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Irradiation and cold plasma technologies for aflatoxin mitigation: a comprehensive review of degradation mechanisms and cytotoxicity profiles

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Aflatoxins (AFs), primarily produced by *Aspergillus* species, are among the most hazardous mycotoxins due to their widespread occurrence in food and feed and their strong mutagenic and carcinogenic potential. Maintaining aflatoxin levels within permissible limits is critical for human and animal health, emphasizing the need for effective detoxification strategies. This review explores irradiation and cold plasma technologies as promising approaches for mitigating aflatoxin contamination. Irradiation methods, including gamma rays, ultraviolet radiation, electron beams, and X-rays, exhibit high reactivity and penetrability, enabling AF degradation and reduction of toxicity. Cold plasma generates reactive oxygen and nitrogen species that induce oxidative degradation under mild processing conditions. The review also summarizes analytical and biological assays used to evaluate the mutagenicity and cytotoxicity of intact aflatoxins and their degradation products. Evidence highlights these technologies as practical detoxification tools; however, gaps remain regarding the detailed mechanisms of aflatoxin degradation and the chemical identity and toxicological profiles of byproducts. Furthermore, although food irradiation up to an average absorbed dose of 10 kGy has been widely recognized as safe, additional toxicological and epidemiological studies on specific aflatoxin degradation products would further strengthen the safety assessment of irradiated commodities. Advancing this knowledge will support the broader adoption of irradiation and cold plasma technologies as viable, sustainable tools for reducing aflatoxin risks in global food and feed supplies.

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Sustainability spotlight

Aflatoxin contamination poses a significant challenge to sustainable food systems by threatening food safety, public health, and global trade. Non-thermal technologies such as irradiation and cold plasma provide residue-free strategies for degrading aflatoxins while preserving the nutritional quality of food commodities. Evidence from cytotoxicity studies indicates that the degradation products formed after treatment exhibit substantially lower toxicity, ensuring consumer safety. Compared with conventional chemical or thermal treatments, these approaches reduce post-harvest losses, improve resource utilization, and minimize environmental impact. By enhancing food safety and supporting efficient use of agricultural products, these technologies contribute to sustainable food processing and align with the United Nations Sustainable Development Goals, particularly SDG 2 Zero Hunger, SDG 3 Good Health and Well-Being, and SDG 12 Responsible Consumption and Production.

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1. Introduction

Fungal contamination of agricultural commodities and associated production of mycotoxins result in significant economic losses and pose severe health risks worldwide. One of the physical processes of food processing, known as food irradiation, involves exposing prepared or bulk foods to radiation treatment. Unlike conventional heat pasteurization, irradiation inhibits microbial growth at low temperatures, a process termed "cold pasteurization." Since 1981, a total dose of up to 10 kGy has been approved for food irradiation by several

international organisations.[1] Later, greater than 10 kGy exposure was deemed safe for a few selected products and markets.[2]

Mycotoxins, toxic secondary metabolites of filamentous fungi, frequently contaminate food and feed crops, threatening both human and animal health.[3] Among these, aflatoxins (AFs)—a group of bifuranocoumarin derivatives—are primarily produced by *Aspergillus* species, including *A. flavus*, *A. parasiticus*, *A. niger*, *A. pseudotamarii*, *A. fumigatus*, and *A. sydowii*.[4] More than 18 AFs have been identified, with aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), G_2 (AFG_2), and M_1 (AFM_1) being the most prevalent; among them, AFB_1 is the most toxic.[5] The International Agency for Research on Cancer classifies AFB_1 as a Group I carcinogen due to its mutagenic, teratogenic, and carcinogenic effects.[6]

Regulatory agencies enforce strict limits on aflatoxin contamination in food and feed. In the European Union, the maximum permitted level of AFB_1 in food for direct human consumption is 2 μg kg^{-1}, with total aflatoxins typically limited to 4–15 μg kg^{-1} depending on the commodity.[7,8] In the United States, the U.S. Food and Drug Administration establishes action levels of 20 μg kg^{-1} total aflatoxins in food and feed and 0.5 μg kg^{-1} AFM_1 in milk products.[9] Similarly, India permits a maximum level of 30 μg kg^{-1} aflatoxins in food commodities under the Food Safety and Standards Regulations (2011), while limits in China range from 0.5 to 20 μg kg^{-1} AFB_1 depending on the product. Brazil and Japan regulate total aflatoxins at approximately 5–20 μg kg^{-1} and 10 μg kg^{-1}, respectively, and many African countries adopt limits within the range of 5–20 μg kg^{-1} total aflatoxins.[10] In this context, "high aflatoxin contamination" generally refers to concentrations exceeding these internationally recognized regulatory thresholds established to protect food safety.[11] However, the contamination of aflatoxins varies significantly across different crop plants. Aflatoxins are an issue not only during cropping but also during handling, processing, storage, and transportation because of their high stability.[3] The countries that are suffering from high aflatoxin contamination in food commodities are shown in Fig. 1.[12–15]

Several physical, chemical, and biological methods have been developed to degrade mycotoxins. Among the physical detoxification methods, irradiation and cold plasma have been widely used for the detoxification of mycotoxins in food and feed. Since the 1970s, research has shown that radiation has several practical uses in the food industry, including the sterilization of fresh and frozen meat products, the preservation of the quality of perishable crops, and the prevention of sprouting in potatoes and onions.[16] Over the last decade, due to its benefits, including high efficiency, sensitivity, high precision, and resolution, high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS) technology has been utilized for the identification of mycotoxin degradation products. Additionally, enhanced determination techniques, such as UPLC-MS, UPLC-Q-TOF-MS/MS, and HPLC-MS-MS, have been developed to identify and detect various products in degraded mixtures.[17]

Fungal contamination and aflatoxin production are strongly influenced by environmental and storage conditions. High temperature (25–35 °C), elevated moisture levels, and water activity above 0.80–0.85 favor the growth of toxigenic fungi such as *Aspergillus flavus* and *A. parasiticus*.[18] Inadequate post-harvest drying, grain moisture above 13–15%, insect damage, and poor storage ventilation further accelerate fungal proliferation and toxin formation in stored commodities.[19] However, in situations where optimal storage conditions cannot be ensured, radiation-based interventions may serve as an additional approach to reduce fungal load and mitigate aflatoxin contamination in stored commodities.

However, most research has not fully understood and defined the mechanism of aflatoxin degradation using irradiation and cold plasma. This review summarizes current knowledge on irradiation and cold plasma induced degradation of aflatoxins, highlights the mutagenicity and cytotoxicity of degradation products, and identifies research gaps to guide future investigations.

2. Detoxification by irradiation

The term "irradiation methods" typically refers to ionizing or partially ionizing treatment using short wavelengths of electromagnetic radiation, such as gamma or UV radiation. A considerable detoxification rate and nearly no quality modification were observed with an adequate dose of gamma irradiation.[20] There are two types of radiation: ionizing (such as X-rays, gamma rays, and electron beams) and non-ionizing (such as ultraviolet rays, microwaves, infrared rays, radio waves, and visible light).[21] In previous years, the use of gamma irradiation and ultraviolet irradiation for aflatoxin decontamination has produced encouraging outcomes. The remaining irradiation technologies are currently being investigated for potential applications in aflatoxin degradation.

Ultraviolet, gamma, and e-beam radiation are the three primary forms of food irradiation techniques used globally. These techniques can eject electrons out of their orbital shells from atoms, thereby "ionizing" the atoms, which is the origin of the term "ionizing radiation".[22] In this context, ionizing radiation technology can be effectively applied in the food sector. The detoxification of aflatoxins by irradiation is primarily attributed to the generation of highly reactive species that attack the molecular structure of the toxin. Gamma and e-beam irradiation act through radiolysis of water, producing hydroxyl radicals ($^{\bullet}OH$), hydrated electrons (e_{aq}^{-}), and hydrogen radicals ($^{\bullet}H$), which interact with the double bonds in the furan and coumarin rings of aflatoxins, leading to bond cleavage and structural rearrangements.[23] UV irradiation, on the other hand, induces photochemical degradation through direct absorption of photons, resulting in electron excitation, bond disruption, and the formation of photoproducts with reduced toxicity.[24] In all cases, the furan double bond at the C_8–C_9 position of AFB_1 is considered the critical target for degradation, as it is responsible for the toxin's mutagenic and carcinogenic activity.[65] Recent studies have demonstrated encouraging results with both gamma and UV irradiation in reducing aflatoxin levels in contaminated commodities.

