Chapter

Promising Detoxification Approaches to Mitigate Aflatoxins in Foods and Feeds

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Abstract

Aflatoxins are a group of naturally occurring carcinogenic mycotoxins produced by certain *Aspergillus* species in nuts, grains, oilseeds and vegetables. Ingestion of aflatoxin contaminated food and feed has extremely negative health implications in humans and livestocks. Additionally, exporting countries face the trade barrier due to strict regulations in international market to maintain food quality. This led to huge economic losses to global exporters. Therefore, there is an urgent need for development of effective methods for detoxification of aflatoxins from food and feed to ensure food security. Till date, numerous methods for detoxification of aflatoxins from foods have been employed. Physical, chemical and biological treatment are the novel promising approaches for partial/complete detoxification of aflatoxins from the foodstuffs. In this chapter, we will address the efficacy and shortcomings of each methods in with respect to economic importance, human health and food security.

Keywords: Aflatoxin, mycotoxin, carcinogenic, detoxification, food security

1. Introduction

Aflatoxins are toxic secondary metabolites, mainly produced by many species of *Aspergillus*, namely *Aspergillus parasiticus, A. flavus, A. nomius,* A. astellatus [1, 2]. They contaminate various crops (wheat, maize, cotton), dried fruits, spices, meat and milk products [3, 4]. Aflatoxins were first discovered as a causative agent of Turkey X disease in early 1960s, led to the death of numerous turkey in England [5]. Generally, *A. flavus* specifically produces B-type aflatoxins while *A. parasiticus* produce B- as well as G-type aflatoxins [6]. Among these four aflatoxins, AFB1 being most dangerous, accounts for more than 75% of all food and feed related aflatoxin contamination [7]. Cytochrome P450 mediated metabolism of AFB1 in liver, resulting in its epoxidation to AFB1-exo-8, 9-epoxide and AFB1-endo-8, 9-epoxide, demethylation to aflatoxin P1 (AFP1) and hydroxylation to aflatoxin Q1 (AFQ1) and aflatoxin M1 (AFM1) [8]. The International Agency for Research on Cancer (IARC) has classified aflatoxin type B and G as Group 1 carcinogen and AF-M1 as Group 2B [9]. Derived from polyketides, consumption of aflatoxin contaminated food and feed cause a range of serious health complications in humans and animals, together named as aflatoxicosis [10, 11]. Short term exposure to high dose of aflatoxins results in jaundice, hemorrhage, liver damage and subsequent death

and long term exposure to sublethal levels of aflatoxins cause nutritional disorders, immunosuppression, cancer [12].

The challenges related to aflatoxins can be overcome by adopting innovative and novel strategies. The risk of aflatoxins can be reduced by preventing the contamination of foods from aflatoxins at the pre-harvest stage and removing/eliminating the aflatoxins from aflatoxin contaminated food at the post-harvest stage. Prevention strategies, including the use of pesticides, fertilizers, maintaining optimum temperature and moisture for storage, right harvesting time, are only partly responsible for achieving aflatoxin-free food and feed. In order to eliminate aflatoxin completely, post-harvest strategies are followed. The post-harvest strategies include cleaning, sorting, milling and dehulling [13], treatment at temperature between 237 and 306°C [14], mineral binders such as, montmorillonite, zeolite, aluminosilicate, bentonite that bind aflatoxins. Such binders may partially or completely conteract the toxicity of dietary aflatoxins [15]. Elliott et al. [16] have reported the cytotoxic effects induced by mineral binders like DNA damage, reduced cell viability, apoptosis, oxidative stress.

2. Novel strategies for aflatoxin degradation

In the past decades, numerous promising novel strategies for aflatoxin mitigation have been developed. They are broadly categorized as physical, chemical and biological approaches. Physical strategies involve the utilization of radiations and cold plasma for the fast aflatoxin degradation [17]. Chemical methods include the treatment with electrolyzed oxidizing water, organic acids, ozone and natural plant extracts. These are methods have been widely used in several countries such as USA and China [18]. Microbial and enzymatic based conversion of highly toxic aflatoxins into less toxic or non-toxic metabolites are included in biological methods [19]. In this chapter, we will address each of these novel technologies for aflatoxin degradation in detail.

