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REVIEW

Reducing aflatoxins (*Aspergillus flavus*) in food and animal feed by physical methods and chemical degradation: An update

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ABSTRACT. We reviewed current challenges and future research directions for aflatoxin degradation based on the existing body of work. The objective of providing this information was to deepen researchers' comprehension of aflatoxin degradation, address present limitations, and promote progress in aflatoxin detoxification methods. Aflatoxins, the most potent naturally occurring mycotoxins identified to date, pose a significant threat to global food safety and international trade, particularly in developing nations. Finding effective detoxification procedures has consistently ranked among the most important concerns worldwide. Among established approaches, physical methods have emerged as the foremost effective in aflatoxin degradation, capable of inducing their rapid and irreversible denaturation. This review provides a concise overview of aflatoxin detection techniques and the structural analysis of degradation products. It also highlights four key aflatoxin safety evaluation methods and assesses the toxicity of degradation products, and provides an update on research developments in aflatoxin decontamination over the past decade. Furthermore, we examine recent applications, mechanisms, and outcomes of physical techniques for aflatoxin decontamination, including microwave heating, irradiation, pulsed light, cold plasma, and ultrasound, offering a detailed analysis of each approach. Regulatory considerations regarding the concept of 'detoxification' are also discussed.

Introduction

Aflatoxins, a diverse class of secondary metabolites, share analogous chemical structure and are primarily synthesised by *Aspergillus flavus* and *Aspergillus parasiticus*. While aflatoxins show limited solubility in water, they readily dissolve in various organic solvents, such as acetonitrile or methanol. They are also characterised by high thermal stability, thus conventional cooking methods are ineffective in eliminating these toxins from contaminated food (Yamada et al., 2020). Based on their fluorescence colour induced by ultraviolet light (UV), aflatoxins are mainly classified into two main groups: B (blue) and G (green) aflatoxins, as proposed by Wogan (1966). These categories include aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG). Aflatoxin M_1

(AFM_1) and M_2 (AFM_2) are generated through *in vivo* hydroxylation in mammals, following their consumption with food or feed contaminated with AFB_1 and AFB_2. Subsequently, these metabolites are eliminated through milk or urine (Rushing and Selim, 2019). Aflatoxins can cause significant liver damage and represent an important factor in the development of primary hepatocellular carcinoma (HCC), a type of malignant liver tumour. Studies attribute nearly 50% of the HCC risk in African and Asian regions to aflatoxins (Mungamuri and Mavuduru, 2020). Of the 18 identified aflatoxin types, AFB_1 has been classified as a Group I carcinogen by the International Agency for Research on Cancer (Peng et al., 2018). It is not only the most potent toxin, surpassing potassium cyanide by a factor of 10, but also the most hazardous to the environment (Ostry et al., 2017). Overall, aflatoxins pose significant risks of inducing cancer, birth defects, and genetic mutations in both humans and livestock (Awuchi et al., 2022). The impact of mycotoxins on the human cellular genome is significant, with exposures linked to DNA damage, kidney injury, DNA/RNA mutations, growth impairment in children, and immune suppression. Despite these known risks, further research is needed to fully elucidate the toxicological mechanisms of mycotoxin action and associated environmental implications. Risk assessment studies focusing on the conditions and factors that contribute to mycotoxin toxicity are essential for advancing this knowledge.

Crops rich in carbohydrates and proteins, including peanuts, maize, wheat, and soybean, are prone to aflatoxin contamination from cultivation to storage. This susceptibility is particularly pronounced in tropical and subtropical regions, where the prevailing temperature and humidity conditions favour the growth of *Aspergillus* fungi, making crops more vulnerable to aflatoxin contamination (Scudamore and Patel, 2000; Lewis et al., 2005). According to the Food and Agriculture Organization (FAO), approximately a quarter of the global food crop supply is contaminated by mycotoxins annually, with aflatoxins considered to be the most harmful (Xiao and Mujumdar, 2020). Alarmingly, more than 5 billion people worldwide are chronically exposed to high aflatoxin levels, exceeding 1000 parts per billion (ppb) (Pandey et al., 2019). To ensure food safety, over 100 countries and regions have implemented specific aflatoxin threshold standards for various food products. The European Union (EU) has enforced relatively stringent regulations in this aspect. Specifically, they stipulate that total levels of aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) in all cereals, except for maize and rice, must not exceed 4 ppb, and AFB_1 alone 2 ppb (Peng et al., 2023). In addition, researchers are actively investigating prevention and management strategies for aflatoxin contamination, aiming to mitigate its global impact (Udovicki et al., 2022). Current approaches to minimise aflatoxin levels typically involve a combination of pre-harvest and post-harvest interventions, including the cultivation of fungal-resistant crop varieties, improved field management practices, post-harvest treatments such as drying and sorting, and appropriate storage techniques. These efforts collectively serve to inhibit fungal growth and the aflatoxin production in crops (Pankaj et al., 2018). Nonetheless, the aforementioned preventive techniques alone cannot fully guarantee aflatoxin-free products, underscoring the importance of post-harvest detoxification methods for controlling aflatoxin contamination. Detoxification plays a key role in aflatoxin control and can be divided into physical, chemical, and biological detoxification methods. These approaches focus on reducing aflatoxins' physiological toxicity by targeting key structural sites, including the C8-C9 double bond of the difuran ring (Site 1) and the coumarin lactone ring showing strong carcinogenic properties (Site 2). Techniques such as photolysis, oxidation, and hydrolysis are employed to transform aflatoxins into less toxic or non-toxic forms (Chang et al., 2013; Wang et al., 2014). However, chemical methods often generate harmful residues or undesirable alterations in the food products. Moreover, certain chemical degradation reactions, such as ammonisation or alkaline degradation, are reversible (Peng et al., 2020). Regarding biological methods, it should be noted that microorganisms can generate new harmful compounds when attempting to detoxify aflatoxins. While a range of enzymes like laccases, peroxidases, oxidases, and reductases can break down aflatoxins, many of them are protein-based compounds and can cause allergies. Consequently, the European Union has not yet approved these enzymes for aflatoxin decontamination (Karlovsky et al., 2016; Adebo et al., 2017). Physical methods remain the preferred choice for aflatoxin degradation, as they offer rapid and efficient results in food production without leaving harmful residues.

To date, several publications have examined a range of well-established traditional physical techniques for aflatoxin decontamination, including traditional methods like activated carbon and extrusion cooking, as well as emerging approaches such as microwave treatment, irradiation, pulsed light,

pulsed electric fields, or cold plasma (Peng et al., 2018; Guo et al., 2021; Wu et al., 2021). Nonetheless, the existing literature lacks a comprehensive analysis of the precise mechanisms and by-products involved in these degradation processes, along with a critical assessment of the toxicity of resulting products. Consequently, the objectives of this review article were as follows: (1) highlight essential methods for safety evaluation, (2) systematically analyse the most recent applications, degradation mechanisms, and by-products associated with leading physical decontamination techniques, (3) provide an overview of regulatory concerns related to 'detoxification', as well as (4) outline current challenges and suggest potential directions for future research. To provide a more comprehensive overview and discussion, the present study also includes some analytical and evaluative approaches, as well as regulatory considerations pertinent to non-physical detoxification methods that can be applied to physical techniques or serve as a benchmark. This information can assist both the academic and industrial sectors improve their understanding of existing aflatoxin degradation methods and develop more practical, effective approaches.