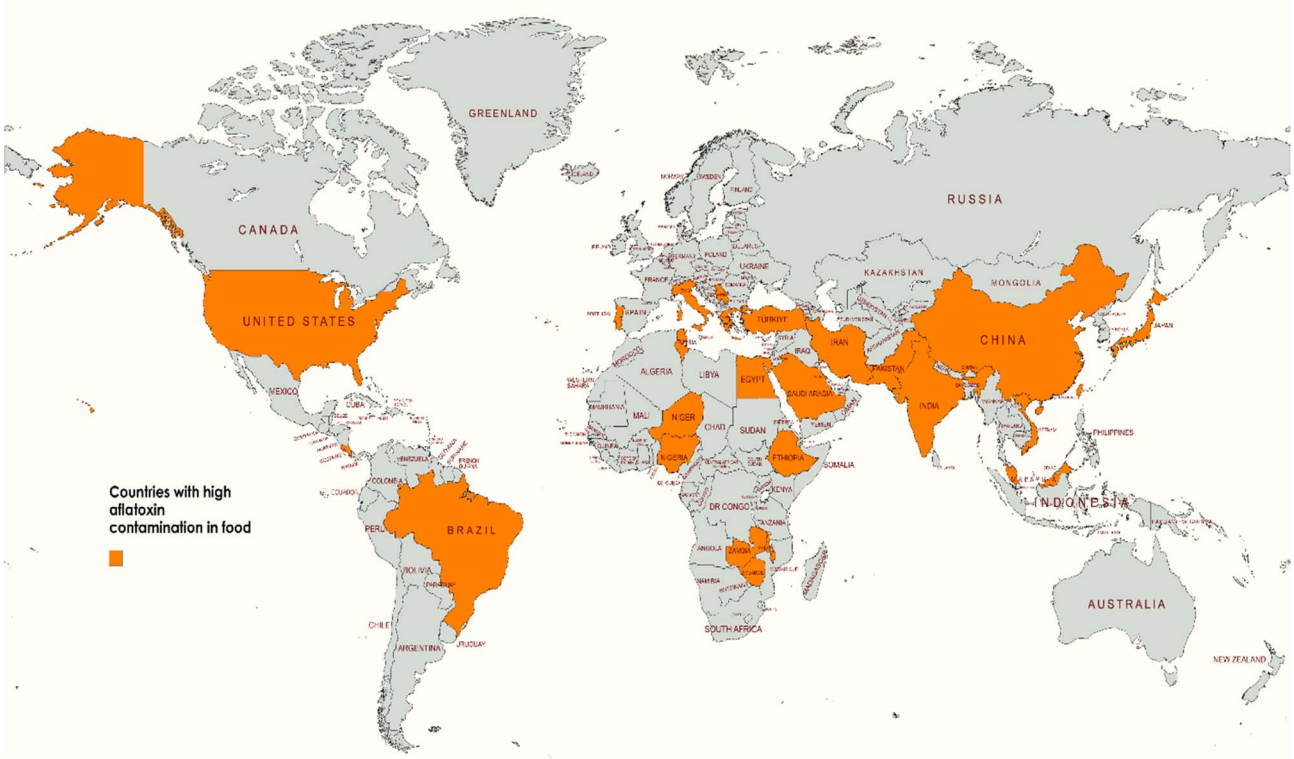

Fig. 1 Countries with prevalence of aflatoxin contamination in food. Countries highlighted in orange represent regions where high aflatoxin contamination has been reported in published literature. Grey-coloured regions do not indicate the absence of contamination but rather limited available data or a lack of reports of high contamination levels (created by the authors using https://www.mapchart.net).

3. Mechanism of radiation interaction with aflatoxin and its degradation

3.1. Gamma irradiation

Gamma rays are electromagnetic radiation that is produced when radioactive isotopes decay (*e.g.*, ^{60}Co, ^{192}Ir, ^{139}Cs, and ^{70}Tm). The high reactivity and penetrability of gamma rays make them the primary source of radiation for food. Up to a total dose of 10 kGy, irradiating food poses no toxicological, microbiological, or nutritional issues.[25]

Both direct and indirect effects are possible from the irradiation. Irradiation can directly harm a variety of microorganisms by damaging their DNA. However, radiolysis of cellular water proceeds when irradiation has an indirect effect. Oxidative radicals are the resultant product. Numerous radiolysis products formed when cells are exposed to radiation exhibit high reactivity with other cellular components, leading to reactions such as ionization and excitation. Hydroxyl radicals can harm DNA by removing hydrogen atoms from sugar and DNA strands (containing four bases), while free radicals can disrupt the structure of organic molecules.[26]

Gamma-ray irradiation significantly affects the germination of *Aspergillus* hyphae. In addition to causing colonies to die after germination, high irradiation doses can also cause mycelium to cease growing altogether, which results in reproductive death.[27] Gamma irradiation has been reported to induce ultrastructural modifications in the cytoplasm and cell wall of *Aspergillus flavus* and *Aspergillus ochraceus* exposed to radiation.[28] In comparison to the control strains, the irradiation strains produced double the number of mycotoxins.[28]

3.1.1. Gamma irradiation effect on aflatoxin degradation. Makari *et al.*[62] conducted a study to investigate the effect of gamma irradiation on *A. flavus* and AFB_1 in pistachio nuts. The findings showed that the viable spore population of *A. favus* was reduced by around 5 logs after the gamma irradiation treatment at dosages of 4 kGy and 6 kGy. The highest levels of AFB_1 degradation in pistachio samples were 73.26% and 83.36%, respectively, at dosages of 4 and 6 kGy.

Tatar *et al.*[36] studied the effect of gamma irradiation in combination with calcium oxide on the detoxification of aflatoxin (AFB_1, AFB_2, AFG_1, and AFG_2) in wheat grains. Wheat samples containing mould spores were combined with varying concentrations of calcium oxide (0, 0.5, and 1%). The samples were subjected to gamma irradiation (0, 5, 10, 15, and 20 kGy) after 20 days. The findings showed that *A. flavus*, AFB_1, and AFB_2 contamination were significantly impacted by calcium oxide, gamma irradiation, and their interactions. In addition, toxins, including AFG_1 and AFG_2, were not detected in the sample. The highest suppression of aflatoxin synthesis was attained at 0.5% CaO, and an additional decrease in AFB_1 and AFB_2 was seen when irradiation was combined with CaO.

3.1.2. Gamma-irradiation aflatoxin degradation product. Aflatoxin degradation depends heavily on the production of highly reactive free radicals, such as hydrogen radical ($H^{\bullet}$),

superoxide radical ($O_2^{\bullet}$), and hydroxyl radical ($OH^{\bullet}$), which can be facilitated by gamma irradiation.[63] Wang *et al.*[64] identified the radiolytic products of AFB_1 using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. The precise mass measurements of the ions and comparable fragmentation routes of AFB_1 contributed to the identification of the radiolytic products. The majority of the radiolytic products were formed because of the addition reaction that the free-radical species in the methanol–water solution induced. Because of the addition reaction that took place on the double bond in the terminal furan ring, the toxicity of radiolytic products was considerably lower than that of AFB_1 (Fig. 2). Domijan *et al.*[65] investigated the composition of AFB_1 radiolytic products, and the findings showed that the insertion of free radicals during gamma irradiation had destroyed the 8,9 double bonds of the terminal furan ring in the majority of radiolytic products. It is well known that the toxicity of AFB_1 is related to the presence of a double bond in the terminal furan ring. Several variables, including irradiation dose, mycotoxin quantity, water content, and matrix composition, influence the detoxification of aflatoxin with the aid of gamma radiation. Gamma irradiation is thus accepted to be a valuable technique for AFB_1 detoxification.

3.2. Ultraviolet irradiation

Ultraviolet (UV) irradiation has long been recognised as an efficient physical technique for mycotoxin eradication due to its photosensitivity towards radiation. It has the benefits of being both cost-effective and environmentally friendly, as it produces no waste or hazardous by-products.[66] Ultraviolet irradiation is a decontamination method that exposes food to radiation with wavelengths ranging from 100 to 400 nm. The effects of UV radiation on decontaminating mycotoxins are comparable to those of gamma radiation. However, the use of UV on solid foods is limited by the "shadow effect" and its low penetrability.[67] A primary source of concern regarding this cutting-edge photochemical detoxification technique is the unknown properties of the radiation-degraded products of aflatoxins and their potential toxicity, despite UV irradiation having long been recognized as an efficient physical method for aflatoxin detoxification due to its sensitivity to light.[63]

3.2.1. Effect of UV irradiation on aflatoxin degradation. Faraji *et al.*[48] observed the highest degradation in Iranian rice at a dose of 4.88 J cm^{-2}, there was a significant decrease in AFG_1 and AFB_2 by 65.2% and 28.9%, respectively. Additionally, UV irradiation with lower energy and reduced risk can decrease the amount of aflatoxin in food.

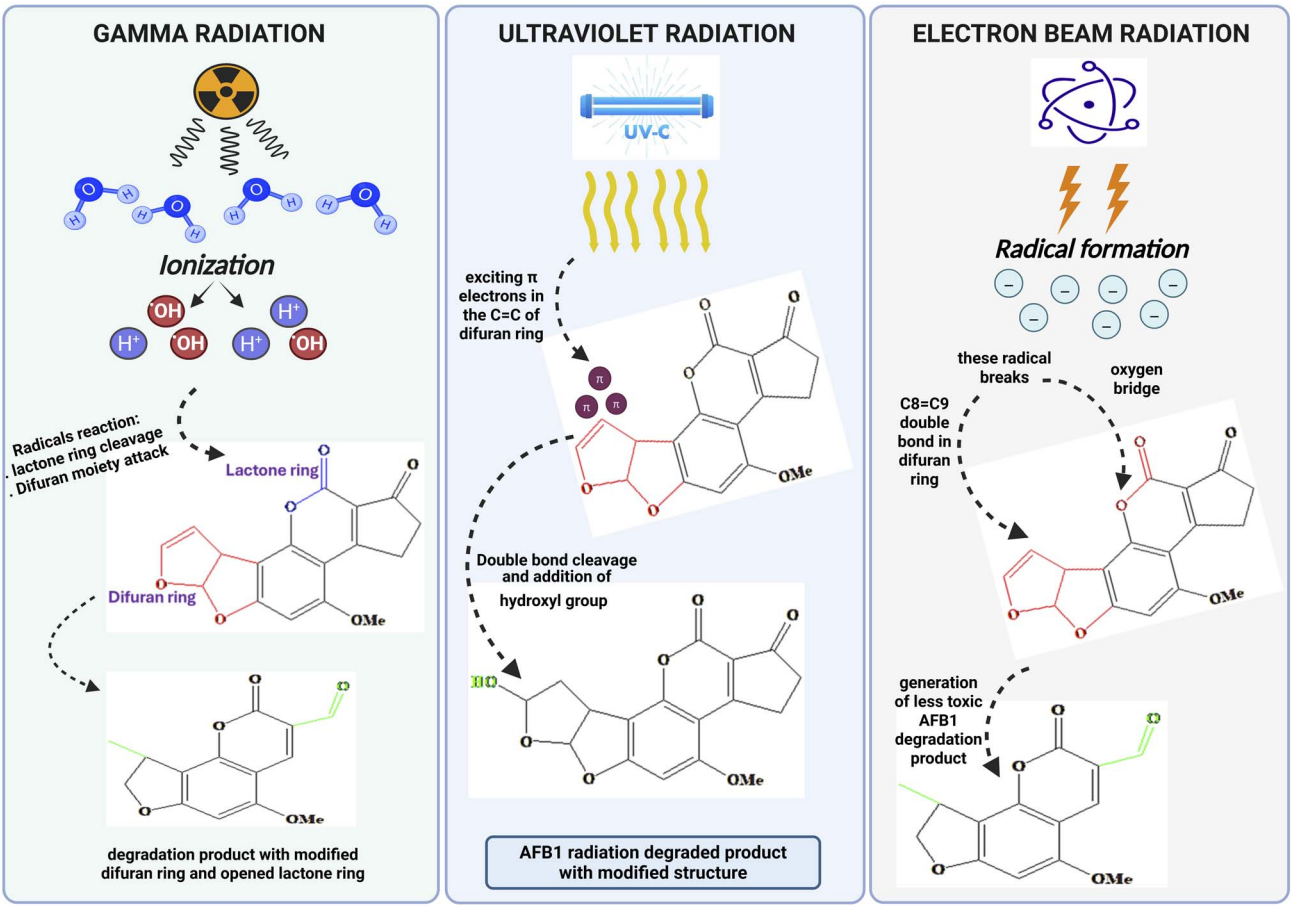

Fig. 2 Graphical representation of the mechanism of AFB_1 degradation product formation after interaction with different types of radiation (created by the authors using BioRender. Chauhan, A. (2025) https://BioRender.com/u2e3v6s).