2.1 Physical treatment for aflatoxins degradation

2.1.1 Irradiation

In the recent times, ionizing irradiation (viz. electron beam, gamma and ultraviolet rays) and nonionizing irradiation (viz. infrared waves, radio waves, visible light waves and microwaves) has been employed extensively for the degradation of aflatoxin present in the food and feed (**Table 1**).

Electron beam irradiation (EBI) technology has great potential for aflatoxin degradation. EBI technology offers the advantage of high effectiveness, low equipment cost, dosage control, short processing time, low heat generation, few variables and in-line processing [36]. EBI technology has been applied for degradation of aflatoxins in coconut agar [37]. Efficiency of EBI technology for degradation of aflatoxin is lesser than that of γ radiation. Assuncao et al. [20] found that EBI at doses of 10 and 5 kGy decreased the content of AFB1 in Brazilnuts by 65.7 and 53.3%, respectively, whereas γ irradiation at same doses led to reduction in AFB1 by 84.2 and 70.6%, respectively. Liu et al. [21] used EBI dose of 300 kGy for reduction of AFB1 by 70%. As the selected dose was ten times of the maximum permissible dosage allowed by FDA, so this method is not highly efficient and preferable in AFB1 degradation in peanut meal.

Gamma (γ) rays has been the most preferred radiation source for the food owing to its high penetrability and reactivity. Treatment of food by gamma rays of

Physical Degradation method	Food product treated	Aflatoxin ($\mu g\ kg^{-1}$ or $\mu g\ L^{-1}$)	Degradation Percentage	Parameters for treatment	Reference
Electron beam	Brazil nut	AFB1 (4.8)	65.7	300 kGy	[20]
	Peanut	AFB1 (1000)	70.0	300 kGy	[21]
^{60} Co gamma Irradiation	Red chili	AFB1 (11–35)	86–98	6 kGy	[22]
	Cattle feed	AFB1 (50)	85	10 kGy	[23]
	Corn	AFB1 (57–1210)	85.6–98.6	10 kGy	[23]
	Brazil nut	AFB1 (4.8)	84.2	10 kGy	[20]
	Peanut	AFB1 (300)	43	9 kGy	[24]
	White pepper	AFB1 (60), AFB2 (18), AFG1 (60) and AFG2 (18)	50.6, 35.2, 47.7 and 42.9	30 kGy	[25]
	Almond	AFB1 (20), AFB2 (20), AFG1 (20) and AFG2 (20)	19.3, 11.0, 21.1and 16.6	15 kGy	[26]
UV irradiation	Peanut	AFB1 (2000)	100	220–400 nm at 0.8 mW cm^{-2} for 80 min	[21]
	Peanut	AFB1 (350)	99.1	254 nm for 10 h	[27]
	Peanut oil	AFB1 (128)	96	365 nm at 55–60 mW cm^{-2} for 20 min	[28]
	Peanut oil	AFB1 (52.0)	86.1	365 nm at 6.4 mW cm^{-2} for 10 min	[29]
	Peanut oil	AFB1 (2000)	100	220–400 nm at 0.8 mW cm^{-2} for 30 min	[30]
	Red chili powder	AFB1 (1872)	87.8	365 nm for 60 min	[31]
Pulsed light	Rice bran	AFB1 (36) and AFB2 (4.4)	90.3 and 86.7	0.52 J cm^{-1} per pulse for 15 s	
	Rough rice	AFB1 (132) and AFB2 (45)	75.0 and 39.2	0.52 J cm^{-1} per pulse for 80 s	[32]
Microwave heating	Peanut	AFB1 (5–183) and AFB2 (7–46.7)	50–60 and 100	Heating in microwave oven at 92°C for 5 min	[33]
	Corn flour	AFB1 (100)	67.7	Heating in microwave oven for 10 min	[34]
	Alkalized corn	AFB1 (22.5) and AFB2 (69.6)	36 and 58	1650 W	[35]

