

Application of novel technologies for the inactivation and reduction of fungi and mycotoxins

Edited by

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Application of novel technologies for the inactivation and reduction of fungi and mycotoxins

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Editorial: Application of novel technologies for the inactivation and reduction of fungi and mycotoxins

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Editorial on the Research Topic

[Application of novel technologies for the inactivation and reduction of fungi and mycotoxins](#)

Fungal contamination and mycotoxin production continue to challenge food safety, agricultural sustainability, and public health worldwide. The vast diversity of mycotoxins, their environmental resilience, and their toxicity demand robust, interdisciplinary solutions. Recently, innovative approaches have emerged, from biological detoxification to predictive AI modeling and structure-based inhibitor design. This editorial synthesizes findings from cutting-edge studies that offer a broad, integrative view of current progress and future potential.

One of the most promising biological tools is probiotic detoxification. [Murtaza et al.](#) demonstrated the ability of *Lactobacillus plantarum* CN1 to remove up to 75.6% of zearalenone (ZEN) from dried distiller's grains and solubles (DDGS). The enhanced detoxification capacity post-autoclaving points to cell wall structural changes facilitating ZEN binding. In addition to toxin removal, the fermentation process enriched feed nutritional value, reinforcing the dual benefit of such bioprocesses.

Equally significant are yeast-based biocontrol strategies. [Zhang et al.](#) showed that xylitol supplementation boosted the antioxidative defenses and antagonistic activity of *Meyerozyma guilliermondii*, improving postharvest resistance against mold infections in apples. This work reflects a paradigm shift-leveraging metabolic priming to increase efficacy under stress conditions, thereby extending shelf-life and reducing reliance on fungicides.

To combat mycotoxins preemptively, understanding fungal ecology in processed foods is vital. [Li et al.](#) characterized fungal diversity in jujube products, revealing *Aspergillus*, *Penicillium*, and *Cladosporium* as dominant genera. Aflatoxin and ochratoxin contamination traced to *Aspergillus flavus* and *Aspergillus niger* highlights the need for continuous monitoring and targeted interventions during processing and storage.

Progress in biodegradation via microbial enzymes also shows strong promise. [Chang et al.](#) used *Bacillus mojavensis* L-4 in semi-solid fermentation of cornmeal to degrade

ZEN, identifying key degradation enzymes and sequencing the strain's genome. Their work provides a foundation for engineering improved detoxifiers with broad-spectrum activity.

A similar direction was pursued by Subagia et al., who applied a recombinant *Bacillus subtilis* laccase (BsCotA) to detoxify aflatoxin B1 (AFB1). The codon-optimized enzyme achieved significant reduction in toxin activity (>80-fold less toxic), and the study highlighted the synergistic role of microaerobic conditions in enhancing enzyme expression and function.

Adding a novel structure-based molecular approach, Wang et al. investigated the cytochrome P450 monooxygenase AflG, a key enzyme in aflatoxin biosynthesis. Using molecular modeling, microsecond-scale molecular dynamics (MD) simulations, and high-throughput virtual screening of over 1.3 million compounds, the authors identified two potent inhibitors of AflG. These molecules disrupted AflG-averantin interactions, significantly reducing aflatoxin production in *A. flavus*. This precision-targeting strategy opens a new frontier for chemical biology in food safety: the possibility of rationally designed inhibitors to prevent toxin biosynthesis at the molecular level.

These molecular strategies are complemented by non-thermal physical methods. Molina-Hernandez et al. reviewed technologies such as high-pressure processing, pulsed electric fields, UV light, ozone, cold plasma, and blue light. These treatments offer effective fungal inactivation and mycotoxin degradation with minimal impact on food quality. However, efficacy remains dependent on fungal strain, food matrix, and specific mycotoxin structure necessitating case-by-case validation.

Among these, cold atmospheric pressure plasma (CAP) stands out. Dias de Oliveira et al. discussed how CAP's reactive species inactivate spores, disrupt membranes, and chemically modify mycotoxins like aflatoxin B1 and deoxynivalenol. CAP treatments preserve food nutrients, making them viable for large-scale postharvest use.

Finally, machine learning and AI-driven predictive models provide tools for preemptive intervention. Castano-Duque et al. used neural networks and gradient boosting to forecast aflatoxin and fumonisin risks in Illinois corn based on environmental and satellite data. With predictive accuracies up to 85% for fumonisin, such models could revolutionize monitoring and risk management systems, giving producers tools to act before contamination occurs.

Together, these studies reveal a converging future for mycotoxin mitigation where bio-based solutions, precision

molecular targeting, non-thermal technologies, and predictive analytics all play synergistic roles. Future success lies in cross-disciplinary integration: microbial engineering, AI, structural biology, and food processing must work hand-in-hand.

Furthermore, regulatory adaptation will be key. As technologies like enzyme detoxification and molecular inhibitors enter the food chain, agencies must develop frameworks to assess efficacy, safety, and consumer acceptance. With supportive policy, the innovations discussed here can transform food systems from reactive containment to proactive protection.

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Dynamic geospatial modeling of mycotoxin contamination of corn in Illinois: unveiling critical factors and predictive insights with machine learning

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Mycotoxin contamination of corn is a pervasive problem that negatively impacts human and animal health and causes economic losses to the agricultural industry worldwide. Historical aflatoxin (AFL) and fumonisin (FUM) mycotoxin contamination data of corn, daily weather data, satellite data, dynamic geospatial soil properties, and land usage parameters were modeled to identify factors significantly contributing to the outbreaks of mycotoxin contamination of corn grown in Illinois (IL), AFL >20 ppb, and FUM >5 ppm. Two methods were used: a gradient boosting machine (GBM) and a neural network (NN). Both the GBM and NN models were dynamic at a state-county geospatial level because they used GPS coordinates of the counties linked to soil properties. GBM identified temperature and precipitation prior to sowing as significant influential factors contributing to high AFL and FUM contamination. AFL-GBM showed that a higher aflatoxin risk index (ARI) in January, March, July, and November led to higher AFL contamination in the southern regions of IL. Higher values of corn-specific normalized difference vegetation index (NDVI) in July led to lower AFL contamination in Central and Southern IL, while higher wheat-specific NDVI values in February led to higher AFL. FUM-GBM showed that temperature in July and October, precipitation in February, and NDVI values in March are positively correlated with high contamination throughout IL. Furthermore, the dynamic geospatial models showed that soil characteristics were correlated with AFL and FUM contamination. Greater calcium carbonate content in soil was negatively correlated with AFL contamination, which was noticeable in Southern IL. Greater soil moisture and available water-holding capacity throughout Southern IL were positively correlated with high FUM contamination. The higher clay percentage in the northeastern areas of IL negatively correlated with FUM contamination. NN models showed high class-specific performance for 1-year predictive validation for AFL (73%) and FUM (85%), highlighting their accuracy for annual mycotoxin prediction. Our models revealed that soil, NDVI, year-specific weekly average precipitation, and temperature were the most important factors that correlated

with mycotoxin contamination. These findings serve as reliable guidelines for future modeling efforts to identify novel data inputs for the prediction of AFL and FUM outbreaks and potential farm-level management practices.

KEYWORDS

Aspergillus, *Fusarium*, machine learning, gradient boosting, neural network, aflatoxin, fumonisin, soil

1. Introduction

Mycotoxin contamination of food and feed crops, such as corn, in the United States (US) leads to annual losses estimated in the range of \$418 million to \$1.66 billion (Vardon et al., 2003; Wu, 2006; Mitchell et al., 2016; Johns et al., 2022). These toxins are produced by distinct fungal species of *Aspergillus* and *Fusarium* (Proctor et al., 2018; Munkvold et al., 2019) and are well characterized by their severe health impacts on humans, livestock, and animals (Vardon et al., 2003). In corn, aflatoxins (AFL) are primarily caused by *A. flavus* and *A. parasiticus*, and fumonisins (FUM) are produced by *F. verticillioides* and *F. proliferatum* (Woloshuk and Shim, 2013). The severity of disease epidemics and associated mycotoxin contamination is driven by environmental and agronomical conditions, with the former being the most relevant. Each fungal species has optimal conditions that favor inoculum formation and dispersal, disease development, and mycotoxin production. For example, for *A. flavus*, the optimal growth temperatures are 30 to 35°C (Abdel-Hadi et al., 2012), while for *F. verticillioides*, temperature ranges between 20 and 25°C (Medina et al., 2014) are ideal for growth. These temperature optima tend to favor the growth of aflatoxin-producing fungal species such as *Aspergillus* spp. in southern latitudes (Klich, 2002; Kerry et al., 2021), whereas these optima favor the growth of *Fusarium* spp. in northern latitudes (Zingales et al., 2022). The geographical distribution of these fungi in the US will most likely change due to climate change leading to an expansion of the temporal and latitudinal temperature growth optima of these mycotoxigenic fungi (Nnadi and Carter, 2021; Yu et al., 2022; Zingales et al., 2022). Additionally, in general, hot conditions favor *A. flavus* conidiation and dispersal and *Fusarium* infection of corn (McMillian et al., 1985; Payne et al., 1986, 1988; Diener et al., 1987; Widstrom et al., 1990; Payne and Widstrom, 1992; Guo et al., 1996; Sétamou et al., 1997; Scheidegger and Payne, 2003; Cotty and Jaime-Garcia, 2007). Furthermore, kernel water activity (0.92–0.97 aw) and insect injury contribute to mycotoxin production (Warfield Colleen and Gilchrist David, 1999; Miller, 2001; Munkvold, 2003; Bush et al., 2004).

The agroecosystem and production of fungal mycotoxins involve soil biogeochemical characteristics that impact both crops and fungi. For example, carbonate content, saturated hydraulic

conductivity, pH, bulk density, and texture, among others, influence soil microbial communities, and which species of fungi, including pathogens, might dominate in a given soil environment (Weil and Brady, 2002). Soil property variability is related to factors such as geologic deposition, age of material, climate, duration of soil development, land use history, and other factors. Much of the soils in Northern IL were formed in glacial and periglacial deposits from the Wisconsin glacial episode of the late Pleistocene associated with various advances and retreats of the continental Laurentide ice sheet, while soils in the more southern portion of the state developed from older parent materials with a difference in soil age of approximately 100,000 years or more (Bergstrom et al., 1976). The soil in Northern IL is richer in clay and carbonate materials. Previous studies in IL have shown a north–south difference in historical mycotoxin contamination (Castano-Duque et al., 2022). The 40° latitude in IL marks a geographical and meteorological boundary in the state that leads to differences in weather from mixed humid to wintry conditions (Köppen, 2011; Beck et al., 2018). Correspondingly, warmer, more humid conditions in Southern IL, provide relatively more favorable conditions for the fungi to infect corn and produce mycotoxins at lower latitudes compared to higher latitudes.

Integrated pest and disease management strategies that aim to minimize economic losses associated with mycotoxin contamination need to account for spatial and temporal-specific factors as well as weather, crop health and genetics, past agronomic practices, and soil characteristics. Mycotoxin contamination assessment tools are key for growers and stakeholders to be informed of the potential probabilities of mycotoxin outbreaks and facilitate the deployment of strategies aimed at controlling disease and grain contamination (Focker et al., 2020). Satellite-acquired databases, including the normalized difference vegetation index (NDVI), have been previously used in studies evaluating mycotoxins in European wheat and led to higher mycotoxin model accuracy (Wang et al., 2022). Several studies have shown significant correlations among climate, soil properties, NDVI, and agricultural management practices with AFL contamination in Africa, as reviewed by Keller et al. (2022). AFL studies conducted in Eastern and Western Kenya (Mutiga et al., 2014) support the utility of remote-sensing data such as NDVI, rainfall, and soil properties (such as organic carbon content, pH, total exchangeable bases, salinity, texture, and soil type) to accurately predict AFL contamination (Smith et al., 2016). Soils and their interactions with hydroclimate are highly variable (Smith et al., 2019; Vergopolan et al., 2022) and influence fungal and microbial populations in soils.

Predicting mycotoxin contamination through models has benefited corn production in other countries, but US corn growers,

Abbreviations: AFL, Aflatoxins; FUM, Fumonisins; ARI, Aflatoxin risk index; GBM, Gradient boosting machine; NN, Neural network; FHB, *Fusarium* head blight; BIC, Bayesian information criterion; NDVI, Normalized difference vegetation index; Veg. Index, Vegetative index; PCRP, Precipitation; TAVG, Average temperature; ML, Machine learning; H, High level; L, Low level.

which produce the most corn in the world, have yet to realize such benefits. Published models assess mycotoxin contamination of milk in Eastern Europe (Van der Fels-Klerx et al., 2019) and mycotoxin risk in small grains, corn, and other cereal crops in Italy (Leggieri et al., 2021), Serbia (Liu et al., 2021), Europe (Wang et al., 2022), and Korea (Lee et al., 2018). Machine learning (ML) technologies such as artificial neural networks have been developed in Europe to identify mycotoxin contamination in corn kernels post-harvest using electronic nose technology in northern Italy (Leggieri et al., 2021). Mycotoxin contamination prediction modeling involves other factors, such as fungal development and dispersal. Spore dispersal modeling is an actively developing field of research worldwide, as reviewed by González-Domínguez et al. (2023). Furthermore, the impact of AFL predictive risk models in sub-Saharan Africa has been reviewed by Keller et al. (2022), showing the economic impact of these technologies. The basis of these models served as a general roadmap to model the prediction of AFL and FUM contamination in IL-US. In the US, modeling of fungal diseases has been done by the *Fusarium* head blight (FHB) risk assessment tool from the US Wheat and Barley Scab Initiative (<https://www.wheatscab.psu.edu/>) that uses modeling approaches to alert wheat and barley growers of FHB risk (Shah et al., 2018). A US-based predictive model based on insurance claims used as a proxy for mycotoxin contamination values is available for corn (Yu et al., 2020, 2022). However, insurance claims are not necessarily equivalent to actual mycotoxin values. Previous US models targeted the prediction of multinomial levels of AFL and FUM contamination and showed that ML approaches can detect weather factors pre-harvest that significantly impact mycotoxin accumulation in IL (Castano-Duque et al., 2022).

The need for US-focused models is even more important when considering that mycotoxin contamination is predicted to increase in the years to come due to climate change (Van der Fels-Klerx et al., 2016, 2019). Climate change models predict increased fungal growth and subsequent toxin production (Battilani et al., 2016) due to negative effects on crop host health and the interaction of multiple abiotic factors such as drought, leading to losses in corn production (Long Stephen et al., 2006; Battilani et al., 2016; Peng et al., 2020). Increasing temperatures, longer drought periods, and rising carbon dioxide levels (Vaughan et al., 2014, 2016) as the effects of climate change are expected to continue leading to increased crop vulnerability combined with favorable conditions for fungi such as *Aspergillus* spp. to grow and produce toxins. These conditions will lead to an increase in plant stress hormones that have been shown to alter plant defense responses (Guo et al., 2008), making the crop more vulnerable to fungal infection and mycotoxin contamination. Therefore, it is predicted that climate change has the potential to negatively impact northern corn-producing states (Corn Belt), as projected weather conditions will increase AFL-related risk (Wu et al., 2011). Risk assessment analysis using 16 climatic models from 2031 to 2040 in the US has shown that 89.5% of corn-growing counties will experience an increase in AFL prevalence, and this projection includes corn in the northern states, suggesting the spread of fungal infection and mycotoxin contamination from southern regions toward the northern Corn Belt (Yu et al., 2022), where it has the potential to be even more devastating to corn production. Models developed

by using mycotoxin data from major US corn-growing states and trained using weather, satellite, and geographically dynamic features for US-focused areas are critically needed. They will benefit corn growers by mitigating losses projected to occur in extreme weather events associated with climate change.

We developed a mycotoxin model that is geospatial dynamic and IL-focused to identify novel risk factors correlated with mycotoxin contamination, we applied machine learning predictive models. We provided new information about the influence of soil properties and remote-sense data (NDVI) on the prediction of AFL and FUM contamination in corn. Understanding the correlations of these factors with mycotoxin contamination can lead to region time-specific targeted integrated pest management practices to prevent AFL and FUM outbreaks, thus serving as guidelines to identify factors that contribute to the contamination risk.

2. Materials and methods

2.1. Mycotoxin data

We used 14 years of historical contamination data, daily county-specific weather data, remotely sensed satellite reflectance data, and data on soil-specific features. Feature engineering (Battilani et al., 2013; Van der Fels-Klerx et al., 2019) was used in combination with gradient boosting models (GBMs) (Friedman, 2001) and neural networks (NNs) to predict (Torgo, 2016) AFL and FUM. In this study, we used 1,772 observations of mycotoxin concentrations, AFL, and FUM from IL-grown corn. Data on the response variable were obtained from historic yearly survey summaries for 2003–2004 and 2008–2021 (data for 2020 were not available due to the COVID-19 pandemic) published by the IL Department of Agriculture. The years used for model validation were selected from these data based on the percentage of incidence of high contamination events. AFL was measured in 2010 (960 data points, 4% incidence of high contamination levels) and FUM was measured in 2016 (960 data points, 8.1% incidence of high contamination levels). The sample collection was done as described by Castano-Duque et al. (2022); briefly, the kernel sample collection was performed at harvest by randomly collecting 2.3–4.5 kg of whole kernels from four corn producers from each county. While mycotoxin analyses were conducted as soon as possible after collection, to minimize post-harvest mycotoxin accumulation, the samples were dried and stored at 4°C. This mycotoxin data set was zero-inflated, with high contamination levels being rare events (Castano-Duque et al., 2022). This issue was considered during data preparation for ML analysis by using the synthetic minority oversampling technique (Torgo, 2016) that allowed us to generate a balanced data set of the high and low contamination events.

2.2. Output variables and correlation analysis

We categorized into discrete variables the AFL and FUM contamination values by following the US Food and Drug Administration's legal threshold limits for mycotoxins in

feed (<https://www.fda.gov/food/natural-toxins-food/mycotoxins>). For AFL, a high category was considered for concentrations >20 ppb (20 ng/g) and low for levels 20 ppb or lower (Supplementary material 1). For FUM, a high contamination level was for concentrations >5 ppm (5,000 ng/g), and the rest of the observations were low (Supplementary material 1). The FUM threshold used is the FDA guidance for equids and rabbits, which is lower than for other classes of livestock. A correlation analysis was performed among all the predictors and output variables using a confidence level of 0.95 for the correlation and clustering method in R (Team, 2017).

2.3. Weather, satellite, and soil data

Soil physical and chemical properties, satellite reflectance data, and soil moisture were analyzed to account for geographic, geomorphic, and surficial geological heterogeneity relating to crop health and soil habitat suitability for mycotoxin-producing fungi. All the satellite data were extracted and summarized by the county after refining them to reflect only land uses for corn and wheat (Boryan et al., 2011). For the extraction of crop specificity of satellite data, a data mask layer was generated for corn acreage for each year of study from the National Agricultural Statistics Service Cropland Data layer (NASS CDL—<https://nassgeodata.gmu.edu/CropScape/>) data set (Boryan et al., 2011). NASS CDL provides 30-meter grid cell estimates of crop type grown for the Conterminous United States for each year starting in 2005 to the present. Each grid cell represents a best estimate of crop type for a 30-meter by 30-meter division of land. To make the data mask, in each year of study, for each grid cell classed as corn in Illinois, a value of 1 was assigned, while a value of 0 was assigned for all other land uses. Each time-series normalized difference vegetation index (NDVI) layer from the MODIS satellite data was multiplied by the mask so that only pixels of MODIS with their centroids falling within corn acreage were tabulated for the county-level NDVI averages (Didan, 2021). The same process was repeated for wheat, with a separate mask file produced from the NASS CDL and separate NDVI county-level averages produced.

Soil properties were similarly aggregated to the county level by using the NASS CDL data mask. In this case, values of 1 were assigned to cropland, and values of 0 were assigned to other land uses such as forest or urban (Boryan et al., 2011). Soil properties were then queried for all pixels with a value >0 in the Soil Properties data layer from UC Davis and USDA NRCS (Walkinshaw et al., 2022). Mean values for all soil properties for cropland were tabulated to represent cropland soil properties by county. Climatological soil moisture estimates were obtained for the top 5 cm of the soil in IL from SMAP-HydroBlocks at 30 m resolution, developed by combining microwave satellite remote sensing, radiative transfer modeling, machine learning, and a high-resolution land surface model (Vergopalan et al., 2020, 2021).

The physical soil properties available for examination within the mycotoxin model context were water-holding capacity, hydraulic conductivity, bulk density, and soil texture (sand, silt, and clay content). Soil chemical properties include calcium carbonate content, cation exchange capacity, electrical conductivity, pH, and

organic matter content. Estimates were determined for the 800 × 800 m pixels for various soil depth increments using measured values and interpolation techniques (Walkinshaw et al., 2022). The soil data were queried for soil properties by examining the intersection of each pixel with the cropland reported in the national cropland data layer of the National Agricultural Statistics Service of the USDA (Boryan et al., 2011). This national cropland data layer (CropScape—<https://nassgeodata.gmu.edu/CropScape/>) provides estimates of cropping land use for 30 m × 30 m land units (pixels) throughout the contiguous continental US for a 5-year summary period prior to the publication date (the summary period used was 2017–2021). For each county, the cropland was characterized for the final 5 years of the study. Only the land area that was planted in crops for the period of study was summarized for its soil properties, which were aggregated to the county-wide level as mean soil property estimates. These estimates excluded urban, forested, and other land uses not pertaining to crop production.

Soil moisture estimates ($\text{m}^3 \text{H}_2\text{O}/\text{m}^3\text{-soil}$) were extracted from the SMAP-HydroBlocks (Vergopalan et al., 2021) 2015 to 2019 soil moisture average. SMAP-HydroBlocks is a satellite-based surface soil moisture product available at 30 m resolution over the conterminous United States. SMAP-HydroBlocks is derived from NASA's Soil Moisture Active-Passive product and the HydroBlocks-Radiative Transfer Model using a “cluster-based merging scheme” (Vergopalan et al., 2020) that is parameterized using machine learning-guided refinements based on relations among other *in situ*, satellite-based observation, and land use-land cover characteristics (Vergopalan et al., 2020). The data product provides soil moisture estimates for the top 5 cm of the soil surface at 6-h intervals. In turn, SMAP-HydroBlocks is sensitive to rainfall, drought, and evapotranspiration dynamics relevant to the microenvironment of mycotoxin-producing fungi.

Historical daily average air temperature, precipitation, and general vegetation index data were obtained from GRO-Intelligence (<https://gro-intelligence.com/>). A non-crop-specific vegetative index from GRO-intelligence was calculated from satellite data by taking into consideration multiple light spectra to enhance the presence of green vegetation by calculating the normalized difference vegetation index (NDVI), and this NDVI will be called *veg_index* to differentiate it from crop-specific NDVI. Crop-specific NDVI was calculated for each pixel of reported corn production for each year studied and for each pixel of reported winter wheat production for each year studied. The NDVI values, which were identical to the pixels of soil property estimates, were also summarized with a county-wide mean to characterize NDVI for corn and wheat-cropped land over time. NDVI was calculated from the MODIS satellite data (Didan, 2021) by obtaining the difference between the near-infrared reflectance and the red reflectance divided by the sum of the near-infrared and the red reflectance given in the MODIS data set. Higher values of NDVI are given when vegetation is healthy and green, as it absorbs red light and reflects near-infrared light. These NDVI values are available every 16 days from the MODIS satellite from February 2000 to 2023 and are reported in a 250-m resolution. We extracted these time-series values from MODIS data for the pixels determined to be growing either corn or wheat for each 16-day

period available using Google Earth Engine. The crop-specific NDVI and *veg_index* (general NDVI) data were then interpolated into weekly summaries for the periods of study.

Historic meteorological data, soil properties data, and NDVI data were linked to the county-level mycotoxin data by using the county and the year as common information. After linking toxin data with weather, NDVI, and soil data, there were 1,772 data points across 99 counties.

2.4. Features engineering and data imputation for the AFL modeling

Daily average precipitation (mm) and air temperature (°C) data, averaged per county for the 14 years of historical data, were acquired from GRO-Intelligence. Furthermore, we used the geographical centroids of each county (latitude and longitude) and the climate zones (Friedman, 2001). For AFL models, we engineered a weekly aflatoxin risk index (ARI) as the cumulative sum per week of daily ARI. The monthly ARI was previously described (Castano-Duque et al., 2022); herein, we modified it to calculate a daily ARI (Eq. 1). These ARI equations have been applied to data from Eastern Europe in particular from Ukraine, which has a climate equivalent to IL (Van der Fels-Klerx et al., 2019).

$$\text{ARI} = \text{growth or weighted_growth} \times \text{afla or weighted_afla} \times \text{dispersal} \times (1 + \text{ECB_damage}) \quad (1)$$

In the daily ARI, fungal growth was calculated on a daily basis (Battilani et al., 2013) as described by Castano-Duque et al. (2022). Weighted fungal growth (10% of original growth) was generated for days of the month throughout the year when there was no corn in the field. The distribution of mycotoxin levels in relation to the thermal climate zone in IL (Friedman, 2001) has been examined and linked to differences in mycotoxin contamination distribution (Castano-Duque et al., 2022). In IL, there are two climate zones: a mixed-humid climate zone with a monthly outdoor temperature below 7°C during the winter and a cold climate zone with 5,900 heating degree days per year (18.3°C basis) (Baechler and Love, 2004). These climatological differences were considered in the model by assuming that the planting date in the mixed-humid zone started about 15th May and in the cold zone in April. This was assumed to be different in the cold and mixed-humid counties due to differences in no-corn in the field times in northern and southern states (January to April, 15th May, and December for cold and January to April and December for mixed-humid). These dates were included by calculating the ARI (Eq. 1). One of the assumptions in our model is that during days of the year when there is no corn in the field, the fungal growth was 90% less. The daily AFL production index was calculated (Battilani et al., 2013) as described by Castano-Duque and Vaughan (Castano-Duque et al., 2022). The spore dispersal was set as an ON/OFF switch (Van der Fels-Klerx et al., 2019; Castano-Duque et al., 2022). The assumption in our model is that if there is any daily precipitation, the dispersal is OFF, and if there is no daily precipitation, the dispersal is ON. Wind and other variables are involved in the spore dispersal of *Aspergillus* (Segers et al., 2023) and *Fusarium* (Hoffmann et al., 2021) species;

nevertheless, only precipitation was used in this model due to the consistency of daily historical records throughout the geographical region studied in this case study. Insect damage was calculated for European corn borer damage by using growing degree days ($T_{base} = 6^\circ\text{C}$ and $T_{cut} = 30^\circ\text{C}$) and the logistic equation (Maiorano et al., 2009) (Eq. 2).

$$\begin{aligned} A &= 7.3 \\ U_m &= 0.013 \\ \text{Lambda} &= 1,236.913 \\ R^2 &= 0.718 \\ e &= \exp(1) \end{aligned}$$

$$\text{ECB_damage} = A \times \exp\{-\exp[(\mu_m \times e/A) \times (\text{lambda} - \text{cumGDD} + 1)]\} \quad (2)$$

where ECB (European corn borer) represents damage to the ear by insects, A represents the asymptotic maximum damage level observed in the field approximated to the higher first decimal, μ_m represents the maximum specific damage rate, λ represents the intersection of the tangent in the inflection point with the x -axis, and accGDD represents the accumulated GDD. Both μ_m and λ are estimated parameters in Maiorano and Reyneri (Maiorano et al., 2009).

For any missing values in the weekly ARI or vegetative index features, we performed imputation using multivariate imputation by chained equations; in specific, the predictive mean matching (PMM) method was used in R (van Buuren and Groothuis-Oudshoorn, 2011; Team, 2017). This imputation method allowed us to determine plausible data values by adding a data point from the original data whose regression-predicted values are closest to the regression-predicted value for the missing data point from the simulated regression model (van Buuren and Groothuis-Oudshoorn, 2011). Due to the high number of missing data for ARI in weeks 8, 16, 24, 32, 40, and 48, we decided to remove these input features from the model. Finally, we linked the AFL data to the feature data set to create a set of 1,772 data points and 153 features or predictors. The inputs for the AFL model were weekly ARI for each county (Supplementary Table 1). We also added to our input features an average bi-weekly NDVI per county during the weeks of the month when corn might not be in the field (below week 13 and above week 45); an average corn and wheat NDVI; soil properties, latitude, and longitude.

2.5. Weather data and imputation for the FUM modeling

For FUM modeling, we could not use feature engineering functions such as the ARI calculated for AFL produced by *Aspergillus* because these equations were generated using *Aspergillus* spp growth curves. The inputs for the FUM model were weekly average temperature and precipitation. We also added to our input features an average bi-weekly *veg_index* per county during the days of the month when there is no corn in the fields; an average corn and wheat-specific NDVI; soil properties; latitude; and longitude. The daily precipitation and daily average temperature data were averaged weekly per county; each calendar year of weather data was kept separate and linked to mycotoxin

yearly data for the 14 years of historical data per year and county (Supplementary Table 1). Average temperatures were expressed in degrees Celsius, and each county was assigned its geographical centroids (latitude and longitude), climate zones (Beck et al., 2018), crop-specific NDVI, and veg_index. For any missing values in our weekly weather data, crop-specific NDVI, and veg_index features, we performed imputation using multivariate imputation by chained equations, specifically the predictive mean matching (PMM) method in R (van Buuren and Groothuis-Oudshoorn, 2011; Team, 2017). Due to the high number of missing data for weather variables in weeks 8, 16, 24, 32, 40, and 48, we decided to remove these input features from the model. Finally, we linked the FUM data to the feature data set to create a set of 1,772 data points and 199 features or predictors.

2.6. Gradient boosting machine learning

GBM was used to perform the prediction of mycotoxin contamination, as GBM allows for the determination of the relative influence of input features on the output variable. The GBM software package in R was used. This software provided extensions to Freund and Schapire's AdaBoost algorithm and Friedman's gradient boosting machine (Friedman, 2001). For performing GBM, we first removed the IL county identifier from the data set and the data for the year that was used for validation, as these would lead to overfitting the model. Next, data were balanced by using the synthetic minority oversampling technique (SMOTE) from the DMwr package in R (Team, 2017), which created a new balanced data set by oversampling observations from the high contamination level class. Finally, the balanced data were partitioned to generate training and testing sets by using a 70 to 30 ratio (Supplementary material 1). We performed GBM for AFL and FUM separately, using the following flags on the training data: a threshold of 500 trees, an interaction depth of 1, a shrinkage of 0.01, and 10 cross-validation folds with the distribution selected as multinomial (Supplementary material 1).

The GBM R package (Ridgeway, 2006) was used to perform prediction analysis using the testing data set and the best fit generated from the training data. GBM uses decision trees in an iterative process to determine the best number of trees leading to the lowest error based on the decline of the loss function. Finally, the GBM package was used to compute the effect values (variable relative influence) for each predictor in the model. The relative influence of each predictor from the tree ensemble is determined as the reduction in the sum of squared error due to the splits on that predictor, then averaging the improvement made by each variable across all the trees to determine the relative effect (Friedman, 2001). Thus, the variables with the largest average decrease in the sum of squared error are considered the most important, expressed as the percentage of the total reduction in the error given by all the predictors. A confusion matrix was developed using the caret package in R that computed the overall statistics, the specific statistics by class, the receiver operating characteristic curve (ROC), and the area under the ROC curve (AUC) values for the multi-class classification to measure the quality of the model's predictions.

For the FUM model, we used the following flags on the training data set: a threshold of 500 trees, interaction depth of 1, shrinkage of 0.01, 10 cross-validation folds, and the distribution was selected as multinomial (Supplementary material 1). The remaining analysis was performed as described for AFL.

2.7. Neural network analysis

NN was selected as a second modeling method because of its high performance in the prediction of rare events (Zamani and Kremer, 2013; Gibson and Kroese, 2022). For performing NN training, we first removed the IL county identifier and the year from the data set to reduce potential overfitting. Then, we selected only the input variables that show a relative influence higher than zero in the GBM and then removed the data for the year that was used for validation. Data without the validation set were balanced using the SMOTE method described for GBM. Data were partitioned to create training and testing sets using a 70-to-30 ratio (Supplementary material 1). The same mean and standard deviation scaling was applied to the testing and validation data sets. We trained the NN model in AFL and FUM separately. The best number of hidden layers and neurons was determined by an exhaustive iterative process that aimed to find the combination of NN parameters with the highest accuracy.

2.8. Validation analysis using GBM and NN

To perform prediction analysis using the GBM and NN models, we used a single year: 2010 for AFL and 2016 for FUM. These years were selected because their incidence of high contamination events was higher than 3% (more than three high contamination events), and the incidence of high contamination events was 4 and 8.1%, respectively. Validation was performed by using the best fit of GBM and the best number of hidden layers (2 layers) and neurons (65 neurons in layer 1 and 15 neurons in layer 2) for AFL and FUM (65 neurons in layer 1 and 30 neurons in layer 2) determined from the training data (Supplementary material 1). All the input features for the validation years were prepared as previously described in the Methods section for the training data. The mycotoxin data for the years 2010 and 2016 included 99 counties and a total of 99 observations.

R code: Available in Supplementary material 1.

3. Results

3.1. AFL and FUM contamination in IL

The AFL data showed that only 3.1% of the samples had concentrations >20 ppb, and only 5.4% of the samples had FUM concentrations >5 ppm (Table 1).

TABLE 1 Incidence of AFL and FUM contamination (high or low) in corn in Illinois from 2003 to 2021.

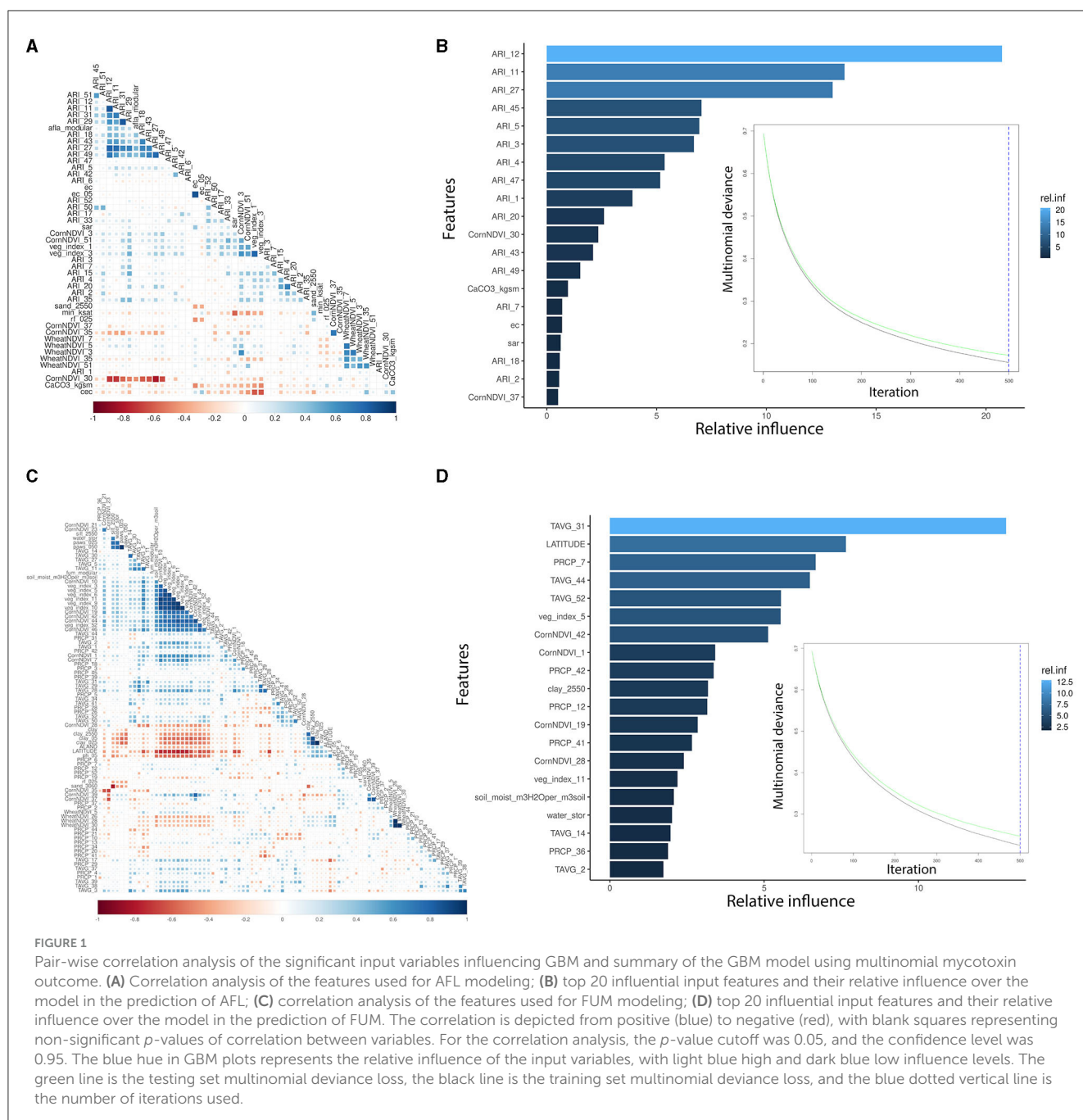
Year	AFL			FUM			N total
	Level	Number of cases	Incidence	Level	Number of cases	Incidence	
2003	High	2	2%	High	16	16.20%	99
	Low	97	98%	Low	83	83.80%	
2004	High	0	0%	High	2	2%	99
	Low	99	100%	Low	97	98%	
2008	High	2	2%	High	25	25.30%	99
	Low	97	98%	Low	74	74.70%	
2009	High	0	0%	High	6	6.10%	99
	Low	99	100%	Low	93	93.90%	
2010	High	4	4%	High	0	0%	99
	Low	95	96%	Low	99	100%	
2011	High	2	2%	High	4	4%	99
	Low	97	98%	Low	95	96%	
2012	High	35	35.40%	High	3	3%	99
	Low	64	64.60%	Low	96	97%	
2013	High	2	2%	High	1	1%	99
	Low	97	98%	Low	98	99%	
2014	High	0	0%	High	1	1%	99
	Low	99	100%	Low	98	99%	
2015	High	0	0%	High	0	0%	99
	Low	99	100%	Low	99	100%	
2016	High	1	1%	High	8	8.10%	99
	Low	98	99%	Low	91	91.90%	
2017	High	4	4%	High	1	1%	99
	Low	95	96%	Low	98	99%	
2018	High	2	2%	High	16	16.20%	99
	Low	97	98%	Low	83	83.80%	
2019	High	0	0%	High	3	3%	99
	Low	99	100%	Low	96	97%	
2021	High	1	0.30%	High	10	2.60%	386
	Low	385	99.70%	Low	376	97.40%	

Data were not collected in 2020 due to COVID-19. For AFL high is ≥ 20 ppb, and for FUM high is ≥ 5 ppm. N is the number of samples per year.

3.2. Weather variables and feature engineering

AFL risk indexes (ARIs) were the main features engineered by linking fungal growth (Battilani et al., 2013; Van der Fels-Klerx et al., 2019; Liu et al., 2021; Castano-Duque et al., 2022) with the weather. Using ARI instead of temperature and precipitation led to a lower number of input variables incorporated into the AFL model, which also decreased the correlation levels among meteorological variables (Figures 1A, C). For the FUM modeling, we could not use feature engineering functions such

as the ARI calculated for AFL produced by *Aspergillus*. FUM output variables showed a significant correlation with general veg_index, corn-specific NDVI, temperature, and several of the soil properties (Figure 1C). Using GBM and only the relative influential variables for the NN led to the inclusion of a lower number of input variables in the models, decreasing the autocorrelation among variables. Furthermore, GBM allows for the control of overfitting caused by high correlation levels among variables by sequentially generating model ensembles that can learn from the errors of previous ensembles (Cooper, 1990; Friedman, 2001).



3.3. GBM analysis for AFL and FUM

We used GBM to model AFL and FUM contamination levels (factorial output variable) with our engineered features for AFL and without engineered features for FUM. The models were used to make predictions for the samples in the testing data set (Table 2, Supplementary Table 1). The optimal number of trees for GBM-AFL and GBM-FUM used was 500, which represents the number of trees at which the cross-validation error is minimized (Figures 1B, D). The McNemar P -value for the GBM-AFL was 0.4862 and for the GBM-FUM was 0.052 (Supplementary Table 1), which indicated that the proportion of type I and type II errors is the same, meaning that there is homogeneity in the proportion

of misclassification. This was expected because the training and testing data sets were balanced by using SMOTE. The overall accuracy of the GBM-AFL model in the test data set (30% of data) was 96%, with a balanced accuracy for high contamination of 96%. For GBM-FUM, the overall accuracy of the test data set was 92%, with a balanced accuracy of 92% (Table 3, Supplementary Table 1). Balanced accuracy or performance is a metric that uses the average of the sensitivity and specificity of the model by considering the predicted classes (high and low contamination). The model performance was the same for the prediction of high and low contamination events. Thus, the balanced accuracy is equal to the overall accuracy. The multi-class area under the curve for GBM-AFL in the testing data set was 0.97 and for GBM-FUM was

TABLE 2 Contingency tables for GBM-AFL, GBM-FUM, NN-AFL, and NN-FUM from the test (30% of the data) and validation (single year) data sets.

Model	Date set	Prediction	Reference	
			High	Low
GBM-AFL	Test	High	455	14
		Low	19	472
	Validation	High	0	0
		Low	4	95
GBM-FUM	Test	High	445	48
		Low	30	427
	Validation	High	6	20
		Low	2	71
NN-AFL	Test	High	472	2
		Low	14	472
	Validation	High	2	4
		Low	2	91
NN-FUM	Test	High	449	12
		Low	26	463
	Validation	High	8	27
		Low	0	64

TABLE 3 Summary statistics of GBM and NN for AFL and FUM.

	Overall accuracy test-set	Balanced accuracy test-set	Overall accuracy validation-set (single year)	Balanced accuracy validation-set (single year)
AFL-GBM	96%	96%	96%	50%
AFL-NN	98%* (46/25/15/2)**	98%*	94%*	73%*
FUM-GBM	92%	92%	78%	76%
FUM-NN	96%* (80/65/30/2)**	96%*	73%*	85%*

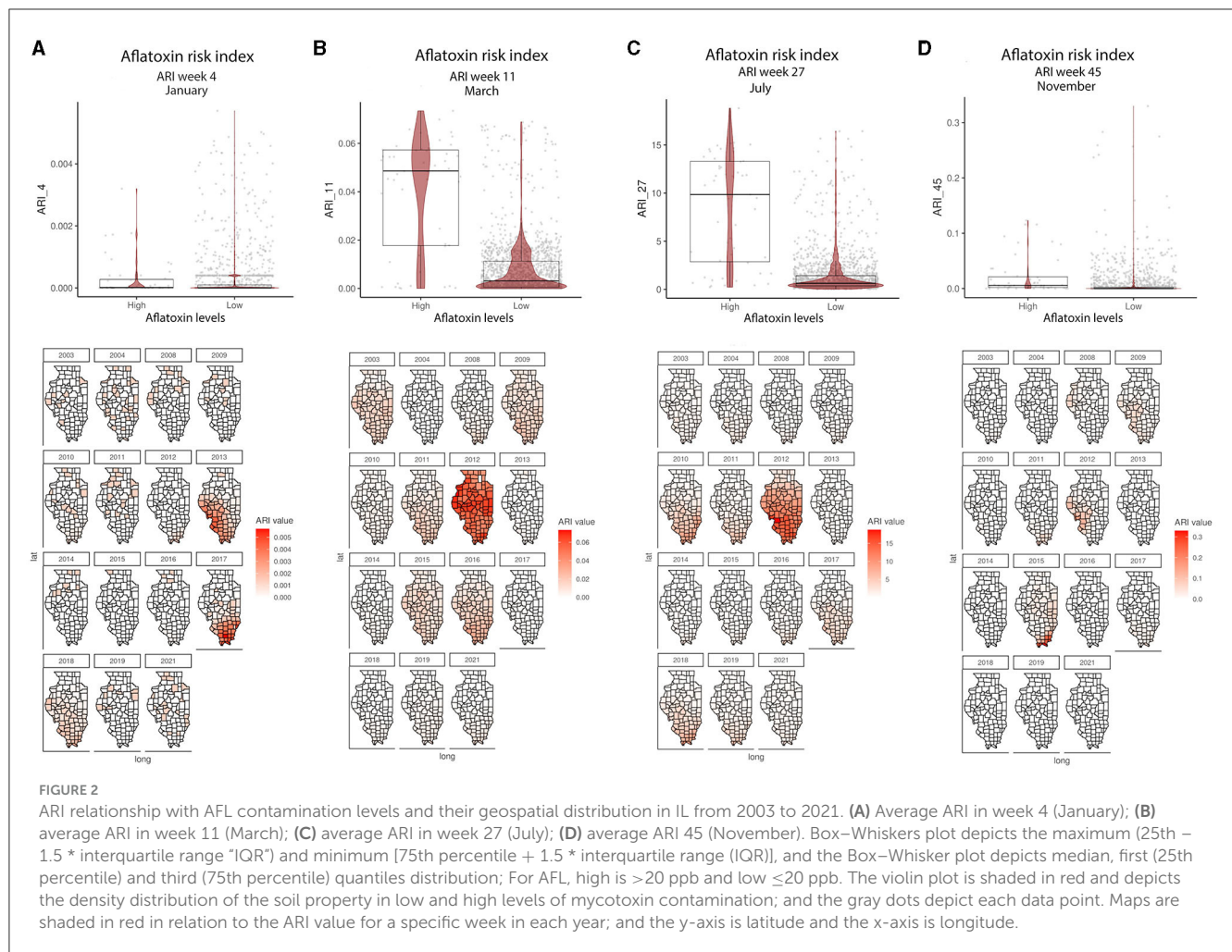
Overall and balanced accuracy for AFL used 2010 as the independent year and FUM used 2016 (Year was not used as an input variable). *Used only input variables with non-zero influence in the GBM. **Number of input variables/number of neurons in the first hidden layer/number of neurons in the second hidden layer/number of outputs.

0.92, which represents the classifier in its ability to distinguish among classes.

The GBM-AFL model showed that of the 153 input features (predictors), only 46 had non-zero influence (Supplementary Table 1). The GBM-FUM showed that of the 199 input features, only 85 had non-zero influence (Supplementary Table 1). For the GBM-AFL model, the top 20 influential features were: ARI in January (Weeks 1, 2, 3, and 4), in February (Weeks 5 and 7), in March (Weeks 11 and 12), in April (Week 18), in May (Week 20), in July (Weeks 27 and 30), in September (Week 37), in October (Week 43), in November (Weeks 45 and 47), and in December (Week 49), soil calcium carbonate (CaCO₃), soil electrical conductivity (ec) and soil sodium absorption ratio (sar) (Figure 2, Supplementary Table 1).

In the GBM-AFL, ARI showed a positive correlation with AFL contamination, meaning that high ARI led to high toxin contamination (Figures 1A, 2). At a geospatial level, the ARI throughout IL tends to be higher in the southern region

except during 2012, the year with the highest incidence of AFL contamination in the historical data set evaluated (35.4%, Table 1), when the ARI levels were the highest throughout the state in March (week 11) and July (week 27) (Figures 1A, 2). Corn NDVI showed a negative correlation with AFL contamination levels in July (week 30) (Figure 3A) and a positive correlation with wheat NDVI values in February (week 5) (Figure 3B). At a geospatial historical scale, corn NDVI values in week 30 were lower in the southern part of IL in 2012 compared to other years (Figure 3A), and PRCP between weeks 28 and 31 was lower in 2012 compared to other years throughout IL. Calcium carbonate levels in the soil had a negative correlation with AFL levels, meaning that low calcium carbonate tends to be associated with high AFL at harvest (Figure 4A). Lower deposits of calcium carbonate were observed in the southern regions of IL compared to the northeast (Figure 4B). To summarize, the GBM-AFL model showed that ARI pre-planting months (January, February, March, and April) had a strong influence over predictions for AFL contamination

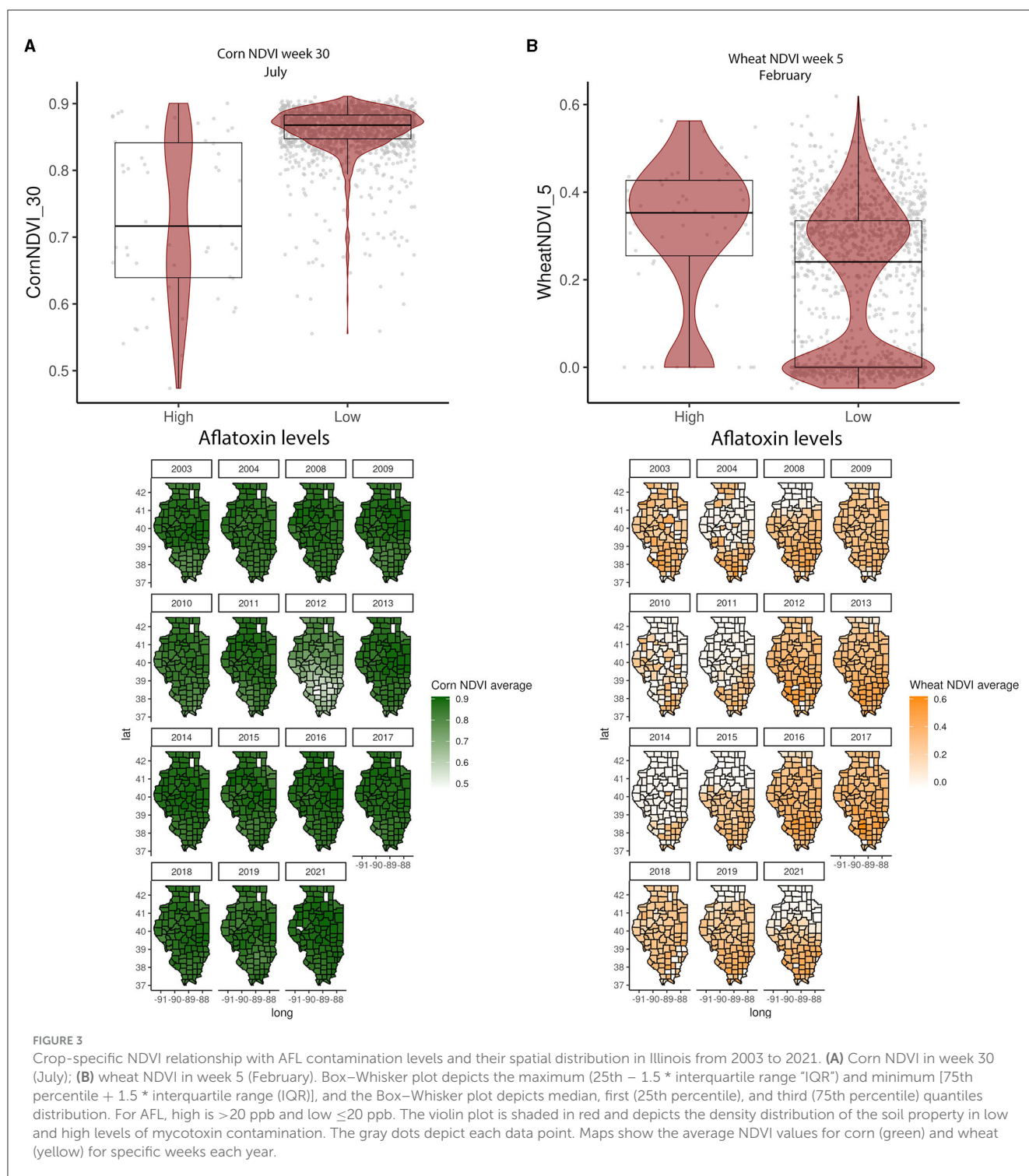


at harvest time, and calcium carbonate levels in the soil influence aflatoxin levels.

The GBM-FUM showed that of the 199 input features (predictors), only 85 had non-zero influence (Table 2, Supplementary Table 1). Among the 85 features, the top 20 were: latitude; temperature in January (week 2), February (week 5), April (week 14), July (weeks 30 and 31), August (week 34), November/December (week 44), and December (week 52); precipitation in February (week 7), March (week 12), September (weeks 36 and 39), and October (weeks 41 and 42); veg_index February (week 5) and March (week 11); corn-specific NDVI January (week 1), May (week 19), July (week 28), and October (week 42); wheat-specific NDVI in June/July (week 26); clay percentage 25–50 cm depth; water storage and soil water capacity (Supplementary Table 1). In the GBM-FUM, temperature in July (Week 31) and October (Week 44) had a positive relationship with FUM contamination levels (Figures 5A, B), similarly for PRCP in February (Week 7), higher precipitation led to higher FUM contamination levels (Figure 5C) at a geospatial historical level, and high PRCP in March throughout IL in 2008 was linked to the highest incidence of FUM (25.6%, Table 1). To summarize, weather patterns such as precipitation levels in week 7 (February)

above 2.5 mm and week 11 (March) above 0.3 mm can lead to higher FUM levels (Figure 5). Furthermore, temperatures in week 31 (July) above 24°C and week 44 (October) above 10°C correlated with high levels of FUM contamination in corn (Figure 5).

General vegetation index in March (Week 11) and corn-specific NDVI in February (week 5), May (week 19), and October (week 42) were positively linked to high FUM contamination, which tends to be observed in the southern parts of IL (Figure 6). While the wheat-specific NDVI showed a negative relationship with FUM contamination levels in June (week 26) (Figure 6D). Soil moisture and available water-holding capacity showed a positive correlation with FUM contamination levels, while clay at 0–5 and 25–50 cm showed a negative correlation (Figure 7). At a geospatial scale, both soil moisture and water-holding capacity tend to be higher in the southwest areas of IL, while the clay content tends to be higher in the northeast regions of the state (Figure 7). GBM-FUM showed that pre-planting precipitation and temperature influence the prediction of FUM contamination levels. Furthermore, crop-specific NDVI is a high influencer, as are several soil characteristics linked to water present in the soil.



3.4. NN analysis for AFL and FUM

We used NN to model AFL and FUM contamination levels (factorial output variable) by using the input features with non-zero relative influence values from the GBM results (Supplementary Table 1). The McNemar *P*-value for testing NN-AFL was 0.007 and for testing NN-FUM was 0.035 (Supplementary Table 1), which indicated that the proportion

of type I and type II errors is not the same, meaning that there is a disproportion of misclassification. This misclassification disproportionality can be seen in the NN-AFL model, which classified 14 data points of 486 as low when they were high and 2 data points of 474 as high when they were low contamination levels (Table 2, Supplementary Table 1). Similarly, the NN-FUM classified 26 data points as low when they were high and 12 as high when they were low (Table 2, Supplementary Table 1). The

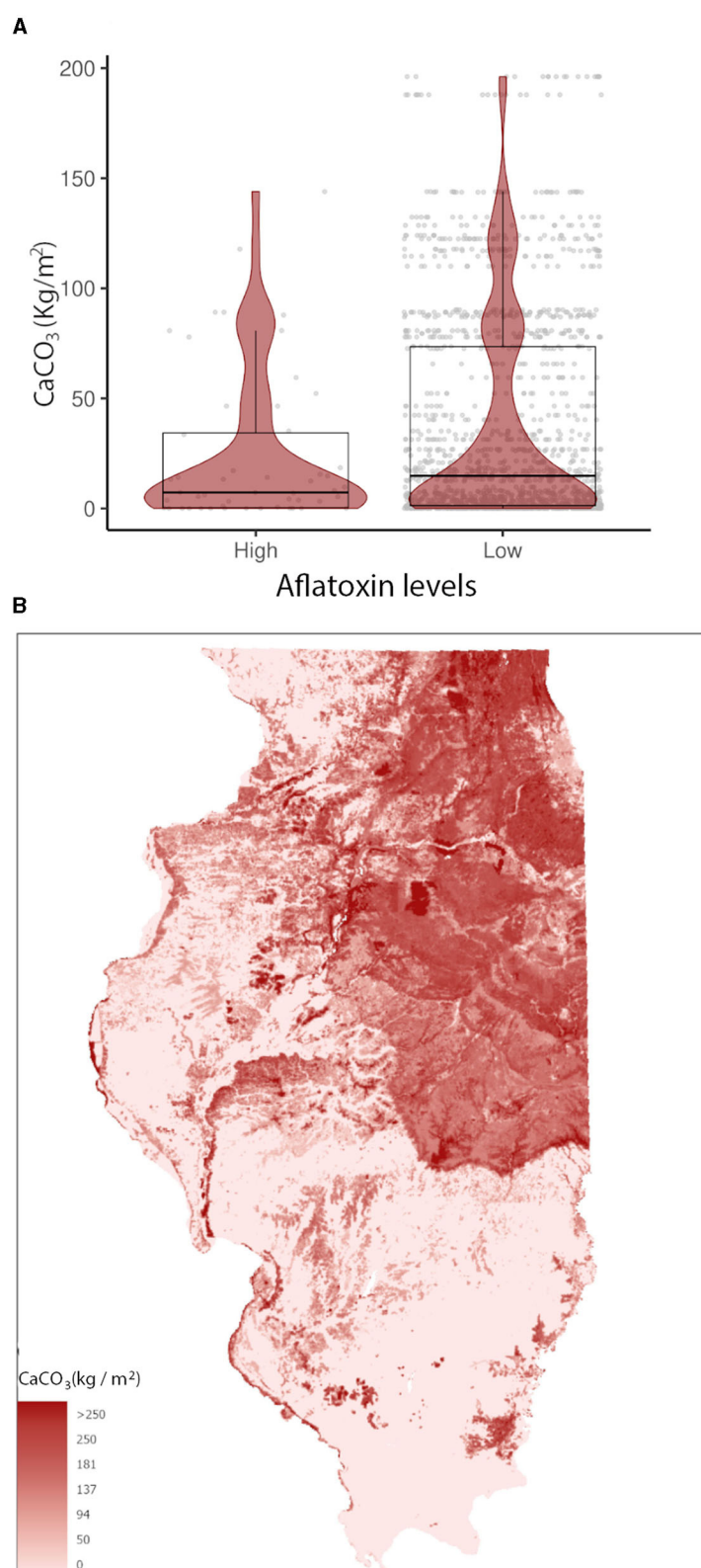
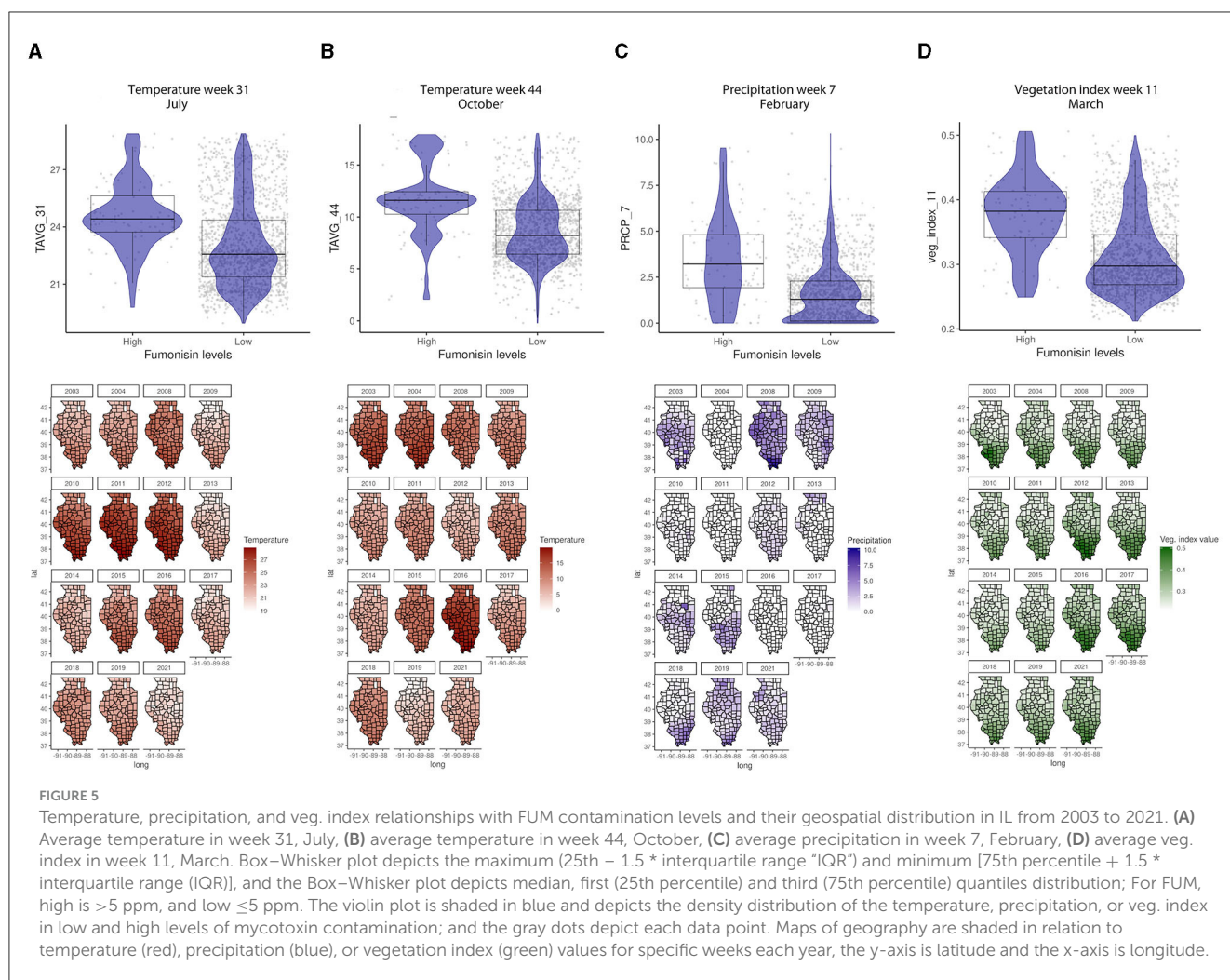


FIGURE 4

Calcium carbonate (CaCO_3 Kg/m²) relationship with **(A)** AFL contamination levels from 2003 to 2021 and its **(B)** spatial distribution in IL. Box-Whisker plot depicts the maximum (25th + 1.5 * interquartile range "IQR") and minimum [75th percentile + 1.5 * interquartile range (IQR)], and the Box-Whisker plot depicts median, first (25th percentile), and third (75th percentile) quantiles distribution. For AFL, high is >20 ppb and low is ≤20 ppb. The violin plot is shaded in red and depicts the density distribution of calcium carbonate in low and elevated levels of mycotoxin contamination; and the gray dots depict each data point. Maps show the weight percentage of calcium carbonate values. For color legend, a non-linear ramp was used.



overall accuracy of the NN-AFL model was 98%, with a balanced accuracy for high contamination of 98%. For NN-FUM, the overall accuracy was 96% and the balanced accuracy was 96% (Table 3, Supplementary Table 1). The multi-class area under the curve for NN-AFL in the testing data set was 0.9835, and for NN-FUM, it was 0.96.

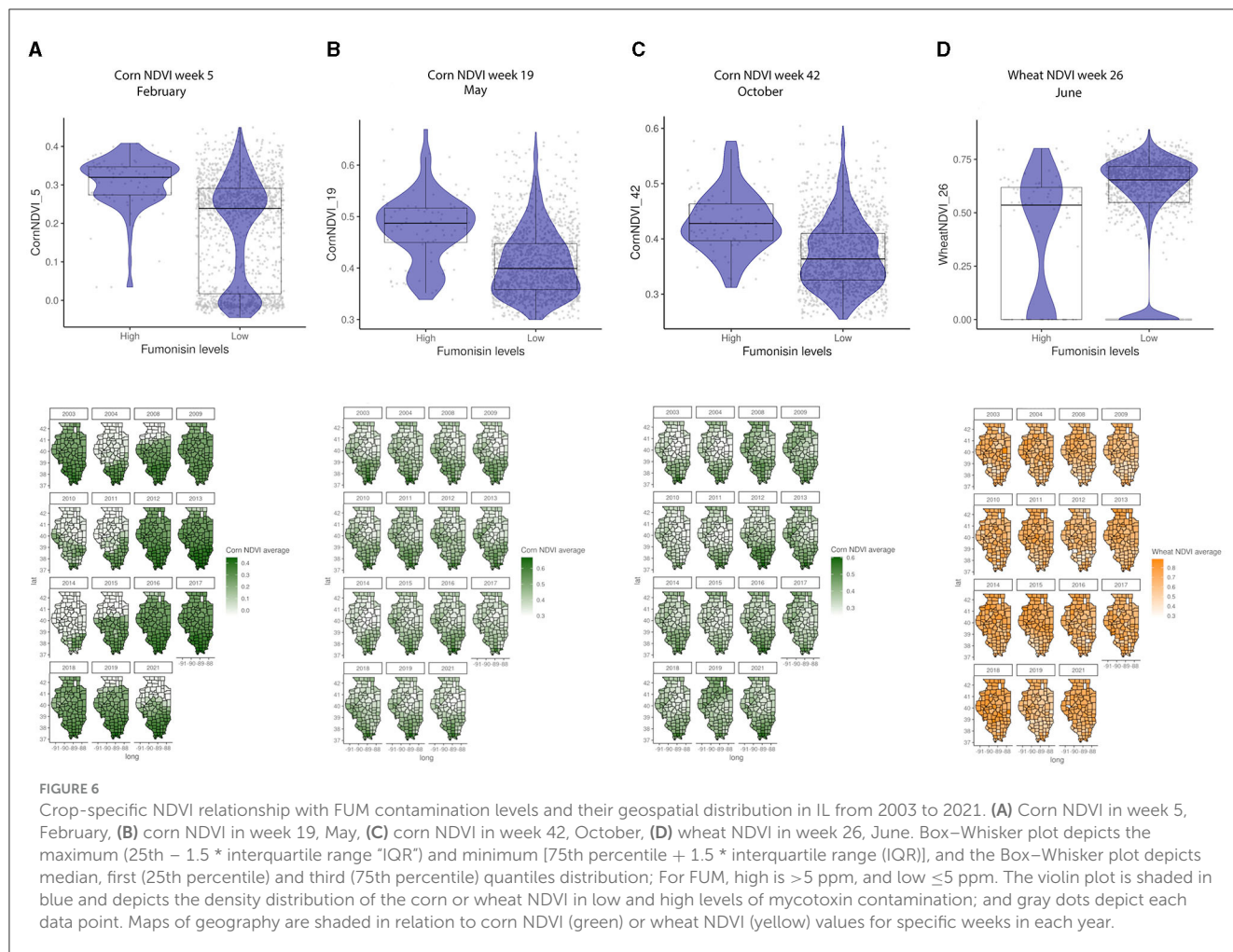
3.5. GBM and NN model validation

Model validation was performed by using mycotoxin data from 2010 for AFL (4% incidence) and 2016 for FUM (8.1% incidence) (Table 1). The data included 99 counties and a total of 99 observations in each year (except 2021 with 386 observations). The GBM-AFL model successfully explained low contamination levels of AFL with an accuracy of 96% (Table 3, Supplementary Table 1). The model was not able to correctly predict high contamination events of AFL from the validation data set, which had four observations labeled as high AFL. The overall accuracy of GBM-FUM was 78%, with a specificity of 75% and a balanced accuracy for high levels of 76% (Table 3, Supplementary Table 1). The NN models showed higher balanced accuracy for both AFL and FUM. The NN-AFL model explained high contamination levels with an

overall accuracy of 94% and a balanced accuracy of 73% (Table 3, Supplementary Table 1). The NN-AFL model correctly classified 2 out of 4 high contamination events in the validation data set, and only 4 out of 95 data points were misclassified as high when they were low. The McNemar *P*-value for validating NN-AFL was 0.68 (Supplementary Table 1). The NN-FUM model explained high contamination levels with an overall accuracy of 73% and a balanced accuracy of 85% (Table 3, Supplementary Table 1). The NN-FUM model successfully classified all the high contamination events in the validation data set, but it had 27 data points misclassified as high when they were low. The McNemar *P*-value for validating NN-FUM was close to zero (0.0000005, Supplementary Table 1).

4. Discussion

Mycotoxin contamination in the US is a pervasive problem that negatively impacts human and animal health and causes losses in the agriculture industry (Vardon et al., 2003; Wu, 2006; Mitchell et al., 2016; Winter and Pereg, 2019). AFL and FUM predictive modeling has been recently studied in the US using machine learning tools (Castano-Duque et al., 2022) that linked

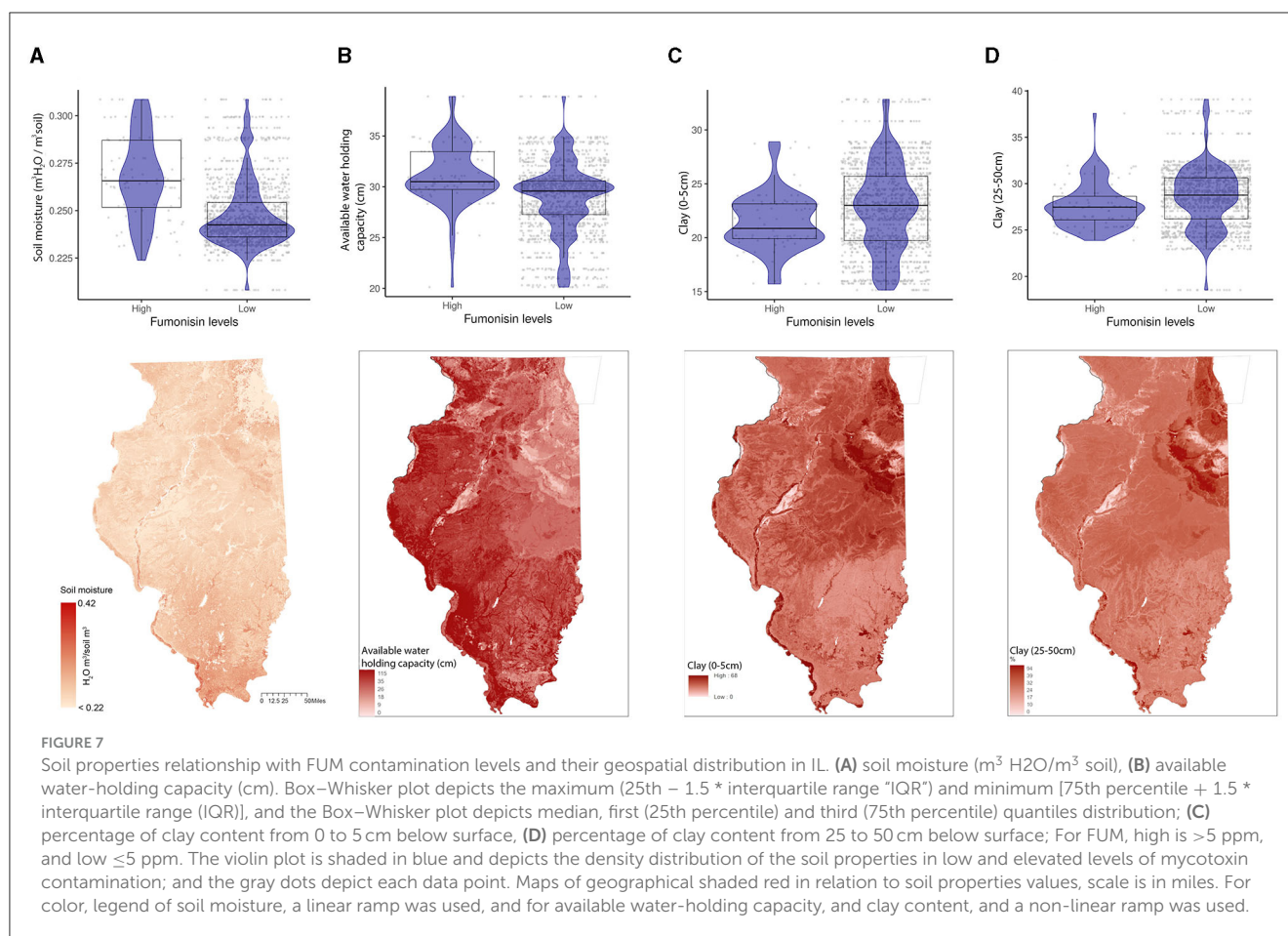


weather parameters and latitudinal location in the IL state with historical contamination events of these two mycotoxins. This study builds upon this initial model (Castano-Duque et al., 2022), which provided an IL state case study to account for daily changes in historical weather data (instead of monthly). We also added geospatial soil mineralogical chemical/physical properties and land usage parameters to the models for AFL and FUM. Similarly, predictive mycotoxin models have been developed for northern Italy, linking cropping system factors, and weather variables with deep neural network (DNN) models (Leggieri et al., 2021). Our NN models are unique because we included geospatial dynamic soil and land features linked to the GPS center coordinates of the IL counties. Land usage and soil mineralogical physical/chemical properties are variable for the specific geospatial locations in the US, at both state and county specificity levels (Smith et al., 2019). Using dynamic geospatial data in our models led to the identification of new insights into factors that influence mycotoxin contamination. These factors could be used for risk assessment and to implement timely and region-specific mycotoxin control management practices.

GBM-AFL identified that higher ARI in January, March, July, and November corresponds to higher AFL contamination, meaning that pre-sowing and post-harvest environmental

conditions have a strong influence on AFL levels at harvest time. Given that the harvest takes place prior to November, our model assumes that post-harvest input features will influence next year's mycotoxin contamination. Similar correlations were previously proposed by research conducted in the US (Castano-Duque et al., 2022), Africa (Keller et al., 2022), and Serbia (Liu et al., 2021). GBM-FUM showed that high precipitation events during February and March (pre-sowing time), as well as elevated temperatures in July (flowering time) and October (harvest time), influence FUM contamination. Consistently, previous model reports have shown that warmer temperatures early in the planting season (January–April) lead to higher AFL contamination levels in the US (Yu et al., 2022). GBM-AFL showed that corn-specific NDVI at week 30 (July—within flowering time) also influenced AFL contamination; this feature is linked to plant health as NDVI represents a composite satellite measurement of plant greenness. Lower NDVI translates into less green, unhealthy and/or lower density plants, and lower NDVI in week 30 would suggest that the plants were stressed possibly due to abiotic and/or biotic factors.

Previous studies have suggested that crop stress in general also correlates with mycotoxin contamination (Kebede et al., 2012). In 2012, when corn NDVI at week 30 was lower than in other years, the corn crops suffered from extreme drought conditions (NOAA,



2013), which were reflected in lower NDVI values and likely contributed to AFL (contamination levels at harvest time). Studies conducted in Kenya found that AFL contamination is positively associated with high rainfall and NDVI during pre-flowering time while negatively associated during post-flowering time and at harvest (Smith et al., 2016). Our findings agree with published logistic regression models that show that drought stress in corn during flowering time (prior to and beyond mid-silk) is critical for higher contamination at harvest (Damianidis et al., 2018). US-IL models have shown that weather and ARI between corn-growing seasons significantly influence the contamination at the end (Castano-Duque et al., 2022). It is possible that environmental conditions (weather conditions or other agricultural practices) during the spring favor fungal growth (Borràs-Vallverdú et al., 2022), leading to higher corn contamination at the end of the growing season. The development of pest management strategies that take into consideration climate change, such as warmer and wetter spring seasons and drought during the flowering time, is relevant for the management of mycotoxins.