Methods for the analysis and measurement of aflatoxins and their degradation products

Accurate quantification of aflatoxins

The primary indicator for assessing the effectiveness of a given treatment is the change in toxin content. The American Association of Official Analytical Chemists (AOAC International, 2023) has released over 10 methods for detecting aflatoxins in food and feed. Several commonly used aflatoxin determination techniques are outlined in Table 1, which also provides an overview of their working principles, advantages, and drawbacks. Among these methods, ultra-high performance liquid chromatography (UPLC/HPLC) has gained significant popularity in recent years due to its precision and sensitivity (Miklós et al., 2020). In particular, this method can accurately measure aflatoxin contents when their concentration exceeds 0.015 ppb (Rahmani et al., 2013).

Analysis and structural characterisation of aflatoxin degradation products

Determining the structure of degradation products is crucial for understanding the process of decomposition. The challenge in identifying these products lies in the fact that they are often completely unknown compounds, with many degradation products often sharing similar structures. Liquid chromatography-multistage mass spectrometry (LC-MS/MS) is one of the most commonly used and well-established methods for both qualitative and quantitative analysis of organic compounds. This method has exceptional separation and identification capabilities and has become a standard technique for detecting unknown metabolites and degradation products (Wang et al., 2011; Diao et al., 2012; Malachová et al., 2018). In a study by Mao et al. (2016), a total of 13 chromatographic peaks were observed in the ion chromatogram with six of them representing primary products. Further analysis using ultra-performance liquid chromatography coupled with thermo quadrupole exactive focus mass spectrometry (UPLC-TQEF-MS/MS) revealed two degradation products of AFB_1 in peanut oil following exposure to ultraviolet irradiation. These products, with mass-to-charge ratios (m/z) of 340 and 227, were identified as derivatives of AFB_1 formed through a series of reactions, including dehydroxylation and amination. Additionally, Iram et al. (2015) found that extracts from *Corymbia citriodora* leaves were highly effective in degrading AFB_1 and AFB_2, achieving reductions of up to 95.21 and 92.95%, respectively. These authors were able to identify ten degradation products of AFB_1 and AFB_2, with eight of them involving the removal of the double bond in the terminal furan ring. These findings underscore the remarkable analytical and structural capabilities of the tandem mass spectrometry system.

Methods for assessing the safety of aflatoxin degradation products

Following the detoxification of aflatoxin-contaminated raw materials, even though the chemical structure of the toxin is altered, the resulting product may still retain some toxicity. Therefore, the safety assessment of these degradation products remains very important. Table 2 summarises the evaluation methods frequently reported in the literature over the past decade.

Ames Test

Figure 1a illustrates the *Salmonella* reverse mutation test, commonly referred to as the Ames test, developed by Ames et al. (1975). This assay employs histidineauxotrophic strains of (his-) *Salmonella typhimurium* to evaluate the genotoxicity

Table 1. Comparison of frequently employed methods for aflatoxin determination

Sensor technologies	Principle of operation	Benefits	Drawbacks	References
Time resolved fluorescence immunoassay (TRFIA)	Extended fluorescence lifetime and significant Stokes shift of rare earth elements immobilised on antigens or antibodies, effectively filtering out the aflatoxin spectrum	High sensitivity and stability, with a broad detection range	Susceptible to contamination with rare earth elements from the surroundings; substantial cost	Wang et al. (2017)
Enzyme-linked immunosorbent assay (ELISA)	Targeted interaction of antibodies with antigens; chromogenic reactions occur between enzymes and substrates	Rapid and responsive, high specificity, making it suitable for screening large sample batches	Issues with cross-reactivity and matrix effects, leading to limited reproducibility	Peng et al. (2020)
Liquid chromatography-mass spectrometry (LC-MS)	The LC system is used for sample extraction, and after ionisation, the parent ions and fragments are separated based on their mass-to-charge ratio by the mass analyser in the MS system	High selectivity and quantitative accuracy; simultaneous detection of substances with varying mass-to-charge ratios	Susceptible to ion suppression; requires additional steps to remove impurities, increasing complexity and cost	Xie et al. (2016); Chen et al. (2019)
Ultrahigh performance liquid chromatography (UPLC/HPLC)	Separates aflatoxins in a chromatographic column based on physical and chemical differences, such as molecular weight, solubility, and molecular structure. The detector then transforms this separation into a photochemical signal	High precision, sensitivity, and reproducibility	Complex procedure, typically involves derivatisation steps, resulting in higher costs	Xie et al. (2016); Zareshahrabadi et al. (2021)
Thin-layer chromatography (TLC)	Aflatoxins are distinguished on a thin plate based on their varying adsorption properties. Aflatoxins emit fluorescence when exposed to ultraviolet light at a wavelength of 365 nm	Affordable and easy to promote; suitable for preliminary screening of large sample batches	Limited sensitivity and specificity; labour-intensive and time-consuming procedure	Hoeltz et al. (2010); Silva et al. (2015)

of chemical substances (Zhang et al., 2021a). The Ames test was utilised to assess the detoxification potential of AFB_1 in alkali-refined peanut oil. The results indicated that the presence of the S9 activator led to a higher average number of revertants in the four strains compared to samples without the S9 activator. Additionally, alkali refining led to a significant reduction in the average number of revertants in the four strains compared to the positive control (Ji et al., 2016; Liu et al., 2016). The Ames test has also been used to confirm the safety of AFB_1 degradation byproducts following exposure to electron beam irradiation (EBI). This test remains a widely accepted method for screening and identifying mutagens and it is the preferred approach for rapid assessment of the mutagenic properties of various chemicals.

Brine shrimp lethality bioassay

Brine shrimps (*Artemia* sp.) are highly sensitive to toxic substances, making them an ideal test organism for assessing acute biological toxicity, as demonstrated in the brine shrimp lethality bioassay (Figure 1b). In the context of aflatoxin degradation, the concentration of AFB_1 at which 50% mortality of *Artemia nauplii* occurred increased from 0.32 to 0.58 μg/ml after treatment with microorganisms isolated from Kangpucha culture (Taheur et al., 2020). Similarly, cold plasma treatment of AFM_1 for 5 min led to a notable reduction in toxicity, resulting in a 16.1% mortality rate and an 83.7% increase in brine shrimp survival (Nguyen et al., 2022). A brine shrimp bioassay was also applied by Anjum et al. (2020) to evaluate the detoxifying effects of water-based extracts from *Acacia nilotica* on AFB_1 and AFB_2. Their findings demonstrated that treating solutions containing 300 μg/l AFB_1 and 13 μg/l AFB_2 with *A. nilotica* leaf extract resulted in a 72.5% reduction in larval mortality after a 96-h incubation period. Due to the elevated sensitivity of brine shrimp to harmful substances, this method can detect weak toxicity that might not cause immediate harm to higher organisms. Therefore, this method has been successfully employed to assess toxicity changes in agricultural residues, food additives, and pollutants