Table 1.
Physical methods for aflatoxin degradation in food and feed.

upto 10 kGy has no toxicological, or microbiological hazards [38]. Additionally, γ irradiation results in the interaction of high energy of γ rays with the water present in the food products. This produces highly reactive free radicals such as superoxide radical ($O2^{\bullet -}$), hydrogen (H•) radical and hydroxyl ion (OH^-) that in turn destroy

aflatoxins and also attack DNA of pathogenic microbes [14, 39]. Markov *et al.* [23] have used high-energy photons from cobalt-60 (gamma source) for destruction of pathogenic microbes by directly damaging DNA of microbial cells.

Many studies in the literature showed that treatment of food commodities with γ rays ranging from 5 to 10 kGy led to degradation of significant amount of aflatoxins. For instance, irradiation with γ rays at low dose of upto 6 kGy has reduced Aflatoxin B1 (AFB_1) level in red chillies and fruits for around 90% [22, 40]. The AFB_1 level reduced for about 95% in maize seed samples with γ irradiation dose of 10 kGy [23]. AFB1 in Brazil nuts can be eliminated upto 84.2% by irradiation of γ rays at 5 to 10 kGy [20]. Likewise, γ irradiation at dose of 10 kGy decreased ochratoxin A (OTA) in coffee beans and dry-cured meat for almost 100% and 22.5% respectively [41, 42]. Nevertheless, few studies in the literature concluded that γ irradiation could not effectively eliminate aflatoxins in food. For e.g. irradiation of black and white pepper with γ rays at 10 kGy dose has no significant effect on its aflatoxins content [25]. Gamma irradiation of poultry feed at 15 kGy dose resulted in 13.6, 11.0, 21.1, 18.2% decrease in AFG2, AFB2, AFG1 and AFB1, respectively [43]. However, the efficiency of γ rays - mediated aflatoxin degradation depends on numerous factors such as concentration of mycotoxin, dose of radiation, content of water, air humidity, composition of food and type and number of fungal strains [25, 39].

The advantage γ irradiation offers is high capacity for microbial inactivation that reduces the microbial load and increases shelf life of food. Gamma irradiation technology has been approved by more than 55 countries such as Japan, USA, European countries, China for food processing [44]. This technology is not preferable with high vitamin and lipid content because polyunsaturated fatty acids undergo extensive peroxidation in unsaturated bonds, leading to increased oxidative rancidity [45].

Apart from being an economical non-thermal technology for Alfatoxin decontamination, Ultraviolet (UV) irradiation is also highly cost effective and eco-friendly [46]. Treatment of food products with moderate doses of UV rays has no negative impact on its sensory and physicochemical properties [47]. Though UV rays can efficiently penetrate into transparent or clear liquids, its penetration efficiency through solids is limited. Therefore, granular or opaque foods has to be in the form of thin layer for decontamination by UV rays [48]. UV light effectively removed of Patulin (PAT) from apple cider and juice. Zhu et al. [49] have used different wavelengths of UVC for PAT reduction in apple juice. They found UVC of 222 nm wavelength was most effective. Exposure to UV affected the taste of apple cider and juice. Intensity and duration of UV irradiation are important factors that affect the elimination efficiency of aflatoxins elimination efficiency Irradiation with short wavelenght (254 nm) and long wavelength (362 nm) UV rays for 30 minutes resulted in complete elimination of AF-B1 and AF-G1 in wheat grains, whereas exposure to same dose of short and long wavelength of UV rays for 2 hours reduced AF-B2 in wheat grains by 50 and 74% respectively [50]. Exposure of pistachio, almond and groundnut with UVC at 265 nm for 15 minutes led to 100% removal of AF-G2 from all the nut samples and complete degradation of AF-G1 in pistachio and almond. UV-C irradiation at 265 nm for 45 minutes showed 97% degradation of AF-B1 [51]. Treatment with UV-A and UV-B rays can also be used for reduction of mycotoxins produced from *A. parasiticus* and *A. carbonarius* in pistachio and grape media [52].