Shen and Singh[8] examined how three different types of low-pressure (LP) UV lamps covering the UV-A (maximum emission: 365 nm), UV-B (maximum emission: 310 nm), and UV-C (maximum emission: 254 nm) ranges, as well as a 2000 W medium-pressure UV lamp covering the UV-A to UV-CLP range, affected the reduction of AF and the change in oil quality in peanuts. In artificially spiked, peeled kernels, UV-A lamp treatment has demonstrated the maximum AF reduction, with no apparent oil damage. A 40% reduction in AF was observed after peeled kernels inoculated with *Aspergillus nomius* were exposed to two LP UV-A lamps (2.76 mW cm^{-2}) for 1 h.[8]

After ten days of incubation and UV irradiation treatment with a dose of 8370 mJ cm^{-2}, the highest reduction in the *Aspergillus flavus* count was observed, attaining a reduction of 4.4 log CFU per g in maize and 3.1 log CFU per g in peanuts. AFB_1 degradation levels in maize and peanuts varied from 17 to 43% and 14 to 51%, respectively, depending on the treatment.[68]

3.2.2. Aflatoxin UV-degradation product. The lactone ring in the coumarin moiety and the C_8–C_9 double bond are key structural features of aflatoxins that contribute to their reactivity and toxicity.[66] Ultraviolet radiation attacks the double bond in the terminal furan ring and the methoxy group, attaching a hydroxyl group, which leads to the formation of three different degradation products. It has been demonstrated that UV treatment converts AF into less hazardous molecules.[69] Further in AFB_2, UV interacts with the methoxy group, and it is replaced by a hydroxyl group, leading to the formation of a degradation product ($C_{16}H_{12}O_6$). The same phenomenon as in AFB_2 was also observed in AFG_1, where a hydroxyl group replaced the methoxy group during UV radiation treatment, resulting in the formation of a degradation product ($C_{16}H_{14}O_7$).[66] The effect of UV radiation treatment on the aflatoxin structure is shown in Fig. 3. According to the findings, AFB_2 demonstrated greater resistance to UV treatment than AFB_1 and AFG_1. AFB_1 may be more susceptible to photodegradation and photo-oxidation due to the C_8–C_9 double bond in its terminal furan ring than AFB_2, which is more stable due to the absence of this double bond.[70]

Similar outcomes were found in another study, where UPLC-MS was used to determine the detoxification products and potential degradation process of AFB_1 and AFM_1 exposed to UV-A LED in water. Furthermore, the toxicity of UV-A-exposed ultrapure water containing AFB_1 or AFM_1 to the Hep-G_2 cell line was reduced due to the damage caused by $C_8 = C_9$.[71]

Another study conducted photolysis of AFB_1 using ultraviolet light in methanol and acetone solutions for analysis of UV-treated AFB_1 effects in various solutions and showed how the matrix has a diverse impact on the resultant detoxification pathways and end-products. In this investigation, the photodegraded products of AFB_1 were identified using nuclear magnetic resonance spectroscopy (NMR). Using HPLC purification, many end-products and stereoisomers were produced, and UPLC-Q-TOF-MS/MS technology was used to hypothesize their origins. The findings showed that the free radicals generated by ultraviolet irradiation might target the double bond on the bifuran moiety of AFB_1 more efficiently in methanol and acetone solutions.[72]

The physicochemical and sensory properties of food and feed are not significantly adversely affected by exposure to moderate amounts of ultraviolet light.[73] However, due to its limited ability to penetrate solid materials, food products with a high quantity of suspended solids are decontaminate with low efficiency.[74]

In peanut samples, Chang *et al.*[75] assessed the effectiveness of UV irradiation on AFB_1. According to UPLC-Q-TOF/MS analysis, the O_3 that was converted from O_2 under UV treatment was crucial in destroying AFB_1's hazardous sites and facilitating the complete breakdown of AFB_1. The O_3's dipole nature suggests that a 1,3-cycloaddition of O_3 at the C_8–C_9 double bond in AFB_1 could be the mechanism of degradation. This product may regroup into a molozonide derivative after the primary ozonide is formed, producing a range of carbonyl compounds (aldehydes and ketones). According to the suggested photodegradation mechanism, the investigation did not detect R–COOH, R–CHO, R–CO–R′, or CO_2.

3.3. Electron beam irradiation

Electron beam irradiation (EBI) is a cutting-edge and innovative nuclear processing technique that can be used to destroy organic molecules through direct or indirect oxidation. In the direct oxidation mode, organic molecules are directly destroyed by radiation. In the indirect oxidation mode, tiny molecules and water are exposed to radiation, which produces free radicals that damage organic molecules.[69]

Soft electron irradiation represents a variation of conventional electron-beam processing in which low-energy electrons are applied to the sample. Due to their lower energy, soft electrons exhibit a limited penetration depth compared with traditional high-energy e-beam irradiation and are therefore mainly suitable for surface or near-surface decontamination applications. As a result, the sample's sensory and nutritional value is less affected. However, because the e-beam lacks the energy to reach the sample's deeper layers, this method can only be used as a surface treatment.[76]

Aflatoxin degradation by EBI has also been demonstrated, offering the benefits of quick processing, affordable equipment, and precise dose control. By-products that were formed because of the EB irradiation are due to the removal of the double bond in the terminal furan ring of aflatoxins, which might be the cause of detoxification of AFB_1 in an aqueous solution.[77] Nonetheless, EB irradiation has a lower disinfection effectiveness than gamma irradiation. Short processing times, excellent efficacy, lower heat requirements, and dose control are some benefits of employing EBI.[78]

3.3.1. Effect of EBI on aflatoxin degradation. Electron beam irradiation (EBI) has demonstrated significant antifungal effects against *Aspergillus flavus*. Following EBI treatment, thallus morphology was damaged, mycelial growth was suppressed, and both spore germination and aflatoxin biosynthesis were reduced. According to these results, *A. flavus* normal growth and toxin production can be successfully inhibited by EBI. Radiation reduced the integrity of the cell wall and increased the permeability of the cell membrane. High-dose EBI

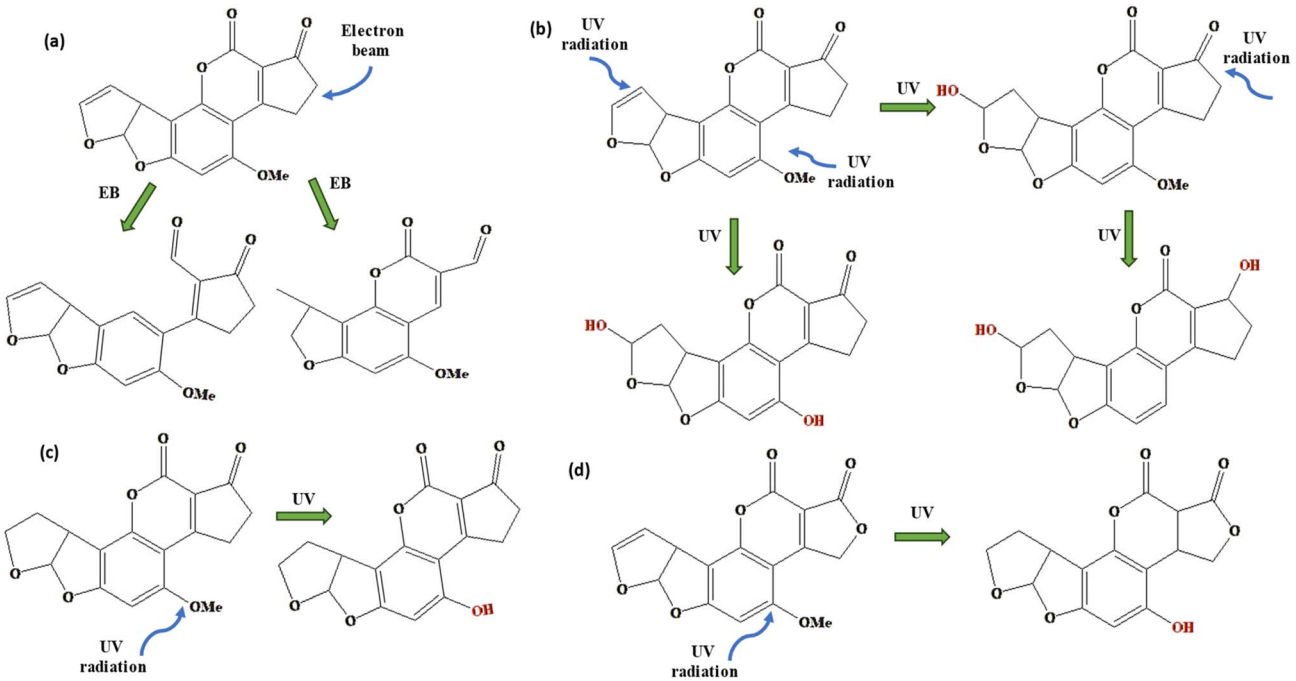

Fig. 3 Effect of irradiation treatment on aflatoxin and how radiation causes changes in the structure of aflatoxin. (a) Degradation phenomena of aflatoxin B_1 (AFB_1) under electron beam (EB) irradiation, (b) degradation phenomena of AFB_1 under ultraviolet (UV) irradiation, (c) degradation phenomena of AFB_2 under ultraviolet (UV) irradiation, and (d) degradation phenomena of AFG_1 under ultraviolet (UV) irradiation.[66,72] (Chemical structures and degradation pathways were illustrated by the authors using ChemDraw based on information compiled from the cited literature).

successfully suppressed growth compared to unirradiated *A. flavus*.[79]

Another aspect of this method that has recently been studied is the breakdown of fungal toxins. The generated electrons are the primary cause of EBI detoxifying activity. There were two mechanisms in this regard: direct electron contacts with mycotoxins, which destroyed their structure directly, and indirect degradation brought about by reactive species, specifically $^{\cdot}H$ radicals and $^{\cdot}OH$ radicals generated during the radioactive degradation of water molecules present in a mycotoxin solution or the matrix of plant, animal, and food materials. Electron energies cause molecular bonds to break down and form free radicals, which can reduce the microbial loads of food products.[80] At 10 kGy, electron beam irradiation treatment decreased mycotoxins by 71% and 78% in both cold-pressed and hot-pressed groundnut cake, respectively. However, some deteriorative changes in the rate of amino acids and proteins were observed.[81]

3.3.2. EBI – aflatoxin degradation product. According to an investigation carried out by Wang *et al.*[70] using ultra-performance liquid chromatography quadruple time-of-flight mass spectrometry (UPLC-Q-TO-MS), an aflatoxin B_1 solution in acetonitrile exposed to an electron beam broke down into two main components. Accurate masses and the suggested chemical formula for the degradation products, 261.1233 m/z ($C_{14}H_{13}O_5$) and 299.1104 m/z ($C_{17}H_{15}O_5$), were also determined. According to the UPLC-Q-TOF-MS result, the degradation of AFB_1 was caused by damage to $C_8 = C_9$ on the furan ring and lactone ring.[70] These findings necessitate additional research in the future to demonstrate EBI's better effectiveness over alternative decontamination technologies. Table 1 presents various recent studies on the detoxification of aflatoxins in food and feed commodities using different irradiation techniques and cold plasma.