AFL and FUM-GBMs showed that soil properties are also influential factors contributing to the mycotoxin contamination of corn across spatiotemporal scales. Higher calcium carbonate content patterns throughout IL soil were negatively correlated with historical AFL contamination levels. It has been demonstrated that the calcium carbonate content in soils influences soil pH (Weil and

Brady, 2002), whereby greater dissolved calcium increases buffering capacity and generates more alkaline soils. Geospatial differences in calcium carbonate in IL soils are due to the advance and retreat of the Laurentide glacier which deposits carbonate-rich materials in the northern half of the state. The negative correlation between AFL levels and calcium carbonate suggests that regions with lower calcium carbonate are associated with higher AFL contamination (Chang and Lynd, 1970), and therefore, calcium carbonate is an important factor in AFL contamination risk.

The abundance of calcium is modulated by both abiotic and biotic factors. In terms of abiotic processes, soils with greater concentrations of CaCO_3 , such as Northern Illinois, typically exhibit greater Ca^{2+} concentrations through the dissolution of parent CaCO_3 and other Ca-bearing mineralogy via infiltrating rainwater (Smith et al., 2019). In terms of biotic factors, such as soil bacteria, they can also modulate the calcium carbonate content of soil by dissolving calcium carbonate into Ca^{2+} ions and other carbonate species. Some of these "Calcite Dissolving Bacteria" are *Serratia marcescens*, *Sphingomonas changbaiensis*, or *Novosphingobium panipatense* (Peper et al., 2022). Furthermore, the soil microbiome can become dominated by microbial assemblages that suppress the growth of AFL-producing fungi with increasing Ca^{2+} (Zhang et al., 2021), FUM-producing fungi (Srinivasan et al., 2009), and suppression of mycotoxin-producing fungi strains by non-toxin-producing fungi (Mehl et al., 2012;

Ehrlich, 2014). The presence of both growth-suppressive bacteria and fungi that dissolve calcium carbonate would potentially lead to positive feedback in a calcium carbonate-rich soil. Fungi are involved in carbonate rock destruction, dissolution, precipitation (Verrecchia et al., 2003), and biomineralization (Bindschedler et al., 2016) in laboratory environments. Other environmental factors, such as wet and dry periods, affect the precipitation and accumulation of calcium carbonate in soil (Birkeland, 1974). It is possible that fungal communities and weather parameters co-influence levels of calcium carbonate and calcium ions in the soil, and these compounds can act as a feedback loop that modulates the fungal communities present in the soil. Understanding the native microbial communities and mycotoxin fungi/contamination could aid in understanding the interaction of soil microbiome, calcium carbonate, and AFL contamination. This knowledge of microbiome diversity could be useful for the development of region-specific biocontrol strategies.

The addition of calcium carbonate in the form of limestone to soils is a major agricultural practice in humid climates worldwide, used to directly change the chemical makeup of the rooting zone and improve soil fertility (Weil and Brady, 2002). Greenhouse experiments with peanut plants (*Arachis hypogaea* L.) showed that adding calcium to the soil leads to a significant reduction in AFL contamination of seeds (Uppala, 2011). Calcium-deficient soils cause calcium deficiency in the corn plant, which can lead to the failure of the upper leaves to unwrap at tasseling time (Melsted, 1953), shorter roots, chlorosis, and necrosis of leaves (do Moraes Gatti et al., 2023). These calcium-deficient symptoms might increase the propensity of fungal infections to thrive, as calcium is an important component of pathogen-associated molecular-triggered immunity (Fountain et al., 2016) and maize defense responses to pathogens (Jiang and Zhang, 2003; Hu et al., 2007; Ma and Berkowitz, 2011). We hypothesize that Northern IL soils, which are richer in CaCO₃ and Ca, are conducive to healthier plants capable of greater resistance to fungal disease and supporting microbiomes that also suppress AFL growth. The characterization of IL native microbiome and fungi soil populations linked to their geospatial location in calcium carbonate-rich or poor zones is not known, although this could aid in understanding the interaction of soil microbiome, calcium carbonate, and AFL contamination. This knowledge of microbiome diversity could be useful for the development of region-specific biocontrol strategies and mycotoxin management alongside external limestone application in areas such as Southern IL or other areas with elevated AFL risks, e.g., a corn-growing season with a hot/wet spring followed by drought during flowering time.

GBM-FUM showed that FUM contamination is correlated with soil moisture, available water-holding capacity, and a high percentage of clay. These soil hydrology properties vary across IL (Smith et al., 2019). Southern IL tends to have higher soil moisture levels compared to the north, with the southern area of the state having historically higher percentages of FUM contamination (Castano-Duque et al., 2022). Correspondingly, a positive correlation was observed between soil moisture and FUM contamination. Other soil properties, such as the percentage of clay, tend to be higher in the northeastern areas of IL (Smith et al., 2019), which historically tend to have lower FUM contamination

(Castano-Duque et al., 2022) as demonstrated by the negative correlation between these two variables.

Clay is the smallest mineral component of soil with the highest surface area per unit mass, in general, it has a high water-holding capacity and low hydraulic conductivity compared to other soil textures, making it prone to becoming waterlogged in moist conditions (Libohova et al., 2018), such as heavy rains. Clay's high water-holding capacity and poor permeability to water and gases (Weil and Brady, 2002) can lead to flood stress and waterlogging of plants during high precipitation events. While the relationship between flood-stressed corn and *Fusarium* spp. is not fully understood, it has been established that *Fusarium* is a strongly aerobic fungus (Stover, 1953), and it has been shown that among sandy loam, silt loam, or clay soil types, *Fusarium solani* and *Fusarium tricinctum* have higher disease severity on soybeans grown in sandy and silt loam soil (Yan and Nelson, 2022). Clay soils have high infiltration capacities as water moves into the shrinkage cracks, but when cracks are not present, their infiltration rate is characteristically slow. The small pores of clay solids when filled with water (saturated and under waterlogging conditions) could also influence *Fusarium* spp by limiting oxygen availability. It would be key to predict FUM outbreaks to understand the biological effect of clay-rich soils and high precipitation events on *Fusarium* growth and the diversity of species in different geospatial locations in the US.

Soils in IL with prominent levels of clay, low soil moisture, and low water-holding capacity offer conditions that historically might lead to lower FUM contamination. Clay-rich soils bind strongly to organic matter, contributing to soil aggregation, soil fertility, and cation exchange. Therefore, clay influences soil fertility by modulating cation exchange capacity and soil physical properties (Weil and Brady, 2002). The capacity of clay to hold water and bind to organic matter affects water in the soil and nutrients available for crops. High precipitation events early in the year (February), when there is no corn in the field, could lead to higher FUM contamination. We hypothesize that there must be some biological and chemical interaction between clay-rich soils early in the season and snow and rain events that influence fungal growth in the fields.

The primary abiotic factors contributing to the presence of FUM are water activity and temperature (Sanchis et al., 2006; Picot et al., 2010), but the nature of this relation is complex in the context of soil, hydroclimate variability, and plant water use. In optimal temperatures, increased water activity promotes FUM production, but at temperatures above the thermal optimum of 25°C, decreased water activity also promotes greater FUM production (Marin et al., 2004; Samapundo et al., 2005; Sanchis et al., 2006; Picot et al., 2010). Furthermore, *F. verticillioides* growth has been shown to be limited by low water activity; however, the FUM1 gene expression was significantly greater with lower water activity (Jurado et al., 2008). Therefore, favorable conditions for FUM production may occur under two different hydroclimate conditions: (1) a wet, i.e., high water activity, period with temperatures in the thermal optimum range; and (2) temperatures above the thermal optimum but dry, i.e., low water activity. Some field-based evidence corroborates the latter, where Roucou et al. (2021) noted increased FUM risk with greater water stress, but only when temperatures were elevated. In this study, the greater historical occurrence of FUM in Southern

IL, where soils have less water and a propensity for water stress, would support the second condition. Another biophysical feedback between crop and soil may also drive greater water stress as starches are produced during kernel ripening (Jurado et al., 2008; Picot et al., 2010). As such, positive feedback driving greater risk for FUM production into the latter part of a growing season may develop when: (1) temperatures are elevated; (2) kernel ripening begins and plant water use increases; (3) water stress is persistent, e.g., drought-like conditions; and (4) crops are grown in soils that exasperate water-holding capacity.

Depending on the soil type, complex plant–water interactions can affect plant health positively or negatively. Soils conducive to drought or flooding stress can result in crops with a lower ability to fight fungal infections and increased susceptibility to AFL (Kebede et al., 2012) and FUM (Parsons and Munkvold, 2010; Vaughan et al., 2016) contamination. Furthermore, relative humidity affects fungal sporulation and growth (Foister, 1946); therefore, both soil–fungi–water interactions and plant–fungi–water interactions will affect mycotoxin outbreaks. Further research that includes field trials from different regions of the US that have variable soil types and includes year-round time frames to account for the variation of precipitation and temperature would aid in understanding the causality that these spatial-temporal variables have on fungal load and mycotoxin contamination.

The AFL and FUM-GBM models showed better accuracy values than previously reported in the monthly-based models that did not include geospatial soil dynamic features (Castano-Duque et al., 2022). NN models showed high class-specific performance for 1-year predictive validation for AFL (73%) and FUM (85%), thus the NN models are recommended for annual mycotoxin prediction. The observed overall and specific accuracy levels are at par when compared to other mycotoxin non-US models that showed ranges from 90 to 99% general accuracy for wheat models in Europe (Wang et al., 2022), 75% general accuracy for corn using AFL–maize models in Italy (Battilani et al., 2013) and >75% general accuracy using machine learning models for toxin prediction in corn in northern Italy (Leggieri et al., 2021). Because GBM and NN models showed high-performance levels (Table 3), both can be used for annual mycotoxin prediction. To be implemented, these models would need to use input features for the months of October–December from the previous year's post-harvest and projected weather and NDVI values for a maximum of a month prior to harvest.

Our dynamic geospatial models offer additional information about soil properties and remote-sensed data (NDVI) that influence AFL and FUM contamination in corn. *Aspergillus* growth begins in the soil, which is the habitat for the fungus; thus, the production of AFL by fungi will be influenced by agricultural practices, environmental conditions, and fungal interaction with soil and plants (Winter and Pereg, 2019). Several studies have shown a significant correlation between climate, soil properties, NDVI, and agricultural management practices and AFL contamination in several countries in sub-Saharan Africa, such as Kenya, Zambia, and others, as reviewed by Keller et al. (2022). AFL surveys and studies conducted in Eastern and Western Kenya (Köppen, 2011) support the utility of remote-sensed data such as NDVI, rainfall, and soil properties to predict aflatoxin contamination (Smith et al., 2016). Satellite-generated databases have been previously used in wheat studies in Europe and led to higher model accuracy (Wang

et al., 2022). Previous studies in Africa found that soil organic carbon content, pH, total exchangeable bases, salinity, texture, and soil type were significantly associated with AFL (Smith et al., 2016). Soil data throughout IL embody important geographic variability related to geologic deposition, age of material, climate, duration of pedogenesis, natural history, and other factors. Soil conditions strongly influence which species of fungi dominate in a given soil environment (Weil and Brady, 2002). Our research shows that in the US, specific properties available from geospatial data such as calcium carbonate, pH, clay content, and soil moisture are key factors correlated with historical AFL and FUM outbreaks. Our dynamic geospatial models can guide research and identify multiple factors contributing to the risk of AFL or FUM contamination in a geographical region and within weekly time frames.

Some agronomic practices take place between corn-growing seasons, such as tilling and drilling (Borràs-Vallverdú et al., 2022), while other factors are constant throughout the year, such as soil properties (Smith et al., 2016) and other socio-economical parameters such as training farmers on the detection of contaminated crops in the field and mitigation strategies, and the involvement of the private sector to provide incentives for farmers to adopt aflatoxin management techniques (Titilayo, 2018) that influence mycotoxin contamination. Several of these factors, such as soil properties, were included in our models, although much information on other agronomic practices is not available at the historical geospatial scale needed for this mycotoxin data set. Nevertheless, our models were able to determine that input features linked to weekly temporal zones between corn-growing seasons have a significant influence on the end-of-year mycotoxin contamination. Among these input features, soil properties were a novel addition to the IL models. It remains to be evaluated if there are differences between the diverse fungi *Aspergillus* (AFL producer) and *Fusarium* (FUM producer) (Samapundo et al., 2005) in relation to growth, development, and toxin production associated with environmental factors in the field, such as crop rotation using winter wheat. For future applications, quarterly historical data should be used to create a quarterly predictive model. These models would be beneficial for farmers as they will be alerted by the prediction of impending contamination several months in advance before harvest, thus providing ample time to deploy mitigation measures such as biocontrol, irrigation, and/or fungicide application to avoid contamination of their crops.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.gro-intelligence.com/>, <https://doi.org/10.5067/MODIS/MOD13A2.061>, Cropscape: <https://nassgeodata.gmu.edu/CropScape/>.

Author contributions

LC-D: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing. EW: Data

curation, Methodology, Software, Visualization, Writing—review & editing. JB: Data curation, Methodology, Software, Visualization, Writing—review & editing. CL: Conceptualization, Resources, Software, Supervision, Writing—review & editing. NV: Data curation, Methodology, Writing—review & editing. MF: Conceptualization, Methodology, Resources, Software, Writing—review & editing. KB: Data curation, Investigation, Resources, Writing—review & editing. PO: Project administration, Resources, Supervision, Writing—review & editing. HF-K: Conceptualization, Project administration, Resources, Supervision, Writing—review & editing. MV: Conceptualization, Investigation, Project administration, Resources, Supervision, Writing—review & editing. KR: Conceptualization, Investigation, Project administration, Resources, Supervision, Writing—review & editing.

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References

- Abdel-Hadi, A., Schmidt-Heydt, M., Parra, R., Geisen, R., and Magan, N. (2012). A systems approach to model the relationship between aflatoxin gene cluster expression, environmental factors, growth and toxin production by *Aspergillus flavus*. *J. R. Soc. Interface* 9, 757–767. doi: 10.1098/rsif.2011.0482
- Baechler, M. C., and Love, P. M. (2004). *Building America Best Practices Series: Volume 1: Builders and Buyers Handbook for Improving New Home Efficiency, Comfort, and Durability in the Hot and Humid Climate*. Golden, CO, USA: National Renewable Energy Lab (NREL). doi: 10.2172/15011705
- Battilani, P., Leggieri, M. C., Rossi, V., and Giorni, P. (2013). AFLA-maize, a mechanistic model for *Aspergillus flavus* infection and aflatoxin B1 contamination in maize. *Comput. Electr. Agric.* 94, 38–46. doi: 10.1016/j.compag.2013.03.005
- Battilani, P., Toscano, P., Van der Fels-Klerx, H. J., Moretti, A., Leggieri, M., Brera, C., et al. (2016). Aflatoxin B1 contamination in maize in Europe increases due to climate change. *Sci. Rep.* 6, 24328. doi: 10.1038/srep24328
- Beck, H. E., Zimmermann, N. E., McVicar, T. R., Vergopolan, N., Berg, A., Wood, E. F., et al. (2018). Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Scient. Data* 5, 180214. doi: 10.1038/sdata.2018.214
- Bergstrom, R. E., Piskin, K., and Follmer, L. R. (1976). *Geology for Planning in the Springfield-Decatur Region*. Champaign, IL: Illinois State Geological Survey, Prairie Research Institute.
- Bindschedler, S., Cailleau, G., and Verrecchia, E. (2016). Role of fungi in the biomineralization of calcite. *Minerals* 6, 41. doi: 10.3390/min6020041
- Birkeland, P. W. (1974). *Pedology, Weathering, and Geomorphological Research*. Oxford: Oxford University Press.
- Borràs-Vallverdú, B., Ramos, A. J., Cantero-Martínez, C., Marín, S., Sanchis, V., Fernández-Ortega, J., et al. (2022). Influence of agronomic factors on mycotoxin contamination in maize and changes during a 10-day harvest-till-drying simulation period: a different perspective. *Toxins* 14, 620. doi: 10.3390/toxins14090620
- Boryan, C., Yang, Z., Mueller, R., and Craig, M. (2011). Monitoring US agriculture: the US Department of Agriculture, National Agricultural Statistics Service, cropland data layer program. *Geocarto Int.* 26, 341–58. doi: 10.1080/10106049.2011.562309
- Bush, B. J., Carson, M. L., Cubeta, M. A., Hagler, W. M., and Payne, G. A. (2004). Infection and fumonisin production by *Fusarium verticillioides* in developing maize kernels. *Phytopathology* 94, 88–93. doi: 10.1094/PHYTO.2004.94.1.88
- Castano-Duque, L., Vaughan, M., Lindsay, J., Barnett, K., and Rajasekaran, K. (2022). Gradient boosting and bayesian network machine learning models predict aflatoxin and fumonisin contamination of maize in Illinois – First USA case study. *Front Microbiol.* 13, 1039947. doi: 10.3389/fmicb.2022.1039947
- Chang, C. M., and Lynd, J. Q. (1970). Soil microfloral interactions with aflatoxin synthesis and degradation. *Mycologia* 62, 978–986. doi: 10.1080/00275514.1970.12019039
- Cooper, G. F. (1990). The computational complexity of probabilistic inference using bayesian belief networks. *Artif. Intell.* 42, 393–405. doi: 10.1016/0004-3702(90)90060-D
- Cotty, P. J., and Jaime-Garcia, R. (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *Int. J. Food Microbiol.* 119, 109–115. doi: 10.1016/j.jfoodmicro.2007.07.060
- Damianidis, D., Ortiz, B. V., Windham, G. L., Bowen, K. L., Hoogenboom, G., Scully, B. T., et al. (2018). Evaluating a generic drought index as a predictive tool for aflatoxin contamination of corn: From plot to regional level. *Crop. Prot.* 113, 64–74. doi: 10.1016/j.cropro.2018.07.013
- Didan, K. (2021). *MODIS/Terra Vegetation Indices 16-Day L3 Global 1km SIN Grid V061: distributed by NASA EOSDIS Land Processes DAAC*. Available online at: <https://lpdaac.usgs.gov/products/mod13a2v061/> (accessed November 15, 2022).
- Diener, U. L., Cole, R. J., Sanders, T. H., Payne, G. A., Lee, I. S., Klich, M. A., et al. (1987). Epidemiology of aflatoxin formation by *aspergillus flavus**. *Annu. Rev. Phytopathol.* 25, 249–270. doi: 10.1146/annurev.py.25.090187.001341
- do Moraes Gatti, V. C., da Silva Barata, H., Silva, V. F., da Cunha, F. F., de Oliveira, R. A., de Oliveira, J. T., et al. (2023). Influence of calcium on the development of corn plants grown in hydroponics. *AgriEngineering* 5, 623–630. doi: 10.3390/agriengineering5010039

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2023.1283127/full#supplementary-material>

- Ehrlich, K. C. (2014). Non-aflatoxigenic *Aspergillus flavus* to prevent aflatoxin contamination in crops: advantages and limitations. *Front. Microbiol.* 5, 50. doi: 10.3389/fmicb.2014.00050
- Focker, M., van der Fels-Klerx, H. J., Magan, S. G. E. N., Grahovac, F. B., Budakov, M. D., Suman, M., et al. (2020). The impact of management practices to prevent and control mycotoxins in the European food supply chain: MyToolBox project results. *World Mycotoxin J.* 14, 139–154. doi: 10.3920/WMJ2020.2588
- Foister, C. E. (1946). The relation of weather to fungus diseases of plants. *II Botan. Rev.* 12, 548–591. doi: 10.1007/BF02861806
- Fountain, J. C., Bajaj, P., Nayak, S. N., Yang, L., Pandey, M. K., Kumar, V., et al. (2016). Responses of *Aspergillus flavus* to oxidative stress are related to fungal development regulator, antioxidant enzyme, and secondary metabolite biosynthetic gene expression. *Front. Microbiol.* 7, 2048. doi: 10.3389/fmicb.2016.02048
- Friedman, J. H. (2001). Greedy function approximation: A gradient boosting machine. *Ann. Stat.* 29, 1189–1232. doi: 10.1214/aos/1013203451
- Gibson, L. J., and Kroese, D. P. (2022). “Rare-Event Simulation via Neural Networks,” in *Advances in Modeling and Simulation: Festschrift for Pierre L'Ecuyer*, eds. Z. Botev, A. Keller, C. Lemieux, B. Tuffin (Cham: Springer International Publishing), 151–68. doi: 10.1007/978-3-031-10193-9_8
- González-Domínguez, E., Caffi, T., Rossi, V., Salotti, I., and Fedele, G. (2023). Plant disease models and forecasting: changes in principles and applications over the last 50 years. *Phytopathology* 113, 678–693. doi: 10.1094/PHYTO-10-22-0362-KD
- Guo, B., Chen, Z. Y., Lee, R. D., and Scully, B. T. (2008). Drought stress and preharvest aflatoxin contamination in agricultural commodity: genetics, genomics and proteomics. *J. Integr. Plant Biol.* 50, 1281–1291. doi: 10.1111/j.1744-7909.2008.00739.x
- Guo, B. Z., Russin, J. S., Brown, R. L., Cleveland, T. E., and Widstrom, N. W. (1996). Resistance to aflatoxin contamination in corn as influenced by relative humidity and kernel germination. *J. Food Prot.* 59, 276–281. doi: 10.4315/0362-028X-59.3.276
- Hoffmann, A., Funk, R., and Müller, M. E. H. (2021). Blowin' in the wind: wind dispersal ability of phytopathogenic *Fusarium* in a wind tunnel experiment. *Atmosphere* 12, 1653. doi: 10.3390/atmos12121653
- Hu, X., Jiang, M., Zhang, J., Zhang, A., Lin, F., Tan, M., et al. (2007). Calcium-calmodulin is required for abscisic acid-induced antioxidant defense and functions both upstream and downstream of H₂O₂ production in leaves of maize (*Zea mays*) plants. *New Phytol.* 173, 27–38. doi: 10.1111/j.1469-8137.2006.01888.x
- Jiang, M., and Zhang, J. (2003). Cross-talk between calcium and reactive oxygen species originated from NADPH oxidase in abscisic acid-induced antioxidant defence in leaves of maize seedlings. *Plant Cell Environ.* 26, 929–939. doi: 10.1046/j.1365-3040.2003.01025.x
- Johns, L. E., Bebb, D. P., Gurr, S. J., and Brown, N. A. (2022). Emerging health threat and cost of *Fusarium* mycotoxins in European wheat. *Nat. Food* 3, 1014–1019. doi: 10.1038/s43016-022-00655-z
- Jurado, M., Marín, P., Magan, N., and González-Jaén Maria, T. (2008). Relationship between solute and matrix potential stress, temperature, growth, and FUM1 gene expression in two *Fusarium verticillioides* strains from Spain. *Appl. Environ. Microbiol.* 74, 2032–2036. doi: 10.1128/AEM.02337-07
- Kebede, H., Abbas, H. K., Fisher, D. K., and Bellaloui, N. (2012). Relationship between aflatoxin contamination and physiological responses of corn plants under drought and heat stress. *Toxins* 4, 1385–1403. doi: 10.3390/toxins4111385
- Keller, B., Russo, T., Rembold, F., Chauhan, Y., Battilani, P., Wemndt, A., et al. (2022). The potential for aflatoxin predictive risk modelling in sub-Saharan Africa: a review. *World Mycotoxin J.* 15, 101–118. doi: 10.3920/WMJ2021.2683
- Kerry, R., Ingram, B., Garcia-Cela, E., Magan, N., Ortiz, B. V., Scully, B., et al. (2021). Determining future aflatoxin contamination risk scenarios for corn in Southern Georgia, USA using spatio-temporal modelling and future climate simulations. *Sci. Rep.* 11, 13522. doi: 10.1038/s41598-021-92557-6
- Klich, M. (2002). Biogeography of *Aspergillus* species in soil and litter. *Mycologia* 94, 21–27. doi: 10.1080/15572536.2003.11833245
- Köppen, W. (2011). The thermal zones of the Earth according to the duration of hot, moderate and cold periods and to the impact of heat on the organic world. *Meteorol. Zeitschrift* 20, 351–360. doi: 10.1127/0941-2948/2011/105
- Lee, H.-S., Kwon, N. J., Kim, Y., and Lee, H. (2018). Prediction of mycotoxin risks due to climate change in Korea. *Appl. Biol. Chem.* 61, 389–396. doi: 10.1007/s13765-018-0370-8
- Leggieri, M. C., Mazzoni, M., and Battilani, P. (2021). Machine learning for predicting mycotoxin occurrence in maize. *Front. Microbiol.* 12, 661132. doi: 10.3389/fmicb.2021.661132
- Libohova, Z., Schoeneberger, P., Bowling, L. C., Owens, P. R., Wysocki, D., Wills, S., et al. (2018). Soil systems for upscaling saturated hydraulic conductivity for hydrological modeling in the critical zone. *Vadose Zone J.* 17, 170051. doi: 10.2136/vzj2017.03.0051
- Liu, N., Liu, C., Dudaš, T. N., Loc, M. C., Bagi, F. F., van der Fels-Klerx, H. J., et al. (2021). Improved aflatoxins and fumonisins forecasting models for maize (PREMA and PREFUM), using combined mechanistic and bayesian network modeling—serbia as a case study. *Front. Microbiol.* 12, 643604. doi: 10.3389/fmicb.2021.643604
- Long Stephen, P., Ainsworth Elizabeth, A., Leakey Andrew, D. B., Nösberger, J., and Ort Donald, R. (2006). Food for thought: lower-than-expected crop yield stimulation with rising CO₂ concentrations. *Science* 312, 1918–1921. doi: 10.1126/science.1114722
- Ma, W., and Berkowitz, G. A. (2011). Ca²⁺ conduction by plant cyclic nucleotide gated channels and associated signaling components in pathogen defense signal transduction cascades. *New Phytol.* 190, 566–572. doi: 10.1111/j.1469-8137.2010.03577.x
- Maiorano, A., Reyneri, A., Sacco, D., Magni, A., and Ramponi, C. (2009). A dynamic risk assessment model (FUMAgain) of fumonisin synthesis by *Fusarium verticillioides* in maize grain in Italy. *Crop. Prot.* 28, 243–256. doi: 10.1016/j.cropro.2008.10.012
- Marin, S., Magan, N., Ramos, A. J., and Sanchis, V. (2004). Fumonisin-producing strains of *Fusarium*: a review of their ecophysiology. *J. Food Prot.* 67, 1792–1805. doi: 10.4315/0362-028X-67.8.1792
- McMillian, W. W., Widstrom, N. W., and Wilson, D. M. (1985). Insect damage and aflatoxin contamination in preharvest corn: Influence of genotype and ear wetting. *J. Entomol. Sci.* 20, 66–68. doi: 10.18474/0749-8004-20.1.66
- Medina, A., Rodriguez, A., and Magan, N. (2014). Effect of climate change on *Aspergillus flavus* and aflatoxin B₁ production. *Front. Microbiol.* 5, 348. doi: 10.3389/fmicb.2014.00348
- Mehl, H. L., Jaime, R., Callicott, K. A., Probst, C., Garber, N. P., Ortega-Beltran, A., et al. (2012). *Aspergillus flavus* diversity on crops and in the environment can be exploited to reduce aflatoxin exposure and improve health. *Ann. N. Y. Acad. Sci.* 1273, 7–17. doi: 10.1111/j.1749-6632.2012.06800.x
- Melsted, S. W. (1953). Some observed calcium deficiencies in corn under field conditions. *Soil Sci. Soc. Am. J.* 17, 52–54. doi: 10.2136/sssaj1953.03615995001700010013x
- Miller, J. D. (2001). Factors that affect the occurrence of fumonisin. *Environ. Health Perspect.* 109, 321–324. doi: 10.1289/ehp.01109s2321
- Mitchell, N. J., Bowers, E., Hurburgh, C., and Wu, F. (2016). Potential economic losses to the US corn industry from aflatoxin contamination. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* 33, 540–550. doi: 10.1080/19440049.2016.1138545
- Munkvold, G. (2003). Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. *Eur. J. Plant Pathol.* 109, 705–713. doi: 10.1023/A:1026078324268
- Munkvold, G. P., Arias, S., Taschl, I., and Gruber-Dorninger, C. (2019). “Chapter 9 - Mycotoxins in Corn: Occurrence, Impacts, and Management,” in *Corn* (Third Edition), ed. S. O. Serna-Saldivar (Oxford: AACC International Press), 235–87. doi: 10.1016/B978-0-12-811971-6.00009-7
- Mutiga, S. K., Were, V., Hoffmann, V., Harvey, J. W., Milgroom, M. G., Nelson, R. J., et al. (2014). Extent and drivers of mycotoxin contamination: inferences from a survey of kenyan maize mills. *Phytopathology* 104, 1221–1231. doi: 10.1094/PHYTO-01-14-0006-R
- Nnadi, N. E., and Carter, D. A. (2021). Climate change and the emergence of fungal pathogens. *PLoS Pathog.* 17, e1009503. doi: 10.1371/journal.ppat.1009503
- NOAA (2013). *National Centers for Environmental Information, Monthly Drought Report for Annual 2012*. Available online at: <https://www.ncei.noaa.gov/access/monitoring/monthly-report/drought/201213> (accessed August 2023).
- Parsons, M. W., and Munkvold, G. P. (2010). Associations of planting date, drought stress, and insects with *Fusarium* ear rot and fumonisin B₁ contamination in California maize. *Food Addit. Contam. Part A* 27, 591–607. doi: 10.1080/19440040903456337
- Payne, G. A., Cassel, D. K., and Adkins, C. R. (1986). Reduction of aflatoxin contamination in corn by irrigation and tillage. *Phytopathology* 76, 679–684. doi: 10.1094/Phyto-76-679
- Payne, G. A., Thompson, D. L., Lillehoj, E. B., and Zuber, M. S. (1988). Effect of temperature on the preharvest infection of maize kernels by *Aspergillus flavus*. *Phytopathology* 78, 1376–1380. doi: 10.1094/Phyto-78-1376
- Payne, G. A., and Widstrom, N. W. (1992). Aflatoxin in maize. *Crit. Rev. Plant Sci.* 10, 423–440. doi: 10.1080/07352689209382320
- Peng, B., Guan, K., Tang, J., Ainsworth, E. A., Asseng, S., Bernacchi, C. J., et al. (2020). Towards a multiscale crop modelling framework for climate change adaptation assessment. *Nat. Plants* 6, 338–348. doi: 10.1038/s41477-020-0625-3
- Peper, A., Brennen, T., and Yang, L. (2022). Calcite dissolving bacteria from peanut (*Arachis hypogaea*) pegging zone influences soil calcium level. *Front. Microb.* 1, 1019134. doi: 10.3389/fmicb.2022.1019134
- Picot, A., Barreau, C., Pinson-Gadais, L., Caron, D., Lannou, C., Richard-Forget, F., et al. (2010). Factors of the *Fusarium verticillioides*-maize environment modulating fumonisin production. *Crit. Rev. Microbiol.* 36, 221–231. doi: 10.3109/10408411003720209
- Proctor, R. H., McCormick, S. P., Kim, H.-S., Cardoza, R. E., Stanley, A. M., Lindo, L., et al. (2018). Evolution of structural diversity of trichothecenes, a family of toxins produced by plant pathogenic and entomopathogenic fungi. *PLoS Pathog.* 14, e1006946. doi: 10.1371/journal.ppat.1006946

- Ridgeway, G. (2006). *Generalized boosted models: A guide to the GBM package*. Available online at: <https://cran.r-project.org/web/packages/gbm/vignettes/gbm.pdf> (accessed January 2023).
- Roucou, A., Bergez, C., Méléard, B., and Orlando, B. A. (2021). Fumonisin prevention tool for targeting and ranking agroclimatic conditions favoring exposure in french maize-growing areas. *Toxins* 13, 214. doi: 10.3390/toxins13030214
- Samapundo, S., Devlieghere, F., De Meulenaer, B., and Debevere, J. (2005). Effect of water activity and temperature on growth and the relationship between fumonisin production and the radial growth of *Fusarium verticillioides* and *Fusarium proliferatum* on corn. *J. Food Prot.* 68, 1054–1059. doi: 10.4315/0362-028X-68.5.1054
- Sanchis, V., Marín, S., Magan, N., and Ramos, A. J. (2006). "Ecophysiology of fumonisin producers in *Fusarium* section Liseola," in *Advances in Food Mycology* (Boston, MA: Springer US), 115–122. doi: 10.1007/0-387-28391-9_6
- Scheidegger, K. A., and Payne, G. A. (2003). Unlocking the secrets behind secondary metabolism: a review of *Aspergillus flavus* from pathogenicity to functional genomics. *J. Toxicol.* 22, 423–459. doi: 10.1081/TOX-120024100
- Segers, F. J. J., Dijksterhuis, J., Giesbers, M., and Debets, A. J. M. (2023). Natural folding of airborne fungal spores: a mechanism for dispersal and long-term survival? *Fungal Biol. Rev.* 44, 100292. doi: 10.1016/j.fbr.2022.10.005
- Sétamou, M., Cardwell, K. F., Schulthess, F., and Hell, K. (1997). *Aspergillus flavus* infection and aflatoxin contamination of preharvest maize in benin. *Plant Dis.* 81, 1323–1327. doi: 10.1094/PDIS.1997.81.11.1323
- Shah, D. A., De Wolf, E. D., Paul, P. A., and Madden, L. V. (2018). Functional data analysis of weather variables linked to *Fusarium* head blight epidemics in the United States. *Phytopathology* 109, 96–110. doi: 10.1094/PHYTO-11-17-0386-R
- Smith, D. B., Solano, F., Woodruff, L. G., Cannon, W. F., and Ellefsen, K. J. (2019). *Geochemical and mineralogical maps, with interpretation, for soils of the conterminous United States*. Report. Reston, VA: Report No.: 2017–5118. doi: 10.3133/sir20175118
- Smith, L. E., Stasiewicz, M., Hestrin, R., Morales, L., Mutiga, S., Nelson, R. J., et al. (2016). Examining environmental drivers of spatial variability in aflatoxin accumulation in Kenyan maize: Potential utility in risk prediction models. *Afr. J. Food, Agric. Nutr. Dev.* 16, 11086–11105. doi: 10.18697/ajfand.75.ILRI09
- Srinivasan, K., Gilardi, G., Garibaldi, A., and Gullino, M. L. (2009). Efficacy of bacterial antagonists and different commercial products against *Fusarium* wilt on rocket. *Phytoparasitica* 37, 179–188. doi: 10.1007/s12600-009-0024-9
- Stover, R. H. (1953). The effect of soil moisture on *Fusarium* species. *Canad. J. Botany*. 31, 693–697. doi: 10.1139/b53-050
- Team, R. C. (2017). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available online at: <http://www.R-project.org> (accessed July 2023).
- Titilayo, F. (2018). "Aflatoxin management strategies in sub-saharan Africa," in *Mycotoxins*, eds. N. Patrick Berka, S. Francois (Rijeka: IntechOpen), 6.
- Torgo, L. (2016). *Data Mining with R: Learning with Case Studies*. 2 ed. New York, NY: Chapman and Hall/CRC.
- Uppala, L. (2011). *Factors Affecting Pre-Harvest Aflatoxin Contamination of Peanut (Arachis hypogaea L.)*. Auburn, AL: Auburn University.
- van Buuren, S., and Groothuis-Oudshoorn, K. (2011). Mice: multivariate imputation by chained equations in R. *J. Stat. Softw.* 45, 1–67. doi: 10.18637/jss.v045.i03
- Van der Fels-Klerx, H., Liu, C., and Battilani, P. (2016). Modelling climate change impacts on mycotoxin contamination. *World Mycotox. J.* 9, 717–726. doi: 10.3920/WMJ2016.2066
- Van der Fels-Klerx, H. J., Vermeulen, L. C., Gavai, A. K., and Liu, C. (2019). Climate change impacts on aflatoxin B1 in maize and aflatoxin M1 in milk: A case study of maize grown in Eastern Europe and imported to the Netherlands. *PLoS ONE* 14, e0218956. doi: 10.1371/journal.pone.0218956
- Vardon, P., McLaughlin, C., and Nardinelli, C. (2003). "Mycotoxins: Risks in plant, animal, and human systems," in *Potential economic costs of mycotoxins in the United States* (Ames, Iowa, USA: Council for Agricultural Science and Technology), 136–42.
- Vaughan, M. M., Huffaker, A., Schmelz, E. A., Dafoe, N. J., Christensen, S., Sims, J., et al. (2014). Effects of elevated [CO₂] on maize defence against mycotoxigenic *Fusarium verticillioides*. *Plant Cell Environ.* 37, 2691–2706. doi: 10.1111/pce.12337
- Vaughan, M. M., Huffaker, A., Schmelz, E. A., Dafoe, N. J., Christensen, S. A., McAuslane, H. J., et al. (2016). Interactive effects of elevated [CO₂] and drought on the maize phytochemical defense response against mycotoxigenic *Fusarium verticillioides*. *PLoS ONE* 11, e0159270. doi: 10.1371/journal.pone.0159270
- Vergopolan, N., Chaney, N. W., Beck, H. E., Pan, M., Sheffield, J., Chan, S., et al. (2020). Combining hyper-resolution land surface modeling with SMAP brightness temperatures to obtain 30-m soil moisture estimates. *Rem. Sens. Environ.* 242, 111740. doi: 10.1016/j.rse.2020.111740
- Vergopolan, N., Chaney, N. W., Pan, M., Sheffield, J., Beck, H. E., Ferguson, C. R., et al. (2021). SMAP-HydroBlocks, a 30-m satellite-based soil moisture dataset for the conterminous US. *Scient. Data* 8, 264. doi: 10.1038/s41597-021-01050-2
- Vergopolan, N., Sheffield, J., Chaney, N. W., Pan, M., Beck, H. E., Ferguson, C. R., et al. (2022). High-resolution soil moisture data reveal complex multi-scale spatial variability across the United States. *Geophys. Res. Lett.* 49, eGL098586. doi: 10.1029/2022GL098586
- Verrecchia, E. P., Loisy, C., Braissant, O., and Gorbushina, A. A. (2003). "The Role of Fungal Biofilm and Networks in the Terrestrial Calcium Carbonate Cycle," in *Fossil and Recent Biofilms: A Natural History of Life on Earth*, eds. W. E. Krumbein, D. M. Paterson, G. A. Zavarzin (Dordrecht: Springer Netherlands), 363–9. doi: 10.1007/978-94-017-0193-8_25
- Walkinshaw, M., O'Geen, A. T., and Beaudette, D. E. (2022). *Cartographers*. California: California Soil Resources Lab.
- Wang, X., Liu, C., and van der Fels-Klerx, H. J. (2022). Regional prediction of multi-mycotoxin contamination of wheat in Europe using machine learning. *Food Res. Int.* 159, 111588. doi: 10.1016/j.foodres.2022.111588
- Warfield Colleen, Y., and Gilchrist David, G. (1999). Influence of kernel age on fumonisin B1 production in maize by *Fusarium moniliforme*. *Appl Environ Microbiol.* 65, 2853–2856. doi: 10.1128/AEM.65.7.2853-2856.1999
- Weil, R. R., and Brady, N. C. (2002). *The Nature and Properties of Soils*. 13 ed Upper Saddle River, New Jersey: Prentice Hall.
- Widstrom, N. W., McMillian, W. W., Beaver, R. W., and Wilson, D. M. (1990). Weather-associated changes in aflatoxin contamination of preharvest maize. *J. Prod. Agric.* 3, 196–199. doi: 10.2134/jpa1990.0196
- Winter, G., and Pereg, L. (2019). A review on the relation between soil and mycotoxins: Effect of aflatoxin on field, food and finance. *Eur. J. Soil Sci.* 70, 882–897. doi: 10.1111/ejss.12813
- Woloshuk, C. P., and Shim, W. B. (2013). Aflatoxins, fumonisins, and trichothecenes: a convergence of knowledge. *FEMS Microbiol. Rev.* 37, 94–109. doi: 10.1111/1574-6976.12009
- Wu, F. (2006). Mycotoxin reduction in Bt corn: potential economic, health, and regulatory impacts. *Transg. Res.* 15, 277–289. doi: 10.1007/s11248-005-5237-1
- Wu, F., Bhatnagar, D., Bui-Klimke, T., Carbone, I., Hellmich, R., Munkvold, G., et al. (2011). Climate change impacts on mycotoxin risks in US maize. *World Mycotox. J.* 4, 79–93. doi: 10.3920/WMJ2010.1246
- Yan, H., and Nelson, B. (2022). Effects of soil type, temperature, and moisture on development of *Fusarium* root rot of soybean by *Fusarium solani* (FSSC 11) and *Fusarium tricinctum*. *Plant Dis.* 106, 2974–2983. doi: 10.1094/PDIS-12-21-2738-RE
- Yu, J., Hennessy, D. A., Tack, J., and Wu, F. (2022). Climate change will increase aflatoxin presence in US Corn. *Environ. Res. Lett.* 17, 054017. doi: 10.1088/1748-9326/ac6435
- Yu, J., Hennessy, D. A., and Wu, F. (2020). The impact of Bt corn on aflatoxin-related insurance claims in the United States. *Sci. Rep.* 10, 10046. doi: 10.1038/s41598-020-66955-1
- Zamani, M., and Kremer, S. C. (2013). "Neural networks in bioinformatics," in *Handbook on Neural Information Processing*, eds. M. Bianchini, M. Maggini, L. C. Jain (Berlin, Heidelberg: Springer Berlin Heidelberg), 505–25. doi: 10.1007/978-3-642-36657-4_15
- Zhang, W., Zhang, B.-w., Deng, J.-f., Li, L., Yi, T.-y., and Hong, Y.-y. (2021). The resistance of peanut to soil-borne pathogens improved by rhizosphere probiotics under calcium treatment. *BMC Microbiol.* 21, 299. doi: 10.1186/s12866-021-02355-3
- Zingales, V., Taroncher, M., Martino, P. A., Ruiz, M.-J., and Caloni, F. (2022). Climate change and effects on molds and mycotoxins. *Toxins* 14, 445. doi: 10.3390/toxins14070445



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Structure-guided discovery of novel AflG inhibitors for aflatoxin contamination control in *aspergillus flavus*

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Aflatoxins (AFs) are highly carcinogenic metabolites produced by *Aspergillus* species that can contaminate critical food staples, leading to significant health and economic risks. The cytochrome P450 monooxygenase AflG catalyzes an early step in AF biosynthesis, resulting in the conversion of averantin (AVN) to 5'-hydroxy-averantin. However, the molecular mechanism underlying the AflG-AVN interaction remains unclear. Here, we sought to understand the structural features of AflG in complex with AVN to enable the identification of inhibitors targeting the AflG binding pocket. To achieve this goal, we employed a comprehensive approach combining computational and experimental methods. Structural modeling and microsecond-scale molecular dynamics (MD) simulations yielded new insights into AflG architecture and unveiled unique ligand binding conformations of the AflG-AVN complex. High-throughput virtual screening of more than 1.3 million compounds pinpointed specific subsets with favorable predicted docking scores. The resulting compounds were ranked based on binding free energy calculations and evaluated with MD simulations and *in vitro* experiments with *Aspergillus flavus*. Our results revealed two compounds significantly inhibited AF biosynthesis. Comprehensive structural analysis elucidated the binding sites of competitive inhibitors and demonstrated their regulation of AflG dynamics. This structure-guided pipeline successfully enabled the identification of novel AflG inhibitors and provided novel molecular insights that will guide future efforts to develop effective therapeutics that prevent AF contamination.

KEYWORDS

aflatoxins, *aspergillus flavus*, P450 monooxygenase AflG, structure-guided inhibitor design, contamination control

1 Introduction

Aflatoxins (AFs) are highly toxic, carcinogenic secondary metabolites primarily produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Caceres et al., 2020). These fungi are ubiquitous in the environment, leading to widespread aflatoxin contamination in agricultural products (Liao et al., 2020). When temperature and humidity conditions are favorable, AFs

are produced in large volumes, contaminating supplies of staple foods such as maize, peanuts, and other nuts. This can occur at multiple points in production, including the growth, harvest, storage, and processing stages (Jallow et al., 2021). Consumption of AF-contaminated foods by humans or other animals can result in various disorders and diseases, such as nutritional malabsorption, infertility, endocrine disruption, congenital fetal malformations, and immune deficiencies (Razzaghi-Abyaneh et al., 2014; Caceres et al., 2020). Mitigating AF contamination in crops is crucial to alleviate the associated health risks and economic impacts.

AF biosynthesis is tightly regulated by various factors, including nutrient sources, oxidative stress responses, ambient temperature, pH, and light conditions. Favorable conditions induce cellular signaling pathways that up-regulate genes involved in AF production (Bhatnagar et al., 2003; Georgianna and Payne, 2009; Caceres et al., 2020; Wu et al., 2022, 2023). The AF biosynthetic pathway contains 30 genes encoding both enzymes and regulatory factors. The enzymes in this pathway catalyze at least 27 reactions, including numerous oxidative rearrangements, making AF biosynthesis one of the most extensive and intricate biological processes known (Chang, 2003; Price et al., 2006; Caceres et al., 2020; Wang et al., 2022). Over a decade ago, researchers embarked on efforts to understand the functional mechanisms of enzymes in the AF biosynthetic pathway. For example, Crawford et al. solved the structure of the product template (PT) domain within PksA, a multidomain iterative polyketide synthase that initiates AF biosynthesis in *A. parasiticus*, and revealed the PT domain's capacity to bind both linear and bicyclic polyketides (Crawford et al., 2009). Subsequently, the thioesterase/Claisen cyclase domain in PksA was found to have an α/β -hydrolase fold in the catalytic closed form, with a distinct hydrophobic substrate-binding chamber (Korman et al., 2010). Despite such mechanistic insights, the functional mechanisms of most enzymes in the AF biosynthetic pathway have remained elusive.

Previous studies in other biochemical pathways of interest have demonstrated the capacities of targeted inhibitors to regulate metabolic processes. For instance, asparaginase and glutaminase inhibitors have been utilized to modify amino acid metabolism in hematologic malignancies, effectively reversing immune suppression (Tabe et al., 2019). Inhibitors of monoamine oxidases (MAOs), which catalyze oxidative deamination of several neurotransmitters, modulate neurotransmitter metabolism and may act as therapeutics in those with Alzheimer's disease by reducing harmful by-products and the associated oxidative stress (Manzoor and Hoda, 2020). Furthermore, several categories of compounds (namely amidepsines, rosipins, and xanthohumol) have been shown to inhibit the activity of diacylglycerol acyltransferase produced by the marine fungus *Gliocladium roseum*, regulating triacylglycerol biosynthesis (Sharma et al., 2020). These studies collectively indicate that inhibitors targeting key enzymes in specific metabolic pathways can effectively modulate the production of these metabolites, suggesting that AF production could be curtailed via targeted inhibition of critical enzymes in the AF biosynthetic pathway.

Building upon this concept, recent research has employed *in silico* docking methodologies to identify potential inhibitors of the AF biosynthetic enzyme PksA. In one such study, researchers conducted a virtual screening of 623 natural compounds from the South African natural compound database, identifying 10 molecules with predicted favorable binding energies (Labib et al., 2022). However, the multiple subunits of PksA, which catalyze various reactions, pose a challenge to effective PksA inhibition (Crawford et al., 2009). Thus, it may prove

more fruitful to target other enzymes in the AF biosynthetic pathway. Earlier investigations have underscored the central roles of cytochrome P450s, a Heme-type superfamily of enzymes with a highly conserved basic fold, in AF biosynthesis (Uka et al., 2020). P450s have been rigorously investigated in prior studies as prospective targets for inhibitor design, laying the groundwork for future efforts (Sezutsu et al., 2013; Hussain et al., 2020; Stout et al., 2021). The P450 AflG is involved in an early step of AF biosynthesis, in which it catalyzes the conversion of averantin (AVN) to 5'-hydroxy-averantin (Yu et al., 1997; Uka et al., 2020). Thus, AflG may be a viable target for inhibitor design to prevent AF biosynthesis.

In the present study, we investigated the AflG-AVN interaction and identified potential AflG inhibitors using structural modeling, molecular dynamics simulations, virtual screening, binding free energy calculations, and experimental validation. This approach was designed to yield novel insights into the structural architecture of the substrate and enzyme and to identify crucial residues that govern their interaction. High-throughput docking was then used to screen a vast library of millions of compounds, allowing the identification of molecules with favorable docking scores. Subsequent binding free energy calculations were conducted to narrow the selection to a handful of candidate compounds that could be tested *in vitro*. This study establishes an effective framework for further development and optimization of effective AflG inhibitors, promoting the establishment of effective agricultural therapeutics to mitigate AF contamination.

2 Materials and methods

2.1 Structural modeling and molecular docking

The *A. flavus* strain NRRL 3357, obtained from the United States Department of Agriculture Agricultural Research Service (USDA-ARS) culture collection (Peoria, IL, United States), was selected for this study due to its extensively characterized genome and widespread use as a model organism in investigations of secondary metabolite regulation and biosynthesis (Nierman et al., 2015; Skerker et al., 2021). The AflG amino acid sequence from *A. flavus* strain NRRL 3357 and the corresponding structural model (predicted with AlphaFold2) were obtained from UniProt (ID: B8NHZ0) (The UniProt Consortium, 2023). The validity of the model was evaluated using predicted local-distance difference test (pLDDT) scores and visual inspection. The initial 35 amino acids, predicted to interact with membranes, were excised from the AlphaFold2 structural model (Kufareva et al., 2014). The revised structure was then employed for subsequent construction steps. The structure of the closely related human enzyme P450 3A5 (PDB ID: 7sv2) was predicted with SWISS-MODEL and aligned to the AflG model using PyMOL (v2.5 Educational Edition, Schrödinger, LLC) (Waterhouse et al., 2018). The Heme cofactor from the aligned P450 3A5 structure was incorporated into the AflG model, forming the AflG-Heme complex.

CavityPlus was employed to detect potential binding pockets in the AflG-Heme complex, into which the native substrate AVN was docked (Wang et al., 2023). Polar hydrogens and Gasteiger charges were added to the receptor structure using the AutodockTools v4.2 package (Morris et al., 2009). The AVN structure was obtained from PubChem and processed using the Python script "mk_prepare_ligand.py" from Meeko (Holcomb et al., 2022). The grid spaces around

potential AflG binding pockets were defined in AutodockTools. The number of points was set to 50 Å in x-, y-, and z-dimensions. The center coordinates were −10.35 Å, 36.078 Å, and 40.881 Å on the x-, y-, and z-axes, respectively. The grid spacing was 0.375 Å. The AutoDock force field affinity maps were generated using AutoGrid4 in AutodockTools. Molecular docking was conducted using Autodock-Vina and the AutoDock4 forcefield with default parameters (Eberhardt et al., 2021). A high-scoring docking pose, in which the AVN alkyl chain was positioned proximate to the Heme iron center, was selected as the AflG-Heme-AVN complex model.

2.2 MD simulations for AflG and the AflG-Heme-AVN complex

Two simulation systems were established to analyze AflG dynamics and interactions with AVN: AflG alone and the AflG-Heme-AVN complex. The initial structures for AflG and AflG-Heme-AVN were obtained as described in section 2.1. Water, Na⁺, and Cl[−] were added to solvate and neutralize the systems at physiological salinity (150 mM). MD simulations were conducted in GROMACS 2020 using the all-atom CHARMM36m force field and the TIP3P water model (Abraham et al., 2015; Huang et al., 2017). Topology and parameter files for AVN were generated with a CHARMM generalized force field (CGenFF) (Vanommeslaeghe et al., 2010). The parameter file for Heme, specifying an Fe(II) oxidation state advantageous for oxygen binding, was sourced from CHARMM-GUI (Jo et al., 2008). The steepest descent algorithm was used for energy minimization over 50,000 steps. The system equilibration lasted 100 ps with constraints on the hydrogen bonds in the isothermal-isobaric (NPT) ensemble (Deift and Zhou, 1993). The semi-isotropic Parrinello-Rahman method was used to maintain the pressure at 1 bar with a time constant of 2 ps. The v-rescale method was used with a time constant of 0.1 ps to maintain a constant temperature of 298 K (Parrinello and Rahman, 1980). Trajectories were produced with constraints on the H-bonds and lasted 1 μs for both AflG and AflG-Heme-AVN complex. H-bonds were constrained using the LINCS algorithm at 2 fs intervals (Hess et al., 1997). The threshold values for electrostatic and van der Waals interactions were both 1.2 nm. Long-range electrostatic interactions were computed using the particle mesh Ewald (PME) method (Essmann et al., 1995). The root mean square deviation (RMSD), root mean square fluctuation (RMSF), and interaction fingerprint values of the MD trajectories were calculated with GROMACS modules and MD-IPF (Kokh et al., 2020). The Gromos algorithm was employed for cluster analysis, using a backbone RMSD threshold of 0.3 nm for AflG and 0.2 nm for the AflG-Heme-AVN complex.

2.3 Large-scale virtual screening

AVN was removed from the central structure of the top cluster in the AflG-Heme-AVN ensemble, and the resulting structure was used as the receptor in virtual screening. The screening compound library comprised 1.3 million molecules from the ZINC20 database with Log(*p*) values ≤3.5 and 32,000 molecules from TopScience Co. Ltd. (Shanghai, China). The docking-ready pdbqt files for each compound were downloaded from the ZINC20 database. The two-dimensional structures of molecules obtained from TopScience were converted to three-dimensional structures using OpenBabel v.3.1.0 and then

processed with the Python script “mk_prepare_ligand.py” (O’Boyle et al., 2011). The receptor structure was prepared, the grid space was set, and the affinity maps were generated as described in section 2.1 with several exceptions: the point settings of the x-, y-, and z-dimensions were 52 Å, 32 Å, and 22 Å with centers at 4.057 Å, 2.767 Å, and 7.66 Å, respectively. The structure derived from the MD simulation exhibited a systematic deviation in position compared to the docked AflG-Heme-AVN model, rendering the grid box center positions incomparable. Autodock-GPU was used to dock molecules from the compound library using the default parameters (Santos-Martins et al., 2021). Molecules that exhibited a docking energy < −7 kcal/mol were classified as highly ranked compounds for further analysis.

2.4 Molecular mechanics/generalized born surface area calculations

Binding free energy was calculated for over 4,000 highly ranked compounds. For each compound, the docking pose with the lowest docking energy was selected. Hydrogen atoms were then added, and the structure was converted to mol format using OpenBabel v.3.1.0. MM-GBSA calculations for the highly ranked compounds were conducted in an automated workflow (Uni-GBSA) with default parameters (Yang et al., 2023). Compounds with a binding free energy < −53 kcal/mol were prioritized for further analysis as prioritized hit compounds against AflG.

2.5 MD simulations of AflG complex with hit compounds

Of the compounds classified as hits, eight were commercially available. MD simulations using Uni-GBSA and GROMACS 2020 were performed using the top-ranked docking poses of these compounds. The protein was described with the amber99sb force field, while the compounds were described with gaff2. Both were done using default parameters in Uni-GBSA. The simulation parameters were similar to those used for the AflG-Heme-AVN system, except the temperature was maintained at 300 K, and the threshold values for electrostatic and van der Waals interactions were 1.0 nm. Simulations for all systems were run for 100 ns, and trajectories were analyzed as described above. Cluster analysis were conducted for all AflG-compound complexes with the Gromos algorithm and a backbone RMSD threshold of 0.2 nm.

2.6 *In vitro* validation of AF biosynthesis inhibition

The *A. flavus* strain NRRL 3357 was used to validate AF biosynthesis inhibition using the previously established liquid incubation system in our lab (Yan et al., 2015; Wu et al., 2022). The eight commercially available candidate inhibitor compounds, identified by PubChem Compound Identifications (CIDs) 50782408, 53209539, 57336812, 53151533, 20880420, 50748540, 91904139, and 54761306, were procured from TopScience Co. Ltd. (Shanghai, China) and had >95% purity. Stock solutions of each compound were prepared in dimethyl sulfoxide (DMSO), except compound 54761306, which was prepared

in liquid glucose mineral salt (GMS) medium (Yan et al., 2012). To analyze *A. flavus* physiology and AF production under control (CK) conditions and in the presence of each putative inhibitor, flasks were prepared with 18 mL of liquid GMS medium, 2 mL of spore suspension (0.8×10^7 spores/mL), and 100 μ L of putative inhibitor stock solution, DMSO, or GMS medium, yielding an initial fungal density of 0.8×10^6 spores/mL, and the final concentrations of eight putative inhibitors 50782408, 53209539, 57336812, 53151533, 20880420, 50748540, 91904139, and 54761306 were 80, 148, 96, 30, 147, 167, 167, and 48 μ M, respectively. All GMS liquid cultures were grown at 28°C under continuous darkness with shaking at 180 rpm (Yan et al., 2012, 2015). The *A. parasiticus* NRRL 2999 strain, obtained from USDA-ARS, was further used to validate the inhibitory effect of compound 50782408 using the same procedure as for the *A. flavus* NRRL 3357 strain. AF contents were measured using thin-layer chromatography (TLC) as previously described (Yan et al., 2012). The relative intensities on the TLC plates were quantified using GelAnalyzer 19.1 (www.gelanalyzer.com) and subsequently normalized to a maximum sample intensity of 1, allowing for the comparison of relative concentrations of AF.

3 Results

3.1 Atomic-level architecture of AflG and the cofactor-substrate complex

Comprehensive structural modeling was undertaken to elucidate the key architectural features of AflG and to provide details of its

cofactor and substrate interactions. Bioinformatics analysis revealed that the full-length AflG amino acid sequence (450 residues) contained a highly conserved ExxR motif (E³⁵³SLR³⁵⁶) and an active-site motif (E⁴²⁹SIGPRNCIG⁴³⁸), both of which are characteristic of cytochrome P450 (Figure 1A) (Yu et al., 1997; Yang et al., 2008; Syed and Mashele, 2014). These findings validated the initial classification of AflG as a member of the cytochrome P450 superfamily. AlphaFold2 was next used to generate a high-confidence three-dimensional AflG structure. This demonstrated that the secondary structure was primarily composed of α -helices (~70%) (Figure 1B), consistent with other well-characterized P450 enzymes. High-confidence pLDDT scores (> 90) for a significant portion of the structure indicated the accuracy of the AflG structural model (Supplementary Figure S1).

In this study, our primary focus was directed toward elucidating the essential residues of AflG that govern its interactions with the substrate, as these aspects are pivotal for understanding the enzymatic function of AflG and facilitating inhibitor screening. Consequently, we omitted the N-terminal 35 amino acids, which were predicted to interact with membranes, from the structural model (Kufareva et al., 2014). Subsequently, we aligned the obtained AflG structure with a closely related human enzyme, P450 3A5, to uncover the binding mode and position of the Heme cofactor (Waterhouse et al., 2018). The superposition of the two structures revealed a close alignment of the Heme-binding α -helical regions (Figure 1C). We, therefore, transferred the Heme position from P450 3A5 to the AflG structure to generate an AflG-Heme complex model. Cavity analysis of the AflG-Heme model demonstrated the presence of a suitable internal binding pocket proximal to the Heme iron center (Supplementary Figure S2) (Gay

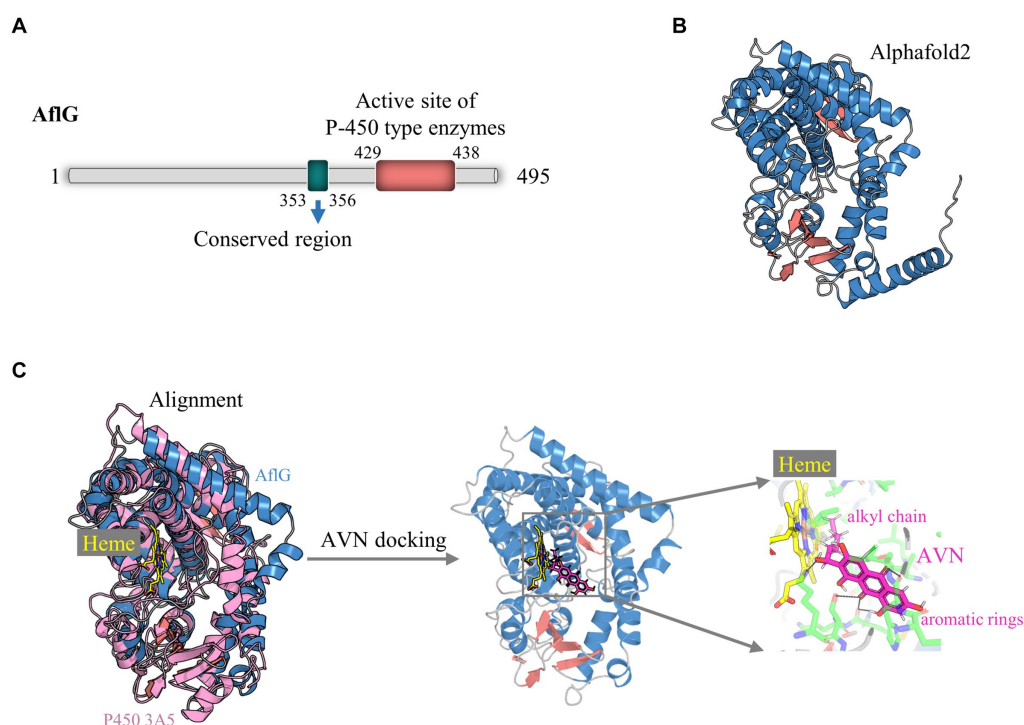


FIGURE 1

Structural modeling of AflG and the AflG-Heme-averantin (AVN) complex. (A) Schematic diagram of the AflG amino acid sequence with conserved cytochrome P450 motifs highlighted. (B) AflG structure as predicted with AlphaFold2. Secondary structures are shown: α -helices (blue), β -sheets (red), and loops (gray). (C) Structural model of the AflG-Heme-AVN complex. The model was generated by the superposition of AflG and human P450 3A5 complexed with Heme, followed by simulated AVN docking.

et al., 2010). The native AflG substrate AVN was then computationally docked into this pocket. A high-scoring pose that positioned the substrate near the Heme with ideal geometry for P450-mediated catalysis was selected as the final AflG-Heme-AVN complex model (Figure 1C) (Lee et al., 2003). Overall, this modeling exercise provided unprecedented atomic-level insights into AflG architecture and the cofactor-substrate complex, enabling further analyses.

3.2 MD simulations revealed AflG dynamics and distinct substrate conformational states

To gain insights into the conformational dynamics of AflG and its interaction with the substrate AVN, MD simulations at the

microsecond timescale were performed on both AflG alone and the AflG-Heme-AVN ternary complex. Equilibration and stability analysis using RMSD measurements revealed that AflG and the AflG-Heme-AVN complex reached equilibrium after approximately 200 ns (Figure 2A). The RMSD of the Heme cofactor remained consistently low throughout each simulation, indicating stable Heme binding and validating the accuracy of the Heme placement. There were periodic fluctuations in the AVN RMSD as the simulations progressed, suggesting that the substrate held various conformational states within the binding pocket. AflG had smaller, more stable RMSD values in the context of the AflG-Heme-AVN complex compared to AflG alone, suggesting that substrate and cofactor binding had a stabilizing effect on AflG. RMSF analysis was conducted to further probe residue

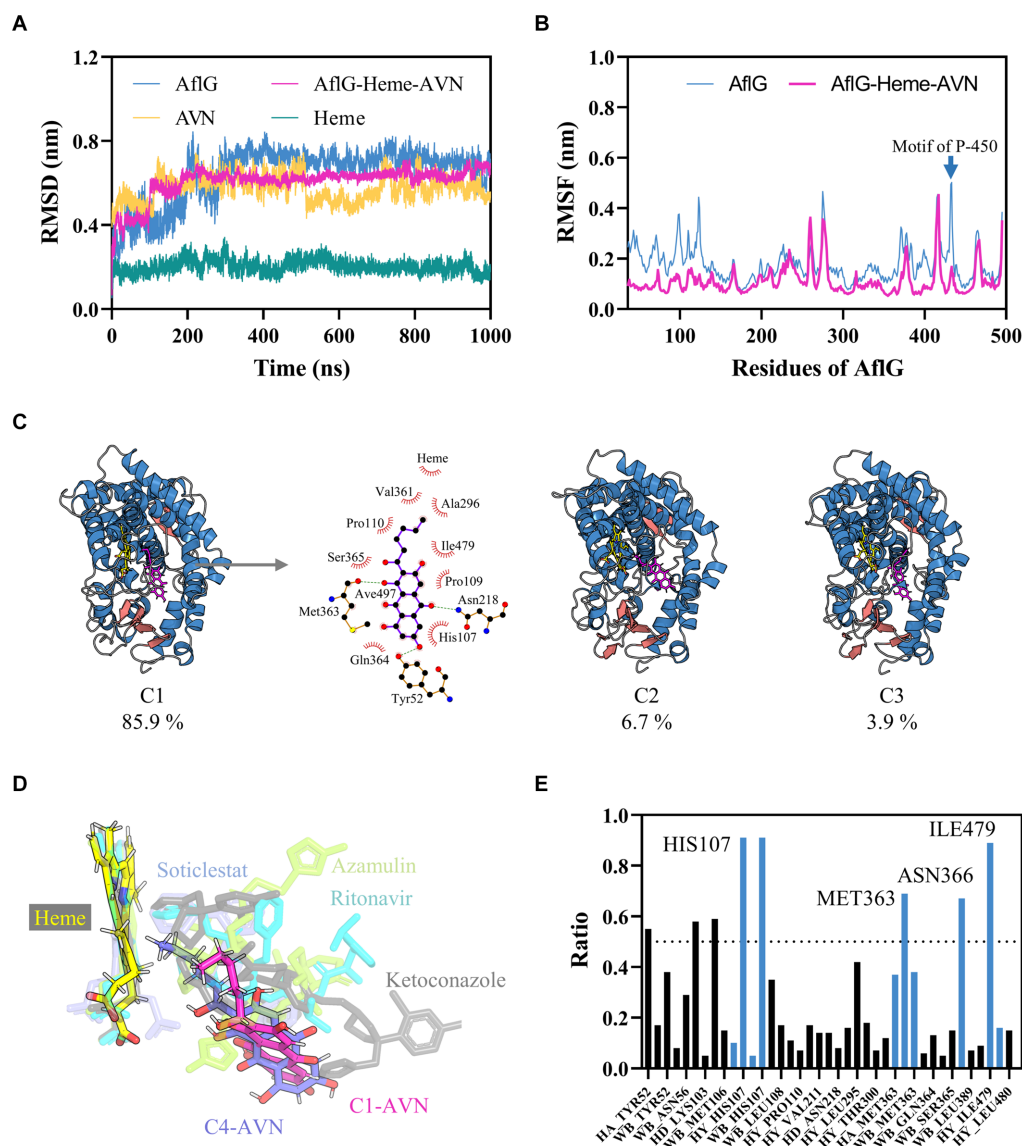


FIGURE 2

Characterization of interactions between AflG and averantin (AVN) by molecular dynamics simulations. (A) Root mean square deviation (RMSD) analysis of AflG and ligands over 1-μs simulations of AflG alone and of the AflG-Heme-AVN complex. (B) Root mean square fluctuation (RMSF) values showing AflG residue flexibility with and without AVN bound. (C) Representative structures from the top three conformational clusters of the AflG-Heme-AVN complex ensemble, along with two-dimensional interaction diagram illustrating the substrate's interactions with the protein and Heme. (D) Geometric configuration of the Heme cofactor and substrates in the AflG complex compared to related cytochrome P450 structures. (E) Ratios of simulation times for specific residue interactions between AflG and AVN.

flexibility in AflG. This showed that several regions of AflG (namely the N-terminus, residues 260–280, and loops surrounding the active site) were highly flexible in the apo system. In comparison, these regions displayed significantly reduced flexibility in the AflG-Heme-AVN complex (Figure 2B), confirming the stabilizing effects of the substrate and cofactor binding.

In-depth conformational analysis via clustering of the simulation ensembles revealed that the most populated structural clusters exhibited relatively low structural heterogeneity for AflG in both systems (Figure 2C; Supplementary Figure S3). However, more significant N-terminal fluctuations were evident in AflG alone (Figure 2B). As suggested by the RMSD profiles, the representative structures of the most populated clusters in the AflG-Heme-AVN

complex clearly showed the AVN substrate shifting between dramatically different conformational states within the binding pocket, with the alkyl chain periodically transitioning between proximal and distal poses with respect to the Heme cofactor (Figure 2C). The two-dimensional interaction diagram of the representative structure from cluster C1 of the AflG-Heme-AVN complex illustrates the interaction of several AflG residues with AVN through hydrogen bond and hydrophobic interactions (Figure 2C). Representative AVN conformations were structurally aligned to crystal structures of other P450 substrate complexes to validate the distinct substrate-binding poses. The simulated AVN conformations were positioned highly similar to known crystal structures of bound ligands in other P450 systems (Figure 2D),

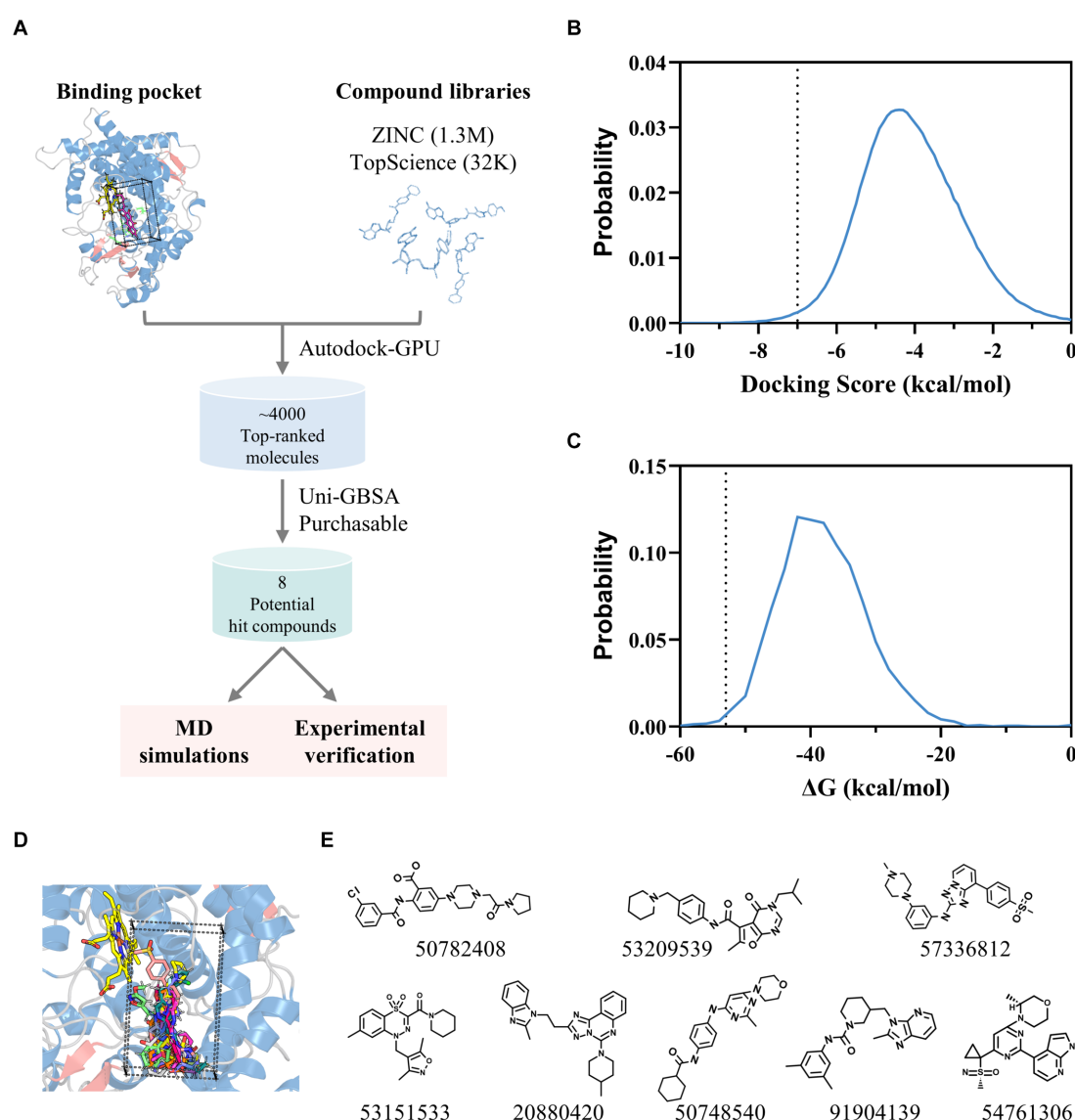


FIGURE 3

Identification of potential hit compounds against AflG with structure-based virtual screening and MM-GBSA calculations. (A) Virtual screening workflow. (B) Distribution of docking scores for all screened compounds. The dashed line indicates the threshold (-7 kcal/mol). (C) Distribution of calculated binding free energies for highly ranked compounds from the docking simulation. The dashed line indicates the threshold (-53 kcal/mol) for further analysis. (D) Superposition of screened compounds with the most favorable binding free energies. (E) Molecular diagrams of the eight commercially available top-ranked compounds selected as potential hit compounds against AflG, corresponding labeled by PubChem CID numbers, are shown below each compound.

indicating the validity of the predicted substrate binding locations in this context.

An interaction fingerprint analysis was then conducted to identify critical intermolecular interactions between AflG and the AVN substrate over the course of the simulations (Kokh et al., 2020). This revealed eight specific interactions that recurred with a contact ratio exceeding 50% of the simulation time. These interactions included hydrogen bonds, hydrophobic contacts, and water bridges. Notably, the AflG residues His107, Met363, Asn366, and Ile479 interacted with AVN during >65% of the simulation time (Figure 2E), highlighting these residues as potentially crucial contact points for substrate binding.

3.3 Large-scale virtual screening yielded potential hit compounds against AflG

Leveraging the AflG-AVN structural model and the identified key binding residues, we next conducted a systematic, structure-based virtual screening to identify potential hit compounds against AflG (Figure 3A). Using two separate compound libraries, we screened over 1.3 million molecules from the ZINC20 database and an additional 32,000 molecules. This comprehensive screening yielded more than 4,000 top-ranked compounds with favorable docking scores (< -7 kcal/mol) (Figure 3B), indicating significant predicted binding affinity. Free energy calculations were next conducted for the top-ranked compounds using MM-GBSA. This revealed 15 compounds with remarkably low binding free energies (< -53 kcal/mol) (Figure 3C; Table 1), which were selected as hits for further analyses.

Structural analysis indicated that these 15 compounds consistently occupied a highly similar position within the AVN substrate binding pocket (Figure 3D). Eight of the compounds were commercially available and therefore prioritized for validation (Figure 3E).

3.4 MD simulations verified the binding stability of prioritized hit compounds

To characterize interactions between AflG and the prioritized hit compounds, 100-ns MD simulations were performed for each AflG-hit complex. AflG RMSD plots revealed minimal structural deviations (~ 0.2 nm) across all of the simulations (Figure 4), indicating that the binding of each compound did not dramatically alter the overall AflG conformation compared to AflG in the AVN-bound state. Ligand RMSD measurements showed similarly small deviations in compounds 53209539, 57336812, 53151533, and 54761306, suggesting that these compounds were stably bound (Figures 4B–D, H). Compounds 50782408, 20880420, 50748540, and 91904139 displayed larger RMSD fluctuations, indicating significant changes in their poses from the initial docked conformations (Figures 4A, E–G).