Non-thermal Pulsed light (PL) technology has been used for degradation of aflatoxins in food and feed. This FDA-approved technology generates short, high-intensity flashes of broad spectrum light (100–1100 nm) including UV, visible and IR radiation that destroy the nucleic acid and cell wall structure of microbes within

few seconds [53]. Eight PL flashes of 1 J cm^{-2} during 300 ms flash resulted in degradation of AFB1 in water by 92.7% [54]. In another study, PL at 0.52 J cm^{-1} per pulse was applied for 80 seconds and 15 seconds to treat rough rice and bran, respectively. It was observed that on 15 seconds of PL treatment AFB1 and AFB2 in rice bran reduced by 90.3 and 86.7% respectively, while on 80 seconds of treatment AFB1 and AFB2 in rough rice reduced by 75.0 and 39.2% respectively [32]. PL treatment also inactivated the mutagenicity and cytotoxicity of these aflatoxins. Abuagela et al. [55] treated dehulled peanuts with PL at 0.4 J cm-1 per pulse. No significant variation in the chemical properties including acidity value, fatty acid content and peroxide value of oil obtained from PL treated peanuts. Aflatoxins levels dignificantly decreased by 91% in PL treated dehulled peanuts. For large industrial scale application of PL technology would require the development of cost- effective equipment for PL treatment.

Microwave heating generates a high temperature (130°C or higher) that is required for aflatoxin reduction in peanut and corn [56, 57]. Aflatoxin contaminated corn was microwave heated at 1650 W power for 5.5 minutes, resulted in reduction in AFB1, AFB2 by 36 and 58%, respectively [35]. Mobeen et al. [33] microwave cooked peanut and its products and reported reduction in AFB1 level by 50–60%, while level of AFB2 reduced to non-detectable limits. Treatment of corn flour by microwave heat for 10 minutes duration led to decrease in AFB1 content up to 67.7% [34]. Major drawback of aflatoxin decontamination by this method is the uneven distribution of temperature during microwave heating. This results in the generation of hot and cold spots in the treated food product [58]. Overheating in the hot spot may lead to loss of nutritional value and quality whereas lesser temperature in cold spot may not be sufficient for degradation of aflatoxins. In view of this, aflatoxin detoxification using microwave heating method has moderate success and limited application.

2.1.2 Cold plasma

The fourth state of matter, plasma predominantly consists of UV rays, ions, electrons, reactive nitrogen species (RNS), reactive oxygen species (ROS) [59]. Based on its temperature, plasma can be categorized into thermal and non-thermal (cold) plasma. Cold plasma is generated through electrical discharges in gases at temperature between 30 and 60°C and atmospheric pressure [60]. Cold plasma technology has been employed for aflatoxin degradation at ambient pressure and temperature [61, 62]. Ouf et al. [63] used argon cold plasma at atmospheric pressure for 9 minutes on *Aspergillus niger* spore and mycotoxin production in palm fruits. Authors found that mycotoxin fumonisin B2, OTA content reduced from 6 and 25 μg/100 mm^2 respectively and all the spores were killed. Another study used dielectric barrier discharge nitrogen plasma (1150 W) for detoxification of aflatoxin inoculated dehulled hazelnuts. Authors demonstrated that after 12 minutes treatment around 70% of AFB1 was detoxified. They also showed that AFB1 and AFG1 were more sensitive whereas AFB2 and AFG2 was less sensitive to nitrogen plasma treatment [64]. Treatment with corona discharge plasma jet (CDPJ) for 30 minutes on AFB1 spiked rice and wheat samples and AFB1 on glass slides reduced the AFB1 concentration by 56.6, 45.7, 95% respectively [65]. Authors suggested the inconsistency in detoxification of AFB1 is due to possible chemical interaction of toxin with the food matrix. The effectiveness of cold plasma technology for aflatoxin degradation depends on the type of food commodities, kind of plasma system, operating parameters used (energy input, moisture, working gas) and the time of plasma exposure [60]. This novel technology has great potential for detoxification of aflatoxin in food and feed. However, it is still in its infancy and there is a need

to standardize conditions for plasma treatment suitable to decontaminate various foods. Further research is required to study the effect of plasma on the nutritional quality and organoleptic characteristics of food.