3.4. X-ray irradiation

X-ray irradiation is another form of ionizing radiation increasingly investigated for food decontamination and mycotoxin mitigation. Similar to gamma radiation, X-rays produce high-energy photons capable of penetrating food matrices and inducing molecular damage in aflatoxin structures, leading to toxin degradation.[63] Studies have reported significant reductions in aflatoxin levels in commodities such as grains, peanuts, and spices following X-ray exposure. Compared with gamma irradiation, X-ray systems offer advantages including on–off operation, absence of radioactive isotopes, and easier regulatory handling. Although industrial application is still developing, X-ray irradiation is considered a promising alternative technology for enhancing food safety and reducing mycotoxin contamination in food supply chains.[82]

3.4.1. Effect of X-ray irradiation on aflatoxin degradation. X-ray irradiation induces structural modifications in aflatoxin molecules primarily through ionization and generation of free radicals. These reactive species can attack the double bond in the terminal furan ring and the lactone moiety of aflatoxin B_1, which are responsible for its toxicity. As a result, several degradation products such as hydrated derivatives and lower molecular weight fragments have been reported.[82] Studies

Table 1 Summary of research carried out for the detoxification of aflatoxins in food materials

Type of aflatoxin	Irradiation method used	Matrix	Effect	Treatment conditions	References
AFB_1, AFB_2, AFG_1 and AFG_2	Gamma	Almond	At 8 kGy, reduction was AFB_1 62%, AFB_2 22% and AFG_1 20.5%	1, 2, 4 and 8 kGy	29
AFB_1	Gamma	Poultry meat, skin, and liver	At 10 kGy, reduction was 100% in skin, 88.2% in liver and 100% in meat	0, 5 and 10 kGy	30
AFB_1, AFB_2, AFG_1 and AFG_2	Gamma	Sorghum	Gamma rays reduced aflatoxin levels (<0.15 μg kg^{-1} for AFB_1 and AFB_2 and <0.13 μg kg^{-1} for AFG_1 and AFG_2)	2, 4, 6, 8, and 10 kGy	31
Aflatoxin	Gamma	Red pepper	10.96%, 34.25%, and 34.65% aflatoxin reductions at 2, 4, and 6 kGy were found	2, 4, and 6 kGy	32
AFB_1 and AFB_2	Low level gamma	Corn	Reduction in AFB_1 and AFB_2 concentrations (9.11% and 9.40% in 1 day of irradiation) and enhanced antioxidant properties	Placed on a 5 kg radioactive granite bed for 3, 5, 6 and 9 days	33
AFB_1, AFB_2, AFG_1 and AFG_2	Gamma	Roasted peanut	5 kGy dose was ineffective, 10 and 20 kGy caused 59% reduction	5, 10 and 20 kGy	34
AFB_1, AFB_2	Gamma	Maize	The best reduction rates were found at 20 kGy, *i.e.*, for AFB_1 40.1% and AFB_2 33.3%	20 kGy	35
AFB_1, AFB_2, AFG_1, and AFG_2	Gamma with calcium oxide	Wheat	Reduction of aflatoxins at 10 kGy was found	0, 5, 10, 15, and 20 kGy (with 0.5–1% CaO)	36
Aflatoxin	Gamma	Hemp flour	A dose of 4 kGy was found sufficient to reduce aflatoxin content below 4 μg kg^{-1}	1, 2, 3, 4, 6, 8, and 10 kGy	37
Aflatoxin	Gamma	Peanut	Aflatoxin concentration at 10 and 25 kGy was 4.73 and 33.01 ppb; no significant differences in color, hardness, oil content	10 and 25 kGy	38
Aflatoxin	Gamma	Red pepper	100% reduction, at 3.5 kGy no *Aspergillus flavus* spores detected	0.5 to 3.5 kGy	39
AFB_1	Gamma	Peanut	43% reduction found	9 kGy	40
AFB_1	Gamma	Hazelnut	47% reduction found	10 kGy for 10 min	41
Aflatoxin	Electron beam	Pistachio	Doses ≥2 kGy can reduce AFB_1 contamination. The % reduction of AFB_1 at 4 and 6 kGy was 99.69% and 100%	1, 2, 4, and 6 kGy	42
Aflatoxin	Electron beam	Maize slurry	Reduction of 0.3 log (ng g^{-1}) in aflatoxin at 20 kGy	5, 10 and 20 kGy	43
Aflatoxin	Electron beam	Pistachio	Reduction at the specified doses was 38.84%, 48.79%, 53.50%, and 77.17%. Color, texture, and overall palatability of pistachios changed after a dose of 5 kGy	1, 3, 5 and 7 kGy	44
AFB_1	Electron beam	Peanut	70% reduction was found	10 kGy	45
AFB_1	Electron beam	Brazil nut	84.2% reduction was found	10 kGy	46
AFB_1	Ultraviolet	Chili pepper	58% of AFB_1 degradation was observed	10 kGy	47
AFB_1, AFB_2, AFG_1 and AFG_2	Ultraviolet	Iranian rice	AFG_1 65.2%, AFB_2 28.9%, and AFB_1 >70% degradation observed	4.88 J cm^{-2} dose for 40 min	48
AFB_1 and AFM_1	Ultraviolet	Whole milk	Max. dose reduced AFB_1 by 78.2% and AFM_1 by 65.7%	The highest dose for AFB_1 was 836 mJ cm^{-2} and for AFM_1 857 mJ cm^{-2}	49
AFB_1	Ultraviolet	Whole peanut	Detoxification rate increased from 60.8 to 75.0 pmol g^{-1} h^{-1}	15 W UV-C lamp (2.3 mW cm^{-2} UV for 2 h)	50

Table 1 (*Contd.*)

Type of aflatoxin	Irradiation method used	Matrix	Effect	Treatment conditions	References
AFB_1 + AFB_2 + AFG_1 + AFG_2	Ultraviolet	Peanut	79% degradation was observed No significant changes were observed in peroxide value and polyphenolic component	5 mg L^{-1} ozone under UV irradiation (254 nm, 0.35 mW cm^{-2} for 30 min)	51
AFB_1 AFB_2	Ultraviolet	Sudanese peanut oil	≥99.4% ≥99.2% No significant changes were observed in the physico-chemical properties of oil	500 W high-pressure UV lamp (200 mW cm^{-2} for 4 min) with titanium dioxide as a photocatalyst	52
AFB_1	Ultraviolet	Peanut oil	35.1% reduction was found	254 nm for 120 min	53
AFB_1	X-ray irradiation	AFB_1 solution in acetonitrile	81% degradation at 5 μg mL^{-1} (10 kGy), 77% degradation at 10 μg mL^{-1} (10 kGy), and 38% degradation at 20 μg mL^{-1} (10 kGy)	X-ray irradiation up to 10 kGy	54
AFB_1	X-ray irradiation	Maize	No significant reduction in AFB_1 concentration	X-ray irradiation of 0–3 kGy applied to maize contaminated with ∼300 ppb AFB_1	55
AFB_1	Cold plasma (dielectric barrier discharge)	Sesame seeds	27.7% reduction with air plasma (65 → 47 ppb) and 68.6% reduction with argon plasma at 56 W for 8 min (63.4 → 19.9 ppb) observed	DBD cold plasma; air (25% ambient humidity), wet argon (75% humidity), and argon (25% humidity); power 45–56 W; treatment time 1–8 min	56
AFB_1	DBD cold plasma	Peanuts	71.3% reduction (2 min treatment at 80% RH)	HVACP treatment at 90 kV, 2–10 min, air with RH 5–80%	57
AFB_1, AFB_2, AFG_1, and AFG_2	Gliding arc cold plasma	Wheat grains	At 10.88 W for 12 min, cold plasma reduced AFB_1 (64%), AFB_2 (41%), AFG_1 (59%), AFG_2 (40%), and total AFs (61%)	Humid air cold plasma; power 5.39–10.88 W; treatment time 2–12 min	58
AFB_1 and total aflatoxin	Cold plasma (dielectric barrier discharge)	Rice	AFB_1 reduction: 55.34%; total AFs: 56.37% (at 20% moisture content, 65% oxygen content, 100 kV for 60 min)	Parameters varied (moisture content, oxygen level, voltage, and treatment time)	59
AFM_1	Cold plasma	Skim milk	65.0% reduction (air, 20 min) and 78.9% reduction (MA65, 20 min) was observed	HVACP treatment; gases air or MA65 (65% O_2); voltage 60–80 kV; treatment time 1–20 min	60
Aflatoxin	Cold plasma (dielectric barrier discharge)	Corn	62 and 82% decrease in aflatoxin in corn with 1 and 10 min treatment at 40% humidity	Air or modified atmosphere (65% O_2, 30% CO_2, and 5% N_2); power 200 W; frequency 50 Hz; voltage 90 kV; discharge gap 4.5 cm; treatment time 1–30 min	61

indicate that the opening of the lactone ring and disruption of the furan structure significantly reduce the mutagenic and carcinogenic potential of the toxin. Although the exact degradation pathways may vary depending on the irradiation dose and food matrix, the resulting products generally exhibit lower toxicity compared with the parent aflatoxin molecule.[54]