Binding free energy calculations for representative cluster structures showed improved affinity compared to the docked poses for all compounds (Supplementary Table S1), confirming that each compound was predicted to bind to AflG with a much stronger affinity than the native substrate (AVN). Interaction fingerprint analysis revealed key sustained contacts of most of the compounds with the AflG residues His107, Met363, and Ile479 (Figure 5; Table 2). Compounds 50782408 and 20880420 showed more persistent

TABLE 1 Docking scores and binding free energies of averantin (AVN) and the potential hit compounds against AflG from virtual screening and MM-GBSA calculations.

Compound	Docking score (kcal/mol)	Binding free energy (kcal/mol)
AVN	−5.88	−47.50
50782408	−7.12	−60.25
92857748	−7.2	−58.47
53209539	−7.36	−58.31
95894266	−7.12	−58.29
57336812	−8.79	−56.25
124366745	−7.04	−55.79
53151533	−8.06	−55.46
20880420	−9.16	−55.30
92408135	−7.85	−54.80
124366746	−7.17	−54.65
124893813	−7.07	−54.39
135404553	−7.1	−54.31
50748540	−8.39	−54.14
91904139	−8.28	−53.27
54761306	−7.09	−53.02

AVN is the native substrate. Other compounds are identified by their PubChem CID numbers.

interactions within the binding pocket than AVN did, supporting the validity of the predicted competitive binding modes. However, compounds 50748540 and 91904139 showed fewer contact points compared to AVN. Overall, these MD simulations verified the stable binding of several high-priority hit compounds to AflG, adopting binding modes similar to that of the native substrate AVN, indicating their potential as AflG inhibitors.

3.5 Experimental validation of putative AflG inhibitors

The effects of putative AflG-inhibitory compounds on AF biosynthesis were explored experimentally using *A. flavus* strain NRRL 3357. Mycelial phenotypes and AF levels in the growth medium were monitored over 6 days in CK cultures and those with selected compounds added. There were no significant differences in mycelial phenotypes between any of the treated cultures and the CK (Figure 6), demonstrating a lack of toxicity to the fungus. However, compounds 50782408 and 54761306 notably inhibited AF production (Figures 6A,B). Specifically, compound 50782408 significantly inhibited AF production over the entire culture time (Figure 6A), whereas compound 54761306 had a remarkable effect on the third day of growth (Figure 6B). Contrary to our expectations based on the predictions, the remaining six compounds (53151533, 57336812, 53209539, 20880420, 50748540, and 91904139) had no measurable inhibitory impacts on mycelial growth or AF production (Figures 6C,D; Supplementary Figure S4).

Given the promising inhibitory effects of compound 50782408 on AF biosynthesis observed in the above experiments, we further

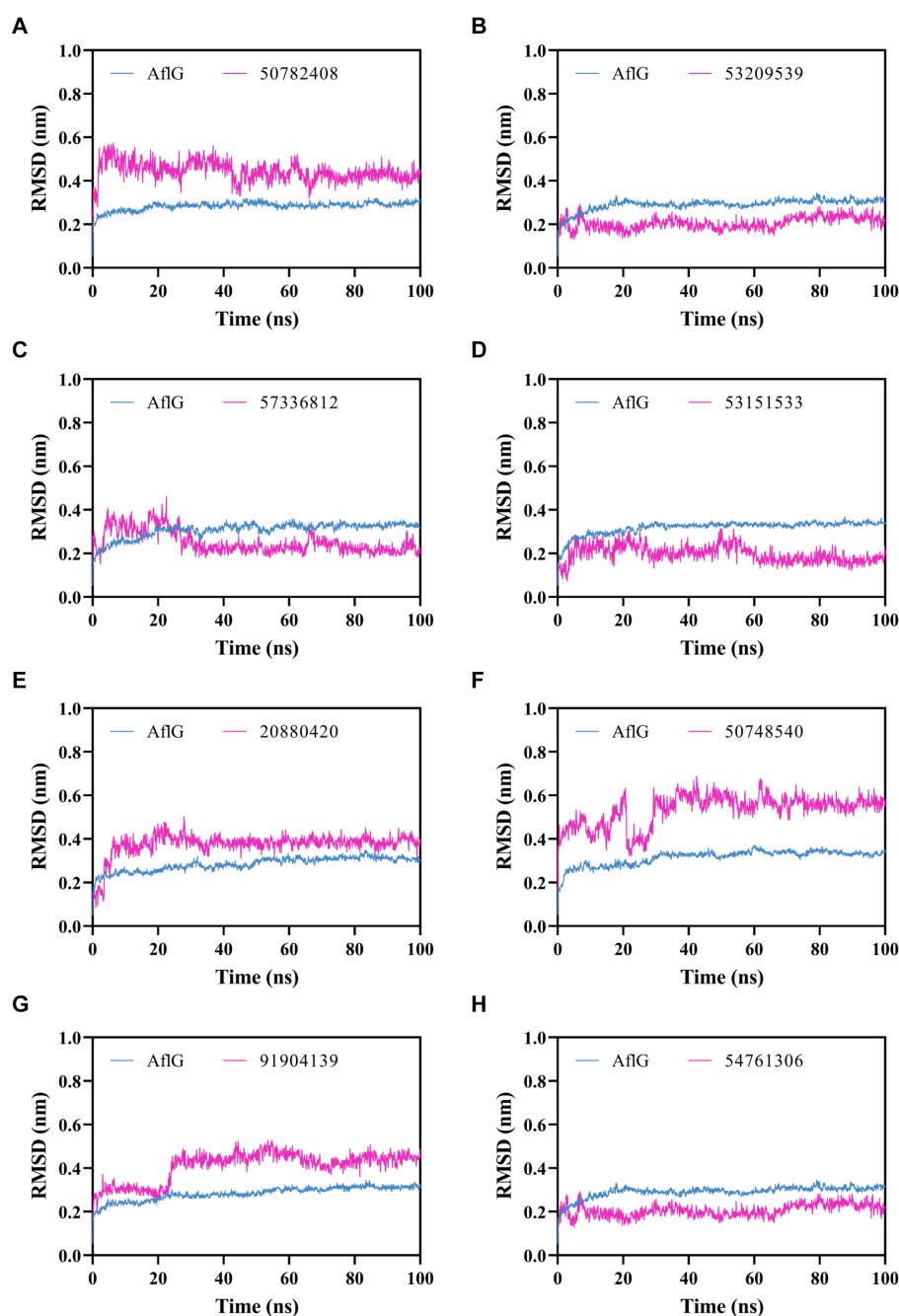


FIGURE 4

Molecular dynamics simulations of AflG bound to prioritized hit compounds. RMSD values of AflG (blue) and ligand (purple) in complex are shown over the course of the 100-ns simulation time for compounds (A) 50782408, (B) 53209539, (C) 57336812, (D) 53151533, (E) 20880420, (F) 50748540, (G) 91904139, and (H) 54761306.

conducted a concentration titration experiment to validate its efficacy. We observed a decrease in AF production with increasing concentrations of compound 50782408 (Supplementary Figures S5, S6). Additionally, we treated another aflatoxigenic strain, *A. parasiticus* NRRL 2999, with compound 50782408. The results showed that, consistent with observations in *A. flavus* NRRL 3357, compound 50782408 exhibited a significant inhibitory effect on the strain *A. parasiticus* NRRL 2999, affirming its potent inhibitory action (Supplementary Figure S7). Collectively, the experimental validations of candidate AflG

inhibitors pinpointed two compounds that inhibit AF biosynthesis, consistent with our computational predictions.

3.6 Structural and dynamic analyses revealed the distinct binding modes underlying functional inhibitory effects

Structural comparisons and dynamics analyses were undertaken to elucidate the molecular mechanisms underlying the distinct effects

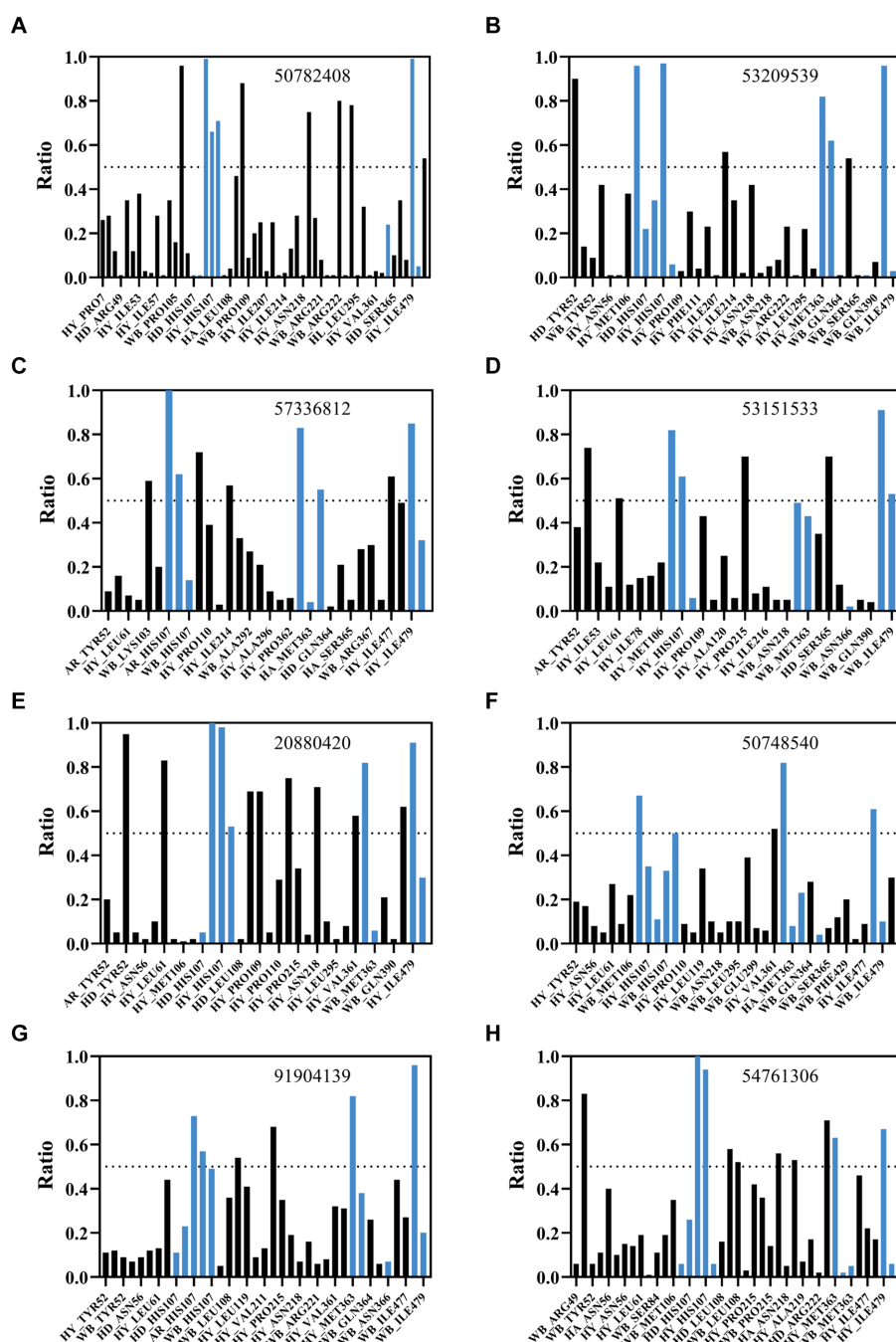


FIGURE 5

Analysis of interactions between AfIG and prioritized hit compounds. The ratios of specific residue interaction times were analyzed between AfIG and compounds (A) 50782408, (B) 53209539, (C) 57336812, (D) 53151533, (E) 20880420, (F) 50748540, (G) 91904139, and (H) 54761306. The Y-axis represents the proportion of interaction time to the total simulation time. The X-axis represents different interaction types, including water bridge (WB), hydrophobic interaction (HY), hydrogen bond acceptor (HA), and hydrogen bond donor (HD), involving different residues.

of candidate compounds on AF biosynthesis. Structural alignment of the top cluster for each AfIG-compound ensemble revealed that the active inhibitor 50782408 occupied the AfIG binding pocket comparably to the native substrate AVN (Figure 7A). Meanwhile, the binding location of compound 54761306 was comparable to that of the AVN aromatic rings only, despite the inhibitory activity of compound 54761306 (Figure 7C). The inactive compound 53151533 demonstrated a comparable proximal localization to the aromatic core

of AVN but lacked functional efficacy (Figure 7E). Additionally, the inactive compound 57336812 displayed a similar proximity to AVN but had no impact on AF production (Figure 7G).

Dynamic analyses based on RMSF values indicated that interactions with most of the candidate inhibitor compounds enhanced AfIG rigidity and stability, consistent with enzyme characteristics in an inhibitor-bound state. Specifically, interactions with compounds 50782408 and 91904139 increased the flexibility of

TABLE 2 Key AflG residues predicted to interact with ligands.

Ligand	Binding free energy (kcal/mole)	Hydrogen-bond	Hydrophobic interaction	Water bridge	Aromatic interaction	Number of interactions
AVN	−47.50	Tyr52	His107, Ile479, Met363	Asn366, Glu82, His107, Lys103		8
50782408	−60.25	Met106	His107, Ile479, Leu480, Pro109, Pro7	Asn218, His107, Tyr223	His107	10
53209539	−58.31	Ser365, Tyr52	His107, Ile479, Met363, Val211	Met106, Met363	His107	9
57336812	−56.25		His107, Ile214, Ile477, Ile479, Met363, Pro109	Lys103, Met363	His107	9
53151533	−55.46	Ser365	His107, Ile479, Leu61, Pro215, Tyr52	Ile479	His107	8
20880420	−55.30	Tyr52	Asn218, His107, Ile214, Ile477, Ile479, Leu61, Met363, Pro109, Val361	His107, Leu108	His107	13
50748540	−54.14	His107	Ile479, Met363, Pro109, Val361			6
91904139	−53.27		His107, Ile214, Ile479, Met363, Pro109		His107	6
54761306	−53.02	Leu108	Asn218, His107, Ile479, Met363, Tyr52	Asn218, Leu108	His107	9

AflG residues included here showed intermolecular contacts with the indicated compound for >50% of the molecular dynamics simulation time. Averantin (AVN) is the native substrate. Other compounds are identified by their PubChem CID numbers.

the AflG N-terminal region compared to the substrate AVN (Figure 7B; Supplementary Figure S8D). Other compounds (e.g., 54761306, 53209539, and 20880420) had a milder impact on AflG N-terminal dynamics (Figure 7D; Supplementary Figure S8). Several compounds, including 54761306, 57336812, 20880420, 53151533, and 50748540, also increased movement dynamics of the active site motif (residue 426–442), whereas only compounds 50782408 and 50748540 reduced flexibility within the 375–381 loop near the active site (Figure 7B, F, H; Supplementary Figure S8C). Despite the adoption of unique binding poses by each compound, these analyses thus revealed that compounds 50782408 and 54761306 inhibited AF biosynthesis while subtly modulating AflG dynamics through localized stabilization or destabilization of specific regulatory regions. Despite affecting similar dynamic changes and engaging in numerous intermolecular contacts with AflG, the ineffectiveness of compound 20880420 as an AflG inhibitor highlights the complexity of correlating interactions and protein dynamics with downstream functional impacts (Supplementary Figure S8B; Table 2). Further biophysical and structural studies are essential to increase our understanding of the ways in which interactions with specific compounds modulate AflG structure and dynamics and inhibit AF biosynthesis.

4 Discussion

Crop contamination with AFs impacts both the food and feed supply chains, exposing humans and livestock to these harmful compounds (Caceres et al., 2020; Uka et al., 2020). Such exposure not only jeopardizes human health but also induces substantial economic losses across the world. It is thus imperative to devise strategies that curtail AF biosynthesis in agricultural products. Enzyme inhibitors, which can be applied to regulate metabolic activity, offer a potential

method for achieving this goal (Tabe et al., 2019; Manzoor and Hoda, 2020; Sharma et al., 2020; Labib et al., 2022). Identification of inhibitors that target critical enzymes in the AF biosynthetic pathway is a promising strategy for minimizing AF contamination.

In the present study, we harnessed an integrated computational-experimental strategy, incorporating structural modeling, MD simulations, virtual screening, binding free energy calculations, and *in vitro* experimental validation to explore the structure, dynamics, and potential inhibition mechanisms of the critical AF biosynthetic enzyme AflG. Advanced structural prediction and modeling techniques enabled the construction of highly accurate AflG models, both of the enzyme alone and in complex with its native substrate (Kufareva et al., 2014; Waterhouse et al., 2018; The UniProt Consortium, 2023). Microsecond-scale MD simulations provided remarkable insights into the conformational landscape and dynamic motions involved in substrate binding (Abraham et al., 2015; Huang et al., 2017). The ligand AVN was observed to hold distinct poses within the binding pocket, periodically transitioning between conformations in which it was proximal and distal to the Heme cofactor. Furthermore, AflG displayed modulated dynamics upon substrate binding, with reduced structural fluctuations, particularly in the N-terminus and the loops surrounding the active site. These simulations demonstrated the power of molecular modeling to capture the inherent flexibility and transient motions governing this molecular system.

Building upon these initial results, we conducted a large-scale virtual screening of over 1.3 million compounds to identify potential hit compounds against AflG (Santos-Martins et al., 2021). Stringent binding free energy calculations yielded 15 high-confidence candidates, and subsequent MD simulations corroborated the robust binding attributes of specific compounds that shared key interaction residues with AVN (namely His107, Met363, and Ile479) (Yang et al., 2023). *In vitro* experiments with *A. flavus* strain NRRL 3357 showed

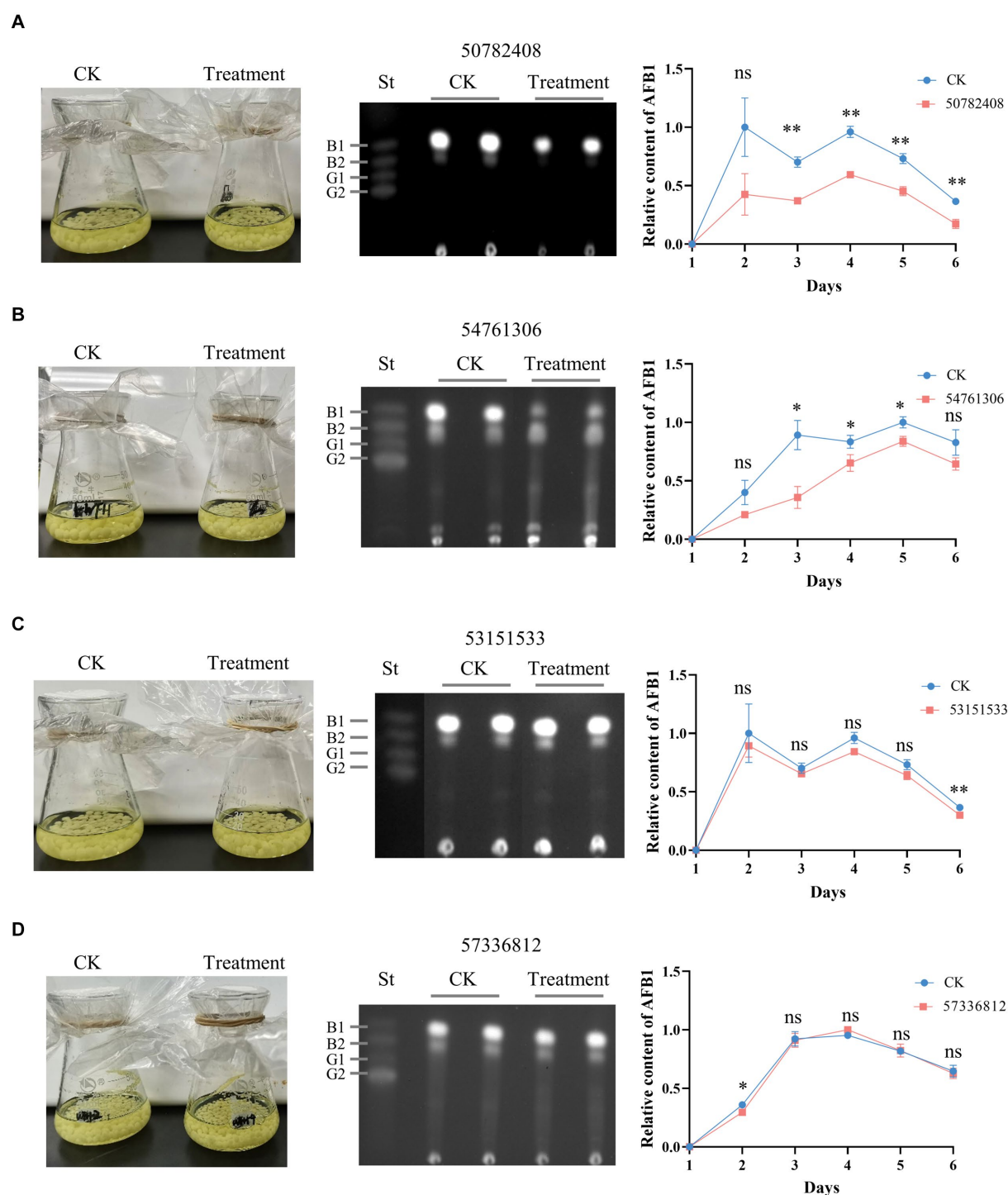


FIGURE 6

Effects of candidate AflG inhibitors on aflatoxin (AF) biosynthesis in *Aspergillus flavus*. (A–D) *A. flavus* control cultures (CK) and *A. flavus* cultures treated with the compounds, TLC of extracted AFs, and quantified relative intensity of AF production in *A. flavus* treated with compounds (A) 50782408, (B) 54761306, (C) 53151533, or (D) 57336812. Left, culture appearance after growth for 3-d. Center, visualization of extracted AF via thin-layer chromatography (TLC) from 3-d cultures, where 'St' represents aflatoxin standards. Right, relative content of AF quantified from TLC plates. The figures show the experimental results from two independent biological replicates. Error bars indicate the standard deviation (St) of the measurements. Significance was determined by the *t*-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

that two of these compounds, 50782408 and 54761306, markedly inhibited AF biosynthesis throughout a 6-d growth period. These results highlight the potential of compounds 50782408 and 54761306 as targeted modulators of the AF metabolic pathway.

Although several of the candidate compounds demonstrated potent inhibition of AF biosynthesis, consistent with the computational predictions, several other candidates proved ineffective *in vitro*, emphasizing the challenges in extrapolating predicted binding affinities

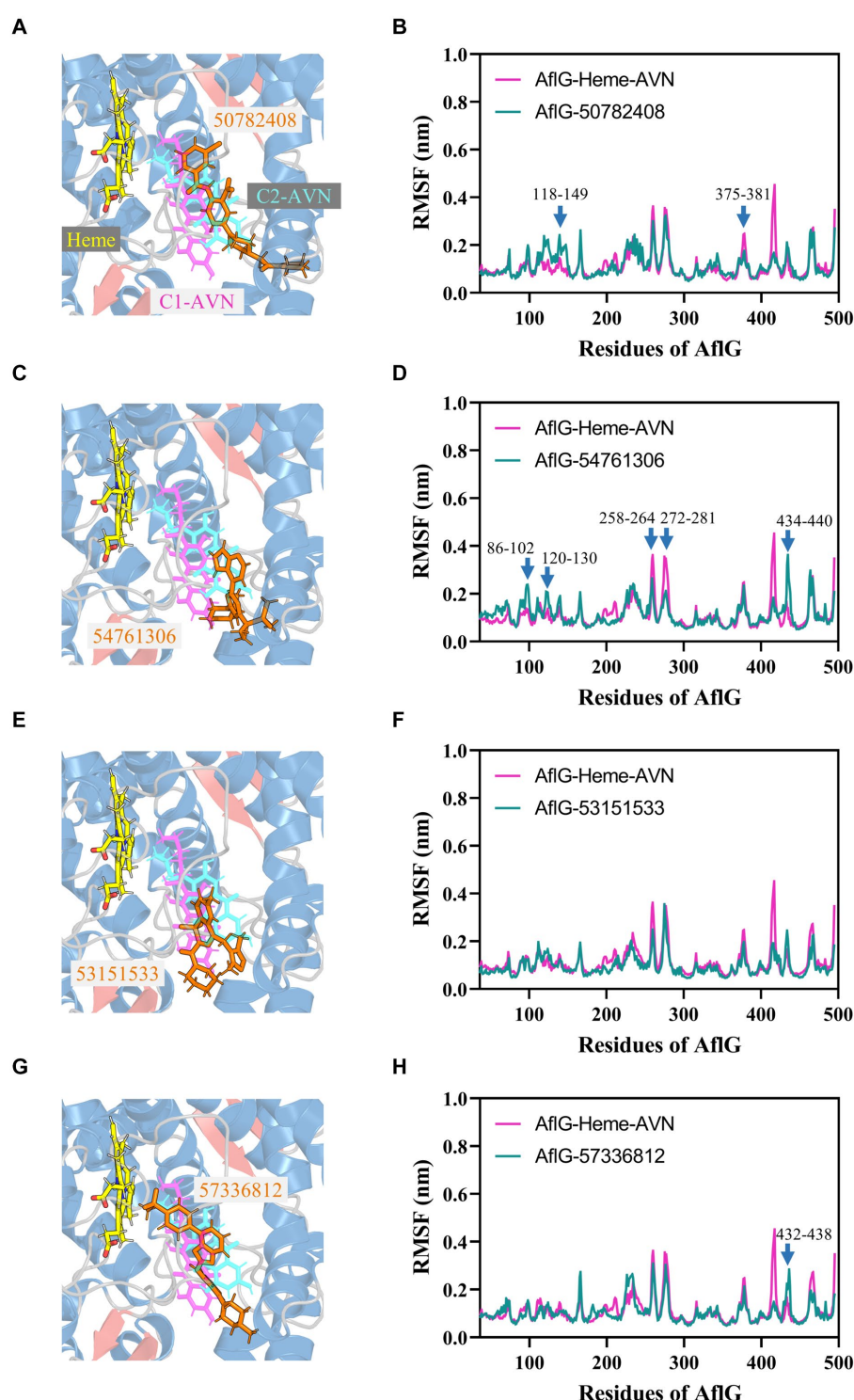


FIGURE 7

Binding modes of putative AflG inhibitors and their effects on AflG flexibility. (A,C,E,G) AflG-ligand complex structures showing the binding orientations of compounds (A) 50782408, (C) 54761306, (E) 53151533, and (G) 57336812. Each candidate inhibitor compound structure is overlaid with the substrate averantin (AVN) in the AflG-Heme-AVN complex. (B,D,F,H) Root mean square fluctuation (RMSF) plots comparing fluctuations in AflG residues between the AflG-Heme-AVN complex and AflG complex with compounds (B) 50782408, (D) 54761306, (F) 53151533, and (H) 57336812.

to functional outcomes. For example, compound 53151533 was predicted to have a low binding free energy but exhibited no discernible influence on AF levels. Conformational analysis revealed that compounds 50782408 and 54761306, which functionally inhibited

AflG, occupied binding regions close to that of the native substrate AVN or its aromatic rings. In contrast, the inactive compound 53151533 was positioned near the binding regions associated with the AVN aromatic rings despite having a similar predicted affinity as those

of compounds 50782408 and 54761306. These findings further highlight the complexity of predicting AF production effects from AflG-inhibitor interaction paradigms and binding energy calculations. This discrepancy could stem from limitations of *in silico* modeling in accounting for complex *in vivo* conditions, differences between kinetic and thermodynamic aspects of inhibition, potential compensatory mechanisms in the fungal system, and bioavailability issues affecting the compounds' ability to reach their target (Xu et al., 2021). To address these challenges, further efforts should prioritize the iterative refinement of structure–activity correlations, leveraging an integrated blend of computational and biophysical methodologies to enhance predictive accuracy continuously (Wu et al., 2020). Future studies could also integrate artificial intelligence (AI) technologies, such as machine learning algorithms, to improve the efficiency and reliability of the screening process (Patel et al., 2020).

Overall, this study establishes a powerful framework, including multi-scale modeling, high-throughput screening, and experimental validation to identify inhibitors of key AF biosynthetic enzymes. We successfully identified several compounds that functionally inhibited AF biosynthesis *in vivo*, and subsequent iterative improvements could be used to screen compounds with more pronounced pesticide-like attributes. Furthermore, the high-resolution models of AflG, both alone and in the substrate-bound form, provide a wealth of information to guide structure-based design. Ultimately, mitigating AF contamination at all stages of agricultural production requires collaborative solutions across the fields of agriculture, toxicology, and public health. Unraveling the biological mechanisms underlying AF biosynthesis is a cornerstone of any approach seeking to minimize AF contamination.

5 Conclusion

We here integrated advanced computational modeling approaches with *in vitro* validation of AF production inhibitory activity to explore functional mechanisms and identify inhibitors of a key enzyme in AF biosynthesis. Predictive structural modeling yielded detailed structural insights into the hitherto unknown structure of AflG. Furthermore, MD simulations revealed unique binding conformations in AflG that had remained undiscovered in static structural representations of enzyme–substrate interactions. A high-throughput virtual screening strategy was used to identify hit compounds against AflG, which were then validated *in vitro*. This structure-guided pipeline yielded promising candidate inhibitors, compounds 50782408 and 54761306. Importantly, our approach offered unprecedented insights into the atomistic characteristics of a critical AF biosynthetic enzyme both alone and in complex with substrates, enabling future optimization of efforts to identify compounds to combat AF contamination in agricultural products. The integrated strategy described here could also be employed to unearth putative inhibitors of other mycotoxin biosynthetic pathways, contributing to decreased economic losses and disease burden arising from fungal specialized metabolite contamination in agricultural products.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding authors.

Author contributions

FW: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. WZ: Investigation, Validation, Writing – original draft. MY: Data curation, Methodology, Writing – original draft. JN: Data curation, Writing – original draft. WH: Data curation, Writing – original draft. ZC: Data curation, Writing – original draft. YC: Investigation, Writing – original draft. DW: Methodology, Writing – original draft. JZ: Funding acquisition, Supervision, Writing – original draft. SW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft. SY: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

MY and DW were employed by DP Technology.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2024.1425790/full#supplementary-material>

References

- Abraham, M. J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B., et al. (2015). GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* 1, 19–25. doi: 10.1016/j.softx.2015.06.001
- Bhatnagar, D., Ehrlich, K. C., and Cleveland, T. E. (2003). Molecular genetic analysis and regulation of aflatoxin biosynthesis. *Appl. Microbiol. Biotechnol.* 61, 83–93. doi: 10.1007/s00253-002-1199-x
- Caceres, I., Khoury, A. A., Khoury, R. E., Lorber, S., Oswald, I. P., Khoury, A. E., et al. (2020). Aflatoxin biosynthesis and genetic regulation: a review. *Toxins (Basel)* 12:150. doi: 10.3390/toxins12030150
- Chang, P. K. (2003). The *aspergillus parasiticus* protein AFLJ interacts with the aflatoxin pathway-specific regulator AFLR. *Mol. Gen. Genomics* 268, 711–719. doi: 10.1007/s00438-003-0809-3
- Crawford, J. M., Korman, T. P., Labonte, J. W., Vagstad, A. L., Hill, E. A., Kamari-Bidkorphe, O., et al. (2009). Structural basis for biosynthetic programming of fungal aromatic polyketide cyclization. *Nature* 461, 1139–1143. doi: 10.1038/nature08475
- Deift, P., and Zhou, X. (1993). A steepest descent method for oscillatory Riemann–Hilbert problems. Asymptotics for the MKdV equation. *Ann. Math.* 137, 295–368. doi: 10.2307/2946540
- Eberhardt, J., Santos-Martins, D., Tillack, A. F., and Forli, S. (2021). AutoDock Vina 1.2.0: new docking methods, expanded force field, and python bindings. *J. Chem. Inf. Model.* 61, 3891–3898. doi: 10.1021/acs.jcim.1c00203
- Essmann, U., Perera, L., Berkowitz, M. L., Darden, T., Lee, H., and Pedersen, L. G. (1995). A smooth particle mesh Ewald method. *J. Chem. Phys.* 103, 8577–8593. doi: 10.1063/1.470117
- Gay, S. C., Roberts, A. G., and Halpert, J. R. (2010). Structural features of cytochromes P450 and ligands that affect drug metabolism as revealed by X-ray crystallography and NMR. *Future Med. Chem.* 2, 1451–1468. doi: 10.4155/fmc.10.229
- Georgianna, D. R., and Payne, G. A. (2009). Genetic regulation of aflatoxin biosynthesis: from gene to genome. *Fungal Genet. Biol.* 46, 113–125. doi: 10.1016/j.fgb.2008.10.011
- Hess, B., Bekker, H., Berendsen, H. J., and Fraaije, J. G. (1997). LINCS: a linear constraint solver for molecular simulations. *J. Comput. Chem.* 18, 1463–1472. doi: 10.1002/(SICI)1096-987X(199709)18:12<1463::AID-JCC4>3.0.CO;2-H
- Holcomb, M., Santos-Martins, D., Tillack, A. F., and Forli, S. (2022). Performance evaluation of flexible macrocycle docking in AutoDock. *QRB Discov.* 3:e18. doi: 10.1017/qrd.2022.18
- Huang, J., Rauscher, S., Nawrocki, G., Ran, T., Feig, M., de Groot, B. L., et al. (2017). CHARMM36m: an improved force field for folded and intrinsically disordered proteins. *Nat. Methods* 14, 71–73. doi: 10.1038/nmeth.4067
- Hussain, R., Ahmed, M., Khan, T. A., and Akhter, Y. (2020). Fungal P₄₅₀ monooxygenases - the diversity in catalysis and their promising roles in biocontrol activity. *Appl. Microbiol. Biotechnol.* 104, 989–999. doi: 10.1007/s00253-019-10305-3
- Jallow, A., Xie, H., Tang, X., Qi, Z., and Li, P. (2021). Worldwide aflatoxin contamination of agricultural products and foods: from occurrence to control. *Compr. Rev. Food Sci. Food Saf.* 20, 2332–2381. doi: 10.1111/1541-4337.12734
- Jo, S., Kim, T., Iyer, V. G., and Im, W. (2008). CHARMM-GUI: a web-based graphical user interface for CHARMM. *J. Comput. Chem.* 29, 1859–1865. doi: 10.1002/jcc.20945
- Kokh, D. B., Doser, B., Richter, S., Ormersbach, F., Cheng, X., and Wade, R. C. (2020). A workflow for exploring ligand dissociation from a macromolecule: efficient random acceleration molecular dynamics simulation and interaction fingerprint analysis of ligand trajectories. *J. Chem. Phys.* 153:125102. doi: 10.1063/5.0019088
- Korman, T. P., Crawford, J. M., Labonte, J. W., Newman, A. G., Wong, J., Townsend, C. A., et al. (2010). Structure and function of an iterative polyketide synthase thioesterase domain catalyzing claisen cyclization in aflatoxin biosynthesis. *Proc. Natl. Acad. Sci. USA* 107, 6246–6251. doi: 10.1073/pnas.0913531107
- Kufareva, I., Lenoir, M., Dancea, F., Sridhar, P., Raush, E., Bissig, C., et al. (2014). Discovery of novel membrane binding structures and functions. *Biochem. Cell Biol.* 92, 555–563. doi: 10.1139/bcb-2014-0074
- Labib, M. M., Amin, M. K., Alzohairy, A. M., Elashokhy, M. M. A., Samir, O., and Hassanein, S. E. (2022). Inhibition analysis of aflatoxin by *in silico* targeting the thioesterase domain of polyketide synthase enzyme in *aspergillus ssp.* *J. Biomol. Struct. Dyn.* 40, 4328–4340. doi: 10.1080/07391102.2020.1856186
- Lee, D. S., Yamada, A., Sugimoto, H., Matsunaga, I., Ogura, H., Ichihara, K., et al. (2003). Substrate recognition and molecular mechanism of fatty acid hydroxylation by cytochrome P450 from *Bacillus subtilis*: Crystallographic, spectroscopic, and mutational studies. *J. Biol. Chem.* 278, 9761–9767. doi: 10.1074/jbc.M211575200
- Liao, J., He, Z., Xia, Y., Lei, Y., and Liao, B. (2020). A review on biosynthesis and genetic regulation of aflatoxin production by major *aspergillus* fungi. *Oil Crop Sci.* 5, 166–173. doi: 10.1016/j.ocsci.2020.11.001
- Manzoor, S., and Hoda, N. (2020). A comprehensive review of monoamine oxidase inhibitors as anti-Alzheimer's disease agents: a review. *Eur. J. Med. Chem.* 206:112787. doi: 10.1016/j.ejmech.2020.112787
- Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., et al. (2009). AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.* 30, 2785–2791. doi: 10.1002/jcc.21256
- Nierman, W. C., Yu, J., Fedorova-Abrams, N. D., Losada, L., Cleveland, T. E., Bhatnagar, D., et al. (2015). Genome sequence of *aspergillus flavus* NRRL 3357, a strain that causes aflatoxin contamination of food and feed. *Genome Announc.* 3:e00168-15. doi: 10.1128/genomeA.00168-15
- O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., and Hutchison, G. R. (2011). Open babel: an open chemical toolbox. *J. Cheminform.* 3:33. doi: 10.1186/1758-2946-3-33
- Parrinello, M., and Rahman, A. (1980). Crystal structure and pair potentials: a molecular-dynamics study. *Phys. Rev. Lett.* 45, 1196–1199. doi: 10.1103/PhysRevLett.45.1196
- Patel, L., Shukla, T., Huang, X., Ussery, D. W., and Wang, S. (2020). Machine learning methods in drug discovery. *Molecules* 25:2277. doi: 10.3390/molecules25222777
- Price, M. S., Yu, J., Nierman, W. C., Kim, H. S., Pritchard, B., Jacobus, C. A., et al. (2006). The aflatoxin pathway regulator AflR induces gene transcription inside and outside of the aflatoxin biosynthetic cluster. *FEMS Microbiol. Lett.* 255, 275–279. doi: 10.1111/j.1574-6968.2005.00084.x
- Razzaghi-Abyaneh, M., Chang, P. K., Shams-Ghahfarokhi, M., and Rai, M. (2014). Global health issues of aflatoxins in food and agriculture: challenges and opportunities. *Front. Microbiol.* 5:420. doi: 10.3389/fmicb.2014.00420
- Santos-Martins, D., Solis-Vasquez, L., Tillack, A. F., Sanner, M. F., Koch, A., and Forli, S. (2021). Accelerating AutoDock4 with GPUs and gradient-based local search. *J. Chem. Theory Comput.* 17, 1060–1073. doi: 10.1021/acs.jctc.0c01006
- Sezutsu, H., Le Goff, G., and Feyereisen, R. (2013). Origins of P450 diversity. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 368:20120428. doi: 10.1098/rstb.2012.0428
- Sharma, S., Ahmed, M., and Akhter, Y. (2020). Fungal acetyltransferases structures, mechanisms and inhibitors: a review. *Int. J. Biol. Macromol.* 157, 626–640. doi: 10.1016/j.ijbiomac.2019.11.214
- Skerker, J. M., Pianalto, K. M., Mondo, S. J., Yang, K., Arkin, A. P., Keller, N. P., et al. (2021). Chromosome assembled and annotated genome sequence of *Aspergillus flavus* NRRL 3357. *G3 (Bethesda)* 11:jkab213. doi: 10.1093/g3journal/jkab213
- Stout, S. M., Nemerovski, C. W., Streetman, D. S., Berg, M., Hoffman, J., Burke, K., et al. (2021). Interpretation of cytochrome P-450 inhibition and induction effects from clinical data: current standards and recommendations for implementation. *Clin. Pharmacol. Ther.* 109, 82–86. doi: 10.1002/cpt.1918
- Syed, K., and Mashele, S. S. (2014). Comparative analysis of P450 signature motifs EXXR and CXG in the large and diverse kingdom of fungi: identification of evolutionarily conserved amino acid patterns characteristic of P450 family. *PLoS One* 9:e95616. doi: 10.1371/journal.pone.0095616
- Tabé, Y., Lorenzi, P. L., and Konopleva, M. (2019). Amino acid metabolism in hematologic malignancies and the era of targeted therapy. *Blood* 134, 1014–1023. doi: 10.1182/blood.2019001034
- The UniProt Consortium (2023). UniProt: the universal protein knowledgebase in 2023. *Nucleic Acids Res.* 51, D523–d531. doi: 10.1093/nar/gkac1052
- Uka, V., Cary, J. W., Lebar, M. D., Puel, O., De Saeger, S., and Diana Di Mavungu, J. (2020). Chemical repertoire and biosynthetic machinery of the *aspergillus flavus* secondary metabolome: a review. *Compr. Rev. Food Sci. Food Saf.* 19, 2797–2842. doi: 10.1111/1541-4337.12638
- Vanommeslaeghe, K., Hatcher, E., Acharya, C., Kundu, S., Zhong, S., Shim, J., et al. (2010). CHARMM general force field: a force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *J. Comput. Chem.* 31, 671–690. doi: 10.1002/jcc.21367
- Wang, S., Xie, J., Pei, J., and Lai, L. (2023). CavityPlus 2022 update: an integrated platform for comprehensive protein cavity detection and property analyses with user-friendly tools and cavity databases. *J. Mol. Biol.* 435:168141. doi: 10.1016/j.jmb.2023.168141
- Wang, P., Xu, J., Chang, P. K., Liu, Z., and Kong, Q. (2022). New insights of transcriptional regulator AflR in *aspergillus flavus* physiology. *Microbiol. Spectr.* 10:e0079121. doi: 10.1128/spectrum.00791-21
- Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., et al. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res.* 46, W296–w303. doi: 10.1093/nar/gky427
- Wu, S., Huang, W., Wang, F., Zou, X., Li, X., Liu, C. M., et al. (2023). Integrated metabolomics and lipidomics analyses suggest the temperature-dependent lipid desaturation promotes aflatoxin biosynthesis in *aspergillus flavus*. *Front. Microbiol.* 14:1137643. doi: 10.3389/fmicb.2023.1137643
- Wu, S., Zhang, Q., Zhang, W., Huang, W., Kong, Q., Liu, Q., et al. (2022). Linolenic acid-derived oxylipins inhibit aflatoxin biosynthesis in *aspergillus flavus* through activation of iminoquin biosynthesis. *J. Agric. Food Chem.* 70, 15928–15944. doi: 10.1021/acs.jafc.2c06230
- Wu, F., Zhou, Y., Li, L., Shen, X., Chen, G., Wang, X., et al. (2020). Computational approaches in preclinical studies on drug discovery and development. *Front. Chem.* 8:726. doi: 10.3389/fchem.2020.00726

- Xu, Y., Shrestha, N., Pr  at, V., and Belouqui, A. (2021). An overview of in vitro, *ex vivo* and *in vivo* models for studying the transport of drugs across intestinal barriers. *Adv. Drug Deliv. Rev.* 175:113795. doi: 10.1016/j.addr.2021.05.005
- Yan, S., Liang, Y., Zhang, J., Chen, Z., and Liu, C. M. (2015). Autoxidated linolenic acid inhibits aflatoxin biosynthesis in *aspergillus flavus* via oxylipin species. *Fungal Genet. Biol.* 81, 229–237. doi: 10.1016/j.fgb.2014.11.005
- Yan, S., Liang, Y., Zhang, J., and Liu, C. M. (2012). *Aspergillus flavus* grown in peptone as the carbon source exhibits spore density- and peptone concentration-dependent aflatoxin biosynthesis. *BMC Microbiol.* 12:106. doi: 10.1186/1471-2180-12-106
- Yang, M., Bo, Z., Xu, T., Xu, B., Wang, D., and Zheng, H. (2023). Uni-GBSA: an open-source and web-based automatic workflow to perform MM/GB(PB)SA calculations for virtual screening. *Brief. Bioinform.* 24:bbad218. doi: 10.1093/bib/bbad218
- Yang, P., Tanaka, H., Kuwano, E., and Suzuki, K. (2008). A novel cytochrome P450 gene (CYP4G25) of the silkworm *Antheraea yamamai*: cloning and expression pattern in pharate first instar larvae in relation to diapause. *J. Insect Physiol.* 54, 636–643. doi: 10.1016/j.jinsphys.2008.01.001
- Yu, J., Chang, P. K., Cary, J. W., Bhatnagar, D., and Cleveland, T. E. (1997). *avna*, a gene encoding a cytochrome P-450 monooxygenase, is involved in the conversion of averantin to averufin in aflatoxin biosynthesis in *aspergillus parasiticus*. *Appl. Environ. Microbiol.* 63, 1349–1356. doi: 10.1128/aem.63.4.1349-1356.1997



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Detoxification of aflatoxin B1 by a *Bacillus subtilis* spore coat protein through formation of the main metabolites AFQ1 and epi-AFQ1

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A variety of important agricultural crops host fungi from the *Aspergillus* genus can produce cancerogenic secondary metabolites such as aflatoxins. Consequently, novel strategies for detoxification and their removal from food and feed chains are required. Here, detoxification of Aflatoxin B1 (AFB1) by the *Bacillus subtilis* multi-copper oxidase CotA (BsCotA) was investigated. This laccase was recombinantly produced in *E. coli* while codon optimization led to duplication of the amount of active protein obtained. CuCl₂ was added to the cultivation medium leading to a 25-fold increase of V_{max} corresponding to improved incorporation of Cu²⁺ into the enzyme protein which is essential for the catalytic reaction. To avoid potential cytotoxicity of Cu²⁺, cultivation was performed at microaerobic conditions indeed leading to 100x more functional protein when compared to standard aerobic conditions. This was indicated by an increase of V_{max} from 0.30 ± 0.02 to 33.56 ± 2.02 U/mg. Degradation kinetics of AFB1 using HPLC with fluorescence detection (HPLC-FLD) analysis indicated a theoretical substrate saturation above solubility in water. At a relatively high concentration of 500 µg/L, AFB1 was decomposed at 10.75 µg/Lh (0.17 nmol*min⁻¹*mg⁻¹) at a dosage of 0.2 µM BsCotA. AFQ1 and epi-AFQ1 were identified as the initial oxidation products according to mass spectrometry (i.e., HPLC-MS, HPLC-QTOF). None of these molecules were substrates for laccase but both decomposed in buffer. However, decomposition does not seem to be due to hydration of the vinyl ether in the terminal furan ring. Genotoxicity of the formed AFB1 was assessed in several dilutions based on the de-repression of the bacterial SOS response to DNA damage indicating about 80-times reduction in toxicity when compared to AFQ1. The results of this study indicate that BsCotA has high potential for the biological detoxification of aflatoxin B1.

KEYWORDS

mycotoxins, laccase, enzymatic degradation, genotoxicity, copper incorporation

1 Introduction

Aflatoxins are cancerogenic secondary metabolites produced by several *Aspergillus* species, predominantly *A. flavus* and *A. parasiticus* (Fouché et al., 2020). A number of agricultural relevant crop species such as corn and peanuts are common hosts to these fungi, leading to frequent contaminations throughout the globe, but predominantly in warm and humid climates. Aflatoxins and their derivatives share a planar difuranocoumarin core structure with partially strong affinities to DNA (Stone et al., 1988; Neto and Lapis, 2009; Mariën et al., 1987). These vary considerably depending on how well the distinct molecular structures can intercalate with DNA (Iyer et al., 1994; Stone et al., 1988). Aflatoxins are activated into a short-lived *exo*-8,9-epoxide via oxidation of the vinyl ether in the terminal furan ring by liver P450-dependent monooxygenases (Shimada and Guengerich, 1989). The highly reactive metabolite readily forms covalent bonds to the guanine N7 of DNA, which further reacts to a formamidopyrimidine adduct (Klvana and Bren, 2019; Croy et al., 1978; Essigmann et al., 1977). Aflatoxin B1 induces misincorporations during DNA replication, in particular the guanine to thymine transversion in the human p53 tumor suppressor gene, which is linked to the initiation of hepatocarcinoma formation (Aguilar et al., 1993). Aflatoxin B1 (AFB1) is the predominant metabolite and the most toxic representative of aflatoxins, and while the contamination levels are generally much lower than for other frequent toxic fungal contaminants on crops such as deoxynivalenol or fumonisin B1, chronic exposure is associated with higher risk of development of liver cancer. Consequently, very low maximum levels in food and feed stuffs have been issued by all major authorities (EUR-Lex - 02006R1881-20140701 - EN - EUR-Lex, 2023).

Strategies to remove AFB1 from the food and feed chain have been studied extensively. AFB1 can be decomposed by physical and chemical methods, such as treatment with hydrogen peroxide or ammonium (Rushing and Selim, 2019). However, neither these approaches are cost-effective, nor they are well-applicable in industry. Silicate-based binders can absorb AFB1 in feed, but the efficacy highly depends on the chemical composition and administration dosage of the respective product (Vekiru et al., 2015). Alternatively, a number of reports exist on biological degradation of AFB1 by microbes reported from a plethora of different genera (Mishra and Das, 2003; Vanhoutte et al., 2016; Taheur et al., 2020; Mwakinyali et al., 2019; Li et al., 2018; Arimboor, 2024; Gu et al., 2024; Wu et al., 2024; Tang et al., 2024). However, most of these reports lack the experimental details to unambiguously document biological/enzymatic degradation in contrast to frequently observed absorption effects. Enzymes as biocatalysts allow detoxification of AFB1 in food and feed products at mild conditions (temperature, pH) and are highly specific which avoids potential damage of valuable ingredients that could happen with other treatments (e.g., high temperature). Only recently, the degradation of aflatoxin B1 by the lipase and protease of *Humicola lanuginosa* was reported (Al-Rajhi et al., 2024). Enzymatic degradation is well-documented for members of the phyla *Actinomycetales* (Ciegler et al., 1966; Teniola et al., 2005) in which $F_{420}H_2$ -dependent reductases catalyze the reduction of a double bond in the AFB1 lactone core, which enables further

decomposition of the otherwise rigid molecular structure (Bashiri, 2022; Taylor et al., 2010). Other reports on enzymatic degradation refer to manganese peroxidases (Wang et al., 2019a, 2011; Loi et al., 2020) and laccases predominantly from filamentous fungi (Alberts et al., 2009; Guo et al., 2020). Manganese peroxidases transfer electrons from hydrogen peroxide to reduce Mn^{2+} . AFB1 degradation by peroxidases thus again requires addition of hydrogen peroxide and hence may be more a function of the initial peroxide concentration in the assay, than related to an enzymatic process.

Laccases (EC 1.10.3.2) are multicopper oxidases which can act on a broad range of substrates (Giardina et al., 2010; Pardo and Camarero, 2015). The ability to oxidize a substrate is limited by steric and electrostatic hindrance but appears to primarily depend on the enzymes specific redox potential presented by a type 1 (T1) copper, which acts as a primary acceptor of electrons from the ligand (Tadesse et al., 2008; Xu et al., 1998). A general classification organizes laccases into high redox potential enzymes mostly from fungi such as *Trametes versicolor* (~780 mV) and medium/low redox potential laccases originating from plants and prokaryotes. High laccase activities in several fungal culture supernatants were associated with the higher ability to degrade AFB1 and a commercial batch of *T. versicolor* laccase did degrade AFB1 into a non-toxic product (Alberts et al., 2009). However, laccases can not only convert AFB1, but also the metabolites AFB2, AFG1 and AFG2, as molecular docking studies have indicated using the laccase from *Saccharomyces cerevisiae* (Liu et al., 2020). Additionally, Loi et al. (2016) assessed the degradation of AFM1 by Lac2 from fungi *Pleurotus pulmonarius*. The activity in AFB1 is not restricted to high redox potential laccases. High redox compounds were oxidized at higher rates by the low redox potential CotA laccase of *Bacillus subtilis* (525 mV) (Martins et al., 2015).

In 2019 (Kunz-Vekiru et al., 2019), we have shown that the laccase CotA from *Bacillus subtilis* is able to degrade AFB1 without the need for mediators. Mediators are small organic compounds that can act as electron carriers between the enzyme and the substrate. Oxidation of the mediator by laccase leads to the formation of highly active cation radicals that oxidize compounds that laccase alone cannot oxidize (Munk et al., 2018). Albeit using mediators, in the same year, likewise Wang et al. (2019b) reported a decrease of the AFB1 concentration upon incubation with this laccase. However, this activity was based on the decrease of AFB1 determined by HPLC while potential degradation products were not identified. Afterwards, other working groups have reported the activity of a different CotA enzymes from another *Bacillus* species, namely *B. licheniformis* on AFB1 (Wang et al., 2019b; Sun et al., 2023; Liu et al., 2023; Wang et al., 2024; Guo et al., 2020). However, this enzyme shows very low identity (65%) and homology (78%) to CotA from *B. subtilis*. Hence, it would be interesting to elucidate to which degradation products these different laccases lead and how this laccase could potentially be produced in higher yields. The results could provide important insights into how strongly the activity on AFB1 is conserved in *Bacillus*. Therefore, in this study, we have studied in detail the catalytic detoxification of AFB1 by the recombinantly produced and purified *B. subtilis* CotA (BsCotA) laccase without the use of mediators. For the first time, aspects are highlighted that have previously received only minor or no

attention, such as gene design and expression optimization for the best possible incorporation of copper into the laccase or the instability of the degradation product AFQ1. We have identified aflatoxin Q1 (AFQ1) and its epimere (epi-AFQ1) as the main reaction products by LC-MS and LC-HRMS and determined the residual mutagenicity compared to AFB1.

2 Materials and methods

2.1 Materials

LB media premix, containing 10 g/L tryptone, 5 g/L yeast extract, 5 g/L sodium chloride (NaCl), for culturing *E. coli* BL21-Gold (DE3) was purchased from Roth, Germany. The following chemicals (all purchased from Sigma-Aldrich, Germany) were dissolved in Milli-Q and sterile filtered: 50 mg/mL kanamycin, 1 M CuCl_2 and 1M IPTG. AFB1 powder was purchased from Fermentek (Jerusalem, Israel) and dissolved in acetonitrile to a stock solution of 900 mg/mL. AFQ1 and epi-AFQ1 were purchased from Toronto Research Chemicals (Toronto, Canada) as solid substances and dissolved to 250 and 100 $\mu\text{g/mL}$ stock solutions, respectively, in 100% acetonitrile.

LC gradient grade acetonitrile as well as formic acid (p.a.) were purchased from Merck (Darmstadt, Germany). A Purelab Ultra system (ELGA LabWater, Celle, Germany) was used for further purification of reverse osmosis water. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained as solid substance from Roche, Germany and dissolved to 10 mM in water.

2.2 Cloning, expression, purification and stabilization of CotA

A codon optimized version of the gene encoding the *Bacillus subtilis* CotA (Uniprot P07788) was synthesized in fusion to a C-terminal Strep-tag II (GenScript, Piscataway, NJ, USA) and cloned downstream of the T7 promoter into the *Escherichia coli* expression vector pET26b(+) over the NdeI and HindIII restriction sites. Chemically competent *E. coli* BL21-Gold (DE3) cells (Invitrogen, Carlsbad, CA, USA) were transformed with the plasmid and the laccase expression induced by addition of IPTG. Therefore, freshly transformed cells were cultured overnight at 37°C and 150 rpm (HT Multitron Pro, Infors, Bottmingen, Schweiz) in LB media containing 40 $\mu\text{g/mL}$ kanamycin. The overnight culture was used to inoculate 300 mL LB media supplemented with 40 $\mu\text{g/mL}$ kanamycin to a starting OD_{600} of 0.1 in a 1 L shake flask. Cells were grown aerobically at 30°C and 120 rpm until an OD_{600} of 0.6 was reached, after which the incubation temperature was reduced to 25°C followed by addition of 0.25 mM CuCl_2 and 0.05 mM IPTG to the culture media. Aerobic incubation was continued further for 4 h, and the change to microaerobic conditions was achieved by switching off the shaking function of the incubator. Cells were harvested after 20 h of microaerobic expression by centrifugation at 3,700 rpm for 20 min at 4°C (Eppendorf centrifuge 5920 R, Germany). 1 g cell pellet was resuspended in 5 mL buffer W (100 mM Tris/HCl pH 8.0 and 150 mM NaCl) and disrupted by sonication (Digital sonifier, Branson, CT, USA) by applying 10

rounds $\times$ 10 s pulses of 60% amplitude with 2 min rest between rounds. The lysate was centrifuged again, and sterile filtrated.

BsCotA_Strep was purified by affinity chromatography (Äkta pure, GE Healthcare, Chalfont St. Giles, UK) from the cleared lysate according to manufacturer's protocol (IBA GmbH, Göttingen, Germany). Briefly, 20 mL of the cleared lysate were loaded onto a 1 mL Strep-Tactin XT cartridge. The column was washed with buffer W (1 mL/min) until the baseline is reached and the enzyme was eluted during a 3-column volume gradient from 0 to 100% of buffer BXT (100 mM Tris/HCl pH 8.0, 150 mM NaCl and 50 mM biotin). Buffer was exchanged to 100 mM Tris/HCl pH 7 using PD-10 desalting columns (GE Healthcare, Chalfont St. Giles, UK) and the protein was stored at -20°C until further use. To improve enzyme stability, bovine serum albumin (BSA) was added making an end concentration of 2 mg/mL in the storage buffer. BsCotA_Strep expression levels were analyzed by SDS PAGE, and protein concentrations calculated from the absorbance values obtained at 280 nm via molecular extinction coefficient of 93,740 $\text{M}^{-1} \text{cm}^{-1}$ and molecular weight of 59.831 kDa (NanoDrop Spectrophotometer NP80 Touch, Implen, München, Germany).

Untagged BsCotA was expressed in the same way as BsCotA_Strep but purified by cation exchange chromatography. By using a washing buffer (20 mM Tris HCl pH 7.6) with a pH lower than the theoretical pI of CotA (pI 7.7), the laccase became positively charged and attached to the cation exchanger column (GE HiTrap SP FF 1 mL). Subsequently, elution was carried out with 20 mM Tris HCl pH 7.6 containing 1 M NaCl. After the pellet was resuspended in washing buffer and extracted with a sonicator (10 cycles of 10 s at 60% amplitude with 2 min rest between runs), the cell lysate was centrifuged for 25 min at $20,817 \times g$. The resulting cell lysate was then filtered and loaded onto the column at a flow rate of 1 mL/min. Following sample loading, the column was further washed with washing buffer to remove nonspecifically bound proteins, indicated by a decrease in UV absorbance at 280 nm. Elution of additional impurities was achieved by applying elution buffer at 10% for 10 column volumes (CV), while elution of the target protein occurred by applying elution buffer as a gradient toward 50% over 10 CV. Finally, 100% elution buffer was applied for 10 CV to remove any remaining attached proteins.

2.3 Determination of copper incorporation rates

The emitted signal of the complex formed between reduced copper released by the BsCotA protein with Bicinchoninic acid (BCA) was photometrically measured at two different wavelengths. The Cu(I)BCA complex is generally read at 562 nm at which the reading is exceptional tolerant to non-ionic detergents, competing cations, a variety of buffer salts and organic compounds, while at 359 nm the sensitivity of BCA is about 2.5 times greater than commonly used reagents for colorimetric determinations of copper (Brenner and Harris, 1995). A series of standard dilutions ranging from 200 to 300 $\mu\text{g/dL}$, and BsCotA concentration of 1 mg/mL was processed according to the copper assay kit protocol from Sigma-Aldrich, Germany. Calculation using higher standard in comparison to copper concentration of the sample was suggested by the manufacturer.

2.4 ABTS standard activity assay

BsCotA and BsCotA_Strep activity was quantified on the model substrate ABTS using a setup consisting of 20 μL enzyme ($\text{Vol}_{\text{Enzyme}}$), 150 μL buffer (not oxygen saturated), and 50 μL ABTS (10 mM). The components were consecutively added, and the reaction started by the addition of ABTS. The increase of absorbance of oxidized ABTS per min ($\Delta\text{Absorbance}$) at 420 nm in 96 well plate was photometrically measured at 405 nm at room temperature (Plate Reader Tecan Infinite M200 Pro, Switzerland). In order to achieve a linear increase of oxidized ABTS, CotA stock solution of 5 mg/mL (C_{Enzyme}) was diluted 400 times (df) before added to the setup, representing BsCotA concentration of 0.0189 μM in the end volume of 220 μL (Vol_{End}). Volumetric and specific activity were calculated with the following formula, via ϵ [molar extinction coefficient of ABTS in 100 mM buffer of choice ($\text{mM}^{-1}\text{cm}^{-1}$)] and d (pathlength in 96 well plate: 0.5785 cm):

$$\text{Volumetric activity } [\text{U mL}^{-1}] = \frac{\Delta\text{Absorbance} \cdot \text{Vol}_{\text{End}} \cdot \text{df}}{\epsilon \cdot d \cdot \text{Vol}_{\text{Enzyme}}}$$

$$\text{Specific activity } [\text{U mg}^{-1}] = \frac{\text{Volumetric activity}}{C_{\text{Enzyme}}}$$

Based on ABTS activity assay, different ABTS stock concentration of 0.5, 1, 5, 7.5, 10, 12.5, 15, and 20 mM were used and measured for 4 min. The calculated specific activity values from triplicates of each concentration at 37°C and the respective pH were plotted against its corresponding substrate concentration yielding the Michaelis-Menten saturation curve. Kinetic values were determined via non-linear least square fitting.

2.5 AFB1 degradation assays and enzyme kinetics

AFB1 concentration used for the determination of kinetic values of BsCotA_Strep ranged from 50 to 15,000 $\mu\text{g L}^{-1}$, with the higher levels set around the estimated solubility limits in water (PubChem). The reactions containing different BsCotA_Strep concentration (0.1, 0.02, 0.01 μM) were conducted in triplicates at 37°C in 100 mM sodium phosphate pH 7 with acetonitrile to a final concentration of 2%. After starting the reaction via enzyme addition, samples were drawn at 0, 1, 2, 3, 4, 5, 6, and 24 h. The reaction was stopped by addition of 99% ACN, diluting the sample to an ACN end concentration of 30%. Samples were further diluted with 30% ACN resulting in total sample dilution of 1:3. After centrifugation (14,000 rpm, 10 min), samples were stored at -20°C for following HPLC-FLD analysis. Kinetic parameters were generated from data obtained within the first 4 h for each data series and plotted against the measured starting concentration. These data were used to determine kinetic values using non-linear least square fitting and weighted least square regression models obtained in R (R Core Team, 2014) based on which specific activities were calculated.

Assays (total volume 1,280 μL) for the determination of the reaction products were conducted similarly at 37°C using 0.2 μM of BsCotA_Strep, and 5 $\mu\text{g/mL}$ of AFB1 (experiment in duplicate) and 1 $\mu\text{g/mL}$ AFQ1 in sodium phosphate buffer (100 mM, pH 7) as time course experiments with extended sampling (180 μL) intervals

(4, 24, 48, and 72 h). The acetonitrile concentration in the reaction was fixed to 1%. In parallel, controls including 5 ppm of AFB1 and 1 ppm of AFQ1 as well as the BsCotA_Strep itself without the presence of toxin were incubated under identical conditions. After enzyme inactivation resulting in 1:1.4 dilution to 30% ACN, samples were centrifuged (14 k rpm, 10 min) and stored at -20°C for HPLC-FLD and HPLC-MS analysis as described below. In a further experiment, 5 ppm of AFQ1 and epi-AFQ1 were treated with and without BsCotA_Strep and samples were collected as described above. After dilution to 30% ACN and centrifugation (14,000 rpm, 10 min) samples were stored at -20°C for HPLC-MS and HPLC-QTOF analysis.

The pH profile of BsCotA_Strep on AFB1 was determined at pH 3, 7, and 8. The assays were conducted in duplicates at 37°C using 0.01 μM BsCotA_Strep and 9 $\mu\text{g/mL}$ AFB1. Acetonitrile concentration was fixed to 1%. Samples were drawn at 0, 2, 4, 24, and 48 h. After enzyme, samples were centrifuged (14,000 rpm, 10 min) stored at -20°C for HPLC-FLD analysis.

2.6 HPLC-FLD analysis

AFB1 levels were routinely checked using a 1290 Infinity II HPLC system coupled to a FLD detector (Agilent, Waldbronn, Germany). Six microliter of the samples were injected and separated at 40°C on a Kinetex Biphenyl, 1.7 μm , 30 $\times$ 2.1 mm column (Phenomenex, Torrance, CA, USA; Part-Nr: 00A-4628-AN) coupled to a Kinetex UHPLC biphenyl 2.1 mm precolumn (Phenomenex, Torrance, CA, USA; Part-Nr: AJO-9209). The 3.5 min method (flow rate 1 mL/min) comprised a gradient of eluent A with 5% methanol, 95% water and 0.1% acetic acid and eluent B with 100% methanol and 0.1% acetic acid, starting from a 70% A:30% B ratio and ending with 25% A:75% B, followed by 40 seconds of column equilibration with the initial ratio at the end of the run. Excitation/emission wavelengths for detecting the analyte were 365 and 460 nm, respectively. Data was evaluated against AFB1 standard series in similar concentrations of solvent using the OpenLab CDS 2.4 software (Agilent, Waldbronn, Germany).

2.7 HPLC-MS analysis

Detection and semi-quantification of AFB1 and putative reaction products were performed on a QTrap 5500 MS/MS system (Applied Biosystems, Foster City, CA, USA) equipped with a TurboV electrospray ionization (ESI) source and a 1290 Infinity I HPLC series (Agilent, Waldbronn, Germany). Samples were thawed at room temperature and vortexed. Three microliter were injected and separated at 25°C on a X-Bridge C18-column (150 $\times$ 2.1 mm i.d., 3.5 μm particle size, Waters, Milford, MA, USA) coupled to a pre-column (C18, 4 $\times$ 3 mm i.d., Security Guard Cartridge, Phenomenex, Torrance, CA, USA; Part-Nr: AJO-4287). Eluents comprised water with 0.1% formic acid (FA, v/v) as eluent A and methanol (0.1% FA, v/v) as eluent B at a 250 $\mu\text{L/min}$ flow rate. The 45 min gradient used, started at 10% eluent B for 2 min, followed by linear increase to 100% within 30 min and hold for 5 min at 100% eluent B going back to 10% eluent B in 8 min. The

LC-stream was directed into the mass spectrometer between 2.0 and 33 min. Q1 scan data (scan rate 1,000 Da/s) were recorded in the positive and negative ionization mode in the mass range between 100 and 850 Da. ESI source (Turbo Spray) depending on the ionization mode were: source temperature 500°C, curtain gas (CUR) 40 psi, nebulizer gas (GS1) and heater gas (GS2) 50 psi, ion-spray voltage $\pm 4,500$ V, declustering potential (DP) ± 110 V, entrance potential (EP) ± 10 . Data capture and analysis was performed using Analyst 1.6.2 (Sciex, Foster City, CA, USA). A quantitation method (details not shown) extracting a defined mass range from the full scan data collected in positive and negative Q1 scan mode was used for semi-quantification of the samples and data evaluation was based on quadratic, $1/\times$ weighted, calibration curves. Solutions for calibration were in the range of 25–3,000 ppb for AFB1 and 25–1,000 ppb for AFQ1 in 30% acetonitrile. Assuming similar ionization behavior, epi-AFQ1 was quantified based on the AFQ1 calibration data. Signal matrix enhancement or suppression was not evaluated.

2.8 HPLC-QTOF measurements

For QTOF-MS/MS calibration purposes, reserpine (m/z 609, 28,066) included in the ESI Positive Calibration Solution for the SCIEX X500R System (SCIEX Framingham, MA) was used. Analysis was carried out on a 1290 Infinity II HPLC series coupled to a QTOF-MS system X500R, SCIEX (Framingham, MA) equipped with an electrospray ionization (ESI) turboVTM source operated in the positive and negative ESI mode. TOF-MS over an m/z range from 100 to 1,000 and TOF-MS/MS data were acquired. The sprayer probe includes an independent channel for the delivery of a calibration solution (reserpine), that allows to correct any drift in the mass accuracy of the mass analyzer. This calibration was run every five samples during the batch analysis. Source conditions were: ion spray voltage: 5,500 V; source temperature: 500°C; nitrogen gas flows (GS1 and GS2): 50 psi; and curtain gas: 35 psi, Ionspray voltage (V): 5,500, CAD gas: 7, Accumulation time (s): 0.15, Declustering potential (V): 80, Collision energy (V): 10. Chromatographic conditions (column, eluents, gradient etc.) remained as already described for the LC-MS analysis. However, here only 1 μ L of each sample was injected. For qualitative and quantitative data processing Sciex O.S. software V 1.5 (SCIEX) was used.

2.9 Mutagenicity tests

The genotoxic potential of AFB1 and AFQ1 was assessed using the SOS Chromotest (EPBI, Ontario, Canada). All required materials including lyophilized cells and S9 homogenate were provided by the manufacturer. The assay was conducted following the provided instructions. Briefly, serial dilution of all analytes was assessed in triplicates in a range from 100 μ g (10 ppm) to 625 ng. Ten microliter of the analyte in 50% acetonitrile and 100 μ L of the bacterial suspension and S9 mixture were incubated for 2 h at 37°C in 96-well microtiter plates (Greiner Bio-one, Austria). Fifty microliter were transferred to a new plate containing 50 μ L of the

substrates. The plates were incubated at 37°C and measurements were taken at 410 nm (X-Gal)/600 nm (pNPP) in regular intervals on a Synergy H1 plate reader (BioTek, VT, USA).

3 Results and discussion

3.1 Optimization of the BsCotA expression strategy

Laccase CotA from *B. subtilis* (BsCotA) is a structural component of the endospore coat of the Gram-positive bacterium and has only 65% identity and 78% homology to CotA from *Bacillus licheniformis*. In contrast to CotA from *B. licheniformis*, considerably less is known about recombinant production for decomposition of the mycotoxin AFB1 by BsCotA and about underlying mechanisms. Therefore, in this study, the expression of BsCotA was optimized before investigation of decomposition of AFB1, which is in contrast to a substantially different CotA laccase from *B. licheniformis*, which was applied as wild-type enzyme in the reaction. Laccases are copper-dependent enzymes and therefore, it is extremely important that the catalytic metal atom is properly incorporated into the enzyme. When choosing the expression conditions, care must therefore be taken to ensure that Cu^{2+} is sufficiently available in *E. coli*. However, also other expression parameters are important for production of a fully functional enzyme. During overexpression, the folding and solubility of the enzyme is significantly influenced by the translation rate, which in turn might depend on the codon usage of the coding gene in the expression host. In addition, short peptide tags can increase the solubility of proteins and provide additional purification benefits.

For investigation of the expression parameters, BsCotA was produced based on the naturally occurring gene on the one hand and, on the other hand, using a codon usage-adapted gene for expression in *E. coli*. Subsequently, a codon-optimized gene for the expression of BsCotA with a C-terminal StrepTag (BsCotA_Strep) was designed, which not only enabled rapid purification by affinity chromatography, but might also contribute to better solubility of the enzyme. Expression of BsCotA_Strep was performed with and without addition of 0.25 mM CuCl_2 . To further improve the Cu^{2+} availability in the *E. coli* cells, the oxygen supply during the enzyme induction was reduced since it is known that intracellular levels of the cytotoxic Cu^{2+} are maintained in *E. coli* at low levels by an elaborate efflux system, resulting in low holoprotein titers (Rensing and Grass, 2003). To increase the ratio of functional protein, the induced culture was maintained at reduced oxygen supply, which led to highly improved cytoplasmic copper levels (Durão et al., 2008). The influence of the expression conditions on the enzyme quality was assessed based on the kinetic parameters of the purified enzymes with ABTS at pH 7 and 37°C as these conditions would meet the application conditions in the food and feed industry (Table 1).

As seen in Table 1, the codon usage optimization for *E. coli* led to an almost doubling of V_{max} and k_{cat} in the case of the untagged BsCotA whereas K_m remained almost unchanged. The higher activity could be due to better folding as a consequence of improved translation through optimized codons. In the case of BsCotA_Strep, the influence of the tested parameters became even more visible.

TABLE 1 Kinetic parameters of BsCotA laccase variants on ABTS at pH 7 and 37°C.

Enzyme	Codon usage optimization	Aeration	Addition CuCl ₂	V _{max} [U/mg]	K _m [mM]	k _{cat} [s ⁻¹]
BsCotA	No	Microaerobic	Yes	8.84 ± 0.18	0.22 ± 0.01	9.58 ± 0.20
BsCotA	Yes	Microaerobic	Yes	15.21 ± 0.22	0.19 ± 0.01	16.48 ± 0.24
BsCotA_Strep	Yes	Microaerobic	Yes	33.56 ± 2.02	0.78 ± 0.03	36.36 ± 2.19
BsCotA_Strep	Yes	Microaerobic	No	1.38 ± 0.12	0.50 ± 0.10	1.49 ± 0.13
BsCotA_Strep	Yes	Aerobic	Yes	0.30 ± 0.02	0.53 ± 0.09	0.33 ± 0.02

All experiments were carried out in triplicates.

Compared to BsCotA_Strep from a fully aerated expression culture, the micro-aerobe culture with addition of CuCl₂ showed about 100x increased activity against the standard substrate ABTS and demonstrated the distinctive blue color indicating the inclusion of the T1 copper atom. Addition of CuCl₂ during expression further improved the kinetics due to higher incorporation of the metal atom into the laccase. The intramolecular copper levels were photometrically determined from a complex formed with BCA. The readings at both wavelengths, 359 and 562 nm, established a ratio of statistically 1.8 Cu atoms per BsCotA_Strep molecule. Adding additional CuCl₂ after enzyme purification did not significantly improve this ratio. The higher activity could be due to the gentler conditions during purification by affinity chromatography or to increased solubility due to the Strep tag. Remarkably, BsCotA_Strep yielded 26% more biomass in comparison to the expressed wild-type enzyme, which allowed production of 6.6 mg BsCotA_Strep/g biomass. Interestingly, fusion of the C-terminal StrepTag led to a shift in pH optima from 4 to 5 determined for the untagged BsCotA to a broadened optimal range of pH 5–7 (Figure 1). In comparison, Guo et al. (2020) determined the kinetic constants $K_m = 34.9 \mu\text{M}$ and $k_{cat} = 9.4 \text{ s}^{-1}$ for CotA from *B. licheniformis* using ABTS at optimal conditions which were pH 4.2 and 80°C. However, the authors fused the laccase with a HisTag that could have leached out the copper from the enzyme. Consequently, they had to use significantly more enzyme in the reactions with AFB1.

For degradation of AFB1, longer reaction times were expected. However, the activity of BsCotA_Strep against ABTS diminished to 25% of the initial velocity within 24 h. In contrast, almost all the activity was retained in similar assays including varying amounts of BSA added to the reaction buffer or storage buffer. To limit any effects of BSA on AFB1 due to competitive binding, BSA was only added to the BsCotA_Strep solution in concentrations of 2 mg/mL, yielding an effective BSA concentration in assay of $0.7 \mu\text{g} \cdot \text{mL}^{-1}$ (0.01 μM).