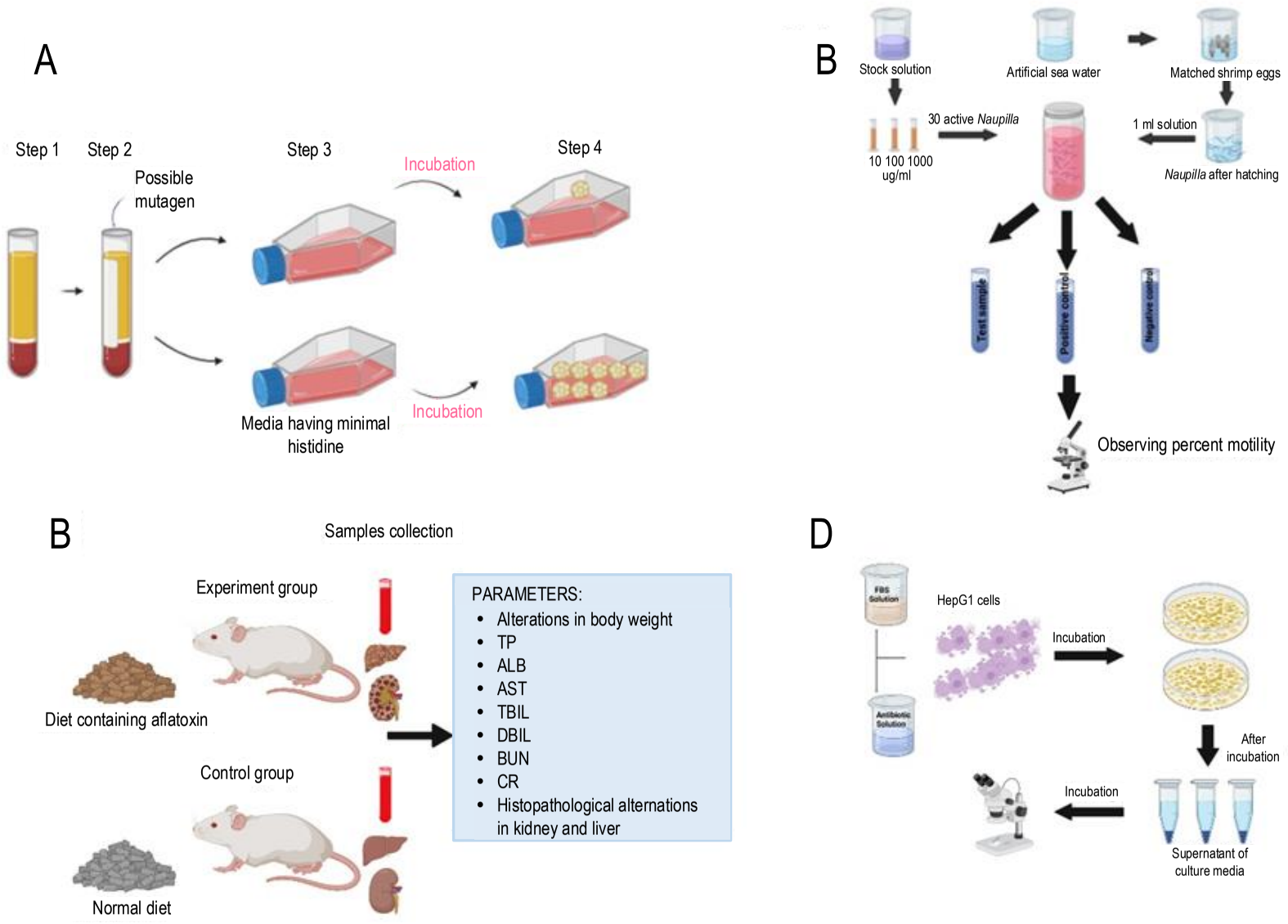

Figure 1. Four effective approaches for evaluating the safety of aflatoxin degradation products: (a) Ames test, (b) brine shrimp lethality bioassay, (c) toxicological assessment in rats, and (d) cytotoxicological assay using MTT

TP – total protein, ALB – albumin, AST – aspartate aminotransferase, TBIL – total bilirubin, DBIL – direct bilirubin, BUN – blood urea nitrogen, CR – creatinine

Assessment of toxicity in rats

Adult mammals may exhibit responses to aflatoxin that more closely resemble those in humans compared to smaller organisms. As depicted in Figure 1c, rats were administered a diet containing the experimental material to evaluate its effects on general toxicity symptoms, body weight changes, and pathological effects on the liver and kidneys. Diao et al. (2013) demonstrated that rats of the same sex, fed exclusively with AFB_1-contaminated peanuts (ACPs), showed a significant reduction in body weight gain and feed conversion efficiency, along with notable changes in several serum biochemical markers. In contrast, rats consuming a standard diet supplemented with ACPs treated with ozone for 120 h exhibited fewer of these effects. Wei et al. (2014) reported that low concentrations of AFB_1 did not cause poisoning or lower weight gain in rats; however, serum aspartate aminotransferase (AST) activity levels, an indicator of liver health status, were significantly elevated on days 7 and 15 in rats exposed to AFB_1. Irreversible histological lesions may develop in advanced stages of aflatoxin-induced liver damage. For instance, rats given an oral dose of 250 μg/kg AFB_1 body weight per day displayed significant pathological changes in the entire hepatic lobule, including extensive vacuolar degeneration, abnormal mitosis, and localised hepatocellular necrosis within four weeks. After eight weeks of exposure, a substantial number of megalocytes, binuclear cells, and necrotic hepatocytes emerged (Ali et al., 2021). Repeated exposure to AFB_1 resulted in widespread haemorrhage and cell necrosis in rat livers, accompanied by an increase in glutathione S-transferase (GST-P)-positive cells and the formation of foci. Incorporating a comprehensive set of monitoring indicators in this assessment system could serve as a scientifically robust approach for predicting the effects of degradation products in both humans and animals (Poapolathep et al., 2015).

Cytotoxicity assessment

The $HepG_2$ cell line, derived from human hepatic tumour tissue, retains a relatively intact set of phase I and II enzymes involved in the biotransformation process (Zeidan et al., 2019). At present, human hepatoma $HepG_2$ cells are considered one of the most suitable *in vitro* models for assessing aflatoxin hepatotoxicity. Cytotoxicity assays are a valuable tool for examining the detrimental effects of aflatoxins and their degradation products on cellular activity (Sobral et al., 2018). The primary objective of these assays is to measure cell viability using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) reagent, as illustrated in Figure 1D. For example, Domijan et al. (2019) conducted MTT assays using Pk15, HepG2, and SH-SY5Y cell lines to determine the safety of gamma-radiolytic products derived from AFB_1. Their findings indicated that $HepG_2$ cells exhibited the highest sensitivity to AFB_1, with byproducts showing reduced toxicity to these cell lines when compared to AFB_1. In addition, cold plasma treatment at 85 kV for a duration of 20 min significantly lowered the cytotoxic effects of AFB_1. Viability, caspase-3 activity, and DNA fragmentation levels in $HepG_2$ cells exposed to AFB_1 following this treatment showed significant differences compared to AFB_1-exposed cells that did not undergo this treatment (Nishimwe et al., 2021). Similarly, studies on AFB_1 degradation through ultraviolet irradiation demonstrated an approx. 40% reduction in the cytotoxicity of photodegradation products in both water and peanut oil. Moreover, p53 protein expression in cells treated with these degradation products was significantly higher compared to cells directly exposed to AFB_1 (Liu et al., 2012).