4. Detoxification by cold plasma

Cold plasma, also referred to as non-thermal plasma, is typically generated through electrical discharges in gases at atmospheric or reduced pressure. When a sufficiently high electric field is applied, the gas undergoes electrical breakdown, producing a mixture of charged particles, electrons, ions, and reactive chemical species. These reactions generate reactive oxygen species (ROS) such as atomic oxygen, hydroxyl radicals, superoxide ions, and ozone, along with reactive nitrogen species (RNS) and ultraviolet radiation.[83] These reactive components play a significant role in microbial and fungal inactivation. In food systems, plasma-generated species interact with fungal cells by damaging cell membranes, disrupting cell wall integrity, and inducing leakage of intracellular components, ultimately leading to loss of viability.[84]

4.1. Effect of cold plasma on aflatoxin degradation

A recent study by Shi *et al.*[85] reported similar degradation mechanisms of aflatoxin B_1 when treated with a high-voltage dielectric barrier discharge (DBD) plasma system. Reactive species generated in humid air plasma, particularly ozone (O_3), hydroxyl radicals ($^{\bullet}OH$), singlet oxygen ($^1O_2$), and superoxide anions ($O_2^{\bullet-}$) formed from the ionization of oxygen, water vapor, and carbon dioxide, play a major role in aflatoxin degradation. The proposed mechanism mainly involves ozonolysis reactions characterized by sequential addition and cleavage processes. These reactions include the addition of water molecules, hydrogen atoms, or aldehyde groups to AFB_1, as well as oxidation and epoxidation mediated by hydroperoxyl radicals ($HO_2^{\bullet}$). Oxidative stress reactions also promote the cleavage of the C_8–C_9 double bond in the dihydrofuran ring of AFB_1, leading to structural degradation of the toxin.

The oxidative reactions induced by plasma species can lead to several structural modifications, including epoxidation, hydroxylation, and cleavage of the furan ring (Fig. 4).[86] In addition, plasma-generated ozone can participate in ozonolysis reactions that result in the formation of unstable ozonide intermediates, which subsequently decompose into smaller oxygenated compounds such as aldehydes, ketones, and carboxylic derivatives. These structural transformations significantly reduce the biological activity and toxicity of the original aflatoxin molecule.[87]

Furthermore, reactive nitrogen species produced during plasma discharge, such as nitric oxide ($NO^{\bullet}$) and nitrogen dioxide ($NO_2^{\bullet}$), may contribute to nitration or oxidative degradation reactions that further destabilize the aflatoxin structure. The combined action of ROS and RNS promotes extensive oxidative stress on the toxin molecule, leading to disruption of the lactone ring and bifuran moiety, which are essential for aflatoxin toxicity.[88]

The efficiency of cold plasma-induced degradation depends on several parameters, including plasma power, treatment time, gas composition, humidity, and the physicochemical properties of the food matrix. Increased humidity generally enhances the generation of hydroxyl radicals, thereby improving aflatoxin degradation efficiency.[89] Consequently, optimization of plasma

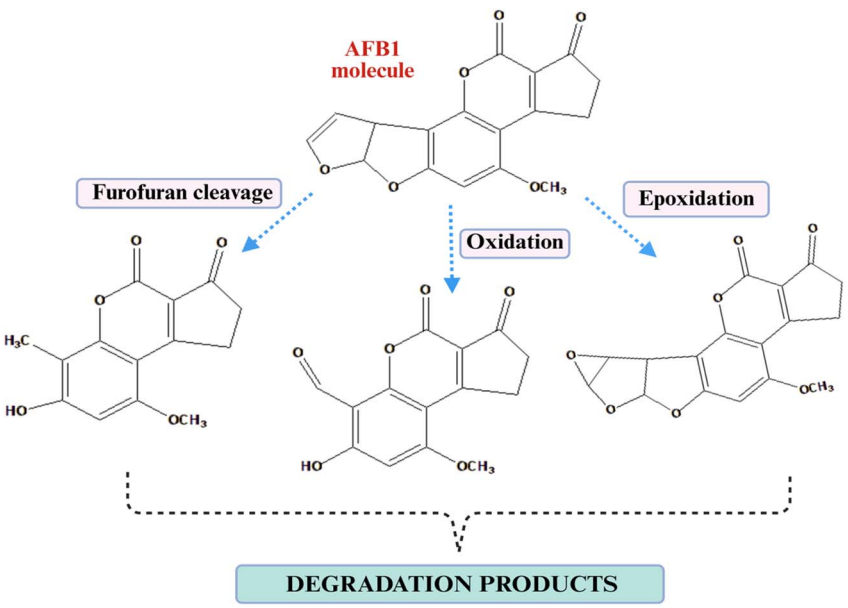

Fig. 4 Proposed degradation pathways of aflatoxin B_1 under cold plasma exposure illustrating key structural transformations including epoxidation and furofuran ring cleavage.[85] (Chemical structures and degradation pathways were illustrated by the authors using ChemDraw based on information compiled from the cited literature).

Table 2 Comparative summary of irradiation and cold plasma treatments for aflatoxin detoxification

Technology	Degradation mechanism	Advantages	Limitations	References
Gamma irradiation	Radical generation (˙OH, ˙H) causing C_8–C_9 bond degradation	High penetration; suitable for bulk foods	Requires a radioactive source and strict regulatory control	90
Ultraviolet irradiation	Photochemical oxidation and bond cleavage	Low cost; simple operation	Low penetration; mainly surface treatment	90
Electron beam irradiation	Electron-induced ionization and radical formation	Rapid treatment; precise dose control	Limited penetration depth than gamma radiation	79
X-ray irradiation	High-energy photon ionization and radical generation	Deep penetration; on–off operation without radioactive isotopes	Higher equipment and operational cost	91
Cold plasma	ROS/RNS-mediated oxidative degradation	Non-thermal; minimal chemical residues	Surface treatment; limited penetration	92 and 93

processing conditions is essential to maximize detoxification while maintaining the nutritional and sensory quality of food products. A comparative summary of irradiation and cold plasma treatments used for aflatoxin detoxification is shown in Table 2.

5. Impact on food quality and organoleptic properties

Although irradiation-based technologies and cold plasma have demonstrated considerable potential for reducing aflatoxin contamination, complete detoxification often requires relatively high irradiation doses and plasma intensities. Several studies have reported that while increasing radiation dose enhances aflatoxin degradation efficiency, excessive exposure may adversely affect the physicochemical and organoleptic properties of treated food matrices. Xu *et al.*[94] reported that irradiation significantly promoted aflatoxin degradation in peanut press cake, with higher doses and moisture content improving efficiency, while only minor changes in nutritional and sensory attributes were observed. Bozinou *et al.*[29] observed that gamma irradiation effectively reduced aflatoxins in almonds with no significant changes in protein and fat content, although α-tocopherol decreased at higher doses. Gamma irradiation and electron beam irradiation reduced *A. flavus* (>4 log) in red pepper powder and gochujang without significant physicochemical changes, while X-ray irradiation showed limited microbial reduction. Sensory evaluation indicated minor flavor changes but no difference in overall acceptability.[39] High-dose irradiation has been associated with lipid oxidation, pigment degradation, textural modifications, and the development of undesirable off-flavors, which may ultimately compromise consumer acceptance.[95] Gamma irradiation (1–10 kGy) in peanuts significantly increased lipid oxidation markers such as peroxide value and malondialdehyde in a dose-dependent manner.[96] Higher doses (10 kGy) also resulted in reductions in fat and protein contents along with changes in fatty acid and amino acid composition.[96] A 2 kGy gamma irradiation dose improved the bacteriological quality of raw whole milk without significantly affecting sensory characteristics during 60 days of refrigerated storage.[97] Gamma irradiation increased TBARS values in raw whole milk, indicating dose-dependent lipid oxidation. NMR analysis confirmed the formation of aldehydes and ketones, with complete degradation of milk fat observed at 3 kGy.[98]

Studies indicate that cold plasma treatment can effectively reduce microbial contamination in food products; however, its impact on quality attributes varies depending on the plasma source and operating conditions. For instance, Lacombe *et al.*[99] reported no significant changes in the respiration rate, color, or firmness in plasma-treated strawberries. Similarly, Yong *et al.*[100] observed substantial mold reduction in beef jerky inoculated with *A. flavus* without significant changes in physicochemical parameters such as shear force, myofibrillar fragmentation index, and metmyoglobin, although slight alterations in sensory attributes were detected after prolonged exposure. Park and Ha[101] reported increased lipid oxidation, indicated by elevated TBARS values, in plasma-treated dried filefish despite moderate reductions in mold counts.

Therefore, although irradiation and cold plasma represent promising non-thermal interventions for aflatoxin mitigation, optimization of treatment parameters is essential to achieve an effective balance between toxin degradation and preservation of food quality attributes.

6. Safety and regulatory considerations in radiation-based aflatoxin mitigation

6.1. Integration with food safety frameworks

Although irradiation technologies show significant potential for aflatoxin mitigation, their application should be integrated within established food safety frameworks. Fungal conidial spores are generally sensitive to radiation and can be inactivated at relatively low doses (<1–3 kGy); however, aflatoxin contamination usually occurs after extensive fungal growth and mycelial development in food commodities.[23] Such contamination reflects failures in preventive measures including good manufacturing practices (GMP), good hygiene practices (GHP), and hazard analysis and critical control points (HACCP). Therefore, irradiation processing should be considered

a complementary intervention rather than a substitute for preventive systems. International guidelines from the Food and Agriculture Organization, International Atomic Energy Agency, and World Health Organization indicate that irradiation doses up to 10 kGy are generally regarded as safe for food processing when applied for microbial decontamination without compromising wholesomeness.[2] Similarly, the Codex Alimentarius Commission recommends that irradiation should be performed under validated conditions using the minimum effective dose required to achieve the intended technological objective.[102] Accordingly, irradiation treatments should follow the As Low As Reasonably Achievable (ALARA) principle to minimize potential impacts on food quality and nutritional integrity.[103] Moreover, the efficiency of radiation-induced aflatoxin degradation is influenced by factors such as food matrix composition, moisture content, and storage conditions, highlighting that irradiation should function as a complementary post-harvest intervention within an integrated food safety strategy.

