2O_4$ under high pressure,[96] and a 95% reduction of aflatoxin in butter bean being achieved by boiling with 0.5% $Na_2S_2O_4$.[97] Treatment of DON-contaminated wheat with 1% sodium metabisulfite for 15 min at 100 °C reduced DON contamination by >95%,[98] and the transformation product was considered to be the same as DON-S. In pig trials with DON-contaminated wheat, feed intake and weight gain of piglets fed $Na_2S_2O_5$-treated contaminated wheat was significantly improved compared to those fed untreated contaminated wheat and was similar to that with nontoxic diet. DON was not detected in serum, and the authors deduced DON-S was stable in the acid condition in the stomach and in neutral or weak alkaline conditions in the small intestine.[98] Sodium metabisulfite treatment was concluded to be effective in reducing DON-induced depression of feed intake in pigs.

In addition to its direct effect on mycotoxins, bisulfite is also known to inhibit growth of some toxigenic fungi. In an in vitro study, sodium bisulfite used as a preservative at 0.1% limited germination of *Aspergillus sulphureus* and *Penicillium viridicatum* by 46 and 90%, respectively, and ochratoxin A production by each fungus was reduced by 97 and 99%, respectively.[99] The salt was also tested along with other salts including ammonium carbonate and sodium chloride, with concentrations of 2% inhibiting growth of *Aspergillus parasiticus* and subsequent production of aflatoxins by the fungus.[100] Another study found that treating *Aspergillus flavus*-inoculated groundnut cake with 1% sodium bisulfite at 10% moisture content achieved

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complete inhibition of both mold growth and aflatoxin production at room temperature.[101]

Organic acids have also been tested as mycotoxin-degrading agents. Lactic acid and citric acid have different degrees of action against some mycotoxins depending on experimental conditions.[102−104] Treating maize containing 29 mg/kg aflatoxin with aqueous citric acid resulted in complete degradation, and when the initial contamination was 93 mg/kg, 97% degradation was observed.[105] Ghosh et al.[101] showed that treatment of *Aspergillus parasiticus*-inoculated groundnut cake with 0.5% propionic acid at 10% moisture completely inhibited mold growth and aflatoxin biosynthesis at room temperature. Pons et al.[106] studied the mechanism of acid conversion of aflatoxins B_1 and G_1 to aflatoxins B_{2a} and G_{2a}, respectively, and found that strong acids convert the aflatoxins through hydration to their hemiacetal forms with the rate of reaction increasing with increase in temperature or decrease in pH. Hydrochloric acid (5 M) was shown to destroy aflatoxin B_1 completely by hydrolysis in peanut flour sealed in culture tubes at 100 °C.[107] Despite the effective degradation of aflatoxin B_1 shown by strong acids, the produced aflatoxin B_{2a} is still toxic, although not as potent as aflatoxin B_1.[108]

"Active chlorines" such as hypochlorite, chlorine, and chlorine dioxide as sanitizing agents in food processing have also demonstrated some potential in mycotoxin reduction. Sodium hypochlorite (NaOCl) degraded verrucarol (**15**) (a trichothecene similar to T-2 toxin (**12**)) into two products at room temperature and, similarly, DON into a single product (Figure 4).[109,110] It has been likewise shown that treatment

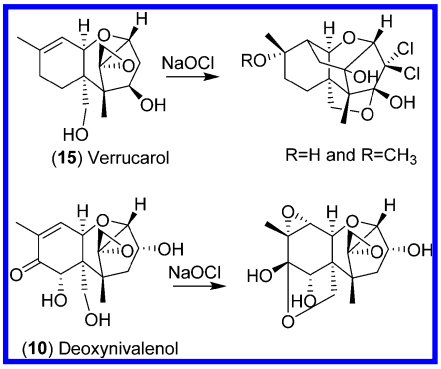

Figure 4. Chemical reaction pathways for degradation of verrucarol and DON by NaOCl.

with alkaline hypochlorite (0.25% NaOCl plus 0.025 M NaOH) for 4 h achieved a high degree of inactivation of T-2 toxin in maize.[111] Chlorine at concentrations >1% (v/v nitrogen) has been shown to significantly reduce DON levels in contaminated maize.[38] Chlorine dioxide (ClO_2) in solution form was shown to be effective in detoxification of trichothecenes verrucarin A and roridin A, the reaction being dependent on the concentration of the gas and exposure time.[112]