This assay provides a direct *in vitro* evaluation of the cytotoxicity of degradation products, offering a means to predict their potential harm to organisms. This approach solves the limitation of traditional zoological methods, which are often labour-intensive and can yield inconsistent results. In assessing the safety of products resulting from aflatoxin degradation, the concurrent use of two or more methods is recommended. This combined approach aims to provide a comprehensive understanding of the potential harm to both humans and livestock by closely simulating biological responses and generating results consistent with *in vivo* effects.

Applying physical techniques for aflatoxin degradation

Microwave radiation heating: Principles and applications

Microwaves are electromagnetic waves with a frequency range of 300 MHz – 300 GHz and wavelengths ranging from 1 mm to 1 m. When materials are exposed to a rapidly alternating microwave field, the polarisation motion of polar molecules and oscillation of ions within the heated object transform the energy of the electromagnetic field into heat (Soni et al., 2020). This method of volumetric heating enables faster selective

heating of greater efficiency compared to conventional heat sources (Chang et al., 2013; Pandiselvam et al., 2020). The Federal Communications Commission has designated the 915 MHz and 2450 MHz microwave frequencies for use in industrial, scientific, and medical applications. The 915 MHz band is primarily utilised in industrial microwave systems, whereas the 2450 MHz band serves a dual purpose, including applications in domestic devices (Pankaj et al., 2018).

In recent years, researchers have studied the efficiency of microwave heating on aflatoxin removal. Table 2 presents the results of various studies applying microwave power, processing duration, or maximum heating temperature as main experimental factors. Temperature is a crucial determinant of the degradation effect, and temperatures in the range of 130–150°C or higher are necessary for effective AFB_1 breakdown. Comparable degradation ratios for AFB_1 can be obtained in shorter times by increasing microwave power or extending the exposure at lower power levels (Patil et al., 2019; Zhang et al., 2020). Additionally, the moisture content of materials during microwave heating can have a significant influence on the degradation process. The presence of water can help to open the lactone ring in aflatoxins, thereby lowering the temperature required for aflatoxin decomposition (Kabak, 2009; Gómez-Bombarelli et al., 2013). In terms of microwave degradation of the AFB_1 standard substance, the study found that a temperature of 150 °C was necessary to induce AFB_1 decomposition under dry heating conditions. However, when heated with moisture at 80 °C for 1 h, 73% of AFB_1 was degraded (Shi, 2016).

Mechanisms and resulting degradation products

The primary method of AFB_1 degradation by microwaves involves hydrolysis reactions, with two studies detailing the mechanisms of these reactions. Zhang et al. (2021b) examined the impact of water-assisted microwave radiation on AFB_1 decontamination and found that six degradation by-products were generated at 500 W and 140 °C. The main product $C_{16}H_{14}O_5$ forms initially through lactone ring hydrolysis and subsequent decarboxylation. Two additional products are generated by conversion of OCH_3• into OH• or by hydration of the double bond in the furan ring. Hydrolysis of the furan rings in AFB_1 and $C_{15}H_{12}O_5$ begins when the temperature exceeds 120 °C, producing $C_{12}H_{12}O_4$ at higher temperatures. Four final by-products result from the hydrolysis of both the furan structure and the lactone ring, followed by decarboxylation. These findings confirm that hydrolysis, with water attacking the lactone ring, is a key reaction in microwave-induced AFB_1 degradation (Shi, 2016). Microwaves provide the necessary energy for the reaction and increase the efficiency of molecular collisions by continuously shifting electric field orientation, thus inducing the reaction. Additionally, the furan ring is particularly susceptible to damage during microwave heating, resulting in the formation of less toxic products. However, while microwave heating is effective for aflatoxin degradation, careful attention is required due to potential uneven temperature distribution and the impact of high temperatures on the physicochemical properties of food.

Irradiation with ultraviolet light: Principles and applications

Ultraviolet (UV) radiation is a form of light invisible to human eye, as it spans the wavelength range from 100 to 400 nm. This spectrum can be further subdivided into UVA (315–400 nm), UVB (280–315 nm), and UVC (200–280 nm) based on their specific positions on the electromagnetic spectrum (Delorme et al., 2020). UV radiation is widely used in a non-thermal food processing method to remove aflatoxins, mainly because they are sensitive to light. The unique structure of all aflatoxins contributes to their varying sensitivity to different wavelengths (Diao et al., 2013). AFB_1 absorbs UV at 222 nm, 265 nm, and 362 nm, with the highest absorption occurring at 362 nm, which is attributed to the presence of the C8–C9 double bond in the terminal furan ring (Samarajeewa et al., 1990). AFB_2 and AFG_2, lacking this double bond structure, are more susceptible to UVC radiation, particularly at 254 nm (Jubeen et al., 2012; Babaee et al., 2022). Moreover, the effectiveness of aflatoxin detoxification is directly proportional to the duration and intensity of UV exposure. AFB_1 present in maize and peanuts, when subjected to 45 min of UVC irradiation at 31 W, was shown to be degraded by 43.2% and 50.8%, respectively. Conversely, when exposed to 12 W for 15 min, the degradation rate for both products remained below 20% (Udovicki et al., 2022). Exposing whole milk to UVA radiation at 365 nm and doses of 836 mJ/cm^2 and 857 mJ/cm^2, resulted in degradation rates of 78.2% for AFB_1 and 65.7% for AFM_1 (Kurup et al., 2022). Moreover, the effect of UVC radiation at 254 nm was assessed for both aflatoxin degradation and its impact on pistachio quality. This treatment achieved over 90% degradation of four aflatoxins while preserving the overall quality of pistachio nuts. Notably, sensory attributes of the treated pistachios were rated higher than those of the control group

(Babaee et al., 2022). Mild exposure to UV light is unlikely to cause substantial deterioration in the physical and chemical properties of food (Delorme et al., 2020). However, it should be noted that UV light has limited penetration and is typically used for solid surfaces or transparent liquids (Guo et al., 2021).

Mechanisms and outcomes of degradation

Numerous studies on the UV photolysis of AFB_1 in a water environment have consistently concluded that ultraviolet exposure generates OH• radicals, which subsequently target the C8–C9 double bond located on the terminal furan ring of AFB_1. This, in turn, increases the degradation rate of AFB_1 and ultimately leads to the formation of two primary by-products in processes involving hydration and demethoxylation (Diao et al., 2013; Patras et al., 2017; Stanley et al., 2020). What is more, demethylation and elimination reactions generate further by-products, likely influenced by variations in ultraviolet wavelength and intensity (Liu et al., 2010; Patras et al., 2017). In more complex matrices, additional factors such as oxygen and other substituents may influence the UV treatment process. For instance, ozone, produced by the reaction of UV light with oxygen, contributes to AFB_1 decomposition in peanuts, leading to the formation of a range of degradation products containing carbonyl groups (Chang et al., 2013). In peanut oil subjected to UV irradiation, two products, $C_{18}H_{33}N_3O_3$ and $C_{12}H_{22}N_2O_2$, were identified, resulting from reactions involving nitrogen-containing compounds (R-NH_2 and -NH_2), with processes including addition and substitution reactions (Mao et al., 2016). In summary, UV irradiation proves to be an effective method for eliminating the toxic sites of AFB_1, producing degradation products with significantly reduced toxicity compared to the original protoxin.