2.2 Chemical detoxification of aflatoxins

2.2.1 Electrolyzed oxidizing water

Electrolyzed oxidizing water (EOW) or electro-activated water is produced by the electrolysis of water containing 1% sodium chloride (NaCl). The resulting water is an electrolyzed one that can be used as a disinfectant. EOW can be categorized into two major types according to its pH and oxidation–reduction potential (ORP): a) neutral electrolyzed oxidizing water (NEOW) with pH of 5.0–6.5, ORP of 800–900 mV; b) acidic electrolyzed oxidizing water (AEOW) with pH of less than 3.0, ORP of more than 1000 mV [66]. Major advantage of EOW is that it turns to ordinary water after use that has no potential threat to animals and environment. Treatment of aflatoxin contaminated corn with NEOW at room temperature for 15 minutes, resulted in significant reduction in the genotoxicity and cytotoxicity of aflatoxins in HepG2 cells [67]. Gomez- Espinosa et al. [68] further confirmed that NEOW treatment of aflatoxin contaminated corn significantly reduces the aflatoxicosis in turkey.

2.2.2 Organic acids

Organic acids have been widely used for aflatoxin degradation in food industry. AFB1 contaminated soyabean soaked in 1.0 N tartaric acid, lactic acid and citric acid,, and at room temperature for 18 hours, resulted in reduction in AFB1 level by 95.1, 92.7 and 94.1%, respectively [69]. Acidulation with lemon juice for 60 minutes at 120°C for decontamination of AFB1 in roasted pistachio nuts, reduced the AFB1content by 50.2% [70]. Acidulation can be combined with other decontamination technologies for better results. For instance, acidulation coupled with pulsed light technique led to aflatoxin degradation in peanuts up to 98.3%. On the other hand, pulsed light and citric acid treatment separately decreased aflatoxins by 78.1 and 20.2%, respectively [71]. Organic acid treatment results in leaching of nutrients such as water-soluble proteins, starch, minerals. However, it has several health benefits on livestocks. The only drawback of this method is the high cost of organic acids.

2.2.3 Ozone

Ozone (O3), a most powerful oxidizing, antimicrobial and disinfecting agent, can be used directly for decontaminating various food products [72]. FDA has granted ozone as Generally Recognized As Safe (GRAS) status for water and food industry [73]. Ozone cause the progressive oxidation of sulfhydryl group of amino acids of proteins, peptides and enzymes or polyunsaturated fatty acids into shorter molecular fragments. Ozone also result in degradation of unsaturated lipids in cell wall envelope, disruption and leakage of microbial cellular contents [74]. Ozone degrade aflatoxins AF-B1 and AF-G1 through an electrophilic attack on C8-C9 double bond of difuran ring, resulting in ozonide formation. This is followed by rearrangement into molozonide derivatives like organic acids, ketones and aldehydes [75]. On the contrary, AF-B2 and AF-G2 are more resistant to ozonisation as they lack C8-C9 double bond in their structure [76]. Ozone treatment of groundnut samples increased the rate of aflatoxin detoxification and has no effect

on the peroxide, resveratrol, acids and polyphenol content [77]. Treatment of AFB1- contaminated maize with ozone resulted in decreased toxicity of the treated samples [78].

Major advantages of using ozone as aflatoxin detoxification method are (i) ozone can be applied in gaseous as well as liquid forms (ii) no leftover residue after contact and no hazardous disposal (iii) easy on-site generation of ozone [72, 79]. Till date several studies have been conducted on ozonation at laboratory scale. There is a need to develop efficient eqipments to scale up the process for successful commercial application of this technology for detoxification of aflatoxin contaminated food and feed.