Essential oils have generated increasing interest as agents for food preservation due to their antimicrobial properties, although there are concerns about their incorporation as food additives.[113] The ability of essential oils from plants to also suppress mycotoxins has been tested showing varying effectiveness. The oils have been shown to achieve a combination of effects by inhibiting fungal growth, reducing production of mycotoxins, and/or degrading the toxins.[114] Studies indicated activity of essential oils against fungal growth and mycotoxin production in *A. flavus*,[115,116] *A. parasiticus*,[115−117] *Penicillium* sp.,[118] and *Fusarium graminearum*.[119] Direct effects of essential oils on mycotoxins have been reported on the degradation of fumonisin B_1[120] and the inhibition of cytotoxic effects of zearalenone.[121]

The effectiveness of different food additives in degrading mycotoxins greatly depends on conditions of treatment, especially temperature and concentration of the additive.[97,122] In some cases the required concentration of additive is high and can cause negative organoleptic effects. This can be particularly true for essential oils,[113] and further investigations are required to assess synergistic effects of these agents with other antimicrobial compounds.

PHYSICAL METHODS FOR MYCOTOXIN DETOXIFICATION

Irradiation. Irradiation is considered a physical process in detoxification of mycotoxins even though the process gives energy to the reacting compounds and can also facilitate chemical changes. Irradiation can be effective either by modifying the chemical structure of mycotoxins or by inhibiting growth and subsequent production of mycotoxins by fungi. The use of advanced photochemical degradation methods in agriculture for degrading and transforming the photosensitive materials is an emerging area showing commercial promise.[123,124]

Photodegradation of mycotoxins is generally found to be effective with light in the UV region. From the first isolation of aflatoxin, irradiation has been shown to be an effective method to destroy the toxin through its photosensitive property.[125] Aflatoxin B_1 absorbs UV light at 222, 265, and 362 nm. Maximum absorption occurs at 362 nm, with irradiation of the toxin resulting in the formation of up to 12 degradation products.[126] Liu et al.[127] demonstrated that exposing aflatoxin B_1 to UV light greatly reduced the mutagenicity of the toxin when tested with HepG2 cells and by the Ames test. Irradiation of contaminated tree nuts resulted in detoxification of >95% of aflatoxins B_1, B_2, and G_1 in almond and pistachio, whereas aflatoxin G_2 was degraded completely.[128] A low dose of 0.5 kGy of γ-rays was adequate to achieve 99.7% degradation of fumonisin B_1 in aqueous solution,[129] but for complete degradation of the toxin in wheat and maize a dose as high as 7 kGy was required.[130] Photodegradation of trichothecenes in grains has been assessed, and the amount of energy required to degrade DON, 3-acetydeoxynivalenol, and T-2 toxin in dry grain was found to be higher than in moist grain.[32] Unlike aflatoxin, most trichothecenes are not photosensitized by irradiation directly but, rather, radiolysis of water produces radicals that react with the toxins.[131,132] Complete degradation of citrinin and degradation of ochratoxins A and B into products that do not contain phenylalanine was possible by using irradiation with blue light at wavelengths of 470 and 455 nm.[133] Deberghes et al.[134] reported inactivation of ochratoxin A was possible by γ-irradiation from 2 to 5 kGy in liquid medium. This was further confirmed by Refai et al.,[135] who reported complete inhibition of both *A. ochraceus* growth and ochratoxin secretion in chicken feeds with 4 kGy of γ-irradiation. However, maximum degradation of the already produced toxin in dry animal feeds was 21.9% using 15 kGy of γ-rays.