Pulsating light: principles and applications

Recently, pulsed light has emerged as an innovative approach for effectively decontaminating food surfaces by degrading aflatoxins (Wang et al., 2016). It works based on a rapid discharge of a capacitor that energises an inert gas, such as xenon, within the lamp, emitting intense bursts of intense white light (Deng et al., 2020). The efficacy of pulsed light in degrading contaminants is significantly affected by factors such as time of exposure, irradiation intensity, and specific food properties. For example, treating peanuts with pulsed light for 4 minutes resulted in a 78% reduction in B-aflatoxin content, showing similar efficacy for both AFB_1 and AFB_2 (Abuagela et al., 2019). Following a pulsed light treatment with 40 flashes, the degradation rates of AFB_1, AFB_2, AFG_1, and AFG_2 in apple juice were 72.09% for AFB_1, 73.65% for AFB_2, 57.06% for AFG_1, and 69.69% for AFG_2. Importantly, this treatment did not significantly affect the quality of the apple juice (Qi et al., 2023). Exposing red pepper powder to 61 light flashes (with a total light energy of 9.1 J/cm^2), resulted in a 67.2% reduction in AFB_1 content and a marked increase in total phenolic content (Woldemariam et al., 2022). Interestingly, aflatoxin reduction in rice bran versus brown rice varied significantly. A 15-s pulsed light treatment with 0.52 J/cm^2 per pulse reduced AFB_1 in rice bran by over 90%, whereas in brown rice, AFB_1 levels decreased by only 75% after 80 s at the same energy input (Wang et al., 2016).

Degradation mechanisms and resulting products

The breakdown of aflatoxins in pulsed light treatment was attributed not only to the photochemical effects of light exposure but also to a significant temperature increase induced by the photothermal effect (Abuagela et al., 2018a). In apple juice, two degradation products ($C_{16}H_{14}O_6$ and $C_{17}H_{14}O_7$) of AFB_1 were detected after pulsed light treatment (Qi et al., 2023). The authors determined that these products formed through hydrolysis of the lactone ring and an addition reaction involving the C8–C9 double bond on the terminal furan ring of AFB_1, respectively. Importantly, the toxicity of these two products was found to be lower than that of AFB_1, as indicated by a structure-activity relationship analysis (Qi et al., 2023). The combination of the photochemical impact of ultraviolet photons combined with the photothermal effect of intense broad-spectrum light results in the disruption of the furan terminal double bond and the lactone ring, both of which contribute to the toxic activity of aflatoxins (Abuagela et al., 2018b). The Ames test and brine shrimp lethality assay results have further confirmed that pulsed light treatment effectively deactivates the mutagenic properties and toxicity of aflatoxins (Moreau et al., 2013; Wang et al., 2016). Hence, pulsed light emerges as a promising approach for aflatoxin degradation, as it can alter structural components responsible for aflatoxins' toxicity, significantly reducing their harmful physiological effects

Gamma ray bombardment: Principles and applications

Gamma rays are a form of electromagnetic radiation generated by the nuclear decay of unstable

radioisotopes, such as ^{60}Co and Cs. They are considered one of the types of ionising radiation approved by the FDA (2016) for the decontamination of food products. With wavelengths shorter than 0.01 nanometres, gamma rays have exceptional penetrating abilities, and their highly energetic photons, although not directly ionising aflatoxin, can indirectly degrade it. This occurs through the radiolysis of water or other components, generating free radicals, such as H•, O2•, and OH• (Pankaj et al., 2018). Table 2 summarises various applications of gamma irradiation for aflatoxin degradation. The available data demonstrate that the irradiation dose is a key factor affecting degradation efficiency. For instance, exposing naturally contaminated maize to a 10 kGy irradiation dose using a ^{60}Co source led to a significant 94.5% decrease in the overall average content of AFB_1, while a 5 kGy exposure resulted in a significantly lower degradation of only 69.8% (Markov et al., 2015). In many countries, the maximum permissible dose for commercial food irradiation is 10 kGy. However, caution is advised when using this method to foods with high vitamin and lipid content, as these components are susceptible to oxidation by free radicals, which can lead to food quality deterioration (Calado et al., 2014). An irradiation dose of 6 kGy with ^{60}Co, significantly adversely affected the antioxidant activity, colour, as well as chlorophyll and carotenoids contents of pistachio nuts (Makari et al., 2021a). The effectiveness of irradiation is also influenced by the properties of the food matrix. In the case of ground red chili, a ^{60}Co irradiation dose of 6 kGy was sufficient to reduce the AFB_1 content by more than 90% in and total aflatoxin content by 80% (Iqbal et al., 2013). Nevertheless, even with a gamma-ray (^{60}Co) dose as high as 15 kGy, the maximum degradation observed for the four aflatoxins in commercial animal feed reached only 21.1% (Di Stefano et al., 2014). Moreover, due to the indirect radiolysis process, the effectiveness of decontamination by gamma irradiation is dependent on factors such as the moisture content of the food and the initial concentration of mycotoxins. The average decrease in AFB_1 levels resulting from gamma irradiation showed a negative correlation with the initial concentration of AFB_1 in naturally contaminated samples (Markov et al., 2015). As the moisture content of black and white pepper increased from 12 to 18%, reductions in AFB_1 levels also significantly increased. Specifically, AFB_1 degradation rose from 35.2 to 47.2% for black pepper and from 39.6 to 50.6% for white pepper when subjected to 30 kGy of gamma irradiation (Jalili et al., 2012).

Degradation mechanisms and resulting products

Utilising ^{60}Co as the radiation source, Wang et al. (2011) investigated the radiation products of AFB_1 in a methanol-water solution. The results suggested that all seven main degradation products formed because of reactions between AFB_1 and free radicals. While the degradation pathway was not explicitly provided, it can be inferred that free radicals, such as OH• and H• produced by the radiolysis of water, and reactive methoxy species (OCH_3) generated by methanol radiolysis, played a role in generating the radiolytic products, including $C_{17}H_{14}O_8$ (a), $C_{17}H_{14}O_7$, and $C_{18}H_{16}O_8$. Subsequent reactions, including the addition of the lactone ring and demethylation, gave rise to two additional products, namely $C_{16}H_{14}O_7$ and $C_{17}H_{14}O_8$ (b). Additionally, $C_{16}H_{10}O_5$ and $C_{14}H_{10}O_6$ were formed through demethoxylation, elimination reactions, and the modification of H• on the C8–C9 double bond of AFB_1. Analysing the structure of these degradation products, it is evident that the toxicity of most of them was reduced due to the addition reactions involving the C8–C9 double bond at the end of the furan ring and the substituents. However, one product ($C_{16}H_{10}O_5$) retained both the C8–C9 double bond and the lactone ring in its structure.