2.2.4 Plant extracts

Natural plant extracts are considered as food additives by food industry worldwide. They are well known for their anti-inflammatory and anti-microbial properties. Incubation of AFB1 with *Rosmarinus officinalis* aqueous leave extracts at different time intervals showed 60.3% reduction in AFB1 after 48 hours of incubation [80]. Iram, *et al.* [81, 82] studied the effectiveness of aqueous extracts of *Corymbia citriodora* and *Trachyspermum ammi* for detoxification in AFB1 and AFB2 spiked corn. After treatment with *C. citriodora* leaf extract, AFB1 and AFB2 levels were reduced to to 91.7 and 88.8%, respectively. AFB1 and AFB2 were degraded by 89.6 and 86.5%, respectively, following *T. ammi* seeds extract treatment. These results were in consistent with the findings of Velazhahan *et al.* [83]. In another study, authors tested the aqueous extracts from 31 medicinal plants for their AFB1 detoxifying ability. *Adhatoda vasica* Nees leaf extract showed the highest AFB1 degradation ability (98%) at 24 hours after incubation at 37°C [84]. The same group found that *Adhatoda vasica* Nees leaf extract contained the partially purified alkaloids that was responsible for its strong AFB1 detoxification ability. Brinda *et al*. [85] fed rats with spraydried formulation of *Adhatoda vasica* Nees leaf extract and exposed them to AFB1. They found that such pre-fed rats counteracted hepatotoxicity induced by AFB1 exposure. To date, there is a meager knowledge on the active compounds present in the plant extracts that is responsible for their aflatoxins detoxification ability. Further research is needed to gain insights into the aflatoxin detoxifying compounds, their mode of action and complex interaction with the food matrices.

2.3 Biological decontamination of aflatoxins

Biological degradation of aflatoxins involves microorganism or enzyme based detoxification of aflatoxins into less toxic or non-toxic metabolites. This method has emerged as an efficient and eco-friendly strategy for degradation of aflatoxins.

2.3.1 Microbial degradation

Various microorganisms isolated from different sources can degrade aflta-toxins present in food and feed. Risa *et al.* [86] investigated the effectiveness of 42 *Rhodococcus* strains for detoxification of AFB1 and zearalenone (ZON). 18 *Rhodococcus* strains were capable of degrading more than 90% of AFB1 and 15 strains could cease the genotoxicity in 72 hours. Only one of these strains, namely *R. percolatus JCM 10087 T* was capable of detoxifying ZON by more than 90% and decreasing the oestrogenicity by 70%. Another group studied the role of *R. pyridinivorans* K408 strain in detoxification of AFB1 in corn based stillage and revealed that the level of AFB1in liquid and solid phases of whole stillage was

degraded by 75 and 63%, respectively [87]. A number of *Bacillus* strains such as *B. subtilis* ANSB060 [88], *B. subtilis* UTBSP1 [89], *B. subtilis* JSW-1 [90], *B. licheniformis* CFR1 [91], *B. licheniformis* BL010 [92], *B. velezensis* DY3108 [93] and *Bacillus* sp. TUBF1 [94] have been reported to degrade aflatoxins. *B. subtilis* has been approved as Generally Recognized As Safe (GRAS) strains of bacteria that is safe for commercial pharmaceutical and nutritional purpose.