3.2 Degradation of AFB1 and characterization of reaction products

BsCotA_Strep was investigated for the ability to metabolize AFB1 without the presence of a mediator which would further add cost and complexity for industrial applications. The reaction kinetics was determined in three experiments with enzyme concentrations of 0.01, 0.02, and 0.1 μM using increasing

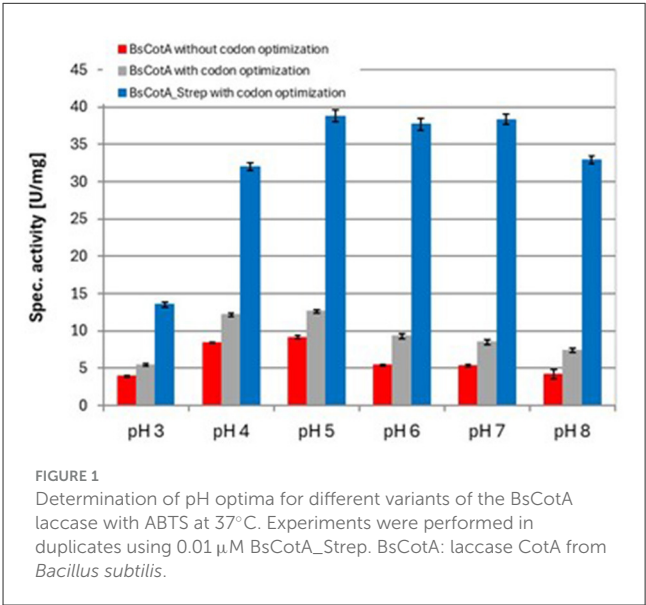
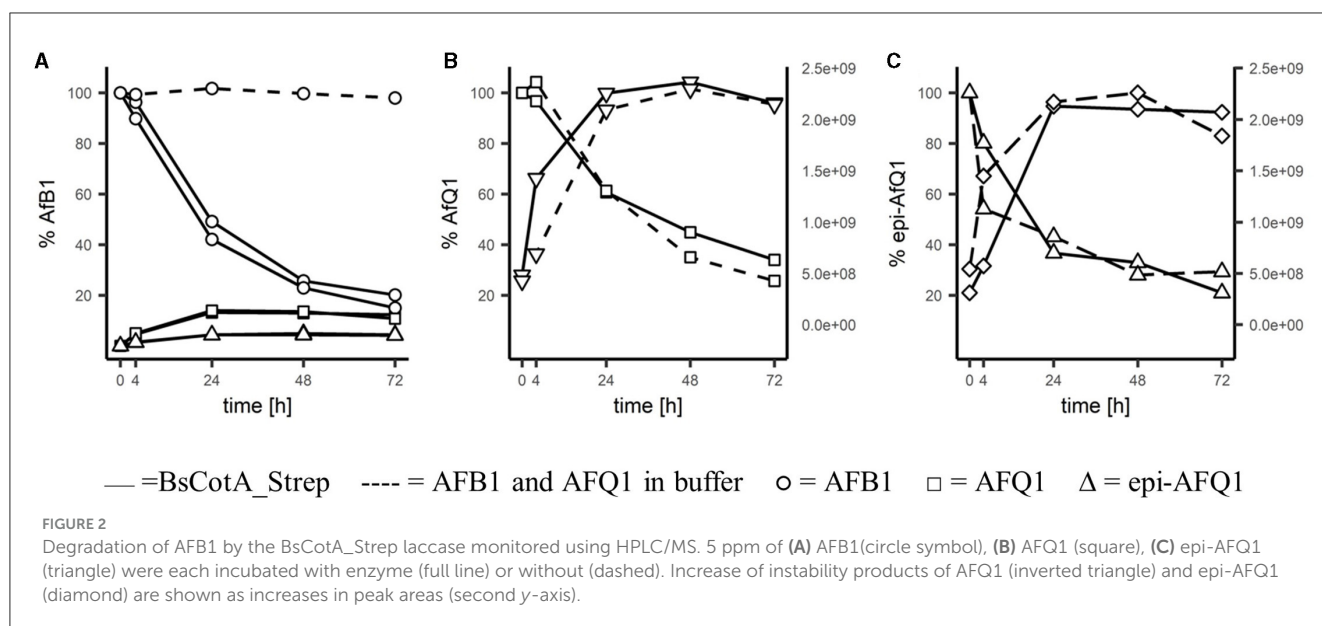


FIGURE 1 Determination of pH optima for different variants of the BsCotA laccase with ABTS at 37°C. Experiments were performed in duplicates using 0.01 μM BsCotA_Strep. BsCotA: laccase CotA from *Bacillus subtilis*.

concentrations of AFB1 up to the limit of reported solubility in water. Up to the highest concentration of $15,000 \mu\text{g L}^{-1}$ AFB1, the reaction velocity calculated from the first 4 h of incubation increased linearly, indicating that substrate saturation for AFB1 to reach V_{max} is above solubility in water. Instead, specific activities have been determined from data sets obtained with 0.1 and 0.02 μM BsCotA_Strep. Based on a weighted linear fit of the reaction velocity at different substrate levels, velocity did not scale relatively with increased enzyme concentrations. The specific activity at 500 $\mu\text{g/L}$ —a representative high contamination level in feed—with 0.2 μM BsCotA_Strep was determined at 10.75 $\mu\text{g/Lh}$ ($0.17 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$) and 20.11 $\mu\text{g/Lh}$ ($0.35 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$) with 0.1 μM BsCotA_Strep. Comparison with data from literature is very difficult since unequal concentrations of AFB1 and laccase have been used for the reactions and applied at various pH and temperature conditions. Sun et al. (2023), for instance, incubated 40 μg of *B. licheniformis* CotA in 0.5 ml reaction volume at pH 7.5 and 37°C with 2 $\mu\text{g/ml}$ AFB1 and detected 90% AFB1 decrease after 24 h of incubation which corresponds to a much lower turnover rate compared to BsCotA_Strep.

For identification of the reaction products, 0.2 μM BsCotA_Strep was incubated with 5,000 $\mu\text{g/L}^{-1}$ of AFB1 at $T = 37^\circ\text{C}$ in sodium phosphate buffer (100 mM, pH 7). LC-MS analysis and comparison with the commercially available standards



revealed a decrease of AFB1 and appearance of AFQ1 and epi-AFQ1 (Figure 2). The retention time was determined to be 17.5 min, 14.90 and 14.35 min for AFB1, AFQ1, and epi-AFQ1, respectively. Prolonged incubation led to losses in AFB1 recovery of up to 80% within 48 h at a starting concentration of $5,000 \mu\text{g L}^{-1}$ AFB1 and $0.2 \mu\text{M}$ BsCotA_Strep (Figure 2A). The AFB1 standard in phosphate buffer (100 mM, pH 7) without BsCotA_Strep did not reveal any significant changes in this time frame. The recovered concentrations were below the nominal AFB1 concentration ($4,200 \mu\text{g L}^{-1}$ instead of $5,000 \mu\text{g L}^{-1}$ at the starting time point), which may be owed to matrix effects that were not further evaluated.

HPLC-FLD analysis showed similar concentration ranges and relative increases/decreases in aflatoxin abundances (data not shown) and the relative changes in time of the analyte remained accurate regardless of the total recovered concentrations. AFQ1 was the predominant product with $365 \mu\text{g L}^{-1}$ formed after 72 h compared to $131 \mu\text{g L}^{-1}$ epi-AFQ1 at this timepoint. The recovery of the initial AFB1 concentration in form of the detected metabolites was low, but this could be at least partly explained e.g., by the instability and rapidly degradation of the detected metabolite itself.

AFQ1 standard in acetonitrile/water (20/80) solution was shown to be stable. However, AFQ1 and epi-AFQ1 incubated in phosphate buffer were shown to be unstable and only 29 and 26% of $5,000 \mu\text{g L}^{-1}$ of either remain after 72 h of incubation. With the decline of substance, the accumulation of single products of instability for AFQ1 and epi-AFQ1 at RT 10.24 and 9.9 min, respectively, were observed (Figures 2B, C). Addition of BsCotA_Strep to AFQ1 or epi-AFQ1 did not lead to markedly different degradation slopes or additional reaction products in the full scan mass spec data, thus we do not consider AFQ1 and epi-AFQ1 substrates of BsCotA_Strep.

Moreover, the full scan mass spectra of the AFB1 samples incubated with BsCotA_Strep revealed the formation of putative reaction products at RT 10.01, 10.23, 12.45, 14.35, 14.90, and 16.20 min (Figure 3), with eventually potential minor reaction

products remaining probably undetected. AFB1 appeared at a retention time of 17.50 min. The predominant formed products eluted at retention times 14.90 and 14.35 min and were by the comparison to the respective standards unambiguously assigned to AFQ1 and epi-AFQ1, respectively. The peak at 10.23 min was the respective product of instability AFQ1 in the buffer medium used for incubation, whereas the peak at 10.01 is the respective product of instability of epi-AFQ1.

Based on the collected LC-MS data—after background subtraction in the resulting full-scan mass spectrum of AFB1 in the pos. ionization mode—we could observe the formation of ions at m/z 313, 335, 642, and 647 which were assigned to $[M+H]^+$, $[M+Na]^+$, $[2M+NH_4]^+$, and $[2M+Na]^+$, respectively, while in the neg. ionization mode mainly formation of $[M-H]^-$ at m/z 311 could be observed. After background subtraction the full-scan mass spectrum in the pos. ionization mode of AFQ1 and epi-AFQ1 revealed identical ions with the following m/z : $[M+H]^+ = 329$, $[M+NH_4]^+ = 346$, $[M+Na]^+ = 351$, $[M+K]^+ = 367$, $[2M+NH_4]^+ = 674$, $[2M+Na]^+ = 679$. In the Q1 spectrum in the neg. ionization mode we observed the ions $[M-14]^- = 313$, $[M-H]^- = 327$, $[M+HCOOH-H]^- = 373$.

For both peaks at 10.23 min and 10.01 min the assignment of the most intense ions was not univocal. In order to clarify this observation and to also determine the sum formula of these products we conducted additional HR-MS measurements. We assigned as $[M-H-CO_2]^- = 301$ and $[M-H]^- = 345$ (negative ionization mode) and $[M+H]^+ = 329$, $[M+Na]^+ = 351$, $[M+K]^+ = 367$, $[2M+Na]^+ = 679$ (positive ionization mode). However, then the observed ions with m/z 347 and m/z 369 could not be assigned. Another assignment of the signals could be that $[M+H]^+ = 347$ and $[M+Na]^+ = 369$. Then by combination with the mass spectrum data obtained in the neg. ionization mode the peak at 10.23 min would have a MW of 346 g/mol.

The peak at 12.45 could be assigned to BsCotA_Strep itself while the origin of the peak at 17.50 remains elusive. It was assigned to $M = 316$ (observed mass signals in pos. ionization mode were

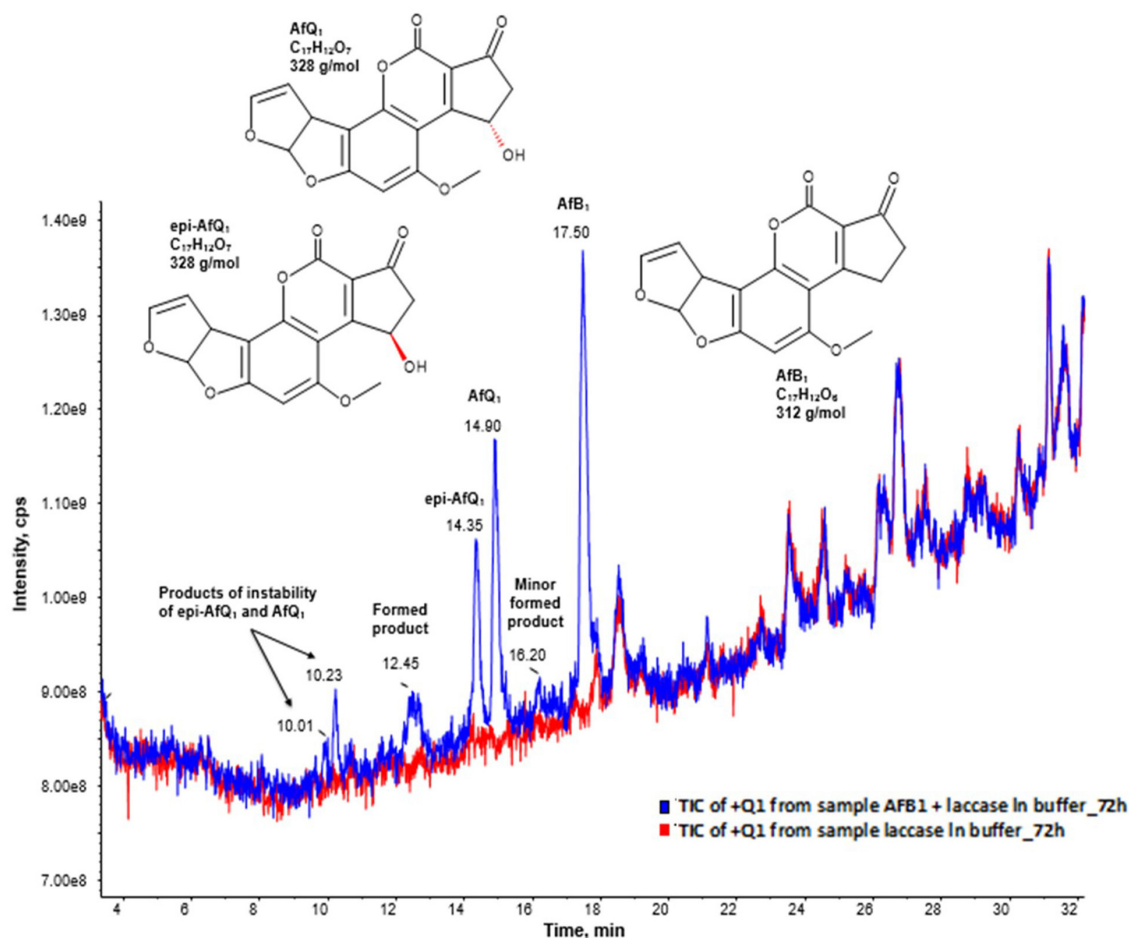


FIGURE 3

AFB1 degradation products after enzymatic treatment. Total ion chromatogram (TIC) of the Q1 scan in positive ionization mode after 48 (blue) hours of incubation with BsCotA_Strep. The blue curve displays the sample of AFB1 after enzymatic treatment and the red curve the sample of the laccase in buffer without AFB1 after 72 of incubation. The peaks 14.35, 14.90, and 17.50 have their origin to epi-AFQ1, AFQ1, and AFB1. The peaks at 10.23 min and 10.01 min have their origin to the instability of AFQ1 in the buffer used for incubation.

at 317 [M+H]⁺ and in neg. ionization mode at m/z 271.3 [M-CO₂-H]⁻ and 315.3 [M-H]⁻).

Molecular weights of +18 suggest the addition of water. Hydration of the vinyl ether in the terminal furan ring under strong acidic conditions are described for AFB1, AFQ1, and AFM1 forming the AFB2a, AFG2a, and AFM2a (Martínez-Ruiz et al., 2018; Fuentes D et al., 2018). Such a reaction forming the undescribed (epi)-AFQ2a should be possible for (epi)-AFQ1 as well. To test whether AFQ2a is identical with the instability product of AFQ2, we diluted 20 μ L of a 125 μ g L⁻¹ AFQ1 solution to 250 μ L with 2.5 N HCl and analyzed the solution after overnight incubation. Ion source fragments of AFQ2a captured at RT 10.01, did however not match fragmentation patterns observed for the instability product of AFQ1 with this retention time RT.

AFQ1 and epi-AFQ1 were also reported by Guo et al. (2020) as main degradation products of AFB1 after incubation with CotA, determined by LC-MS, NMR, and CD. However, they applied CotA from *B. licheniformis* ANSB821 in their study. Mutagenesis of the enzyme led to the identification of mutant Q441A, which had a 1.73-fold higher catalytic efficiency (k_{cat}/K_m)

than the wt enzyme (Liu et al., 2023). Similarly, CotA from *B. licheniformis* ZOM-1 was heterologously expressed in *Escherichia coli* (*E. coli*) and investigated for its activity on AFB1 (Sun et al., 2023). Here, the degradation products AFB1-diol, AFQ1 or epi-AFQ1 were identified by UPLC-HRMS/MS. However, none of these studies mentioned and discussed the instability of the degradation products.

3.3 Genotoxicity assessment of AFQ1

The genotoxicity assay relies on the quantitative de-repression of the bacterial SOS response to DNA damage. The test employs an engineered *E. coli* strain, which expressed lacZ—the structural gene for β -galactosidase—in fusion with the SOS-responsive *sfiA*, involved in regulating cell division. Thus, the genotoxicity of target compounds can be directly determined in a photometric assay measuring LacZ activity (substrate X-Gal). Endogenous phosphatase released from damaged cells is determined simultaneously with *para*-nitrophenylphosphat

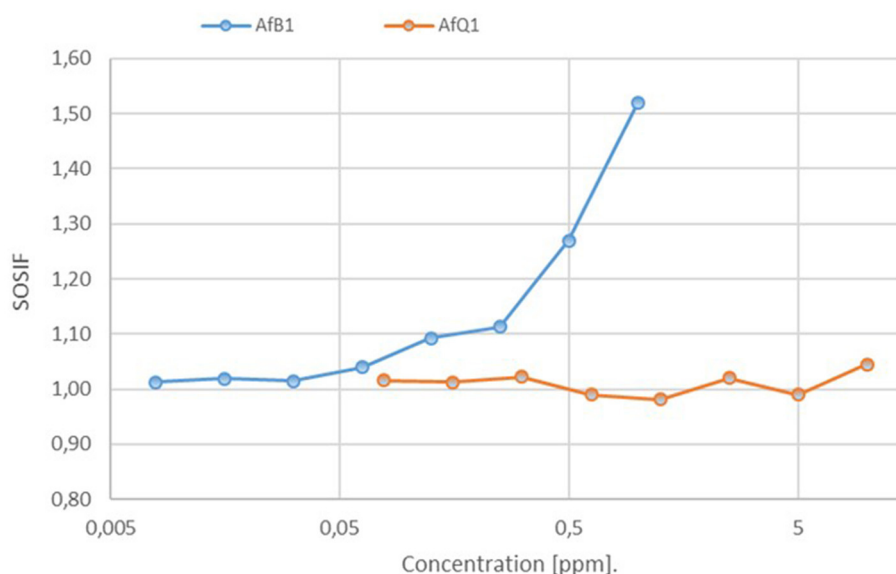


FIGURE 4

Assessment of the genotoxic potential of AFB1 and AFQ1 by the SOS Chromotest based on the measurement of the primary response of *E.coli* cells to genetic damage. Estimated SOSIF values for AFB1 against AFQ1 resulting after decomposition by laccase. SOSIF, SOS induction factor.

(pNPP) as substrate to assess additional cell toxicity. Compounds such as aflatoxins require metabolic activation by hepatic cytochrome P450 oxidoreductases. To this end S9 rat-liver homogenate including NADPH and a corresponding enzymatic regeneration system are added to the assay.

AFQ₁ is expected to be less or even non-toxic according to Hsieh et al. (1974), who have used the Ames test with a single indicator strain (*Salmonella typhimurium* TA1538). To our knowledge no experiments have been conducted to clearly explain the reduced toxicity of AFQ₁. The laccase facilitates the oxidation of the C-14 position. This additional hydroxyl moiety could reduce toxicity by either limiting the substrate affinity of liver monooxygenases that activate AFB1 by epoxidizing the 8,9-furan ring, and/or its increased hydrophilic properties and steric alterations reduce successful migration between DNA sheets.

To reassess AFQ₁ toxicity with the SOS Chromotest, we included a range of dilutions in acetonitrile up to 10 ppm and compared readings to controls including only the solvent at both wavelengths, yielding a “SOSIF” value corrected for background activity:

$$SOSIF = \frac{(OD_{600, i}) / (OD_{420, i})}{(OD_{600, negative}) / (OD_{420, negative})}$$

Similar measurement on dilutions were conducted with the positive control, 4-nitroquinoline 1-oxide (4-NQO, up to 10 ppm) and AFB₁ (up to 1 ppm) in three technical replicates. The recorded SOSIF values for the positive control 4-NQO (not shown) and AFB₁ show a clear dose-dependent response (Figure 4). In comparison for AFQ₁ no significant changes were observed between the individual concentrations up to 10 ppm. The observed values compare to a AFB₁ concentration of 0.125 ppm. We therefore conclude that 10 ppm AFQ₁ is at best at the

toxicity level of 0.125 ppm AFB₁. Its genotoxicity is at 80-times lower (10/0.125) when compared to the toxicity of AFB₁ according to the measurements.

The results are in accordance with Hsieh et al. (1974) and have been evaluated in the past also by other authors using various methods. Wang et al. (2019b) used growth of hydra in a culture containing AFB₁ treated or non-treated with one of the BsCotA/mediators and observed that the hydra collapsed after 18 h when incubated with non-treated AFB₁ whereas the hydra remained alive when they were incubated with AFB₁ treated with BsCotA in the presence of methyl syringate. Guo et al. (2020) treated human liver cells (L-02) with AFQ₁ and epi-AFQ₁ and observed that the viability of the cells decreased with increasing concentrations of AFB₁ from 0 to 100 μM after incubation for 48 h whereas the cells were not reduced after exposure to AFQ₁ or epi-AFQ₁. According to further studies, AFQ₁ is approximately eighteen times less toxic and approximately eighty-three times less mutagenic than AFB₁ (Popescu et al., 2022).

4 Conclusion

The contamination of feed and food with mycotoxins is a major health problem for humans and animals, which is predicted to worsen due to climate change (Leggieri et al., 2021). In addition to the high health risk, contamination with mycotoxins also causes considerable economic losses for the food and animal feed industry. The detoxification of mycotoxins has therefore become a major problem that could be reduced by biological methods such as enzymes, as they are highly specific and can work under environmentally friendly conditions. Oxidative enzymes such as laccases are designed from nature to transform highly complex molecules due to their high oxidative potential and have also shown

activity on aflatoxin B1. Laccases use molecular oxygen as a co-substrate and produce only water as a by-product, which makes their application environmentally friendly. In addition, they can be produced in large quantities and at low cost. Laccases are therefore already used in various processes in the food industry, for example, to stabilize wine and beer, or to give doughs in the baking industry the necessary strength and stability. In this study, we examined the activity of the laccase CotA from *Bacillus subtilis* on aflatoxin B1 and elucidated for the first time the reaction products of this laccase, which has only 65% identity to CotA from *B. licheniformis*, using MS analysis. For this purpose, BsCotA was optimized with regard to the maximum incorporation of the catalytically active copper atoms leading to substantially more active enzyme. The optimized BsCotA was incubated with AFB1 and the reaction products AFQ1 and epi-AFQ1 were identified by MS. The fact that the conversion to the less toxic metabolites takes place without the addition of mediators makes the use of laccase in food and feed particularly attractive. The recovery of the initial AFB1 concentration in the form of detected metabolites was investigated in more detail as well as instability of the metabolites was demonstrated, which were no substrates for BsCotA. Unambiguous identification of the reaction products is an important topic as themselves may be equally or even more toxic than the parent toxin. Therefore, both metabolites were examined in a genotoxicity test and an 80-fold lower toxicity was found compared to AFB1. Due to the conversion of AFB1, the laccase could have the potential to convert other toxic secondary metabolites and thus represent a broader approach for the biological detoxification of mycotoxins in the future. In further studies, however, the specificity and turnover rates of the laccase should be improved in order to successfully degrade the strongly matrix-associated aflatoxins at very low concentrations.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

References

- Aguilar, F., Hussain, S. P., and Cerutti, P. (1993). Aflatoxin B1 induces the transversion of G → T in codon 249 of the P53 tumor suppressor gene in human hepatocytes. *Proc. Natl. Acad. Sci. U. S. A.* 90, 8586–8590. doi: 10.1073/pnas.90.18.8586
- Alberts, J. F., Gelderblom, W. C. A., Botha, A., and van Zyl, W. H. (2009). Degradation of aflatoxin B1 by fungal laccase enzymes. *Int. J. Food Microbiol.* 135, 47–52. doi: 10.1016/j.ijfoodmicro.2009.07.022
- Al-Rajhi, A. M. H., Ganash, M., Alshammari, A. N., Alsalamah, S. A., and Abdelghany, T. M. (2024). *In vitro* and molecular docking evaluation of target proteins of lipase and protease for the degradation of aflatoxins. *BioResources* 19, 2701–2713. doi: 10.15376/biores.19.2.2701-2713
- Arimboor, R. (2024). Metabolites and degradation pathways of microbial detoxification of aflatoxins: a review. *Mycotoxin Res.* 40, 71–83. doi: 10.1007/s12550-023-00515-0
- Bashiri, G. (2022). Cofactor F420, an emerging redox power in biosynthesis of secondary metabolites. *Biochem. Soc. Trans.* 50, 253–267. doi: 10.1042/BST20211286
- Brenner, A. J., and Harris, E. D. (1995). A quantitative test for copper using bicinchoninic acid. *Anal. Biochem.* 226, 80–84. doi: 10.1006/abio.1995.1194
- Ciegler, A., Lillehoj, E. B., Peterson, R. E., and Hall, H. H. (1966). Microbial detoxification of aflatoxin. *Appl. Environ. Microbiol.* 14, 934–939. doi: 10.1128/am.14.6.934-939.1966
- Croy, R. G., Essigmann, J. M., Reinhold, V. N., and Wogan, G. N. (1978). Identification of the principal aflatoxin B1-DNA adduct formed *in vivo* in rat liver. *Proc. Natl. Acad. Sci. U. S. A.* 75, 1745–1749. doi: 10.1073/pnas.75.4.1745
- Durão, P., Chen, Z., Fernandes, A. T., Hildebrandt, P., Murgida, D. H., Todorovic, S., et al. (2008). Copper incorporation into recombinant CotA laccase from *Bacillus subtilis*: characterization of fully copper loaded enzymes. *J. Biol. Inorg. Chem.* 13, 183–193. doi: 10.1007/s00775-007-0312-0
- Essigmann, J. M., Croy, R. G., Nadzan, A. M., Busby, W. F., Reinhold, V. N., Büchi, G., et al. (1977). Structural identification of the major DNA adduct formed by aflatoxin B1 *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* 74, 1870–1874. doi: 10.1073/pnas.74.5.1870

Author contributions

RS: Data curation, Methodology, Validation, Writing – original draft. WS: Conceptualization, Methodology, Project administration, Supervision, Writing – original draft. EK-V: Conceptualization, Data curation, Methodology, Validation, Writing – original draft. DW: Data curation, Methodology, Writing – review & editing. GS: Conceptualization, Resources, Writing – review & editing. DR: Conceptualization, Methodology, Supervision, Writing – original draft. GG: Conceptualization, Resources, Writing – review & editing.

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Conflict of interest

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- EUR-Lex - 02006R1881-20140701 - EN - EUR-Lex (2023). Commission Regulation (EU) 2023/915 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. *Off. J. Eur. Union L* 119, 103–157.
- Fouché, T., Claassens, S., and Maboeta, M. (2020). Aflatoxins in the soil ecosystem: an overview of its occurrence, fate, effects and future perspectives. *Mycotoxin Res.* 36, 303–309. doi: 10.1007/s12550-020-00393-w
- Fuentes D, S., Carvajal, M., Ruiz, S., Martínez, N. C., Azucena Gomez, A., and Rojo, F. (2018). Presence of mutagens and carcinogens, called aflatoxins, and their hydroxylated metabolites in industrialized food for dogs. *J. Microb. Biochem. Technol.* 10:1000399. doi: 10.4172/1948-5948.1000399
- Giardina, P., Faraco, V., Pezzella, C., Piscitelli, A., Vanhulle, S., and Sannia, G. (2010). Laccases: a never-ending story. *Cell. Mol. Life Sci.* 67, 369–385. doi: 10.1007/s00018-009-0169-1
- Gu, R., Yang, X., Cheng, L., Zhang, Q., Li, P., and Mao, J. (2024). Screening, identification, and application of *Bacillus velezensis* NWPZ-8 probiotics to aflatoxin detoxification in peanut meal. *ACS Food Sci. Technol.* 4, 2155–2166. doi: 10.1021/acsfds.3c00371
- Guo, Y., Qin, X., Tang, Y., Ma, Q., Zhang, J., and Zhao, L. (2020). Cota laccase, a novel aflatoxin oxidase from *Bacillus licheniformis*, transforms aflatoxin B1 to aflatoxin Q1 and epi-aflatoxin Q1. *Food Chem.* 325:126877. doi: 10.1016/j.foodchem.2020.126877
- Hsieh, D. P. H., Salhab, A. S., Wong, J. J., and Yang, S. L. (1974). Toxicity of aflatoxin Q1 as evaluated with the chicken embryo and bacterial auxotrophs. *Toxicol. Appl. Pharmacol.* 30, 237–242. doi: 10.1016/0041-008X(74)90095-7
- Iyer, R. S., Coles, B. F., Raney, K. D., Thier, R., Peter Guengerich, F., and Harris, T. M. (1994). DNA adduction by the potent carcinogen aflatoxin B1: mechanistic studies. *J. Am. Chem. Soc.* 116, 1603–1609. doi: 10.1021/ja00084a001
- Klvana, M., and Bren, U. (2019). Aflatoxin B1-formamidopyrimidine DNA adducts: relationships between structures, free energies, and melting temperatures. *Molecules* 24:150. doi: 10.3390/molecules24010150
- Kunz-Vekiru, E., Subagia, R., Wolfsberger, D., Schatzmayr, G., Ribitsch, D., Guebitz, G., et al. (2019). "Mycotoxins and phycotoxins conference in GRC," in *The B. Subtilis Cota Laccase Metabolizes Aflatoxin B1 into Aflatoxin Q1 and Epi-Aflatoxin Q1*. (Stonehill College Massachusetts).
- Leggieri, M. C., Toscano, P., and Battilani, P. (2021). Predicted aflatoxin B1 increase in Europe due to climate change: actions and reactions at global level. *Toxins* 13:292. doi: 10.3390/toxins13040292
- Li, J., Huang, J., Jin, Y., Wu, C., Shen, D., Zhang, S., et al. (2018). Aflatoxin B1 degradation by salt tolerant tetragenococcus halophilus CGMCC 3792. *Food Chem. Toxicol.* 121, 430–436. doi: 10.1016/j.fct.2018.08.063
- Liu, Y., Guo, Y., Liu, L., Tang, Y., Wang, Y., Ma, Q., et al. (2023). Improvement of aflatoxin B1 degradation ability by *Bacillus licheniformis* Cota-laccase Q441A mutant. *Heliyon* 9:e22388. doi: 10.1016/j.heliyon.2023.e22388
- Liu, Y., Mao, H., Hu, C., Tron, T., Lin, J., Wang, J., et al. (2020). Molecular docking studies and in vitro degradation of four aflatoxins (AFB1, AFB2, AFG1, and AFG2) by a recombinant laccase from *Saccharomyces cerevisiae*. *J. Food Sci.* 85, 1353–1360. doi: 10.1111/1750-3841.15106
- Loi, M., Fanelli, F., Zucca, P., Liuzzi, V. C., Quintieri, L., Cimmarusti, M. T., et al. (2016). Aflatoxin B1 and M1 degradation by Lac2 from pleurotus pulmonarius and redox mediators. *Toxins* 8:245. doi: 10.3390/toxins8090245
- Loi, M., Renaud, J. B., Rosini, E., Pollegioni, L., Vignali, E., Haidukowski, M., et al. (2020). Enzymatic transformation of aflatoxin B1 by Rh_DypB peroxidase and characterization of the reaction products. *Chemosphere* 250:126296. doi: 10.1016/j.chemosphere.2020.126296
- Mariën, K., Moyer, R., Loveland, P., Van Holde, K., and Bailey, G. (1987). Comparative binding and sequence interaction specificities of aflatoxin B1, aflatoxicol, aflatoxin M1, and aflatoxicol M1 with purified DNA. *J. Biol. Chem.* 262, 7455–7462. doi: 10.1016/S0021-9258(18)47588-6
- Martínez-Ruiz, N. C., Carvajal-Moreno, M., Rojo-Callejas, F., Fuentes-Daza, S., Gómez-Carrión, A., and Ruiz-Velasco, S. (2018). Mutagens and carcinogens called aflatoxins and their hydroxylated metabolites in food for domestic cats. *Biochem. Anal. Biochem.* 7:366. doi: 10.4172/2161-1009.1000366
- Martins, L. O., Durão, P., Brissos, V., and Lindley, P. F. (2015). Laccases of prokaryotic origin: enzymes at the interface of protein science and protein technology. *Cell. Mol. Life Sci.* 72, 911–922. doi: 10.1007/s00018-014-1822-x
- Mishra, H. N., and Das, C. (2003). A review on biological control and metabolism of aflatoxin. *Crit. Rev. Food Sci. Nutr.* 43, 245–264. doi: 10.1080/1040869030826518
- Munk, L., Andersen, M. L., and Meyer, A. S. (2018). Influence of mediators on laccase catalyzed radical formation in lignin. *Enzyme Microb. Technol.* 116, 48–56. doi: 10.1016/j.enzmictec.2018.05.009
- Mwakinyali, S. E., Ming, Z., Xie, H., Zhang, Q., and Li, P. (2019). Investigation and characterization of myroides odoratimimus strain 3J2MO aflatoxin B1 degradation. *J. Agric. Food Chem.* 67, 4595–4602. doi: 10.1021/acs.jafc.8b06810
- Neto, B. A. D., and Lapis, A. A. M. (2009). Recent developments in the chemistry of deoxyribonucleic acid (DNA) intercalators: principles, design, synthesis, applications and trends. *Molecules* 14, 1725–1746. doi: 10.3390/molecules14051725
- Pardo, I., and Camarero, S. (2015). Laccase engineering by rational and evolutionary design. *Cell. Mol. Life Sci.* 72, 897–910. doi: 10.1007/s00018-014-1824-8
- Popescu, R. G., Rădulescu, A. L., Georgescu, S. E., and Dinischiotu, A. (2022). Aflatoxins in feed: types, metabolism, health consequences in swine and mitigation strategies. *Toxins* 14:853. doi: 10.3390/toxins14120853
- R Core Team (2014). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available at: <http://www.R-project.org/>
- Rensing, C., and Grass, G. (2003). *Escherichia coli* mechanisms of copper homeostasis in a changing environment. *FEMS Microbiol. Rev.* 27, 197–213. doi: 10.1016/S0168-6445(03)00049-4
- Rushing, B. R., and Selim, M. I. (2019). Aflatoxin B1: a review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. *Food Chem. Toxicol.* 124, 81–100. doi: 10.1016/j.fct.2018.11.047
- Shimada, T., and Guengerich, F. P. (1989). Evidence for cytochrome P-450(NF), the nifedipine oxidase, being the principal enzyme involved in the bioactivation of aflatoxins in human liver. *Proc. Natl. Acad. Sci. U. S. A.* 86, 462–465. doi: 10.1073/pnas.86.2.462
- Stone, M. P., Gopalakrishnan, S., Harris, T. M., and Graves, D. E. (1988). Carcinogen-nucleic acid interactions: equilibrium binding studies of aflatoxins B1 and B2. *J. Biomol. Struct. Dyn.* 5, 1025–1041. doi: 10.1080/07391102.1988.10506447
- Sun, F., Yu, D., Zhou, H., Lin, H., Yan, Z., and Wu, A. (2023). Cota laccase from *Bacillus licheniformis* ZOM-1 effectively degrades zearalenone, aflatoxin B1 and alternariol. *Food Control* 145:109472. doi: 10.1016/j.foodcont.2022.109472
- Tadesse, M. A., D'Annibale, A., Galli, C., Gentili, P., and Sergi, F. (2008). An assessment of the relative contributions of redox and steric issues to laccase specificity towards putative substrates. *Org. Biomol. Chem.* 6, 868–878. doi: 10.1039/b716002j
- Taheur, F. B., Mansour, C., Jeddou, K. B., Machreki, Y., Kouidhi, B., Abdulkhakim, J. A., et al. (2020). Aflatoxin B1 degradation by microorganisms isolated from kombucha culture. *Toxicon* 179, 76–83. doi: 10.1016/j.toxicon.2020.03.004
- Tang, Y., Liu, X., Tang, L., and Dong, J. (2024). Investigating the mechanism of *Bacillus amyloliquefaciens* YUAD7 degrading aflatoxin B1 in alfalfa silage using isotope tracing and nuclear magnetic resonance methods. *Chem. Biol. Technol. Agric.* 11:102. doi: 10.1186/s40538-024-00619-4
- Taylor, M. C., Jackson, C. J., Tattersall, D. B., French, N., Peat, T. S., Newman, J., et al. (2010). Identification and characterization of two families of F420H2-dependent reductases from mycobacteria that catalyze aflatoxin degradation. *Mol. Microbiol.* 78, 561–575. doi: 10.1111/j.1365-2958.2010.07356.x
- Teniola, O. D., Addo, P. A., Brost, I. M., Färber, P., Jany, K. D., Alberts, J. F., et al. (2005). Degradation of aflatoxin B1 by cell-free extracts of rhodococcus erythropolis and *Mycobacterium fluoranthenorans* Sp. Nov. DSM44556T. *Int. J. Food Microbiol.* 105, 111–117. doi: 10.1016/j.ijfoodmicro.2005.05.004
- Vanhoutte, L., Audenaert, K., and De Gelder, L. (2016). Biodegradation of mycotoxins: tales from known and unexplored worlds. *Front. Microbiol.* 7:561. doi: 10.3389/fmicb.2016.00561
- Vekiru, E., Fruhauf, S., Rodrigues, I., Ottner, F., Krska, R., Schatzmayr, G., et al. (2015). In vitro binding assessment and in vivo efficacy of several adsorbents against aflatoxin B1. *World Mycot. J.* 8, 477–488. doi: 10.3920/WMJ2014.1800
- Wang, J., Ogata, M., Hirai, H., and Kawagishi, H. (2011). Detoxification of aflatoxin B1 by manganese peroxidase from the white-rot fungus *phanerochaete sordida* YK-624. *FEMS Microbiol. Lett.* 314, 164–169. doi: 10.1111/j.1574-6968.2010.02158.x
- Wang, X., Bai, Y., Huang, H., Tu, T., Wang, Y., Wang, Y., et al. (2019a). Degradation of aflatoxin B1 and zearalenone by bacterial and fungal laccases in presence of structurally defined chemicals and complex natural mediators. *Toxins* 11:609. doi: 10.3390/toxins11100609
- Wang, X., Cui, L., Liu, M., Qi, Z., Luo, H., Huang, H., et al. (2024). Theoretical insights into the mechanism underlying aflatoxin B1 transformation by the BsCotA-methyl syringate system. *Ecotoxicol. Environ. Saf.* 272:116049. doi: 10.1016/j.ecoenv.2024.116049
- Wang, X., Qin, X., Hao, Z., Luo, H., Yao, B., and Su, X. (2019b). Degradation of four major mycotoxins by eight manganese peroxidases in presence of a dicarboxylic acid. *Toxins* 11:566. doi: 10.3390/toxins11100566
- Wu, J., Wang, Z., An, W., Gao, B., Li, C., Han, B., et al. (2024). *Bacillus subtilis* simultaneously detoxified aflatoxin B1 and zearalenone. *Appl. Sci.* 14:1589. doi: 10.3390/app14041589
- Xu, F., Berka, R. M., Wahleithner, J. A., Nelson, B. A., Shuster, J. R., Brown, S. H., et al. (1998). Site-directed mutations in fungal laccase: effect on redox potential, activity and pH profile. *Biochem. J.* 334, 63–70. doi: 10.1042/bj3340063



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Application of cold atmospheric plasma for decontamination of toxigenic fungi and mycotoxins: a systematic review

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Introduction: Microbial contamination remains a vital challenge across the food production chain, particularly due to mycotoxins—secondary metabolites produced by several genera of fungi such as *Aspergillus*, *Fusarium*, *Alternaria*, and *Penicillium*. These toxins, including aflatoxins, fumonisins, ochratoxins, and trichothecenes (nivalenol, deoxynivalenol, T2, HT-2). These contaminants pose severe risks to human and animal health, with their potential to produce a variety of different toxic effects. Notably, up to 50% of global cereal production is affected by mycotoxin contamination, leading to significant economic losses. Current research focuses on innovative technologies to mitigate mycotoxins, with cold atmospheric pressure plasma emerging as a promising decontamination method.

Method: This systematic review aimed at describing recent advances in the application of cold atmospheric plasma for the decontamination of toxigenic fungi and mycotoxins.

Results and discussion: Cold atmospheric plasma offers a sustainable and cost effective solution to preserve food quality while inactivating toxigenic fungi and degrading mycotoxins. Through the generation of reactive oxygen and nitrogen species, cold plasma disrupts fungal cell integrity, hinders spore germination, and inhibits toxin biosynthesis. Additionally, cold atmospheric plasma-driven degradation of mycotoxins involves structural modifications, breaking key molecular bonds that reduce toxicity. The effectiveness of cold plasma depends on operational parameters and the specific characteristics of the treated food, with notable efficacy in degrading aflatoxin B₁ and deoxynivalenol by converting them into less toxic substances and inhibiting their spores and DNA responsible for their biosynthesis. While the data demonstrates that cold atmospheric plasma has minimal impact on food composition, further research is needed to fully assess the nature of the degradation products of mycotoxins, its influence on food quality attributes and to optimize application strategies for different products.

KEYWORDS

CAP technology, mycotoxins, contamination, foodstuffs, detoxificationprotect

1 Introduction

Microbial contamination is a major concern throughout the food supply chain, posing significant challenges to food production (Khaneghah et al., 2019). Among microbial contaminants, filamentous fungi are found on various food and feed products and are capable of producing toxic secondary metabolites known as mycotoxins, which present a significant threat to human health. The most common mycotoxigenic fungi include genera such as *Aspergillus*, *Fusarium*, *Alternaria*, and *Penicillium* (Khaneghah et al., 2019; Nesic et al., 2021).

Mycotoxin contamination remains a persistent challenge in agriculture, especially in cereal grains, and can occur during pre- and post-harvest stages, as well as during processing, packaging, and storage of products (Ali et al., 2023a). The most common mycotoxins found in food and feed products include aflatoxins (AFs), fumonisins (FBs), ochratoxins (OTs), zearalenone (ZEN), patulin (PAT), and trichothecenes such as nivalenol, deoxynivalenol (DON), HT-2 and T-2 toxins (Nesic et al., 2021). *Fusarium* mycotoxins, such as DON, FBs, and ZEN, are known to contaminate grains in the field during the pre-harvest stage of crop cultivation. In contrast, AFs produced by *Aspergillus* and OTs produced by *Penicillium* may appear later due to improper post-harvest practices, including inadequate storage and transport (Neme and Mohammed, 2017). The growth of mycotoxigenic fungi and the production of mycotoxins are influenced by increased temperature and moisture. As a result, higher levels of mycotoxin contamination are strongly correlated with global climate change, which also impacts the global economy. Food transported over long distances is exposed to varying local climates, extended transport, and prolonged storage times, all of which contribute to increased contamination risks (Moretti et al., 2019). It is estimated that 25–50% of global cereal production is affected by significant mycotoxin levels, with 5–10% reaching concentrations considered irremediable (Haque et al., 2020; Abrunhosa et al., 2016). Such contamination not only compromises product quality but also poses serious safety risks and is therefore subject to strict regulatory standards (Khaneghah et al., 2019). Recently, there has been increased focus on so-called emerging mycotoxins, which are either masked or modified forms of already identified types or have been newly discovered. These toxic compounds are not yet regulated but, like other mycotoxins, can occur frequently and at high concentrations in food and feed. Emerging mycotoxins include *Fusarium* metabolites such as enniatins (ENNs), fusaproliferin (FP), and beauvericin (BEA), as well as *Alternaria* spp. mycotoxins and ergot alkaloids produced by *Claviceps* spp (Gruber-Dorninger et al., 2017).

When mycotoxin levels exceed the recommended limits (EFSA, 2004; EU, 2023), they can cause acute or chronic toxic effects, with carcinogenicity being one of the most concerning outcomes (Richard, 2007). From a risk perspective, several mycotoxins have been classified based on their carcinogenic potential by the International Agency for Research on Cancer (IARC, 2023). Aflatoxin B₁ (AFB₁) is categorized as Group 1 (carcinogenic to humans), fumonisin B₁ (FB₁) and ochratoxin A (OTA) as Group 2B (possibly carcinogenic to humans), while ZEN and DON are placed in Group 3 (not classifiable as to their carcinogenicity to humans) (IARC, 2023; Schrenk et al., 2020; Ostry et al., 2017;

EFSA, 2004). In addition to genotoxicity and carcinogenicity, mycotoxins are known to cause a range of other toxic effects. Human exposure, particularly in young children, is especially concerning due to their immature detoxification systems and increased vulnerability to these toxins (Knutsen et al., 2017; Fakhri et al., 2019). Exposure to FB₁ has been linked to neural tube defects and cancer (Alvito and Pereira-da-Silva, 2022; Schrenk et al., 2022), while ZEN is known to cause estrogenic disturbances and degenerative syndromes, affecting both growth and reproductive health in humans and animals (Borutova et al., 2012; Schoevers et al., 2012). The T-2 toxin can inhibit protein, RNA, and DNA synthesis, leading to apoptosis, necrosis, lipid peroxidation, and adverse hematopoietic effects (Arcella et al., 2017). DON disrupts eukaryotic cells and mitochondria, with chronic exposure linked to intestinal microbiota imbalance and health complications (Knutsen et al., 2017; Ali et al., 2024; Arce-López et al., 2021; Akbari et al., 2017). OTA is recognized for its nephrotoxic, neurotoxic, teratogenic, and carcinogenic properties (Clark and Snedeker, 2006; Majeed et al., 2017).

Preventive measures including good agriculture practices, appropriate grain handling after harvest and proper storage are key factors to avoid fungal development and mycotoxin production (Haque et al., 2020). However, these practices may not be effective once mycotoxins have formed. Therefore, efforts have been directed toward developing effective strategies to alleviate or eliminate toxins and their metabolites from food materials (Neuenfeldt et al., 2023). Effective decontamination approaches must minimize both toxigenic fungi and mycotoxins levels, without compromising food quality and safety. So far, various methods—including chemical, biological, physical—have been explored in the food industry for mycotoxin detoxification (Marshall et al., 2020; Basso et al., 2023; Ali et al., 2023b; Naeem et al., 2024). Chemical treatments, such as ammonization, ozone, hydrogen peroxide, and sodium bisulfite among others, have been widely applied to detoxify mycotoxins in various food matrices. However, these methods may reduce nutritional quality, leave harmful residues, or only partially remove toxins, raising concerns over safety and consumer acceptance (Nahle et al., 2022; Mir et al., 2021; Khan, 2024). Biological methods involving microorganisms or enzymes to adsorb or degrade toxins offer safer alternatives but can face challenges with production consistency (Loi et al., 2023; Mir et al., 2021). Physical methods, such as thermal treatments, can effectively reduce microbial loads and toxins but may alter sensory and nutritional properties, or achieve uneven heating in grains (Naeem et al., 2024; Anderson, 2019). In contrast, innovative approaches to mycotoxin detoxification such as non-thermal technologies are anticipated to have a pivotal role in the food supply chain, ensuring the preservation of food quality while minimizing environmental impacts and maintaining economic viability (Hojnik et al., 2020). Recently, research has increasingly focused on cold atmospheric pressure plasma (CAP) as a promising approach for mycotoxin decontamination of food, aligning with these critical requirements (Patriarca and Pinto, 2017; Gavahian et al., 2021). CAP has demonstrated effectiveness in reducing microbial loads and detoxifying mycotoxins across diverse food types, especially in low-moisture products like cereal grains (Sun et al., 2014; Mir et al., 2021; Naeem et al., 2024). Although some studies have outlined CAP applications for fungi and mycotoxin decontamination (Gavahian and Cullen, 2019; Neuenfeldt et al.,

2023; Misra et al., 2018; Hojnik et al., 2017), there is no available systematic reviews on this subject. Therefore, the objective of the present study was to systematically review recent literature on the application of CAP for the decontamination of commonly occurring mycotoxins in foodstuffs, discussing its potential and challenges for broader adoption in the food industry.

2 Search strategy

A literature search was conducted according to Cochrane protocols and the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines (Shamseer et al., 2015) across PubMed, ScienceDirect, Web of Science, Scopus and Google Scholar to identify relevant studies on the use of CAP for decontamination of main mycotoxins found in food products published in the past 10 years. The following key terms were employed: “Mycotoxins” OR “Aflatoxins” OR “Fumonisin” OR “Ochratoxin A” OR “Zearalenone” OR “Patulin” OR “Trichothecenes” OR “Deoxynivalenol” OR “T-2 Toxin” OR “HT-2 Toxin” OR “Atmospheric pressure plasma technology” OR “Cold atmospheric plasma” OR “Decontamination” OR “Detoxification” OR “Degradation.”

To optimize search precision and account for database-specific limitations, the terms were grouped into five search combinations:

1. “Cold atmospheric plasma” AND “Mycotoxins” AND “Aflatoxins” OR “Deoxynivalenol” AND “Degradation.”
2. “Atmospheric pressure plasma technology” AND “Mycotoxins” OR “Fumonisin” OR “Patulin” AND “Decontamination.”
3. “Cold atmospheric plasma” AND “Ochratoxin A” OR “Zearalenone” AND “Degradation” OR “Detoxification.”
4. “Atmospheric pressure plasma technology” AND “Mycotoxins” OR “Trichothecenes” OR “Toxin HT-2” AND “Detoxification.”
5. “Cold atmospheric plasma” AND “Mycotoxins” OR “Toxin T-2” OR “Trichothecenes” AND “Decontamination.”

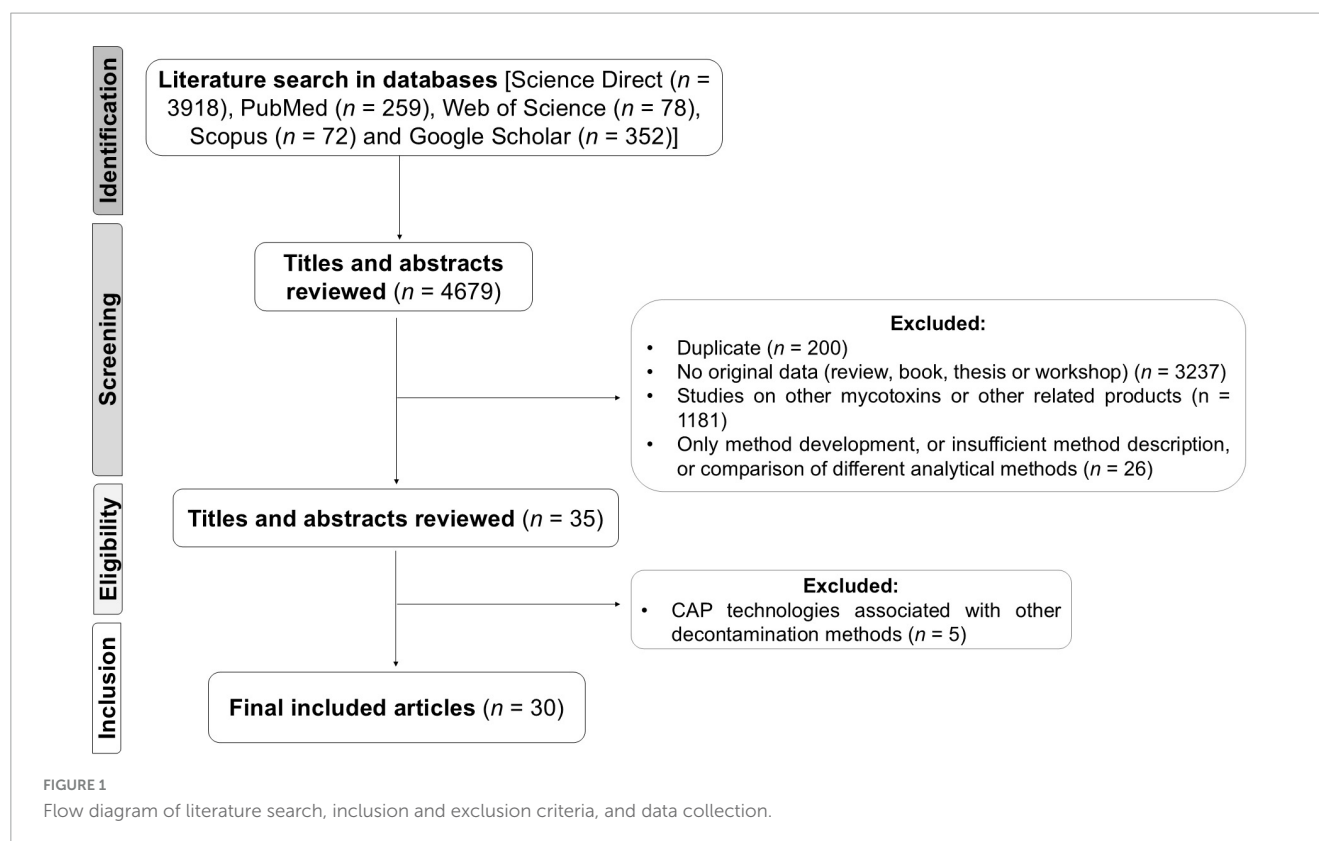
After screening titles and abstracts for relevance, full-text articles were assessed for eligibility based on the following criteria: (1) availability of full text, (2) original research studies (excluding reviews), (3) detailed experimental procedures, (4) precise analytical methods and (5) publication in English. The initial search yielded 4,679 articles, from which 3,237 articles were excluded immediately for not meeting criterion 2, as they were not original studies. Additionally, 200 articles were removed as duplicates, resulting in a total of 1,242 unique publications on the application of CAP. Following another screening, 1,181 articles were excluded for describing CAP applications for secondary fungal metabolites other than main mycotoxins that commonly occur in food products or involving products unrelated to the food sector. Further 26 articles were excluded due to incomplete descriptions of the methods used or because they focused on comparisons between different analytical methods. Finally, 5 articles that used CAP in combination with other decontamination methods were also excluded. At the end of the screening process, a total of 4,650 articles were excluded, as outlined in Figure 1.

Ultimately, 30 studies met the inclusion criteria and were included in this review.

3 Principles of cold atmospheric plasma technologies

Plasma, known as the 4th state of matter, is created when sufficient energy, in the form of heat or an electric field, is applied to a gas, causing free electrons to be accelerated to high energies. Collisions between electrons and neutral gas atoms or molecules can result in ionization, if a sufficient number of ionization events occur the process becomes self-sustaining and a plasma is formed (Neuenfeldt et al., 2023). Atmospheric pressure plasmas can be categorized as either “thermal,” where a local thermodynamic equilibrium is maintained among the electrons and heavy particles (e.g., ions and neutrals), or “non-thermal,” characterized by thermodynamic imbalance, whereby electrons are at a significantly higher temperature. CAP falls within the non-thermal category (Pankaj and Keener, 2017).

Two essential components are required for plasma generation: a high-voltage source and a reactor. The high-voltage source is used to establish a high electric field, which accelerates electrons to the high energies needed to cause ionization, this is typically done in a reactor employing two electrodes, a powered electrode and ground (Niemira, 2012; Ojha et al., 2021). Key parameters affecting the efficacy of the system relate to the form of the applied voltage (e.g., direct current, radio-frequency, pulsed, microwave etc.), and the particular configuration of the reactor (e.g., plasma jet, parallel plate etc.). Once plasma forms in the reactor, the energetic electrons take part in a plethora of reactions causing dissociation and excitation of the background gas creating a complex mixture including ions, free radicals, electrons, and UV photons. For example, in humid air plasma it is known that over 50 unique chemical species are created, which take part in over 600 reactions (Sakiyama et al., 2012). Of particular importance are the reactive neutral species, commonly termed reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Hertwig et al., 2018; Misra and Jo, 2017). Beyond the key physical parameters of the plasma system, a large number of factors affect ROS and RNS generation and therefore the efficacy of the plasma in degrading mycotoxin contamination. For example, plasma generation parameters, such as energy input, play a key role, as the electrical power used for plasma generation directly affects the energy available for the chemical ionization, dissociation, and excitation processes. These energy-dependent processes determine the types and densities of reactive species formed (Shimizu et al., 2012). The energy distribution of electrons is another vital factor, as it governs the nature of reactions within the plasma. Specifically, the electron energy distribution function (EEDF) influences whether molecular dissociation or ionization occurs (Abdel-Fattah, 2013). The frequency of the applied voltage, whether direct current (DC), radio frequency (RF), or microwave, also impacts the energy distribution of electrons and ions. Abdel-Fattah (2013) demonstrated that higher frequencies led to increased peak electron densities, a finding supported by Naidis (2014), who observed elevated densities of reactive species at higher voltage amplitudes and frequencies. In a helium-air plasma model, Naidis (2014) noted that higher voltage amplitudes and frequencies increased NO and



O₃ densities while reducing O atom concentrations, whereas the hydroxy radical (OH) density remained relatively stable.

The feed gas composition is another critical determinant of plasma chemistry, as it defines the atoms and molecules participating in plasma formation. Variations in feed gases, such as helium (He), argon (Ar), nitrogen (N₂), air, or oxygen (O₂), result in distinct reactive species profiles (Naidis, 2014; Han et al., 2016; Dünnebier et al., 2013). The introduction of specific gases can significantly influence reactive species generation. For example, Han et al. (2016) observed a gradual increase in hydrogen peroxide (H₂O₂) production with the addition of oxygen, a process linked to enhanced generation of ROS, including OH radicals. Gas flow rate also affects the formation and distribution of reactive species such as NO and HO₂. Increased flow rates promote the spread of OH radicals, affecting their interaction sites (Hasan and Walsh, 2017). Interaction with ambient air further modulates plasma chemistry, introducing nitrogen, oxygen, and water vapor into the system, which contributes to RNS and ROS formation (Naidis, 2014; Morabit et al., 2021).

Electrode geometry and material significantly impact the electric field distribution, which influence plasma characteristics. Changes in electrode geometry, for instance diameter, alter the breakdown voltage and power deposition within the plasma discharge (Hasan and Walsh, 2017). The chemical composition of plasma also depends on the distance between electrodes and the target. Variations in these distances modify the ionization region, affecting the generation and transport of ROS and RNS (Morabit et al., 2021). Moreover, the density of reactive species decreases as the distance between the plasma jet and the target sample increases (Zhang et al., 2019). Plasma interactions with

either a biological, liquid, or polymeric sample, can catalyze or inhibit specific reactions, altering the chemical composition near the sample surface (Morabit et al., 2021; Kovačević et al., 2018). In some types likely liquid samples, these effects are particularly pronounced, as the presence of the liquid alters reactive species composition and modifies the potential gap near the target surface (Kovačević et al., 2018).

Historically, CAP generation was constrained to low-pressure conditions, requiring the use of a vacuum chamber and thus limiting its applications for the treatment of food products. However, advancements in technology now allow for the stable and non-thermal generation of plasma at atmospheric pressure, leading to extensive research across various scientific fields (Pankaj and Keener, 2017). In specific reactor configurations, such as plasma jets, the mass transport of reactive species is facilitated by gas flow, enabling the effects of plasma to extend to regions well beyond the electrodes. Due to the limited area covered by a single plasma jet, multiple jets or motion systems are often used to treat larger areas or volumes (Feizollahi et al., 2020).

The effectiveness of CAP in fungal inactivation depends on several factors, including the type of plasma generator, the gas utilized, the fungal strains involved, and the test matrix (Mošovská et al., 2019; Wu et al., 2021). Additionally, specific equipment parameters, such as frequency (ranging from 0 Hz to several GHz), discharge voltage (1 kV to over 100 kV), and power (ranging from W to kW), significantly influence CAP's impact (Ojha et al., 2021). While numerous studies highlight the importance of these parameters, there remains no consensus among recent research regarding the optimal ranges for CAP treatment. The variability in these parameters, as applied to mycotoxin degradation in food, is

further discussed according to the specific CAP types examined in the studies.

3.1 Indirect plasma treatment

As previously described, a wide range of different plasma reactors have been used to examine the impact of plasma exposure on mycotoxin contamination. Typically, these can be categorized as either direct—meaning the plasma directly interacts with the target, or indirect—meaning the plasma is generated remotely from the target and only the neutral species interact with the target. This is a fundamental difference, as the spatial separation between plasma and target acts as a spatial filter for reactive species, meaning only the less reactive agents, such as ozone, play a role in the degradation process.

Of the many indirect plasma treatment systems reported in the literature, the surface barrier discharge (SBD) has been widely adopted. This particular configuration belongs to the dielectric barrier discharge (DBD) family of devices, and typically employs a dielectric material sandwiched between a powered and grounded electrode, illustrated in Figure 2A. In this indirect scenario, the plasma region is in the form of a thin layer situated mm to cm from the target. When ambient air is used as the working gas, highly reactive species such as H, N and O form in the discharge layer and are transported toward the target via diffusion and convection (Dickenson et al., 2018; Hasan et al., 2017; Dickenson et al., 2017; Hasan and Walsh, 2017). Owing to their high chemical reactivity, they rapidly react beyond the discharge region to form more stable states such as O₃ and N₂O. Other indirect treatment modalities include plasma jets where the target is placed at a distance from the generated plasma plume and plasma “activated” liquids (Naidis, 2014; Morabit et al., 2021). In a plasma activated liquid, either direct or indirect plasma exposure can be used to first treat a liquid, such as water, introducing a complex chemical cocktail; with the liquid being subsequently applied to the target. Typically, this process generates an acidic solution, owing to abundant nitrogen-based species whilst containing hydrogen peroxide and others. Such solutions have been shown to possess disinfectant properties, making them effective for surface decontamination (Neuenfeldt et al., 2023; Han et al., 2023; Perinban et al., 2022).

3.2 Direct plasma treatment

In contrast to the in-direct treatment scenario, the direct application of CAP to a target enables the entire reactive chemistry created within the discharge to play a role in the decontamination process (Figure 2B). Plasma reactors, such as plasma jets and parallel plates, facilitate the direct exposure of a target to a plasma (Harikrishna et al., 2023). Both plasma jets and parallel plates can take the form of a DBD or employ other approaches to inhibit the glow-to-arc transition, such as radio-frequency excitation (Park et al., 2000; Polito and Kushner, 2024). Through these methods it is possible to maintain discharge stability and a non-thermal characteristic. While such systems can offer enhanced efficacy over their indirect counterparts in microbial inactivation (Wan et al., 2019), due primarily to the enhanced flux and composition of

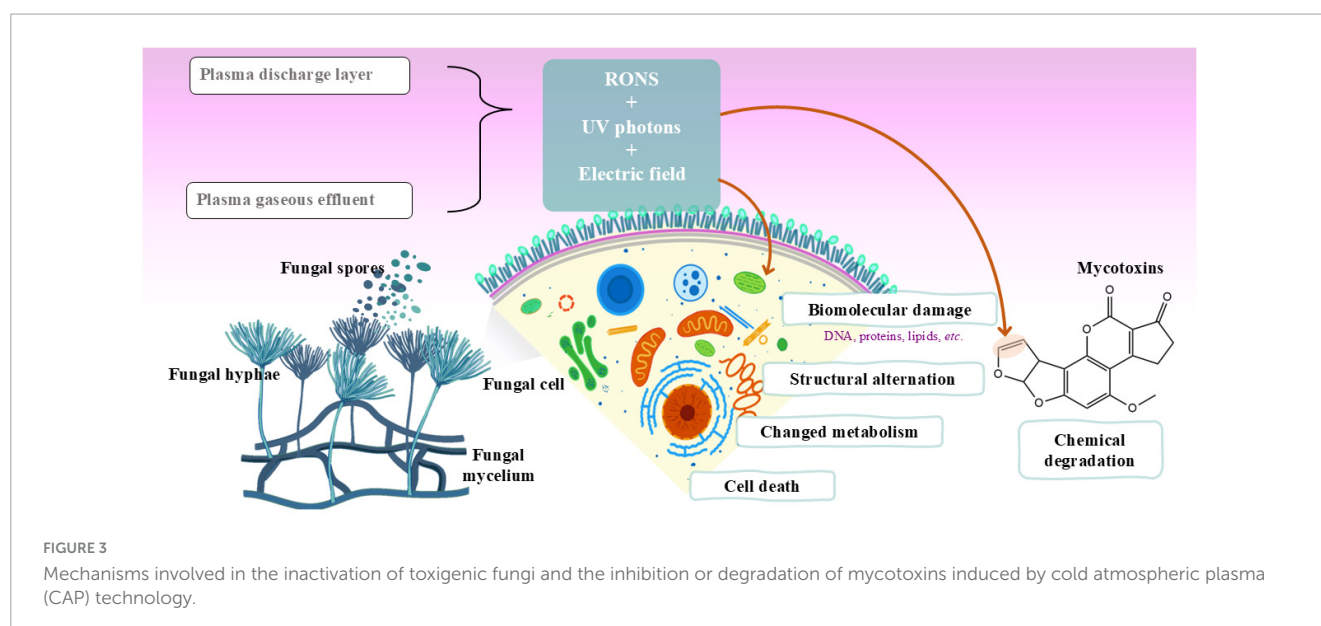
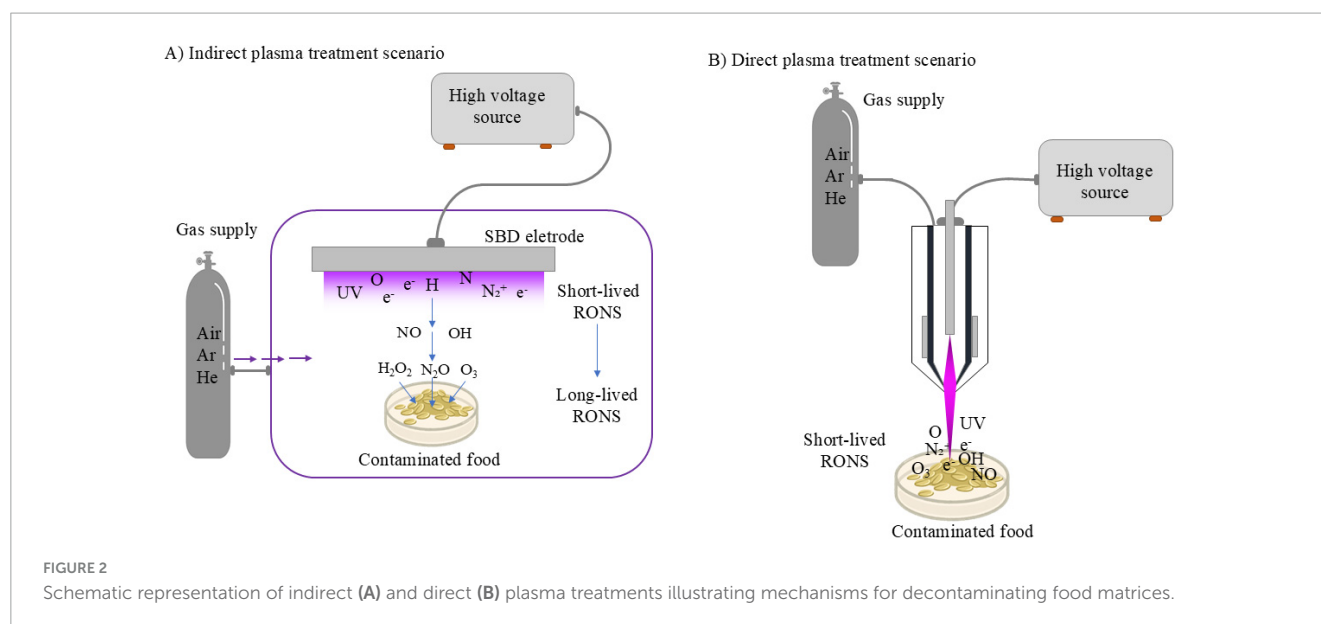
IONS arriving at the target, they are also more complex and typically more difficult to control. When a target is electrically conductive, such as most food stuffs, it is inherently part of the electrical circuit and therefore plays a role in dictating the specific composition of the plasma. Plasma jets can overcome this by physically separating the plasma generation region from the application region, but typically require significant gas flows and/or operation in noble gases such as Argon and Helium (Morabit et al., 2021).

4 Application of cold atmospheric plasma in inactivating toxigenic fungi and mycotoxin biosynthesis

In foodstuffs and other substrates, fungal sporulation and germination evolve until a fully developed fungus. For this process, the fungus and its spores nurture on the target food matrix and introduce it with different chemical agents such as damaging enzymes, and self-defense metabolites including mycotoxins (Ali et al., 2023a). The fungus cellular integrity, its cellular metabolic processes and the enzymatic events are essential in establishing this cycle. However, injury to any or all the essential components, leads to an end with the fungus sporulation and germination with no more production of toxins. Inhibiting such mechanisms has been tempted with modern technologies including CAP application on certain foods. Figure 3 highlights the mechanisms underlying the inactivation of toxigenic fungi and inhibition of mycotoxin biosynthesis by CAP technologies.

The antimicrobial properties of CAP are well-established, making this technology an appealing option for controlling mycotoxigenic fungi and decontaminating the mycotoxins they produce. Table 1 summarizes the main findings from studies focused on the inactivation of toxigenic fungi and inhibition of mycotoxin biosynthesis. CAP generates different reactive species like ROS, RNS and radicals (Hertwig et al., 2018; Misra and Jo, 2017), which are capable of damaging spores and avoid their germination. To do so, the chemical species are directed to induce oxidative stress on the outer layers of the target spores. The oxidative stress further causes a disruption to the cellular metabolic events, and cellular membrane together with its essential DNA, protein, and lipid components, resulting in declined growth of fungus and a reduced production of toxins (Zhao X. et al., 2024). The exposure of *A. ochraceus* to CAP caused ruptures and desiccations in its spores, with significant decline in their viability (Hoppanová et al., 2020). Membrane rupture and desiccation allow the cellular contents to escape from the damaged cell and eventual cell death occurs. In addition, CAP can lead to poor production or lack of energy, ultimately causing spore prevention and cell death, as described for *Clostridioides difficile* (Kaur et al., 2020). CAP can also interfere in the metabolic pathways and enzyme events needed to spore development (Hojnik et al., 2017).

The inhibition efficacy of CAP created with DBD was evaluated in a case of decontaminating *A. niger*, *P. verrucosum*, *F. graminearum*, and *Rhizopus oryzae* on rice grains (Guo et al., 2023). Promising results were achieved after 4 min of exposure, significantly reducing fungal mycelial growth across all tested species, with increased efficacy observed at a longer exposure time



(Table 1). The study demonstrated a significant inhibitory effect on spore germination, particularly in the case of *F. graminearum* and *R. oryzae*, for which a 4-min exposure resulted in a 65–80% reduction in spore viability (Guo et al., 2023). However, the efficiency of CAP against spores varies according to the fungal genus and spore size (Wang Y. et al., 2022; Zhao X. et al., 2024). The larger spore sizes are shown to correlate well with a lower CAP inactivation efficiency (Wen et al., 2017; Wen et al., 2019; Wen et al., 2020). By studying the inactivation ability of CAP on *Fusarium* species, Wang Y. et al. (2022) found that smaller sized spores were more susceptible to CAP reactive species due to their larger specific surface area, resulting in more effective inactivation. Additionally, spore germination rates dropped below 10% following to 2 min of exposure to CAP generated with a dielectric barrier surface micro-discharge (SMD). Regarding DON levels relative to the dry weight of mycelium ($\mu\text{g/g}$), CAP treatment resulted in a 30–48% reduction (Wang Y. et al., 2022).

CAP's action can alter spore morphology, leading to structural damage such as increased roughness surface, membrane fractures, and size reduction. The observed membrane damage, confirmed by monitoring intracellular nucleic acid and protein leakage, indicates that increasing CAP exposure beyond 2 min progressively compromises membrane integrity (Wang Y. et al., 2022). CAP also inhibits mycotoxin biosynthesis by downregulating the expression of the genes responsible for toxin production. Ouf et al. (2014) found that CAP generated with a double atmospheric pressure argon plasma jet (DAPACP) or plasma jet affected the biosynthesis of FB₂ and OTA in treated *A. niger* spores, through disruptions in the microbial DNA. This was also investigated in a study by Zhao et al. (2023), where aflatoxin production was analyzed at the biomolecular level, focusing on the gene expressions involved in aflatoxin biosynthesis. Among the 12 genes associated with AFB₁ production, CAP generated with the dielectric barrier (DB) specifically reduced the expression of *aflE* and *aflM*, indicating

TABLE 1 Summary of main outcomes from studies using cold atmospheric pressure plasma (CAP) in decontaminating toxigenic fungi.

Fungal species	Matrix type	CAP source (process parameters)	Main outcomes	References
<i>Aspergillus niger</i> , <i>Rhizopus oryzae</i> , <i>Penicillium verrucosum</i> , and <i>Fusarium graminearum</i>	Rice grain	DBD (Distance: 20 mm; voltage: 25 kV; time: 2, 4, 6, 8 min)	↓ 65–80% spore formation, ≠ DON biosynthesis	Guo et al., 2023
<i>F. graminearum</i> HX01, <i>F. graminearum</i> LY26, <i>F. pseudograminearum</i> , and <i>F. moniliforme</i>	Spore suspensions in carboxymethylcellulose liquid medium	SMD (Frequency: 7 kHz; discharge power: 5 ± 0.15 W; distance: 3 mm; time: 0.5, 1, 1.5, 2, 2.5, 3, 3.5 min; gases: O ₂ and N ₂ ; indirect exposure)	(I). <i>F. moniliforme</i> ↓ at 6.0 log ₁₀ , <i>F. graminearum</i> HX01 ↓ at 5.1 log ₁₀ , <i>F. graminearum</i> LY26 ↓ at 2.5 log ₁₀ , <i>F. pseudograminearum</i> ↓ at 2.0 log ₁₀ (II). <i>F. graminearum</i> LY26 ↓ at 4.9 log ₁₀ CFU. mL ⁻¹ , <i>F. pseudograminearum</i> ↓ at 3.9 log ₁₀ CFU. mL ⁻¹	Wang Y. et al., 2022
<i>A. niger</i> and <i>P. verrucosum</i>	Barley	DCSBD 400 (Power: 350 W; gas flow rate: 10 L/min; time: 1 or 3 min; gas: 100% CO ₂ + 80% CO ₂ + 20% O ₂)	(III). ↓ mold count of <i>A. niger</i> and <i>P. verrucosum</i> by 2.5–3 log cycles	Durek et al., 2018
<i>A. flavus</i>	Corn	DBD (Frequency: 45–250 Hz; voltage: 60–160 kV; distance: 30 mm; time: 4, 8, 12 min)	(IV). <i>A. flavus</i> spores ↓ by 0.96–3.20 log ₁₀ CFU/g (V). AFB ₁ produced by <i>A. flavus</i> ↓ by 96.16%	Zhao L. et al., 2024
<i>A. flavus</i>	<i>A. flavus</i> spore suspension in sterile water	DBD (Voltage: 50, 60, 70 V; distance: 6 mm; time: 0, 1, 2, 3, 4, 5, 6 min)	(VI). 88% ≠ for <i>A. flavus</i> , ↓ <i>A. flavus</i> spores by 4.47 log ₁₀ CFU/mL	Zhao L. et al., 2024
<i>A. flavus</i>	Fungal malt extract agar with <i>A. flavus</i> spores	HVCAP (Frequency: 50 Hz; voltage: 70, 80, 85 kV; time: 1, 2, 5, 10 min; gases: O ₂ , N ₂ ; direct exposure)	50% ≠ of <i>A. flavus</i> spores, ↓ > 99% of DON (in aqueous suspension), ↓ 33% of DON (in powdered form)	Ott et al., 2021
<i>A. flavus</i> and <i>A. parasiticus</i>	Wheat grains	Gliding arc cold plasma [Frequency: 20 kHz; power: 5–10 W; gases: dry air (21% O ₂ , 79% N ₂); time: 2, 6, 12 min]	57% ↑ in lag time, 68% ↓ in growth rate and 78% ↓ of <i>A. flavus</i> 70% ↑ in lag time, 55% ↓ in growth rate and 68% ↓ of <i>A. parasiticus</i>	Rahnavard et al., 2024
<i>A. niger</i>	<i>A. niger</i> spore suspension from contaminated date palm fruits	Plasma jet (Voltage: 25 kV; frequency: 25 kHz; dist.: 12 mm; time: 7.5 min; gas: argon; indirect exposure)	<i>A. niger</i> spores ↓ from 1000 CFU/100 mm ² (control) to 20 CFU/100 mm ²	Ouf et al., 2014
<i>A. niger</i>	Spore suspension of <i>A. flavus</i> cultured on PDA	DB (Frequency: 45–250 Hz; voltage: 60–160 Kv; distance: 14 mm; time: 2 min; gas: O ₂ , N ₂ ; direct exposure)	(VII) ↓ 5.39 Log ₁₀ CFU/cm ² , 17–66% ≠ in <i>aflE</i> and <i>aflM</i> genes	Zhao et al., 2023

CFU/g, colony-forming unit per gram; CFU/mL, colony-forming unit per milliliter; DBD, dielectric barrier discharge; DCSBD, diffuse coplanar surface barrier discharge; I: results obtained with 3 min time of exposure; II: results obtained with 3.5 min time of exposure; III: results obtained with 3 min time of exposure; IV: results obtained with 8 min time of exposure; V: results obtained with 12 min time of exposure; VI: results obtained with 6 min exposure time of plasma at 60 V; VII: results obtained with 2 min time of exposure; SDBD, surface dielectric barrier discharge; ↓ reduction; ≠ inhibition or inactivation; ↑ increase. NI, not informed.