Also allowing mold contamination to progress to the stage of aflatoxin production and subsequently attempting toxin removal is generally considered an undesirable strategy in food safety management. Extensive fungal growth in food commodities may lead not only to the formation of aflatoxins but also to the production of several other mycotoxins and secondary metabolites that can adversely affect food safety, flavor, and nutritional quality. Gomes *et al.*[104] reported a higher prevalence of mycotoxigenic fungi such as *Aspergillus*, *Penicillium*, and *Fusarium* in organic corn, with fungal growth observed in about 70% of samples. The study also detected higher frequencies and co-occurrence of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2) and fumonisin B_1 in organic products, highlighting potential public health risks and the need for effective monitoring. Therefore, preventive measures aimed at inhibiting fungal growth during cultivation, storage, and processing remain the most effective strategy for controlling mycotoxin contamination. Nevertheless, irradiation technologies have been investigated as post-harvest corrective interventions for contaminated commodities; however, the suitability of such treated products for food or feed applications requires careful safety evaluation. The toxicological safety and cytotoxicity of aflatoxin degradation products formed after irradiation treatments are discussed in detail in Section 7.

6.2. Environmental and occupational safety

Environmental and occupational safety in food irradiation facilities is ensured through internationally recognized standards. According to guidelines from the International Atomic Energy Agency (IAEA), the facilities must incorporate thick concrete shielding, restricted access zones, and automated interlocks to prevent accidental exposure.[105] As recommended by the International Commission on Radiological Protection (ICRP), the occupational radiation exposure should not exceed 20 mSv per year (averaged over five years), while public exposure should remain below 1 mSv per year.[106] According to the Codex Alimentarius Commission and the World Health Organization, safe operation of irradiation facilities requires continuous radiation monitoring, personal dosimeters, environmental surveillance, routine equipment inspection, protective clothing, and periodic medical examinations for personnel.[107] These measures collectively ensure the safe application of irradiation technologies in food processing.

7. Mutagenicity and cytotoxicity assay

The ability to damage living cells, including by disrupting protein synthesis or breaking the cell membrane, which eventually results in apoptotic cell death, is known as cytotoxicity. The ability of chemical agents or pharmacological compounds to cause genetic mutation is referred to as mutagenicity.[108]

Mammalian cells have been shown to exhibit reduced cell survival when AFB_1 is present. This could be due to either direct impairment of protein functions, such as cyclic nucleotide phosphodiesterase activity or protein phosphorylation in the liver, or DNA damage brought about by the toxin.[109] Aflatoxin B_1 is a major public health concern due to its widespread presence and status as a hazardous metabolite. By generating a single strand break in DNA and bioactivating AFB_1 to its epoxide metabolite, it is believed that AFB_1 actively contributes to hepatic and extrahepatic carcinogenesis in both humans and animals.[110] Because degradation products of AFB_1 may also interact with DNA or proteins, assessing their cytotoxicity and mutagenicity is essential for evaluating the safety of irradiation-based detoxification methods.

7.1. Mutagenesis pathway of aflatoxins

The biological activity of AFB_1 arises after metabolic activation, which can occur through both chemical and enzymatic pathways. In chemical activation systems, oxidizing agents such as dimethyl dioxirane convert the C_8–C_9 double bond of AFB_1 into the reactive AFB_1-8,9-epoxide. In biological systems, enzymatic activation is primarily mediated by cytochrome P450 enzymes, which similarly generate the epoxide intermediate responsible for DNA and protein adduct formation.[111]

As a liposoluble substance, AFB_1 can enter the bloodstream throughout the body after being absorbed from the site of exposure. Epoxidation, or the oxidation of the furan ring, results from aflatoxin derivatives covalently binding to macromolecules *via* the unsaturated furan ring. Reactive 8,9-exoepoxide and 8,9-*endo*-epoxide are produced from AFB_1 by CYP3A4 and CYP1A2 of liver enzyme cytochrome P450 (CYPs), which are abundantly found in the liver; the exo-epoxide is the toxic species that gives AFB_1 its genotoxic characteristics.[112] Aflatoxin-N7-guanine, which results from the extremely unstable aflatoxin-8, 9-epoxide binding to the DNA molecule with high affinity, causes a guanine (G) to thymine (T) transversion mutation to form the primary 8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB_1 (AFB_1-N7-Gua) adduct. This codon's mutation appears as a transversion in its third position, resulting in the insertion of a serine at position 249 in the mutant protein. Apart from the G → T transversion, AFB_1 also causes G → T and C → A transversions into nearby codons, though these occur less

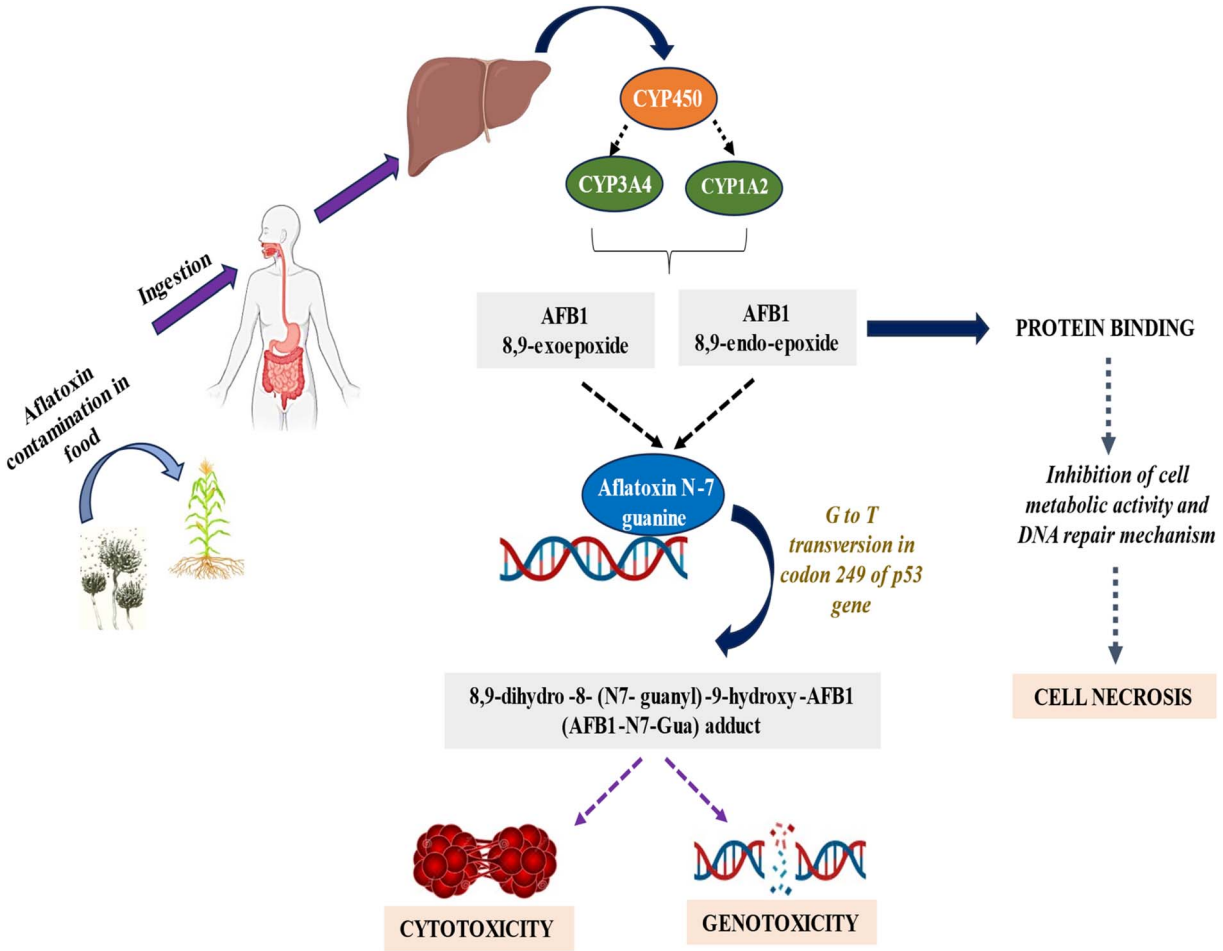

Fig. 5 Mutagenesis pathway of aflatoxin B_1 (AFB_1).

frequently. However, only the G → T transversion at codon 249 has been demonstrated.[113] This mutation directly affects the cell cycle by influencing the p53 gene, which encodes tumour suppressor proteins that prevent the growth of tumours. It turns out that the p53 gene mutation in HCC caused by AFB_1 in regions where AFB_1 contamination in food has been reported is associated with the mutant serine 249 p53 protein. This demonstrated the hepato-carcinogenicity of AFB_1.[114,115] The mutagenesis pathway of AFB_1 is shown in Fig. 5. Additionally, it was proposed that AFB_1 induces oxidative stress through the generation of reactive oxygen species (ROS) and that these further trigger signal pathways dependent on ROS in the mitochondria, ultimately leading to apoptosis.[116] There are numerous cell lines in which aflatoxin has shown a cytotoxic effect (Fig. 6).

