

## Mechanisms and application of mycotoxin decontamination techniques in stored grains

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### ABSTRACT

Ensuring the safe storage of food grains is paramount for global food security. However, mycotoxin contamination poses a significant threat by compromising grain quality and consumer health. Various decontamination techniques are employed to inactivate toxins, each with distinct mechanisms of toxin inactivation. This review examines the pivotal mechanisms in reducing mycotoxin levels in stored grains, elucidating the principles and pathways underlying novel decontamination techniques such as cold plasma, ozone, photocatalysis, nanoparticle adsorbents, and microbial enzymes, and assesses their practical application and industrial feasibility. Our thorough investigation reveals that the effectiveness of decontamination techniques relies on three fundamental mechanisms: adsorption, treatment with reactive chemical species, and biotransformation. Several novel technologies are highly effective in laboratory tests, but face challenges at the industrial scale. Current research indicates that novel decontamination techniques, including pulsed light, photocatalysis, and microbial enzymes, hold much promise in significantly reducing fungal growth and mycotoxin contamination in grains. However, it is also evident that techniques with high efficacy in reducing fungal infestations are not necessarily effective in eradicating mycotoxin contamination. A combinatory approach to these techniques is the way forward, and future research should focus on hybrid treatments to enhance the effectiveness of these technologies on an industrial scale. This review aims to bolster food safety and mitigate economic losses linked to mycotoxin contamination in grains by offering a theoretical basis for developing and implementing effective decontamination strategies.

### 1. Introduction

Cereal grains are vital staple foods that significantly contribute to the energy and nutrient intake of humans. The estimated global grain production for 2021-22 was 2799 million metric tons, approximately 4.5% higher than in 2019-20 (USDA, 2023). This increase in grain production has necessitated a need for larger storage structures. However, the increased size of storage structures poses practical challenges in conducting routine checks on grain quality, making the grain vulnerable to localized pockets of high moisture and temperature (called hotspots) embedded deep in grain bulks (Asefi et al., 2017). Hotspots in storage bins develop primarily due to temperature gradients, water condensation, sub-optimal aeration, fungal infections, and insect/pest infestations, providing ideal conditions for further fungal invasion and

subsequent mycotoxin production. Mycotoxins are toxic secondary metabolites found in food and agricultural products at any stage of production, processing, and storage. There are approximately 20 mycotoxins that are most commonly found in stored grains, including aflatoxins (AF), ochratoxins (OTA), fumonisins (FMN), trichothecenes, zearalenone (ZEN), sterigmatocystin, penicillic acid, patulin, kojic acid, citroviridin, gliotoxin, ergot alkaloids, luteoskyrin, chetomin, cladosporin, rubratoxin, rugulosin, diplodiatoxin, enniatin B, and citrinin (Chandravarnan et al., 2022). The first five mycotoxins listed above are responsible for most food safety concerns. Most of these toxins are produced by *Aspergillus*, *Penicillium* and *Fusarium* species (Bullerman and Bianchini, 2014; Neme and Mohammed, 2017) (Table 1). These toxins have some common characteristics: 1) small molecular size (<1000 Da), aiding penetration through biological barriers; 2) heterocyclic rings

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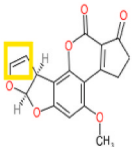
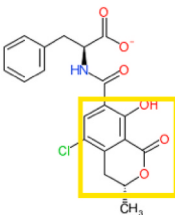
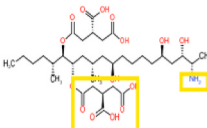
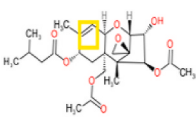
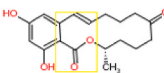
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**Table 1**  
Major mycotoxins in stored grain, their chemical structure, critical active site, properties, legal limits and toxic effect on human beings.

Mycotoxin	Chemical structure <sup>a</sup>	Critical active site	Molecular weight (Dalton) <sup>b</sup>	EU limits (µg/kg)	Melting point (°C) <sup>c</sup>	Producer fungi	Crops <sup>d</sup>	Toxic effects	Major decontamination techniques
Aflatoxin		Furan ring	312–330	2–8	268–269	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i>	Wheat, maize, peanut, pistachio, mushroom, legumes	Cancer, liver diseases, immune disorders	Ultraviolet irradiation, adsorbents, and cold plasma
Ochratoxin		Isocoumarin moiety	403.83	3–10	169	<i>Penicillium verrucosum</i> and <i>Aspergillus ochraceus</i>	Coffee, soybean, grapes, raisins	Liver and kidney diseases, nausea, vomiting	Adsorbents, and ozonation
Fumonisin		Two tricarballic acid side chains and free amino groups	705	200–1000	100–120	<i>Fusarium verticillioides</i> and <i>F. proliferatum</i>	Rice, maize, canola, soybean, peanuts, sorghum	Lung, kidney diseases, cancer, neurotoxicity	UV photolysis, ozonation, antagonistic microbes
Trichothecene		Double bond at the C9-C10 position and epoxide ring	200–500			<i>Fusarium</i> , <i>Trichoderma</i> , <i>Myrothecium</i> , <i>Trichothecium</i> , <i>Stachybotrys</i> , and <i>Spicellum</i>	Barley, oats, rice, rye, wheat, barley	Cytotoxicity, teratogenicity, skin necrosis, GIS lesions	Adsorbents, ozonation, NaClO treatment, and treatment with <i>Lactobacillus</i> spp
Zearalenone		Lactone ring	318.4	75–200	164–165	<i>Fusarium graminearum</i> and <i>F. culmorum</i>	Corn, wheat, barley, sorghum	Hyper-estrogenic effect, abortion, cancer	Oxidizing agents, irradiation

<sup>a</sup> The yellow frame indicates the critical toxicity site.

<sup>b</sup> IARC. [https://publications.iarc.fr/\\_publications/media/download/1372/292f829f75e2d9bd641611e2768b3db005839ad2.pdf](https://publications.iarc.fr/_publications/media/download/1372/292f829f75e2d9bd641611e2768b3db005839ad2.pdf).

<sup>c</sup> Kabak (2009).

<sup>d</sup> Major infested crops.

with oxygen, nitrogen, and/or sulfur atoms, enhancing reactivity and toxicity; 3) conjugated systems with alternating single and double bonds, facilitating absorption, fluorescence, and molecular interactions; and 4) a lipophilic nature, promoting accumulation in lipid-rich foods and animal tissues. The main toxicity groups, toxic effects, and thermal stabilities of the most prevalent mycotoxins are summarised in Table 1.

Mycotoxins have a detrimental impact on grain quality, appearance, and nutritional value. They contribute to significant economic losses in international trade by compromising food safety. According to the Food and Agriculture Organization (FAO) of the United Nations, mycotoxin contamination adversely affects almost a quarter of global crop productivity (Eskola et al., 2018; Mannaa and Kim, 2017; Sirohi et al., 2021). Annual economic losses of around a billion US dollars are attributed to post-harvest losses caused by fungal contamination of grains worldwide (Grenier et al., 2014; Shen and Singh, 2021). Furthermore, consumption of toxic food has been associated with carcinogenic, estrogenic, nephrotoxic, and immunosuppressive effects on human and animal health (Table 1). Thus, various regulatory agencies worldwide have established maximum limits on mycotoxins in food commodities (Table 1). Consequently, there has been a growing demand for decontamination techniques to obtain cleaner and toxin-free grains in recent years.

Mycotoxin decontamination techniques can be broadly classified into three categories: physical (sorting, milling, heating, irradiation, etc.), chemical (ammonification, ozonation, organic acids, etc.), and biological (microbial and enzymatic control) techniques. The degradation mechanism of mycotoxins by each of these treatments is different. To date, most reviews have focused on evaluating the effectiveness of decontamination methods for degrading mycotoxins in grains. However, there remains a lack of an in-depth and comprehensive explanation of the mechanisms of mycotoxin degradation in foods, particularly stored grains. It is essential to understand the underlying principles of decontamination techniques as this 1) may aid in targeting and breaking the specific toxic sites in a given mycotoxin's structure and 2) allows for an understanding of the factors affecting the decontamination efficiency and optimizing those factors to achieve maximum decontamination efficacy.

Researchers have developed various detection techniques such as gas chromatography-mass spectrometry (GC-MS), ultra-high performance liquid chromatography-mass spectrometry (UPLC-MS), high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS), and ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS) to identify and quantify different mycotoxins and their degradation products based on their unique mass-to-charge ( $m/z$ ) ratios and retention times. These highly sensitive analytical techniques can detect toxin levels far below minimum safe thresholds. However, this sensitivity may not always align with the actual risk posed. With global warming and increasing pest resistance expected to alter fungal populations and drive disease and mycotoxin production in crops worldwide, it is crucial to develop effective decontamination methods to prevent fungal growth and mycotoxin formation in stored grains. Therefore, this review explores the underlying principles of toxin degradation by some common decontamination techniques and synthesizes understandings derived from studies that have explained possible mechanisms of mycotoxin degradation. It also discusses the status, implication, and prospects of recent techniques like magnetic nanoparticles and cold plasma for controlling mycotoxins in grain. Furthermore, we discuss the current industrial applications of these techniques. This review provides a theoretical basis for developing and applying decontamination techniques for controlling mycotoxins in stored grains.