CAP’s role in inhibiting aflatoxin synthesis. These findings highlight the importance of advancing our understanding of CAP’s impact on fungal activity and mycotoxin production at the molecular level.

5 Mycotoxins degradation by cold atmospheric plasma

Mycotoxin reduction through the application of CAP technologies occur mainly by degradation where a target toxin is broken down into less or non-toxic chemical components (Wang et al., 2024). Mycotoxins’ degradation through CAP is primarily

influenced by the type of chemical reactions, the reactive species generated by the apparatus, and UV radiation. This combination leads to cleavage of the molecular structure of toxin, resulting in the formation of transformation products (TPs) (Feizollahi et al., 2020). When CAP induces the cleavage of the specific site on the mycotoxin molecule responsible for its toxicity, it can be assumed that this process leads to the degradation of the mycotoxin and the formation of less toxic TPs. In line, reduction effects following CAP application have been demonstrated for AFs and some other mycotoxins (Figures 4–8), as shown in Tables 2, 3, respectively.

The toxicity of AFB₁ is attributed to the double bond between carbons 8 and 9 (C8 = C9) in furan ring, and disrupting this bond produces less toxic degraded products (Hojnik et al., 2021), depicted in Figure 4. In a study by Nguyen et al. (2022), CAP’s effect

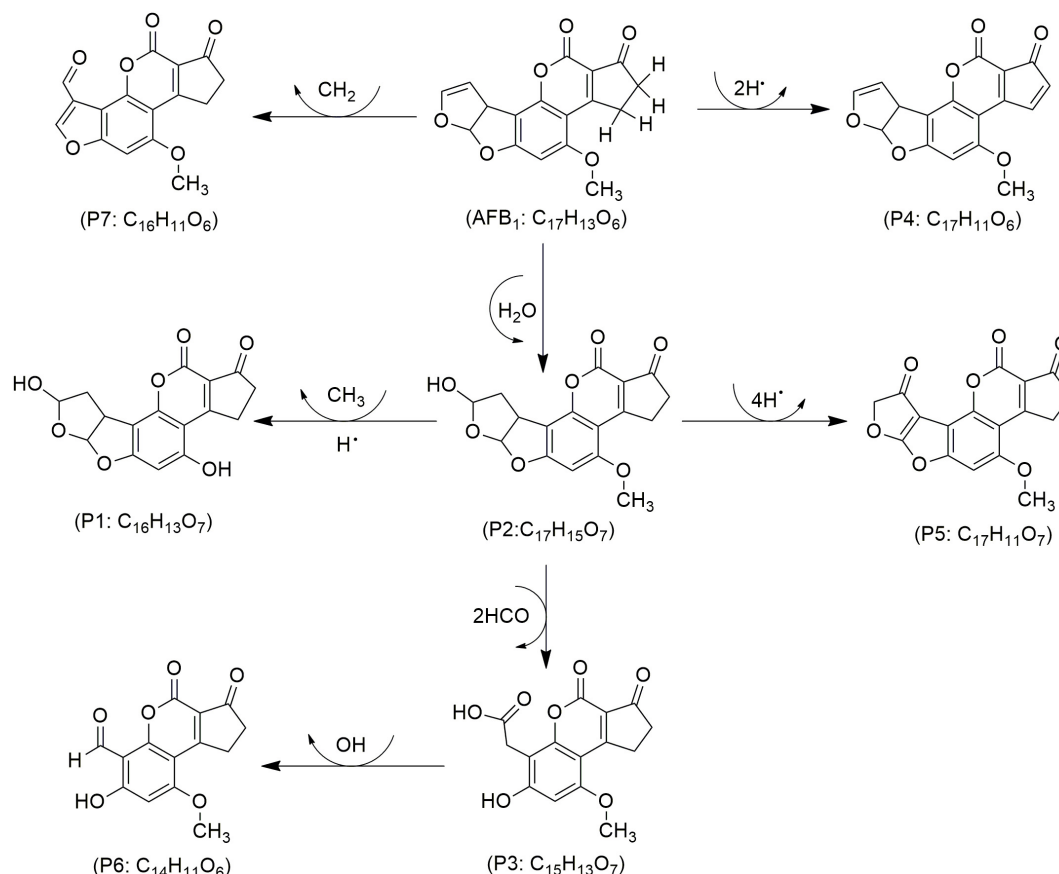


FIGURE 4

Proposed mechanisms and degradation products (P) of aflatoxin B₁ (AFB₁) in food under cold atmospheric plasma (CAP) treatment, adapted from Zhao et al. (2023).

on aflatoxin M₁ (AFM₁) was investigated, reporting reductions across all treatments, with the most significant decrease of 60% occurring after 20 min of exposure to a high voltage generated CAP (Table 2). Similarly, Nikmaram and Keener (2022) achieved an even higher AFM₁ degradation rate of 87% with just 4 min of CAP exposure (30–130 kV, 60 Hz) in milk. The analysis in this study indicated three TPs resulted from AFM₁ degradation (i.e., C₁₅H₁₁O₇, C₁₇H₁₅O₉, and C₁₅H₁₃O₇). Each TP was formed as a result from CAP induced chemical modifications in furofuran ring, particularly by the disruption of the double bond between C8 and C9, leading to a reduction in toxicity of the molecule (Nikmaram et al., 2023).

In pistachio kernels treated with ozone-based CAP, AFB₁ and AFG₁ were reduced by 81 and 82% within 15 min, respectively, reaching 99% after 60 min, while CAP with NO_x reduced AFB₁ and AFG₁ by 64 and 63%, respectively (Laika et al., 2024). In wheat, CAP treatment led to 61% reduction in AFs, with specific decreases of 64, 41, 59 and 40% for AFB₁, AFB₂, AFG₁ and AFG₂, respectively (Rahnavard et al., 2024). The reduction rate of AFB₁ increased from 62 to 82% in corn powder treated by a high voltage CAP for one to 10 min (Shi et al., 2017a). A 5-min treatment of high voltage CAP, revealed 76% degradation in AFB₁ in standard solutions, suggesting ozonolysis and epoxidation that led to the breakdown of C8 = C9 in furofuran, which resulted in six TPs—e.g., C₁₆H₁₆O₆, C₁₇H₁₄O₇,

C₁₄H₁₂O₅, C₁₄H₁₀O₆, C₁₇H₁₂O₇, and C₁₉H₁₈O₈ (Shi et al., 2017b; Table 2).

Hojnik et al. (2020) achieved complete AFB₁ degradation in corn grains after 5–10 min treatment of CAP generated with SBD. Except for a negligible alteration in sample morphology, CAP application led to AFB₁ degradation based on its four TPs such as C₁₇H₁₅O₇, C₁₆H₁₁O₆, C₁₅H₁₃O₇, and C₁₄H₁₀O₆ (Hojnik et al., 2020). In another study, a 30-s treatment of CAP with SDBD demonstrated 99% reduction in AFB₁ and 70% in AFB₂, while 100 and 74% reductions were observed for AFG₁ and AFG₂, respectively (Hojnik et al., 2019). In rice inoculated with *A. parasiticus*, CAP reduced AFs by 56.4% after 60 min with a DBD system using a mixture of O₂, N₂, and CO₂. However, minor fat oxidation was noted probably because of insource generated reactive species (Zhi et al., 2023). In another study, corn inoculated with *A. flavus* and treated with CAP at intervals of 2, 4, 8, and 12 min showed progressive AFB₁ reductions of 23.4, 41.8, 64.4, and 80.0% (Zhao L. et al., 2024). Additionally, AFB₁ degradation by CAP in an *A. niger* cell suspension reached 95.3% within 30 s. The data indicated a disintegrated chemical structure of AFB₁, resulting in seven TPs designated as products (Ps) in Figure 4, e.g., C₁₆H₁₃O₇ (P1), C₁₇H₁₅O₇ (P2), C₁₅H₁₃O₇ (P3), C₁₇H₁₁O₆ (P4), C₁₇H₁₁O₇ (P5), C₁₄H₁₁O₆ (P6) and C₁₆H₁₁O₆ (P7) (Zhao et al., 2023). Previously, Wielogorska et al. (2019) obtained a 66% reduction of AFB₁ after treatment by CAP generated with

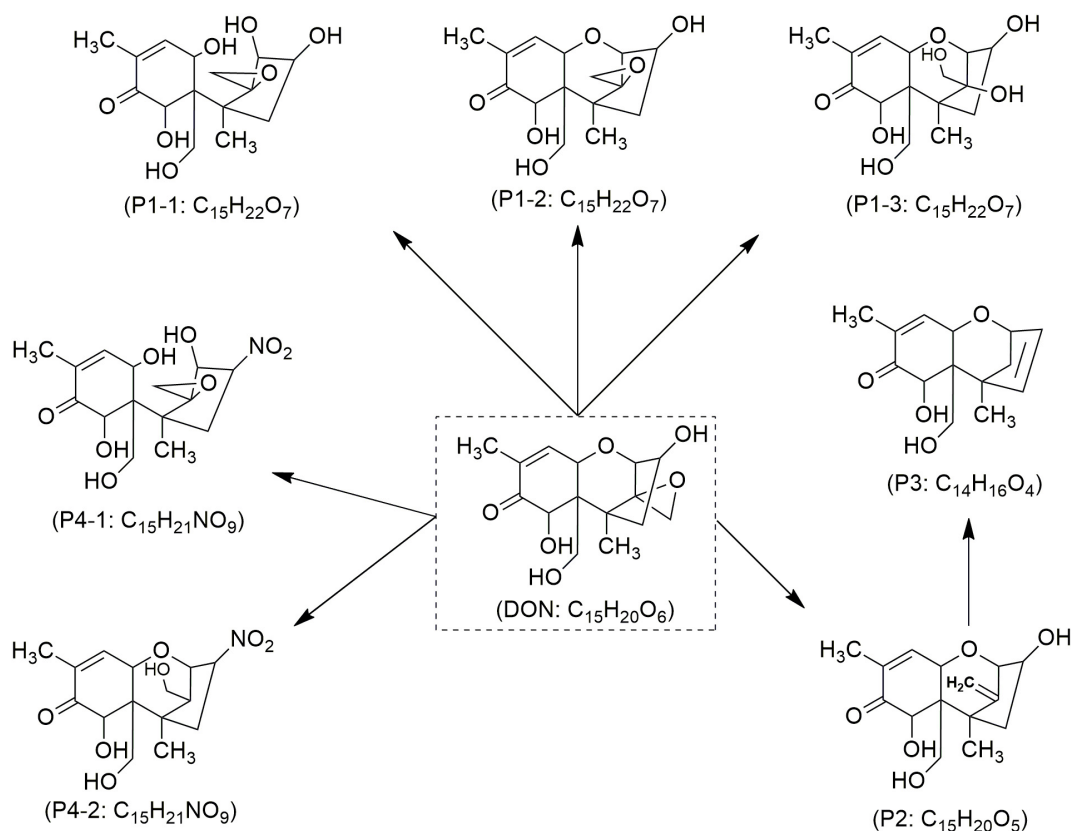


FIGURE 5

Proposed mechanisms and degradation products (P) of deoxynivalenol (DON) in food under cold atmospheric plasma (CAP) treatment, adapted from [Chen et al. \(2022\)](#).

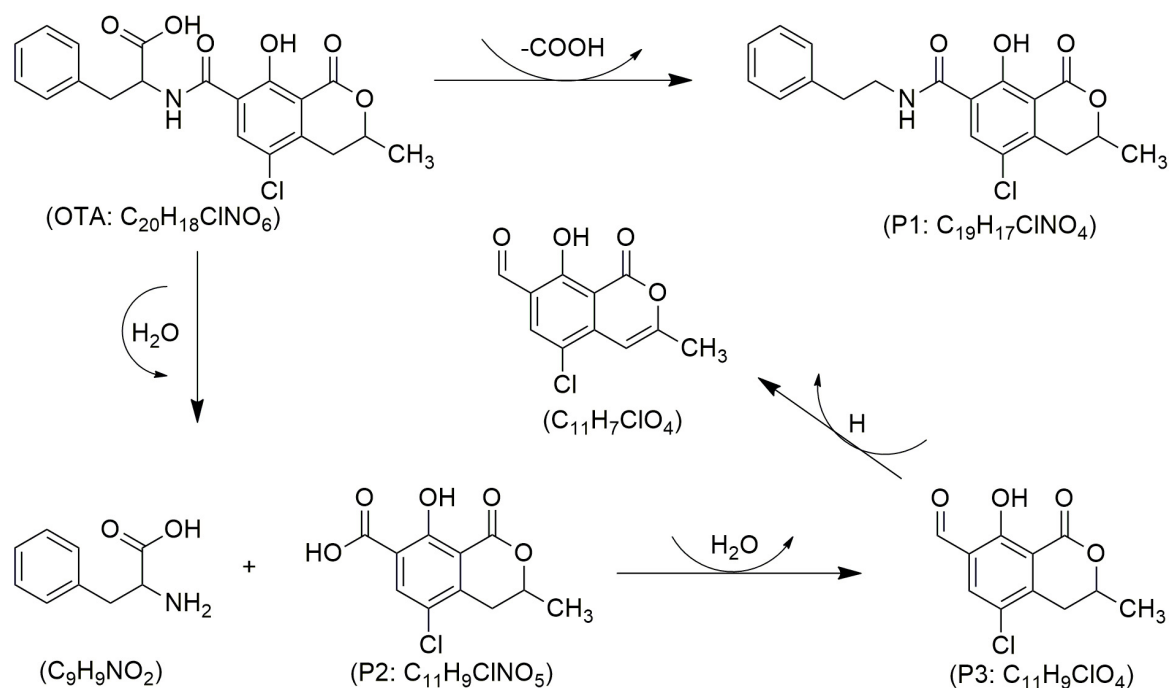


FIGURE 6

Proposed mechanisms and degradation products (P) of ochratoxin A (OTA) in food under cold atmospheric plasma (CAP) treatment, adapted from [Wang et al. \(2024\)](#).

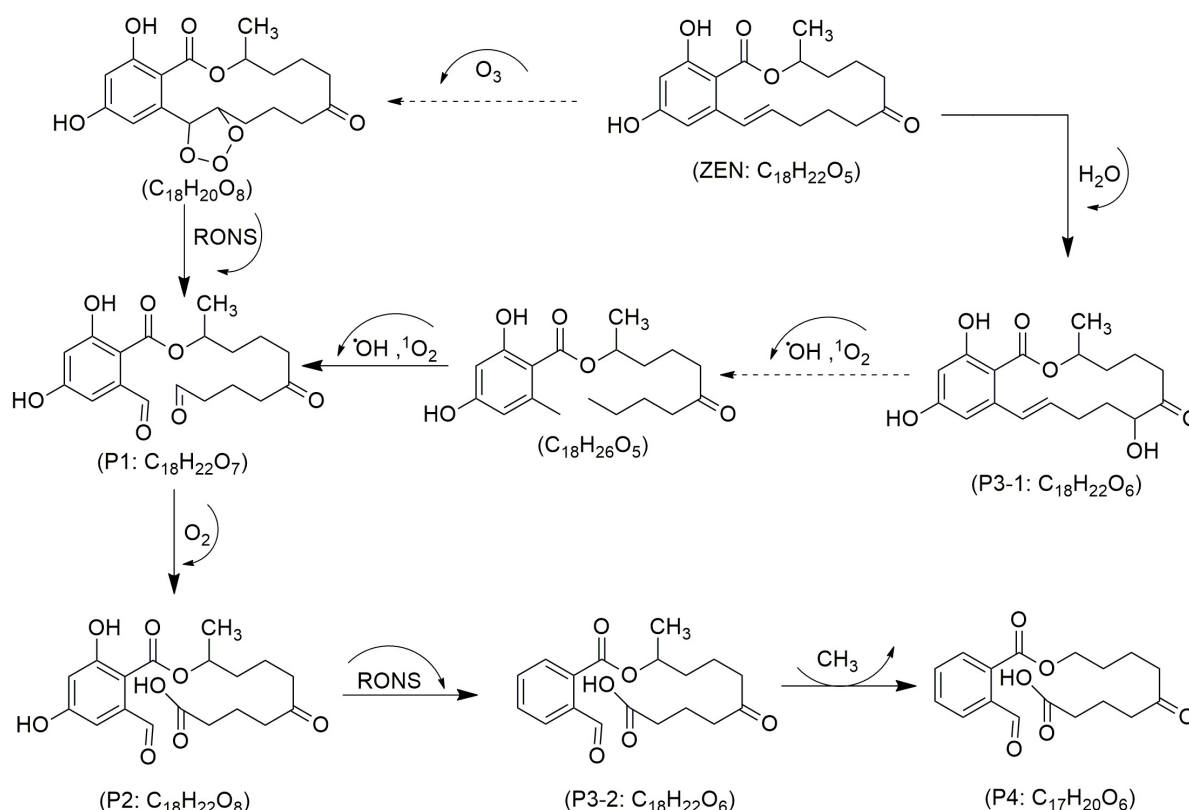


FIGURE 7

Proposed mechanisms and degradation products (P) of zearalenone (ZEN) in food under cold atmospheric plasma (CAP) treatment, adapted from Liu et al. (2024).

DBD demonstrated, mainly due to a high voltage in oxygen-rich environment that boosted oxygen radical and ozone formation, thereby enhancing peroxidation. This reaction primarily modified the terminal furans, with lactone and methoxy groups unchanged, indicating selective site reactivity with a net result of seven TPs such as C₁₇H₁₂O₆, C₁₇H₁₄O₈, C₁₆H₁₂O₇, C₁₅H₁₂O₇, C₁₅H₁₀O₅, C₁₅H₁₀O₇, and C₁₄H₁₂O₅, respectively (Wielogorska et al., 2019). Similarly, wheat and rice inoculated with *A. flavus*, treated with CAP generated by CDPJ using variable frequency, current and electrodes distance, revealed remarkably a reduction of 95% of AFB₁ of which 45 to 56% were assigned to wheat and rice samples (Puligundla et al., 2020). Moreover, DBD-based CAP for a 12 min treatment of hazelnuts showed the reduction rates between 43 and 100% for AFs, and 75 to 100% for AFB₁ (Siciliano et al., 2016). Sakudo and Yagyu (2024) reported time-dependent reductions in AFB₁, achieving 75.8% decreased within 1 min and up to 82.5% over 30 min of CAP exposure.

Studies have demonstrated the ability of CAP to decontaminate other types of mycotoxins (Table 3). Wang Y. et al. (2022) investigated the impact of CAP generated with SMD on *Fusarium graminearum* and observed a significant reduction in DON production across all samples evaluated, with results varying according to exposure duration. Notably, a 6-min CAP treatment resulted in over 50% reduction in DON levels. Similarly, Chen et al. (2022) reported that applying CAP generated with DBD at 50 kV to wheat for 8 min led to a DON degradation of over 62–84%. The authors mentioned that seven TPs of DON (Figure 5) were

confirmed according to four molecular formulas, e.g., C₁₅H₂₂O₇ (P1/1–3), C₁₅H₂₀O₅ (P2), C₁₄H₁₆O₄ (P3), and C₁₅H₂₁NO₉ (P4/1–2) (Chen et al., 2022). Among these, C₁₅H₂₀O₅ (P2), known as de-epoxy deoxynivalenol (DOM-1), was noted for its reduced toxicity compared to its parent DON molecule (Figure 5). DON's toxicity is primarily associated with the hydroxyl (-OH) group at C3, the double bond between C9 and C10, and the sigma bond between C12 and C13 in epoxy group. Since the degradation products lack these or some of these chemical features, are considered as less harmful (Chen et al., 2022). Notably, the breakdown of the C12-C13 epoxy ring may be more prevalent in acidic environments, suggesting that food matrices with lower pH may facilitate this process (Guo et al., 2023). In the same study, it was also demonstrated that CAP generated with DBD effectively reduces the levels of both DON and OTA. The highest reduction was obtained after 8 min of treatment, reaching 61 and 56% reduction rates for DON and OTA, respectively (Guo et al., 2023). Regarding OTA, CAP treatment promotes the hydrolysis of the amide bond, thus resulting in the formation of less toxic compounds like phenylalanine (C₉H₉NO₂) and ochratoxin-α (OT-α: P2) (Figure 6). It is also notable that ozone generated by CAP can oxidize the chlorinated ring in the OTA moiety, which leads to its breakdown into amino acids or free chlorine, further contributing to detoxification (Guo et al., 2023; Wang L. et al., 2022). Wang et al. (2024) demonstrated the effectiveness of CAP generated with GPSDP in degrading OTA in raisin, achieving 62% degradation at a four-min treatment and reaching 100% with a 10-min treatment (Table 3), without

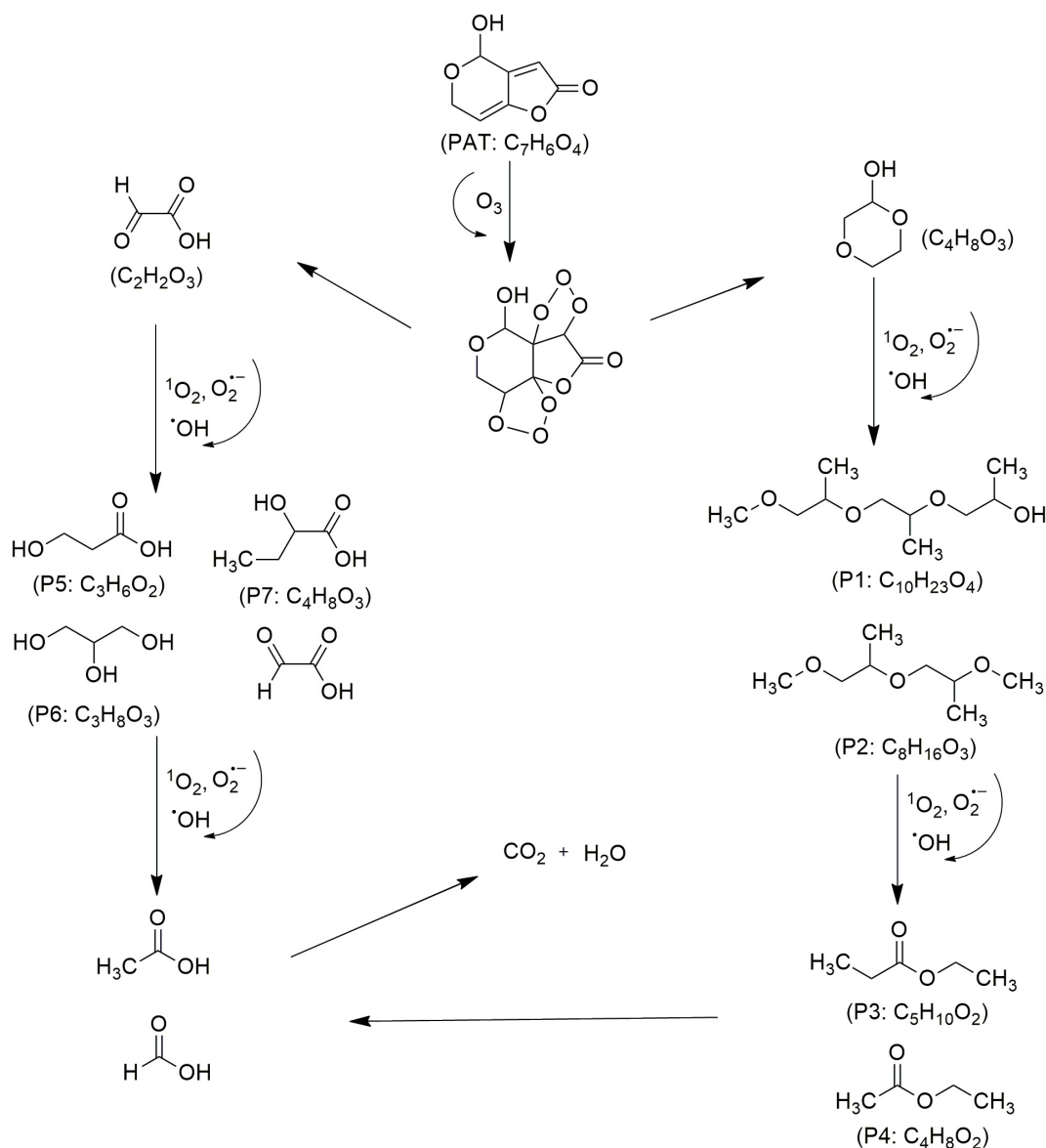


FIGURE 8

Proposed mechanisms and degradation products (P) of patulin (PAT) in food under cold atmospheric plasma (CAP) treatment, adapted from Shirazi et al. (2025) and Xue et al. (2021).

affecting matrix quality. Three OTA degradation products such as $C_{19}H_{17}ClNO_4$ (P1), $C_{11}H_9ClNO_5$ (P2) and $C_{11}H_9ClO_4$ (P3) were identified (Figure 6), likely resulting from ozone, peroxides, and free radicals, as detailed elsewhere (Guo et al., 2023; Wang L. et al., 2022; Wang et al., 2024).

In corn containing six mycotoxins and treated with CAP using a DBD system, Wielogorska et al. (2019) observed 60–66% reductions of AFB₁ and FB₁ within 10 min. The study led to the identification of TPs only for AFB₁ (e.g., $C_{17}H_{12}O_6$, $C_{17}H_{14}O_8$, $C_{16}H_{12}O_7$, $C_{15}H_{12}O_7$, $C_{15}H_{10}O_5$, $C_{15}H_{10}O_7$ and $C_{14}H_{12}O_5$) and ZEN (e.g., $C_{18}H_{22}O_5$, $C_{18}H_{22}O_6$ and $C_{21}H_{24}O_8$). Helium plasma enhanced the degradation efficiency when compared with mixed nitrogen-oxygen, primarily due to increased oxygen radical formation and a preference for peroxidation reactions (Wielogorska et al., 2019). A three-min CAP treatment using SDM

at 30 watts achieved 96.2% degradation of ZEN in corn and wheat kernels, as well as their flour. However, individual treatments of wheat and corn alone over 20 min showed ZEN reductions of 50.5–58.1%, with no significant changes to gluten in either matrix. Four ZEN degradation products, presented as $C_{18}H_{22}O_7$ (P1), $C_{18}H_{22}O_8$ (P2), $C_{18}H_{22}O_6$ (P3/1–2) and $C_{17}H_{20}O_6$ (P4) in Figure 7, were identified, primarily from oxidative cleavage of the olefinic C11 = C12 bond, following well-explored pathways (Liu et al., 2024). Key factors in ZEN degradation included irradiation distance, treatment time, RONS, and free radical oxidation.

The use of CAP for mycotoxin decontamination has primarily focused on the widely consumed cereals, grains, and their derivatives, with limited attention given to perishable products like fruits and vegetables. Research on perishable matrices has mainly addressed microbial control and shelf-life extension,

TABLE 2 Summary of main outcomes from studies using cold atmospheric pressure plasma (CAP) in decontaminating aflatoxins.

Aflatoxin	Initial concentration	Type of matrix	CAP source (process parameters)	Main outcomes	Degradation products	References
AFB ₁	10 µL	<i>In vitro</i> Rice and wheat (<i>A. flavus</i>)	CDPJ (Voltage: 20 kV; frequency: 58 kHz; current: 1.00, 1.25, 1.50 A; distance: 15, 25, 35 mm)	95% AFB ₁ Φ, 56% AFB ₁ ↓ (rice) 45% AFB ₁ ↓ (wheat)	NI	Puligundla et al., 2020
AFM ₁	0.1, 1, and 50 µg/L	Milk (standard solution)	HVCAP (Voltage: 80 kV; frequency: 60 Hz; power: 200 W; time: 20 min; direct and indirect exposure)	80% AFM ₁ ↓ in milk using MA 65 operating gas, 65.0% AFM ₁ ↓ in milk using air, no change in milk color	NI	Nguyen et al., 2022
AFB ₁	50 µg/mL*	Corn grain (standard solution)	SBD (Distance: 5 mm; low power: 0.18 W/cm; high power: 0.31 W/cm; time: 5, 10 min; gas: O ₂ and N ₂ ; indirect exposure)	100% AFB ₁ decontamination, negligible change in corn morphology	C ₁₇ H ₁₅ O ₇ C ₁₆ H ₁₁ O ₆ C ₁₅ H ₁₃ O ₇ C ₁₄ H ₁₀ O ₆	Hojnik et al., 2020
AFB ₁	0.1 µg/mL	<i>In vitro</i> Corn (standard solution)	DBD (Voltage: 6 kV; frequency: 20 kHz; distance: 12 mm; time: 10 min; gas: He, N ₂)	66% AFB ₁ ↓, No toxic by-products formation of matrix constituent	C ₁₇ H ₁₂ O ₆ C ₁₇ H ₁₄ O ₈ C ₁₆ H ₁₂ O ₇ C ₁₅ H ₁₂ O ₇ C ₁₅ H ₁₀ O ₅ C ₁₅ H ₁₀ O ₇ C ₁₄ H ₁₂ O ₅	Wielogorska et al., 2019
AFB ₁	0.05 µg/mL	<i>In vitro</i>	HVCAP (Voltage: 30–130 kV; frequency: 60 Hz; time: 1–5 min; gas: O ₂ and N ₂ ; direct and indirect exposure)	77% and 93.3% ↓ in AFM ₁ (I) 56.4% and 89.6% ↓ in AFM ₁ (II)	NI	Nikmaram and Keener, 2023
AFB ₁ AFs	AFB ₁ : 20 µg/kg, AFB ₂ : 50 µg/kg, AGB ₁ : 100 µg/kg, AGB ₂ : 150 µg/kg	Rice (<i>A. parasiticus</i>)	DBD [Voltage: 28–169 kV; frequency: 50–200 Hz; distance: 35 mm; time: 5–60 min; gas: O ₂ (0–65%), N ₂ (5–70%), CO ₂ (30%); direct and indirect exposure]	55.34% ↓ in AFB ₁ , 56.37% ↓ in AFs, no change in rice quality except fat oxidation, ↓ AFB ₁ (66.12–29.19 µg/kg) and ↓ AFs (96.93–208.58 µg/kg) (III)	NI	Zhi et al., 2023
AFB ₁	1 mg/L	Corn (<i>A. flavus</i>)	DBD (Frequency: 45–250 Hz; voltage: 60–160 kV; distance: 30 mm; time: 12 min)	80% Φ of AFB ₁	NI	Zhao L. et al., 2024
AFM ₁	0.05 mg/L	Milk (standard solution)	HVCAP [Frequency: 60 Hz; voltage: 30–130 kV; time: 1, 3, and 5 min; gas: O ₂ (65%), N ₂ (5%), CO ₂ (30%); direct and indirect exposure]	41.9% and 37.8% AFM ₁ ↓ in skim milk and whole milk (IV), 87% AFM ₁ ↓ with 4 h of post treatment (V)	NI	Nikmaram and Keener, 2022
AFB ₁	50 µg/mL	<i>In vitro</i>	DBD (Frequency: 10 kHz; voltage: 10 kV; distance: 30 mm; time: 1, 2, 5, 15, 30 min; indirect exposure)	75.8% ↓ after 1-min 80.9% ↓ after 2-min 81.8% ↓ after 5-min 82.3% ↓ after 15-min 82.5% ↓ after 30-min	NI	Sakudo and Yagyu, 2024
AFM ₁	10 µg/mL	<i>In vitro</i> (Petri dish contaminated with AFM ₁)	HVCAP [Frequency: 60 Hz; voltage: 30–130 kV; time: 1, 3, and 5 min; gas: O ₂ (65%), N ₂ (5%), CO ₂ (30%); direct and indirect exposure]	Φ of AFM ₁ (VI), ↓ in AFM ₁ bioactivity due to loss of C8–C9 double bond in furofuran ring	C ₁₅ H ₁₁ O ₇ C ₁₇ H ₁₅ O ₉ C ₁₅ H ₁₃ O ₇	Nikmaram et al., 2023

(Continued)

TABLE 2 (Continued)

Aflatoxin	Initial concentration	Type of matrix	CAP source (process parameters)	Main outcomes	Degradation products	References
AFB ₁ AFB ₂ AFG ₁ AFG ₂	100 µg/kg	Pistachio kernels (standard solution)	SDBD (Frequency: 23 kHz; voltage: 6 kV; power: 42–425 W; time: 0–60 min; gas: O ₃ and NO _x ; indirect exposure)	Experiment under O ₃ : ↓ in AFB ₁ (81%) and AFG ₁ (82%) on 15 min, 99% ↓ in AFB ₁ + AFG ₁ on 60 min, 60% ↓ in AFB ₂ + AFG ₂ Experiment under NO _x : ↓ in AFB ₁ (64%), AFG ₁ (63%), AFB ₂ (17%), AFG ₂ (19%)	NI	Laika et al., 2024
AFB ₁ AFB ₂ AFG ₁ AFs	NI	Wheat grains (<i>A. flavus</i> and <i>A. parasiticus</i>)	Gliding arc cold plasma [Frequency: 20 kHz; power: 5–10 W; time: 12 min; gas: O ₂ (21%), N ₂ (79%)]	↓ in AFB ₁ (64%), AFB ₂ (41%), AFG ₁ (59%), AFG ₂ (40%), TAF (61%)	NI	Rahnavard et al., 2024
AFB ₁ AFB ₂ AFG ₁ AFG ₂	AFB ₁ : 0.2 µg/mL AFB ₂ : 0.2 µg/mL AFG ₁ : 0.05 µg/mL AFG ₂ : 0.05 µg/mL	<i>In vitro</i> (Stock solutions of mycotoxins)	SDBD (Frequency: 40 kHz; voltage: 7–10 kV; distance: 5 mm; time: 1, 2, 4, 8 min; gas: O ₂ , N ₂ ; indirect exposure)	99% ↓ in AFB ₁ , at 30 s AFB ₁ values ↓ from 18 µg/kg to < 0.006 µg/kg, 70% ↓ in AFB ₂ , 100% ↓ in AFG ₁ , 74% ↓ in AFB ₂	NI	Hojnik et al., 2019
AFB ₁	NI	<i>A. niger</i>	DB (Frequency: 45–250 Hz; voltage: 60–160 kV; distance: 14 mm; time: 30 sec; gas: O ₂ and N ₂ ; direct exposure)	95.33% Φ efficiency of AFB ₁ , AFB ₁ toxicity ↓ by changes in –OCH ₃ group, double bond formation in cyclopentanone and disintegration in furan ring	C ₁₆ H ₁₃ O ₇ C ₁₇ H ₁₅ O ₇ C ₁₅ H ₁₃ O ₇ C ₁₇ H ₁₁ O ₆ C ₁₇ H ₁₁ O ₇ C ₁₄ H ₁₁ O ₆ C ₁₆ H ₁₁ O ₆	Zhao et al., 2023
AFB ₁	4 µL/g (Solution 50 µg/mL)	Corn (Powdered)	HVCAP [Frequency: 50 Hz; voltage: 90 kV; power: 200 W; time: 1, 2, 5, 10, 20, 30 min; gas: O ₂ (22%), N ₂ (78%); direct and indirect exposure]	62% and 82% ↓ in AFB ₁ at 1-min and 10-min	NI	Shi et al., 2017a
AFB ₁	50 µg/mL	<i>In vitro</i> (Standard AFB ₁ solution in chloroform)	HVCAP [Frequency: 50 Hz; voltage: 90 kV; power: 200 W; distance: 4.44 cm; time: 1, 2 or 5 min; gas: O ₂ (22%), N ₂ (78%); direct exposure]	76% ↓ in AFB ₁ on 5-min treatment, AFB ₁ ↓ bioactivity was to disappearance of furofuran ring's C8-C9 double bond, Observed pathways of degradation (1) ozonolysis, (2) epoxidation, Six Φ products of AFB ₁	C ₁₆ H ₁₆ O ₆ C ₁₇ H ₁₄ O ₇ C ₁₄ H ₁₂ O ₅ C ₁₄ H ₁₀ O ₆ C ₁₇ H ₁₂ O ₇ C ₁₉ H ₁₈ O ₈	Shi et al., 2017b
AFB ₁ TAFs	10 mg/mL	<i>In vitro</i> Hazelnut (standard solutions)	DBD [Frequency: 100–150 kHz; power: 0.4–2 kW; distance: 50 mm; time: 1, 2, 4, 12 min; gas: O ₂ (0.1, 1, or 21%), N ₂]	70% ↓ of AFB ₁ and TAFs on 12 min treatment at 1 kW; 75–100% ↓ in AFB ₁ ; 43–100% ↓ in TAF; 0–70.9% ↓ in AFB ₁ ; 2.3–69.4% ↓ in TAFs	NI	Sciliano et al., 2016

*20 µL of the solution was added to each sample; AFs, aflatoxins (AFB₁ + AFB₂ + AFG₁ + AFG₂); AFB₁, aflatoxin B₁; AFB₂, aflatoxin B₂; AFG₁, aflatoxin G₁; AFG₂, aflatoxin G₂; AFM₁, aflatoxin M₁; TAF, total aflatoxins. CDPJ: corona discharge plasma jet; cm, centimeters; DBD, dielectric barrier discharge; I: results obtained after 1 and 5 min as well as on direct exposure; II: results obtained after 1 and 5 min as well as on indirect exposure; III: results obtained after 60 min time of exposure; IV: results obtained with 1 min time of plasma exposure; V: results obtained on 3 min time of plasma exposure; VI: results obtained after 3 and 5 min time of exposure; NO_x, nitrogen oxide; O₃, ozone; SDBD, surface dielectric barrier discharge; SDM, dielectric barrier surface micro-discharge; ↓ reduction or decrease; Φ degradation; NI, not informed.

TABLE 3 Summary of main outcomes from studies using cold atmospheric pressure plasma (CAP) in decontaminating other mycotoxins.

Type of mycotoxin	Initial concentration of mycotoxin	Matrix type	CAP source (process parameters)	Main outcomes	Degradation products	References
OTA and DON	OTA: 50 ng/mL DON: 10 µg/mL	Rice grain	DBD (Voltage: 25 kV; distance: 20 mm; time: 2, 4, 6, 8 min)	↓ in OTA (55%) and DON (61%), ↑ in rice protein and in prolamin contents	NI	Guo et al., 2023
DON	NI	<i>Fusarium</i>	SMD (Frequency: 7 kHz; power: 5 ± 0.15 W; distance: 3 mm; gas: O ₂ , N ₂ ; indirect exposure)	↓ of DON in flour: 6.4% (3 min), 52.9% (6 min), 54.7% (9 min) ↓ of DON in fungal mycelia: 30.0% (3 min), 48.4% (6 min), 48.5% (9 min)	NI	Wang Y. et al., 2022
DON	2,000–2,500 µg/kg	Wheat (standard solution)	DBD (Voltage: 50 kV; time: 8 min; CO ₂ , N ₂ , O ₂ and atmospheric air)	83.99% DON Φ on 8 min, ↑ in wheat quality and slight ↓ whiteness in wheat powder	C ₁₅ H ₂₂ O ₇ C ₁₅ H ₂₀ O ₅ C ₁₄ H ₁₆ O ₄ C ₁₅ H ₂₁ NO ₉	Chen et al., 2022
OTA	Fungal contamination	Barley (<i>A. niger</i> and <i>P. verrucosum</i>)	DCSBD 400 [Power: 350 W; gas flow rate: 10 sL/min; gases: CO ₂ (80%) + O ₂ (20%); time: 1 or 3 min]	OTA on inoculated barley ↓ from 38.9 to 17.5 and 17 ng/g on 1 min (55% ↓) and 3 min (56.2% ↓).	NI	Durek et al., 2018
DON	200 µL/495–505 g (Solutin 50 µg/mL)	Barley grain (standard solution)	DBD (Frequency: 3,500 Hz; voltage: 0–34 kV; power: 300 W; current: 1 A; distance: 2 mm; time: 0, 2, 4, 6, 8, 10 min; gas: O ₂ , N ₂)	48.9% DON ↓ (6-min), 54.4% DON ↓ (10 min)	NI	Feizollahi et al., 2020
FB ₁ ENB OTA ZEN DON	FB ₁ : 2 µg/mL ENB: 0.5 µg/mL OTA: 0.5 µg/mL ZEN: 2 µg/mL DON: 10 µg/mL	<i>In vitro</i> Corn (standard solution)	DBD (Frequency: 20 kHz; voltage: 6 kV; distance: 12 mm; time: 10 min; gas: He and O ₂)	66% ↓ in FB ₁ and ENB ↓ (1.1 min half-life), OTA and ZEN ↓ (2.6 min half-life), slow ↓ in DON (74 min half-life)	AFB ₁ : C ₁₇ H ₁₂ O ₆ C ₁₇ H ₁₄ O ₈ C ₁₆ H ₁₂ O ₇ C ₁₅ H ₁₂ O ₇ C ₁₅ H ₁₀ O ₅ C ₁₅ H ₁₀ O ₇ C ₁₄ H ₁₂ O ₅ ZEN: C ₁₈ H ₂₂ O ₅ C ₁₈ H ₂₂ O ₆ C ₂₁ H ₂₄ O ₈	Wielogorska et al., 2019
ZEN	15, 10, 15 and 20 µg/mL	<i>In vitro</i> Corn kernels and flour (standard solution) Wheat kernels and flour (standard solution)	SDM (Frequency: 7 kHz; power: 10–30 W; distance: 1, 2, 3, 5 mm; indirect exposure)	96.18% ↓ in ZEN (3-min at 30 W), ↑ in wheat and corn flour glutens, 50.55% Φ of ZEN in wheat (20 min), 58.07% Φ of ZEN in corn (20 min)	C ₁₈ H ₂₂ O ₇ C ₁₈ H ₂₂ O ₈ C ₁₈ H ₂₂ O ₆ C ₁₇ H ₂₀ O ₆	Liu et al., 2024

(Continued)

TABLE 3 (Continued)

Type of mycotoxin	Initial concentration of mycotoxin	Matrix type	CAP source (process parameters)	Main outcomes	Degradation products	References
OTA	20, 30 and 50 µg/mL (solution 100 µg/mL)	Raisin (standard solution)	GPSDP (Voltage: 0–30 kV; time: 4, 10 min; gas: Atmospheric air; indirect exposure)	62% Φ in OTA (4 min), 100% Φ of OTA (10 min), OTA Φ in non-toxic PheA, no ↑ in raisin quality attributes	C ₁₉ H ₁₇ ClNO ₄ C ₁₁ H ₉ ClNO ₅ C ₁₁ H ₉ NO ₄	Wang et al., 2024
OTA	100 µg/kg	Pistachio kernels (standard solution)	SDBD (Frequency: 23 kHz; voltage: 6 kV; power: 42–425 W; time: 4, 60 min; gas: O ₃ and NO _x ; indirect exposure)	37% ↓ in OTA	NI	Laika et al., 2024
DON	100 µg/mL	DON in aqueous suspension and in powdered form	HVCAP (Frequency: 50 Hz; voltage: 70, 80, 85 kV; time: 0, 5, 10, 20 min; gas: O ₂ and N ₂ ; direct exposure)	99% ↓ in DON in aqueous suspension (20 min), 33% ↓ in DON in powdered state (20 min)	NI	Ott et al., 2021
OTA FB ₂	NI	<i>A. niger</i>	Plasma jet (Frequency: 25 kHz; voltage: 25 kV; distance: 12 mm; time: 6, 7.5 min; gas: Ar; indirect exposure)	No FB ₂ and OTA were detected	NI	Ouf et al., 2014
DON Trichothecenes FBs ZEN	DON and Trichothecenes: 27 µg/mL, FBs: 25 µg/mL, ZEN: 27 µg/mL	<i>In vitro</i>	SDBD (Frequency: 40 kHz; voltage: 7–10 kV; distance: 5 mm; time: 8 min; gas: O ₂ , N ₂ ; indirect exposure)	2.7 to 0.11 mg/kg ↓ in DON; 90% ↓ in Trichothecenes, 93% ↓ in FBs, 97% ↓ in DAS, 100% ↓ of ZEN	NI	Hojnik et al., 2019
T-2 HT-2	T-2: ~85 µg/kg HT-2: ~87 µg/kg	Oat flour	DBD (Frequency: 25 kHz–2,5 kV; power: 6 W; time: 10, 20 and 30 min; gas: O ₂ , N ₂ , Ar and Air)	43% Φ of T-2 (30 min), 38% Φ of HT-2 (30 min)	NI	Kiš et al., 2020
FB ₁ DON ZEN T2	1.5 µL (solution 100 µg/mL)	<i>In vitro</i> Pure standards of FB ₁ , DON, ZEN, T2	DBD (Frequency: 17 kHz; voltage: 38 kV; distance: 2 mm; power density: 4 W/cm ² ; waveform: pulsed sine; time: 5 sec, 10 sec, 20 sec, 30 sec, 1 min; gas: O ₂ , N ₂)	99% Φ of FB ₁ , DON, T2, ZEN (1 min)	NI	Ten-Bosch et al., 2017
PAT	100 µg/kg	Apple	DBD (Frequency: 10–50 kHz; voltage: 17–23 kV; time: 10 min; gas: O ₂ , N ₂)	55% ↓ in PAT (2.5 and 8.5 min); 99% ↓ in PAT (10 min)	NI	Shirazi et al., 2025

ENB, enniatin B; DAS, diacetoxyscirpenol; DON, deoxynivalenol; FBs, fumonisins; FB₁, fumonisin B₁; FB₂, fumonisin B₂; PTA, patulin; TEN, tentoxin; OTA, ochratoxin A; T2, T2 toxin; ZEN, zearalenone; CO₂, carbon dioxide; DBD, dielectric barrier discharge; DCSBD, diffuse coplanar surface barrier discharge; GPSDP, gas-phase surface discharge plasma; O₂, oxygen molecule; PheA, phenylalanine; SDBD, surface dielectric barrier discharge; SMD, dielectric barrier surface micro-discharge; ↑ represents small change or affects; ↓ reduction or decrease; Φ degradation; NI: not informed.

leaving the potential for mycotoxin mitigation largely unexplored (Yinxin et al., 2022; Pinela and Ferreira, 2017; Yarabbi et al., 2023). A recent study highlighted the effectiveness of CAP generated with DBD in degrading PAT in fresh-cut apple slices while simultaneously enhancing their quality attributes (Shirazi et al., 2025). Treatment with 23 kV for 2.5 and 8.5 min gave 55 to 99% reduction of PAT to the undetectable levels, demonstrating the importance of high voltage and optimized exposure time. The degradation was attributed to ROS produced during plasma discharge, which facilitated oxidative breakdown into seven TPs (Figure 8) including $C_{10}H_{23}O_4$ (P1), $C_8H_{16}O_3$ (P2), $C_5H_{10}O_2$ (P3), $C_4H_8O_2$ (P4), $C_3H_6O_2$ (P5), $C_3H_8O_3$ (P6), and $C_4H_8O_3$ (P7) (Shirazi et al., 2025; Xue et al., 2021). These findings highlight the dual benefits of CAP in mitigating mycotoxins and enhancing food quality. The demonstrated efficacy of CAP for perishable products like fresh-cut fruits provides a foundation for broader applications and indicates the potential for further research and development in food safety and preservation strategies.

The efficiency of CAP in degrading different mycotoxins and reducing their toxicity depends on its ability to induce structural modifications in the target mycotoxin molecules, particularly at the molecular sites responsible for their toxicity. Degradation is confirmed only when less toxic molecules are generated. However, despite the significance of understanding the toxicity of degradation products, only 7 out of the 30 studies reviewed provided data on the by-products of the given toxins degraded by the CAP technology. In conformity with these results, the TPs mentioned and those newly emerging should be comprehensively studied for their potential toxicities and risks. While challenging, accurately measuring the maximum number of detectable TPs requires highly sensitive methods, likely the use of both high-resolution mass spectrometry (HRMS) and low-resolution MS, for inclusive proof of identities, validation, and quantification of all novel TPs. To ensure compliance with the guidelines set by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), both *in vitro* and *in vivo* studies are essential to assess the overall toxicological profiles of the TPs generated by CAP treatments.

6 Effects of cold atmospheric plasma on the food matrix

Assessing the impact of CAP on food products is crucial, particularly regarding their physicochemical, sensory, and nutritional properties. Research indicates that CAP treatment does not significantly alter the nutritional composition of food matrices. Studies on raw materials like rice and wheat, as well as processed products like wheat flour, show that starch content remains largely unaffected by CAP exposure (Guo et al., 2023; Chen et al., 2022; Zhi et al., 2023; Zhao L. et al., 2024).

Regarding the sensory attributes, specifically color, CAP treatment of rice revealed no significant changes in its characteristic appearance (Zhi et al., 2023). In the case of milk, however, Nikmaram and Keener (2022) observed a non-perceptible color change in skim milk, while a slight but perceptible yellowing occurred in whole milk. This yellowing effect is likely due to the

oxidation of fats and proteins induced by CAP which is a source of highly reactive species (Ali et al., 2023b; Segat et al., 2015).

The limited impact of CAP on the food matrices may also be attributed to its inability to penetrate deeply into the product. For example, in rice, the presence of husks reduces CAP's effectiveness (Guo et al., 2023). Similar results were observed in corn kernels, where analytical methods such as X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS) demonstrated that 8 min treatment with air plasma generated with SBD led to the slight oxidation of corn kernel surface, while scanning electron microscopy (SEM) showed no significant differences between CAP-treated and control samples (Hojnik et al., 2020).

CAP treatment can have varying effects on the food protein composition. While there were significant differences in specific proteins, such as prolamin and globulin, between the CAP-treated and control samples, the overall protein content remained unaffected. These findings are consistent with those of Zhi et al. (2023), where even prolonged exposure to CAP (60 min) did not result in significant changes. Regarding fatty acid (FA) content, CAP treatment was observed to increase the number of FA types over time, generating 3 to 5 new FAs. However, the primary FA components in wheat showed no significant difference compared to the control, suggesting that CAP does not alter the total FA content (Guo et al., 2023). In contrast, Zhi et al. (2023) reported a significant upsurge of 275.3% in free FA content in rice, indicating that CAP can indeed influence such composition. These findings collectively indicate that the application of CAP using different system parameters and exposure times had none or negligible qualitative effects on food matrices, as described for various items including dairy products (milk), and cereal grains (e.g., rice, wheat, corn).

7 Challenges of cold atmospheric plasma application in the food industry

CAP is an emerging technology in food mycotoxin decontamination due to its high efficiency, environmentally friendly nature, and dual functionality in degrading toxins and inhibiting the growth of toxigenic microorganisms (Marshall et al., 2020; Alizadeh et al., 2021; Ouf et al., 2014; Wang et al., 2020). Despite these advantages, several challenges obstruct its industrial application. Scalability remains a major challenge, while the DBD model, as the easiest of all possible options and most used in research, presents significant obstacles for industrial implementation (Neuenfeldt et al., 2023; Guo et al., 2023). The lack of standardized range for key parameters such as discharge frequency, voltage, and power, also complicate its optimization for large-scale use (Neuenfeldt et al., 2023). Future advancements should prioritize the development of modular, scalable CAP systems and the establishment of standardized operational parameters to broaden CAP's applicability while achieving consistent and reproducible outcomes. The cost of CAP equipment, energy demands, and limited penetration of reactive species in complex food matrices remains another barrier to be addressed (Chakka et al., 2021). Designing cost-effective, energy-efficient CAP devices integrated with mechanisms like agitation

systems or rotating conveyor belts could enhance reactive species distribution, improve decontamination efficiency, and facilitate industrial applications.

The literature highlights a well-understood degradation pathway for mycotoxins like AFB₁ and DON. However, there is limited knowledge regarding the degradation products and cytotoxicity of other important mycotoxins, such as AFM₁ and OTA (Hojnik et al., 2020; Deng et al., 2020). Additionally, research on the effects of CAP on the physicochemical, nutritional, and sensory properties of food matrices is scarce, underscoring the need for further investigation in this area while maximizing CAP efficiency (Guo et al., 2023). Taken together, overcoming these obstacles will need interdisciplinary collaboration across engineering, toxicology, and food science. Such efforts will be pivotal in advancing CAP as a sustainable, scalable, and reliable technology in the food sector.

8 Concluding remarks and future perspectives

This systematic review confirms the effectiveness of CAP in degrading mycotoxins such as AFB₁ and DON. The extensive knowledge of the degradation compounds and their reduced toxicity underscores CAP's potential in food safety applications. However, the review highlights a significant gap in research regarding the cytotoxicity of these degradation by-products. Future studies should focus on the cytotoxicological assessment of by-products from the breakdown of a broader range of mycotoxins, including AFs, FBs, OTs, ZEN, DON, T-2, HT-2 and PAT. CAP also shows promise in preventing mycotoxin contamination by inhibiting the growth of toxigenic fungi. The technology effectively targets fungal spores and damages the DNA responsible for mycotoxin biosynthesis, offering a dual approach to both degrading existing mycotoxins and preventing their formation. Despite its potential, the industrial application of CAP faces several challenges, particularly regarding the practical viability of large-scale implementation. Key obstacles include the need for more robust equipment design and the optimization of operational parameters, as there is currently no consensus on the best practices for these variables. The literature suggests that CAP has a minimal impact on the nutritional and sensory qualities of food, making it an attractive option for the food industry. However, research in this area remains limited, highlighting the need for more studies to fully understand the CAP efficacy on different food matrices. As a cutting-edge method for mycotoxin decontamination, addressing these research gaps is crucial for advancing the development and industrial applicability of CAP. Further exploration will enhance the efficiency of decontamination processes, ultimately contributing to the production of safer food products on a larger scale.

Data availability statement

The original contributions presented in this study are included in this article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

AO: Conceptualization, Investigation, Writing – original draft, Writing – review and editing. SA: Formal Analysis, Writing – review and editing. CC: Validation, Writing – review and editing. SU: Investigation, Writing – original draft. KP: Investigation, Writing – original draft. JW: Visualization, Writing – review and editing. NH: Visualization, Writing – review and editing. CO: Funding acquisition, Supervision, Writing – review and editing.

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Conflict of interest

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References

- Abdel-Fattah, E. (2013). Investigation of capacitively coupled argon plasma driven at various frequencies and validation of surface waves excitation. *Phys. Lett. A*. 377, 297–302. doi: 10.1016/j.physleta.2012.11.014
- Abrunhosa, L., Morales, H., Soares, C., Calado, T., Vila-Chã, A. S., Pereira, M., et al. (2016). A review of mycotoxins in food and feed products in Portugal and estimation of probable daily intakes. *Crit. Rev. Food Sci. Nutr.* 56, 249–265. doi: 10.1080/10408398.2012.720619
- Akbari, P., Braber, S., Varasteh, S., Alizadeh, A., Garssen, J., and Fink-Gremmels, J. (2017). The intestinal barrier as an emerging target in the toxicological assessment of mycotoxins. *Arch. Toxicol.* 91, 1007–1029. doi: 10.1007/s00204-016-1794-8
- Ali, S., Franco, B. B., Rezende, V. T., Freire, L. G. D., de Paiva, E. L., Haikal, M. C. F., et al. (2024). Exposure assessment of children to dietary mycotoxins: a pilot study conducted in Ribeirão Preto, São Paulo, Brazil. *Food Res. Int.* 180:114087. doi: 10.1016/j.foodres.2024.114087
- Ali, S., Freire, L. G. D., Rezende, V. T., Noman, M., Ullah, S., Abdullah, et al. (2023a). Occurrence of mycotoxins in foods: unraveling the knowledge gaps on their persistence in food production systems. *Foods* 12:4314. doi: 10.3390/foods12234314
- Ali, S., Rezende, V. T., Ullah, S., de Paiva, E. L., Tonin, F. G., Abdullah, et al. (2023b). Food processing and challenges in the food production and quality: the foodomics approach. *Food Biosci.* 56:103217. doi: 10.1016/j.fbio.2023.103217
- Alizadeh, A. M., Hashempour-Baltork, F., Khaneghah, A. M., and Hosseini, H. (2021). New perspective approaches in controlling fungi and mycotoxins in food using emerging and green technologies. *Curr. Opin. Food Sci.* 39, 7–15. doi: 10.1016/j.cofs.2020.12.006
- Alvito, P., and Pereira-da-Silva, L. (2022). Mycotoxin exposure during the first 1000 days of life and its impact on children's health: a clinical overview. *Toxins* 14:189. doi: 10.3390/toxins14030189
- Anderson, N. M. (2019). Recent advances in low moisture food pasteurization. *Curr. Opin. Food Sci.* 29, 109–115. doi: 10.1016/j.cofs.2018.11.001
- Arcella, D., Gergelova, P., Innocenti, M. L., and Steinkellner, H. (2017). Human and animal dietary exposure to T-2 and HT-2 toxin. *EFSA J.* 15:e04972. doi: 10.2903/j.efsa.2017.4972
- Arce-López, B., Lizarraga, E., López De Mesa, R., and González-Peñas, E. (2021). Assessment of exposure to mycotoxins in Spanish children through the analysis of their levels in plasma samples. *Toxins* 13:150. doi: 10.3390/toxins13020150
- Basso, A. B. G., Ali, S., Corassin, C. H., Rosim, R. E., and Oliveria, C. A. F. (2023). Individual and combined decontamination effect of fermentation and ultrasound on aflatoxin B1 in wheat-based doughs: a preliminary study. *Qual. Assur. Saf. Crop.* 15, 96–103. doi: 10.15586/qas.v15i3.1244
- Borutova, R., Aragon, Y. A., Nährer, K., and Berthiller, F. (2012). Co-occurrence and statistical correlations between mycotoxins in feedstuffs collected in the Asia–Oceania in 2010. *Anim. Feed Sci. Technol.* 178, 190–197. doi: 10.1016/j.anifeedsci.2012.09.015
- Chakka, A. K., Sriraksha, M. S., and Ravishankar, C. N. (2021). Sustainability of emerging green non-thermal technologies in the food industry with food safety perspective: a review. *Lwt* 151:112140. doi: 10.1016/j.lwt.2021.112140
- Chen, X., Qiu, Y., Zhang, J., Guo, Y., Ding, Y., and Lyu, F. (2022). Degradation efficiency and products of deoxynivalenol treated by cold plasma and its application in wheat. *Food Control* 136:108874. doi: 10.1016/j.foodcont.2022.108874
- Clark, H. A., and Snedeker, S. M. (2006). Ochratoxin A: its cancer risk and potential for exposure. *J. Toxicol. Environ. Health B Crit. Rev.* 9, 265–296. doi: 10.1080/15287390500195570
- Deng, L.-Z., Tao, Y., Mujumdar, A. S., Pan, Z., Chen, C., Yang, X.-H., et al. (2020). Recent advances in non-thermal decontamination technologies for microorganisms and mycotoxins in low-moisture foods. *Trends Food Sci. Technol.* 106, 104–112. doi: 10.1016/j.tifs.2020.10.012
- Dickenson, A., Britun, N., Nikiforov, A., Leys, C., Hasan, M. I., and Walsh, J. L. (2018). The generation and transport of reactive nitrogen species from a low temperature atmospheric pressure air plasma source. *Phys. Chem. Chem. Phys.* 20, 28499–28510. doi: 10.1039/c8cp05762a
- Dickenson, A., Morabit, Y., Hasan, M. I., and Walsh, J. L. (2017). Directional mass transport in an atmospheric pressure surface barrier discharge. *Sci. Rep.* 7:14003. doi: 10.1038/s41598-017-14117-1
- Dünnbier, M., Schmidt-Bleker, A., Winter, J., Wolfram, M., Hippler, R., Weltmann, K.-D., et al. (2013). Ambient air particle transport into the effluent of a cold atmospheric-pressure argon plasma jet investigated by molecular beam mass spectrometry. *J. Phys. D Appl. Phys.* 46:435203. doi: 10.1088/0022-3727/46/43/435203
- Durek, J., Schlüter, O., Roscher, A., Durek, P., and Fröhling, A. (2018). Inhibition or stimulation of ochratoxin A synthesis on inoculated barley triggered by diffuse coplanar surface barrier discharge plasma. *Front. Microbiol.* 9:2782. doi: 10.3389/fmicb.2018.02782
- EFSA (2004). Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to aflatoxin B1 as undesirable substance in animal feed. *EFSA J.* 39, 1–27. doi: 10.2903/j.efsa.2004.39
- EU (2023). *Commission Regulation (EU) 2023/915 of 25 April 2023 on Maximum Levels for Certain Contaminants in Food and Repealing Regulation (EC) No 1881/2006*. Brussels: European Union.
- Fakhri, Y., Rahmani, J., Oliveira, C. A. F., Franco, L. T., Corassin, C. H., Saba, S., et al. (2019). Aflatoxin M1 in human breast milk: a global systematic review, meta-analysis, and risk assessment study (Monte Carlo simulation). *Trends Food Sci. Technol.* 88, 333–342. doi: 10.1016/j.tifs.2019.03.013
- Feizollahi, E., Iqdiar, B., Vasanthan, T., Thilakarathna, M. S., and Roopesh, M. S. (2020). Effects of atmospheric-pressure cold plasma treatment on deoxynivalenol degradation, quality parameters, and germination of barley grains. *Appl. Sci.* 10:3530. doi: 10.3390/app10103530
- Gavahian, M., and Cullen, P. J. (2019). Cold plasma as an emerging technique for mycotoxin-free food: efficacy, mechanisms, and trends. *Crit. Rev. Food Sci. Nutr.* 56, 193–214. doi: 10.1080/87559129.2019.1630638
- Gavahian, M., Mathad, G. N., Oliveira, C. A. F., and Khaneghah, A. M. (2021). Combinations of emerging technologies with fermentation: interaction effects for detoxification of mycotoxins? *Food Res. Int.* 141:110104. doi: 10.1016/j.foodres.2021.110104
- Gruber-Dorninger, C., Novak, B., Nagl, V., and Berthiller, F. (2017). Emerging mycotoxins: beyond traditionally determined food contaminants. *J. Agric. Food Chem.* 65, 7052–7070. doi: 10.1021/acs.jafc.6b03413
- Guo, J., He, Z., Ma, C., Li, W., Wang, J., Lin, F., et al. (2023). Evaluation of cold plasma for decontamination of molds and mycotoxins in rice grain. *Food Chem.* 402:134159. doi: 10.1016/j.foodchem.2022.134159
- Han, L., Boehm, D., Amias, E., Milosavljević, V., Cullen, P. J., and Bourke, P. (2016). Atmospheric cold plasma interactions with modified atmosphere packaging inducer gases for safe food preservation. *Innov. Food Sci. Emerg. Technol.* 38, 384–392. doi: 10.1016/j.ifset.2016.09.026
- Han, Q.-Y., Wen, X., Gao, J.-Y., Zhong, C.-S., and Ni, Y.-Y. (2023). Application of plasma-activated water in the food industry: a review of recent research developments. *Food Chem.* 405:134797. doi: 10.1016/j.foodchem.2022.134797
- Haque, M. A., Wang, Y., Shen, Z., Li, X., Saleemi, M. K., and He, C. (2020). Mycotoxin contamination and control strategy in human, domestic animal, and poultry: a review. *Microb. Pathog.* 142:104095. doi: 10.1016/j.micpath.2020.104095
- Harikrishna, S., Anil, P. P., Shams, R., and Dash, K. K. (2023). Cold plasma as an emerging nonthermal technology for food processing: a comprehensive review. *J. Agric. Food Res.* 14:100747. doi: 10.1016/j.jafr.2023.100747
- Hasan, M. I., and Walsh, J. L. (2017). Influence of gas flow velocity on the transport of chemical species in an atmospheric pressure air plasma discharge. *Appl. Phys. Lett.* 110:134102. doi: 10.1063/1.4979178
- Hasan, M. I., Morabit, Y., Dickenson, A., and Walsh, J. L. (2017). Impact of electrode geometry on an atmospheric pressure surface barrier discharge. *Appl. Phys. Lett.* 110:264101. doi: 10.1063/1.4985030
- Hertwig, C., Meneses, N., and Mathys, A. (2018). Cold atmospheric pressure plasma and low energy electron beam as alternative nonthermal decontamination technologies for dry food surfaces: a review. *Trends Food Sci. Technol.* 77, 131–142. doi: 10.1016/j.tifs.2018.05.011
- Hojnik, N., Cvelbar, U., Tavèar-Kalcher, G., Walsh, J. L., and Kržaj, I. (2017). Mycotoxin decontamination of food: cold atmospheric pressure plasma versus “classic” decontamination. *Toxins* 9:151. doi: 10.3390/toxins9050151
- Hojnik, N., Modic, M., Tavèar-Kalcher, G., Babič, J., Walsh, J. L., and Cvelbar, U. (2019). Mycotoxin decontamination efficacy of atmospheric pressure air plasma. *Toxins* 11:219. doi: 10.3390/toxins11040219
- Hojnik, N., Modic, M., Walsh, J. L., Žigon, D., Javornik, U., Plavec, J., et al. (2021). Unravelling the pathways of air plasma induced aflatoxin B1 degradation and detoxification. *J. Hazard. Mater.* 403:123593. doi: 10.1016/j.jhazmat.2020.123593
- Hojnik, N., Modic, M., Žigon, D., Kovač, J., Jurov, A., Dickenson, A., et al. (2020). Cold atmospheric pressure plasma-assisted removal of aflatoxin B1 from contaminated corn kernels. *Plasma Process. Polym.* 18:2000163. doi: 10.1002/ppap.202000163
- Hoppanová, L., Dylíková, J., Kováček, D., Medvecká, V., Ľurina, P., Kryštofová, S., et al. (2020). The effect of cold atmospheric pressure plasma on *Aspergillus ochraceus* and ochratoxin A production. *Antonie van Leeuwenhoek* 113, 1479–1488. doi: 10.1007/s10482-020-01457-8
- IARC (2023). Agents Classified by the IARC Monographs, 1–134 – IARC Monographs on the Identification of Carcinogenic Hazards to Humans. Available online at: <https://monographs.iarc.who.int/agents-classified-by-the-iarc/> (Accessed November 30, 2024).
- Kaur, M., Hüblerli, D., and Bayliss, K. L. (2020). Cold plasma: exploring a new option for management of postharvest fungal pathogens, mycotoxins and insect pests in Australian stored cereal grain. *Crop Pasture Sci.* 71, 715–724. doi: 10.1071/CP20078

- Khan, R. (2024). Mycotoxins in food: occurrence, health implications, and control strategies—A comprehensive review. *Toxicon* 248:108038. doi: 10.1016/j.toxicon.2024.108038
- Khaneghah, A. M., Fakhri, Y., Gahrue, H. H., Niakousari, M., and Sant'ana, A. (2019). Mycotoxins in cereal-based products during 24 years (1983–2017): a global systematic review. *Trends Food Sci. Technol.* 91, 95–105. doi: 10.1016/j.tifs.2019.06.007
- Kiš, M., Milošević, S., Vuljic, A., Herceg, Z., Vukušić, T., and Pleadin, J. (2020). Efficacy of low pressure DBD plasma in the reduction of T-2 and HT-2 toxin in oat flour. *Food Chem.* 316:126372. doi: 10.1016/j.foodchem.2020.126372
- Knutsen, H. K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., et al. (2017). Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. *EFSA J.* 15:e04718. doi: 10.2903/j.efsa.2017.4718
- Kovačević, V. V., Sretenović, G. B., Slikboer, E., Guaitella, O., Sobota, A., and Kuraica, M. M. (2018). The effect of liquid target on a nonthermal plasma jet—Imaging, electric fields, visualization of gas flow, and optical emission spectroscopy. *J. Phys. D Appl. Phys.* 51:065202. doi: 10.1088/1361-6463/aaa288
- Laika, J., Viteritti, E., Molina-Hernandez, J. B., Sergi, M., Neri, L., Laurita, R., et al. (2024). Efficiency of cold atmospheric plasma under ozone (O₃) and nitrogen oxide (NO_x) regimes on the degradation of aflatoxins and ochratoxin A in solid state and in spiked pistachio kernels. *Food Control* 159:110286. doi: 10.1016/j.foodcont.2024.110286
- Liu, M., Feng, J., Fan, Y., Yang, X., Chen, R., Xu, C., et al. (2024). Application of atmospheric cold plasma for zearalenone detoxification in cereals: kinetics, mechanisms, and cytotoxicity analysis. *J. Adv. Res.* doi: 10.1016/j.jare.2024.04.024 [Epub ahead of print].
- Loi, M., Logrieco, A. F., Pusztahelyi, T., Leiter, É., Hornok, L., and Pócsi, I. (2023). Advanced mycotoxin control and decontamination techniques in view of an increased aflatoxin risk in Europe due to climate change. *Front. Microbiol.* 13:1085891. doi: 10.3389/fmicb.2022.1085891
- Majeed, M., Khaneghah, A. M., Kadmi, Y., Khan, M. U., and Shariati, M. A. (2017). Assessment of ochratoxin A in commercial corn and wheat products. *Curr. Res. Nutr. Food Sci.* 14, 116–120. doi: 10.2174/15734013136661703301558231
- Marshall, H., Meneely, J. P., Quinn, B., Zhao, Y., Bourke, P., Gilmore, B. F., et al. (2020). Novel decontamination approaches and their potential application for post-harvest aflatoxin control. *Trends Food Sci. Technol.* 106, 489–496. doi: 10.1016/j.tifs.2020.11.001
- Mir, S. A., Dar, B. N., Shah, M. A., Sofi, S. A., Hamdani, A. M., Oliveira, C. A. F., et al. (2021). Application of new technologies in decontamination of mycotoxins in cereal grains: challenges, and perspectives. *Food Chem. Toxicol.* 148:111976. doi: 10.1016/j.fct.2021.111976
- Misra, N. N., and Jo, C. (2017). Applications of cold plasma technology for microbiological safety in meat industry. *Trends Food Sci. Technol.* 64, 74–86. doi: 10.1016/j.tifs.2017.04.005
- Misra, N. N., Yadav, B., Roopesh, M. S., and Jo, C. (2018). Cold plasma for effective fungal and mycotoxin control in foods: mechanisms, inactivation effects, and applications. *Comprehens. Rev. Food Sci. Food Saf.* 18, 106–120. doi: 10.1111/1541-4337.12398
- Morabit, Y., Hasan, M. I., Whalley, R. D., Robert, E., Modic, M., and Walsh, J. L. (2021). A review of the gas and liquid phase interactions in low-temperature plasma jets used for biomedical applications. *Eur. Phys. J. D.* 75, 01–26. doi: 10.1140/epjd/s10053-021-00015-1
- Moretti, A., Pascale, M., and Logrieco, A. F. (2019). Mycotoxin risks under a climate change scenario in Europe. *Trends Food Sci. Technol.* 84, 38–40. doi: 10.1016/j.tifs.2018.03.008
- Mošovská, S., Medvecká, V., Gregov, M., Tomeková, J., Valík, L., Mikulajová, A., et al. (2019). Plasma inactivation of *Aspergillus flavus* on hazelnut surface in a diffuse barrier discharge using different working gases. *Food Control* 104, 256–261. doi: 10.1016/j.foodcont.2019.05.003
- Naeem, I., Ismail, A., Riaz, M., Aziz, M., Akram, K., Shahzad, M. A., et al. (2024). Aflatoxins in the rice production chain: a review on prevalence, detection, and decontamination strategies. *Food Res. Int.* 188:114441. doi: 10.1016/j.foodres.2024.114441
- Nahle, S., El Khoury, A., Savvaidis, I., Chokr, A., Louka, N., and Atoui, A. (2022). Detoxification approaches of mycotoxins: by microorganisms, biofilms and enzymes. *Int. J. Food Contamin.* 9:3. doi: 10.1186/s40550-022-00089-2
- Naidis, G. V. (2014). Production of active species in cold helium–air plasma jets. *Plasma Sourc. Sci. Technol.* 23:065014. doi: 10.1088/0963-0252/23/6/065014
- Neme, K., and Mohammed, A. (2017). Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategy. A review. *Food Control* 78, 412–425. doi: 10.1016/j.foodcont.2017.03.012
- Nesic, K., Habschied, K., and Mastanjevic, K. (2021). Possibilities for the biological control of mycotoxins in food and feed. *Toxins* 13:198. doi: 10.3390/toxins13030198
- Neuenfeldt, N. H., Silva, L. P., Pessoa, R. S., and Rocha, L. O. (2023). Cold plasma technology for controlling toxigenic fungi and mycotoxins in food. *Curr. Opin. Food Sci.* 52:101045. doi: 10.1016/j.cofs.2023.101045
- Nguyen, T., Palmer, J., Phan, N., Shi, H., Keener, K., and Flint, S. (2022). Control of aflatoxin M1 in skim milk by high voltage atmospheric cold plasma. *Food Chem.* 386:132814. doi: 10.1016/j.foodchem.2022.132814
- Niemira, B. A. (2012). Cold plasma for sustainable food production and processing. *Annu. Rev. Food Sci. Technol.* 3, 125–142. doi: 10.1146/annurev-food-022811-101132
- Nikmaram, N., and Keener, K. M. (2022). Degradation of aflatoxin M1 in skim and whole milk using high voltage atmospheric cold plasma (HVACP) and quality assessment. *Food Res. Int.* 162:112009. doi: 10.1016/j.foodres.2022.112009
- Nikmaram, N., and Keener, K. M. (2023). Aflatoxin M1 degradation using high voltage atmospheric cold plasma (HVACP) technology. *Crit. Rev. Food Sci. Nutr.* 63, 503–515. doi: 10.1080/01919512.2023.2173556
- Nikmaram, N., Brückner, L., Cramer, B., Humpf, H.-U., and Keener, K. M. (2023). Degradation products of aflatoxin M1 (AFM1) formed by high voltage atmospheric cold plasma (HVACP) treatment. *Toxicon* 230:107160. doi: 10.1016/j.toxicon.2023.107160
- Ojha, S., Fröhling, A., Durek, J., Ehlbeck, J., Tiwari, B. K., Schlüter, O. K., et al. (2021). Principles and application of cold plasma in food processing. *Innov. Food Process. Technol.* 1, 519–540. doi: 10.1016/B978-0-08-100596-5.23033-3
- Ostry, V., Malir, F., Toman, J., and Grosse, Y. (2017). Mycotoxins as human carcinogens—the IARC Monographs classification. *Mycotoxin Res.* 33, 65–73. doi: 10.1007/s12550-016-0265-7
- Ott, L. C., Appleton, H. J., Shi, H., Keener, K., and Mellata, M. (2021). High voltage atmospheric cold plasma treatment inactivates *Aspergillus flavus* spores and deoxynivalenol toxin. *Food Microbiol.* 95:103669. doi: 10.1016/j.fm.2020.103669
- Ouf, S. A., Basher, A. H., and Mohamed, A.-A. H. (2014). Inhibitory effect of double atmospheric pressure argon cold plasma on spores and mycotoxin production of *Aspergillus niger* contaminating date palm fruits. *J. Sci. Food Agric.* 95, 3204–3210. doi: 10.1002/jsfa.7060
- Pankaj, S. K., and Keener, K. M. (2017). Cold plasma: background, applications and current trends. *Curr. Opin. Food Sci.* 16, 49–52. doi: 10.1016/j.cofs.2017.07.008
- Park, J., Henins, I., Herrmann, H. W., Selwyn, G. S., Jeong, J. Y., Hicks, R. F., et al. (2000). An atmospheric pressure plasma source. *Appl. Phys. Lett.* 76, 288–290. doi: 10.1063/1.125724
- Patriarca, A., and Pinto, V. P. (2017). Prevalence of mycotoxins in foods and decontamination. *Curr. Opin. Food Sci.* 14, 50–60. doi: 10.1016/j.cofs.2017.01.011
- Perinban, S., Orsat, V., Lyew, D., and Raghavan, V. (2022). Effect of plasma activated water on *Escherichia coli* disinfection and quality of kale and spinach. *Food Chem.* 397:133793. doi: 10.1016/j.foodchem.2022.133793
- Pinela, J., and Ferreira, I. C. F. R. (2017). Nonthermal physical technologies to decontaminate and extend the shelf-life of fruits and vegetables: trends aiming at quality and safety. *Crit. Rev. Food Sci. Nutr.* 57, 2095–2111. doi: 10.1080/10408398.2015.1046547
- Polito, J., and Kushner, M. J. (2024). A hierarchical model for bacterial cell inactivation in solution by direct and indirect treatment using cold atmospheric plasmas. *J. Phys. D Appl. Phys.* 57:405207. doi: 10.1088/1361-6463/ad5f3b
- Puligundla, P., Lee, T., and Mok, C. (2020). Effect of corona discharge plasma jet treatment on the degradation of aflatoxin B1 on glass slides and in spiked food commodities. *LWT* 124:108333. doi: 10.1016/j.lwt.2019.108333
- Rahnavard, M. A., Zare, D., Nassiri, S. M., Taghvaei, H., and Fazaeli, M. (2024). Impact of gliding arc cold plasma on deactivating aflatoxin and post-treatment fungal growth on wheat grains. *Food Control* 164:110597. doi: 10.1016/j.foodcont.2024.110597
- Richard, J. L. (2007). Some major mycotoxins and their mycotoxicoses—An overview. *Int. J. Food Microbiol.* 119, 3–10. doi: 10.1016/j.ijfoodmicro.2007.07.019
- Sakiyama, Y., Graves, D. B., Chang, H.-W., Shimizu, T., and Morfill, G. E. (2012). Plasma chemistry model of surface microdischarge in humid air and dynamics of reactive neutral species. *J. Phys. D Appl. Phys.* 45:425201. doi: 10.1088/0022-3727/45/42/425201
- Sakudo, A., and Yagyu, Y. (2024). Degradation of toxins derived from foodborne pathogens by atmospheric-pressure dielectric-barrier discharge. *Int. J. Mol. Sci.* 25:5986. doi: 10.3390/ijms25115986
- Schoevers, E. J., Santos, R. R., Colenbrander, B., Fink-Gremmels, J., and Roelen, B. A. (2012). Transgenerational toxicity of Zearalenone in pigs. *Reprod. Toxicol.* 34, 110–119. doi: 10.1016/j.reprotox.2012.03.004
- Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., et al. (2022). Assessment of information as regards the toxicity of fumonisins for pigs, poultry and horses. *EFSA J.* 20:e07534. doi: 10.2903/j.efsa.2022.7534
- Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., Del Mazo, J., Grasl-Kraupp, B., et al. (2020). Risk assessment of aflatoxins in food. *EFSA J.* 18:e06040. doi: 10.2903/j.efsa.2020.6040