Given these findings, further studies are needed to comprehensively assess the toxicity of aflatoxin degradation products following gamma irradiation. Furthermore, it is crucial to recognise the potential food safety concerns and health implications associated with ionising irradiation.

Electron beam exposure: Principles and applications

While the use of gamma irradiation for aflatoxin degradation is widely accepted, concerns persist among consumers regarding the presence of radioactive residues from isotopes used for food irradiation. Electron beam irradiation (EBI), an innovative method for aflatoxin degradation utilising nuclear technology, differs from traditional methods by generating high-energy electron beams through accelerators, thereby avoiding the need of radioactive sources (Liu et al., 2016; Woldemariam et al., 2021). These electrons can penetrate food materials to a depth of up to four inches and breakdown organic molecules by means of both direct and indirect oxidation (Balakrishnan et al., 2021). Based on a compilation of studies presented in Table 2, EBI has proven to be a reliable technique for aflatoxin degradation, offering several advantages,

Table 2. Overview of safety assessment methods for aflatoxins and their degradation products (past decade)

Remedy	Structure	Aflatoxins	Subjects	References
Ultraviolet	H_2O and peanut oil	AFB_1	Cell viability of the HepG2 cell line was assessed by cytotoxicity test using the MTT method. Apoptosis was evaluated using flow cytometry, and P53 expression was determined using ELISA	Liu et al. (2012)
	Blend of DMSO and ethanol	AFB_1	Cell toxicity in A549 and THP-1 cell lines was assessed using the MTT method; Genotoxicity was evaluated by the Alkaline comet assay, and immunomodulatory effects were measured using ELISA	Jakšić et al. (2019)
Ozone	Maize	AFB_1	Cytotoxicity in the HepG2 cell line was examined using the MTT assay; apoptosis was evaluated using Hoechst 33258 staining	Luo et al. (2014)
Ambient plasma	Glass slide	AFB_1	Cytotoxicity assessment on the HepG2 cell line included: determination of cell viability using the MTT method, evaluation of apoptosis using caspase-3 assays, cell death detection using ELISA, and examination of oxidative damage to proteins by the DNPH assay	Nishimwe et al. (2021)
	DMEM	AFB_1	Cytotoxicity assessment on Caco-2 and HepG2 cell lines using the MTT method	Madalena et al. (2018)
Exposure to gamma radiation	Methanol	AFB_1	Cytotoxicity assessment of on Pk15, HepG2, and SY5Y cell lines using the MTT method	Domijan et al. (2019)
Compounds produced by Fusarium	Medium containing nutrient broth	AFB_1	Cytotoxicity assessment using the MTT method on the HepG2 cell line	Wang et al. (2017)
UV radiation	Peanuts	AFB_1	Ames test with *Salmonella typhimurium* test strains TA98 and TA100; Cytotoxicity assessment on the HepG2 cell line using the MTT method	Chang et al. (2013)
Aspergillus niger	PDB, also known as a specialised powdered diet	AFB_1	Ames test using *S. typhimurium* test strains TA97, TA98, TA99, and TA100; Evaluating toxicity in Sprague-Dawley rats	Sun et al. (2016)
	Olive oil	AFB_1	Evaluating toxicity in Wistar rats	Ali et al. (2021)
Curcumin	DMSO	AFB_1	Toxicological assessment in Fischer rats	Poapolathep et al. (2015)
	Feed stuff	AFB_1	Toxicity assessment in Sprague-Dawley rats	Wei et al. (2014)
Ozone	Peanuts	AFB_1	Toxicological assessment in Wistar rats	Diao et al. (2013)
Water extracts of *Acacia nilotica*	Aqueous solution	AFB_1, AFB_2	Lethality bioassay using brine shrimp	Anjum et al. (2020)
Pulsed light technology	Filter paper strips	AFB_1, AFB_2	Lethality bioassay using brine shrimp and an improved version of the Ames test, i.e., the fluctuation test using *S. typhimurium* test strains TA98 and TA100	Wang et al. (2016)
Non-thermal plasma	Acetonitrile-water blend	AFM_1	Lethality bioassay using brine shrimp	Nguyen et al. (2022)
Microbes such as lactic acid bacteria and yeasts	Kombucha culture	AFB_1	Cell viability assessment using the MTT method on the HepG2 cell line and brine shrimp lethality bioassay	Taheur et al. (2020)
Pulsed light irradiation	Methanol	AFB_1	Ames test with *S. typhimurium* test strains TA98, TA100, and TA102	Moreau et al. (2013)
Electron beam irradiation	Aqueous solution		Ames test using *S. typhimurium* test strains TA98 and TA100, and cytotoxicity assessment on the HepG2 cell line using the MTT method	
Bacillus licheniformis	Medium containing nutrient broth	AFB_1	Ames test using the *S. typhimurium* test strain TA100	Rao et al. (2017)
Refining through alkali treatment	Peanut oil	AFB_1	Ames test using *S. typhimurium* test strains TA97, TA98, TA100, and TA102; cytotoxicity assay on the HepG2 cell line using cell viability methods such as propidium iodide dye exclusion technique and flow cytometry	Rao et al. (2017)

DMSO – dimethyl sulfoxide, DMEM – Dulbecco's modified eagle medium, DNPH – 2,4-dinitrophenylhydrazine, AFB_1 – aflatoxin-B_1, AFB_2 – aflatoxin-B_1, AFM_1 – aflatoxin-M_1

including rapid processing, high effectiveness, affordability in terms of equipment, and precise control over dosage (Liu et al., 2018). Increasing electron beam radiation dose effectively degraded aflatoxin in the study of Hashemi et al. (2020). An EBI dose of 7 kGy resulted in a 77.17% reduction of AFB_1 content in pistachio kernels. For Brazil nuts, AFB_1 levels decreased by 53.32% and 65.66% at EBI doses of 5 and 10 kGy, respectively. EBI showed a more significant detoxification effect in peanut meal with elevated AFB_1 levels and increased water content. The highest level of AFB_1 degradation, reaching 70% of its content was achieved in EBI treatment at a dose of 300 kGy (Liu et al., 2018). Furthermore, subjecting food to EBI at doses up to 10 kGy resulted not only in the absence of harmful residues but also minimal nutrient losses (Calado et al., 2014).

Degradation mechanisms and resulting products

The degradation of aflatoxins using EBI can be broadly classified into two categories. In the direct oxidation pathway, electron beam energy causes the breakdown of chemical bonds in molecules, resulting in alterations in atomic and electron positions. In the indirect pathway, irradiation of water and small molecules generates free radicals, which exert strong oxidative effects on organic molecules (Wang et al., 2015a). Only two degradation products ($C_{14}H_{12}O_5$ and $C_{17}H_{14}O_5$) of AFB_1 were identified in acetonitrile, both resulting from the ring structure opening at the AFB_1 toxic sites (Wang et al., 2015a). In a study by Liu et al. (2016), five by-products were identified when AFB_1 was subjected to EBI in an aqueous medium. These by-products were mainly formed in the substitution and addition reactions occurring at the C8C9 double bond and methoxy group of AFB_1. These findings are likely associated with the elevated presence of free radicals in irradiated water, including hydroxyl radicals, hydrogen atoms, and hydrogen peroxide. In summary, the application of EBI led to a reduction in AFB_1 initial toxicity, mainly due to the removal of the double bond at the terminal furan ring.