## 2. Mycotoxin contamination in food grains

Recent advancements in analytical methods have confirmed the widespread presence of mycotoxins in food and feed. In surveys of

mycotoxin incidence in cereals and cereal-based foods within the years 2008–2018, mycotoxins were found to be frequent contaminants across various countries, such as Spain (11–100% incidence rate), Serbia (40–43%), Italy (2–12%), Hungary (17–86%), the Czech Republic (8–83%), China (78–100%), the United States (3.8–42%), Canada (33–57%), Brazil (37–100%), India (16–80%), and Nigeria (33–55%) (Torović, 2018; Carballo et al., 2018; Khodaei et al., 2021; Wan et al., 2020). Lee and Ryu (2017) found the incidence of mycotoxins in cereals and cereal-derived food products in different continents such as Africa, America, Asia, and Europe to be 36–100%, 15–95%, 3–80% and 17–59%, respectively, as per the literature collected.

Fungal growth and mycotoxin production can occur at any stage along the food management chain, i.e., in the field prior to harvest, during storage, and while transporting food grains. The most significant fungal species in food contamination are the genera *Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria*. *Aspergillus* and *Penicillium* are primarily saprophytic, often causing spoilage during storage, while *Alternaria* and *Fusarium* are plant pathogens that typically infect specific hosts, leading to contamination and accumulation of toxins during the pre-harvest stage. Based on their infection environments, fungi responsible for mycotoxin contamination in grains are traditionally categorized into two groups: field fungi (e.g., *Alternaria* and *Fusarium*) that primarily infect crops during growth in the field and storage fungi (e.g., *Aspergillus* and *Penicillium*) that can proliferate during grain storage. However, these categories are not absolute; some *Aspergilli* can invade crops in the field and grain kernels during storage, and *Fusarium* can grow in high-moisture grains in the field and during storage conditions (Bianchini and Bullerman, 2014).

Field fungi commonly infest crops partly due to warm and humid weather conditions before and after flowering and the presence of abundant inoculum because of reduced tillage practices that leave crop residue on the soil (Wegulo et al., 2015). Also, high environmental temperatures, excessive precipitation or prolonged droughts can intensify plant stress, and elevated CO<sub>2</sub> may dilute defensive compounds within plant tissue, thereby increasing the susceptibility of grains to fungal infection and mycotoxin contamination (Bencze et al., 2017). It should also be noted that the presence of fungi doesn't always lead to mycotoxin production, as the conditions conducive to mycotoxin production are specific and may be different than optima for simple fungal growth (Hamad et al., 2023). During harvesting, improper handling of grains can cause mechanical injury, resulting in cracks and breaks in the outer layer of grains that act as entry points for fungi and fungal spores.

Many studies have discussed the factors that favor mycotoxin contamination in stored grains (Akbar and Alam, 2019; Fleurat-Lessard, 2017; Garcia-Cela et al., 2018). Water availability or water activity of grains, environmental temperature and humidity, level of field infection, nutrient availability, physical damage to the kernels, oxygen and carbon dioxide tension, activity of insect pests, and the interactions of all these factors determine the extent of fungal activity and toxin production under storage conditions. Grains are commonly dried before storage, restricting fungal activity and creating a low-moisture environment in grain bins. However, trading grains on a wet weight basis without ensuring proper drying increases the risk of fungal growth and mycotoxin production. Typically, mycotoxigenic fungi thrive at water activities ( $a_w$ ) ranging from 0.85 to 0.99, while mycotoxin production occurs within a narrower range of 0.90–0.99  $a_w$  (Mannaa and Kim, 2017). Mycotoxin production is typically favoured within a temperature range of 25–30 °C. Kos et al. (2023) reported the environmental optima (water activity/temperature) for AF, OTA, DON, and ZEN production to be 0.99/30 °C, 0.98/25 °C, 0.99/25 °C, and 0.99/25 °C; respectively. In another study, Mylona et al. (2012) showed optimum DON production at the wettest condition (0.97  $a_w$ ), highest temperature (30 °C), and highest accumulated CO<sub>2</sub> concentration. Different moisture and temperature conditions for maximal toxin production may be reported among studies because these parameters may differ among fungal strains.

Under high moisture conditions, grain respiration increases, which changes the interstitial gas composition within the grain bulk. Interstitial gases primarily comprise nitrogen, oxygen, water vapour, carbon dioxide, trace gases, and volatile chemical compounds. Mycotoxin production can be associated with CO<sub>2</sub> generation and dry mass losses in grains (Garcia-Cela et al., 2018). The interplay among these factors is intricate and significantly influences fungal activity and subsequent mycotoxin production.

### 3. Grain processing techniques for mycotoxin management

Limiting the fungi growth and multiplication is the most effective way to prevent mycotoxin production. Therefore, keeping grain at low temperatures under dry conditions is the best way to limit mycotoxin production during storage. Any methods limiting fungi growth and multiplication will also prevent mycotoxin production. There are many studies on this topic (Neme and Mohammed, 2017), which is out of scope in this review.

Given the challenges in monitoring and managing mycotoxin production, primary processing of grains becomes crucial in significantly enhancing mycotoxin removal from food grains. Depending on the level of fungal and mycotoxin penetration, mycotoxin solubility and the grain size distribution (small and broken kernels are disproportionately associated with mycotoxin contamination), aspiration, washing/floatation, and sorting can eliminate 2–93% of mycotoxins from grains (Liu et al., 2022; Schaarschmidt and Fauhl-Hassek, 2021). Washing procedures are most effective when followed by high-moisture processing steps, such as cooking, malting, fermentation, or wet milling. Additionally, washing, steeping, and cooking grains at elevated pH levels or using alkaline solutions such as sodium bisulphite and sodium carbonate have been shown to significantly reduce mycotoxin levels (Schaarschmidt and Fauhl-Hassek, 2021).

The conventional sorting techniques are based on gravity separation, centrifugal force, and air flotation (Pascale et al., 2020). However, they are unreliable as the physical properties of contaminated and non-contaminated grains might be the same, making it difficult to separate them (Shanakhat et al., 2018). More advanced separation technologies include automated sorting based on visual and spectral characteristics using optical sorters, which are continuously being improved. Vicens-Sans et al. (2024) showed that near-infrared hyperspectral imaging combined with linear discriminant analysis could effectively sort DON-contaminated wheat batches with 0.71 balanced accuracies. For industrial purposes, high-speed sorters have been developed based on the optical properties of grains (Colović et al., 2019).

For many mycotoxins, a large fraction of the mycotoxin load is carried by the outer parts of the cereal grains (bran, flour shorts, screenings, and middling), and therefore dehulling and other processing can remove parts of the kernel tissues that carry highest mycotoxin content, leaving the endosperm (flour or semolina) intended for human consumption with lower mycotoxin levels (Tibola et al., 2016). Tibola et al. (2016) observed a significant redistribution of DON during wheat milling, with a 57% reduction in DON levels in finished flour and a 117% increase in the bran fraction. Similarly, Savi et al. (2016) reported the highest mean concentration of DON in bran fraction (2278 µg/kg), followed by milled wheat (1895 µg/kg) and the lowest levels in the finished flour (1305 µg/kg). The difference in the concentration of mycotoxins in distinct grain tissues can be associated with the colonization pattern of moulds, susceptibility of cereal cultivars, diffusion of mycotoxins within grain and inhibition of mycotoxin biosynthesis in particular fractions (Schaarschmidt and Fauhl-Hassek, 2018).

On the other hand, thermal food processing methods, such as roasting and baking, have a low to moderate effect on mycotoxin degradation, as most mycotoxins are heat-stable at the temperatures used in food preparation. Some studies have shown that high-temperature treatments (>150 °C), like roasting and extrusion, can

reduce 6–94% of mycotoxins in grains (Neme and Mohammed, 2017). Factors such as type and concentration of mycotoxin, moisture content of grains, pH, time, temperature, and level of heat penetration decide the decomposition extent of mycotoxins during heat treatments (Milani and Maleki, 2014). In a recent study, Kuchenbuch et al. (2018) reported that T-2 toxins are more susceptible to thermal degradation than HT-2. During biscuit preparation, T-2 toxin levels were reduced by 45%, compared to a 20% reduction in HT-2 toxin. Stadler et al. (2019) observed that during industrial baking, higher baking temperature (200 °C) and longer baking time (11 min) with increased concentrations of sodium bicarbonate (0.59%) were directly associated with high degradation (2–21%) of DON.

Though proper grain handling, effective storage practices, and primary processing of grains considerably reduce mycotoxins, these methods become ineffective once mycotoxins have already formed within the portion of the grains used for human consumption (Mir et al., 2021). Therefore, effective decontamination techniques such as physical (UV light, photocatalysts), chemical (ozonation), and biological (microbial enzymes) treatments are essential to degrade mycotoxins and maintain minimum regulatory limits in raw and processed food grains to ensure product safety for consumers.