- Segat, A., Misra, N. N., Cullen, P. J., and Innocente, N. (2015). Atmospheric pressure cold plasma (ACP) treatment of whey protein isolate model solution. *Innovative Food Science & Emerging Technologies*. 29, 247–254. doi: 10.1016/j.ifset.2015.03.014
- Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., et al. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 349:g7647. doi: 10.1136/bmj.g7647
- Shi, H., Cooper, B., Stroshine, R. L., Ileleji, K. E., and Keener, K. M. (2017b). Structures of degradation products and degradation pathways of aflatoxin B1 by high-voltage atmospheric cold plasma (HVACP) treatment. *J. Agric. Food Chem.* 65, 6222–6230. doi: 10.1021/acs.jafc.7b01604
- Shi, H., Ileleji, K., Stroshine, R. L., Keener, K., and Jensen, J. L. (2017a). Reduction of aflatoxin in corn by high voltage atmospheric cold plasma. *Food Bioproc. Technol.* 10, 1042–1052. doi: 10.1007/s11947-017-1873-8
- Shimizu, T., Sakiyama, Y., Graves, D. B., Zimmermann, J. L., and Morfill, G. E. (2012). The dynamics of ozone generation and mode transition in air surface micro-discharge plasma at atmospheric pressure. *N. J. Phys.* 14:103028. doi: 10.1088/1367-2630/14/10/103028
- Shirazi, S., Ramezan, Y., Moslehshad, M., Ghomi Marzdashti, H., and Mirsaeedghazi, H. (2025). Effects of cold atmospheric plasma on patulin degradation, polyphenol oxidase inactivation, and other physicochemical properties of fresh-cut apple slices during storage. *Food Chem.* 465:142017. doi: 10.1016/j.foodchem.2024.142017
- Siciliano, I., Spadaro, D., Prella, A., Vallauri, D., Cavallero, M. C., Garibaldi, A., et al. (2016). Use of cold atmospheric plasma to detoxify hazelnuts from aflatoxins. *Toxins* 8:125. doi: 10.3390/toxins8050125
- Sun, S., Anderson, N. M., and Keller, S. (2014). Atmospheric pressure plasma treatment of black peppercorns inoculated with *Salmonella* and held under controlled storage. *J. Food Sci.* 79, E2441–E2446. doi: 10.1111/1750-3841.12696
- Ten-Bosch, L., Pfohl, K., Avramidis, G., Wieneke, S., Viöl, W., and Karlovsky, P. (2017). Plasma-based degradation of mycotoxins produced by *Fusarium*, *Aspergillus*, and *Alternaria* species. *Toxins* 9:97. doi: 10.3390/toxins9030097
- Wan, Z., Pankaj, S. K., Mosher, C., and Keener, K. M. (2019). Effect of high voltage atmospheric cold plasma on inactivation of *Listeria innocua* on Queso Fresco cheese, cheese model and tryptic soy agar. *LWT* 102, 268–275. doi: 10.1016/j.lwt.2018.11.096
- Wang, L., Liu, X., Cai, R., Ge, Q., Zhao, Z., Yue, T., et al. (2022). Detoxification of ochratoxin A by pulsed light in grape juice and evaluation of its degradation products and safety. *Innov. Food Sci. Emerg. Technol.* 78:103024. doi: 10.1016/j.ifset.2022.103024
- Wang, X., Wang, S., Yan, Y., Wang, W., Zhang, L., and Zong, W. (2020). The degradation of *Alternaria* mycotoxins by dielectric barrier discharge cold plasma. *Food Control* 117:107333. doi: 10.1016/j.foodcont.2020.107333
- Wang, Y., Li, B., Shang, H., Ma, R., Zhu, Y., Yang, X., et al. (2022). Effective inhibition of fungal growth, deoxynivalenol biosynthesis and pathogenicity in cereal pathogen *Fusarium* spp. by cold atmospheric plasma. *Chem. Eng. J.* 437:135307. doi: 10.1016/j.cej.2022.135307
- Wang, Y., Zheng, Y., Wang, J., Mu, L., Zhang, W., Huang, M., et al. (2024). Effect of plasma on ochratoxin degradation and raisin properties. *J. Food Eng.* 375:112051. doi: 10.1016/j.jfoodeng.2024.112051
- Wen, G., Liang, Z., Xu, X., Cao, R., Wan, Q., and Ji, G. (2020). Inactivation of fungal spores in water using ozone: kinetics, influencing factors and mechanisms. *Water Res.* 185:116218. doi: 10.1016/j.watres.2020.116218
- Wen, G., Xu, X., Zhu, H., Huang, T., and Ma, J. (2017). Inactivation of four genera of dominant fungal spores in groundwater using UV and UV/PMS: efficiency and mechanisms. *Chem. Eng. J.* 328, 619–628. doi: 10.1016/j.cej.2017.07.05
- Wen, G., Zhao, D., Xu, X., Chen, Z., Huang, T., and Ma, J. (2019). Inactivation of fungi from four typical genera in groundwater using PMS/Cl₂ system: efficacy, kinetics and mechanisms. *Chem. Eng. J.* 357, 567–578. doi: 10.1016/j.cej.2018.09.195
- Wielogorska, E., Ahmed, Y., Meneely, J., Graham, W. G., Elliott, C. T., and Gilmore, B. F. (2019). A holistic study to understand the detoxification of mycotoxins in maize and impact on its molecular integrity using cold atmospheric plasma treatment. *Food Chem.* 301:125281. doi: 10.1016/j.foodchem.2019.125281
- Wu, Y., Cheng, J.-H., and Sun, D.-W. (2021). Blocking and degradation of aflatoxins by cold plasma treatments: applications and mechanisms. *Trends Food Sci. Technol.* 109, 647–661. doi: 10.1016/j.tifs.2021.01.053
- Xue, M., Wang, T., Sun, Q., Qu, G., Jia, H., and Zhu, L. (2021). Insights into the highly efficient detoxification of the biotoxin patulin in water by discharge plasma oxidation. *Chem. Eng. J.* 411:128432. doi: 10.1016/j.cej.2021.128432
- Yarabbi, H., Soltani, K., Sangatash, M. M., Yavarmansh, M., and Zenoozian, M. S. (2023). Reduction of microbial population of fresh vegetables (carrot, white radish) and dried fruits (dried fig, dried peach) using atmospheric cold plasma and its effect on physicochemical properties. *J. Agric. Food Res.* 14:100789. doi: 10.1016/j.jafr.2023.100789
- Yinxin, L., Can, Z., Menglu, H., Cui, S., Jinping, C., Jingyu, W., et al. (2022). Effect of cold atmospheric plasma on the gray mold rot of postharvest mulberry fruit. *Food Control* 137:108906. doi: 10.1016/j.foodcont.2022.108906
- Zhang, K., Perussello, C. A., Milosavljević, V., Cullen, P. J., Sun, D.-W., and Tiwari, B. K. (2019). Diagnostics of plasma reactive species and induced chemistry of plasma treated foods. *Crit. Rev. Food Sci. Nutr.* 59, 812–825. doi: 10.1080/10408398.2018.1564731
- Zhao, L., Sheng, X., Li, S., Yan, W., Qian, J., Wang, J., et al. (2024). Assessment of non-thermal plasma for decontamination against *Aspergillus flavus* and aflatoxin B1 in maize. *Food Control* 163:110521. doi: 10.1016/j.foodcont.2024.110521
- Zhao, L., Wang, J., Sheng, X., Li, S., Yan, W., Qian, J., et al. (2023). Non-thermal plasma inhibited the growth and aflatoxins production of *Aspergillus flavus*, degraded aflatoxin B1 and its potential mechanisms. *Chem. Eng. J.* 475:146017. doi: 10.1016/j.cej.2023.146017
- Zhao, X., Wang, N., Lu, Y., Li, Y., Zhao, T., Xu, J., et al. (2024). Effects of cold plasma on the growth and aflatoxin production of *Aspergillus flavus*. *Food Biosci.* 61:104552. doi: 10.1016/j.fbio.2024.104552
- Zhi, A., Shi, S., Li, Q., Shen, F., He, X., Fang, Y., et al. (2023). Aflatoxins degradation and quality evaluation in naturally contaminated rice by dielectric barrier discharge cold plasma. *Innov. Food Sci. Emerg. Technol.* 88:103426. doi: 10.1016/j.ifset.2023.103426



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Enhancing postharvest food safety: the essential role of non-thermal technologies in combating fungal contamination and mycotoxins

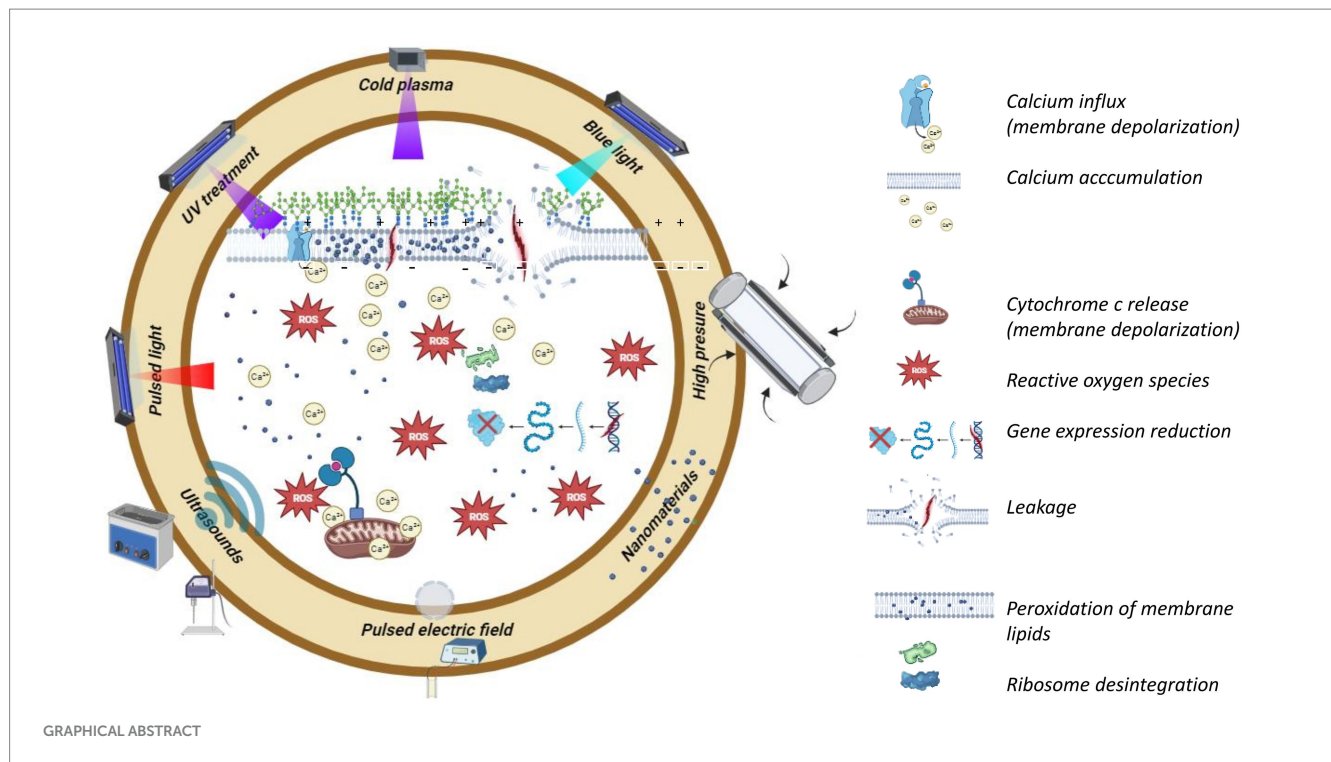
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During the production and storage of agricultural products, molds frequently occur as contaminants that can produce a wide range of secondary metabolites, the most important of which are mycotoxins. To solve these problems, the industry uses various methods, products and processes. This review examines the latest advances in novel non-thermal technologies for post-harvest inactivation of filamentous fungi and reduction of mycotoxins. These technologies include high pressure processes (HPP), ozone treatment, UV light, blue light, pulsed light, pulsed electric fields (PEF), cold atmospheric plasma (CAP), electron beams, ultrasound (US) and nanoparticles. Using data from previous studies, this review provides an overview of the primary mechanisms of action and recent results obtained using these technologies and emphasizes the limitations and challenges associated with each technology. The innovative non-thermal methods discussed here have been shown to be safe and efficient tools for reducing food mold contamination and infection. However, the effectiveness of these technologies is highly dependent on the fungal species and the structural characteristics of the mycotoxins. New findings related to the inactivation of fungi and mycotoxins underline that for a successful application it is essential to carefully determine and optimize certain key parameters in order to achieve satisfactory results. Finally, this review highlights and discusses future directions for non-thermal technologies. It emphasizes that they meet consumer demand for clean and safe food without compromising nutritional and sensory qualities.

KEYWORDS

emerging technologies, post-harvest treatment, filamentous fungal suppression, reduction of mycotoxin, food safety, food preservation, food quality



1 Introduction

The contamination of food and feed by fungi has become a global problem with a significant economic impact worldwide. Airborne fungal spores can easily infect, colonize, and spoil plants and food. The rapid spread and adaptability of the spores in different areas negates all active disease control strategies. In addition, the morphology and stress tolerance of fungal spores, as well as their production process and dormant state, are highly variable. It is estimated that fungi are responsible for losing up to 10–23% of crop products due to disease and additionally, 10 to 20% in the post-harvest processes (Stukenbrock and Gurr, 2023), and climate change can exacerbate the situation by creating conditions that make the occurrence and spread of foodborne hazards more likely because they weaken the resistance of host plants making them more susceptible to fungal diseases. On the other hand, it is possible that there will be a shift in mycotoxin-producing fungi and a change in global patterns of mycotoxin incidence (Casu et al., 2024; Delgado-Ospina et al., 2021). Mycotoxins are known to be dangerous secondary metabolites produced mostly by fungi of the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*. Over 500 mycotoxins have been reported, with aflatoxins, ochratoxin A (OTA), fumonisins, trichothecenes, and zearalenone being the most significant due to their impact on agriculture, economics, and public health (Moretti et al., 2017). They form a chemically heterogeneous family, grouped together by their harmful effects on human and animal's health, including permanent damage. Some of them are potentially mutagenic, teratogenic, carcinogenic, cytotoxic, neurotoxic, nephrotoxic, and estrogenic and contain immunosuppressive compounds (Laika et al., 2024a). European Food Safety Authority (EFSA) regularly publish reports on food safety issues, including mycotoxin-related recalls (www.efsa.europa.eu). According to Wu (2008), the average financial loss due to

an EU border rejection ranges between €7,000 and €11,000. These expenses primarily cover administrative processes such as sampling, transportation, and storage, excluding additional costs related to the final disposal or management of contaminated goods. With climate change expected to increase natural toxin contamination, including mycotoxins, the resulting economic impact is anticipated to be even greater (Wu and Mitchell, 2016). Meanwhile, the European Union's Rapid Alert System for Food and Feed (RASFF) reported that in 2023, mycotoxins ranked as the third most frequently notified hazard category. The majority of these cases involved the detection of aflatoxins, with nuts, nut products, and seeds being the most commonly affected product category. Mycotoxins can be transferred from a rotten part of vegetables and fruit with a high water content into the internal environment by diffusion. The concentration gradient of a penetrating substance through food is usually described by Fick's second law and by the solutions of this differential equation (da Silva Lima et al., 2022). Furthermore, some studies have shown that the integrity of the fruit tissue and the vegetable tissue plays an essential role in the diffusion of mycotoxins; in addition, the skins of the fruit become more susceptible to increasing ripeness (Enikova et al., 2020). Mycotoxin exposure also occurs in animals regardless of the farming system—whether intensive, extensive, or mixed. Consequently, mycotoxin contamination poses a risk in nomadic and integrated crop-livestock systems that depend on forages, crops, and animal byproducts (El-Sayed et al., 2022). Although effective agricultural practices, quality control measures, and proper storage techniques help reduce the risk of mycotoxin exposure in industrialized nations, research indicates that several developing regions still face a significant threat, ranging from severe to extreme levels of contamination. For example, mycotoxins are highly prevalent in North and Central America, as well as South Asia, while other areas, including Southeast Asia, Oceania, and Europe, experience

moderate to high levels of contamination (El-Sayed et al., 2022; Gruber-Dorninger et al., 2019).

Mycotoxins are highly resilient compounds that, once formed, are extremely difficult to eliminate from contaminated food and feed. They are chemically stable and can withstand various food processing methods, including high temperatures, freezing, and chemical treatments. Conventional decontamination techniques, such as washing, milling, or heating, often fail to completely remove them, and some methods may even lead to the formation of toxic byproducts (Awuchi et al., 2021). Consequently, controlling mycotoxins in food can be difficult and requires regular monitoring. Although preservatives and strict cleanliness in the factory (such as using a sterile line) can prevent spoilage by many fungal species, some fungi are highly resistant to preservatives and can persist even in storage conditions that typically inhibit unspecialized microbes. Therefore, alternatives other than antifungal agents must be available to control fungi and to avoid the emergence of resistant species. These alternatives must be able to prevent the proliferation of mycelia and the survival of spores and degrade mycotoxins that may be present in food. In this context, some alternatives to antimicrobials are thermal processing technologies, which are well established and relatively cost-effective, especially in large-scale production, and many types of food can be treated with these methods, from liquids to solids, but the main problem is that the process conditions not only lead to the loss of nutrients, bioactive compounds and sensory properties of the food but can also lead to browning reactions, severe dehydration, the formation of hydroxymethylfurfural, furfural, and acrylamide (Moreno-Vilet et al., 2018). For this reason, innovative non-thermal technologies have become increasingly important in recent years, as they can avoid the detrimental effects of thermal processing. They have low energy consumption, are adaptable, can be used on a larger number of food products and show better results in the control of filamentous fungi, spores and mycotoxins compared to thermal technologies (Yousefi et al., 2021), thus ensuring food safety and delivering a high-quality product. Therefore, this review aims to explore novel non-thermal technologies such as high pressure, ozone, UV treatment, blue light treatment, pulsed electric fields (PEF), cold plasma, electron beam (e-beam), plasma jet, pulsed light and nanomaterials that are becoming increasingly popular in food processing. In addition, using novel technologies that are more environmentally friendly, cleaner, and more efficient than conventional methods could help the food industry reduce its environmental impact. In addition, non-thermal technologies offer several potential benefits, such as improving the health attributes of foods by increasing the bioavailability of micronutrients and phytochemicals from both animal and plant sources, which have nutraceutical applications. Moreover, these technologies are used to enhance the physiological properties of proteins and carbohydrates in food, enabling their use in various food processing applications. This includes improving the release of taste compounds and enhancing the sensory quality of fermented products (Gao et al., 2019). However, there are some practical barriers that may arise for the use of non-thermal technologies, such as higher initial investment costs, high operating efficiency and higher processing costs with a higher energy input than a conventional thermal process with heat recovery capacity. In addition, the industrial scale of these technologies is a challenge as laws and regulations need to be put in place to ensure the safety of the processed food (Chacha et al., 2021).

The use of non thermal technologies to reduce fungal and mycotoxin in foods deserves attention, as demonstrated by some other reviews already published in the scientific literature; however, some of them are limited only to detoxification/removal of mycotoxins with the exclusion of fungi inactivation (Adebo et al., 2021; Wang et al., 2023a; Yousefi et al., 2021); or are not specifically focused on fungi (Lopes et al., 2023) or not updated to the last recent developments in innovative technologies (Deng et al., 2020).

This paper provides a comprehensive overview of using specific non-thermal technologies to reduce fungi and mycotoxins during post-harvest processing to maintain product quality. In addition, the opportunities and limitations of newly developed non-thermal methods are explored, providing valuable insight into emerging trends in food processing over the last 6 years. This paper contributes to understanding these innovations and their practical applications in various post-harvest products.

2 High-pressure processing (HPP)

High-pressure processing or high hydrostatic pressure (HHP) requires the immersion of a product into water and exposure to high hydrostatic pressure (from 100 up to 800 MPa) in a vessel (Aganovic et al., 2021). The product is commonly packed in a high-pressure-suitable packaging and held under pressure for some minutes until decompression. There are two types of HPP techniques depending on the type of food to be treated. Batch-type HPP is used for solid foods or products with big solid particles, whereas semi-continuous HPP is used for liquid and other products that may be pumped because they have the flexibility to be treated by semi-continuous procedures (Huang et al., 2020).

Although HPP is considered a non-thermal process, the increase in pressure causes adiabatic heating with a temperature rise of 2–3°C/100 MPa for water-based foods and up to 9°C/100 MPa for oil-based foods (Sehrawat et al., 2021). HPP is generally applied for food processing and shelf-life extension due to their inactivating effect on pathogenic and spoilage microorganisms. The determining factors are the types of microbes and their growth phases, duration of processing, pressure, food content, temperature, pH and water activity. Overall, HPP affects only non-covalent bonds as these are sensitive to pressure. HPP may be an effective post-harvest strategy to reduce microbial pathogens in fresh produce if plant tissue damage is minimal.

2.1 Mechanisms of action

In general, microorganism cell membrane damage is a critical factor in triggering cell death during HPP treatment and can lead to leakage of intracellular components and loss of homeostasis (Woldemariam and Emire, 2019). These effects lead to impaired cell functions. Four factors contribute to pressure-induced cell death in vegetative microorganisms: (i) unfolding of proteins and enzymes, including partial or complete denaturation; (ii) cell membrane phase transition and change in fluidity; (iii) disintegration of ribosomal subunit; and (iv) changes in intracellular pH related to enzyme inactivation and membrane damage (Gouvea et al., 2023). About fungal spores, HPP also leads to ruptures in the cell wall and membrane and cell membrane of ascospores and the ornamentation

on some of these ascospores, which resemble crowns. Recently, Hsiao et al. (2020) reported a degradation of intracellular and extracellular proteins leading to an inactive state in *Aspergillus flavus* spores and the formation of pores in the cell membrane and wall.

Studies showed that HPP could reduce mycotoxins by altering their chemical structure and inducing changes in noncovalent bonds (Azam et al., 2021). To date, however, only few studies have investigated the sensitivity of mycotoxins to HPP treatments. The majority of studies have been conducted using patulin in liquid media. Overall, evidence suggests that thiol content is critical factor for the HPP assisted degradation of patulin. Avsaroglu et al. (2015) reported the degradation of patulin reduction in apple juice with pressure in the range of 300–500 MPa, and attributed this phenomenon to the formation of adducts, with reduced toxicity, between patulin and environmental compopunds with sulphhydryl groups including cysteine and glutathione.

Research is showing that the effectiveness of HPP varies depending on the type of mycotoxin. For example, aflatoxins and ochratoxins are more resistant to HPP, while patulin and zearalenone show higher susceptibility to pressure treatments (Azam et al., 2021).

2.2 Filamentous fungal suppression and mycotoxin reduction

It has been demonstrated that vegetative fungal cells are easily inactivated by HPP at low temperatures and moderate pressure (300–400 MPa). In contrast, some fungal spores are more resistant (Sehrawat et al., 2021). The primary determinants of ascospore resistance to HPP are age (normally, older ascospores are known to be more resistant than younger ascospores), pH, water activity (a_w), and food composition (Pinto et al., 2020). The body of research produced results indicating that HPP was effective in inhibiting fungal development overall in grains. In this context, Kalagatur et al. (2018) reported high membrane damage and complete inactivation of *Fusarium graminearum* spores inoculated in peptone water treated with HPP at 380 MPa and 60°C for 30 min. However, in maize grains, the total inactivation of spores was detected at 550 MPa and 45°C for 20 min, indicating that water activity and food matrix are important for the HPP efficacy. In this context, the growth *Fusarium culmorum* in winter wheat submerged in distilled water was suppressed after treatments with HPP 100–300 MPa for 10–600 s, for 6 weeks (Schmidt et al., 2019).

On the other hand, molds were completely inactivated by the HPP treatment (600 MPa for 3 min at 4°C) on pitted sour cherries and they stayed at very low levels for the whole 5 months of refrigerated storage (Tenuta et al., 2023). It is to highlight that the low susceptibility of the aged fungal spores to HPP could be a concern for fruit product manufacturers because spores contaminating fruits can be very old. Therefore since industrial-scale HPP machines can operating at maximum pressures of 650 MPa with or without temperatures up to 50°C, scientific challenges related to the inactivation of resistant spores remain unresolved (Echenique-Martínez et al., 2023). The age-related changes in pressure resistance of ascospores may be due to ongoing changes in the ultrastructural state of the multilayer ascospores wall. Low pH has been proven in some cases to increase reductions while higher pH and media rich in nutrients can improve the spore's survival; low water activity (a_w) products offer

baroprotection, with differing results based on the solute. More recently, an emerging pathway that could be interesting for mold spores' inactivation is represented by the induced nutrient-like physiological germination that was reported to occur in spores under low/moderate hydrostatic pressures (150–200 MPa), and that was responsible for inactivation of baroresistant microorganisms after germination (Pinto et al., 2024).

Few studies have investigated the sensitivity of mycotoxins to HPP treatments. Kalagatur et al. (2018) found that the level of deoxynivalenol (DON) and zearalenone (ZEA) produced by *F. graminearum* in maize were completely reduced (550 MPa, 45°C for 20 min). On the other hand, Schmidt et al. (2019) found for cereal that HPP (300 MPa, 30°C for 10 min) reduced the concentration of DON, ochratoxin A (OTA), and ochratoxin B (OTB) below detection limits. Additionally, high-pressure treatments effectively degrade other fungal biomolecules that can deteriorate food quality. Although the results are encouraging, the cereal industry still finds it difficult to implement this technique.

2.3 Drawbacks

One of HPP application's primary limitations in reducing fungi and mycotoxins is its reduced efficacy against dry foods and products with a water activity below 0.8. Additionally, the thick cell walls and dehydrated spore core of fungal spores render them highly resistant to HPP inactivation. High-pressure processing requires a very high level of initial investment and a relatively high maintenance cost (Cacace et al., 2020), which includes energy consumption, equipment maintenance, and the cost of water used to generate the pressure, which can limit its accessibility for small and medium-sized companies. Integrating HPP equipment into existing production lines may require significant modifications. The equipment works only in batch or semi-continuous mode, implying additional costs and downtime. HPP equipment has a limited size and capacity, which can restrict its use for high-volume or irregularly shaped products.

3 Ozone (O₃) treatment

Ozone treatment is considered a safe method for food because it decomposes into oxygen and does not form residues that affect consumers' health. The most common gaseous ozone generators work through Corona Discharge (CD), with 600 to 2 kHz frequencies. Ozone in the aqueous phase (ozonated water) can be used in fresh foods; however, its effect on fungal inactivation is lower due to its low stability in this medium (Davies et al., 2021).

3.1 Mechanisms of action

Ozone can diffuse through the cell wall of filamentous fungi, reach the cytoplasm, and change cell activity (Deng et al., 2020). In the fungal cell envelope, polyunsaturated fatty acids are affected by ozone, whereby the membrane permeability enables electrolytes and the contents of cells to leak out (Ouf and Ali, 2021). One aspect of ozone toxicity is its ability to form reactive oxygen species (ROS), which oxidatively destroy biological components, causing cellular

dysfunction or cell death. Additionally, ozone inhibits the expression of genes involved in ergosterol synthesis (Li et al., 2022), and decreases the quantity of β -1,3-glucan rather than chitin in the inner layer of the cell wall (Ali and Abdallah, 2024); in addition O_3 oxidizes sulfhydryl and amino acid groups of enzymes, resulting in a reduction of spore development and germination and causing rapid cell death (Afsah-Hejri et al., 2020). Fungal spores differ in sensitivity to ozone, and it seems to be directly linked to spore surface (Pagès et al., 2020) and to differences in their component content, which might accelerate or decrease the toxic action of ozone (Ali and Abdallah, 2024).

It is known that the functional groups inside the mycotoxin molecules would be able to interact with the oxidizing agents. It may change their chemical structures, allowing for the creation of products with fewer double bonds, a lower molecular weight, and lower toxicity. Afsah-Hejri et al. (2020) provided a detailed review of the recognized studies on the mechanisms of mycotoxin degradation by O_3 : (i) O_3 reacts with nitrogen heterocycles of fumonisins (F) and forms N-oxide, the primary amine of the molecule; (ii) in the case of ochratoxin A (OTA), O_3 attacks the chlorinated ring structure resulting in amino acids or free chlorine; (iii) in the deoxynivalenol (DON), O_3 attack the C9–C10 double and oxidizes the allylic carbon in the C8; (iv) ozone attacks on the furan ring of the aflatoxin C8–C9 double bond degrading their structure; (v) patulin tend to be more degraded than complex mycotoxins due to its simple structure producing diglycolic acid, oxalic acid, and CO_2 as final products.

3.2 Filamentous fungal suppression and mycotoxin reduction

Research conducted in maize demonstrated the reduction of *Aspergillus* spp. and *Penicillium* spp. when treated with of 2.14 mg/L of the gas up to 50 h (Brito et al., 2018), and 13.5 mg/L for 24 h and 36 h (Ribeiro et al., 2022). The results show that in *Aspergillus* species, the pigmentation is deeper, and their conidia are comparatively thick-walled, which could provide protection against the sun's ultraviolet radiation and possibly against gases such as O_3 . In addition, physiology, morphology among genera, moisture content, and substrate variations may influence the sensitivity or resistance of fungal spores to O_3 (Akbar et al., 2020; Conte et al., 2020). In this context, in coffee beans infected with *Aspergillus westerdijkiae*, *A. ochraceus*, and *A. carbonarius*, treatments with 600 mg/L O_3 at low water activity ($a_w = 0.75$) reduced significantly fungal populations and therefore the mycotoxin content during the storage (12 days). However, when a_w was maintained at values of 0.90 and 0.95, toxigenic fungal populations increased dramatically 48 h after exposure (Akbar et al., 2020). It should be borne in mind that in many products with low water activity, molds are present in the form of spores, which, as already mentioned, are more resistant to ozone. The inhibitory effect of ozone on spore formation has considerable commercial potential, as the treatment breaks the infection cycle. However, the effectiveness of ozone under *in vitro* conditions is not able to reduce fungal growth. It is, therefore, important to optimize the composition or treatment of ozone.

Table 1 depicts the most significant reports, in the last years, of ozone fumigation on postharvest pathogenic fungi in seeds, cereals, and fruits. A significant part of the works demonstrated that O_3

treatment had a clear inhibitory effect on molds in a dose- and time-dependent manner, potentially preventing product decay.

On the other hand, several studies have shown that the O_3 efficacy in decontaminating mycotoxins depends on their concentration, exposure time, ozone state, matrix moisture content, weight, and specific surface area. The treatment efficiency increases with temperature, with increased ozone exposure (higher ozone concentration or longer treatment times), and better ozone diffusion within the matrix. For example, in naturally contaminated ground corn (10% moisture content), treatments of 40 mg/L of O_3 led to a reduction of up to 68.1% of ZEN in 120 min while the 70.3% of OTA reduction required 180 min; on the contrary, DON appeared to be quite difficult to be degraded (Krstović et al., 2021). In contrast, Purar et al. (2022) reported the reduction of DON in naturally contaminated maize to levels below the detection limits (40, 70, and 85 mg/L for 180 min), and differences could be related to the mycotoxin concentration. The ozone efficacy has also been demonstrated in the reduction of aflatoxin B₁ (AFB₁) and aflatoxin G₁ (AFG₁) naturally present in corn grit (9.5% of moisture content), as well as ZEN and AFs content in both naturally and artificially contaminated ground maize with a degradation level that was dependent on ozone exposure (Purar et al., 2022). In wheat bran, ZEN was degraded by a major percentage with respect to DON (Santos Alexandre et al., 2018). Also significant reduction of AFB₁ and AFG₁ was reported in pistachios and almonds (Ali and Abdallah, 2024; Babae et al., 2022). On the other hand, Ozone treatment reduced the accumulation of diacetoxyscirpenol (DAS) by *Fusarium sulphureum* inoculated in potato tuber by down-regulating expression of genes involved in the DAS biosynthesis pathway (Li et al., 2022).

3.3 Drawbacks

Ozone is an unstable gas and decomposes rapidly, so specialized equipment is required to generate it *in situ*, which increases investment and operating costs. In addition, the adaptation of existing production lines, the corrosiveness of ozone towards certain materials, and the health risks for operators require rigorous safety and control measures. Accurate ozone dosing is critical to prevent adverse product and environment effects. Excessive ozone exposure can lead to the degradation of proteins, vitamins, and lipids, resulting in undesirable compounds and altered sensory properties. Moreover, ozone's limited selectivity can generate undesirable by-products, underscoring the importance of precise dosing and control in ozone applications. On the other hand, batch or semi-continuous operation limits the scalability of ozonation processes or limits them to bulk product storage operations.

4 UV treatment

Ultraviolet refers to electromagnetic radiation with a wavelength shorter than visible violet light and is categorized according to its energy level: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). The dose of UV radiation given determines how effectively it disinfects. It is defined as the product of intensity (mW/cm²) and the exposure time (s) and is commonly expressed as mWs/cm² or mJ/cm².

TABLE 1 Summary of recent studies on filamentous fungi reduction by ozone.

Substrate	Process parameters	Observations	References
Brazil nuts	O ₃ concentration: 2.42, 4.38, 8.88, and 13.24 mg O ₃ /L. Time: 0, 60, 120, 180, and 240 min. Flow rate of 3.0 L/min.	CFU count of <i>A. flavus</i> decreased from 5.34 to 2.21 Log CFU/g at 8.88 mg O ₃ /L for 240 min.	de Oliveira et al. (2020)
Wheat seeds	O ₃ concentration: 2000 mg O ₃ /h. Time: 15, 30, and 45 min. Temperature: 30, 40, and 50°C of drying air temperature.	The maximum reduction of fungal count was 92.86%, with reduction from 1.87 CFU/g to 0.13 CFU/g, when the wheat seeds was treated with ozone for 45 min and dried with air temperature at 50°C.	Granelle et al. (2018)
Rice storage	O ₃ concentration: 0.393 kg O ₃ /m ³ . Time: 30, 90, and 180 min.	Ozonation for 180 min caused 90% fungal elimination, and some strains remained resistant.	Savi et al. (2020)
Peanuts	O ₃ concentration: 0.94, 1.89, 2.83, and 3.78 mol O ₃ /kg peanut Airflow rate: 0.19 mol/min.	The higher exposure (240 min = 3.78 mol O ₃ /kg peanut) reached 92% of fungal growth inactivation, including aflatoxigenic fungi. A minimum ozone dosage to eliminate fungi and not cause lipid peroxidation on the peanut surface.	Gomes et al. (2023)
Winter jujube (<i>Ziziphus jujuba</i> Miller cv. Dongzao)	O ₃ : 0, 2.5, 5 and 10 µL/L, 1 h per each day of storage, temperature of storage: 25 ± 0.5°C	The growth of fungi was largely inhibited by ozone, with concentrations being higher than 2.5 µL/L, indicating that ozone could reduce the fruit decay of jujube by inhibiting fungal proliferation on the fruit surface.	Zhang et al. (2022)
Cantaloupe	O ₃ : 0, 6.432, 10.720, and 15.008 mg/m ³ for 1 h. The ozone treatment occurred weekly in a fumigation device with a storage capacity of 1,200 L.	Reduction in the relative content of <i>Alternaria</i> spp. due to the increase in ozone concentration.	Chen et al. (2020)
Muskmelon (<i>Cucumis melo</i> L.)	O ₃ : 1.10 and 2.20 mg/L per 30, 60, and 120 min.	The development of Fusarium rot was effectively controlled in muskmelon inoculated with <i>Fusarium sulphureum</i> after ozone treatment. The concentrations of NEO in the rotten part with ozone treatment after 30, 60, and 120 min were 0.84, 0.17, and 0.14 times lower than those in the control for 8 days of storage.	Hua-Li et al. (2018)
Mango (<i>Mangifera indica</i>) fruit	O ₃ : 0.25 mg/L for 24 or 36 h at 10°C. Experimental fruit were stored at 90% relative humidity and 10°C for three weeks and ripened at ambient temperature for 1 week.	Ozone treatment (24 h) decreased mycelial growth by 60.35%. On day 21, untreated fruit had a high disease incidence <i>Lasiodiplodia theobromae</i> (68.33%) compared to O ₃ (24 h) (53.29%) and O ₃ (36 h) (43.30%).	Bambalele et al. (2023)
Papaya (<i>Carica papaya</i>) fruit	Ozonated water (3 mg/L) at 20 ± 2°C for 300 s.	Reduced the stem-end rot, controlling around 50% of the severity, and delaying the onset of the symptoms in 3 and 4 days. The integrated approach combining heat treatment, by immersing the papaya peduncle in hot water at 70°C for 15 s, followed by immersion in ozonated water (3 mg/L) for 300 s, efficiently controlled the stem-end rot (>90%).	Terao et al. (2019)
Blueberry fruit (<i>Vaccinium corymbosum</i> , cv. O'Neal)	O ₃ : 18 mg O ₃ /L for 10 and 20 min. Exposure to 18 mg O ₃ /L for 10 min	Slight but no significant differences for native mycobiota and <i>Botrytis cinerea</i> incidence reduced the percentage of infected fruit by ~34 and 40%.	Jaramillo-Sánchez et al. (2019)

4.1 Mechanisms of action

UV radiation can denature microorganisms' DNA, proteins, or lipids, causing death or inactivation. When nucleotide bases are exposed to UV light, pyrimidine (6–4) pyrimidone photoproduct [(6–4) PP] and cyclobutene pyrimidine dimer (CPDs) are formed. These may cause mutations and alter patterns of gene expression and cell death. A smaller amount of UV light is also absorbed by other cellular constituents, such as aromatic amino acids, which results in photo-fragmentation, loss of enzyme specificity, and other biological effects (Wong et al., 2019). The wavelength of the incoming photons, the DNA sequence, the flexibility of the DNA structure, and the base locations all have a significant impact on the type and quantity of lesions that occurs. On the other hand, other indirect damages are induced by UV treatment, such as water radiolysis, which can lead to the accumulation of ROS within cells. In addition, UV radiation can also degrade mycotoxins; it is commonly recognized that many mycotoxins with complex structures and more reactive functional groups can absorb UV light, which can trigger photoreaction and reduce mycotoxin.

4.2 Filamentous fungal suppression and mycotoxin reduction

According to Esua et al. (2019), the mechanism of action of UV-C radiation in strawberries, tomatoes, mushrooms, and grapes is related to the activation of regulatory enzymes for the production of phenolic compounds with antifungal properties. Major studies reported that the effect of UV radiation on conidia is easier compared to mycelium. In fact, fungal mycelium has a higher metabolic activity than conidia, which are largely dormant or inactive structures with high resistance against various stresses (Baltussen et al., 2020). Several authors reported the efficacy of UV radiation treatment by inducing DNA damage conidia, preventing its germination and multiplication; nevertheless, the effect is fungal species-dependent, and the spore resistance could be due to the thicker conidial wall. In this context, Nakpan et al. (2019) reported that UV treatment for 10 min against *A. fumigatus* was ineffective on conidia due to their defense mechanisms correlated with melanin, thus suggesting that the exposure to ultraviolet radiation must be more significant to destroy the cellular structure of *Aspergillus* conidia substantially. Simultaneously, other authors reported that *A. fumigatus* and *A. niger* conidia are highly resistant due to the involvement of DHN-melanin against UV-C treatments (Blachowicz et al., 2020; Cortesão et al., 2020).

In situ studies revealed good efficacy of UV treatments to reduce the disease severity (near 90%) of *B. cinerea* in mature strawberry fruits (1.3 mJ/cm² UV-B treatment) (Abd El Hamid et al., 2022), as well as the reduction of the total fungal colonies in the brown, black, and red rice treated with UV-C for 3 h (Ferreira et al., 2021) and the growth of *Penicillium* spp. (79% reduction) and *Fusarium* spp. (62% reduction), following treatment of maize kernels with a UV-C dose of 5,000 mJ/cm² (Popović et al., 2018).

UV irradiation has been shown to be an effective method of destroying mycotoxins in many agricultural products. The studies reported either the inactivation of mycotoxins directly and by means

of the inhibition of the fungal mycotoxin producer, in this context, the efficacy of UV-C irradiation to completely degrade AFB₁, OTA, and aflatoxin B₂ (FB₂), but not against nivalenol (NIV) on semolina has been reported (Shanakhat et al., 2019). Also, a low dose (25 mJ/cm²) of UV-C treatment inhibited the production of Alternariol (AOH), alternariol monomethyl-ether (AME), and tenuazonic acid (TeA) in tomato fruits inoculated with *Alternaria alternata* (Jiang et al., 2019); however a reduction of 70% of patulin in apples inoculated with *P. expansum* required a higher dose (3,510 mJ/cm²) (Nicolau-Lapeña et al., 2023). Aflatoxins (B₁ + B₂ + G₁ + G₂), DON, OTA, and ZEN were reduced in the brown, black, and red rice after UV-C treatment for 1 and 3 h when compared to freshly-harvested grains and those without UV-C treatment (Ferreira et al., 2021). Furthermore, Popović et al. (2018) determined that postharvest UV-C treatment of corn kernels by using 253.7 nm sources was efficacious in reducing DON (30%), OTA (17%) and ZEN (52%) accumulation.

4.3 Drawbacks

Despite its effectiveness, UV disinfection has limitations inherent to the nature of UV radiation. The UV penetration power is insufficient to completely destroy microorganisms when the initial total microbial count is high (Deng et al., 2020). Limited penetration in turbid media, intensity attenuation, and shadow formation restrict its application in heterogeneous systems, thus requiring higher doses or recirculation systems, which increase operating costs. In addition, UV-induced photochemical reactions can generate undesirable by-products that may be toxic or have bad taste and odor (Byun et al., 2020). Industrial implementation requires careful design of reactors, considering factors such as UV dose and energy efficiency. Energy costs associated with operating UV lamps can be high. Strict safety measures, such as physical barriers, interlock systems, and personal protective equipment, must be implemented to protect against the harmful effects that UV radiation can cause to the skin and eyes of operators.

5 Blue light (BL) treatment

The antifungal properties of light are a research area that is gaining more attention due, in part, to the development of resistance to standard control methods. Several researchers have evidenced that among different visible light wavelengths (blue, green, and red light), only the blue spectral range from 400 to 470 nm allowed for detectable antimicrobial effects (Leanse et al., 2022). Alternatively, a low-cost, environmentally friendly, non-thermal sanitization system may be created using light-based technology, specifically light-emitting diodes (LED); it is a semiconductor material doped with impurities that create a boundary or interface (known as a p-n junction) between two types of semiconductor materials, one type (the positive or p-type) having an excess of holes and the other type (the negative or n-type) having an excess of electrons. LEDs function according to the electroluminescence principle, which states that they emit light when an electric or magnetic field is applied. Excited electrons release energy in the form of electromagnetic radiation and produce light when they move toward lower energy states in an electric or magnetic field.

Many microorganisms are sensitive to BL, which can lead to physiological reactions triggered by blue light receptors. In particular, many fungal species have their wavelength receptors, and all of these receptors have an organic compound of low molecular weight, such as flavin, retinal, or tetrapyrroles, for the perception of blue, green, and red light, respectively (Leanse et al., 2022). When fungal cells are exposed to BL, light-sensitive molecules within the cells absorb the light. This absorption stimulates the chromophores, which leads to the production of ROS. Moreover, another proposed mechanism of inactivation for several microorganisms is represented by the natural accumulation of photoactive metal-free porphyrins such as uroporphyrin, coproporphyrin, and to a lesser extent protoporphyrin due to exposure to blue light (Dong et al., 2022).

5.1 Mechanisms of action

The widely accepted mechanism by which blue light (BL) induces fungal cell death involves the photoexcitation of photosensitizing chromophores, such as iron-free porphyrins, flavins, and key proteins like White Collar WC-1, WC-2, and cryptochromes. This process generates intracellular reactive oxygen species (ROS), which compromise cell membrane integrity, damage DNA, trigger lipid peroxidation, and potentially destroy mitochondrial membranes. As a result, mitochondrial dysfunction occurs (Zhu et al., 2023), leading to extensive cellular damage and ultimately cell death through necrosis. Additionally, research by Grangeteau et al. (2018) has shown that BL disrupts the spore cell wall and membrane, reducing spore viability and further contributing to fungal cell death. The proposed mechanism behind the action of blue light is the photoexcitation of endogenous porphyrins, resulting in ROS, loss of cell membrane integrity, and cell death.

Although BL treatment demonstrated a lethal effect for some filamentous fungi, its activity depends on the wavelength, dose, and fungal genus. The studies all clearly agree that BL's shorter wavelengths (e.g., 400–410 nm) had greater microbicidal effects than the others (e.g., 450–470 nm). These results, when applied after spore germination, are crucial for identifying the best BL wavelength for cleanliness or lowering contaminations. On the other hand, the sensibility of *Aspergillus oryzae* to BL has been linked to the presence of a BL receptor that senses blue light irradiation; in this case, the signal is transmitted to the central regulatory module associated with spores (such as the BrlA→AbaA→WetA regulatory cascade, explained below), which likely led to downregulation of the associated genes and ultimately led to a significant reduction in the number of conidia produced (Shangfei et al., 2021). AbaA is a crucial element for the correct differentiation and function of phialides. It is activated by BrlA during the middle stages of conidiophore development, following the differentiation of the metulae. The *brlA* gene in *Aspergillus nidulans* facilitate the transition from the indeterminate apical growth pattern of the conidiophores. Additionally, the *wetA* gene is induced by AbaA in the later stages during of conidiation and is essential for synthesizing the cell wall layers that enable conidia to mature and become impermeable.

5.2 Filamentous fungal suppression and mycotoxin reduction

The efficiency of BL inactivation depends on several variables, including the wavelength of the light, the duration of exposure, the fungal species, and the temperature. Fungal spores are more resistant to environmental stress than vegetative cells, but BL can still impact them. For example, exposure to BL low-intensity suppressed by 81% the development of *Penicillium italicum* spores in mandarin fruits (Bhatta, 2022), controlled *Penicillium expansum* in apples after harvest and affected the expression levels of the virulence genes and, in particular the gene *LaeA* which regulates several secondary metabolite genes, including the patulin gene cluster (Zhu et al., 2023). In addition, it inhibited the growth of *Geotrichum citri-aurantii*, which is responsible for acid rot in citrus fruit, and the mycelial diameter was greatly reduced with increasing BL intensity (Du et al., 2023b).

Blue light can also interfere with the spore germination process, as Trzaska et al. (2017) reported that a 60 min treatment with BL (405 nm) led to a significant inhibition of germination of *Fusarium oxysporum*, *Fusarium solani*, *Scedosporium apiospermum*, and *Scedosporium prolificans* fungal conidia. In contrast, *Rhizopus microsporus* and *Mucor circinelloides* conidia showed the highest resistance. Other authors reported that a treatment duration of 1 and 12 days under BL led to a drastic inhibition of conidia germination of *A. alternata* (Igbalajobi et al., 2019).

The efficacy of BL at room temperature rather than at lower temperatures (4–16°C) to inactivate fungi was reported in the growth of *B. cinerea* and *Rhizopus stolonifera* on tomatoes and strawberries (Ghate et al., 2021), and in *Geotrichum candidum* and *Fusarium* sp. isolated from litchi fruit (Yu et al., 2023). It is likely that the fungi may have a greater metabolic load at room temperature, which could accelerate the rate of cytotoxic response in addition to a greater production of intracellular ROS formation, which in turn accelerates microbial inactivation. The surface texture of food also plays a role in the effectiveness of BL, as light interacts better with smooth surfaces. However, rough surface textures may allow better harboring or shading of fungal spores in surface defects or cracks as demonstrated by the growth of *Geotrichum candidum* on smooth and rough surfaces of litchi fruit (Yu et al., 2023). More recently, Du et al. (2023a) found that blue light not only directly inhibit the mycelium growth of *G. citri-aurantii* in citrus fruit but also induced the activities of defense-related enzymes and antioxidant enzymes in citrus to limiting the development of sour rot caused by this species.

The reduction of mycotoxin formation is critical for protecting human and animal health, maintaining food quality, avoiding economic losses, and promoting sustainability. Blue light was reported as an effective strategy to control mycotoxins. Zhang et al. (2021) reported that OTA production decreased by 66.88%, when *Aspergillus carbonarius* was grown under blue light. Depending on the fungal species or strain, there are differences in the interaction between light irradiation and mycotoxin production. For example, using BL treatments (455–470 nm), OTA production was reduced by controlling the expression of ochratoxin polyketide synthase in *P. expansum*, *Penicillium verrucosum*, and *Penicillium nordicum* (Zhu et al., 2023). Similarly, BL with a wavelength of 455/470 nm alone, in combination with low a_w values (0.95), leads to a significant reduction in AFs and cyclopiazonic acid formation. In addition, reduced

transcriptional activity of genes responsible for mycotoxin biosynthesis of *A. flavus* and *A. parasiticus* was observed (Priesterjahn et al., 2020). Similarly, the expression of the genes PatA, PatE, and PatN related to the patulin production decreased by 90, 50, and 72%, respectively, when the culture of *P. expansum* was exposed to 500 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$ blue LED light treatment (Zhu et al., 2023).

5.3 Drawbacks

Among the most widely used blue light sources in industrial applications are LEDs and laser diodes, which are notable for their high efficiency, low cost, and ease of scaling. Femtosecond lasers, while offering exceptional precision, are more suitable for niche applications due to their complexity and cost. Implementing blue light-based disinfection systems can be limited by factors such as light absorption by plastic materials, heat generation, and the need for specialized equipment. In addition, prolonged exposure to blue light can cause eye damage in workers. The effectiveness of this technology depends on variables such as the power of the source, the distance to the surface to be treated, and the presence of biofilms (Hadi et al., 2020). Successful implementation of blue light technology in the food industry requires a comprehensive evaluation of each specific application, considering factors such as the target microorganisms, surface materials, and environmental conditions. While light-based treatments (LB) are generally considered to have a low risk of resistance development due to their multi-targeted nature, there remains a possibility of surviving cells developing tolerance. If the inflicted damage is insufficient to eliminate all targeted cells, those that survive may adapt and become resistant to future treatments (Rapacka-Zdonczyk et al., 2019).

6 Pulsed light (PL)

A pulsed light system (PL) is also known as pulsed UV light, high-intensity broad-spectrum pulsed light, pulsed white light, or intense light pulses (John and Ramaswamy, 2018). One of the key advantages of pulsed light (PL) over other non-thermal technologies is its emission of highly concentrated, short-duration light bursts. These intense energy pulses can inactivate microorganisms within milliseconds to seconds, making PL a highly time-efficient method for surface treatments. Its broad-spectrum light, which includes UV, visible, and infrared wavelengths, enhances its ability to target and eliminate a wide range of pathogens effectively (Santamera et al., 2020). A typical PL system consists of a high-voltage power supply, a storage capacitor, a pulse-forming network that determines the pulse shape and spectrum characteristics, a gas-discharge flash lamp, and a trigger that initiates the discharging of the electrical energy to the flash lamp. Electrical pulses are applied to excite inert gases, such as xenon in flashlamps, and cause gaseous molecules to collide, producing light pulses. After that, the light energy is emitted in extremely concentrated short-duration light bursts (lasting for a few hundred microseconds, usually 1 to 1,000 μs). The resulting light has an electromagnetic spectrum ranging from ultraviolet (UV) to near-infrared (IR). This range of electromagnetic radiation includes UV rays ($\lambda = 100\text{--}400\text{ nm}$), visible light ($\lambda = 400\text{--}700\text{ nm}$), and infrared

($\lambda = 700\text{--}1,100\text{ nm}$). In order to satisfy the unique process requirements, the PL system can deliver light as a single pulse, a burst of pulses (timed mode) at a frequency of 1–20 Hz with a pulse width of 300 ns to 1 ms, or a continuous array of pulses in random sequences. Optical sensors measure the fluence of the PL irradiation on the sample. Numerous variables, such as the number of flashes, the pulsed energy level, the distance between the sample and the lamps, and the type of sample treated, affect the inhibitory effect of PL (Rifna et al., 2019).

6.1 Mechanisms of action

The effects of PL on microbial cells are divided into three categories: (i) The photochemical effect, caused by PL UV component that can be absorbed by DNA and other cell components, refers to the water vapor-induced damages like disruption in the cell wall, shrinkage in the cytoplasmic membrane, and mesosome rupture, followed by leakage of cell content and genetic material (Contigiani et al., 2021), the absorption of UV fraction initiates the DNA or RNA damage by forming pyrimidine dimers, resulting in mutations, inhibition of DNA, and thus prevents the microorganisms' ability to replicate; (ii) the photothermal effect caused by the visible and near-infrared regions, which only generate heat high enough to kill microorganisms on the surface of the treated substrate (a few μm thick), and (iii) the photophysical effect caused by the high-power pulsing effect, which is constantly disturbing structure damages.

Many factors influence microbial inactivation, such as (i) food surface, in this case, non-uniform exposure of the sample reducing the inactivation efficiency; (ii) food shape, spherical shape is the most suitable shape; (iii) distance of exposure of light (iv) food color media, (v) degree of heat dissipation and its absorption by the food matrix, and (vi) turbidity that may be caused by the presence of particles that have a high UV absorbance, which could lower overall PL efficiency. In addition, after PL treatment, temperature, moisture content, and lighting are considered significant environmental variables that can influence microbial activation and inhibition. However, the intrinsic properties of the microbial cells—that is, the kind of microorganism, the growth stage, and the size of the inoculum—also influence PL lethality. For instance, it is commonly known that microorganisms' PL susceptibility follows the following pattern: Gram-negative bacteria < Gram-positive bacteria < yeasts < bacterial spores < molds < viruses. Recently, transcriptomic analysis on *A. carbonarius* treated with fluences of 9 J/cm^2 (600 s) conducted by Wang et al. (2023b) revealed that the mechanisms of mold suppression are caused by down-regulation of differentially expressed genes related to energy and glucose metabolism, transport, stress response, and secondary metabolism in addition to inhibition of DNA replication and disruption of the cell wall and cell membrane. Transcriptomic analysis of pulsed light inhibition of *Aspergillus carbonarius* growth detected negative effects on DNA replication, glucose metabolism, cell integrity, and secondary metabolism (Wang et al., 2023b). Mycotoxin destruction should be attributed to photochemical mechanisms, which in this case include mycotoxin fragmentation, disruption of the terminal furan double bond, and lactone ring opening (Abuagela et al., 2018).

6.2 Filamentous fungal suppression and mycotoxin reduction

Research on fresh fruits, including melons and strawberries, demonstrated that PL only reduces lesions' size and decay rate. Specifically, the inhibition of *Fusarium pallidroseum* growth in melons (*Cucumis melo* var. Spanish) treated with a dose of pulsed light (PL) of 9 J/cm² (0.3 μs), was linked to the upregulation of some fruit biomarkers, including pipelicolic acid, saponarin, and orientin, which function as key indicators of the fruit's defense mechanism against pathogens (Filho et al., 2020). Romero Bernal et al. (2019) observed that in strawberries artificially contaminated with *B. cinerea*, and there was a 2-day delay in the infection's onset and a lower mold incidence when treated with PL for 1 to 40 s (fluence: 1.2 to 4.78 J/cm²), respectively; compared to the control group in 10 days of cold storage. On the contrary, low efficacy of PL (fluence: 1.9 J/cm²) was observed in fungal spoilage of strawberries (cv. Albion) stored at 5°C (Contigiani et al., 2021).

It is well recognized that PL technology is more suitable for surface decontamination in foods with smooth surfaces, such as fresh whole fruit and vegetable commodities than in cereals, as demonstrated by Zenklusen et al. (2018) on the inactivation of *Aspergillus carbonarius* and *A. flavus* in malting barley. In this case, the authors found that even after extended treatment times, there was a persistent residual population, and a reduction of 1.2–1.7 log cycles up to 5–15 s (fluence: 6.0–18.0 J/cm²) was recorded. The noticeable tailing observed in both molds could be attributed to either the inoculated conidia not receiving a uniform treatment due to one side of the grain being exposed to radiation or the uneven distribution of conidia on the grain surface. The restricted penetration of PL into food, on the other hand, is regarded as a shortcoming of this technology.

The impact of PL treatment on mycotoxin decontamination *in vitro* and on fruits has been the subject of numerous studies. Table 2 summarizes the percentage decrease by light pulses of the most well-known mycotoxins reported in recent years. The studies found that variations in mycotoxins' degradation capacity

are caused by differences in molecular structures, which alter photodegradation efficiency.

6.3 Drawbacks

One of the technique's key limitations is its limited penetrating power; hence, opacity, topography, and matrix composition all significantly impact process effectiveness. If the treated surface has a rough texture or a pored structure, a shadow effect occurs, allowing microorganisms to survive the treatment. The large-scale implementation of PL systems in production environments presents significant challenges. The effectiveness of PL depends on several factors, such as the wavelength of the emitted light (generally between 200 and 300 nm for maximum germicidal action), the fluence (energy per unit area), and the pulse duration. These parameters must be carefully adjusted for each application since excessive fluence can damage materials, and insufficient fluence may not guarantee the complete inactivation of microorganisms. In addition, the generation of ozone as a byproduct of the interaction of UV light with atmospheric oxygen can sometimes limit its application. The costs associated with the acquisition and operation of PL equipment and the need for trained personnel to operate it constitute another obstacle to its widespread adoption. Despite these challenges, ongoing research in the field of PL seeks to optimize this technology, develop more efficient and compact light sources, and explore new applications in the food industry, such as packaging treatment and wastewater decontamination.

7 Pulsed electric fields (PEF)

PEF technology uses strong electric field pulses with durations ranging from a few microseconds to milliseconds and intensities ranging from 10 to 80 kV/cm. The product is positioned between two electrodes, and the number of pulses applied determines its efficacy. Numerous ways, including bipolar waves, exponentially decaying

TABLE 2 Percentage reduction of the best known mycotoxins by treatment with Pulsed Light.

Mycotoxins	Substrate	PL conditions	Decrease (%)	References
DON	Germinating barley	PL intensity 0.528 J/cm ² /pulse (fluence = 1.56 J/cm ² for 60 s)	35.5	Chen et al. (2019)
OTA	Grape juice	PL intensity of 0.98 J/cm ² /pulse (fluence = 39 J/cm ²).	95.29	Wang et al. (2022)
AFB1	Rough rice	PL at intensity of 0.52 J/cm ² /pulse (fluence = 84.4 J/cm ² for 80 s)	75	Wang et al. (2016)
AFB2			39	
AFB1	Rice bran	PL at intensity of 0.52 J/cm ² /pulse (fluence = 16.1 J/cm ² for 15 s)	90.3	
AFB2			86.7	
AFB1	Red pepper powder	PL intensity 0.15 J/cm ² /pulse (fluence = 1.0 to 9.1 J/cm ² for 20 s)	67.2	Woldemariam et al. (2022)
Total aflatoxins (AF)			50.9	
(OTA)			36.9	

AFB: Aflatoxin B₁ and B₂; DON: Deoxynivalenol; OTA: Ochratoxin A.

waves, and oscillatory pulses, can be used to apply the electric field (Abbas Syed, 2017).

7.1 Mechanisms of action

The inactivation of microorganisms occurs by electroporation or permeabilization of membranes caused by the electric field, which allows the movement of extracellular and intracellular molecules. For electroporation to occur, the electric field must be above the threshold value at which irreversible rupture of the cell membrane occurs, leading to loss of microbial homeostasis and, ultimately, cell death (Kranjc and Miklavčič, 2017). This threshold value, or critical electric field strength, is different for each microorganism. However, it is generally at 15–35 kV/cm. Several parameters can influence specific microorganism inactivation during PEF processing. These key parameters include field strength, treatment time, temperature, pulse shape, microbe type, development stage, and treatment substrate properties.

In filamentous fungi, field intensity, number of pulses, and pulse duration and number of hyphae, thickness, size, and shape of the cell membrane are the most important critical factors (Feng et al., 2019). A theoretical study with a cell dielectric model in filamentous fungi found that the optimal pulsed electric field parameters for inactivation are an electric field of 13 kV/cm and a pulse duration of 1,000 ns (Feng et al., 2019).

7.2 Filamentous fungal suppression and mycotoxin reduction

There are few reports of the application of this technology on fungal suppression. Most of the research has been registered in the treatment of juices or liquid products where the incidence of fungi is lower. Some significant advances have been made in applying PEF on food-related fungi. For example, PEF has been investigated on wheat seeds, where it improved vigor and germination but failed to suppress the fungi completely. A maximum reduction of 2.85 log CFU/mL (12 kV/cm) has been reported, while other conditions reduce surface fungi and yeasts by only 1 log CFU/mL (Evrendilek et al., 2021). In sesame seeds, 56.1% of the inactivation of *A. parasiticus* was obtained after PEF treatment using 17.28 J of energy (19.79 s × 180 Hz) (Bulut et al., 2020).

The efficacy of PEF against aflatoxin naturally present in red pepper flakes was studied (Akdemir Evrendilek et al., 2022): a reduction of nearly 98% was obtained in samples containing low quantities (from 14.88 to 43.02 µg/L) of several aflatoxins, while the reduction was lower in samples containing high quantities. Similar results were observed in sesame seeds (Bulut et al., 2020). This reduction could be due to adding OH⁻ and H⁺ reaction groups to double bonds of AFB₂ structure and the loss of methylene group (Pallarés et al., 2021). Very recently, Stranska et al. (2023) demonstrated the potential of PEF to reduce mycotoxins content in cereals via the generation of different reaction products from the parental chemicals and through improved matrix extractability into conductor water. Based on the dry matter of barley, the authors found that PEF-induced reductions in trichothecenes, ZEN, enniatins, beauvericin (BEA), and tentoxin were up to 31, 48, 84, 36, and 46%, respectively. While reductions were also noted for most mycotoxins in water, an increase was noted for BEA, ZEN, enniatins, and type A trichothecenes—likely

due to easier extraction from the matrix. Hydrolysis, elimination, and/or oxidation are primarily responsible for the breakdown and transformation of mycotoxins. The authors demonstrated the existence of ester bond hydrolytic products, specifically for enniatins. They suggested that oxidation broke the bond between the amino acids β-carbon and its α-functionalized carboxylic group.

7.3 Drawbacks

The fundamental disadvantage of PEF technology is that it is only suitable for particles suspended in liquids. Furthermore, the presence of bubbles during treatment and the heterogeneity of foods, especially those with high concentrations of solids or particles, can hinder the uniform distribution of the electric field, leading to operational problems and non-uniform treatment. The large-scale implementation of this technology presents considerable challenges. The effectiveness of PEF depends on several factors, such as the electrical conductivity of the food, the geometry of the treatment chamber, and the frequency of the pulses. In addition, the high costs of PEF equipment, including power supplies, control systems, and specialized electrodes, represent a significant barrier to its widespread adoption in the food industry. To overcome the limitations of PEF technology, researchers are actively exploring innovative solutions, such as developing novel electrode materials, improving control systems, and integrating PEF with other food processing techniques.

8 Cold atmospheric plasma (CAP) treatment

Plasma is a collection of various excited atomic, molecular, ionic, and radical species that coexist with a variety of other particles, such as electrons, ions, free radicals, reactive oxygen/nitrogen species (RONS), gas atoms, molecules in ground or excited state, and electromagnetic radiation (UV photons and visible light) which have a potent oxidizing effect (Laurita et al., 2021; Misra et al., 2019). The production of a variety of components is necessary for the antifungal activity and mycotoxin degradation process, including ultraviolet radiation, reactive oxygen species (ROS) like ozone (O₃), hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂), peroxy (ROO•) and hydroxyl radicals (•OH), reactive nitrogen species (RNS) like nitric oxide (NO•), peroxynitrite (ONOO⁻) or peroxynitrous acid (OON). Although each of these species can act on its own, it has been noted a synergistic interaction of the plasma's constituent.

8.1 Mechanisms of action

This technology presents differences in the mechanisms of action according to the food matrix (liquid or solid) or the way of application. When plasma and liquid media are in contact, RONS are transported into the liquid, and secondary active species are created. Therefore, an effect is generated either inside or on the surface of the food as a consequence of the physical and chemical changes triggered by the production of reactive species (Sharma et al., 2018). For solid foods, the application is limited to the surface, and the effectiveness depends on the severity of the treatment, water activity, and porosity. In the last years plasma activated water (PAW), also called plasma acid, plasma

activated liquids, nutrients broths (Thirumdas et al., 2018), containing mainly reactive species, has been proposed as an alternative method for fungal reduction.

Reactive species produced by CAP can potentially modify the fungal cell wall and membrane, releasing cytoplasm and leading to cell inactivation. This process can cause the cell to shrink, flatten, and rupture, allowing the cytoplasm and DNA to escape (Los et al., 2018). Furthermore, several oxidizing molecules, such as reactive nitrogen species (RNS) and ROS, accumulate inside the cell and result in cell death. Molina-Hernandez et al. (2022a,b) showed that oxidative stress-dependent reaction cascades triggered by membrane depolarization could account for CAP-induced cell death. In actuality, CAP-generated radicals are poisonous and can act quickly, causing depolarization of the cell and mitochondrial membranes, an increase in intracellular calcium levels (Ca^{+2}), DNA damage, and possibly even the induction of cell apoptosis.

Mycotoxin degradation is linked to various mechanisms, including chemical interactions with reactive species and UV radiation created by CAP, which results in molecular cleavage by the treatment (Figure 1).

While the mechanisms of AF degradation by CAP are well studied (Wu et al., 2021), those of the other mycotoxins are less elucidated. Neuenfeldt et al. (2023) reviewed potential metabolite products that may be formed after exposure of mycotoxins to CAP treatments. Their results suggest that the by-products of mycotoxin degradation may be non-toxic or have a lower level of toxicity than the parent compound. However, it is not known if foods treated with CAP are safe.

8.2 Filamentous fungal suppression and mycotoxin reduction

CAP significantly suppresses the number of fungi and their spores on grain (rice, wheat, corn, barley, and oats) surfaces, and its effect is fungal species dependent. A complete inhibition of *B. cinerea*, *M. fructicola*, and *A. carbonarius* spores and a partial inhibition in *A. alternata* was obtained using an Surface Dielectric Barrier Discharge (SDBD); with *A. alternata* and *C. carbonarius* the most resistant probably due to the presence of dihydro naphthalene-type melanin (DHN) (Gerin et al., 2018). Also, the diffuse coplanar surface barrier discharge was helpful in reducing *A. niger* and *P. verrucosum* inoculated onto barley (Durek et al., 2018), and to the complete devitalization of *A. flavus*, *A. alternata*, and *F. culmorum* on maize surface (Zahoranová et al., 2018). Less efficacious resulted in CAP treatment using Dielectric Barrier Discharge (DBD) to control the native microbiota in wheat grains (Los et al., 2018). While PAW was useful for *F. graminearum* inhibition, the primary pathogen for Fusarium head blight (FHB) in spiked samples of wheat (Guo et al., 2022), and it was also useful in field-plot experiments to verify the disease incidence (Ju et al., 2023). On the contrary, no reduction in the grain's natural fungal diversity and abundance was detected using a corona (Blown-Arc) surface (Kaur, 2023). However, Cold Plasma using O_2 as input gas was efficacious in reducing fungal frequency and diversity in common and Tartary buckwheat, in particular fungi of

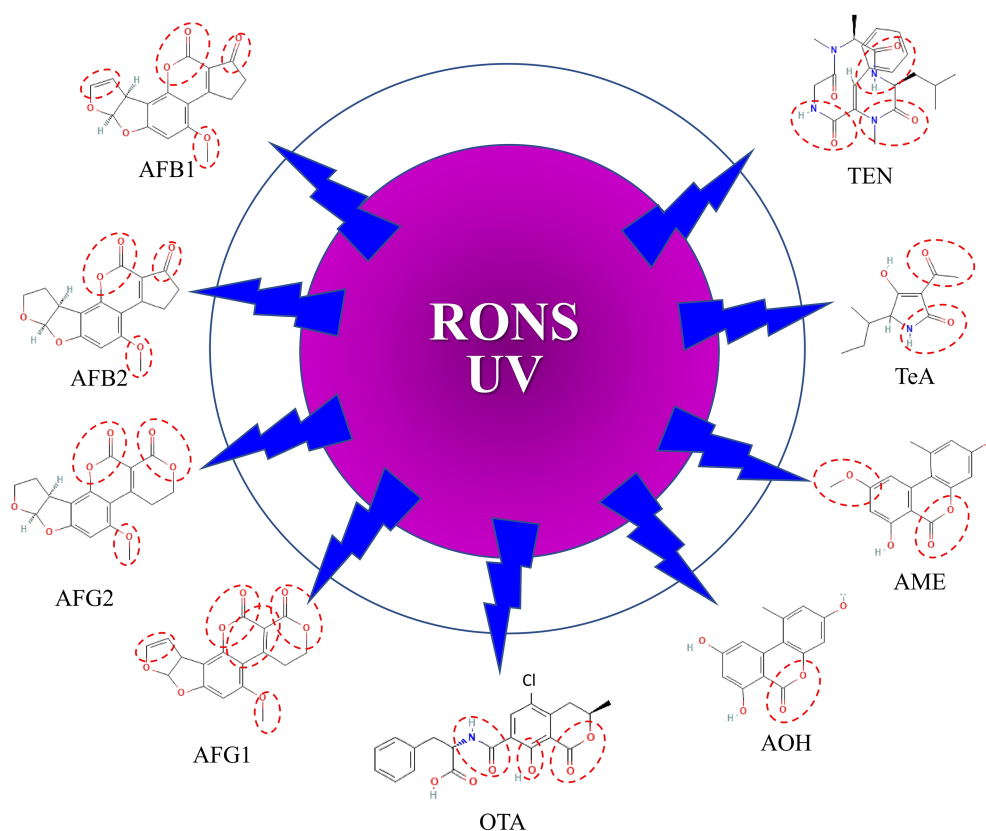


FIGURE 1

Sites susceptible to attack by reactive oxygen/nitrogen species (RONS) and UV in mycotoxins. AFB1, Aflatoxin B1; AFB2, Aflatoxin B2; AFG1, Aflatoxin G1; AFG2, Aflatoxin G2; OTA, Ochratoxin A; AOH, Alternariol; AME, Alternariol monomethyl ether; TeA, Tenuazonic acid; TEN, Tentoxin.

genera *Alternaria* and *Epicoccum*, which proved to be the most resistant (Mravljec et al., 2021).