Low-temperature plasma: Principles and applications

Plasma is considered the fourth state of matter, alongside solids, liquids, and gases. It is a neutral ionised gas composed of highly reactive components, including free radicals (e.g., reactive oxygen or nitrogen species), electrons, charged ions, neutral excited particles, and ultraviolet photons (Sruthi et al., 2022). Plasma can be broadly classified into two groups, i.e., thermal and non-thermal, depending on its temperature (Wu et al., 2021). Thermal plasma remains in a fully ionised, thermally balanced state where electrons and heavier particles exist at temperatures of thousands of degrees Kelvin (Nguyen et al., 2022). Non-thermal plasma, on the other hand, can be further subdivided into local thermal equilibrium plasma and non-equilibrium plasma (Deng et al., 2020). During the discharge of non-equilibrium plasma, while the electrons are at high temperatures, the heavy particles remain at very low temperatures, resulting in the overall low-temperature system. This is why such plasma is often referred to as cold plasma. As a relatively new non-thermal method for food processing, cold plasma has been increasingly employed for the rapid food detoxification from aflatoxins (Hojnik et al., 2017). For instance, subjecting peanuts to radio frequency plasma (RFP) at 60 watts for 12 minutes resulted in a reduction of AFB_1 content by more than 90% (Devi et al., 2017). In addition to RFP, cold plasma can be generated through various apparatuses, including corona discharge (CD), dielectric barrier discharge (DBD), and atmospheric pressure plasma jet (Niveditha et al., 2021). The types of reactive species generated by individual systems can vary significantly, directly affecting the efficiency of aflatoxin degradation. For example, the commonly used DBD system can produce ozone, CHO•, and hydrogen peroxide during the discharge, all of which readily react with the target substance (Shi et al., 2017a). Moreover, the working gas between the electrodes will generate reactive species when ionised by the high voltage, serving as another critical factor influencing the diversity and concentration of reactive species and driving the plasma chemistry. The levels of ozone and NOx produced by high-voltage atmospheric cold plasma (HVACP) using modified atmospheric gas (MA65; 65% O_2, 30% CO_2, 5% N_2) as the filler gas were shown to be significantly higher compared to using air. Employing MA65 as the working gas resulted in a 78.9% reduction in AFM_1 content, whereas only a 38.5% reduction was observed with air as the filler gas (Nguyen et al., 2022). Additionally, an increase in relative humidity led to a greater concentration of OH•, produced either by HVACP or via the ozone-water reaction. This increase in relative humidity, in turn, enhanced the efficiency of aflatoxin degradation, suggesting that relative humidity levels also play a role in regulating reactive species generation.

Reaction mode, exposure time, and material characteristics are additional key factors influencing the effectiveness of cold plasma in degrading aflatoxins (Shi et al., 2017b). Exposure of AFB_1 to nitrogen gas plasma was shown to result in 75% degradation of the toxin within 15 min, and nearly its complete removal after 30 min of treatment (Sakudo et al., 2017). In another study, DBD cold plasma treatment for 180 s resulted in a 65% reduction of AFB1 on glass slides, while only 52% of AFB_1 was degraded in pistachio samples (Makari et al., 2021b).

Degradation mechanisms and resulting products

Reactive species are the main drivers of aflatoxin degradation during cold plasma treatment, initiating a range of potential reactions. During HVACP treatment, a total of six degradation products were identified (Shi et al., 2017a), which were assigned to two distinct degradation pathways. The first pathway mainly consisted of addition reactions, where H_2O, H, or CHO were incorporated into the C8C9 double bond of AFB_1, partly promoted by ozone. The second pathway involved HO2•-induced epoxidation by and oxidation due to the combined effect of OH•, H_2O_2, and O_3 on the difuran moiety of AFB_1. Nevertheless, the choice of working gas may influence degradation products, as Srivastava et al. (2012) observed six by-products that underwent primarily hydroxyl modification and terminal furan cleavage. Wang et al. (2015b) investigated the degradation process of AFB_1 using low-temperature RFP, and showed that the C8-C9 double bond and the lactone ring in five potential AFB_1 degradation products were all decomposed through hydrolysis, addition, and elimination reactions involving H• and OH• radicals. This implies that RFP demonstrates superior efficacy in detoxifying aflatoxins. The detected degradation products were composed solely of C, H, and O elements, indicating that reactive oxygen species play a significant role in the degradation of aflatoxins in cold plasma treatment. Currently, the utilisation of cold plasma for food detoxification remains predominantly in the research phase. The potential food safety concerns and quality implications resulting from complex chemical reactions still require further assessment before approval for industrial food processing.

Ultrasonic waves: principles and application

Ultrasounds are mechanical waves with frequencies above the threshold audible for humans (>20 kHz), generated by converting electrical pulses into acoustic energy using a specialised transducer (Bhargava et al., 2021). In the food industry, ultrasound applications are distinguished based on power and frequency, falling into two categories: low-intensity ultrasound (<1 W/cm^2, >100 kHz) and high-intensity ultrasound (>1 W/cm^2, 20–100 kHz), depending on power and frequency (Taha et al., 2023). Low-intensity ultrasound, primarily used for diagnostic purposes, is typically employed in non-destructive testing to assess the physicochemical quality of foods. In contrast, high-intensity ultrasound is known for its stronger acoustic cavitation effect, which causes bubble formation and energy release when these bubbles expand to a critical size and subsequently collapse. Consequently, high-intensity ultrasound finds broader application in food processing.

Interest in high-intensity ultrasound as a sustainable method for aflatoxin degradation in food is growing. Existing studies summarised in Table 2 indicate that ultrasound-driven degradation of aflatoxins is primarily influenced by four key factors: power intensity, treatment duration, duty cycle, and substrate used. The most effective AFB_1 degradation, reaching 96.5%, was obtained in an aqueous solution with ultrasound applied at a power intensity of 4.4 W/cm^3, a 25% duty cycle, and time of 40 min. In comparison, only 11% degradation of AFB_1 occurred in maize slurry when continuous ultrasound was applied at a power intensity of 1.65 W/cm^3 for 10 min (Liu et al., 2019a). Similarly, a 21.2% reduction in AFB_1 was reported in maize powder exposed to 1.65 W/cm^3 ultrasound with a 50% duty cycle for 10 min. However, the latter treatment had an adverse impact on the nutritional quality of maize, including the content of fatty acids and amino acids (Liu et al., 2022). Further, a study on the effects of 40 kHz ultrasound on AFB_1, AFB_2, AFG_1, and AFG_2 degradation showed that ultrasound was a more efficient method for aflatoxin degradation compared to electrolytic water and UV treatment, reducing the content of all four aflatoxins by over 55% (Castro-Ríos et al., 2021).