### 4. Major mechanisms of mycotoxin detoxification in grains

#### 4.1. Adsorption

Selective adsorption of mycotoxins is one technique that can reduce mycotoxin exposure without affecting grain quality. Three types of adsorbents are primarily used for toxin removal from grains: 1) microbial adsorbents, 2) nanoparticle adsorbents, and 3) magnetic nanoparticle adsorbents (Table 2). Adsorbents reduce the harmful effects of mycotoxins by forming complexes with them, thereby blocking their absorption into the bloodstream and lowering their bioavailability. The stability of this complex between adsorbent and mycotoxin across a wide pH range is crucial to the effectiveness of adsorbents and preventing the desorption of mycotoxins in the gastrointestinal tract. Adsorbents are widely used as feed additives, allowing livestock to experience reduced mycotoxin exposure despite consuming a higher quantity of mycotoxin in their diet.

##### 4.1.1. Mechanism of microorganisms as adsorbents

Certain bacteria, fungi, and yeast can adsorb some mycotoxins, mainly as a function of cell wall components that produce interaction forces with the mycotoxins (Schmidt et al., 2019). Microbial cell walls rich in beta-glucans and chitin have high binding capacity for mycotoxins. The interactions responsible for surface binding of toxins are hydrophobic, electrostatic, and ionic interactions (Liu et al., 2020; Schmidt et al., 2019). For example, cell wall materials produced by some yeast can bind AF by hydrogen bonds and van der Waals interactions. At the same time, lactic acid bacteria (LAB) use electrostatic forces and hydrophobic interactions to bind AF to polysaccharides and peptidoglycans (Gómez-Salazar et al., 2021). Piotrowska (2014) conducted a study to evaluate OTA binding with three lactic acid bacteria, *Lactobacillus plantarum*, *L. brevis*, and *L. sanfranciscensis*. They observed that over a 24 h MRS (de Man, Rogosa, and Sharpe media) culture period, *L. sanfranciscensis* and *L. plantarum* were able to remove 30% of the initial OTA content (5 mg dry weight/ml), while *L. brevis* reduced the initial toxin content by a smaller 20.5%. They attributed this to variation in species growth over 24 h incubation and the effect of hydrophobic, Lewis acid-base and electron donor-acceptor interactions between OTA and bacterial cell wall. Some other LAB species used for mycotoxin adsorption in food and feed include *Streptococcus*, *Lactococcus*, and *Bifidobacterium* (Piotrowska, 2014).

Further, most microorganisms with adsorption capacity are categorized as fermentation microorganisms. Yeast biomass has shown the capacity to adsorb mycotoxins, attributed to the mannoproteins and

**Table 2**  
Recent decontamination techniques used for mycotoxin reduction in grains.

Method	Medium	Mycotoxin	Technological aspect	Reduction %	Reference
<b>Microorganism adsorbent</b>					
<i>Pseudomonas fluorescens</i> strain 3JW1	Peanut	AFB <sub>1</sub>	96 h	88.3	Yang et al. (2017)
<i>Bacillus licheniformis</i>	Soybean	OTA	37 °C, 48 h	92.5	Luo et al. (2018)
<b>Nanoparticles adsorbent</b>					
SEN		FB <sub>1</sub> and DON		63 and 76	Hu et al. (2019)
Carbon nanocomposites	Maize by-products	AFB <sub>1</sub>	pH 7 treatment time 180 min	90	Zhao et al. (2015)
Silver nanoparticles	Nuts	<i>A. flavus</i>	particle size ranges from 5 to 30 nm Ag nanoparticles conc. 50, 100, 150 ppm	48.2–100	Al-Othman et al. (2014)
<b>Photolysis</b>					
Gamma radiation	Sorghum	AFB <sub>1</sub> , OTA	10 kGy	59, 32	Ben Amara et al. (2022)
	Maize	AFB <sub>1</sub>	10 kGy	95	Markov et al. (2015)
	Wheat	ZEN	20 kGy	97	Sebaei et al., 2022
	Yellow corn			51	
	White corn			59.9	
	Wheat flour	OTA	30 kGy	24	Calado et al. (2017)
Pulsed light	Solid medium	AFB <sub>1</sub> , AFB <sub>2</sub>	2.86 W/cm <sup>2</sup> , 12 s	96.6, 91.7	Wang et al. (2018)
<b>UV radiation</b>					
UV radiation	Wheat	AF	0.1 mW/cm <sup>2</sup> , 160 min	65–73	Ghanghro et al. (2016)
	Wheat semolina	AFB <sub>1</sub>	254 nm, 30 W, 15 min, AF level: 2.18 ± 0.92 µg/kg	100	Shanakhath et al. (2018)
	Maize	DON, ZEN, OTA	254 nm, 15000 mJ/cm <sup>2</sup> , initial conc. 2000 µg/kg	30, 52, 17	Popović et al. (2018)
	Wheat	DON, ZEN, OTA	254 nm, 10000 mJ/cm <sup>2</sup> , initial conc. 2000 µg/kg	14, 42, 6	Jajić et al. (2016)
	Almond, pistachio, Maize	DON	254 nm, 120 min, 30 cm from UV source	17	
<b>Photocatalysts</b>					
	Sudanese peanut oil	AFB <sub>1</sub> AFB <sub>1</sub> , AFB <sub>2</sub>	10 mol% Sc-doped SrTi <sub>0.7</sub> Fe <sub>0.3</sub> O <sub>3</sub> , 120 min immobilized TiO <sub>2</sub>	88.2 99	Jamil et al. (2017) Magzoub et al. (2019)
	Wheat	DON	6 mg/ml (Upconversion nanoparticles coated with titanium oxide UCNP@TiO <sub>2</sub> ); 120 min	69.8	Wu et al. (2020)
	Wheat	DON	8 mg/mL (UCNP@TiO <sub>2</sub> ), 90 min	72.8	Wu et al. (2019)
	Wheat	DON	Dielectric barrier discharge, 20% grain m.c., 8 min, 50 kV	36.10	Chen et al. (2021)
<b>Cold plasma</b>					
	Peanut	AFB <sub>1</sub>	Atmospheric pressure plasma	72.5	Ren et al. (2017)
	Peanut	AFB <sub>1</sub>	Glow discharge plasma, 720–900s, 0.04–0.07 kW	95–97	Devi et al. (2017)
	Barley	DON	Gliding arc discharge atmospheric pressure plasma	80	Kriz et al. (2015)
	Rice	DON and OTA	8 min at 25 kV	61, 55	Guo et al. (2023)
	Wheat	DON	Dielectric barrier discharge, 20% grain m.c., 8 min, 50 kV	36.10	Chen et al. (2021)
<b>Ozonation</b>					
	Wheat	DON	10 g/m <sup>3</sup> of gaseous ozone, 30 s	94	Conte et al. (2020)
	Wheat	AFB <sub>1</sub> and AFB <sub>2</sub>	60 µmol/mol; 180 min	95, 85	Savi et al. (2015)
	Wheat grains, pasta, semolina	DON and DON-3-Glc	40 g/m <sup>3</sup> ; 6 h	29, 44	Alexandre et al. (2019)
	Maize	FB <sub>1</sub> , FB <sub>2</sub>	13.5 mg/L, 24h	81.2, 86.2	Ribeiro et al. (2022)
	Wheat	DON	Initial DON content: 0.86 mg/kg, ozone con. 60 mg/L, 2 h	33.33	Zhuang et al. (2020)
	Corn	ZEN, OTA	100 mg/L, 180 min, corn m.c. 19.6%	90.7, 70.7	Qi et al. (2016)
<b>Enzymes</b>					
FUMzyme®	Maize	FB	≥1000 U/L, 1h incubation	80	Alberts et al. (2019)
Carboxypeptidase ( <i>Bacillus amyloliquefaciens</i> ASAG1)	Maize	OTA	31 °C, 10 h, OTA final conc. 1 µg/ml	72	Chang et al. (2015)
TV-AFB1D ( <i>Trametes versicolor</i> )	Rice	AFB <sub>1</sub>	32 °C for 5h500 µl of sodium acetate buffer 1 mM, pH 5; incubation at 25 °C, 72h	67.4	Yang et al. (2022)
ZLHY-6 ( <i>B. subtilis</i> Z6)	Corn	ZEN	37 °C for 12h	92	Wang et al. (2022)
2-Cys peroxiredoxin ( <i>Acinetobacter</i> sp. SM04)	Corn	ZEN	Initial ZEN conc. Nearly 1000µg/ml; 6h at 30 °C with purified Prx and 0.09% H <sub>2</sub> O <sub>2</sub>	90	Loi et al. (2017)

β-glucans in their cell walls, through mechanisms such as physical adsorption, ion exchange, and complexation (Wall-Martínez et al., 2019). Mannoproteins contain hydrophobic domains that facilitate hydrophobic interactions with toxins, while D-glucans enhance van der

Waals bonds with the toxins (Faucet-Marquis et al., 2014). Wall-Martínez et al. (2019) proposed that the primary mechanism of mycotoxin removal during the fermentation of contaminated wort was the adsorption of mycotoxins on the yeast cell wall. They found that



fermenting contaminated wort with *Saccharomyces pastorianus* and *Saccharomyces cerevisiae* resulted in removing 30–70% of ZEN and 10–17% of DON. Moreover, *Saccharomyces* yeasts exhibited a higher absorption rate for ZEN than for DON. Bueno et al. (2007) reported *Saccharomyces cerevisiae* as the more efficient adsorbent of AFB<sub>1</sub> in comparison to LAB. In another study, Mozaffary et al. (2019) found that using 2% *S. cerevisiae* and 30 °C fermentation temperature reduced OTA content by 59.41% during bread-baking. Several yeast strains, such as species of *Candida*, *Rhodotorula*, *Schizosaccharomyces*, *Kloeckera*, and *Pichia*, have been reported to adsorb OTA and patulin (Piotrowska, 2021). In food industries, various probiotics, such as LAB, are used for adsorbing mycotoxins in food (Jafarzadeh et al., 2022). However, some challenges faced in the industrial application of microbial adsorbents of mycotoxins are the extensive process of microbial cell extraction and secondary pollution (Luo et al., 2020).