Adebo *et al.* [95] used the lysates of bacterial strains (*Staphylococcus* sp. VGF2, *Pseudomonas fluorescens* and *Pseudomonas anguilliseptica* VGF1) isolated from goldmine aquifer to study the degradation of AFB1. It was found that *Staphylococcus* sp. VGF2 lysate showed the highest AFB1 degradation capacity of 100% while *P. fluorescens* and *P. anguilliseptica* VGF1 reduced the AFB1 by 63 and 66.5%, respectively. About 124 *Streptomyces* strains were examined for AFB1 degradation capability. It was found that 55% of the *Streptomyces* strains could degrade AFB1 [96]. Eshelli *et al.*, [97] demonstrated that *S. lividans* TK 24 was capable of degrading AFB1 by 88% in liquid culture upon 24 hour incubation. *E. coli* CG1061, AFB1 detoxifying bacterium, was isolated from chicken cecum and found to degrade AFB1 by 93.7% following 72 hours of incubation. It resulted in formation of degradation products of lower molecular weight [98]. The non-toxigenic strains of *Aspergillus niger* with aflatoxin detoxification capability have been used in the food industry. *A. niger* FS-UV1 strain, derived from *A. niger* FS-Z1 wild strain following UV irradiation showed superior AFB1 detoxification capability up to 95.3% and significantly decreased mutagenic activity [99]. A novel thermophilic microbial consortium TADC7 was constructed for efficient, specific and stable AFB_1 degradation. Microbial consortium TADC7 showed the degradation of AFB1 by more than 95% of the toxin after 72 hours of incubation in the temperature range between 50 and 60°C. 16S rRNA sequencing revealed that *Tepidimicrobium* and *Geobacillus* may play a major role in AFB1 degradation [100].

2.3.2 Enzymatic degradation

Recently, the reports on isolation, identification and purification of aflatoxin-degrading enzymes from microorganisms have increased significantly. Various enzymes such as oxidases, peroxidases, reductases and laccases are capable of degrading aflatoxins. Laccases represent a class of multicopper oxidase enzymes widely present in fungi, plants, bacteria and insects. They catalyze oxidation of various phenolic and non-phenolic compounds coupled to reduction of molecular oxygen in water. Alberts *et al*. [101] first proposed the role of fungal laccases in decontamination of AFB1. Enzyme laccase (Lac2) produced and purified from *Pleurotus pulmonarius* showed AFB1 degradation up to 90% with mediator acetoseringone present in the medium [102]. Verheecke *et al.* [103] documented purified enzymes effective for AFB1 detoxification. Guo *et al.* [104] summarized the reaction mechanisms of aflatoxin-degrading enzymes responsible for AFB1 detoxification in the review. Alberts *et al.* [101] purified laccase from *Trametes versicolor* and produced recombinant laccase using *A. niger*. The laccase from *T. versicolor* resulted in reduction in AFB1 pro-oxidative properties by 59% and genotoxicity by 100% [105]. Compared to fungal laccases, bacterial laccases are excellent candidates for xenobiotics degradation because they are extremely thermostable, tolerant to alkaline conditions, wider range of pH and substrate spectrum [106]. Enzyme CotA laccase from *B. licheniformis* catalyze the C3-hydroxylation of AFB1 and transformed toxic AFB1 into products epi-aflatoxin Q1 and aflatoxin Q1 that are non-toxic human liver L-02 cells. Laccase CotA was found to be highly thermostable with the half life of 1 hour. It was capable of degrading AFB1 in the temperature range between 60 and 80°C by more than 70% in a period of 30 minutes [107].

Peroxidases are a class of oxidoreductases that catalyze oxidation of substrates with the use of hydrogen peroxide or organic peroxide. They are mainly heme- proteins with contain iron (III) protoporphyrin IX as the prosthetic group. Peroxidases are widespread in nature, found in both prokaryotes and eukaryotes. Researchers have achieved 65 and 97% reduction in AFM1 and AFB1, respectively in milk with horse radish peroxidase (HRP) treatment at 30°C following 8 hours of incubation [108]. Another group used manganese peroxidase (MnP) isolated from *Phanerochaete sordida* YK-624 for AFB1 degradation. MnP reduced the AFB1 level by 86.0% and mutagenicity by 69.2% [109]. Type B dye decolorizing peroxidase (Rh_DypB) was isolated and purified from *Rhodococcus jostii* and tested for AFB1 degradation. Authors achieved 96% degradation of AFB1 in the sodium malonate buffer [110].