Photodegradation of aflatoxin B_1 (**1**) follows first-order reaction kinetics ($R^2 \geq 0.99$). Three photodegradation products—(**16**) P_1 ($C_{17}H_{14}O_7$), (**17**) P_2 ($C_{16}H_{14}O_6$), and

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(**18**) P_3 ($C_{16}H_{12}O_7$)—were detected and degradation pathways proposed (Figure 5).[136] The structure of the three products are

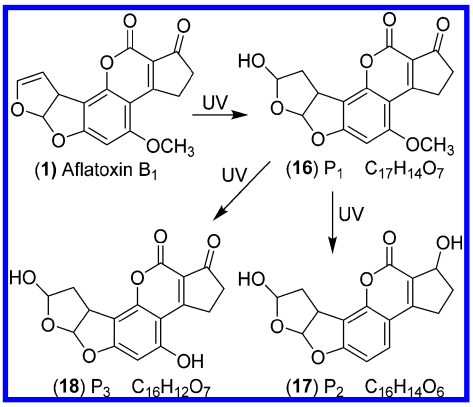

Figure 5. Chemical reaction pathway for UV degradation of aflatoxin B_1.

similar to aflatoxin B_1 (**1**), with initial hydoxylation of the C9−10 double bond in the formation of P1 being largely responsible for the loss of toxicity. In general, the effectiveness of the method on mycotoxin degradation depends on the irradiation dose, physical state of the medium treated, and combination with other methods such as heating.[137]

Thermal Inactivation. Heat treatment of foods and feeds is mostly carried out as processing or flavor-enhancing procedures, which in addition may have degradative effects to contaminating mycotoxins.[138] With heat treatments, the stability and rate of degradation of individual mycotoxins differ according to structure.[139] Thermal degradation of mycotoxins is also influenced by the moisture content of the food product, toxin concentration, food type matrix, and additives present.[140] In all types of cooking or heating processes, degradation of the mycotoxins in contaminated foods depends on the temperature and exposure time. Aflatoxins, for instance, are highly temperature resistant in their pure form and can be denatured only at temperatures ≥250 °C.[139] However, when heating is done in situ, significant degradations are attained at lower temperatures, with heat treatment by roasting reported to have superior degradation effects as compared to boiling or steaming.[141,142] For example, roasting of coffee beans at 200 °C resulted in a reduction of up to 100% of aflatoxins,[143] whereas roasting pistachios at 150 °C for 120 min reduced up to 95% of aflatoxin B_1.[144] The efficacy of thermal degradation of aflatoxins by heating also depends on the moisture content of the food product. A reduction of 85% aflatoxin was recorded following 100 °C heat treatment of a cottonseed meal with 30% moisture content. Only 50% aflatoxin degradation was attained with the same temperature−time combination when the moisture content of the meal was 6.6%.[145] It is logical to conclude that aflatoxin degradation by heating is enhanced at high moisture content. Hale and Wilson[146] reduced aflatoxin levels from 383 to 60 μg/kg by heating corn grain (typically ∼15% moisture) at 160−180 °C for 60 min.

Thermal decomposition of citrinin also depends on moisture content, with heating in water to 175 °C resulting in partial decomposition whereas heating in dry atmosphere to 160 °C resulted into complete decomposition.[147] Kirby et al.[148] reported boiling contaminated maize for 8 min destroyed some citrinin activity but heating at 105 °C for 16 min completely destroyed the diuretic effect of the toxin. Although ochratoxin A is highly stable even at 200 °C,[149,150] heating the compound in the presence of aqueous NaOH (0.1 M) led to decomposition of the toxin and subsequent detoxification.[150] The effect of heat on ochratoxin A contamination in coffee was tested through a collaborative study with nine research groups that indicated a reduction of between 69 and 96% ochratoxin during coffee roasting under a range of conditions.[151] Additionally, other studies have reported that roasting time and temperature correlate with a reduction of ochratoxin A.[152,153] It should, however, be noted that even under wet conditions and high temperatures, complete detoxification of ochratoxin A is not achieved, even at 250 °C.[154] It is also suggested that degradation products of ochratoxin A may be equally toxic,[153] and some beneficial compounds to human health may be destroyed.[155]