#### 4.1.2. Mechanism of nanoparticle adsorbents

Utilizing nanomaterials as adsorbents is highly promising due to their large surface area, strong attraction for organic compounds, and ability to be modified to increase selectivity to specific contaminants (Horky et al., 2018). The most efficient nanomaterials for eliminating mycotoxins are carbon nanostructures, nanodiamonds, chitosan polymeric nanoparticles, nanoclay binders, and metal nanoparticles. Carbon nanoparticles such as graphene, reduced graphene, graphene oxide, fullerenes, and carbon nanotubes bind mycotoxins to the surface, grooves, bundles, or channels via different binding interactions (Horky et al., 2018). Depending on the applied doses, fullerene nanoparticles exhibit dual roles as antioxidants and pro-oxidants (Živančev et al., 2024).

The polarity and arrangement of functional nanoparticle groups determine their binding efficiency to mycotoxins. For example, nanocarbon structures exhibit amphoteric properties, allowing their surface to undergo protonation and deprotonation, thereby enabling them to bind to polar and non-polar mycotoxins (Horky et al., 2018). These adsorbents are only efficient if the complex remains stable in the digestive tract and ensures bound mycotoxins are excreted via urine and feces (Moore et al., 2016).

Živančev et al. (2024) investigated the effect of various concentrations of fullerene nanoparticles (0.16, 0.32, 0.80, 8, 20, 40, and 80 µg/ml) on AF reduction in YES broth and *A. flavus* contaminated maize flour. They found that fullerene nanoparticles at a concentration of 8 µg/ml exhibited the best adsorption of AF, with a 96% reduction in AFB<sub>1</sub> and complete removal of AFB<sub>2</sub> from the solution in YES broth. Similarly, even at low concentrations of fullerene nanoparticles (0.16–0.8 µg/g), AFB<sub>1</sub> levels in contaminated maize flour decreased by up to 42 and 60% after 7 and 14 d incubation, respectively.

Nanodiamonds are another effective adsorbent of OTA and AFB<sub>1</sub> (Gibson et al., 2011). The adsorption of OTA and AFB<sub>1</sub> onto nanodiamonds depends on the aggregate size and electrostatic interactions, which are influenced by the surface functional groups. Gibson et al. (2011) reported that nanodiamonds (approx. 40 nm) exhibit better adsorption capacity for AFB<sub>1</sub> compared to yeast cells and have adsorption capacity comparable to activated charcoal. Additionally, due to the negative charge or presence of the carboxyl group in OTA and electrostatic interactions, positively charged nanodiamonds outperformed negatively charged clay minerals in adsorption capacity. Some studies have reported that chitosan nanoparticles crosslinked with glutaraldehyde can bind AF, OTA, ZEN, and FUM1, while carbon nanotubes can adsorb AFs, trichothecenes, and ZEN (Horky et al., 2018; Zhao et al., 2015).

A significant challenge in utilizing nanoparticles on an industrial scale is transitioning from laboratory-scale to large-scale production. Also, the formation of nanomaterials necessitates considerable solvent consumption, resulting in high costs and significant environmental impact.

#### 4.1.3. Mechanism of magnetic nanoparticle adsorbents

Magnetic nanoparticles are excellent adsorbents and are easily recoverable with external magnetic fields. Magnetic nanoparticle adsorbents are primarily used in processed food. A detailed review on this topic is out of scope; hence, only a few relevant studies have been discussed here. Pirouz et al. (2017) used magnetic graphite oxide nanocomposites as an adsorbent in naturally contaminated palm kernel cake under optimal conditions of pH 6.2 for 5.2 h at 40.6 °C to obtain reduction levels of 67.3, 69.9, 57.4 and 37.2% for ZEA, DON, HT-2, and T-2; respectively. They concluded magnetic graphene oxide (MGO) is an economical adsorbent combining graphene and iron oxide nanoparticles. Similarly, Saminathan et al. (2018) found high efficacy of magnetic graphene oxide with chitosan (MGO-CTS) against AFs (20 ng/g) present in the broiler feed. MGO-CTS has a high adsorption capacity for OTA, AFB<sub>1</sub>, and ZEA at 50 °C and pH 5 (Pirouz et al., 2018). Zahoor and Ali Khan (2016) prepared magnetic carbon nanostructures from maize straw using ethanolic iron chloride (FeCl<sub>3</sub>·6H<sub>2</sub>O). Another commonly used adsorbent is Surface-Active Maghemite Nanoparticles (SAMN). Magro et al. (2016) demonstrated that treating contaminated *Monascus* suspension with 1 g/L SAMN removed citrinin by 70%. In the subsequent treatment, the citrinin level fell below the analytical detection limits (0.25 mg/L) using the same amount of SAMN.

#### 4.1.4. Industrial application and limitations of mycotoxin adsorbents

Duarte et al. (2009) reported that poultry and pork products account for approximately 5% of overall human exposure to OTA. LAB and yeast cells are primarily used for feed decontamination. There are few organic and inorganic adsorbents available commercially. For instance, the Alltech group produces several microbial adsorbents such as yeast cell wall, MTB-100® (polymeric glucomannan adsorbing agent derived from the cell wall of yeast), and Mycosorb™ (mycotoxin adsorbing agent formulated with yeast glucan); polymeric glucomannan for the adsorption of AF, OTA, ZEN and T2 toxins. Promochem INRA Thivernal-Grignon, a French company, produces *Lactobacillus jugurti* 63, *Lactobacillus lactis* 170, *Lactobacillus casei*, *Lactobacillus rhamnosus* GG, *Streptococcus thermophilus* NG40Z, *Lactobacillus helveticus* 46 and 72, and *Lactobacillus paraplantarum* CNRZ 1885, which adsorb DON, ZEN, FB<sub>1</sub>, and FB<sub>2</sub> (Boudergue et al., 2009). *Lactobacillus casei* can markedly decrease AF absorption within the intestinal tract (Liu et al., 2022). However, the use of adsorbents in grain and food industries is still in its infancy, possibly due to the lack of comprehensive safety evaluation and the unavailability of safety thresholds in the food industry.

#### 4.2. Treatment of mycotoxins with reactive oxidative species and ions

Irradiation by ionizing and non-ionizing radiations is effective in reducing mycotoxin levels in grains. Photolysis of water molecules by gamma and UV radiations generates oxidative species for decontaminating grains. Similarly, plasma degradation of mycotoxins can be directly related to free radicals. The degradation pathway of plasma treatment mainly involves ozonolysis, which involves cleavage and sequential addition reactions. Photocatalysts are another means of generating reactive species by exposing catalytic material to light or UV. These reactive species, such as H<sup>+</sup>, OH<sup>-</sup>, HO<sup>•</sup>, HO<sub>2</sub><sup>•</sup>, H<sub>3</sub>O<sup>+</sup>, and H<sub>2</sub>O<sub>2</sub>, can disrupt the chemical structure of mycotoxins by breaking bonds at specific toxicity sites.