The studies on the efficacy of CAP in groundnuts, nuts, peanuts, and pistachios are concentrated on the reduction of *A. flavus* and *A. parasiticus*, two important species involved in the production of aflatoxins (Esmaili et al., 2023; Lin et al., 2022), the results showed that the viable spore population is reduced with the increase of treatment duration. CAP also was efficacious successfully in decreasing the percentage disease index of *Colletotrichum musae*, *Fusarium semitectum*, and *Colletotrichum gloeosporioides* responsible of crown rot on Cavendish banana (Sandanuwan et al., 2020), achieving a shelf life of 42 days. Also, the retardation of anthracnose disease caused by *C. gloeosporioides* or *C. asianum* in Nam Dok Mai mangoes was observed after treatment prolonging shelf life by 8 days (Phan et al., 2019). Less effect (25.8% of decay) was observed in blueberry. Sun et al. (2023), using high-throughput sequencing, observed that the fungi surviving to CAP treatment demonstrated stronger biosynthesis and metabolic activity. In Apricot fruits inoculated with *Rhizopus oryzae* and *A. tenuissima*, treatments with DBD at different voltages significantly inhibited the mycelial and reduced spore germination as well as reduced the incidence of soft rot and black spot (81.9 and 82.3%) (Pan et al., 2023). In sun-dried tomatoes, CAP treatments under the NOx regime had a species and dose-dependent effect; in fact, among 32 different species, *A. niger* was the most resistant one. In addition, studies *in vitro* by the same authors revealed that germination kinetics of the fungal spores as a function of CAP time treatment was: *A. chevalieri* < *A. alternata* < *A. tubingensis* < *A. flavus* < *A. niger*. The authors suggested that major hydrophobicity and the presence of melanin contributed significantly to this resistance (Molina-Hernandez et al., 2023).

On the other hand, CAP produced with helium has been used with success to eliminate *A. niger*, *A. westerdijkiae*, *A. steynii*, and *A. versicolor* in coffee beans (Casas-Junco et al., 2019). Also, in naturally contaminated beans (*Phaseolus vulgaris* L.), the CAP treatments effectively inactivated fungal growth (Rüntzel et al., 2019). Very recently, the effects of PAW on *Colletotrichum gloeosporioides*, identified as a serious concern in papaya (Peralta-Ruiz et al., 2023) and chili (Sawangrat et al., 2022), were quite interesting. Compared to previous treatments, the PAW treatment in chili showed a notable 83% increase in efficacy in reducing anthracnose (Sawangrat et al., 2022). In addition, a gliding arc plasma jet (GAPJ) was used to suppress the brown rot disease caused by *A. alternata* in Gala apples; in this case, the results evidenced that 15-min treatment was efficacious in controlling apple brown rot and inhibited the spore germination (Pereira et al., 2024).

Table 3 presents the mycotoxin inactivation results reported in recent years. Our research group recently investigated the potential of CAP in decreasing mycotoxins AFB₁, AFB₂, AFG₁, AFG₂, and OTA in two different plasma reactive species environments: ozone (O₃) and nitrogen oxides (NOx). The findings demonstrated that CAP treatments significantly decreased all tested pure mycotoxins: It was found that the degradation of mycotoxins depends on the applied regime, molecule chemical structure, distance from the source, and time-dependent (Laika et al., 2024b). At 60 min and 4 cm from the plasma source, the O₃ regime was more effective (AFB₁ and AFG₁ 99% decreased, AFB₂ and AFG₂ 60% decreased, and OTA 70% decreased). In naturally contaminated pistachio

kernels (*Pistacia vera* L. seeds), the trend was similar. However, the mycotoxins in pistachio were reduced to a much lesser extent than pure molecules. A slightly better response was observed for chopped pistachio kernels than whole ones, most likely due to a larger surface area of the food exposed to plasma (Laika et al., 2024b), implying that the food matrix plays an important role in the CAP degradation process. CAP reduces *Alternaria* toxins such as tenuazonic acid (TeA), one with Alternariol (AOH), Tentoxin (TEN), Alternariol monomethyl ether (AME), and Altenuene (ALT) in dried tomatoes. In particular, the degradation rate under O₃ regime increased significantly with 60 min of exposure time, reaching 52.80 ± 1.21% of mycotoxin degradation (Laika et al., 2024a). Additionally, GAPJ effectively reduced the production of *A. alternata* mycotoxins, including aAOH, AME and TeA, after 15 min of treatment. The reactive species OH, NO, NO₂ and NO₃ are believed to be responsible for these effects (Pereira et al., 2024). Finally, some authors have found that that higher efficacy of CAP was achieved with higher concentrations of mycotoxins (Laika et al., 2024a). The authors hypothesized that many active ions are generated in the plasma during discharge. Therefore, the reaction rate increases with increasing substrate concentration. As a result, the system with the higher initial concentration of mycotoxins showed a faster degradation reaction, eventually leading to greater mycotoxin degradation over time. In addition, Liu et al. (2024) demonstrated that 30-W CAP treatment for 180 s achieved a ZEN degradation of 96.18% in cereals. This degradation was attributed to the oxidative destruction of C=C double bond, resulting in four major degradation products: C₁₈H₂₂O₇ (*m/z* = 351.19), C₁₈H₂₂O₈ (*m/z* = 367.14), C₁₈H₂₂O₆ (*m/z* = 335.14), and C₁₇H₂₀O₆ (*m/z* = 321.19), that showed a significantly reduced cytotoxicity.

8.3 Drawbacks

Large-scale implementation involves considerable challenges. During the CAP reaction process, numerous distinct types of active substances are created, which can modify the qualitative features of foods. Several reactions, including accelerated lipid oxidation, may affect the sensory properties of CAP-treated foods. Furthermore, it is possible to identify enzyme breakdown, vitamin, and sensory feature loss. The heterogeneity of foods, especially those with high solids or particulate concentrations, can make uniform plasma distribution difficult. The complexity of scaling plasma generation systems to industrial levels and the costs associated with acquiring and operating CAP equipment, including high-voltage power supplies, plasma generation systems, and control systems, represent a significant barrier to its adoption in the food industry. Reproducibility studies of the results are required. Despite these challenges, ongoing research in CAP seeks to optimize this technology.

9 Electron-beam (E-beam)

During this process, electrons are generated from electricity in a vacuum. The electrons are fired or pulsed from an electron gun (consisting of a cathode, grid, and anode), creating a beam of electrons. The beam of pulsed electrons is carried across a radio frequency wavelength in the linear accelerator, which has positively

TABLE 3 Summary of the conducted research on cold plasma-induced mycotoxin degradation.

Mycotoxin	Food	Plasma type condition	Principal results	References
AOH, AME, TeA, and Ten	Fresh wolfberries	DBD	Approximately 62.84% of reduction for all mycotoxins	Du et al. (2023a)
DON and OTA	Rice grain	DBD	Reduction of the mycotoxin content by up to 61.25% for DON and 55.64% for OTA.	Guo et al. (2023)
DON	Wheat	DBD	Reduction of the mycotoxin content by up to 25.82%	Chen et al. (2022)
AFT	Peanuts	Rotary plasma jets	Reduction of the mycotoxin content by up to 98.25%	Lin et al. (2022)
DON	Wheat grains	DBD micro discharge	Reduction of the mycotoxin content by up to 56%	Feizollahi et al. (2020)
T-2 and HT-2	Barley grains	DBD	Reduction of the mycotoxin content by up to 43.25% for T2 and 29.23% for HT-2.	Kiš et al. (2020)
OTA	Roasted coffee	DBD with radio-frequency generator (RF)	Reduction of the mycotoxin content by up to 50%	Casas-Junco et al. (2019)
AOH, AME, and TEN	Wheat flour	SDBD	Reduction of the mycotoxin content by up to 60.6% for AOH, 73.8% for AME, and 54.5% TEN.	Hajnal et al. (2019)
AFT	Peanuts	Atmospheric-pressure plasma jet	Reduction of the mycotoxin content by up to 38%	Iqdiem et al. (2020)
AFB ₁	Rice and wheat grains	Corona discharge plasma jet	Reduction of the mycotoxin content by up to 56.6% for rice and 45.7% for wheat.	Puligundla et al. (2020)
AFB ₁ and AFB ₂	Hazelnut blend	Cold atmospheric pressure (AP) and low-pressure (LP)	Reduction of the mycotoxin content by up to 71% for both plasmas	Sen et al. (2019)
AFB ₁ and FB ₁	Maize	DBD	Reduction of the mycotoxin content by up to 65 and 64% for AFB ₁	Wielogorska et al. (2019)
AFB ₁	Corn kernels	High-voltage atmospheric cold plasma	Significant degradation of mycotoxin was achieved in modified atmosphere gas composition and higher RH (40 and 80% instead of 5%).	Shi et al. (2017)

AFB: Aflatoxin B₁ and B₂; AOH: Alternariol; AME: Alternariol methyl ether; TeA: Tenuazonic Acid; DON: Deoxynivalenol; OTA: Ochratoxin A; AFT: Aflatoxin; FB₁, Fumonisin B₁; AFT: Aflatoxin; TEN: Tentoxin; T-2: Trichothecene; HT-2: Trichothecene.

and negatively charged cavities that increase the speed of the beam. Unfortunately, its penetration power is low, limiting it to surface disinfection (Hertwig et al., 2018).

9.1 Mechanisms of action

Electrons result in breaking molecular bonds and creating free radicals; e-beam may ionize the water molecules to produce unstable free radicals, which can influence either directly the RNA or DNA molecules or indirectly affect the structure of molecules as a result of radiolysis of water, thus damaging other cellular metabolic pathways promoting intracellular oxidation, which results in cell injury and death.

According to a recent study (Mohamed et al., 2022), *R. oryzae* cell wall structure was destroyed by E-beam, which also increased chitinase activity and decreased chitin content. The integrity of the cell membrane is compromised, leading to a decrease in pH, an increase in relative conductivity, and a reduction in soluble protein. E-beam induces oxidative stress in cells, raising the concentration of increasing H₂O₂ content. This includes the production of free radicals and ROS, which damage membranes and essential enzymes. It also reduces the activity of defense enzymes (CAT and SOD) and DPPH free radical scavenging. This phenomenon suggests that E-beam can induce redox homeostasis disorders, metabolic dysfunction, and cell structural damage.

Microorganisms with large genomes are usually more susceptible to radiation than smaller genomes. Several variables, including

dosage, microorganism species, and food composition, frequently influence the e-beam's ability to decontaminate food. As energetic ions influence the cell membrane and speed up the rate at which microorganisms are destroyed, increasing the irradiation dose can also affect the structure and configuration of RNA (Schmidt et al., 2019).

9.2 Filamentous fungal reduction and mycotoxins degradation

The effect of e-beam treatment doses on the inactivation of molds in different food commodities (cereals, beans, and species) has been investigated. Treatments at 6 kGy applied to red pepper powder led to significant decontamination of yeasts and molds naturally present (Woldemariam et al., 2020). Other studies showed a significant decrease of up to 88% in fungal contamination with increasing irradiation doses of up to 6.2 kg. The mixture of fungal subpopulations on maize seeds with varying radiation sensitivity is responsible for the sigmoidal pattern observed in the survival curve of total fungi as determined by the blotter test. According to the authors, the order of the most common fungi's sensitivity to electron beam treatment was *Penicillium* spp. > *Fusarium* spp. > *Aspergillus* spp., with total inactivation occurring at irradiation doses of 1.7, 2.5, and 4.8 kGy, respectively (Sanaei Nasab et al., 2023). Conversely, in split beans of *Canavalia maritima*, higher doses (10–15 kGy) significantly reduced the fungal incidence and eliminated the mycotoxins. Additionally, beans exposed to 10 kGy radiation had a minimum six-month shelf life (Akhila et al., 2021). However, e-beam irradiation of barley infected with *Fusarium* spp. at 6–10, kGy did not result in a discernible decrease in the fungal incidence or DON contents. However, it was noted that the resulting barley malt had a much lower DON content and fungal occurrence (Schmidt et al., 2018).

The efficacy of e-beam on the mycotoxins reduction depends on the type of mycotoxin: Yang et al. (2020) reported the effect of ozone and e-beam irradiation on the degradation of zearalenone and ochratoxin A, using a dose of 16 kGy, and found degradation of 92% of OTA and 72% the of ZEN when treated in acetonitrile. Similarly, Luo et al. (2017) observed differences in ZEN and OTA reduction in corn; in this case, the reduction was 56% for ZEN and 75% for OTA. Also, in red pepper, OTA was reduced by 25% after treatments of 30 kGy (Woldemariam et al., 2020). After 20 kGy of E-beam treatment, aflatoxin that was naturally present in maize was significantly reduced, but fumonisin was not (Mohamed et al., 2022).

9.3 Drawbacks

The high investment and operating costs associated with electron beam technology represent a significant challenge to its widespread adoption in the food industry. The acquisition and maintenance of electron accelerators, vacuum systems, and radiation shielding entail large investments. In addition, the limited penetration of the electron beam into dense foods requires higher irradiation doses, which can induce undesirable changes in the sensory and nutritional quality of the products. These changes can manifest in color alterations, loss of volatile compounds, protein degradation, and formation of free radicals. Furthermore, the effectiveness of E-beam decontamination

is affected by factors such as beam energy, sample geometry, and packaging material properties. Low beam penetrability, especially for low-energy electrons, and the need to irradiate both sides of the product limit its application in large-sized foods or packaging with low beam permeability.

10 Ultrasound (US) treatments

The US consists of sound waves whose frequency exceeds the limit of human hearing (around ~20 kHz) and is classified into three groups: (i) power frequency (frequencies between 20 and 100 Hz), (ii) high-frequency US (frequencies between 20 kHz and 100 MHz), and (iii) diagnostic US (frequencies >1 MHz). On the other hand, the US, according to its application, is classified into low intensity (less than 1 W/cm²) and high intensity (10–1,000 W/cm²) (Gavahian et al., 2021).

10.1 Mechanisms of action

With high-intensity ultrasound, cavitation bubbles are created through generated pressure cycles, which grow irregularly during the compression/rarefaction cycles, absorbing energy until a maximum when implodingly collapsing, releasing a large amount of energy and, in some instances, producing radicals and ROS. This released energy generates shear forces with very high pressure and temperature (5,000 K and 5,000 atm), capable of destroying any membrane or cell wall of microorganisms (Moosavi et al., 2021) (Figure 2). Three aspects influence the effectiveness of microbial inactivation: the cavitation threshold (intensity, frequency, amplitude, temperature, and external pressure), the medium (viscosity, volume, pH, and initial amount of microorganism), and therefore the properties of the microorganism (cell wall, size, shape, endospore or growth phase, and growth phases) (Evelin and Silva, 2020).

There is evidence that using liquids improves microbial inactivation efficacy: higher water content facilitates the transfer of acoustic waves and high energy, increasing the fungicidal capacity (Liu et al., 2019b). In this sense, foods that contain more water, such as fruits and vegetables, are affected by ultrasound treatment to a greater extent. Moreover, it has been mentioned that water breaks down into hydrogen, hydroxyl, and hydrogen peroxide radicals that can break covalent bonds in mycotoxins (Liu et al., 2019b).

10.2 Filamentous fungal suppression and mycotoxins reduction

The effectiveness of the US in inhibiting fungi and mycotoxin production has also been reported. Rudik et al. (2020) used high-frequency (24–36 kHz) and low-intensity (~1.0 W/cm²) US to reduce fungi and mycotoxins in cereal grains. Villalobos et al. (2019) reported that pretreatment of fresh figs for 30 min at 40 kHz and 60 W combined with osmotic dehydration considerably decreased microbial counts, especially the growth of mycotoxin-producing fungi such as those of the genus *Penicillium* (which produce penicillic acid, griseofulvin, and roquefortine), without affecting the organoleptic parameters of the figs. Nevertheless, Schmidt et al. (2019) reported

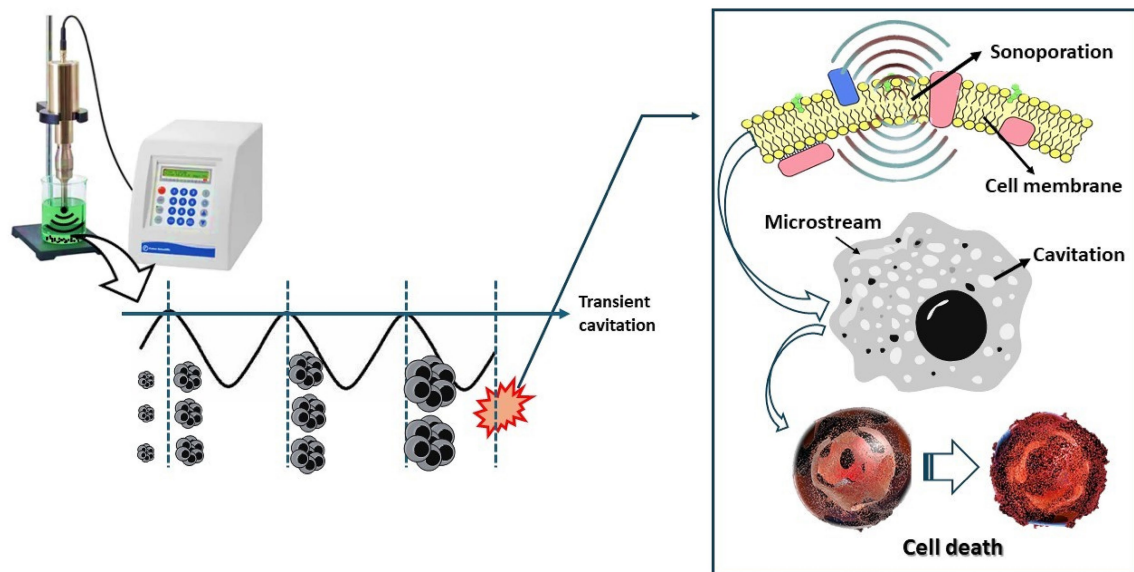


FIGURE 2
Mechanism of action of ultrasound. Cell death can occur by sonoporation of the cell membrane, by cavitation bubbles, or by microstream.

different findings. They demonstrated that US treatment (45 kHz and 120 W for 5–20 min) on cereal grains had no desired results because it could not prevent fungi' growth during storage.

In the study reported by Liu et al. (2019b), degradation of AFB₁ (96.5%), ZEA (95.9%), OTA (91.6%), and DON (60.8%) was achieved using US treatment with intensity between 2.2–11 W/cm² and between 10–50 min. In another study, Liu et al. (2019a) reported a US treatment of 550 W power with a 13 mm-probe, a frequency of 20 kHz, and a power intensity of 6.6 W/cm² for 80 min increased AFB₁ degradation by 85.1%. All these studies demonstrate that the US is an adequate treatment to inactivate pathogenic microorganisms in foods without altering the sensory characteristics and stability.

10.3 Drawbacks

The attenuation of ultrasonic waves in heterogeneous media, such as food, significantly reduces their penetration, requiring longer processing times to ensure adequate decontamination. This, in turn, can negatively affect the sensory and textural quality of products. Furthermore, the heat generation inherent to the ultrasonic process can degrade thermolabile compounds, such as vitamins and enzymes, compromising the nutritional value of foods. The investment and operating costs of ultrasonic equipment, including generators, transducers, and control systems, are high and can represent an economic barrier to its large-scale implementation.

11 Nanoparticles-metal and metal oxide nanostructures-based antimicrobials

Nanomaterials include nanoparticles (NPs), clusters of very thin atoms with dimensions between 1 and 100 nm, and can be classified

into organic, inorganic, and carbon-based (Khan et al., 2019). Metallic nanoparticles of Ag, Zn, Pd, Cu, Fe, Se, and Ni were useful as antifungal agents (Cruz-Luna et al., 2021). The size, shape, and structure of metallic NPs are crucial for antifungal activity. It has been observed that the antifungal effect increases with the decrease in size (3–30 nm) since the surface area/volume ratio increases, which allows the fungal cell membrane to be penetrated more easily and affects the cytosol (Win et al., 2020). Larger sizes of nanoparticles (40–90 nm) do not allow cell membrane penetration but affect hyphae and spores, generating malformations in these structures and inhibiting fungal growth (Bocate et al., 2019).

Also, the antifungal efficacy of nanoparticles of metal oxides, such as TiO₂, CuO, and ZnO, has been reported due to their optical and electrical characteristics (Ajmal et al., 2019). When irradiated with a specific wavelength laser, these NPs can undergo photocatalytic processes and generate antimicrobial effects. In addition, these NPs are considered biocompatible, slightly toxic, very stable, and with greater specificity for their action (Ilyas et al., 2021).

11.1 Mechanisms of action

Among metallic NPs, silver NPs (AgNPs) have been the most widely used thanks to their ability to disrupt the cell membrane and other biological functions (Akpınar et al., 2021; Cruz-Luna et al., 2021). In fact, they can alter the structure of proteins, nucleic acids, ribosomes, and gene expression, affecting such essential processes as ATP synthesis, protein synthesis, and nutrient transport (Na⁺ and K⁺), membrane potential and leading to apoptosis (Sunday et al., 2021). Additionally, AgNPs can generate lipid peroxidation due to ROS generated and affect the post-translational modifications of proteins in the Golgi apparatus (Guo et al., 2022; Rizwana et al., 2021). However, antimicrobial activity is also strongly influenced by the size, morphology, surface charge, and type of material with which the NPs

are produced. Positively charged NPs react more readily to the negatively charged cell membranes of bacteria and fungi, especially if they are tiny and have a large surface area (Cruz-Luna et al., 2021). Molina-Hernandez et al. (2022a,b) confirmed the adaptive fungal responses during AgNPs with catechin (accumulation of trehalose, glycerol, citric and oxalic acids, volatile compounds derived from the α - or β -oxidation of fatty acids as alcohols, aldehydes, alkanes, and acids) and several enzymes correlated with cell growth.

The antimicrobial activity of metal oxide NPs (TiO_2 , ZnO , CuO) comes from the generation of ROS (Zudyte and Luksiene, 2021), which is a function of the valence band and conduction band energies. Thus, for example, in ZnO , any incident radiation with photons of energy above the bandgap (3.3 eV) will cause the formation of photoactivated free electrons in the conduction band from the valence band and holes in the valence band (Figure 3). The interaction of photogenerated free electrons with environmental oxygen forms superoxide radicals, while the holes generated in the valence band interact with water molecules, producing H^+ and $\cdot\text{OH}$. Subsequently, the interaction of these radicals with other H^+ in the medium produces $\text{HO}_2^{\cdot}$ and hydrogen peroxide. Singlet oxygen, a powerful oxidizing agent, can also be produced through aqueous reactions of $\text{O}_2^{\cdot-}$ (Ilyas et al., 2021). These radicals are highly reactive and can interact with biomolecules, such as lipids, carbohydrates, DNA proteins, amino acids, and enzymes, on the cell surface, disrupting their vital functions in the cell and causing cell death (Tralamazza et al., 2016).

11.2 Filamentous fungal reduction

In agriculture, nanomaterials have offered alternatives for the control of phytopathogenic fungi, especially metallic nanoparticles

(Alghuthaymi et al., 2021; Kutawa et al., 2021). In addition, numerous nanomaterials have been created to prevent diseases in citrus, grapes, bananas, apples, mangos, peaches, and nectarine and have demonstrated potential in post-harvest management (Ruffo Roberto et al., 2019).

The antifungal activity of CuNPs with sizes between 11 and 55 nm was studied in tomato crops (*Lycopersicon esculentum*) by comparing their effectiveness with commercial copper-based products (Ashraf et al., 2021). The results showed that a lower concentration was needed in the formulation of antifungals with CuNPs compared to commercial products to achieve a good inhibition of the fungus *Phytophthora infestans* without negatively affecting the plant. Since then, many studies have shown the effectiveness of these nanoparticles against various phytopathogenic fungi (Table 4). On the other hand, nickel nanoparticles (NiNPs) (Ahmed et al., 2016) demonstrated effectiveness against the growth and sporulation capacity of *F. oxysporum* under *in vitro* conditions with 100 ppm of NPs, while under *in vivo* conditions, they reduced the severity up to ca. 60% in tomato and lettuce crops using 50 ppm of NPs. Silver nanoparticles (AgNPs) synthesized with phenolic compounds (PCs) showed antifungal activity against *A. niger* isolated from coffee seeds (Scroccarello et al., 2021), especially on spore germination and mycelial growth, which is in line with the trend of antioxidant capacity (catechin < caffeic acid < myricetin < gallic acid). The strong inhibition was attributed to the ability of the different PCs to generate/stabilize AgNPs with different envelopes, the residual antioxidant capacity, and the ability to interact and aggregate during their “attack” on the hyphae.

Many studies reported the photocatalytic effectiveness of TiO_2 and ZnO NPs in inhibiting fungi (Table 5). Using visible radiation

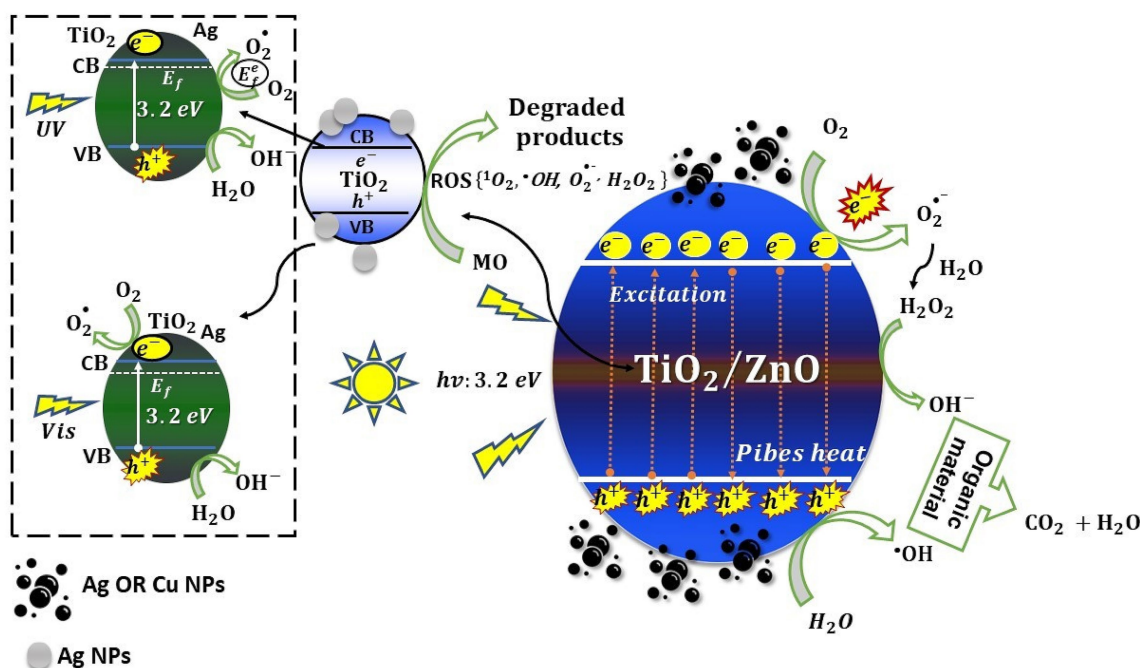


FIGURE 3

Mechanism of photocatalytic activity of Cu/Ag-doped TiO_2 or ZnO NPs and ROS production under irradiation.

TABLE 4 Sample of studies of metal nanoparticles applied for fungi control.

Fungal specie	NP	Synthesis method	Size (nm)	Concentration (µg/g)	Shape	Assay	Antifungal mechanism and MIC	References
<i>A. niger</i> ; <i>A. terreus</i> ; <i>A. fumigatus</i>	Cu	Biological (<i>Talaromyces pinophilus</i>)	10	2000	Spherical	<i>In vitro</i>	ROS. <i>A. niger</i> (62.5 µg/mL); <i>A. terreus</i> (125 µg/mL); <i>A. fumigatus</i> (62.5 µg/mL)	Hasanin et al. (2022)
<i>A. niger</i> ; <i>A. oryzae</i>	Cu	Chemical (hydrothermal)	14 ± 2	0.2–0.8	Spherical	<i>In vitro</i>	N.R. <i>A. niger</i> (20 µg/mL); <i>A. oryzae</i> (20 µg/mL)	Seku et al. (2018)
<i>F. graminearum</i> ; <i>F. poae</i> ; <i>F. avenaceum</i> ; <i>F. sporotrichioides</i>	Ag	Biological (<i>P. ginseng</i> extracts)	50–90	5–100	Spherical	<i>In vitro</i> – <i>In vivo</i>	Only a reduction in the sporulation reported	Yugay et al. (2021)
<i>A. flavus</i> ; <i>A. niger</i> ; <i>A. terreus</i> ; <i>P. notatum</i> ; <i>F. solani</i> ; <i>F. oxysporum</i> ; <i>T. viride</i> ; <i>Verticillium dahliae</i> ; <i>P. spinulosum</i>	Ag	Biological (<i>Bacillus pseudomycolides</i>)	25–43	10–90	Spherical	<i>In vitro</i>	Hyphae damage. <i>A. flavus</i> (85 µg/mL); <i>A. niger</i> (80 µg/mL); <i>A. terreus</i> (80 µg/mL); <i>P. notatum</i> (70 µg/mL); <i>F. solani</i> (90 µg/mL); <i>F. oxysporum</i> (75 µg/mL); <i>T. viride</i> (95 µg/mL); <i>V. dahliae</i> (75 µg/mL); <i>P. spinulosum</i> (75 µg/mL)	El-Saadony et al. (2019)
<i>Sclerospora graminicola</i> ; <i>A. solani</i> ; <i>M. phaseolina</i> ; <i>Diaporthe longicolla</i>	Se	Chemical	< 100	0–1,000	Spherical	<i>In vitro</i>	ROS. Inactivation of Sulfur proteins in membrane and leakage. Between (150 and 350 µg/mL).	Ismail et al. (2016) ; Nandini et al. (2017) ; Vrandečić et al. (2020)
<i>F. oxysporum</i> f. sp. <i>Lactucae</i> ; <i>F. oxysporum</i> f. sp. <i>Lycopersici</i>	Ni	RF-thermal plasma system	< 100	50–100	N.R.	<i>In vitro</i> – <i>In vivo</i>	Inhibition of sporulation and conidia germination. Membrane leakage – DNA, ROS, protein damage. Reduction of 60% of growth with 100 µg/mL.	Ahmed et al. (2016)
<i>A. flavus</i> ; <i>F. verticillioides</i>	*Zn/ CdO; Zn	Chemical (heat treatment)	11.5–29.1	**N.R.	Spherical-Nanorods	<i>In vitro</i>	ROS and liberation of Zn ²⁺ and Cd ²⁺ . Cell membrane and organelles leakage. Cell wall penetration and metabolism disturb. <i>A. flavus</i> (23% of growth inhibition); <i>F. verticillioides</i> (15% of growth inhibition).	Kumar et al. (2020)
<i>A. flavus</i> ; <i>A. niger</i> ; <i>A. fumigatus</i> ; <i>A. oryzae</i> ; <i>A. Terreus</i> ; <i>C. tropicalis</i>	HgS	Chemical–physical (hydrothermal-ultrasound)	72.78	100 (ppb)	Crystalline hexagonal	<i>In vitro</i>	ROS disrupting cell functions, enzymatic system membrane, growth, morphology, and ATP alterations. N.R.	Ibrahim et al. (2021)

Adapted from [Cruz-Luna et al. \(2021\)](#).

ROS: Reactive oxygen species; N.R: Not reported; R.F: Radio Frequency. *CdO:Zn: Zn nanoparticles doped with CdO. ** N.R.: The final concentration of the NP is non-reported. However, the mol ratio of Zn was reported between 3 and 10 mol% to CdO.

TABLE 5 Examples of studies on metal oxide nanoparticles used to reduce filamentous fungi.

Fungal specie	NP	Synthesis method	Size (nm)	Concentration (mg/L)	Shape	Assay	Antifungal mechanism and MIC	References
<i>Arthrographis cuboidea</i> ; <i>A. niger</i> ; <i>A. fumigatus</i>	TiO ₂	Biological (<i>Mentha arvensis</i>)	20–70	10,000–30,000	Spherical	<i>In vitro</i>	Cell wall penetration. ROS production. DNA, RNA, enzymes, and macromolecules damage. <i>Arthrographis cuboidea</i> (10 mg/mL); <i>A. niger</i> (10 mg/mL 74% reduction); <i>A. fumigatus</i> (10 mg/mL)	Ahmad et al. (2020)
<i>A. alternata</i> ; <i>F. verticillioides</i>	ZnO	Chemical (one-pot precipitation synthesis)	65.3	2–5,000	Irregular-shaped	<i>In vitro</i>	Zn ²⁺ interaction with the fungal cell wall, cell metabolism disturbances, DNA, ribosome disassembly protein denaturation, electron chain disruptions. ROS causing lipid peroxidation. <i>A. alternata</i> (5 mg/mL, 34% of inhibition); <i>F. verticillioides</i> (5 mg/mL, 39% of inhibition)	Akpomie et al. (2021)
<i>B. cinerea</i>	ZnO	Biological (<i>Citrus sinensis</i>)	33.1 ± 11.7	100,000–500,000 (<i>In-vitro</i>) – 9.54 Fresh weight of strawberries (<i>In vivo</i>)	Hexagonal	<i>In vitro</i> – <i>In vivo</i>	Size and concentration-dependent effect. Probably cell membrane leakage, cytosolic content affectation, DNA, and protein denaturation. <i>B. cinerea</i> (100 mg/mL, 25.2% of inhibition)	Gao et al. (2020)
<i>P. italicum</i>	CH/ZnO/SEO	Commercial (Sigma Aldrich)	< 50	Film blends (CH 80/ZnO 10/SEO 10)	Slender shape	<i>In vitro</i> – <i>In vivo</i>	Loss of membrane integrity of spores. Intracellular interaction, ionic membrane imbalance, DNA denaturation. Cell membrane destruction, ROS. Fungal cell wall synthesis inactivation. Inhibition of 85% growth and almost 80% spore germination inhibition.	Wardana et al. (2022)
<i>F. oxysporum</i> f. sp. <i>lactucae</i>	CuO-embedded hydrogels	Commercial (Sigma Aldrich)	< 50	31	Spherical	<i>In vitro</i> – <i>In vivo</i>	ROS. Membrane disruption, cell lysis. Up to 74.1% reduction of dehydrogenase activity.	Shang et al. (2021)
<i>P. italicum</i> ; <i>P. digitatum</i>	CH/CuO/CEO Films	Commercial (Sigma Aldrich)	< 50	6–75 NPs; Film blends (CH 80/CuO 10/CEO 10)	Slender shape	<i>In vitro</i> – <i>In vivo</i>	Spore growth is inhibited. Loss of membrane integrity. Metabolic disruption. Synergistic effect between CuO and CEO. Membrane disruption and more effective penetration of CuO. Up to 70% inhibition of spores and up to 80% inhibition of mold infection in vivo.	Wardana et al. (2021)

(Continued)

TABLE 5 (Continued)

Fungal specie	NP	Synthesis method	Size (nm)	Concentration (mg/L)	Shape	Assay	Antifungal mechanism and MIC	References
<i>Penicillium</i> spp.; <i>A. flavus</i>	GO/CuO	Chemical (hydrothermal and Hummer's method)	<100 lengths and 23 size	500–1,000	Cuo (nanorods)/GO (nanosheets)	<i>In vitro</i>	ROS. Membrane penetration of ion release. <i>Penicillium</i> spp. (500 µg/mL); <i>A. flavus</i> (1,000 µg/mL)	Banu et al. (2022)
<i>A. niger</i>	MgO/Cellulose	Chemical (co-precipitation)	21	2,000–8,000	Spherical	<i>In vitro</i>	The nanocomposite between cellulose and MgO NPs prevents the agglomeration of NPs, improving the active surface available. Damage to fungi's membranes. Induction of oxidative stress. Release of toxic ions of Mg ²⁺ , membrane leakage. Affection of cytosolic content. The composite formed by 4 mg/mL MgO and 1 mg/mL cellulose inhibited growth by 85.03%.	Safaei and Taran (2022)
<i>A. fumigatus</i> ; <i>A. niger</i> ; <i>A. cuboidea</i>	CaO	Biological (pomegranate peel extract)	30–50	10,000–30,000	Spherical	<i>In vitro</i>	ROS causes protein, enzymes, and DNA denaturation, affecting several cell processes and causing death. <i>A. fumigatus</i> (30 mg/mL, 19.6% of inhibition); <i>A. niger</i> (30 mg/mL, 18.5% of inhibition); <i>A. cuboidea</i> (30 mg/mL, 29.6% of inhibition)	Ahmad et al. (2022)

N.R: Not reported; US: Ultrasound; CH: Chitosan; SEO: Indonesian sandalwood essential oil; CEO: Indonesian cedarwood essential oil; GO: Graphene oxide.

(irradiation of visible fluorescence light of 8 W, 2950 lux intensity), Ag-doped TiO₂ nanoparticles allowed the control of *F. solani* and *Venturia inaequalis* by generating ROS through a photocatalytic mechanism ([Boxi et al., 2016](#)). Commercial NPs with sizes less than 100 nm with the capacity to inhibit fungi such as *B. cinerea*, *P. expansum*, and *F. oxysporum* have been studied, and the control of *B. cinerea* directly in strawberries through a photocatalytic mechanism, using visible light and 10 min of ultrasound to disperse the ZnO NPs has been reported.

Despite a large number of published reports on the use of metal NPs and metal oxides ([Table 5](#)), few studies of *in situ* NPs demonstrate these systems' applicability directly in foods ([Gao et al., 2020](#); [Luksiene et al., 2020](#); [Maringgal et al., 2020](#); [Rezaei et al., 2020](#); [Shang et al., 2021](#); [Wardana et al., 2021](#); [Zudyte and Luksiene, 2021](#)).

11.3 Drawbacks

Metal and metal oxide nanoparticles safety, especially at high concentrations, is a general concern; since metal and metal oxide nanoparticles can alter the structure of proteins, nucleic acids, ribosomes, and gene expression. It is essential to assess the cytotoxicity in different cell lines or microorganisms and decide the safety of the

nanoparticles used in different environments. Very low doses of metal oxide nanoparticles are preferred to avoid cytotoxicity in non-target microorganisms or cells. Nanoparticles' complete and accurate characterization is complex due to their small size and the diversity of available analytical techniques. In the food matrix, nanoparticles tend to agglomerate due to intermolecular forces, which hinder their dispersion and affect their properties.

12 Concluding remarks

The non-thermal technologies discussed here demonstrated the potential of reducing mycelium proliferation, preventing spore propagation, and degrading mycotoxins during post-harvest storage of cereals, seeds and fruits. The outcomes of the different studies indicate that these non-thermal technologies decrease fungal proliferation by altering the structure of the wall and cell membrane and the DNA structure, disrupting fungal metabolism and leading to rapid microbial cell death. In addition, it is evident from the comparative analysis of the antifungal activity of the non-thermal technologies reported here that they share several similarities, notably in the production of ROS, which induces oxidative stress and cell death, binds DNA, proteins and enzymes to disrupt cell function, prolongs the lag phase associated with microbial

proliferation, and reduces the growth rate. At the same time, these technologies use a multi-targeted mode and prevent microorganisms from developing resistance; some of these technologies, like CAP, PL, and UV light, are limited to a surface-level treatment. Therefore, it is to consider that the small proportion of alive cells that remain can overcome the stress induced by ROS, producing enzymes and compounds like glutathione as well as osmolytes like trehalose that protect lipid membranes, stabilize proteins and act as free radical scavengers, and modulate cell wall carbohydrates such as chitin and glucan. Consequently, it would be very difficult for the organism to mount a stress response, even if it had enough time. It is also important to remember that stress exposure may strengthen memory or priming, making them more resilient to stress in the future.

As far as the detoxification of mycotoxins is concerned, the non-thermal processing methods discussed here employ diverse mechanisms for detoxifying mycotoxins. Certain approaches, including CAP, E-Beam, and O_3 , generate reactive species and deactivate the mycotoxins by altering their structure. Other techniques, such as PL and UV, instigate the fragmentation of the mycotoxins via photochemical reaction and destroy their molecular structure, which is responsible for their toxicity. However, several uncertainties remain regarding the toxicity of the degradation products generated by these treatments within the food matrix. While some authors reported that the degradation products are less toxic than the original molecule, further research is needed to fully understand their safety. For instance, CAP treatment of ZEN resulted in less toxic compounds (Liu et al., 2024). Similarly, CAP treatment of OTA in coffee produces slightly toxic compounds, such as L-phenylalanine, and patulin which can be completely decomposed by O_3 in diglycolic acid, oxalic acid, and CO_2 (Casas-Junco et al., 2019). Additionally, The main ozonolysis products of AFB1 including $C_{17}H_{14}O_{10}$, $C_{18}H_{16}O_{10}$, $C_{16}H_{10}O_6$, $C_{19}H_{15}NO_9$, $C_{17}H_{12}O_9$, and $C_{17}H_{12}O_9$ did not show any toxic effects on animals (Afsah-Hejri et al., 2020).

Even though these non-thermal technologies have numerous benefits for the food industry, some are still exclusively utilized in laboratories and are seldom employed in the field. The total inactivation of spores with some of these technologies (CAP, O_3 , PL) could require a long, prolonged treatment time, which could compromise the color, the integrity of lipids, proteins, and vitamins, and the texture of the product's texture. Thus, for these technologies to be used successfully, it is crucial to identify key parameters that influence both food quality and the inactivation of molds and mycotoxins to optimize the process. However, defining a precise dose–response range is challenging as various factors such as the fungal species, the amount of inoculum, the spore or mycelium structure, and whether the fungi are xerophilic or acidophilic all play a role. The equipment used, such as the CAP, and the presence of certain gases can also influence the effectiveness. The composition of the food, water content, water activity, and structure also play an important role, with treatments generally more effective in liquid than solid media. Additionally, the shape of the samples is a challenge for the application as many of these technologies cannot treat samples with irregular forms uniformly, making large-scale processing challenging. Therefore, one of the most important challenges is to optimize the parameters for non-thermal technologies since it is a complex task that requires careful matching of the technology, food characteristics, and specific mycotoxins and fungi. For this reason, the successful application of these methods in post-harvest scenarios depends on achieving a balance between treatment efficacy and food quality preservation. Further research is necessary to understand the long-term effects on the sensorial and nutritional

properties of the treated products as well as the potential allergenicity in these products. In the case of nanoparticles, it is important to study their migration, toxicity, and permissible limit when using them in foods or food packaging containing nanoparticles.

13 Future trends

The future of non-thermal technologies to eliminate fungal growth and mycotoxin contamination in food is promising. Innovations in this area could include the use of combined non-thermal technologies and natural antifungal compounds to increase the efficacy of fungal control. This approach could lead to the development of safer, more natural methods of food preservation and reduce the need for synthetic chemicals.

Although the ability of microorganisms such as lactic acid bacteria and yeasts to biotransform or adsorb mycotoxins in fermentative processes is well known, synergy with non-thermal low-intensity technologies such as pulsed electric fields, cold plasma, and ultrasound represents an emerging and promising area of research. By enhancing cell permeability, increasing enzymatic activity, and facilitating the transfer of metabolites from beneficial microorganisms, these technologies offer the under-researched potential to reduce mycotoxin contamination in food, thereby preserving nutritional quality and improving food safety.

Studies on the biology of spore resistance, dormancy, and germination could lead to new approaches for food preservation that take advantage of the signaling pathways associated with these phenomena or provide insights into the precise elimination of spore subpopulations. Therefore, it is only logical to further investigate the responses of fungi to sublethal stress caused by these technologies. In addition, global studies have shown that although non-thermal technologies can effectively reduce mold contamination and infections caused by these microorganisms, their effects on mold growth, biosynthesis, and mycotoxin degradation vary depending on the fungal species and mycotoxin structure. Therefore, a large number of fungi still need to be investigated. This area will grow as more and more countries introduce legislation to regulate the handling of mycotoxins and fungal spores. On the other hand, it is important to study microorganisms resistant to harsh environmental conditions, as they are more resilient to various treatments and can provide insights into developing of more robust non-thermal methods. Understanding their resistance mechanisms can lead to innovative solutions that increase product safety and efficacy while minimizing environmental impact. In addition, research into the genetic and metabolic pathways of these microorganisms could reveal new targets for intervention and enable the development of specialized treatments that are both effective and sustainable.

Developments in materials science and engineering may result in discoveries that increase the efficiency of these technologies and open the door for more ecologically friendly solutions.

Author contributions

JM-H: Methodology, Writing – original draft, Writing – review & editing, Investigation. CG-T: Investigation, Methodology,

Writing – original draft, Writing – review & editing. LN: Investigation, Writing – review & editing, Formal analysis. JD-O: Investigation, Writing – review & editing, Methodology, Visualization, Writing – original draft. MR: Formal analysis, Investigation, Writing – review & editing. GC-B: Formal analysis, Investigation, Writing – review & editing. CC-L: Methodology, Writing – original draft, Writing – review & editing, Conceptualization, Project administration, Supervision.

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References

- Abbas Syed, Q. (2017). Pulsed electric field technology in food preservation: a review. *J. Nutr. Health Food Eng.* 6, 168–172. doi: 10.15406/jnhfe.2017.06.00219
- Abd El Hamid, S., Hazman, M., Zayton, M., Elwahi, A., Hamouda, S., and Kabil, F. (2022). Pro-active application of UV-B light could protect strawberry fruits against the postharvest gray mold. *Egypt. J. Agric. Res.* 100, 260–270. doi: 10.21608/ejar.2022.117209.1198
- Abuagela, M. O., Sarnoski, P. J., Iqdiem, B. M., Mostafa, H., Gu, L., and Smith, M. E. (2018). Assessing pulsed light treatment on the reduction of aflatoxins in peanuts with and without skin. *Int. J. Food Sci. Technol.* 53, 2567–2575. doi: 10.1111/ijfs.13851
- Adebo, O. A., Molelekoa, T., Makhuvele, R., Adebisi, J. A., Oyediji, A. B., Gbashi, S., et al. (2021). A review on novel non-thermal food processing techniques for mycotoxin reduction. *Int. J. Food Sci. Technol.* 56, 13–27. doi: 10.1111/ijfs.14734
- Afsah-Hejri, L., Hajeb, P., and Ehsani, R. J. (2020). Application of ozone for degradation of mycotoxins in food: a review. *Compr. Rev. Food Sci. Food Saf.* 19, 1777–1808. doi: 10.1111/1541-4337.12594
- Aganovic, K., Hertel, C., Vogel, R. F., John, R., Schlüter, O., Schwarzenbolz, U., et al. (2021). Aspects of high hydrostatic pressure food processing: perspectives on technology and food safety. *Compr. Rev. Food Sci. Food Saf.* 20, 3225–3266. doi: 10.1111/1541-4337.12763
- Ahmad, W., Jaiswal, K. K., and Soni, S. (2020). Green synthesis of titanium dioxide (TiO₂) nanoparticles by using *Mentha arvensis* leaves extract and its antimicrobial properties. *Inorg. Nano-Metal Chem.* 50, 1032–1038. doi: 10.1080/24701556.2020.1732419
- Ahmad, W., Kamboj, A., Banerjee, I., and Jaiswal, K. K. (2022). Pomegranate peels mediated synthesis of calcium oxide (CaO) nanoparticles, characterization, and antimicrobial applications. *Inorg. Nano-Metal Chem.* 54, 157–164. doi: 10.1080/24701556.2021.2025080
- Ahmed, A. I. S., Yadav, D. R., and Lee, Y. S. (2016). Applications of nickel nanoparticles for control of fusarium wilt on lettuce and tomato. *Int. J. Innov. Res. Sci. Eng. Technol.* 5, 7378–7385. doi: 10.15680/IJIRSET.2016.0505132
- Ajmal, N., Saraswat, K., Bakht, M. A., Riadi, Y., Ahsan, M. J., and Noushad, M. (2019). Cost-effective and eco-friendly synthesis of titanium dioxide (TiO₂) nanoparticles using fruit's peel agro-waste extracts: characterization, in vitro antibacterial, antioxidant activities. *Green Chem. Lett. Rev.* 12, 244–254. doi: 10.1080/17518253.2019.1629641
- Akbar, A., Medina, A., and Magan, N. (2020). Potential control of Mycotoxigenic Fungi and Ochratoxin A in stored coffee using gaseous ozone treatment. *Microorganisms* 8:1462. doi: 10.3390/microorganisms8101462
- Akdemir Evrendilek, G., Bulut, N., Atmaca, B., and Uzuner, S. (2022). Prediction of aspergillus parasiticus inhibition and aflatoxin mitigation in red pepper flakes treated by pulsed electric field treatment using machine learning and neural networks. *Food Res. Int.* 162:111954. doi: 10.1016/j.foodres.2022.111954
- Akhila, P. P., Sunooj, K. V., Aaliya, B., Navaf, M., Sudheesh, C., Sabu, S., et al. (2021). Application of electromagnetic radiations for decontamination of fungi and mycotoxins in food products: a comprehensive review. *Trends Food Sci. Technol.* 114, 399–409. doi: 10.1016/j.tifs.2021.06.013
- Akpınar, I., Unal, M., and Sar, T. (2021). Potential antifungal effects of silver nanoparticles (AgNPs) of different sizes against phytopathogenic *Fusarium oxysporum* f. sp. radicle-lycopersici (FORL) strains. *SN Appl. Sci.* 3, 1–9. doi: 10.1007/s42452-021-04524-5
- Akpomije, K. G., Ghosh, S., Gryzenhout, M., and Conradie, J. (2021). One-pot synthesis of zinc oxide nanoparticles via chemical precipitation for bromophenol blue adsorption and the antifungal activity against filamentous fungi. *Sci. Rep.* 11:8305. doi: 10.1038/s41598-021-87819-2
- Alghuthaymi, M. A., Kalia, A., Bhardwaj, K., Bhardwaj, P., Abd-Elsalam, K. A., Valis, M., et al. (2021). Nanohybrid antifungals for control of plant diseases: current status and future perspectives. *J. Fungi* 7:48. doi: 10.3390/jof7010048
- Ali, E. M., and Abdallah, B. M. (2024). The potential use of ozone as antifungal and antiflatoxigenic agent in nuts and its effect on nutritional quality. *Braz. J. Biol.* 84:e263814. doi: 10.1590/1519-6984.263814
- Ashraf, H., Anjum, T., Riaz, S., Ahmad, I. S., Irudayaraj, J., Javed, S., et al. (2021). Inhibition mechanism of green-synthesized copper oxide nanoparticles from *Cassia fistula* towards *Fusarium oxysporum* by boosting growth and defense response in tomatoes. *Environ. Sci. Nano* 8, 1729–1748. doi: 10.1039/D0EN01281E
- Avsaroglu, M. D., Bozoglu, F., Alpas, H., Largeteau, A., and Demazeau, G. (2015). Use of pulsed-high hydrostatic pressure treatment to decrease patulin in apple juice. *High Pressure Res.* 35:214–222.
- Awuchi, C. G., Ondari, E. N., Ogbonna, C. U., Upadhyay, A. K., Baran, K., Okpala, C. O. R., et al. (2021). Mycotoxins affecting animals, foods, humans, and plants: types, occurrence, toxicities, action mechanisms, prevention, and detoxification strategies—a revisit. *Food Secur.* 10:1279. doi: 10.3390/foods10061279
- Azam, M. S., Ahmed, S., Islam, M. N., Maitra, P., Islam, M. M., and Yu, D. (2021). Critical assessment of mycotoxins in beverages and their control measures. *Toxins* 13:323. doi: 10.3390/toxins13050323
- Babae, R., Karami-Osboo, R., and Mirabolfathy, M. (2022). Evaluation of the use of ozone, UV-C and citric acid in reducing aflatoxins in pistachio nut. *J. Food Compos. Anal.* 106:104276. doi: 10.1016/j.jfca.2021.104276
- Baltussen, T. J. H., Zoll, J., Verweij, P. E., and Melchers, W. J. G. (2020). Molecular mechanisms of conidial germination in *Aspergillus* spp. *Microbiol. Mol. Biol. Rev.* 84:e00049–19. doi: 10.1128/mmbr.00049-19

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- Bambalele, N. L., Mditshwa, A., Mbili, N. C., Tesfay, S. Z., and Magwaza, L. S. (2023). The antifungal effect of gaseous ozone on *Lasiodiplodia theobromae* causing stem-end rot in “Keitt” mangoes. *Food Secur.* 12:195. doi: 10.3390/foods12010195
- Banu, R., Salvi, N., Gupta, S., Ameta, C., Ameta, R., and Punjabi, P. B. (2022). A facile synthesis of GO/CuO nanocomposite with enhancing photocatalytic activity for the degradation of azure-B dye and its antimicrobial behavior. *Arab. J. Sci. Eng.* 47, 365–378. doi: 10.1007/s13369-021-05421-0
- Bhatta, U. K. (2022). Alternative management approaches of Citrus diseases caused by *Penicillium digitatum* (green Mold) and *Penicillium italicum* (blue Mold). *Front. Plant Sci.* 12:833328. doi: 10.3389/fpls.2021.833328
- Blachowicz, A., Raffa, N., Bok, J. W., Choera, T., Knox, B., Lim, F. Y., et al. (2020). Contributions of spore secondary metabolites to UV-C protection and virulence vary in different *Aspergillus fumigatus* strains. *MBio* 11, 1–12. doi: 10.1128/mBio.03415-19
- Bocate, K. P., Reis, G. F., de Souza, P. C., Oliveira Junior, A. G., Durán, N., Nakazato, G., et al. (2019). Antifungal activity of silver nanoparticles and simvastatin against toxigenic species of *aspergillus*. *Int. J. Food Microbiol.* 291, 79–86. doi: 10.1016/j.ijfoodmicro.2018.11.012
- Boxi, S. S., Mukherjee, K., and Paria, S. (2016). Ag doped hollow TiO₂ nanoparticles as an effective green fungicide against *Fusarium solani* and *Venturia inaequalis* phytopathogens. *Nanotechnology* 27:085103. doi: 10.1088/0957-4484/27/8/085103
- Brito, J. G., Faroni, L. R. D., Cecon, P. R., Benevenuto, W. C. A. D. N., Benevenuto, A. A., and Heleno, F. F. (2018). Efficacy of ozone in the microbiological disinfection of maize grains. *Braz. J. Food Technol.* 21:e2017022. doi: 10.1590/1981-6723.02217
- Bulut, N., Atmaca, B., Akdemir Evrendilek, G., and Uzuner, S. (2020). Potential of pulsed electric field to control *Aspergillus parasiticus*, aflatoxin and mutagenicity levels: sesame seed quality. *J. Food Saf.* 40:e12855. doi: 10.1111/jfs.12855
- Byun, K., Park, S. Y., Lee, D., Chun, H. S., and Ha, S. (2020). Effect of UV-C irradiation on inactivation of *Aspergillus flavus* and *Aspergillus parasiticus* and quality parameters of roasted coffee bean (*Coffea arabica* L.). *Food Addit. Contam. Part A* 37, 507–518. doi: 10.1080/19440049.2020.1711971
- Cacace, F., Bottani, E., Rizzi, A., and Vignali, G. (2020). Evaluation of the economic and environmental sustainability of high pressure processing of foods. *Innovative Food Sci. Emerg. Technol.* 60:102281. doi: 10.1016/j.ifset.2019.102281
- Casas-Junco, P. P., Solís-Pacheco, J. R., Ragazzo-Sánchez, J. A., Aguilar-Uscanga, B. R., Bautista-Rosales, P. U., and Calderón-Santoyo, M. (2019). Cold plasma treatment as an alternative for ochratoxin A detoxification and inhibition of mycotoxigenic fungi in roasted coffee. *Toxins* 11:337. doi: 10.3390/toxins11060337
- Casu, A., Camardo Leggeri, M., Toscano, P., and Battilani, P. (2024). Changing climate, shifting mycotoxins: a comprehensive review of climate change impact on mycotoxin contamination. *Compr. Rev. Food Sci. Food Saf.* 23:e13323. doi: 10.1111/1541-4337.13323
- Chacha, J. S., Zhang, L., Ofoedu, C. E., Suleiman, R. A., Dotto, J. M., Roobab, U., et al. (2021). Revisiting non-thermal food processing and preservation methods—action mechanisms, pros and cons: a technological update (2016–2021). *Food Secur.* 10:1430. doi: 10.3390/foods10061430
- Chen, D., Chen, P., Cheng, Y., Peng, P., Liu, J., Ma, Y., et al. (2019). Deoxynivalenol decontamination in raw and germinating barley treated by plasma-activated water and intense pulsed light. *Food Bioprocess Technol.* 12, 246–254. doi: 10.1007/s11947-018-2206-2
- Chen, X., Qiu, Y., Zhang, J., Guo, Y., Ding, Y., and Lyu, F. (2022). Degradation efficiency and products of deoxynivalenol treated by cold plasma and its application in wheat. *Food Control* 136:108874. doi: 10.1016/j.foodcont.2022.108874
- Chen, C., Zhang, H., Zhang, X., Dong, C., Xue, W., and Xu, W. (2020). The effect of different doses of ozone treatments on the postharvest quality and biodiversity of cantaloupes. *Postharvest Biol. Technol.* 163:111124. doi: 10.1016/j.postharvbio.2020.111124
- Conte, G., Fontanelli, M., Galli, F., Crottozzi, L., Pagni, L., and Pellegrini, E. (2020). Mycotoxins in feed and food and the role of ozone in their detoxification and degradation: an update. *Toxins* 12:486. doi: 10.3390/toxins12080486
- Contigiani, E. V., Jaramillo Sánchez, G. M., Castro, M. A., Gómez, P. L., and Alzamora, S. M. (2021). Efficacy of mild thermal and pulsed light treatments, individually applied or in combination, for maintaining postharvest quality of strawberry cv. Albion. *J. Food Process. Preserv.* 45:e15095. doi: 10.1111/jfpp.15095
- Cortês, M., de Haas, A., Unterbusch, R., Fujimori, A., Schütze, T., Meyer, V., et al. (2020). *Aspergillus niger* spores are highly resistant to space radiation. *Front. Microbiol.* 11, 1–12. doi: 10.3389/fmicb.2020.00560
- Cruz-Luna, A. R., Cruz-Martínez, H., Vásquez-López, A., and Medina, D. I. (2021). Metal nanoparticles as novel antifungal agents for sustainable agriculture: current advances and future directions. *J. Fungi* 7:1033. doi: 10.3390/jof7121033
- da Silva Lima, G., Franco dos Santos, G., Ramalho, R. R. F., de Aguiar, D. V. A., Roque, J. V., Maciel, L. I. L., et al. (2022). Laser ablation electrospray ionization mass spectrometry imaging as a new tool for accessing patulin diffusion in mold-infected fruits. *Food Chem.* 373:131490. doi: 10.1016/j.foodchem.2021.131490
- Davies, C. R., Wohlgemuth, F., Young, T., Violet, J., Dickinson, M., Sanders, J.-W., et al. (2021). Evolving challenges and strategies for fungal control in the food supply chain. *Fungal Biol. Rev.* 36, 15–26. doi: 10.1016/j.fbr.2021.01.003
- de Oliveira, J. M., de Alencar, E. R., Blum, L. E. B., de Souza Ferreira, W. F., Botelho, S. D. C. C., Racanicci, A. M. C., et al. (2020). Ozonation of Brazil nuts: decomposition kinetics, control of *aspergillus flavus* and the effect on color and on raw oil quality. *LWT* 123:109106. doi: 10.1016/j.lwt.2020.109106
- Delgado-Ospina, J., Molina-Hernández, J. B., Chaves-López, C., Romanazzi, G., and Paparella, A. (2021). The role of fungi in the cocoa production chain and the challenge of climate change. *J. Fungi* 7:202. doi: 10.3390/jof7030202
- Deng, L.-Z., Tao, Y., Mujumdar, A. S., Pan, Z., Chen, C., Yang, X.-H., et al. (2020). Recent advances in non-thermal decontamination technologies for microorganisms and mycotoxins in low-moisture foods. *Trends Food Sci. Technol.* 106, 104–112. doi: 10.1016/j.tifs.2020.10.012
- Dong, P. T., Zhan, Y., Jusuf, S., Hui, J., Dagher, Z., Mansour, M. K., et al. (2022). Photoinactivation of catalase sensitizes *Candida albicans* and *Candida auris* to ROS-producing agents and immune cells. *Adv. Sci.* 9:2104384. doi: 10.1002/adv.202104384
- Du, Y., Mi, S., Wang, H., Yang, F., Yu, H., Xie, Y., et al. (2023a). Inactivation mechanism of *Alternaria alternata* by dielectric barrier discharge plasma and its quality control on fresh wolfberries. *Food Control* 148:109620. doi: 10.1016/j.foodcont.2023.109620
- Du, Y., Sun, J., Tian, Z., Cheng, Y., and Long, C. (2023b). Effect of blue light treatments on geotrichum citri-aurantii and the corresponding physiological mechanisms of citrus. *Food Control* 145:109468. doi: 10.1016/j.foodcont.2022.109468
- Durek, J., Schlüter, O., Roscher, A., Durek, P., and Fröhling, A. (2018). Inhibition or stimulation of ochratoxin A synthesis on inoculated barley triggered by diffuse coplanar surface barrier discharge plasma. *Front. Microbiol.* 9:2782. doi: 10.3389/fmicb.2018.02782
- Echenique-Martínez, A. A., Ramos-Parra, P. A., Rodríguez-Sánchez, D. G., Troncoso-Rojas, R., Islas-Rubio, A. R., Montoya-Ballesteros, L. D. C., et al. (2023). Botrytis cinerea induced phytonutrient degradation of strawberry puree: effects of combined preservation approaches with high hydrostatic pressure and synthetic or natural antifungal additives. *CyTA J. Food* 21, 451–463. doi: 10.1080/19476337.2023.2222812
- El-Saadony, M. T., El-Wafai, N. A., El-Fattah, H. I. A., and Mahgoub, S. A. (2019). Biosynthesis, optimization and characterization of silver nanoparticles using a soil isolate of *Bacillus pseudomycoides* MT32 and their antifungal activity against some pathogenic fungi. *Adv. Anim. Vet. Sci.* 7, 238–249. doi: 10.17582/journal.aavs/2019/7.4.238.249
- El-Sayed, R. A., Jebur, A. B., Kang, W., and El-Demerdash, F. M. (2022). An overview on the major mycotoxins in food products: characteristics, toxicity, and analysis. *J. Fut. Foods* 2, 91–102. doi: 10.1016/j.jfutfo.2022.03.002
- Enikova, R. K., Stoyanovska, M. R., and Karcheva, M. D. (2020). Mycotoxins in fruits and vegetables. *J. IMAB* 26, 3139–3143. doi: 10.5272/jimab.2020262.3139
- Esmaili, Z., Hosseinzadeh Samani, B., Nazari, F., Rostami, S., and Nemati, A. (2023). The green technology of cold plasma jet on the inactivation of *aspergillus flavus* and the total aflatoxin level in pistachio and its quality properties. *J. Food Process Eng.* 46:e14189. doi: 10.1111/jfpe.14189
- Esua, O. J., Chin, N. L., Yusof, Y. A., and Sukor, R. (2019). Effects of simultaneous UV-C radiation and ultrasonic energy postharvest treatment on bioactive compounds and antioxidant activity of tomatoes during storage. *Food Chem.* 270, 113–122. doi: 10.1016/j.foodchem.2018.07.031
- Evelin, E., and Silva, F. V. M. (2020). Ultrasound assisted thermal inactivation of spores in foods: pathogenic and spoilage bacteria, molds and yeasts. *Trends Food Sci. Technol.* 105, 402–415. doi: 10.1016/j.tifs.2020.09.020
- Evrendilek, G. A., Atmaca, B., Bulut, N., and Uzuner, S. (2021). Development of pulsed electric fields treatment unit to treat wheat grains: improvement of seed vigour and stress tolerance. *Comput. Electron. Agric.* 185:106129. doi: 10.1016/j.compag.2021.106129
- Feizollahi, E., Iqdam, B., Vasanthan, T., Thilakarathna, M. S., and Roopesh, M. S. (2020). Effects of atmospheric-pressure cold plasma treatment on deoxynivalenol degradation, quality parameters, and germination of barley grains. *Appl. Sci. (Switzerland)* 10:3530. doi: 10.3390/app10103530
- Feng, X., Zhu, M., Xu, J., Yin, W., and Hu, F. (2019). Analysis of factors influencing the transmembrane voltage induced in filamentous Fungi by pulsed electric fields. *Microorganisms* 7:307. doi: 10.3390/microorganisms7090307
- Ferreira, C. D., Lang, G. H., Lindemann, I. D. S., Timm, N. D. S., Hoffmann, J. F., Ziegler, V., et al. (2021). Postharvest UV-C irradiation for fungal control and reduction of mycotoxins in brown, black, and red rice during long-term storage. *Food Chem.* 339:127810. doi: 10.1016/j.foodchem.2020.127810
- Filho, F. O., Silva, E. D. O., Lopes, M. M. D. A., Ribeiro, P. R. V., Oster, A. H., Guedes, J. A. C., et al. (2020). Effect of pulsed light on postharvest disease control-related metabolomic variation in melon (*Cucumis melo*) artificially inoculated with *Fusarium pallidoreum*. *PLoS One* 15:e0220097. doi: 10.1371/journal.pone.0220097
- Gao, Y., Xu, D., Ren, D., Zeng, K., and Wu, X. (2020). Green synthesis of zinc oxide nanoparticles using *Citrus sinensis* peel extract and application to strawberry preservation: A comparison study. *LWT* 126:109297. doi: 10.1016/j.lwt.2020.109297

- Gao, X., Zhang, J., Liu, E., Yang, M., Chen, S., Hu, F., et al. (2019). Enhancing the taste of raw soy sauce using low intensity ultrasound treatment during moromi fermentation. *Food Chem.* 298:124928. doi: 10.1016/j.foodchem.2019.05.202
- Gavahian, M., Mathad, G. N., Oliveira, C. A. F., and Khaneghah, A. M. (2021). Combinations of emerging technologies with fermentation: interaction effects for detoxification of mycotoxins? *Food Res. Int.* 141:110104. doi: 10.1016/j.foodres.2021.110104
- Gerin, D., González-Candelas, L., Ballester, A. R., Pollastro, S., De Miccolis Angelini, R. M., and Faretra, F. (2018). Functional characterization of the alb1 orthologue gene in the ochratoxigenic fungus *Aspergillus carbonarius* (Ac49 strain). *Toxins* 10, 1–16. doi: 10.3390/toxins10030120
- Ghate, V., Yew, I., Zhou, W., and Yuk, H.-G. (2021). Influence of temperature and relative humidity on the antifungal effect of 405 nm LEDs against *Botrytis cinerea* and *Rhizopus stolonifer* and their inactivation on strawberries and tomatoes. *Int. J. Food Microbiol.* 359:109427. doi: 10.1016/j.ijfoodmicro.2021.109427
- Gomes, T., Mendes de Souza, H., Savi Bortolotto, G. D., Escobar, B. A., Furtado, B. G., and Angioletto, E. (2023). Application of ozone in peanut kernels: a multiscale model approach and effects on filamentous fungi decontamination. *J. Food Eng.* 357:111649. doi: 10.1016/j.jfoodeng.2023.111649
- Gouvea, F. S., Koutchma, T., Ferreira, E. H. R., Walter, E. H. M., and Rosenthal, A. (2023). Resistance of *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes* in high and low-acidity juices processed by high hydrostatic pressure. *Int. J. Food Microbiol.* 395:110189. doi: 10.1016/j.ijfoodmicro.2023.110189
- Granello, S. J., Christ, D., Werncke, I., Bechlin, T. R., and Machado Coelho, S. R. (2018). Effect of drying and ozonation process on naturally contaminated wheat seeds. *J. Cereal Sci.* 80, 205–211. doi: 10.1016/j.jcs.2018.03.003
- Grangeteau, C., Lepinois, F., Winckler, P., Perrier-Cornet, J.-M., Dupont, S., and Beney, L. (2018). Cell death mechanisms induced by photo-oxidation studied at the cell scale in the yeast *Saccharomyces cerevisiae*. *Front. Microbiol.* 9:2640. doi: 10.3389/fmicb.2018.02640
- Gruber-Dorninger, C., Jenkins, T., and Schatzmayr, G. (2019). Global mycotoxin occurrence in feed: a ten-year survey. *Toxins* 11:375. doi: 10.3390/toxins11070375
- Guo, J., He, Z., Ma, C., Li, W., Wang, J., Lin, F., et al. (2023). Evaluation of cold plasma for decontamination of molds and mycotoxins in rice grain. *Food Chem.* 402:134159. doi: 10.1016/j.foodchem.2022.134159
- Guo, J., Wang, J., Xie, H., Jiang, J., Li, C., Li, W., et al. (2022). Inactivation effects of plasma-activated water on fusarium graminearum. *Food Control* 134:108683. doi: 10.1016/j.foodcont.2021.108683
- Hadi, J., Wu, S., and Brightwell, G. (2020). Antimicrobial blue light versus pathogenic Bacteria: mechanism, application in the food industry, hurdle technologies and potential resistance. *Foods* 9:1895. doi: 10.3390/foods9121895
- Hajnal, E. J., Vukić, M., Pezo, L., Orčić, D., Puač, N., Škoro, N., et al. (2019). Effect of atmospheric cold plasma treatments on reduction of Alternaria toxins content in wheat flour. *Toxins* 11:704. doi: 10.3390/toxins11120704
- Hasanin, M., Al Abboud, M. A., Alawlaqi, M. M., Abdelghany, T. M., and Hashem, A. H. (2022). Ecofriendly synthesis of biosynthesized copper nanoparticles with starch-based nanocomposite: antimicrobial, antioxidant, and anticancer activities. *Biol. Trace Elem. Res.* 200, 2099–2112. doi: 10.1007/s12011-021-02812-0
- Hertwig, C., Meneses, N., and Mathys, A. (2018). Cold atmospheric pressure plasma and low energy electron beam as alternative nonthermal decontamination technologies for dry food surfaces: a review. *Trends Food Sci. Technol.* 77, 131–142. doi: 10.1016/j.tifs.2018.05.011
- Hsiao, Y.-T., Chen, B.-Y., Huang, H.-W., and Wang, C.-Y. (2020). Inactivation mechanism of *Aspergillus flavus* conidia by high hydrostatic pressure. *Foodborne Pathog. Dis.* 18, 123–130. doi: 10.1089/fpd.2020.2825
- Hua, L.-X., Yang, B., Raza, H., Hu-jun, W., Lu-Mei, P., Mi-Na, N., et al. (2018). Detection of NEO in muskmelon fruits inoculated with fusarium sulphureum and its control by postharvest ozone treatment. *Food Chem.* 254, 193–200. doi: 10.1016/j.foodchem.2018.01.149
- Huang, H.-W., Hsu, C.-P., and Wang, C.-Y. (2020). Healthy expectations of high hydrostatic pressure treatment in food processing industry. *J. Food Drug Anal.* 28, 1–13. doi: 10.1016/j.jfda.2019.10.002
- Ibrahim, A. B. M., Mahmoud, G. A.-E., Meurer, F., and Bodensteiner, M. (2021). Preparation and crystallographic studies of a new mercuric salicylaldimine complex for fabrication of microscaled and nanoscaled mercuric sulfide as antimicrobial agents against human pathogenic yeasts and filamentous fungi. *Appl. Organomet. Chem.* 35:e6134. doi: 10.1002/aoc.6134
- Igbalajobi, O., Yu, Z., and Fischer, R. (2019). Red-and blue-light sensing in the plant pathogen *Alternaria alternata* depends on phytochrome and the white-collar protein LreA. *MBio* 10:e00371–19. doi: 10.1128/mbio.00371-19
- Ilyas, M., Waris, A., Khan, A. U., Zamel, D., Yar, L., Baset, A., et al. (2021). Biological synthesis of titanium dioxide nanoparticles from plants and microorganisms and their potential biomedical applications. *Inorg. Chem. Commun.* 133:108968. doi: 10.1016/j.inoche.2021.108968
- Iqdiam, B. M., Abuagela, M. O., Boz, Z., Marshall, S. M., Goodrich-Schneider, R., Sims, C. A., et al. (2020). Effects of atmospheric pressure plasma jet treatment on aflatoxin level, physicochemical quality, and sensory attributes of peanuts. *J. Food Process. Preserv.* 44:e14305. doi: 10.1111/jfpp.14305
- Ismail, A.-W. A., Sidkey, N. M., Arafa, R. A., Fathy, R. M., and El-Batal, A. I. (2016). Evaluation of in vitro antifungal activity of silver and selenium nanoparticles against Alternaria solani caused early blight disease on potato. *Biotechnol. J. Int.* 12, 1–11. doi: 10.9734/BBJ/2016/24155
- Jaramillo-Sánchez, G., Contigiani, E. V., Castro, M. A., Hodara, K., Alzamora, S. M., Loreda, A. G., et al. (2019). Freshness maintenance of blueberries (*Vaccinium corymbosum* L.) during postharvest using ozone in aqueous phase: microbiological, structure, and mechanical issues. *Food Bioprocess Technol.* 12, 2136–2147. doi: 10.1007/s11947-019-02358-z
- Jiang, N., Li, Z., Wang, L., Li, H., Zhu, X., Feng, X., et al. (2019). Effects of ultraviolet-c treatment on growth and mycotoxin production by alternaria strains isolated from tomato fruits. *Int. J. Food Microbiol.* 311:108333. doi: 10.1016/j.ijfoodmicro.2019.108333
- John, D., and Ramaswamy, H. S. (2018). Pulsed light technology to enhance food safety and quality: a mini-review. *Curr. Opin. Food Sci.* 23, 70–79. doi: 10.1016/j.cofs.2018.06.004
- Ju, S., Li, B., Yang, X., Wu, J., Pei, X., Zhuang, J., et al. (2023). Plasma-activated water for inactivation of fusarium graminearum spores: an in-vitro wheat study. *LWT* 183:114815. doi: 10.1016/j.lwt.2023.114815
- Kalagatur, N. K., Kamasani, J. R., Mudili, V., Krishna, K., Chauhan, O. P., and Sreepathi, M. H. (2018). Effect of high pressure processing on growth and mycotoxin production of fusarium graminearum in maize. *Food Biosci.* 21, 53–59. doi: 10.1016/j.fbio.2017.11.005
- Kaur, M. (2023). Cold plasma inhibits *Fusarium graminearum* growth in vitro but does not translate to field-contaminated grain (K. Bayliss (ed.)) [Murdoch University]. Available at: <https://researchportal.murdoch.edu.au/esploro/outputs/doctoral/Cold-plasma-inhibits-Fusarium-graminearum-growth/991005593763707891>
- Khan, I., Saeed, K., and Khan, I. (2019). Nanoparticles: properties, applications and toxicities. *Arab. J. Chem.* 12, 908–931. doi: 10.1016/j.arabjchem.2017.05.011
- Kiš, M., Milošević, S., Vulić, A., Herceg, Z., Vukušić, T., and Pleadin, J. (2020). Efficacy of low pressure DBD plasma in the reduction of T-2 and HT-2 toxin in oat flour. *Food Chem.* 316:126372. doi: 10.1016/j.foodchem.2020.126372
- Kranjc, M., and Miklavčič, D. (2017). “Electric field distribution and electroporation threshold” in Handbook of electroporation. ed. D. Miklavcic (Switzerland AG, Cham: Springer Cham), 1043–1058.
- Krstović, S., Krulj, J., Jakšić, S., Bočarov-Stančić, A., and Jajić, I. (2021). Ozone as decontaminating agent for ground corn containing deoxynivalenol, zearalenone, and ochratoxin A. *Cereal Chem.* 98, 135–143. doi: 10.1002/cche.10289
- Kumar, P., Mathpal, M. C., Inwati, G. K., Ghosh, S., Kumar, V., Roos, W. D., et al. (2020). Optical and surface properties of Zn doped CdO nanorods and antimicrobial applications. *Colloids Surf. A Physicochem. Eng. Asp.* 605:125369. doi: 10.1016/j.colsurfa.2020.125369
- Kutawa, A. B., Ahmad, K., Ali, A., Hussein, M. Z., Abdul Wahab, M. A., Adamu, A., et al. (2021). Trends in nanotechnology and its potentialities to control plant pathogenic fungi: A review. *Biology* 10:881. doi: 10.3390/biology10090881
- Laika, J., Annunziata, L., Molina-Hernandez, J. B., De Flaviis, R., Campana, G., Ricci, A., et al. (2024a). Emerging Alternaria toxins present in naturally contaminated dried tomatoes and their reduction using cold atmospheric plasma. *Food Control* 164:110563. doi: 10.1016/j.foodcont.2024.110563
- Laika, J., Viteritti, E., Molina-Hernandez, J. B., Sergi, M., Neri, L., Laurita, R., et al. (2024b). Efficiency of cold atmospheric plasma under ozone (O3) and nitrogen oxide (NOx) regimes on the degradation of aflatoxins and ochratoxin A in solid state and in spiked pistachio kernels. *Food Control* 159:110286. doi: 10.1016/j.foodcont.2024.110286
- Laurita, R., Gozzi, G., Tappi, S., Capelli, F., Bisag, A., Laghi, G., et al. (2021). Effect of plasma activated water (PAW) on rocket leaves decontamination and nutritional value. *Innov. Food Sci. Emerg. Technol.* 73:102805. doi: 10.1016/j.ifset.2021.102805
- Leanse, L. G., dos Anjos, C., Mushtaq, S., and Dai, T. (2022). Antimicrobial blue light: A ‘magic bullet’ for the 21st century and beyond? *Adv. Drug Deliv. Rev.* 180:114057. doi: 10.1016/j.addr.2021.114057
- Li, L., Xue, H., Bi, Y., Zhang, R., Kouasseu, C. J., Liu, Q., et al. (2022). Ozone treatment inhibits dry rot development and diacetoxyscirpenol accumulation in inoculated potato tuber by influencing growth of *Fusarium sulphureum* and ergosterol biosynthesis. *Postharvest Biol. Technol.* 185:111796. doi: 10.1016/j.postharvbio.2021.111796
- Lin, C.-M., Patel, A. K., Chiu, Y.-C., Hou, C.-Y., Kuo, C.-H., Dong, C.-D., et al. (2022). The application of novel rotary plasma jets to inhibit the aflatoxin-producing aspergillus flavus and the spoilage fungus, *Aspergillus niger* on peanuts. *Innovative Food Sci. Emerg. Technol.* 78:102994. doi: 10.1016/j.ifset.2022.102994
- Liu, M., Feng, J., Fan, Y., Yang, X., Chen, R., Xu, C., et al. (2024). Application of atmospheric cold plasma for zearalenone detoxification in cereals: kinetics, mechanisms, and cytotoxicity analysis. *J. Adv. Res. (In press)*. doi: 10.1016/j.jare.2024.04.024