Most of the *Fusarium* toxins are known to be thermally resistant. Temperatures as high as 150 °C for 10 min were required for 50% denaturation of fumonisin B_1 in dry maize grains.[156] A study to determine the thermal stability of fumonisins in processed maize products found a decrease of 11−15% of fumonisins in canned whole-kernel maize when heated.[157] Extrusion of fumonisin-contaminated corn grits with 10% added glucose resulted in a 75−85% reduction in fumonisin B_1 levels.[140] Jackson et al.[158] reported that, similar to fumonisin B_1, fumonisin B_2 was also thermally stable, especially in moist conditions. Minimal effects on fumonisin content are observed under heat treatments such as retorting or boiling at temperatures around 125 °C.[158] DON, zearalenone, and nivalenol are reported to have similar thermal stability, and baking at 170 °C was found to have an insignificant effect on the toxins.[159,160] Despite the stability of DON on baking, a study has indicated that a modified product, deoxynivalenol-3-glucoside, increases during bread baking using DON-contaminated flour,[161] which might as a result increase the assimilated DON burden when ingested.[162]

The use of microwave heating has also been investigated for control of mycotoxins. Aflatoxins are destroyed when exposed to microwaves at rates proportional to microwave power and exposure time.[163] Luter et al.[164] reported a 95% reduction of aflatoxin levels in peanuts after they had been microwaved at 3.2 kW for 5 min or at 1.6 kW for 16 min.

Despite the good outcomes that can be obtained, thermal decontamination of food and feeds sometimes results in undesired effects on food quality. For example, in the study that achieved a reduction of aflatoxin from 383 to 60 μg/kg by heating contaminated corn grains at 106−180 °C,[146] two important amino acids, methionine and lysine, were also significantly reduced. Additionally, the process resulted in reduced digestion and absorption of crude fiber and nitrogen in the treated food. It is therefore essential that heating to destroy mycotoxins should take into consideration potential side effects on food quality and nutrition.

Adsorbents. Adsorbents such as clay minerals are primarily used in animal feeds to act as anticaking and pelletizing agents, to regulate digestion, to control gastrointestinal disorders, and to provide trace elements to the animals.[165] The use of binding agents has also been shown to limit mycotoxin absorption in the gastrointestinal tract and uptake into the body, hence reducing observed adverse effects on the animals consuming contaminated feeds, especially in poultry and swine.[5] Incorporation of specific clay minerals in contaminated animal feeds to reduce the bioavailability of fumonisins and aflatoxins has been tested and proven effective.[166,167] Whereas different

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types, forms, and preparations of clay minerals are commercially available only for use with animal feeds, studies on human and laboratory animals have also indicated significant reduction in aflatoxins and fumonisins. For example, NovaSil is an encapsulated clay preparation that is applied to enhance reduction of aflatoxins in humans exposed to naturally contaminated foods.[168−170]

Activated charcoal, silicates, humic substances, and plant and fungal extracts have shown potential in reducing mycotoxin toxicity as binders in foods and feeds.[171] In vitro studies to test the concept of mycotoxin adsorption have shown that activated charcoal and certain clay minerals can efficiently bind aflatoxins.[172,173] Although activated charcoal was shown to be effective in absorbing ochratoxin A in growing chicken feeds, the alteration of the feed color and reduced intake by the chicks made it difficult to adopt the technique.[174] Attempts to adsorb DON and zearalenone using metallic and mineral-adsorbing materials such as phyllosilicate minerals, activated charcoal, or synthetic resins have been often less encouraging; however, significant levels of zearalenone were adsorbed using smectite clay, humic substances, and yeast cell wall-derived materials.[175]

The use of some natural additives to facilitate adsorption of mycotoxins and their associated complexes has been shown to be effective in controlling exposure to the toxins. Addition of chlorophyllin to the diet was shown experimentally to be a potent inhibitor of aflatoxin B_1 hepatocarcinogenesis in rainbow trout and rats.[176,177] In these studies, it was hypothesized the green plant pigment binds to aflatoxin and inhibits absorption and distribution to different body organs. When trialed with volunteer humans using a low dose of 30 ng of aflatoxin B_1 (equivalent to 1.5 g of peanut butter with the FDA allowable limit of 20 μg/kg), the pharmacokinetics of the toxins was shown to be influenced by chlorophyll *a* (Chl *a*) and chlorophyllin.[178] This result suggested co-consumption of the two green plant pigment extracts may limit the bioavailability of ingested aflatoxin in humans, as they do in animal models.