##### 4.2.1. Mechanism of ionizing and non-ionizing radiations

Both ionizing (gamma rays and electron beams) and non-ionizing (ultraviolet) forms of radiation have been used for food preservation and remediation of mycotoxin contamination. These forms of radiation exert direct and indirect effects on fungal inactivation and mycotoxin degradation. The direct impact of irradiating grains depends on the specific wavelengths of light that a given mycotoxin can absorb, as this is crucial to the extent of mycotoxin degradation (Diao et al., 2015). If mycotoxins have strong absorption in the UV or visible range, direct

photolysis might be possible. The indirect method involves photochemical effects, such as forming free radicals and reactive species, that induce mycotoxin degradation (Calado et al., 2014). Mycotoxins that are more photostable are less likely to degrade directly under light exposure, making degradation by reactive species more critical. Ionization of water molecules generates  $\text{H}_2\text{O}^+$  radicals and free electrons, which, by recombination and cross-combinations, generate various reactive species such as  $e_{\text{aq}}^-$ ,  $\text{H}^\bullet$ ,  $\text{OH}^\bullet$ ,  $\text{HO}_2^\bullet$ ,  $\text{OH}^-$ ,  $\text{H}_3\text{O}^+$ ,  $\text{H}_2$ , and  $\text{H}_2\text{O}_2$  that can modify the chemical structure of mycotoxins, influencing their toxicity (Calado et al., 2014). Gamma radiation has a high penetration capacity and effectiveness in inactivating microorganisms (Calado et al., 2017). Ben Amara et al. (2022) reported that 10 kGy gamma radiation degraded 59 and 32% of AFB<sub>1</sub> and OTA, respectively, in naturally contaminated sorghum. Similarly, Mehrez et al. (2016) showed that 8 kGy gamma radiation reduced OTA by 35.5–47.2% in artificially contaminated wheat grains. In contrast, some studies have shown that only high doses (>10 kGy) of gamma radiation can degrade mycotoxins in grains (Stefano et al., 2014). Calado et al. (2017) reported a 24% reduction in OTA in wheat flour at 32% moisture when exposed to 30.5 kGy gamma radiation. Variation in results among studies could be due to differences in initial mycotoxin concentrations, the chemical structure of the particular mycotoxins that were studied, the radiation dose, the moisture content of the substrate, and the type of irradiated food matrix (Ben Amara et al., 2022; Calado et al., 2017).

Electron beam radiation is another irradiation method but has limited penetrability. Luo et al. (2017) reported that an irradiation dose of 50 kGy degraded 71.7 and 67.9% ZEN and OTA, respectively, in maize kernels. They explained that the limited degradation of mycotoxins was mainly due to the high initial concentration of mycotoxins (2812.5 and 60.18  $\mu\text{g}/\text{kg}$  of ZEN and OTA, respectively) and the low moisture content (11.9%) of the grains used in their study. Because of the lower penetrability of electron beam radiation, it is primarily used in combination with food processing techniques and additives for food preservation. For example, electron beam irradiation combined with probiotics effectively inhibits pathogenic microorganisms (Balayan et al., 2019).

Ultraviolet (UV) radiation has a relatively lower energy intensity at the molecular level to stimulate ions or electrons than ionizing radiation. The decontamination efficiency of UV radiation depends on factors such as wavelength, dosage, exposure time, and the intensity of light source. Various studies have reported UV irradiation as an effective method to remediate mycotoxin contamination that has already occurred in cereal grains (Popović et al., 2018; Udovicki et al., 2022), nuts (Babae et al., 2022), and milk (Nguyen et al., 2022). Ghanghro et al. (2016) reported a 65–73% reduction in AF in stored wheat when exposed to 0.1  $\text{mW}/\text{cm}^2$  UV at 254 nm for 160 min. In another study, Popović et al. (2018) reported a reduction of 30% DON, 52% ZEN, and 17% OTA in maize, while 14% DON, 42% ZEN, and 6% OTA in wheat using a UV-C dose of 10,000  $\text{mJ}/\text{cm}^2$  for 444s. They explained that due to the presence of double bond conjugations in DON and OTA, the UV sensitivity of these compounds decreases because radiation gets absorbed before reaching the breakable or low-energy bonds.

Pulsed light (PL) emits UV light in short intervals of high intensity, spanning a broad spectrum of wavelengths from 100 to 1100 nm (Mirza Alizadeh et al., 2021). This spectrum includes short wavelengths known for their potent bactericidal effects. Wang et al. (2018) found that the most effective reduction rates for AFB<sub>1</sub> and AFB<sub>2</sub> in rough rice occurred with a pulsed light treatment duration of 80 s, resulting in a 75 and 39% decrease in AFB<sub>1</sub> and AFB<sub>2</sub>, respectively. Contrarily, a treatment duration of 15 s resulted in a 90 and 86.7% reduction in AFB<sub>1</sub> and AFB<sub>2</sub>, respectively, in rice bran. The cytotoxic and mutagenic activity of AFB<sub>1</sub> and AFB<sub>2</sub> were also eliminated using pulsed light treatment. Moreover, this study demonstrates that UV irradiation is more successful on surfaces than it is for whole-grain decontamination. Pulsed light has a higher photodegradation reaction rate compared to UV-C, primarily due to its capability to generate high-intensity light over brief intervals

(Wang et al., 2018).

#### 4.2.2. Industrial application and limitations of ionizing and non-ionizing radiation

Commercial-scale disinfection primarily utilizes UV germicidal lamps. They are used for disinfecting surfaces, preventing microorganism buildup on food surfaces, sterilizing air, disinfecting packaging materials, and providing a convenient and effective method of purifying water without toxic chemicals. Recently, food industries have utilized UV-LED lamps, which are much smaller than traditional UV lamps, enabling their use with various devices. They are environmentally friendly, mercury-free, emit high-intensity radiation, and require no warm-up time (Chawla et al., 2021). Despite its efficacy, UV irradiation has several limitations, such as the 'shadow effect,' i.e., uneven UV irradiation distribution due to the irregular shape of the grains, low penetration capacity in grains, lamp, and sample heating, and the inability to reduce internal microbial contamination (Shen and Singh, 2021). Furthermore, despite being approved by the FDA, UV light is not often used in the grain industry due to a lack of guidelines on compatible exposure conditions.

#### 4.2.3. Mechanism of photocatalysis

Researchers have recently investigated photocatalytic technology due to its high efficiency, mild reaction conditions, lack of secondary pollution, and low energy consumption for mycotoxin degradation. The process combines light (including UV wavelengths) and a catalytic material. Light with energy levels equal to or greater than the bandgap of the photocatalyst generates electrons ( $e^-$ ) in the conduction band and holes ( $h^+$ ) in the valence band within the photocatalytic material (Murugesan et al., 2021). The generated electrons and holes result in redox reactions on the surface of photocatalytic material and the formation of reactive oxygen species (ROS) such as hydroxyl radical ( $\text{OH}^\bullet$ ), superoxide ( $\text{O}_2^-$ ), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), which act as oxidizing agents that can degrade mycotoxins into less hazardous compounds (Fig. 1). Different semiconductor materials, such as metal oxides, carbon, and hybrid materials, are used for photocatalytic applications. Oxide semiconductors such as  $\text{TiO}_2$  and  $\text{ZnO}$  are commonly used for food mycotoxin degradation. Sun et al. (2019) found that under UV–Vis light, within 120 min, an activated carbon-supported  $\text{TiO}_2$  catalyst ( $\text{AC}/\text{TiO}_2$ ) at a dose of 6  $\text{mg}/\text{ml}$  in methanol resulted in a 95% reduction of AFB<sub>1</sub>. In contrast, UV–Vis irradiation alone led to a decrease in AFB<sub>1</sub> of only 50%.

Further, semiconductors can be doped with composite materials to enhance their photocatalytic efficiency. He et al. (2021) demonstrated the superior photocatalytic activity of a cerium-doped titanium dioxide ( $0.5\text{Ce}-\text{TiO}_2$ ) catalyst compared to traditional pure  $\text{TiO}_2$  treatment, where the former achieved 96% DON degradation in 4 h in an aqueous solution under UV light irradiation. Similarly, Chen et al. (2021) demonstrated superior degradation rates of DON using a protonated graphite carbon nitride/oxygen-doped graphite-phase carbon nitride homojunction ( $\text{CNH}/\text{OCN}$ ) compared to  $\text{OCN}$ ,  $\text{CNH}$ , graphite carbon nitride ( $g\text{-C}_3\text{N}_4$ ),  $\text{ZnO}$  and  $\alpha\text{-Fe}_2\text{O}_3$  catalysts in barley malt.

Wang et al. (2019) and Chen et al. (2021) identified two photocatalytic degradation products of DON, i.e.,  $\text{C}_{12}\text{H}_{18}\text{O}_4$  ( $m/z$  227) and  $\text{C}_{12}\text{H}_{16}\text{O}_3$  ( $m/z$  209.17), following photocatalysis with dendritic-like  $\alpha\text{-Fe}_2\text{O}_3$  and oxygen-doped graphite carbon nitride ( $g\text{-C}_3\text{N}_4$ ). They observed that photocatalysts deoxidized the C12-13 epoxy group to a carbon-carbon double bond and oxidized the double bond at C9,10 to form C-OH in degraded products. In another study, Mao et al. (2019) fabricated a Z-schematic system of clew-like  $\text{WO}_3$  decorated with CdS nanoparticles paired with visible light irradiation to reduce the toxicity of AFB<sub>1</sub> in aqueous solution significantly. The primary mechanism involved the inactivation of the C8-C9 double bond in AF by the additional reaction of hydroxyl radicals.