Scientists are working constantly towards identification, isolation and purification of novel aflatoxin detoxifying enzymes from wide variety of organisms. For instance, an extracellular enzyme MADE was purified from *Myxococcus fulvus* ANSM068 from a culture supernatant. At the temperature of 35°C and pH 6.0, degraded AFB1 by 71.89% following 48 hours of incubation. The purified MADE also degraded AFM1 and AFG1 by 96 and 97%, respectively [111]. Xu *et al*. [112] purified enzyme MADE from *Bacillus shackletonii* that resulted in degradation of AFB1 by 48% with 72 hours of incubation at 70°C. BADE was found to be extremely thermostable, even at 100°C for 10 minutes. Nine F420H2- dependent reductases (FDRs) were identified and characterized from *Mycobacterium smegmatis*. FDRs catalyze the α, β-unsaturated ester reduction in aflatoxins, using deazaflavin as cofactor. They are categorized into two classes, namely FDR-A and FDR-B with FDR-A class of enzymes being 100 times more active against aflatoxins than FDA-B [113].

The main advantage of cell free enzyme based aflatoxin degradation is that it has no negative impact on the degradation ability and organoleptic properties of food products [95]. Although cell free aflatoxin degradation enzymes are extremely effective, however their application in food and feed industry is still limited due to certain shortcomings. Firstly, the low yield of aflatoxin-degrading enzymes in the native hosts. This can be solved by the intervention of recombinant DNA technology. Secondly, food processing generally requires the use of solvents, extreme temperature and pH conditions. This can effect the catalytic efficiency and stability of the wild type enzymes. The use of enzyme engineering technologies such as random or site directed mutagenesis could address these issues [114].

In comparison to natural enzymes, nanozymes are more robustness, cost effective, stabile and durable. Nanozymes are the nanomaterials with intrinsic enzyme like properties that catalyze the substrates of natural enzymes following the same catalytic mechanisms and kinetics under physiological conditions [115]. The laccase-mimicking nanozyme was prepared by coordinating guanosine monophosphate (GMP) with Cu^{2+} at room temperature. This led to formation of amorphous metal–organic framework (MOF) nanomaterial. Cu/GMP nanozyme has the same catalytic efficiency as the natural laccase but it is 2400-fold more cost-effective and more robust against extreme temperature, pH salt and storage conditions [116]. Nanozymes with peroxidase-like activity such as $CuMnO_2$ nanoflakes [117], $FeMnO_3$ nanoparticle-filled polypyrrole nanotubes [118], FePt nanoparticle-decorated graphene oxide nanosheets [119], $Pt_{74}Ag_{26}$ nanoparticle-decorated ultrathin MoS_2 nanosheets [120] have been prepared. The recent technological advancements will open the gate for the development and application of nanozymes to aflatoxin detoxification in food and feed industry.

3. Conclusion

Contamination caused by aflatoxins in food and feed poses a great threat to human and animal health and result in considerable economic loss to the agriculture production of the country. To produce healthy, high quality, aflatoxin free food products is the worldwide concern. Researchers have been working continuously for the development of effective aflatoxin decontamination strategies since decades. Despite all the efforts, there still a need to come up with an efficient decontamination technology that meets all these criteria for industrial commercialization: (i) Retain/enhance the nutritional quality of the food (ii) Efficiently reduce aflatoxins to the safe limits without leaving toxic residues (iii) cost effective and eco-friendly. So far, no aflatoxin decontamination strategy has been developed that meet all the mentioned criteria. For instance, physical and chemical methods may change the organoleptic properties and chemical composition of food and feed. Biological approaches are more specific, effective with more control over the generated bio-products of aflatoxin detoxification. Use of pure enzyme for aflatoxin degradation has no negative impact on the degradation efficiency, chemical composition and organoleptic properties of food products. Further, adoption of enzyme engineering technology would provide highly efficient and specific aflatoxin detoxifying enzymes in the near future.

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Conflict of interest

Authors have no competing interests.

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