Other Physical Methods. Studies have demonstrated that sorting out physically damaged, distorted, and discolored grains can substantially reduce mycotoxin levels in the remaining maize products.[179,180] Such sorting is often done manually, although the use of automated equipment has been successfully employed, especially with peanuts.[181−183] Pearson et al.[184] achieved reductions of about 81% of aflatoxins (initially 53 μg/kg) and 85% of fumonisins (initially 17 mg/kg) in maize using a high-speed dual-wavelength sorter. Effective sorting of contaminated food products is advantageous in that it can reduce mycotoxin concentrations to safe levels without producing toxin degradation products or negatively affecting the nutritional value of the food. The reliability of sorting and physical separation to minimize mycotoxins is, however, challenged by the fact that in most cases, contaminated products are not visually distinguishable from uncontaminated ones. In single-kernel analysis, it was observed that the distribution of aflatoxins in the kernel lots was extremely heterogeneous, in the range of 100−80,000 μg/kg, and highly contaminated kernels on a cob were frequently adjacent to aflatoxin-free ones and could not be detected visually.[185] The presence of visible fungal hyphae, physical damage, insect infestations, and discoloration does not always correlate with the levels of mycotoxins in food.[186,187] Traditional maize sorting practiced by consumers in Kenya, which removes debris and damaged grains, was shown to significantly reduce fumonisin B_1 contamination but failed to sort out aflatoxin B_1-contaminated material.[188]

Mycotoxin reduction can also be achieved during food processing and preparation, particularly in processes that include soaking and washing as has been observed in aflatoxin-reduction in maize.[189,190] The effectiveness of washing and soaking is maximized when done in combination with removal of debris, damaged grains, and surface parts, which often are more infested with fungi and mycotoxins.[180,191] A significant reduction of DON is achieved by cleaning and milling wheat with the resulting flour free of up to 80% of the original concentration of the toxin in the grain.[192,193] Trenholm et al.[194] reported that washing barley and corn contaminated with DON and zearalenone with sodium carbonate solution considerably reduced the contamination. In this study, the efficiency of decontamination was higher if the grains were stirred with the salts solution, with 100% decontamination achieved after 4 h of stirring.

In some foods removal of mycotoxins can also be achieved by solvent extraction. Steeping corn in water significantly reduced fumonisin concentration levels,[95] although this outcome could not be achieved in rice.[195] Because aflatoxins are not soluble in water, the toxins are extracted using organic solvents or organic/water mixes. Several studies have reported significant reductions of aflatoxins from different food samples using organic/water extraction.[196−198] There are obvious disadvantages and limitations associated with solvent extraction, ranging from the removal of essential nutritional components to introduction of undesirable residues, as well as additional costs. In most cases, solvent extraction requires highly specialized equipment and incorporation of safe procedures to handle the mycotoxin extracts.

SELECTING THE MOST APPROPRIATE METHOD

As can be seen from this discussion, nonbiological processing methods can achieve remarkable reductions in mycotoxin contamination of food and feeds. The relative outcomes depend on the given food or feed commodity, type of mycotoxin, and method applied. Degradation products of mycotoxins in some chemical and physical treatments might still be potentially toxic, and this issue is important for further studies. Although applying a method primarily for mycotoxin control is an option, the majority of these processing methods are integral food preparation processes intended for grading, sanitation, cooking, nutritional enhancement, flavoring, packaging, customer attraction, and preservation, so mycotoxin reduction presents an added bonus. In developed countries people and livestock can benefit from reduced mycotoxin exposure risks achieved by some industrial processing and packaging of foods. However, a large proportion of agricultural crops in developing countries is stored and consumed directly from the farm; hence, household level applications aimed at detoxification of mycotoxin-contaminated products will be more useful in these regions.