Recently, studies have focussed on using nanomaterials as photocatalysts due to their unique optoelectronic properties. Zhou et al. (2020) reported complete degradation of DON within 120 min using

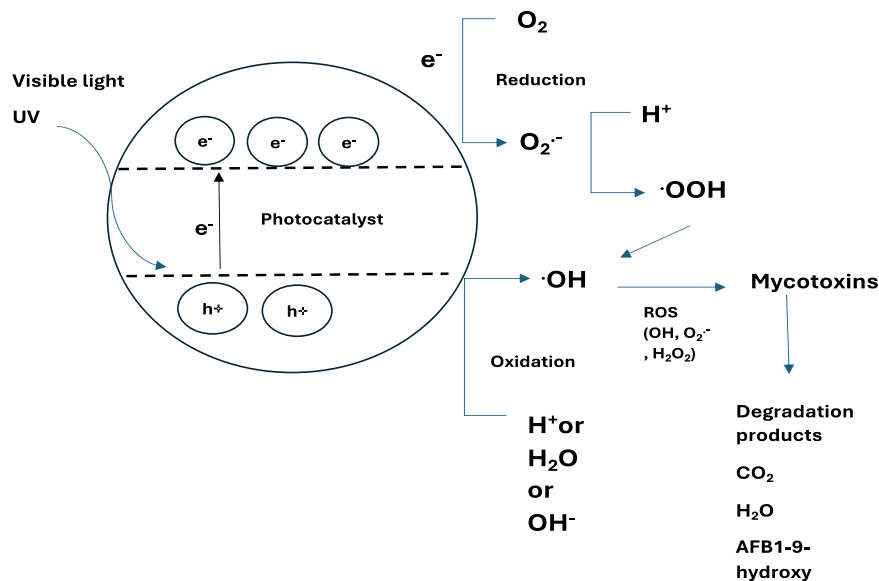


Fig. 1. Mechanism of photocatalytic degradation of mycotoxins by photocatalyst (Adapted from Jing et al., 2024).

upconversion nanoparticles@TiO<sub>2</sub> (UCNP@TiO<sub>2</sub>) composite. Additionally, they found much lower toxicity in degraded products than in DON. Similar findings were reported by Jamil et al. (2017), who achieved an 88.2% reduction of AFB<sub>1</sub> within 120 min using 10 mol% Sc-doped SrTi<sub>0.7</sub>Fe<sub>0.3</sub>O<sub>3</sub> under visible light. They demonstrated that the oxidation mechanism and resulting byproducts suggested ring breaks, forming short-chain aliphatic alcohols that could lead to complete degradation.

#### 4.2.4. Industrial application and limitations of photocatalysis

Although photocatalysis with metal oxide nanoparticles has shown promise in degrading toxins, there are significant challenges to implementing them practically in food systems. Over the past few decades, researchers have explored the application of photocatalysis in various areas, such as wastewater treatment, incorporation into packaging materials, food coatings, and microbial decontamination. However, some limitations hinder its utilization, including developing robust coatings with strong antimicrobial properties to prevent nanoparticle leaching. Additionally, limited information on the stability and safety of photocatalysts used for mycotoxin decontamination is available.

#### 4.2.5. Mechanism of plasma treatment

Applying energy to gases partially or completely ionizes gases to form plasma, the fourth state of matter. Plasma is produced by applying an electric field to gases at ambient pressure, forming reactive species such as hydroxy radicals, superoxide anions, electrons, and ozone, depending on the type of gas used (Deng et al., 2020). These reactive species and their combinations through epoxidation and oxidation reactions degrade the chemical structure of mycotoxins (Gavahian and Cullen, 2019).

Cold plasma is generated by different electrical discharges. The most common method is dielectric barrier discharge, effective at a frequency range between 50 and 10 MHz and pressures from 10<sup>4</sup>-10<sup>6</sup> Pa (Feizollahi et al., 2021). Other production methods include radiofrequency, microwave-induced argon plasma, and gliding arc discharge treatment. Generally, a plasma-producing device consists of a high-voltage source to generate an electric field and a reactor that utilizes this energy to ionize a specific volume of gas to produce plasma.

Utilization of different plasma designs and experimental parameters such as treatment dose and time, type of working gas, mycotoxin and matrix identity, and working gas can vary the efficiency of mycotoxin

degradation significantly. Guo et al. (2023) reported a significant reduction of 61 and 55% in DON and OTA levels, respectively, by exposing contaminated rice grains to cold plasma for 8 min at 25 kV discharge voltage and high voltage AC power. Their findings suggested that longer treatment time could significantly reduce toxin levels. Additionally, they indicated that the higher reduction of DON compared to OTA in cold plasma water treatment could be attributed to the chemical structure and acidification induced by the process, as the C12, 13 epoxy ring in DON is more likely to be broken in an acidic environment. Similar results were reported by Devi et al. (2017), who demonstrated the effect of different treatment times (0, 12, and 15 min) and plasma power (40 and 60 W) on AFB<sub>1</sub> reduction in groundnuts. They found that higher plasma power (60 W) and longer treatment time (15 min) enhanced AFB<sub>1</sub> degradation.

Grain characteristics also play an essential role in determining the degradation efficiency of cold plasma. Ten Bosch et al. (2019) demonstrated that the presence of a matrix hinders toxin degradation by cold plasma. They studied the effect of substrate matrix (rice extract) on the degradation of DON, T-2 toxin, enniatin, and *Alternaria* toxins by plasma treatment. They found that all four mycotoxins in the matrix were more resistant to plasma treatment than pure toxins. This resistance can be attributed to components of the matrix scavenging plasma-generated reactive species, providing alternative targets for attack. Furthermore, cold atmospheric pressure plasma has a low penetration depth, limiting degradation to thin surface layers and protecting nutrient loss in grains.

#### 4.2.6. Industrial application and limitations of plasma treatment

Leap100 (PlasmaLeap Technologies, Sydney, Australia) has been reported to prepare plasma-activated water by generating cold plasma with atmospheric air at 60 kHz resonance frequency and 50 μs duty cycle (Barrales Astorga et al., 2022). The same company developed a large gap pin-to-plate plasma reactor with two steel plates as electrodes with high voltage comprising pin array (11\*8) and flat ground electrode (Venkataratnam et al., 2020). However, this technology lacks regulatory approval and has only been used for research and development. Heniker Plasma Company (Runcorn, UK) also offers plasma equipment for surface decontamination and removal of organic contaminants (Laroque et al., 2022).

However, the application of cold plasma technology is still limited in handling bulk grains in food industries due to other practical challenges. It is a surface treatment, so treating irregularly shaped bulk grains is



challenging due to the limited penetration of reactive species. Moreover, for large-scale industrial applications, continuous processing and upscaling of this technology is complex with the current design of plasma equipment. The operating parameters, barrier discharge configuration, material, and geometry of electrodes need to be optimized for all grain types. Further, there are limited publications regarding the potential cytotoxicity of partially modified mycotoxins in treated grains (Neuenfeldt et al., 2023).

#### 4.2.7. Mechanism of ozonation

Ozonation is a sustainable technology that leaves no residue after its application. Ozone can be generated by exposing dry oxygen to electric corona discharge, UV radiation, and electrolytic and chemical processes (Chandravarnan et al., 2022). Ozone is a powerful oxidant with high reactivity, making it one of the most potent detoxification agents in the

food industry. However, it is highly unstable at room temperature and needs to be continuously generated at the treatment site.

As ozone is also generated by plasma treatment, both techniques have the same mechanisms. Ozone has a high oxidation potential (2.07 V), which facilitates the inactivation of fungi and the degradation of mycotoxins (Afsah-Hejri et al., 2020). The toxicity of mycotoxins is attributed to specific toxic sites present in their chemical structure; for example, double bond at C8-C9 in AF, C12,13 epoxide in DON, etc. Ozone interacts with the functional groups in mycotoxins, altering their molecular structures and producing compounds with reduced molecular weight, fewer double bonds, and decreased toxicity. In DON, ozone targets the C9-C10 double bond via an electrophilic mechanism, whereas in AFB<sub>1</sub>, the epoxidation and oxidation of the C8-C9 double bond decrease toxicity (Li et al., 2015; Porto et al., 2019) (Fig. 2). Furthermore, in ZEN, the oxidation of the double bond at C10-C11 aids