7.2. Cytotoxicity of UV-irradiation degraded aflatoxin

UV irradiation reduces the toxicity of AFB_1 primarily by structural modification of the lactone ring, which is essential for both fluorescence and toxicity.[122] Loss of fluorescence upon lactone ring disruption suggests reduced mutagenic potential (Fig. 7). Toxicological assays confirm this reduction. Chang *et al.*[75] demonstrated that UV-treated AFB_1 in peanuts exhibited markedly fewer revertant colonies in *Salmonella typhimurium* strains TA98 and TA100 compared to untreated controls, indicating lower mutagenicity. Similarly, Diao *et al.*[123] showed that $HepG_2$ cell viability increased from ~52% in non-irradiated samples to ~95% following 10 min of UV exposure, with no further improvements beyond that point. Another study was conducted on degradation of AFB_1 in red chilli powder using enzymatic treatment coupled with UV irradiation. It was shown that the suppression of bacterial growth occurred at 4.5 mg mL^{-1} of AFB_1 and that, as the concentration of AFB_1 increased, the bacterium cell growth was completely inhibited (>92%) at about 10 mg mL^{-1} of AFB_1. In comparison to the control and enzyme-treated sample alone, the enzyme + UV treatment caused various modifications in the AFB_1 molecule that resulted in its conversion to a less hazardous compound that led to maximal bacterial growth or the least growth inhibition percentage. Further, the result of the Ames test demonstrated that each fraction examined showed some level of carcinogenicity and mutagenicity and it can be concluded that they were all noticeably less mutagenic than regular AFB_1. Following the AFB_1–POX–UV reaction, the mutagenic effect of AFB_1 on *S. typhimurium* TA 98 was significantly reduced, as evidenced by a lower number of revertant colonies enumerated.[124]

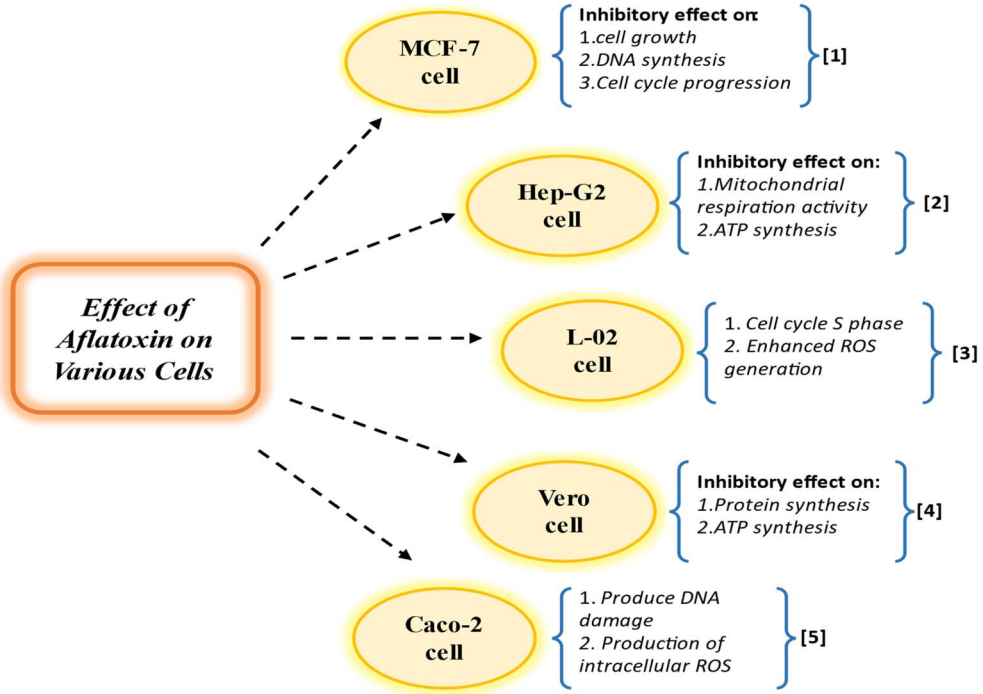

Fig. 6 Inhibitory effect of aflatoxins on different cells leading to cytotoxicity. [1] Yip *et al.*,[117] [2] Chen *et al.*,[118] [3] Zhang *et al.*,[119] [4] Rasooly *et al.*,[120] and [5] Zhang *et al.*[121]

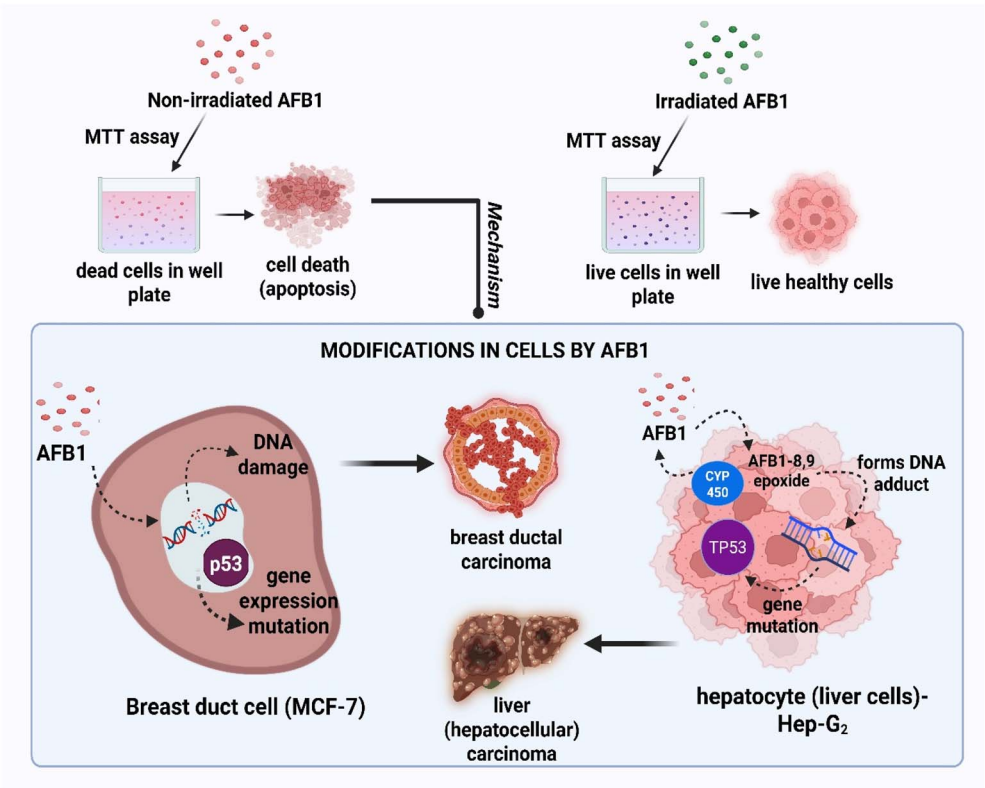

Fig. 7 Effect of non-irradiated and irradiated AFB_1 on a breast duct cell (MCF-7) and a liver cell ($HepG_2$) (created by the authors using BioRender. Chauhan, A. (2025) https://BioRender.com/whqch9e).

Animal studies also support these findings. AFB_{2a}, a major photodegradation product, was >200 times less toxic to ducklings than AFB_1.[125] Its reduced toxicity may be explained by reversible conversion to dialdehydes, which interact with amino groups in food matrices, thereby reducing bioavailability.[126] Cell-based assays reinforce these protective effects. Chang *et al.*[75] observed that $HepG_2$ cells exposed to AFB_1 showed vacuolation and death, whereas cells exposed to photodegradation products remained viable. Mao *et al.*[127] further confirmed reduced cytotoxicity using MTT and CCK-8 assays in L-02 liver cells, where UV-induced products showed negligible time-dependent toxicity compared to AFB_1. UPLC-TQEF-MS/MS revealed two distinct photodegradation products ($C_{18}H_{33}N_3O_3$ and $C_{12}H_{22}N_2O_2$), suggesting structural modifications underlie reduced toxicity. Overall, UV irradiation substantially decreases the cytotoxicity and mutagenicity of AFB_1 through photochemical breakdown of its active moieties, with both *in vitro* and *in vivo* studies confirming reduced toxicological risk.

7.3. Cytotoxicity of gamma irradiation degraded aflatoxin

Additionally, gamma radiation may cause mycotoxins to break down into potentially hazardous byproducts.[128] To assess the health risk of radiation-degraded aflatoxin end products, toxicological assays should be incorporated into investigations involving the irradiation of mycotoxins.

Gamma irradiation also reduces AFB_1 toxicity, although concerns remain about potential harmful byproducts.[129] Domijan *et al.*[65] reported that AFB_1 irradiated at 10 kGy exhibited lower cytotoxicity across cell lines (Pk15, $HepG_2$, and SH-SY5Y) compared to untreated toxin, with cell viability reductions of 36%, 14%, and 16%, respectively. These results indicate that degradation products were less harmful than native AFB_1. Similarly, Calado *et al.*[130] demonstrated that irradiation doses between 1 and 10 kGy significantly reduced cytotoxicity in $HepG_2$ cells, with higher doses correlating with improved cell viability. Mechanistically, Zhao *et al.*[131] suggested that degradation products lacking the lactone ring are less detrimental and may even display growth-promoting effects. Thus, while gamma irradiation does not eliminate cytotoxicity, evidence suggests that its degradation products are considerably less hazardous than the parent toxin.

7.4. Cytotoxicity of EBI degraded aflatoxin

Electron beam irradiation (EBI) effectively reduces the mutagenicity and cytotoxicity of AFB_1 by disrupting key structural groups, including the furofuran and lactone rings.[132] Ames and MTT assays confirmed that EB-degraded AFB_1 exhibited significantly lower toxicity compared to the untreated toxin. However, reduced mutagenicity was not eliminated.[133]

Liu *et al.*[77] reported that *S. typhimurium* strains TA98 and TA100 still produced revertants, although at levels markedly lower than those induced by native AFB_1. Since AFB_1 is a promutagen that becomes carcinogenic after metabolic activation, the presence of residual mutagenicity underscores the importance of cautious interpretation.

Overall, EBI significantly decreases the toxicological risk of AFB_1, though complete detoxification may not be achieved, necessitating further evaluation of its degradation products. A summary of studies evaluating the cytotoxicity of aflatoxin and its radiation-degraded products is presented in Table 3.

7.5. Cytotoxicity of X-ray irradiation degraded aflatoxin

Limited studies have evaluated the cytotoxicity of aflatoxin degradation products formed after X-ray irradiation. Available evidence suggests that X-ray treatment can reduce the toxicity of AFB_1 by inducing oxidative degradation and structural modifications of the toxin molecule. *In vitro* hepatocyte studies have shown that AFB_1 exposed to X-ray irradiation exhibits lower cytotoxicity toward human liver cells compared with untreated AFB_1, indicating that the resulting degradation products are less toxic.[54]

7.6. Cytotoxicity of cold plasma degraded aflatoxin

Cold plasma treatment has also been reported to reduce the cytotoxic effects of aflatoxins through the generation of reactive oxygen and nitrogen species that oxidatively degrade the toxin structure. Several studies using cell-based assays, including $HepG_2$ liver cells, have demonstrated that plasma-treated aflatoxin exhibits significantly reduced cytotoxicity and oxidative damage compared to untreated toxin. The decrease in toxicity is generally attributed to the modification of key structural features such as the C_8–C_9 double bond and lactone ring.[134] Despite these promising findings, additional studies are still required to identify the degradation products and to confirm their toxicological safety.