The highlighted simple applications such as nixtamalization and other flavor-enhancing methods, thermal and microwave heating, sorting, washing, and dehulling of grains are, for example, applicable to different degrees and capacities at the household level. Some of these processes in more or less similar extent and scope play an integral part in traditional food preparations in developing countries, although not primarily for addressing mycotoxin decontamination. It can also be assumed that in the same communities different practices employed

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basically for cleaning, cooking, and flavor enhancement may also have potential in mycotoxin detoxification. The co-occurrence of mycotoxins in foods is not uncommon, and hence methods that have effects on multiple toxins such as sorting and combining different methods when possible should be advocated. Although industrial food processing is slowly but encouragingly growing in developing countries, efforts to reduce mycotoxin exposure should also include household level food processing. Further studies should be done to investigate common food-processing and preparation practices in these countries and what can be improved, adopted, and promoted.

Tables 1 and 2 summarize the potential applications, challenges, and possible improvements for the reviewed nonbiological procedures and the mycotoxins against which they have demonstrated the greatest efficacy.

In conclusion, although further investigations are needed, this review has highlighted potential nonbiological mycotoxin degradation strategies that may be combined with other degradation and fungal decontamination techniques to achieve significant postharvest mycotoxin control. Traditional methods such as nixtamalization, sorting, and cleaning have demonstrated utility at the household level. Large-scale techniques such as ammoniation are acceptable in some countries, especially for treatment of animal feeds. Studies are continuing to find safer and more economical ways to increase both efficacy and acceptance of the more controversial treatments such as ozonation and irradiation in mycotoxin control. The employment of natural adsorbent additives to reduce mycotoxin bioavailability are of particular interest in some sectors and warrants continued investigation due to their ready applicability at both the small-holder and household levels. More studies on the utility of home-based food processing in reducing mycotoxin contaminations should be done, especially in developing countries.

Table 1. Chemical Degradation Methods and Their Application in the Control of Mycotoxins in Foods and Animal Feeds

method	application, challenges, and possible improvements
ozonation	• commonly used in food industry for sanitization • effective in degrading aflatoxins and trichothecenes • mainly applicable in animal feeds (grains) • limited use in human foods due to effect on organoleptic properties and uncertain health effects of degradation products • best applicable in large-scale industrial animal feed processing • can be combined with ammoniation and thermal treatment to improve efficacy
ammoniation	• effective against aflatoxins, ochratoxin A, citrinin, penicillic acid • mainly applied in animal feeds (corn, wheat, barley) for controlling mycotoxins and nutritional enhancement • possible unpleasant chemical residue • best applicable in large-scale industrial cattle feed processing • application at farm level limited by technical requirements • can be combined with ozonation and adsorptive clay additives to improve results
nixtamalization	• effective in reducing aflatoxins, fumonisins, zearalenone, and deoxynivalenol • applicable in reducing aflatoxins and fumonisins in maize meals at household level with traditional cooking methods • prior cleaning of the maize by sorting, steeping, and washing may increase efficacy
chemical additives	• primarily used as preservatives and artificial flavors • sulfites and organic acids are effective against DON in wheat and, to lesser extent, aflatoxins; can be the best method of reducing DON in wheat at both household and industrial levels • minor modifications in routine procedure of applying additives to attain maximum mycotoxin reduction

Table 2. Physical Degradation Methods and Their Application in the Control of Mycotoxins in Foods and Animal Feeds

method	application, challenges and possible improvements
irradiation	• common in sanitization of food products and packaging and readily adapted for mycotoxins control • applicable at industrial level to degrade aflatoxins and trichothecenes in packaged foods, juices, and grains • challenged by consumer perception on food irradiation
thermal treatment	• best results are demonstrated in products processed by roasting • successful in controlling ochratoxin A in roasted coffee and aflatoxins in roasted nuts • microwave heating has shown promise in degrading aflatoxins • warrants investigation as household-level treatment due to widespread adoption of microwave cooking
adsorbents	• proven to bind and reduce bioavailability of aflatoxins, fumonisins, and zearalenone • **clay minerals** enhance nutrition and digestibility of animal feeds; hence, easy adoption for mycotoxin control applicable at both home and industrial feed compoundings • **clay capsules for humans** are potential aflatoxin control remedy but face acceptability challenge for routine use • **chlorophyll** provides promising area for further investigation
physical separation	• washing and removing spoiled food materials efficiently reduce majority of mycotoxins, but less effective with aflatoxins in maize • easiest and best method applicable at household level, but does not provide 100% reduction • an important preparatory method before applying other processing methods

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