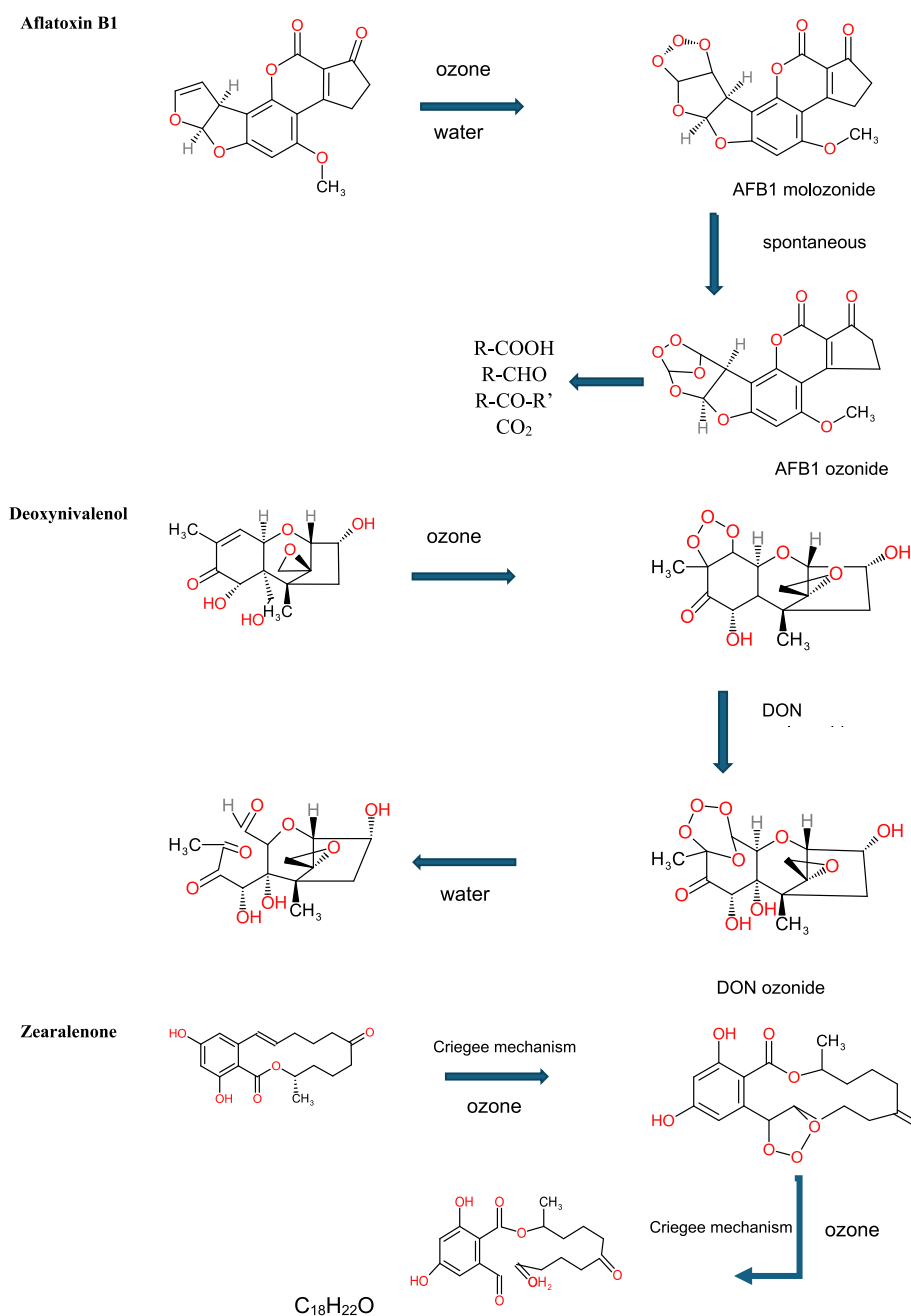


Fig. 2. Degradation mechanism of mycotoxins by ozone treatment (Conte et al., 2020; Feizollahi and Roopesh, 2022; Xu et al., 2019).

the ozonolysis process (Qi et al., 2016).

The effectiveness of ozone degradation depends on ozone concentration and treatment time, the type and concentration of mycotoxin, treatment temperature, and grain parameters such as moisture, porosity, and roughness (Ahmad Mir et al., 2023). The effectiveness of ozone increases with an increase in the moisture content of grains (Chandravarnan et al., 2022). Li et al. (2014) showed that the degradation rate of DON increased from 19.5 to 57.3% in wheat samples with 9.5 and 17% moisture content, respectively, when exposed to 60 mg/L ozone for 12 h. This is because ozone is transported via diffusion, and the presence of water in the grain retards ozone diffusion, leading to prolonged exposure of microflora to ozone. Increasing ozone concentration and processing time improves the degradation of mycotoxins (Porto et al., 2019).

Porto et al. (2019) studied the effect of different ozone concentrations (20–60 mg/L) and treatment time (120–480 min) to reduce AF levels in contaminated maize grits. They found reductions up to 57% in AF levels by applying 60 mg/L ozone for 480 min in a kilogram of maize grit. In another study, Wang et al. (2016) reported almost a double increase in DON degradation, from 26 to 53%, in naturally contaminated wheat treated with 75 mg/L ozone for 30 and 90 min, respectively.

The application of ozone can cause substantial oxidative damage in cereal flour at high ozone concentrations (>50 ppm) (Tiwari et al., 2010). Zhuang et al. (2020) showed reduced sedimentation volume, gluten index, and gluten content of wheat flour exposed to 60 mg/L ozone for 2 h. In another study, the overall quality of ozonized wheat flour was improved due to increased whiteness and tenacity compared to the control sample (Wang et al., 2016). Similarly, Dodd et al. (2011) reported no negative effect of gaseous ozone on the quality parameters of final malt. Alexandre et al. (2019) studied the nutritional profile of ozonated flour and found no significant difference except for an increase in palmitic acid content.

#### 4.2.8. Industrial application and limitations of ozonation

Many companies, such as Spartan Environmental Technologies Ltd., Oxidation Technologies Ltd., Sihon Ltd., and Absolute Ozone Ltd., produce commercial ozonators. An ozonator converts oxygen from the air into ozone using electrical discharges or ultraviolet light. The equipment is used in textile industries for wastewater treatment, in food industries for sanitization and prevention of foodborne illness, and in the medical sector for sterilization and disinfection. However, the grain industry does not widely use ozonation due to limitations such as high capital cost and oxidative deterioration of product quality. Furthermore, using ozone in stored grains can cause corrosion of metal storage bins, as ozone is a strong oxidizer. Only high-quality food-grade stainless steel can withstand ozonation, making the material cost-prohibitive for storage bins. Ozone decomposes rapidly, which necessitates onsite production of ozone. Additionally, safety procedures are mandatory while working with ozone as it irritates the human respiratory tract. Moreover, the formation of mycotoxin degradation by-products after oxidation may be more toxic or persistent than the target compound (Jevtić et al., 2021). Hence, toxicity assessment of degradation products is critical while determining the utility of any oxidative decontamination technique.

#### 4.3. Biotransformation

Biotransformation is the conversion of one mycotoxin form to another through biological processes. Here, we consider biotransformation to encompass the effects of microbial enzymes in transforming mycotoxins into less toxic compounds. Biotransformation is an environment-friendly and effective way of controlling fungal growth and grain mycotoxin contamination.

##### 4.3.1. Mechanism of enzymes

Enzymes are easy to handle, pose no risk of contamination, offer

good repeatability and uniformity, and do not raise safety risks for operators, unlike adsorbent microorganisms (Li et al., 2023; Loi et al., 2017). The functionality of enzymes heavily relies on the configuration and layout of their active site, i.e., a minute region within the protein molecule that accommodates and causes chemical change to very specific substrates. This active site comprises a catalytic center and a binding site, which are integral for enzyme activity. Some chemical reactions involved in enzymatic biotransformation include ring cleavage, hydrolysis, decarboxylation, acetylation, deamination, oxidation, hydroxylation, and glucosylation (Adegoke et al., 2023).

Various enzymes can degrade mycotoxins in real matrices and in vitro (Loi et al., 2017). Most enzymes known for degrading AFs belong to the oxidoreductase category, including peroxidases and laccases (Loi et al., 2023). One of the mechanisms hypothesized for AF degradation by oxidases involves nucleophilic attack on the furan and lactone rings, resulting in their opening (Kumar et al., 2022). Loi et al. (2020) utilized recombinant type B dye decolorizing peroxidase to convert 96% AFB<sub>1</sub> into AFQ1 with lower acute toxicity at 25 °C and 4 days. Zeinvand-Lorestani et al. (2015) observed that a laccase degraded 67% of the total AFB<sub>1</sub> amount within two days under optimal conditions. Also, the degraded product exhibited reduced prooxidative properties and mutagenicity compared to AFB<sub>1</sub>. Furthermore, laccases have been found capable of degrading ZEN. Banu and Lupu (2013) conducted reactions with 62 mg/mL of ZEN, incubating them with 0.4 mg/mL of laccase at 30 °C for 4 h, leading to a peak degradation rate of 81.7%. Similar results were found by Loi et al. (2018), who assessed the effectiveness of laccase and laccase-mediator systems derived from *Pleurotus eryngii* and observed simultaneous degradation of 86% AFB<sub>1</sub> and 100% ZEN. Besides laccase, ZEN can be degraded by lactase and peroxidase.

Recent studies suggest that the current approach to degrading ZEN involves degrading its lactone structure using lactonase or laccase and oxidizing or modifying the C6 ketone and hydroxyl groups at C2 and C4. Zhang et al. (2019) cloned a lactonase from *Gliocladium roseum*, named ZENG, which effectively degrades ZEN. The optimal conditions for recombinant ZENG were pH 7 and 38 °C, achieving high degradation rates for  $\alpha$ -ZOL and  $\alpha$ -ZAL.

The toxicity of DON is mainly linked to its epoxides group, so breaking this ring can significantly mitigate its toxicity. The *Coriobacteriaceae* family (Biomin® BBSH® 797, DSM 11798) synthesizes de-epoxidase capable of detoxifying trichothecenes (Loi et al., 2023). Another degradation pathway of DON is the oxidation of hydroxyl groups at the C3 position. Shanakhat et al. (2022) reported that fungal laccase paired with redox mediator TEMPO can alter the chemical structure of DON by oxidizing hydroxyl groups at C3 and C15 positions and covalently linking the chemical mediator at the C4 position.