Degradation mechanisms and resulting products

Liu et al. (2019b) investigated the degradation products and pathways of AFB_1 using ultrasound and identified specific transformations resulting from these interactions. Moreover, the cavitation effect produced by ultrasound can break covalent bonds in water molecules, generating free radicals such as OH•, H•, and H_2O_2 (Ince, 2018). These free radicals subsequently react with aflatoxins (Liu et al., 2019b).

However, it is worth noting that currently the research on the safety of sonolysis products of aflatoxins remains limited, given the early-stage development of ultrasound applications for aflatoxin degradation. The cavitation effect occurring in liquid media may significantly alter the physicochemical properties of foods. Moreover, combining ultrasound with other techniques such as thermosonication and manosonication has shown potential to produce synergistic effects, improving the degradation of aflatoxins in food products (Condón-Abanto et al., 2016).

Regulatory concerns associated with the detoxification process

The FDA considers aflatoxin contamination in food and feed to be unavoidable, prompting countries, regions, and international organisations to implement regulations aimed primarily at minimising its presence (Jallow et al., 2021). In developed nations, food and feed production systems are largely operated by a few major industrial processors. This enables effective implementation of preventive measures and stringent regulations, consequently, aflatoxin contamination poses limited health risks. The opposite is true in developing countries, where economic constraints and wide-spread small-scale storage at households and farms hinder effective management of aflatoxin contamination (Williams et al., 2004). The development of detoxification methods mainly focuses on addressing these issues in developing countries, and target crops that, though contaminated with aflatoxins, retain commercial value as they are not severely infected by mould. The European Food Safety Authority (EFSA) assesses applications related to procedures and products designed to decontaminate aflatoxin in animal feeds. To determine the eligibility of detoxification methods, Commission Regulation (EU) 2015/786, enacted on May 19, 2015, established criteria for the acceptability of detoxification procedures applied to animal feed products. It specified that only detoxification processes that have undergone scientific evaluation by EFSA with positive results and have been approved by the relevant authority may be used to decontaminate affected feeds (Knutsen et al., 2017). For physical detoxification, the criteria are as follows: (a) the process must be effective, (b) it should not negatively affect the characteristics and nature of the feed, and (c) there must be a safe disposal method for the removed portion of the feed. For chemical or microbiological detoxification processes, the criteria include: (a) use of a well-characterised and approved chemical substance or microbiological agent, (b) effectiveness and irreversibility, (c) absence of harmful residues of the chemical or microbiological agent used in the detoxification process, (d) no reaction products or metabolites that endanger animal or public health or the environment, and (e) no adverse effects on the feed's characteristics and nature.

EFSA evaluated an application for detoxification of groundnut press cake to reduce aflatoxin levels through ammoniation. The Panel on Contaminants in the Food Chain (CONTAM) concluded that the proposed detoxification process could not be endorsed, primarily due to the lack of genotoxicity studies on processed feed extracts and identified degradation products (Pankaj et al., 2018). In contrast, although many novel degradation techniques are still in their early stages of development and have not yet been commercialised, certain technologies, including electron beam, pulsed light, and ozone, have received FDA approval for the purification of food and feed products (Guo et al., 2021). By examining the acceptance criteria for the three categories of detoxification processes, it becomes apparent that physical methods have an advantage in preventing the formation of harmful residues or contaminants, which are more likely to occur with most chemical and biological approaches during detoxification. As research on the efficacy of aflatoxin degradation, product safety, and the effects on materials continues to advance for the discussed physical degradation methods, it is expected that these techniques will eventually be adopted commercially for the detoxification of feeds and potentially even human food products contaminated with aflatoxins.

Future challenges

As mentioned earlier, decontamination methods have made significant progress in aflatoxin degradation in recent years. Although many of the developed solutions remain in the laboratory phase, the theoretical foundations of each technology underlying the mechanism of aflatoxin degradation and the safety of degradation products continue to improve. However, challenges remain that require further investigation to ensure the successful transition of these methods into more established industrial applications.

The structures and degradation pathways of the products are hypothetical. Even though LC-MS/MS can significantly narrow down potential molecular structures based on information about the parent molecule and characteristic ion fragments, there is no guarantee that the resulting molecular structure of the degradation product precisely matches the actual structure (Diao et al., 2012). Furthermore, aflatoxins generate numerous degradation products, most of which are trace amounts of unidentified compounds with similar molecular weight and structure. Consequently, isolating a specific pure degradation product is difficult, while comprehensive characterisation using methods like near-infrared spectroscopy and nuclear magnetic resonance spectroscopy is unlikely (Kwaśniewicz and Czarnecki, 2018).

Currently, degradation products and pathways are mainly investigated in controlled, isolated systems. Nevertheless, it should be noted that the products formed in different systems can vary significantly, and the detoxification of food products can potentially lead to the formation of even more complex substances compared to those observed in simplified systems consisting of a single substance (Mao et al., 2016). Future research should prioritise the analyses of toxicity and the mechanisms underlying the production of degradation products in real food matrices. Such studies would serve as a valuable reference for identifying appropriate treatments tailored to specific food products.

Toxicological assessments of aflatoxin degradation products have been restricted to *in vitro* experiments and acute *in vivo* studies (Ali et al., 2021). This limitation leads to an insufficient understanding of the safety implications associated with chronic, low-level exposure, as current safety evaluations do not address the long-term consequences of consuming food containing degradation product residues in both humans and animals. Therefore, it is crucial to develop innovative chronic toxicity tests to strengthen the existing toxicological assessment framework for aflatoxin degradation products and expand our knowledge of their potential toxicity.

The industrialisation of most detoxification methods has been impeded by concerns such as undesirable nutrient loss, limited penetration, and food safety issues associated with handling (Ismail et al., 2018). To address the global challenge of aflatoxin contamination effectively, a promising approach involves integrating different detoxification techniques. By combining these methods to leverage their complementary strengths, it may be possible to overcome the limitations of individual approaches and achieve more efficient detoxification.

Conclusions

Aflatoxin contamination poses a significant and persistent threat to both human and animal health, leading to substantial crop losses and economic ramifications for the food industry. Therefore, a comprehensive understanding of the toxicological risks associated with aflatoxins is required. To this end, this study emphasised the development of safety evaluation methods for detoxification. Among the various techniques developed to neutralise toxins, conventional physical detoxification is widely recognised in industrial production. Emerging technologies, such as pulsed light, cold plasma, and ultrasound, while still in the laboratory developmental stage, have shown promising results. However, each technology has its inherent limitations, including challenges like restricted penetration of ultraviolet and pulsed light, uneven microwave heating, and safety concerns related to ionising radiation. A more thorough understanding of the degradation mechanism is essential for improving the rationale and applicability of detoxification methods. This knowledge can help preserve the physicochemical properties and nutritional value of processed foods, while transforming degradation products into non-toxic or less toxic forms. Future research efforts should concentrate on the development of combined detoxification techniques that address the weaknesses and limitations of individual methods. The objective is to achieve a synergistic detoxification effect that exceeds the capabilities of individual methods.

Conflict of interest

The authors declare that there is no conflict of interest.

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