8. Sustainability potential of irradiation and cold plasma technologies in aflatoxin control

Irradiation represents a sustainable alternative to conventional detoxification methods because it avoids the use of chemical fumigants and high-temperature treatments that may generate toxic residues, degrade nutrients, or require significant energy input.[136] Unlike chemical detoxification processes, irradiation does not introduce additional compounds into food systems, thereby minimizing environmental contamination and eliminating the need for residue management. In addition, irradiation can be applied to commodities such as maize, peanuts, spices, and dried fruits during post-harvest handling and storage, allowing contaminated batches to be detoxified rather than discarded. This approach can significantly reduce post-harvest losses and improve food availability in regions where aflatoxin contamination frequently affects staple crops.

For example, irradiation treatments have been successfully used to reduce aflatoxin contamination in peanuts and maize without significantly affecting their nutritional quality, enabling these commodities to remain suitable for consumption or further processing.[137] Similarly, irradiation has been widely applied in the spice industry to control microbial

Table 3 Summary of research carried out to test the cytotoxicity of aflatoxins and their radiation degraded products

Irradiation method used	Type of AF	Matrix	Testing conditions	Cell line/test used	Result	References
Ultraviolet	AFB_1 and AFM_1	Whole milk	The highest dose for AFB_1 was 777 mJ cm^{-2} and for AFM_1 838 mJ cm^{-2}	$HepG_2$ cell line	No reduction in the viability of cells was found at the highest UV doses of 777 (AFB_1), 838 (AFM_1), and 746 (total AFs) mJ cm^{-2}	40
Ultraviolet	AFB_1	Methanol and acetone	365 nm, 20 °C, 72 μW cm^{-1}, 45 h	$HepG_2$, MCF-7 and LO2 cells	Showed greater cytotoxicity towards the AFB_1 standard than from the AFB_1 degradation product	53
Ultraviolet	AFB_1, AFM_1	Aqueous	365 nm, 4 °C, 1200 mJ cm^{-1}, 156 s	Hep-G_2 cells	At a UV dose of 200 mJ cm^{-2}, no toxicity on the cell line due to aflatoxins was reported	63
Ultraviolet	AFB_1, AFB_2, AFG_1	Pure water	UV doses varied from 0, 1.22, 2.44, 3.66, and 4.88 J cm^{-2}	Hep-G_2 cells. Cell viability was assessed using the XTT assay	Survival percentage of cells was found to be maximum (93.5%) at a dose of 4.88 J cm^{-2}. So, the % survival of cells decreased as the aflatoxin amount increased	49
Electron beam	AFB_1	Aqueous	5 MeV, 2 kGy, 4 °C	$HepG_2$ cell line	In comparison to control cells, *i.e.*, not treated with AFB_1, the survival of $HepG_2$ cells decreased by >50% compared to those treated with 100 mM AFB_1	63
Ultraviolet	AFB_1	Peanut oil	365 nm, 26 °C, 55–60 mw cm^{-2}, 30 min	L-02 cells	The MTT and CCK-8 test results were comparable; the vitality of cells declined linearly as the concentration of AFB_1 increased	116
Gamma	AFB_1	Methanol: water solution	1 to 10 kGy	$HepG_2$ cells	Significant reduction in cytotoxicity with an increased dose of radiation was observed	119
Ultraviolet	AFB_1	Peanut oil	UV lamps with power: 36 W; λ: 365 nm; intensity: 6.4 mW cm^{-1}	Ames test (*Salmonella typhimurium* tester strains TA98 and TA102) Hep G_2 cells	The cytotoxicity of AFB_1 was considerably decreased after 10 min of radiation exposure 95% survival rate at 10 min of UV exposure was observed	113
Ultraviolet	AFB_1	Peanut	220 to 400 nm, 800 μW cm^{-2}, 80 min	$HepG_2$ cells	As the concentrations of AFB_1 (0–100 μM) and its photodegradation products increased, the survival of $HepG_2$ cells declined	66
X-ray irradiation	AFB_1	AFB_1 solution (acetonitrile)	X-ray irradiation at different doses: 0.4, 0.7, 1.0, 3.0, 5.0, 7.0, and 10.0 kGy	$HepG_2$ cells	Irradiated AFB_1 showed lower cytotoxicity than the untreated toxin as the irradiation dose increased	54
DBD cold plasma	AFB_1	Bread (inoculated with *Aspergillus flavus*)	DBD cold plasma; gases air, N_2, Ar, N_2 + Ar; 0–35 min treatment time; gas flow rate 10 L min^{-1}	$HepG_2$ cells	Air plasma (25 min) showed the highest AFB_1 degradation and reduced cytotoxicity and bioaccessibility	135
Atmospheric cold plasma	AFB_1	AFB_1 solution (100 μM)	HVACP treatment at 85 kV for 0, 2, 5, 10, and 20 min	$HepG_2$ cells	20 min treatment significantly reduced AFB_1 cytotoxicity and oxidative stress	134

contamination while preserving product quality, demonstrating its practical feasibility for large-scale food processing. From an environmental perspective, technologies such as electron beam and X-ray irradiation operate using electricity and can be integrated with renewable energy sources, potentially lowering the carbon footprint of food processing operations. Furthermore, by extending the shelf life of stored commodities through the reduction of microbial growth and toxin levels, irradiation can contribute to more sustainable supply chains and improved storage management.

Similarly, emerging non-thermal technologies such as cold plasma offer additional sustainability advantages because they operate at ambient temperatures, require minimal water or chemical inputs, and generate reactive oxygen and nitrogen species capable of degrading aflatoxins on food surfaces.[89] Therefore, the integration of irradiation and cold plasma with other non-thermal technologies into food safety management systems may support the development of environmentally responsible, resource-efficient, and sustainable strategies for controlling aflatoxin contamination in global agri-food systems.

9. Challenges and future research

Although different non-thermal treatments have shown potential for aflatoxin detoxification, further research is required to optimize these techniques. To accelerate the detoxification process and minimize the impact on the quality of food, it is required to combine irradiation and cold plasma treatment with other detoxification strategies. In addition, regulatory limits for aflatoxins in foods such as cereals, nuts, and spices are extremely stringent in many countries, typically ranging from 2 to 10 $\mu g\ kg^{-1}$ depending on jurisdiction. Since radiation treatments often result in partial rather than complete degradation of aflatoxins, the treated commodities must still comply with these regulatory limits before being considered suitable for human consumption. Therefore, irradiation should be regarded as a complementary mitigation strategy rather than a standalone solution for aflatoxin control.

Future research should focus on the detailed identification of radiation-induced aflatoxin degradation products using advanced analytical techniques such as high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance spectroscopy (NMR). Previous studies have assessed the safety of irradiated foods using assays such as the Ames test, toxicity assays in various cell lines, and experiments involving animal embryos or organs. In addition, integrated toxicological assessments combining *in vitro*, *in vivo*, and computational approaches are required to comprehensively identify degradation products, evaluate the biological safety of these degradation products, and better understand the molecular mechanisms underlying radiation-induced detoxification of aflatoxins.

In addition, consumer acceptance of irradiated foods and the high cost of irradiation facilities remain important challenges for large-scale implementation. In practical settings, strategies for controlling AFB_1 contamination primarily focus on preventive measures during crop growth, harvesting, and storage, while relatively limited research has addressed post-harvest degradation techniques. Future studies should therefore focus on optimizing irradiation parameters, understanding the influence of food matrix composition on degradation efficiency, and integrating irradiation with existing food safety management systems to develop more effective and practical aflatoxin mitigation strategies.

10. Conclusions

The review provides detailed information on the detoxification of mycotoxins using non-thermal technologies, particularly irradiation and cold plasma technologies, and it has been observed that optimization of the treatment process is needed to lower mycotoxin levels below the necessary regulatory limits. The gamma and ultraviolet irradiation methods have shown promising results in degrading aflatoxin. Still, the low penetration levels and the need for suitable accelerators remain limitations of the electron beam irradiation method. Compared with gamma irradiation, fewer studies have examined the effectiveness of X-ray irradiation for aflatoxin degradation; however, existing research indicates its potential for toxin reduction and highlights the need for further investigation. Cold plasma has also emerged as a promising approach, as the reactive oxygen and nitrogen species generated during treatment can effectively induce oxidative degradation of aflatoxins under mild processing conditions. Numerous research studies have been conducted to suggest the structures of aflatoxin degradation by-products using various analytical techniques. The main objective of the future research should be to address the knowledge gap about the identification and confirmation of the structures of mycotoxin degradation. Although the theoretically degraded molecules should be less hazardous or perhaps non-toxic, further research is still needed to determine the residual toxicity following treatment. The broader adoption of irradiation and other non-thermal detoxification technologies may significantly reduce aflatoxin-related health effects, including immunosuppression, growth impairment, and malnutrition. Furthermore, integrating these technologies into food safety management systems could help reduce food losses and strengthen food security, particularly in regions where aflatoxin contamination is prevalent.

Author contributions

Muskan Kumari: conceptualization; investigation; formal analysis; writing – original draft; writing – review & editing. Digvir S. Jayas: writing – review & editing; supervision; visualization. Anil Kumar Chauhan: conceptualization; writing – review & editing; resources; supervision. Deepak Choudhury: writing – review & editing. Rakesh K. Singh: writing – review & editing. Shraddha Prakash: formal analysis; visualization. Kaustubh Singh: formal analysis; visualization.

Conflicts of interest

There are no conflicts to declare.

Abbreviations

AF	Aflatoxin
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFM_1	Aflatoxin M_1
DBD	Dielectric barrier discharge
DNA	Deoxyribonucleic acid
EB	Electron beam
EBI	Electron beam irradiation
EC	European Commission
FAO	Food and Agriculture Organization
HCC	Hepatocellular carcinoma cell line
HPLC	High-performance liquid chromatography
HPLC-MS	High-performance liquid chromatography-tandem mass spectrometry
IAEA	International Atomic Energy Agency
IARC	International Agency for Research on Cancer
LC-MS	Liquid chromatography-mass spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMR	Nuclear magnetic resonance spectroscopy
SEM	Scanning electron microscopy
UPLC-MS	Ultra-performance liquid chromatography-mass spectrometry
UPLC-Q-TOF	MS ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry
UV	Ultraviolet
UV-A LED	Ultraviolet-A light emitting diode
WHO	World Health Organization

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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