Loi et al. (2017) reviewed the degradation of OTA as being linked to two classes of carboxypeptidases: carboxypeptidase A (CPA) and carboxypeptidase Y (CPY). CPY extracted from *Saccharomyces cerevisiae* was shown to degrade OTA optimally at pH 5.6 and 37 °C (Abrunhosa et al., 2010). However, the efficiency of CPY was considerably lacking, with just 52% of OTA transformed into OT $\alpha$  following a five-day incubation period. *Trichosporon mycotoxinivorans*, a non-pathogenic yeast, also produces peptidases that detoxify OTA. However, both OTA hydrolase and OTase enzymes are up to 600 times more active than carboxypeptidase A for the hydrolysis of OTA. Some studies have demonstrated amidase reduces OTA toxicity by hydrolyzing the toxic amide bond and cleaving OTA into OT $\alpha$  and L- $\beta$ -phenylalanine (Zhang et al., 2017; Sun et al., 2023). Other enzymes associated with OTA degradation are lipases and commercial proteases. Enzymatic removal of the free amino group at C-2 and de-esterifying the ester bonds at C-14 and C-15 are used to detoxify fumonisins (Alberts et al., 2019). Some microorganisms and enzymes that cause fumonisin detoxification are amino oxidase enzymes of *Exophiala spinifera* ATCC 74269, carboxypeptidase (*Bacillus amyloliquefaciens* ASAG1), recombinant carboxylesterase from the bacterium *Sphingopyxis* sp., *Lactococcus lactis*, *Komagataella phaff*, and laccase from *Pleurotus eryngii* (Li et al., 2020);

Alberts et al., 2019; Adegoke et al., 2023).

#### 4.3.2. Industrial application and limitations of enzymes

Due to the discovery of very few effective enzymes to date, only a limited number of commercially available enzymes exist. Some commercial enzymes are fumonisin esterase FumD (FUMzyme®; BIOMIN, Tulln, Austria) and ZENzyme®. The former has been developed to hydrolyze the tricarballic acid groups of fumonisin B1. It has been approved by the European Food Safety Authority (EFSA) as safe for the environment, humans, and animals (Alberts et al., 2019). ZENzyme® is a hydrolase recognized for its efficacy in detoxifying zearalenone, and it has recently gained approval for use in all terrestrial animal species (Loi et al., 2023). Mycofix® Plus 5.Z (DSM, Tulln Austria) is an EU-authorized and patented product that can deactivate ZEN, FMN, AF, OTA, trichothecenes and ergot alkaloids. Carboxylesterase (Biomin, Austria) is another commercially available enzyme obtained from fumonisins degrading bacterium *Sphingopyxis macrogoltabida*. Recently, some companies have come up with additives that contain both enzymes and adsorbents to increase the efficacy of mycotoxin decontamination. For example, VemoZyme Detox® contains alkaline proteases (peptidase), neutral protease, acid protease, glucose oxidase from *Aspergillus niger*, bentonite (90% montmorillonite), and activated charcoal. Enzyme application has been primarily seen in animal feed industries, with enzymes used in animal diets in order to reduce the risk posed by contaminated feed. It is important that such additives be stabilized by suitable formulations. However, this is not a new challenge as a wide range of other enzymes (including proteases,  $\alpha$ -amylases, xylanases, phytases, polygalacturonases,  $\alpha$ -galactosidases, and glucanases) are increasingly utilized in animal nutrition, yielding increased nutritional value and micronutrient accessibility. However, enzymes can convert mycotoxins to less or more toxic compounds, so newly discovered mycotoxin-modifying enzymes must be well characterized in terms of whether actual detoxification is achieved. Furthermore, extracting and purifying enzymes is time-consuming and labor-intensive (Li et al., 2023), creating a challenge for affordability in the resulting products.

## 5. Remarks and conclusions

Decontamination techniques have different mechanisms to detoxify mycotoxins associated with grains. This review reveals three primary mechanisms for remediating grains contaminated with mycotoxins: 1) adsorption using microbial and physical adsorbents, 2) generating reactive oxidative species and ions and 3) biotransformation using enzymes. Detoxifying methods dismantle the molecular structures of mycotoxins that are responsible for their toxic effects. According to the literature, these techniques show varying efficiency in detoxifying mycotoxins, with some reported methods even demonstrating complete decontamination. Techniques like gamma and UV irradiation are widely used to deactivate fungi and their toxins. Irradiation techniques are most effective in high-moisture grains, inhibiting fungal growth and directly ionizing mycotoxins under specific conditions, facilitating their elimination. Adsorbents are predominantly utilized in livestock feed industries, although select ones, including *S. cerevisiae*, *Lactobacillus*, and clay nanoparticles, are deemed safe for human food application, depending upon their purity, composition, and dosage. Similarly, food industries widely use oxidative and photolysis techniques for water treatment or sanitation.

Furthermore, irradiation emerges as a potential solution for mycotoxin decontamination in the grain industry due to its low cost, moderate efficiency and lack of residue. Some recent decontamination techniques discussed in this review, such as nanoparticles, cold plasma and pulsed light irradiation, have considerable decontamination efficiency but no broad industrial applicability. For example, reactive species produced by cold plasma and ozonation are short-lived and have low penetrability, which limits their industrial application. Most techniques are still in the initial stages of development and require further

optimization and validation before they can be commercially utilized in the grain industry (Table 3). Emerging technologies for grain decontamination are currently being investigated at the laboratory level. It is essential to evaluate these technologies at an industrial scale to determine their feasibility and suitability for commercial application. In this review, we compared each technique's effectiveness in degrading fungi and mycotoxins, specifically within a grain matrix, and its economic feasibility. Using these criteria, we ranked each technique's potential for decontamination efficacy and scalability as high, medium or low (Table 3). Additionally, we identified knowledge gaps and limitations from the current literature to assess the need for further research (Table 3).

Until now, none of the individual decontamination techniques have been able to remove mycotoxins in food or feed completely, nor are they standard for all food matrices (Hamad et al., 2023; Afsah-Hejri et al., 2020). Therefore, recent research has focused on synergistic or integrated approaches of combining multiple decontamination techniques to produce additive or synergistic effects, improving their detoxification efficiency and better adaptability to different food matrices (Hamad et al., 2023). However, significant knowledge gaps remain in optimizing the treatment parameters when multiple techniques are used simultaneously or sequentially.

Another vital knowledge gap that future research needs to address is the need for toxicity tests for derived decontaminated products. To resolve discrepancies and ensure consistent degradation mechanisms, toxicity assessments of degraded products under different treatment methods and for various mycotoxins must be conducted. Effective diagnostic tools that can be used to monitor and quantify mycotoxins in stored grains are required. Research must also be directed to creating models predicting mycotoxigenic mold activity and the conditions hindering mycotoxin production.

This review critically discusses the recent findings of mycotoxin decontamination techniques and examines their mode of action and current and potential industrial applications. These techniques operate via distinct decontamination mechanisms, with some focussed solely on surface decontamination, while others show greater efficacy in penetrating deeper within grains. Irradiation treatments such as gamma and UV radiation are highly effective in mycotoxin decontamination but may negatively affect grain appearance and functional properties. On the other hand, cold plasma, ozone, and pulsed light irradiation use short treatment times and are non-thermal techniques that do not degrade the nutritional quality of grains. Similarly, biological treatments such as enzymes are safe and effective in reducing mycotoxins without affecting grain quality. Despite showing promise in effectively decontaminating mycotoxins, the practicality of these methods depends on scalability. Given their potential for commercialization, it is imperative to promote the adoption of these technologies on a large scale, particularly in developing countries where mycotoxin contamination is widespread. Additional research should explore the synergistic effects of combining two or more techniques.

### CRedit authorship contribution statement

**Aanchal Pande:** Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Jitendra Paliwal:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Fuji Jian:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Matthew G. Bakker:** Writing – review & editing, Resources.

### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Aanchal Pande reports financial support was provided by Natural

Table 3

Summary of the efficacy, scalability, research needed, and cost of important mycotoxin decontamination technologies.

Technique	Efficacy		Upscalability	Research needed	Cost
	Fungal disinfection	Mycotoxin degradation			
Thermal treatment	Moderate	Low	Moderate	No	Moderate
Adsorbents nanoparticles	Moderate	Low	Low	Yes, for specific binding of mycotoxins	Low
Irradiation	Moderate	Moderate	Moderate	No	Moderate
Ozone	High	Moderate	Moderate	Yes, for toxicity study of degraded products	Moderate
Cold plasma	High	Moderate	Low	Yes, for toxicity study of degraded products	High
Photocatalyst	Moderate	Low	Low	Yes, for safety studies	Low-moderate
Enzymes	High	Moderate	Low	Yes, for residual toxicity of degradation products and need research effort to discover new enzymes	High
Microorganism	Moderate	Moderate	Moderate	Yes, for in vivo studies on bioavailability and toxicity	Moderate
Plant extracts	High	Moderate	Low	No	High

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### Data availability

No data was used for the research described in the article.

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