- Liu, Y., Li, M., Liu, Y., Bai, F., and Bian, K. (2019a). Effects of pulsed ultrasound at 20 kHz on the sonochemical degradation of mycotoxins. *World Mycotoxin J.* 12, 357–366. doi: 10.3920/WMJ2018.2431
- Liu, Y., Li, M., Liu, Y., and Bian, K. (2019b). Structures of reaction products and degradation pathways of aflatoxin B1 by ultrasound treatment. *Toxins* 11:526. doi: 10.3390/toxins11090526
- Lopes, S. J. S., Sant'Ana, A. S., and Freire, L. (2023). Non-thermal emerging processing technologies: mitigation of microorganisms and mycotoxins, sensory and nutritional properties maintenance in clean label fruit juices. *Food Res. Int.* 168:112727. doi: 10.1016/j.foodres.2023.112727
- Los, A., Ziuzina, D., Akkermans, S., Boehm, D., Cullen, P. J., Van Impe, J., et al. (2018). Improving microbiological safety and quality characteristics of wheat and barley by high voltage atmospheric cold plasma closed processing. *Food Res. Int.* 106, 509–521. doi: 10.1016/j.foodres.2018.01.009
- Luksiene, Z., Rasiukeviciute, N., Zudyte, B., and Uselis, N. (2020). Innovative approach to sunlight activated biofungicides for strawberry crop protection: ZnO nanoparticles. *J. Photochem. Photobiol. B Biol.* 203:111656. doi: 10.1016/j.jphotobiol.2019.111656
- Luo, X., Qi, L., Liu, Y., Wang, R., Yang, D., Li, K., et al. (2017). Effects of Electron beam irradiation on zearalenone and ochratoxin A in naturally contaminated corn and corn quality parameters. *Toxins* 9:84. doi: 10.3390/toxins9030084
- Maringgal, B., Hashim, N., Tawakkal, I. S. M. A., Hamzah, M. H., and Mohamed, M. T. M. (2020). Biosynthesis of CaO nanoparticles using *Trigona* sp. honey: physicochemical characterization, antifungal activity, and cytotoxicity properties. *J. Mater. Res. Technol.* 9, 11756–11768. doi: 10.1016/j.jmrt.2020.08.054
- Misra, N. N., Yadav, B., Roopesh, M. S., and Jo, C. (2019). Cold plasma for effective fungal and mycotoxin control in foods: mechanisms, inactivation effects, and applications. *Compr. Rev. Food Sci. Food Saf.* 18, 106–120. doi: 10.1111/1541-4337.12398
- Mohamed, A. B., Chavez, R. A., Wagacha, M. J., Mutege, C. K., Muthomi, J. W., Pillai, S. D., et al. (2022). Efficacy of electron beam irradiation in reduction of mycotoxin-producing fungi, aflatoxin, and fumonisin, in naturally contaminated maize slurry. *Toxicol* 16:100141. doi: 10.1016/j.toxcx.2022.100141
- Molina-Hernandez, J. B., Capelli, F., Laurita, R., Tappi, S., Laika, J., Gioia, L., et al. (2022a). A comparative study on the antifungal efficacy of cold atmospheric plasma at low and high surface density on *Aspergillus chevalieri* and mechanisms of action. *Innovative Food Sci. Emerg. Technol.* 82:103194. doi: 10.1016/j.ifset.2022.103194
- Molina-Hernández, J. B., Scroccarello, A., Della Pelle, F., De Flaviis, R., Compagnone, D., Del Carlo, M., et al. (2022b). Synergistic antifungal activity of catechin and silver nanoparticles on *Aspergillus niger* isolated from coffee seeds. *LWT* 169:113990. doi: 10.1016/j.lwt.2022.113990
- Molina-Hernandez, J. B., Tappi, S., Gherardi, M., de Flaviis, R., Laika, J., Peralta-Ruiz, Y. Y., et al. (2023). Cold atmospheric plasma treatments trigger changes in sun-dried tomatoes mycobiota by modifying the spore surface structure and hydrophobicity. *Food Control* 145:109453. doi: 10.1016/j.foodcont.2022.109453
- Moosavi, M. H., Khaneghah, A. M., Javanmardi, F., Hadidi, M., Hadian, Z., Jafarzadeh, S., et al. (2021). A review of recent advances in the decontamination of mycotoxin and inactivation of fungi by ultrasound. *Ultrason. Sonochem.* 79:105755. doi: 10.1016/j.ultsonch.2021.105755
- Moreno-Vilet, L., Hernández-Hernández, H. M., and Villanueva-Rodríguez, S. J. (2018). Current status of emerging food processing technologies in Latin America: novel thermal processing. *Innovative Food Sci. Emerg. Technol.* 50, 196–206. doi: 10.1016/j.ifset.2018.06.013
- Moretti, A., Logrieco, A. F., and Susca, A. (2017). "Mycotoxins: an underhand food problem" in *Mycotoxigenic fungi: methods and protocols*, eds A. Moretti and A. Susca (New York: Springer), 3–12. doi: 10.1007/978-1-4939-6707-0_1
- Mravljje, J., Regvar, M., Starič, P., Mozetič, M., and Vogel-Mikuš, K. (2021). Cold plasma affects germination and fungal community structure of buckwheat seeds. *Plan. Theory* 10:851. doi: 10.3390/plants10050851
- Nakpan, W., Yermakov, M., Indugula, R., Reponen, T., and Grinshpun, S. A. (2019). Inactivation of bacterial and fungal spores by UV irradiation and gaseous iodine treatment applied to air handling filters. *Sci. Total Environ.* 671, 59–65. doi: 10.1016/j.scitotenv.2019.03.310
- Nandini, B., Hariprasad, P., Prakash, H. S., Shetty, H. S., and Geetha, N. (2017). Trichogenic-selenium nanoparticles enhance disease suppressive ability of Trichoderma against downy mildew disease caused by *Sclerospora graminicola* in pearl millet. *Sci. Rep.* 7:2612. doi: 10.1038/s41598-017-02737-6
- Neuenfeldt, N. H., Silva, L. P., Pessoa, R. S., and Rocha, L. O. (2023). Cold plasma technology for controlling toxigenic fungi and mycotoxins in food. *Curr. Opin. Food Sci.* 52:101045. doi: 10.1016/j.cofs.2023.101045
- Nicolau-Lapeña, I., Rodríguez-Bencomo, J. J., Colás-Medà, P., Viñas, I., Sanchis, V., and Alegre, I. (2023). Ultraviolet applications to control Patulin produced by *Penicillium expansum* CMP-1 in apple products and study of further Patulin degradation products formation and toxicity. *Food Bioprocess Technol.* 16, 804–823. doi: 10.1007/s11947-022-02943-9
- Ouf, S. A., and Ali, E. M. (2021). Does the treatment of dried herbs with ozone as a fungal decontaminating agent affect the active constituents? *Environ. Pollut.* 277:116715. doi: 10.1016/j.envpol.2021.116715
- Pages, M., Kleiber, D., and Violleau, F. (2020). Ozonation of three different fungal conidia associated with apple disease: importance of spore surface and membrane phospholipid oxidation. *Food Sci. Nutr.* 8, 5292–5297. doi: 10.1002/fsn3.1618
- Pallarés, N., Berrada, H., Tolosa, J., and Ferrer, E. (2021). Effect of high hydrostatic pressure (HPP) and pulsed electric field (PEF) technologies on reduction of aflatoxins in fruit juices. *LWT* 142:111000. doi: 10.1016/j.lwt.2021.111000
- Pan, Y., Li, T., Wu, C., Guo, S., Fan, G., Li, X., et al. (2023). Subcellular damages of pathogenic fungi combined with gene expression analysis reveals mechanisms that cold plasma controlling apricot disease. *Food Biosci.* 53:102728. doi: 10.1016/j.fbio.2023.102728
- Peralta-Ruiz, Y., Rossi, C., Grande-Tovar, C. D., and Chaves-López, C. (2023). Green management of postharvest anthracnose caused by *Colletotrichum gloeosporioides*. *J. Fungi* 9:623. doi: 10.3390/jof9060623
- Pereira, V. L., dos Santos Caramés, E. T., Almeida, N. A., Chiappim, W., Pessoa, R. S., Petraconi Filho, G., et al. (2024). Gliding arc plasma jet for inhibiting mycotoxin production and apple brown rot by *Alternaria alternata*. *Food Control* 155:110108. doi: 10.1016/j.foodcont.2023.110108
- Phan, K. T. K., Phan, H. T., Brennan, C. S., Regenstein, J. M., Jantanasakulwong, K., Boonyawan, D., et al. (2019). Gliding arc discharge non-thermal plasma for retardation of mango anthracnose. *LWT* 105, 142–148. doi: 10.1016/j.lwt.2019.02.012
- Pinto, C. A., Moreira, S. A., Fidalgo, L. G., Inácio, R. S., Barba, F. J., and Saraiva, J. A. (2020). Effects of high-pressure processing on fungi spores: factors affecting spore germination and inactivation and impact on ultrastructure. *Compr. Rev. Food Sci. Food Saf.* 19, 553–573. doi: 10.1111/1541-4337.12534
- Pinto, C. A., Mousakhani Ganjeh, A., Barba, F. J., and Saraiva, J. A. (2024). Impact of pH and high-pressure pasteurization on the germination and development of *Clostridium perfringens* spores under hyperbaric storage versus refrigeration. *Food Secur.* 13:1832. doi: 10.3390/foods13121832
- Popović, V., Fairbanks, N., Pierscianowski, J., Biancaniello, M., Zhou, T., and Koutchma, T. (2018). Feasibility of 3D UV-C treatment to reduce fungal growth and mycotoxin loads on maize and wheat kernels. *Mycotoxin Res.* 34, 211–221. doi: 10.1007/s12550-018-0316-3
- Priesterjahn, E.-M., Geisen, R., and Schmidt-Heydt, M. (2020). Influence of light and water activity on growth and mycotoxin formation of selected isolates of *Aspergillus flavus* and *Aspergillus parasiticus*. *Microorganisms* 8:2000. doi: 10.3390/microorganisms8122000
- Pulgundla, P., Lee, T., and Mok, C. (2020). Effect of corona discharge plasma jet treatment on the degradation of aflatoxin B1 on glass slides and in spiked food commodities. *LWT* 124:108333. doi: 10.1016/j.lwt.2019.108333
- Purar, B., Djalovic, I., Bekavac, G., Grahovac, N., Krstović, S., Latković, D., et al. (2022). Changes in *Fusarium* and *Aspergillus* mycotoxin content and fatty acid composition after the application of ozone in different maize hybrids. *Food Secur.* 11:2977. doi: 10.3390/foods11182877
- Rapacka-Zdonczyk, A., Wozniak, A., Pieranski, M., Woziwodzka, A., Bielawski, K. P., and Grinholc, M. (2019). Development of *Staphylococcus aureus* tolerance to antimicrobial photodynamic inactivation and antimicrobial blue light upon sub-lethal treatment. *Sci. Rep.* 9:9423. doi: 10.1038/s41598-019-45962-x
- Rezaei, M., Pirs, S., and Chavoshizadeh, S. (2020). Photocatalytic/antimicrobial active film based on wheat gluten/ZnO nanoparticles. *J. Inorg. Organomet. Polym. Mater.* 30, 2654–2665. doi: 10.1007/s10904-019-01407-6
- Ribeiro, D. F., Faroni, L. R. D. A., Pimentel, M. A. G., Prates, L. H. F., Heleno, F. F., and De Alencar, E. R. (2022). Ozone as a fungicidal and detoxifying agent to maize contaminated with fumonisins. *Ozone Sci. Eng.* 44, 38–49. doi: 10.1080/01919512.2021.1924616
- Rifna, E. J., Singh, S. K., Chakraborty, S., and Dwivedi, M. (2019). Effect of thermal and non-thermal techniques for microbial safety in food powder: recent advances. *Food Res. Int.* 126:108654. doi: 10.1016/j.foodres.2019.108654
- Rizwana, H., Bokahri, N. A., Alkhattaf, F. S., Albasher, G., and Aldehaish, H. A. (2021). Antifungal, antibacterial, and cytotoxic activities of silver nanoparticles synthesized from aqueous extracts of mace-arils of *Myristica fragrans*. *Molecules* 26:7709. doi: 10.3390/molecules26247709
- Romero Bernal, A. R., Contigiani, E. V., González, H. H. L., Alzamora, S. M., Gómez, P. L., and Raffellini, S. (2019). *Botrytis cinerea* response to pulsed light: cultivability, physiological state, ultrastructure and growth ability on strawberry fruit. *Int. J. Food Microbiol.* 309:108311. doi: 10.1016/j.ijfoodmicro.2019.108311
- Rudik, F. Y., Morgunova, N. L., and Krasnikova, E. S. (2020). Decontamination of grain by ultrasound. *IOP Conf. Ser.: Earth Environ. Sci.* 421:022022. doi: 10.1088/1755-1315/421/2/022022
- Ruffo Roberto, S., Youssef, K., Hashim, A. F., and Ippolito, A. (2019). Nanomaterials as alternative control means against postharvest diseases in fruit crops. *Nano* 9:1752. doi: 10.3390/nano9121752
- Rüntzel, C. L., da Silva, J. R., da Silva, B. A., Moecke, E. S., and Scussel, V. M. (2019). Effect of cold plasma on black beans (*Phaseolus vulgaris* L.), fungi inactivation and micro-structures stability. *Emir. J. Food Agric.* 31, 864–873. doi: 10.9755/efja.2019.v31.i11.2029

- Safaei, M., and Taran, M. (2022). Preparation of bacterial cellulose fungicide nanocomposite incorporated with MgO nanoparticles. *J. Polym. Environ.* 30, 2066–2076. doi: 10.1007/s10924-021-02329-6
- Sanaei Nasab, S., Zare, L., Tahmouzi, S., Nematollahi, A., Mollakhalili-Meybodi, N., Abedi, A.-S., et al. (2023). Effect of irradiation treatment on microbial, nutritional and technological characteristics of cereals: a comprehensive review. *Radiat. Phys. Chem.* 212:111124. doi: 10.1016/j.radphyschem.2023.111124
- Sandanuwana, T., Attygalle, D., Amarasinghe, S., Weragoda, S. C., Ranaweera, B., Rathnayake, K., et al. (2023). Shelf life extension of Cavendish Banana fruit using cold plasma treatment. *2020 Moratuwa Engineering Research Conference (MERCon)*, 182–186. doi: 10.1109/MERCon50084.2020.9185237
- Santamera, A., Escott, C., Loira, I., del Fresno, J. M., González, C., and Morata, A. (2020). Pulsed light: challenges of a non-thermal sanitation Technology in the Winemaking Industry. *Beverages* 6:45. doi: 10.3390/beverages6030045
- Santos Alexandre, A. P., Vela-Paredes, R. S., Santos, A. S., Costa, N. S., Canniatti-Brazaca, S. G., Calori-Domingues, M. A., et al. (2018). Ozone treatment to reduce deoxynivalenol (DON) and zearalenone (ZEN) contamination in wheat bran and its impact on nutritional quality. *Food Addit. Contam. Part A* 35, 1189–1199. doi: 10.1080/19440049.2018.1432899
- Savi, G. D., Gomes, T., Canever, S. B., Feltrin, A. C., Piacentini, K. C., Scussel, R., et al. (2020). Application of ozone on rice storage: A mathematical modeling of the ozone spread, effects in the decontamination of filamentous fungi and quality attributes. *J. Stored Prod. Res.* 87:101605. doi: 10.1016/j.jspr.2020.101605
- Sawangrat, C., Phimmolsiripol, Y., Leksakul, K., Thanapornpoonpong, S., Sojithamporn, P., Lavilla, M., et al. (2022). Application of pinhole plasma jet activated water against *Escherichia coli*, *Colletotrichum gloeosporioides*, and decontamination of pesticide residues on chili (*Capsicum annuum* L.). *Food Secur.* 11:2859. doi: 10.3390/foods11182859
- Schmidt, M., Zannini, E., and Arendt, E. K. (2018). Recent advances in physical post-harvest treatments for shelf-life extension of cereal crops. *Food Secur.* 7:45. doi: 10.3390/foods7040045
- Schmidt, M., Zannini, E., and Arendt, E. K. (2019). Screening of post-harvest decontamination methods for cereal grains and their impact on grain quality and technological performance. *Eur. Food Res. Technol.* 245, 1061–1074. doi: 10.1007/s00217-018-3210-5
- Scroccarello, A., Molina-Hernández, B., Della Pelle, F., Ciancetta, J., Ferraro, G., Frattini, E., et al. (2021). Effect of phenolic compounds-capped AgNPs on growth inhibition of *Aspergillus niger*. *Colloids Surf. B: Biointerfaces* 199:111533. doi: 10.1016/j.colsurfb.2020.111533
- Sehrawat, R., Kaur, B. P., Nema, P. K., Tewari, S., and Kumar, L. (2021). Microbial inactivation by high pressure processing: principle, mechanism and factors responsible. *Food Sci. Biotechnol.* 30, 19–35. doi: 10.1007/s10068-020-00831-6
- Seku, K., Reddy Ganapuram, B., Pejaji, B., Mangatayaru Kotu, G., and Golla, N. (2018). Hydrothermal synthesis of copper nanoparticles, characterization and their biological applications. *Int. J. Nano Dimension* 9, 7–14.
- Sen, Y., Onal-Ulusoy, B., and Mutlu, M. (2019). Detoxification of hazelnuts by different cold plasmas and gamma irradiation treatments. *Innov. Food Sci. Emerg. Technol.* 54, 252–259. doi: 10.1016/j.ifset.2019.05.002
- Shanakhat, H., Sorrentino, A., Raiola, A., Reverberi, M., Salustri, M., Masi, P., et al. (2019). Technological properties of durum wheat semolina treated by heating and UV irradiation for reduction of mycotoxin content. *J. Food Process Eng.* 42:e13006. doi: 10.1111/jfpe.13006
- Shang, H., Ma, C., Li, C., Zhao, J., Elmer, W., White, J. C., et al. (2021). Copper oxide nanoparticle-embedded hydrogels enhance nutrient supply and growth of lettuce (*Lactuca sativa*) infected with *Fusarium oxysporum* f. sp. *lactucae*. *Environ. Sci. Technol.* 55, 13432–13442. doi: 10.1021/acs.est.1c00777
- Shangfei, L., Haokuan, Q., Xiaolin, Z., Wenqi, L., and Muqing, L. (2021). Inhibition of *aspergillus oryzae* mycelium growth and Conidium production by irradiation with light at different wavelengths and intensities. *Microbiology. Spectrum* 9:e00213–21. doi: 10.1128/Spectrum.00213-21
- Sharma, R. R., Reddy, S. V. R., and Sethi, S. (2018). “Cold plasma technology for surface disinfection of fruits and vegetables” in *Postharvest disinfection of fruits and vegetables*. ed. M. W. Siddiqui. (Cambridge, Massachusetts, United States: Elsevier Inc.).
- Shi, H., Ileleji, K., Strohshine, R. L., Keener, K., and Jensen, J. L. (2017). Reduction of aflatoxin in corn by high voltage atmospheric cold plasma. *Food Bioprocess Technol.* 10, 1042–1052. doi: 10.1007/s11947-017-1873-8
- Stranska, M., Prusova, N., Behner, A., Dzuman, Z., Lazarek, M., Tobolkova, A., et al. (2023). Influence of pulsed electric field treatment on the fate of fusarium and alternaria mycotoxins present in malting barley. *Food Control* 145:109440. doi: 10.1016/j.foodcont.2022.109440
- Stukenbrock, E., and Gurr, S. (2023). Address the growing urgency of fungal disease in crops. *Nature* 617, 31–34. doi: 10.1038/d41586-023-01465-4
- Sun, H., Duan, Y., Li, H., Hu, X., Li, B., Zhuang, J., et al. (2023). Microbiota characterization of atmospheric cold plasma treated blueberries. *LWT* 180:114720. doi: 10.1016/j.lwt.2023.114720
- Sunday, O. E., Chidike, E. T. P., Mao, G., Chen, Y., Feng, W., and Wu, X. (2021). Nano-enabled agrochemicals/materials: potential human health impact, risk assessment, management strategies and future prospects. *Environ. Pollut.* 295:118722. doi: 10.1016/j.envpol.2021.118722
- Tenuta, M. C., Artoni, E., Fava, P., Bignami, C., and Licciardello, F. (2023). Shelf life extension and nutritional quality preservation of sour cherries through high pressure processing. *Food Secur.* 12:342. doi: 10.3390/foods12020342
- Terao, D., de Lima Nechet, K., Shiraishi Frighetto, R. T., and Cerqueira Sasaki, F. F. (2019). Ozonated water combined with heat treatment to control the stem-end rot of papaya. *Sci. Hortic.* 257:108722. doi: 10.1016/j.scienta.2019.108722
- Thirumdas, R., Kothakota, A., Annapure, U., Silveru, K., Blundell, R., Gatt, R., et al. (2018). Plasma activated water (PAW): chemistry, physico-chemical properties, applications in food and agriculture. *Trends Food Sci. Technol.* 77, 21–31. doi: 10.1016/j.tifs.2018.05.007
- Tralamazza, S. M., Bemvenuti, R. H., Zorzete, P., de Souza Garcia, F., and Corrêa, B. (2016). Fungal diversity and natural occurrence of deoxynivalenol and zearalenone in freshly harvested wheat grains from Brazil. *Food Chem.* 196, 445–450. doi: 10.1016/j.foodchem.2015.09.063
- Trzaska, W. J., Wrigley, H. E., Thwaite, J. E., and May, R. C. (2017). Species-specific antifungal activity of blue light. *Sci. Rep.* 7, 1–7. doi: 10.1038/s41598-017-05000-0
- Villalobos, M. C., Serradilla, M. J., Martín, A., Ruiz-Moyano, S., Casquete, R., Hernández, A., et al. (2019). Use of efficient drying methods to improve the safety and quality of dried fig. *J. Food Process. Preserv.* 43:e13853. doi: 10.1111/jfpp.13853
- Vrandečić, K., Čosić, J., Ilić, J., Ravnjak, B., Selmani, A., Galić, E., et al. (2020). Antifungal activities of silver and selenium nanoparticles stabilized with different surface coating agents. *Pest Manag. Sci.* 76, 2021–2029. doi: 10.1002/ps.5735
- Wang, L., Liu, X., Cai, R., Ge, Q., Zhao, Z., and Yue, T. (2022). Detoxification of Ochratoxin A by pulsed light in grape juice and evaluation of its degradation products and safety. *Innov. Food Sci. Emerg. Technol.* 78:103024. doi: 10.1016/j.ifset.2022.103024
- Wang, B., Mahoney, N. E., Pan, Z., Khir, R., and Wu, B. (2016). Effectiveness of pulsed light treatment for degradation and detoxification of aflatoxin B1 and B2 in rough rice and rice bran. *Food Control* 59, 461–467. doi: 10.1016/j.foodcont.2015.06.030
- Wang, Y., Shang, J., Cai, M., Liu, Y., and Yang, K. (2023a). Detoxification of mycotoxins in agricultural products by non-thermal physical technologies: a review of the past five years. *Crit. Rev. Food Sci. Nutr.* 63, 11668–11678. doi: 10.1080/10408398.2022.2095554
- Wang, Y., Zheng, Y., Zhou, A., Neng, J., Wu, D., Li Shen, X., et al. (2023b). Transcriptomic analysis reveals the inhibition mechanism of pulsed light on fungal growth and ochratoxin A biosynthesis in *Aspergillus carbonarius*. *Food Res. Int.* 165:112501. doi: 10.1016/j.foodres.2023.112501
- Wardana, A. A., Kingwascharapong, P., Tanaka, F., and Tanaka, F. (2021). CuO nanoparticles/Indonesian cedarwood essential oil-loaded chitosan coating film: characterisation and antifungal improvement against *Penicillium* spp. *Int. J. Food Sci. Technol.* 56, 4224–4238. doi: 10.1111/ijfs.15195
- Wardana, A. A., Kingwascharapong, P., Wigati, L. P., Tanaka, F., and Tanaka, F. (2022). The antifungal effect against *Penicillium italicum* and characterization of fruit coating from chitosan/ZnO nanoparticle/Indonesian sandalwood essential oil composites. *Food Packag. Shelf Life* 32:100849. doi: 10.1016/j.fpsl.2022.100849
- Wielogorska, E., Ahmed, Y., Meneely, J., Graham, W. G., Elliott, C. T., and Gilmore, B. F. (2019). A holistic study to understand the detoxification of mycotoxins in maize and impact on its molecular integrity using cold atmospheric plasma treatment. *Food Chem.* 301:125281. doi: 10.1016/j.foodchem.2019.125281
- Win, T. T., Khan, S., and Fu, P. (2020). Fungus- (*Alternaria* sp.) mediated silver nanoparticles synthesis, characterization, and screening of antifungal activity against some Phytopathogens. *J. Nanotechnol.* 2020:8828878. doi: 10.1155/2020/8828878
- Woldemariam, H. W., and Emire, S. A. (2019). High pressure processing of foods for microbial and mycotoxins control: current trends and future prospects. *Cogent Food Agric.* 5:1622184. doi: 10.1080/23311932.2019.1622184
- Woldemariam, H. W., Harmeling, H., Admassu, S., Paulos, E., Teshome, G., Toepfl, S., et al. (2022). Pulsed light treatment reduces microorganisms and mycotoxins naturally present in red pepper (*Capsicum annuum* L.) powder. *J. Food Process Eng.* 45:e13948. doi: 10.1111/jfpe.13948
- Woldemariam, H. W., Kießling, M., Emire, S. A., Teshome, P. G., Töpfl, S., and Aganovic, K. (2020). Influence of electron beam treatment on naturally contaminated red pepper (*Capsicum annuum* L.) powder: kinetics of microbial inactivation and physicochemical quality changes. *Innov. Food Sci. Emerg. Technol.* 67:102588. doi: 10.1016/j.ifset.2020.102588
- Wong, H. J., Mohamad-Fauzi, N., Rizman-Idid, M., Convey, P., and Alias, S. A. (2019). Protective mechanisms and responses of micro-fungi towards ultraviolet-induced cellular damage. *Pol. Sci.* 20, 19–34. doi: 10.1016/j.polar.2018.10.001
- Wu, F. (2008). A tale of two commodities: how EU mycotoxin regulations have affected US tree nut industries. *World Mycotoxin J.* 1, 95–102. doi: 10.3920/WMJ2008.x011
- Wu, Y., Cheng, J.-H., and Sun, D.-W. (2021). Blocking and degradation of aflatoxins by cold plasma treatments: applications and mechanisms. *Trends Food Sci. Technol.* 109, 647–661. doi: 10.1016/j.tifs.2021.01.053
- Wu, F., and Mitchell, N. J. (2016). How climate change and regulations can affect the economics of mycotoxins. *World Mycotoxin J.* 9, 653–664. doi: 10.3920/WMJ2015.2015

- Yang, K., Li, K., Pan, L., Luo, X., Xing, J., Wang, J., et al. (2020). Effect of ozone and Electron beam irradiation on degradation of Zearalenone and Ochratoxin A. *Toxins* 12:138. doi: 10.3390/toxins12020138
- Yousefi, M., Mohammadi, M. A., Khajavi, M. Z., Ehsani, A., and Scholtz, V. (2021). Application of novel non-thermal physical technologies to degrade mycotoxins. *J. Fungi* 7:395. doi: 10.3390/jof7050395
- Yu, X., Chen, J., Zhong, J., Deng, W., Zhang, Z., Wu, Y., et al. (2023). Antifungal efficacy of LEDs against major postharvest pathogens of litchi fruit in vitro and in vivo. *Food Control* 154:110019. doi: 10.1016/j.foodcont.2023.110019
- Yugay, Y., Rusapetova, T., Mashtalyar, D., Grigorichuk, V., Vasyutkina, E., Kudanova, O., et al. (2021). Biomimetic synthesis of functional silver nanoparticles using hairy roots of *Panax ginseng* for wheat pathogenic fungi treatment. *Colloids Surf. B: Biointerfaces* 207:112031. doi: 10.1016/j.colsurfb.2021.112031
- Zahoranová, A., Hoppanová, L., Šimončicová, J., Tučeková, Z., Medvecká, V., Hudecová, D., et al. (2018). Effect of cold atmospheric pressure plasma on maize seeds: enhancement of seedlings growth and surface microorganisms inactivation. *Plasma Chem. Plasma Process.* 38, 969–988. doi: 10.1007/s11090-018-9913-3
- Zenklusen, M. H., Coronel, M. B., Castro, M. Á., Alzamora, S. M., and González, H. H. L. (2018). Inactivation of *aspergillus carbonarius* and *aspergillus flavus* in malting barley by pulsed light and impact on germination capacity and microstructure. *Innovative Food Sci. Emerg. Technol.* 45, 161–168. doi: 10.1016/j.ifset.2017.09.003
- Zhang, Y., Masum, M. M. I., Gao, C., Cheng, Y., and Guan, J. (2022). Ozone reduces the fruit decay of postharvest winter jujube by altering the microbial community structure on fruit surface. *Microbiol. Res.* 262:127110. doi: 10.1016/j.micres.2022.127110
- Zhang, H., Wang, G., Yang, Q., Yang, X., Zheng, Y., Liu, Y., et al. (2021). Effects of light on the Ochratoxigenic Fungi *aspergillus ochraceus* and *A. carbonarius*. *Toxins* 13:251. doi: 10.3390/toxins13040251
- Zhu, R., Wang, W., Luo, Z., Lin, H., Li, Y., Lu, W., et al. (2023). Blue LED light treatment inhibits virulence and patulin biosynthesis in *Penicillium expansum*. *Postharvest Biol. Technol.* 200:112340. doi: 10.1016/j.postharvbio.2023.112340
- Zudyte, B., and Luksiene, Z. (2021). Visible light-activated ZnO nanoparticles for microbial control of wheat crop. *J. Photochem. Photobiol. B Biol.* 219:112206. doi: 10.1016/j.jphotobiol.2021.112206



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Biodegradation of ZEN by *Bacillus mojavensis* L-4: analysis of degradation conditions, products, degrading enzymes, and whole-genome sequencing, and its application in semi-solid-state fermentation of contaminated cornmeal

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Zearalenone (ZEN), a naturally occurring estrogenic mycotoxin prevalent in cereals and animal feed, poses significant challenge to livestock industry owing to its detrimental effects on animal reproduction. In this study, the strains with high degradation rate were screened through co-culture with ZEN, and identified by bacterial morphology, 16S rDNA sequencing and whole genome sequencing. The detoxification effect of L-4 strain on ZEN was evaluated under different ZEN concentration, treatment time, pH value and temperature, the degradation products were identified, and the degradation effect of L-4 strain on ZEN contaminated corn meal was evaluated. The ZEN degrading enzyme sequence was obtained through the whole genome protein sequence analysis of strain L-4, and the ZEN degrading enzyme was verified by molecular binding and addition of catalase. We isolated *Bacillus mojavensis* L-4 from the cecal content of laying hens, which demonstrated exceptional ZEN-degrading efficiency. Under optimized conditions (pH 7.0, 37 °C), L-4 completely degraded 0.5–1.0 µg/mL ZEN into less toxic 15-OH-ZEN within 24 h. Importantly, L-4 achieved a 49.41% degradation rate for ZEN in cornmeal. Whole-genome sequencing of L-4 revealed the presence of ZEN-degrading genes and enzymes. In particular, efeB 3668, a peroxidase-like enzyme with high homology (95.91%) to BsDyP from *Bacillus subtilis*, played a key role in ZEN detoxification primarily through hydrogen bonding and hydrophobic interactions. Thus, the rapid and effective degradation of ZEN by *B. mojavensis* L-4, coupled with its adaptability to diverse environments, underscores its potential application in safeguarding animal health and mitigating environmental pollution.

KEYWORDS

zearalenone, *Bacillus mojavensis* L-4, biodegradation, degradation products, peroxidase efeB 3,668

1 Introduction

Zearalenone (ZEN), commonly known as F-2 toxin, is a non-steroidal estrogenic mycotoxin produced by various *Fusarium* and *Gibberella* spp. It is one of the most pervasive contaminants found in grains and animal feed, posing a formidable challenge to food safety and livestock health (Han et al., 2022). Owing to its structural similarity to the endogenous hormone 17 β -estradiol, ZEN exhibits potent reproductive toxicity, capable of disrupting the endocrine balance in animals, inducing hormonal imbalances, and adversely affecting growth and reproductive performance (Zheng et al., 2018). There is an alarming increase in ZEN contamination in agricultural products and feedstocks, with detection rates as high as 45% across Europe, America, and Asia and an astonishing 96.9% in Chinese feed samples (Gruber-Dorninger et al., 2019; Zhao et al., 2021), thus highlighting the urgent need for developing efficient strategies to mitigate the toxic effects of ZEN.

One of the primary challenges in ZEN management arises from its remarkable thermal stability, allowing it to persist even at temperatures as high as 220°C for an hour (Murtaza et al., 2022). Consequently, conventional thermal processing methods are ineffective in eliminating ZEN. Current ZEN detoxification approaches encompass physical, chemical, and biological methods, each with their own limitations. Physical adsorption and chemical detoxification techniques often exhibit low efficiency, can potentially compromise the nutritional value and palatability of feeds, and can accidentally introduce harmful compounds (Shanakhata et al., 2018; Zheng et al., 2018; Yang et al., 2020). In contrast, biological methods exploit the innate decomposing abilities of microorganisms, offering a highly efficient, specific, and environmental-friendly alternative for ZEN degradation. By disrupting the toxic moieties within the ZEN molecule, these microorganisms or their enzymes can convert ZEN into non-toxic or less toxic compounds (Ji et al., 2022). However, it is crucial to note that ZEN degradation may not always lead to complete detoxification, because it can sometimes yield estrogenic analogs such as α -zearalenol and β -zearalenol, which retain toxic properties (Zhang et al., 2020). Numerous bacteria and fungi, including *Bacillus* spp., *Lactobacillus plantarum*, and *Saccharomyces cerevisiae*, along with their associated enzymes, have demonstrated the ability to transform ZEN into less harmful products (Gao et al., 2017; Medeiros et al., 2018; Chlebicz and Śliżewska, 2020; Møller et al., 2021). For example, Chlebicz and Śliżewska (2020) reported good ZEN adsorptive capacity of *S. cerevisiae*, while Møller et al. (2021) isolated a *L. plantarum* strain from Brazilian cheese with robust ZEN degradation capabilities.

In the present study, a novel *Bacillus mojavensis* strain with remarkable ZEN-degrading capabilities was isolated. Subsequently, comprehensive investigation of the optimal degradation conditions, enzymes involved, and products generated from ZEN degradation by this novel strain was performed. A ZEN-degrading peroxidase, designated as efeB 3,668, was identified in *B. mojavensis* L-4, and its kinetic properties and crucial amino acid residues were further examined. Mass spectrometry analysis of the degradation products confirmed that *B. mojavensis* L-4 and its secreted enzyme efeB 3,668 could successfully convert ZEN into 15-OH-ZEN, a derivative with significantly reduced estrogenic toxicity.

Specifically, our study advances previous research in the following ways: Unique Strain Identification: We identified and characterized a

novel strain, L-4, which exhibits superior ZEN degradation efficiency compared to previously reported strains. The content of ZEN was measured using HPLC, and it was found that 0.5 to 1.0 μ g/mL of ZEN in liquid medium could be completely degraded at 24 h.

Mechanistic Insights: Unlike prior studies that primarily focused on ZEN degradation pathways, our work provides new insights into the enzymatic mechanisms, particularly the role of Dyp-peroxidase, in ZEN degradation. After identification, prediction and molecular binding, we found that efeB3668 has a high homology with the peroxidase BsDyP, and there is a good interaction between the peroxidase and ZEN, which fully indicates that the peroxidase can effectively degrade ZEN. However, the ability of efeB3668 to degrade ZEN needs further exploration.

Practical Application: Previous studies have often only focused on the degradation efficiency of degrading bacteria in liquid media. We demonstrated the potential of strain L-4 for practical application in reducing ZEN contamination in cornmeal, offering a promising solution for improving food safety.

These findings provided a solid foundation for exploring innovative ZEN biodegradation technologies, offering promising avenues for mitigating the detrimental effects of this persistent mycotoxin.

2 Materials and methods

2.1 Chemicals, media, and test strains

ZEN (purity $\geq 99.4\%$) was procured from Qingdao PureBio Bioengineering Co., Ltd. HPLC-grade methanol and acetonitrile were obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. The bacterial growth and ZEN degradation medium consisted of 15.0 g/L peptone, 5.0 g/L yeast extract, 2.0 g/L potassium dihydrogen phosphate, 10.0 g/L glucose, 2.5 g/L tomato extract powder, and 1.0 g/L Tween 80, with pH adjusted to 7.0. The fungal growth and ZEN degradation medium contained 20.0 g/L yeast extract and 20.0 g/L peptone, with a pH of 6.5. For obtaining solid medium, agar (12.0 g/L) was added to the liquid medium and sterilized at 121°C and 0.1 MPa for 20 min in an autoclave (Shanghai Boxun Industrial Co., Ltd., China). Template DNA, upstream primers, downstream primers, 10 $\times$ PCR Buffer, dNTP, Taq Plus DNA Polymerase, MgCl₂, catalase (2000 IU) and ddH₂O was purchased from Sangon BioEngineering (Shanghai) Co., LTD. All strains used in this study were isolated and preserved in the Laboratory of Animal Nutrition, Metabolism, and Toxicology, Qingdao Agricultural University, with the following codes: E-1 (*Enterococcus faecalis*), L-2 (*Lactobacillus rhamnosus*), L-3 (*Lactobacillus delbrueckii* subsp. *bulgaricus*), L-4 (*Bacillus mojavensis*), L-5 (*Lactobacillus plantarum*), L-6 (*Lactobacillus parasei*), B-7 (*Streptococcus thermophilus*), B-8 (*Bacillus licheniformis*), B-9 (*Bacillus cereus*), S-10 (*Saccharomyces cerevisiae*), B-11 (*Bacillus subtilis*).

Animal ethics statement: The L-4 strain used in the experiment was isolated from the cecal contents of healthy Hy-line brown laying hens by previous researchers in our laboratory. The animal experiments were approved by the Qingdao Agricultural University Animal Care and Use Committee (Qingdao, China) in accordance with Laboratory Animal Guidelines for the Ethical Review of Animal Welfare (GB/T35892-2018, National Standards of the People's Republic of China).

2.2 Preliminary evaluation of ZEN-degrading strains

Eleven candidate strains were inoculated in liquid medium for resuscitation. In this experiment, 3.8 mL liquid medium and 200 μ L fermentation bacteria solution were added to a 10 mL sterile enzyme-free centrifuge tube to control the ZEN concentration at 0.5 μ g/mL. The centrifuge tubes for ZEN degradation by 10 bacterial strains were incubated at 37°C and 160 rpm for 48 h, respectively. In the control group, only the medium containing ZEN was added. The centrifuge tubes for ZEN degradation by one strain of fungi were incubated at 30°C and 160 rpm for 72 h. The control group contained only ZEN without any strain. After incubation, 460 μ L of the samples were mixed with 460 μ L of acetonitrile and 80 μ L of methanol in sterile centrifuge tubes, vortexed for 5 min, and filtered through a 0.22- μ m PTFE membrane. The presence of ZEN in the cultures was detected using HPLC with a UV detector (LC-20A, SHIMADZU CORPORATION, Japan) at 274 nm (Xu et al., 2020). Separation was performed on a C18 column (150 mm $\times$ 4.6 mm, 5 μ m, Yuexu Technology Shanghai Co., Ltd., China) with an injection volume of 20 μ L and a column temperature of 30°C. The mobile phase consisted of acetonitrile-water-methanol (46: 46:8, V/V/V) at a flow rate of 0.8 mL/min, with ZEN eluted using 100% methanol for 5 min. A standard curve was generated based on the retention time and peak area of the ZEN standard stock solution to determine ZEN residues. All samples were performed three times, and the average values were then calculated. Strain L-4, which exhibited the highest ZEN degradation rate, was selected for further study and stored at -80°C in liquid medium containing 40% glycerol.

2.3 Morphological identification of ZEN-degrading bacterium

These strains were inoculated onto solid medium by the three-zone streaking method and incubated at 37°C for 24 h. Solid medium includes bacterial solid medium formula (peptone 15.0 g, yeast extract powder 5.0 g, potassium dihydrogen phosphate 2.0 g, glucose 10.0 g, tomato extract powder 2.5 g, Tween 80 1.0 g, Agar 12.0 g) and fungal solid medium formula (peptone 10.0 g, glucose 20.0 g, yeast extract powder 5.0 g, Agar 14.0 g). Subsequently, the color, size, shape, texture, transparency, and edge elevation of individual colonies were recorded. Then, a well-grown single colony was subjected to Gram staining and examined under a microscope (CK40-SL, Olympus, Japan) to observe the cellular morphology.

2.4 The 16S rDNA identification

DNA was extracted from strain L-4 using a DNA extraction kit, and PCR amplification of the 16S rDNA region was performed using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), as described by Wang et al. (2019). The PCR reaction system consisted of 1.0 μ L template DNA, 1.0 μ L 27F, 1.0 μ L 1492R, 0.5 μ L Taq DNA Polymerase, 0.5 μ L dNTP, 2.0 μ L MgCl_2 , 2.5 μ L 10 $\times$ PCR Buffer, and 16.5 μ L ddH₂O. The conditions were predenatured at 94°C for 5 min. 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min were followed by an extension of 8 min. Among them, the concentration of DNA was 50 ng/ μ L, and the working concentration of forward and reverse primers was 10

μ M. The PCR products were verified by 1.2% agarose gel electrophoresis at 120 V for 40 min. The positive bands were sequenced by Shanghai Sangon Biotech Co., Ltd. (Shanghai, China), and the 16S rDNA sequence of strain L-4 was submitted to the National Center for Biotechnology Information (NCBI) with GenBank ID OR 649301.1. The closest related sequences were retrieved from the NCBI database and a phylogenetic tree was constructed using the maximum likelihood method by comparing homology with selected *Bacillus* model strains by utilizing MEGA 5.10 software.

2.5 ZEN degradation efficiency under different cultivation conditions

To investigate the degradation rate of ZEN by L-4 strain under different conditions, 1 mL of fermentation bacteria solution was added to a conical flask containing 19 mL of liquid medium, shaken at 240 rpm, and samples were collected at regular intervals. HPLC was used to quantify the amount of ZEN remaining, and the degradation rate was calculated to evaluate the degradation of ZEN by the L-4 strain. Culture conditions included time (3 h, 6 h, 9 h, 12 h, 15 h, 18 h, and 21 h), temperature (25°C, 37°C, 40°C, 42°C, and 45°C), initial pH (2.5, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0) and different initial ZEN concentrations (0.5 μ g/mL, 1.0 μ g/mL, and 5.0 μ g/mL). A control group was set up, and only ZEN standards were added to the liquid medium. All samples were performed three times, and the average values were then calculated.

2.6 Evaluation of ZEN degradation by different components of strain L-4

The ZEN degradation efficacies of different components of strain L-4, including viable bacteria, heat-inactivated bacteria, bacterial intracellular fluid, bacterial debris, bacteria-free supernatant, heat-inactivated bacteria-free supernatant, and bacteria-free supernatant treated with proteinase K/SDS, were assessed. The bacterial cultures were centrifuged at 8,000 rpm for 20 min to obtain cell-free supernatants, and the bacterial precipitates were resuspended in PBS to obtain live and heat-inactivated bacterial suspensions. Intracellular fluid was extracted from the suspension of live bacteria subjected to ultrasonic fragmentation and the fragments were resuspended in PBS. All the treatment groups were mixed with 0.5 μ g/mL ZEN, whereas the control comprised PBS or liquid medium containing ZEN. All the samples were incubated at 37°C and 240 rpm for 72 h. After centrifugation and filtration, the ZEN residues were detected by HPLC.

To determine whether ZEN degradation was enzyme-dependent, cell-free supernatants were heat-treated at 100°C for 10 min. Then, SDS (final concentration of 1%), proteinase K (1 mg/mL), and a combination of proteinase K + SDS were added to the samples and incubated for 72 h, and the samples were analyzed by HPLC to evaluate ZEN degradation.

2.7 Analysis of ZEN degradation products

In order to observe the final phase of ZEN presence, we chose to sample the reaction at 20 h for subsequent analysis of degradation products by UPLC-Q-Exactive-MS/MS. The strain L-4 and ZEN were

added to the liquid medium and incubated at 37°C and 240 rpm for 20 h. The reaction was terminated by adding an equal volume of methanol, followed by vortex mixing for 10 min. The mixture was filtered through a 0.22- μ m PTFE membrane into a liquid chromatography vial. The control group consisted of liquid medium and ZEN. Identification and analysis of the samples were performed using UPLC-Q-Exactive-MS/MS (Thermo Fisher Scientific, United States).

Chromatographic Conditions: An Agilent Poroshell 120 EC-C18 column (2.1 mm $\times$ 100 mm, 2.7 μ m) was employed, with a column temperature of 30°C, an injection volume of 5 μ L, and a flow rate of 0.2 mL/min. The mobile phases consisted of methanol (A) and water containing 0.1% formic acid and 5 mmol/L ammonium formate (B). The gradient elution program was set as follows: 0–0.5 min, 90% B and 10% A; 0.51–5 min, 90–5% B and 10–95% A; 5.01–8 min, 5% B and 95% A; and 8.01–9 min, 90% B and 10% A.

Mass Spectrometry Conditions: An electrospray ionization source was used in both positive and negative ion scanning modes with multiple reaction monitoring. The ion source temperature, drying gas flow rate, nebulizer gas pressure, capillary voltage, and scan range were set at 350°C, 8 L/min, 25 psi, 3,000 V, and m/z 50–1,000, respectively. For MS/MS analysis, the induced collision dissociation energy was set at 20 eV.

2.8 Degradation of ZEN in cornmeal by strain L-4

To investigate the effectiveness of strain L-4 in degrading ZEN in corn products, a degradation experiment was conducted using cornmeal. In this experiment, to ensure that the added bacterial solution was a single variable. A total of 10.0 ± 0.01 g of finely ground cornmeal were added to a 250-mL Erlenmeyer flask and sterilized in an autoclave at 121°C and 0.1 MPa for 20 min. Subsequently, ZEN standard was added under aseptic conditions to achieve a final concentration of 532.91 μ g/kg, and the flasks were inoculated with 10% L-4 bacterial suspension (1.5×10^9 cfu/mL). The control group only contained 10% sterile liquid medium. Each treatment comprised three replicates. All the flasks were incubated at 37°C for 5 days, and samples were collected at 0, 1, 2, 3, 4, and 5 days, respectively. The residual ZEN content in the cornmeal was detected using the up-converting phosphor technology-based lateral flow assay (Zhu et al., 2021), and the degradation rate was calculated as follows: Degradation rate = [(ZEN content in control group - ZEN content in experimental group)/ZEN content in control group] $\times$ 100%.

2.9 Whole-genome sequencing, degradative enzyme prediction, and molecular docking

2.9.1 Whole-genome sequencing of strain L-4

The genomic DNA of strain L-4 was extracted using a bacterial DNA extraction kit (Omega, USA), and its concentration and purity were determined with a Thermo Scientific NanoDrop 2000 microspectrophotometer. Genome sequencing of strain L-4 was performed on the PacBio Sequel II platform (Shanghai Yuanxin Biomedicine Technology Co., Ltd., China). Filtered subreads were assembled using Canu v1.6 software, and the assembly was further corrected with Racon v1.4.20 utilizing third-generation subreads.

Gene prediction was conducted with Prodigal v2.6.3 and Island Path-DIMOB v1.0. (She et al., 2009) The predicted gene-encoded proteins were compared with Clusters of Orthologous Groups of proteins (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases in BLASTP (BLAST 2.2.28+) and reported with functional annotations. A genome circle plot of strain L-4 was generated using Circos software (Krzywinski et al., 2009). The mechanisms underlying ZEN degradation were explored from aspects such as cell motility and degradation-related gene prediction in strain L-4.

2.9.2 Degradative enzyme prediction and molecular docking

The complete protein sequences obtained by sequencing were aligned with the six known ZEN degradation protein sequences in NCBI, and the sequence with the highest homology was identified using DNAMAN software. NCBI accession numbers and details for these sequences are as follows: Esterase ZHD101 (*Clonostachys rosea* IFO 7063, Accession Number: ALI16790.1), Phosphoric acid transferase ZPH (*Bacillus subtilis* Y816, Accession Number: QVL29807.1), Laccase CotA (*Bacillus licheniformis*, Accession Number: QAX90317.1), Peroxidase BsDyP (*Bacillus subtilis*, Accession Number: 7PKX_A), ZENC (*Neurospora crassa*, Accession Number: 1547034726), and ZLHY-6 (*Pichia pastoris* GSZ, Accession Number: QHW12260.1).

The subcellular localization of efeB 3,668 was predicted using PSORT server (Lau et al., 2021), and its hydrophobicity was assessed using ProtScale. SignalP 6.0 and TMHMM 2.0 servers were utilized to predict the presence of signal peptides and transmembrane regions in the amino acid sequence of efeB 3,668, respectively (Almagro Armenteros et al., 2019). The secondary structure of efeB 3,668 was predicted by SOMPA, and functional sites were analyzed using PROSITE and NCBI conserved domain search tools. Protein tertiary structure homology modeling of efeB 3,668 was conducted on the SWISS-MODEL web server using 7pkx.1.A as a template, and the reliability of the model was evaluated by the Ramachandran Plot server.

The 2D structures of small-molecule ligands were retrieved from the PubChem database,¹ ZEN database link to <https://pubchem.ncbi.nlm.nih.gov/compound/5281576> and converted into 3D structures in Chem Office 20.0 software (CambridgeSoft, United States). The docking receptor is the 3D structure of efeB 3,668 derived from homology modeling. Water molecules and phosphate groups were removed from the proteins using PyMOL 2.6.0 (DeLano Scientific LLC, United States; El-Hachem et al., 2017). Compounds were energy-minimized, and target proteins were preprocessed to identify active pockets using Molecular Operating Environment 2019 software (Chemical Computing Group Inc., Canada). Molecular docking was performed with MOE 2019, with 50 runs set for the calculation. Binding affinity was evaluated to assess the binding activity, and the results were visualized using PyMOL 2.6.0 and Discovery Studio 2019 (BIOVIA, United States).

2.9.3 Validation of the major ZEN degrading enzymes

In order to test that the main degradation component of ZEN by the fermentation supernatant of strain L-4 was peroxidase, we added 10 μ g/mL catalase to the culture supernatant of strain L-4 to remove H₂O₂ generated during the reaction, and sampled the remaining ZEN

¹ <http://pubchem.ncbi.nlm.nih.gov/>

after 72 h to measure the degradation rate according to the method as described in section 2.8.

2.10 Data statistics and analysis

Statistical analysis was conducted using Microsoft Excel 2019 (Microsoft, United States) and SPSS 22.0 (IBM, United States; Dong et al., 2024). One-way ANOVA was employed for comparisons, and multiple comparisons of means were performed using the least significant difference test. The results are presented as the “mean $\pm$ standard deviation (SD), “with statistical significance indicated by $p < 0.05$. The phylogenetic tree of strain L-4 was constructed using MEGA 5.10. Mass spectrometry analysis of the degradation products was performed with Qualitative Analysis B.05.00 software (Agilent Technologies, United States). Degradative enzyme sequences were predicted using DNAMAN software (Lynnon Biosoft, United States), and graphs were generated with GraphPad Prism 9 (GraphPad Software, United States).

3 Results

3.1 Screening and identification of ZEN-degrading bacteria

As shown in Figure 1A, among the 11 strains, L-4, S-10, and S-11 presented better ZEN-degrading ability. In particular, strain L-4 exhibited

the highest ZEN degradation rate of 81.04%, which was further investigated. As illustrated in Figure 1B, the colonies of strain L-4 were yellowish, opaque, rough, and wrinkled. Gram-staining further confirmed that strain L-4 was Gram-positive with rod-like morphology (Figure 1C).

Sequence homology analysis revealed a 99.65% similarity between strain L-4 and *B. mojavensis* D50 (GenBank ID: MZ165352.1) in the NCBI database, positioning strain L-4 within the same clade as *B. mojavensis* D50. Based on both colony morphology and 16S rDNA sequence analysis, strain L-4 was identified as *B. mojavensis*. The sequence obtained by 16S rDNA sequencing was submitted to GenBank and the accession number was OR 649301.1.

3.2 Degradation of ZEN by strain L-4

As shown in Figure 2, the ZEN degradation rate of *B. mojavensis* L-4 was that after 24 h of incubation, ZEN was completely degraded in liquid medium. By optimizing pH, temperature and initial ZEN concentration, it was found that 0.5 and 1.0 $\mu\text{g/mL}$ ZEN could be completely degraded after 24 h at pH 7 and 37°C.

3.3 Degradation of ZEN by different components of strain L-4

ZEN degradation rates of each components of strain L-4, including live bacteria, heat-inactivated bacteria, bacterial

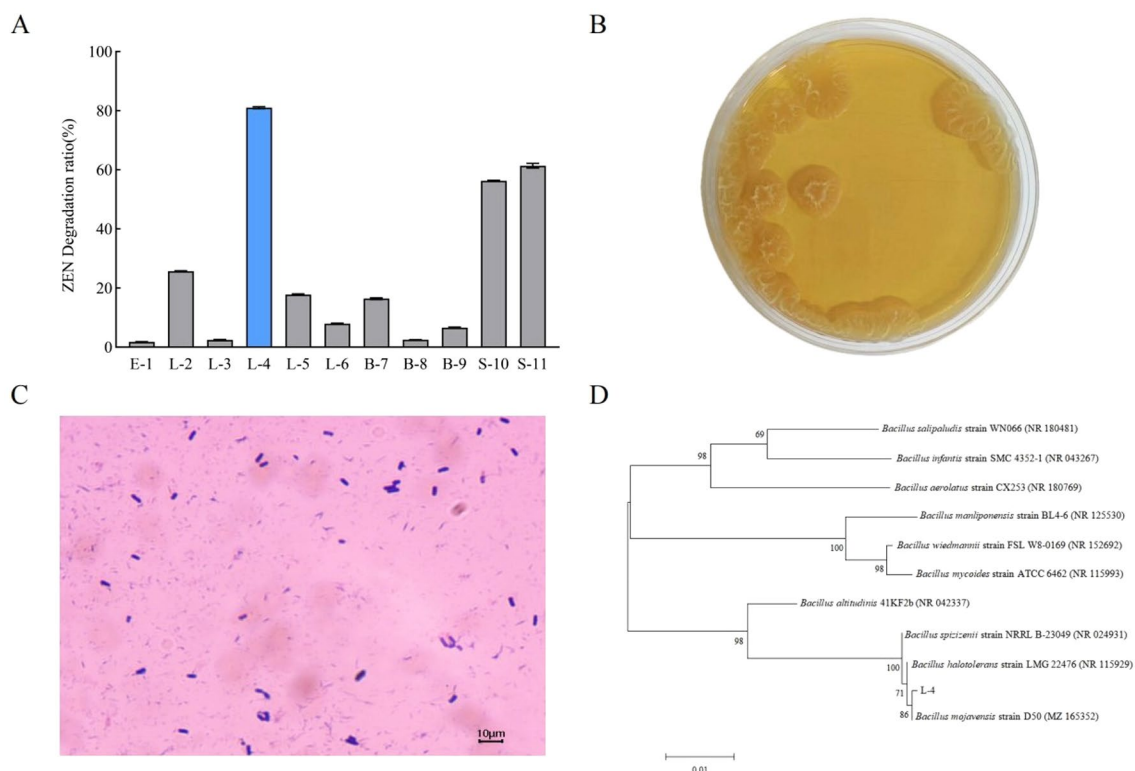


FIGURE 1

(A) Screening of ZEN-degrading bacteria. (B) Colony morphology and (C) cell morphology of strain L-4. (D) Phylogenetic tree of strain L-4 based on 16S rDNA gene sequences. Data are mean $\pm$ SD of three independent replicates.

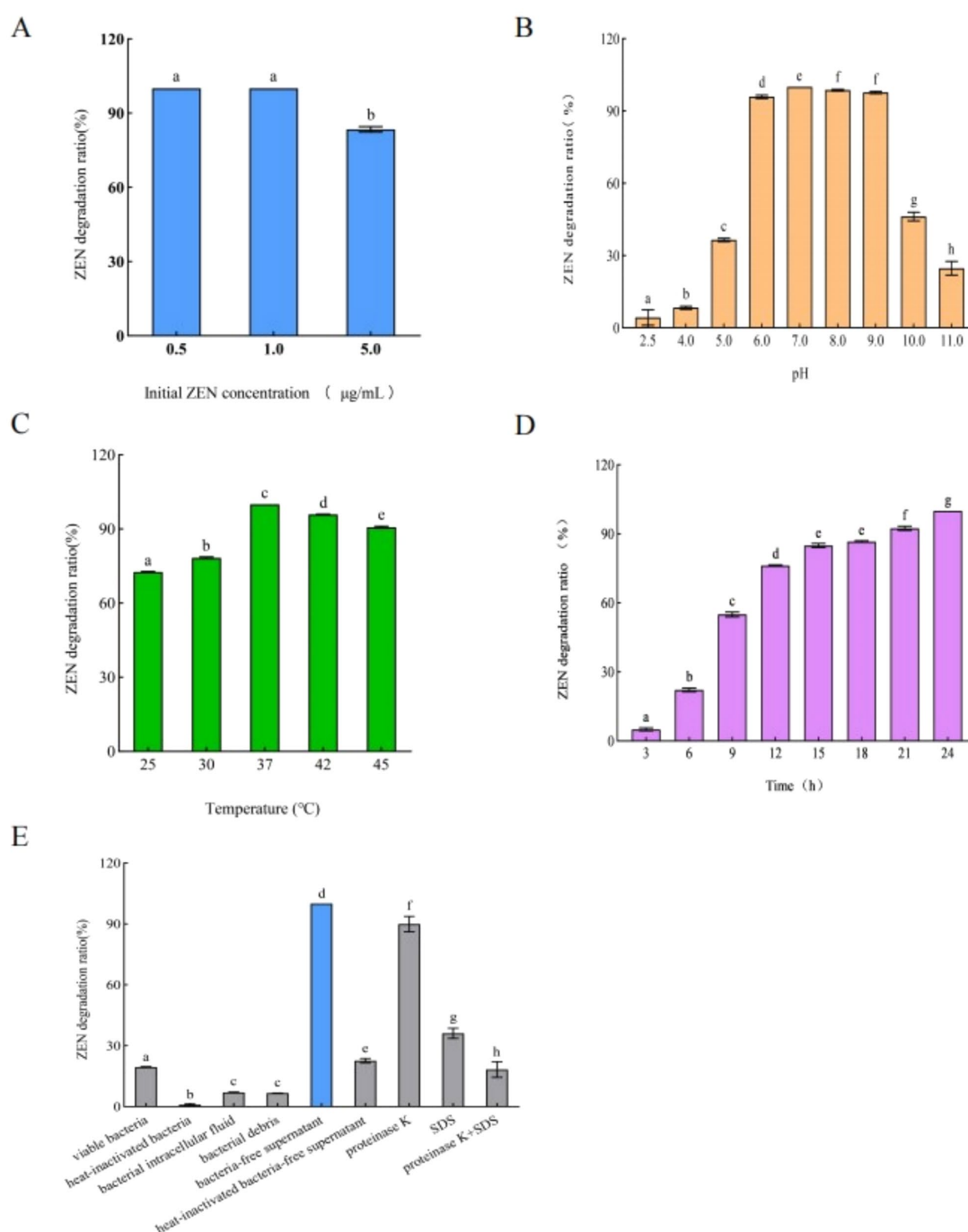


FIGURE 2

Effects of (A) initial concentration of ZEN, (B) pH of liquid medium, (C) temperature, and (D) time on the ZEN degradation rate. (E) ZEN degradation rates of different components of strain L-4. Data are the mean $\pm$ SD of three independent replicates.

intracellular fluid, bacterial debris, and bacteria-free supernatant, were shown in Figure 2E. The ZEN degradation rate of sterile supernatant was the highest, reaching 100% at 48 h, while the ZEN degradation rates of bacterial intracellular fluid and live bacteria were 7.06 and 19.50%, respectively (Figure 2E). After inactivating the sterile supernatant and adding protease K (1 mg/mL), SDS (final concentration 1%) or SDS + protease K, the ZEN degradation rates declined to 22.69, 89.88, 36.13, and 18.13%, respectively (Figure 2E). The degradation rate of ZEN was significantly reduced after treatment of bacteria-free supernatant with protease K, SDS, protease K + SDS,

and 100°C heat treatment, which strongly suggested that strain L-4 produced an extracellular enzyme responsible for ZEN degradation. However, further research is needed to fully understand the specific degradation mechanism.

3.4 Analysis of ZEN degradation products

By analyzing the degradation products, a potential degradation product was detected in the negative ion mode, which we designated

L-4-1 (Figure 3A). L-4-1 was not detected in the liquid medium containing only ZEN, confirming that the substance was not derived from the liquid medium and ZEN. However, L-4-1 was found in the sample of L-4 bacterial solution +ZEN, indicating that L-4-1 was produced by L-4 degradation of ZEN. The results of mass spectrometry were shown in Figures 3B,C. The predicted molecular weight of L-4-1 was 333.1000 g/mol, which was consistent with the molecular formula $C_{18}H_{23}O_6$ (OH-ZEN), but the ion fragment $m/z201$ was found in the secondary mass spectrometry analysis. Hildebrand et al. (2012) found that ionic fragments of $m/z201$ are present only in 15-OH-ZEN; therefore, we suggest that strain L-4 and the extracellular enzymes it produces can degrade ZEN to the product L-4-1 at low concentrations.

3.5 Detoxification effect of strain L-4 on ZEN-contaminated cornmeal

As shown in Figure 4, the ZEN detoxification rate of strain L-4 against ZEN-contaminated cornmeal reached 49.41%. After 5 days of incubation, the ZEN concentration in cornmeal decreased from 532.91 $\mu\text{g/kg}$ to 269.60 $\mu\text{g/kg}$.

3.6 Whole-genome analysis of strain L-4

3.6.1 Basic genome annotation analysis

As shown in Figure 5A, the total length of the L-4 genome is 4,027,871 bp, with a GC content of 43.78% and 3,659 predicted protein-coding genes (CDS).

(1) COG annotation.

Functional classification and prediction of the genome of strain L-4 were performed using COG to infer protein functions. As shown in Figure 5B, 3,080 genes were annotated into 22 COG categories. The most abundant functional genes were “Function unknown (354 CDSs),” “Amino acid transport and metabolism (286 CDSs),” “Carbohydrate transport and metabolism (282 CDSs),” “Transcription (275 CDSs),” “Cell wall/membrane/envelope biogenesis (194 CDSs),” and “Inorganic ion transport and metabolism (190 CDSs).”

(2) KEGG metabolic pathway analysis.

Whole-genome sequencing of strain L-4 facilitated the investigation of the ZEN degradation mechanism. Figure 5C showed the KEGG

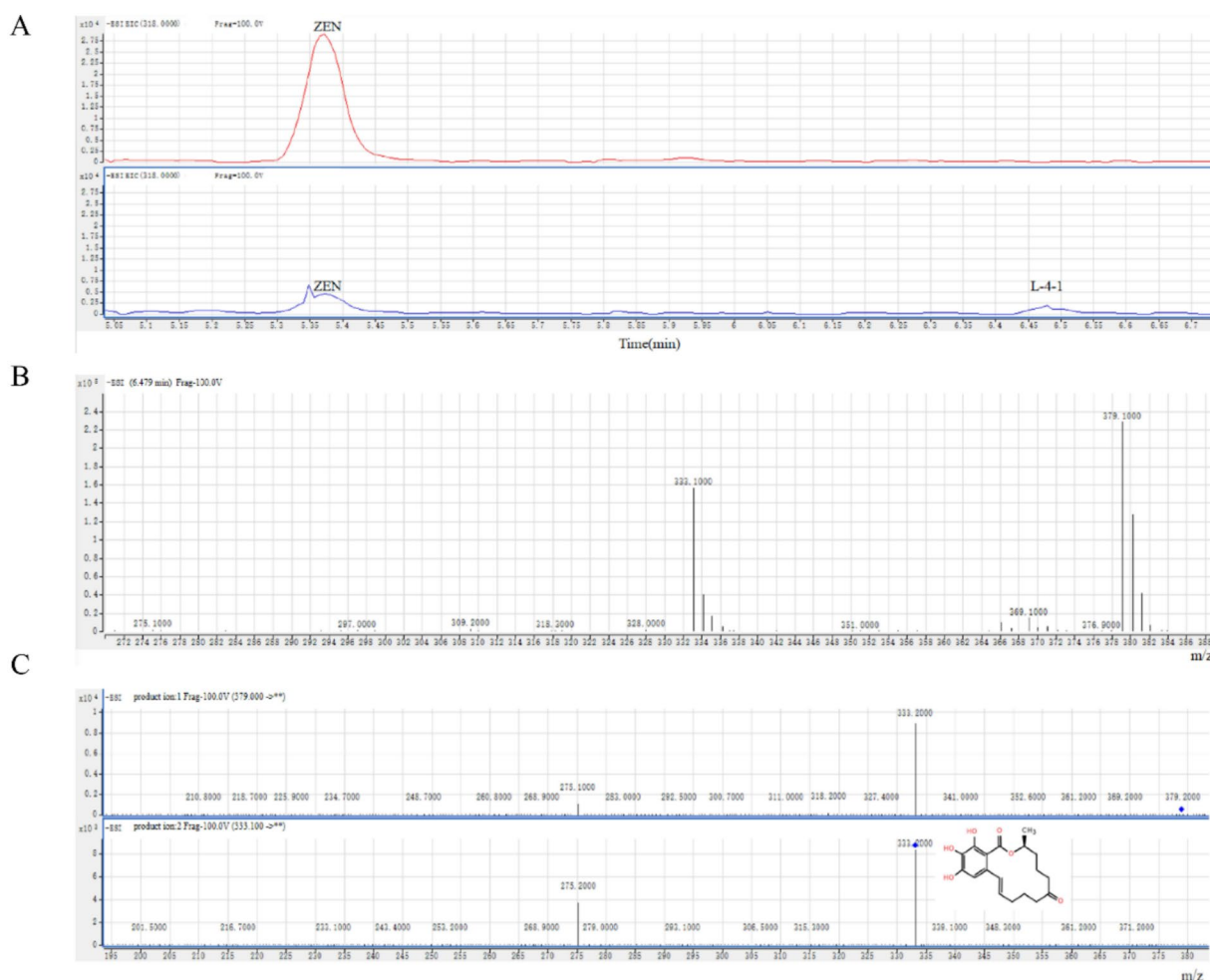


FIGURE 3
(A) ZEN degradation products produced by strain L-4. (B) Mass spectrometry and (C) secondary mass spectrometry analyses of the degradation product L-4-1.

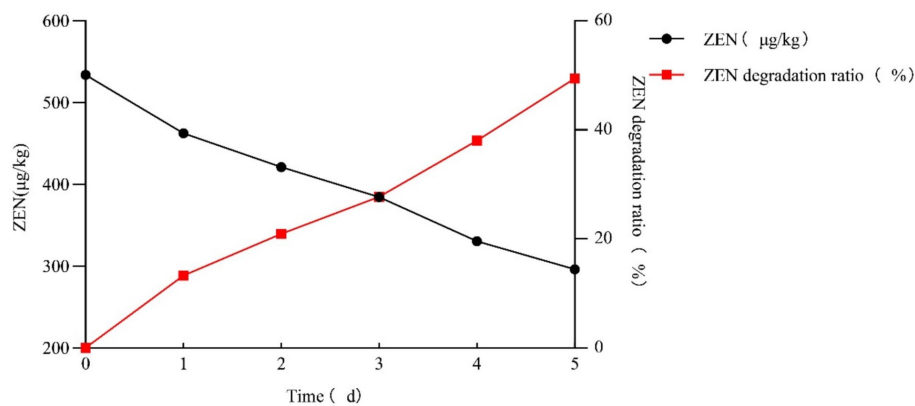


FIGURE 4

Rate of ZEN degradation by strain L-4 in cornmeal. Data are the mean $\pm$ SD of three independent replicates. As SD < 0.01, bar is not shown in the diagram.

annotation classification statistics of the L-4 genome, revealing 2,637 annotated genes. Most of the genes in strain L-4 were found to be related to metabolism, with 558 genes involved in carbohydrate, amino acid, and lipid metabolism. In particular, amino acid and lipid metabolism (such as β -oxidation of fatty acids) may be associated with the formation of hydroxyl groups in ZEN degradation products. Moreover, strain L-4 was noted to possess complete pathways for glycolysis, transmembrane transport, oxidative phosphorylation, tricarboxylic acid cycle, and pyruvate metabolism, indicating its robust metabolic capabilities conducive to ZEN degradation.

3.6.2 Analysis of degradation-related features in genome annotation

(1) Genes associated with ZEN degradation.

Identifying enzymes involved in ZEN degradation is crucial for understanding the environmental transformation of ZEN and management of the degradation processes. As shown in Figure 6A, comparison of the 3,659 protein sequences annotated in strain L-4 with known ZEN-degrading enzyme sequences using DNAMAN software revealed a protein sequence with 95.91% homology to BsDyP from *Bacillus subtilis*.

(2) Flagella formation and chemotaxis.

Bacterial chemotaxis, encompassing flagellar motility and signal transduction, is essential for strain L-4 to navigate toward favorable conditions, enhancing ZEN degradation and bioavailability. As depicted in Figures 6B,C, strain L-4 exhibited complete flagellar assembly and chemotaxis-related genes.

3.6.3 Subcellular localization, transmembrane region, signal peptide, hydrophobicity, and secondary structure of efeB 3,668

By using PSORT, efeB 3,668 was predicted to primarily exist in the cytoplasm, presenting the following distribution: cyto (22.5%), cyto_nucl (20%), nucl (12.5%), mito (12.5%), plas (10%), extr (7.5%), cysk (7.5%), pero (5%), and E.R. (2.5%). SignalP 6.0 analysis of the amino

acid sequence of efeB 3,668 (Figure 7A) identified a signal peptide. Furthermore, TMHMM 2.0 predicted a transmembrane sequence in efeB 3,668 (Figure 7B), suggesting its role as a transporter protein. ProtScale analysis showed Ala at position 25 with the highest hydrophobic value of 2.133 and Lys at position 53 with the maximum hydrophilic value of -3.633 (Figure 7C). In particular, the number of hydrophilic amino acid residues significantly exceeds that of hydrophobic amino acid residues throughout the entire polypeptide chain, suggesting that efeB 3,668 was a hydrophilic protein.

SOPMA predicted that the secondary structure of efeB 3,668 consisted of 29.33% α -helix, 14.42% extended strand, 5.29% β -turn, and 50.96% random coil (Figure 7D). ScanProsite software predicted the presence of two active centers in efeB 3,668: a TAT active site spanning amino acids 1–44 and a dyp_peroxidase active site spanning amino acids 63–408 (Figure 7E). NCBI analysis revealed two conserved domains in efeB 3,668: the TAT small family and Dyp_perox superfamily (Figure 7G).

ProtParam software analysis demonstrated the molecular weight of the protein to be approximately 45 kD, with a molecular formula of $C_{2024}H_{3192}N_{556}O_{618}S_{15}$. The theoretical isoelectric point was 8.86, indicating alkaline characteristic. The number of negatively charged amino acid residues (Asp+Glu) was 48, while that of positively charged amino acid residues (Arg + Lys) was 54. The extinction coefficient was 6,405, instability index was 34.09, and the aliphatic index was 70.67.

3.6.4 Molecular docking model representing the binding of efeB 3,668 (tertiary structure) to ZEN

By employing the 7pkx.1.A structure as a template, homology modeling of efeB 3,668 was conducted using SWISS-MODEL, which yielded a sequence identity of 95.91% and similarity score of 0.90. The functional prediction for efeB 3,668 was peroxidase. Subsequently, the 3D structure of efeB 3,668, obtained through homology modeling, was utilized to investigate its interaction with ZEN via molecular docking simulations. As shown in Figure 7F, the docking pattern revealed a binding energy of -6.9557 kcal/mol between ZEN and efeB 3,668, indicating a strong binding interaction. ZEN primarily interacted with efeB 3,668 through the formation of hydrogen bonds and hydrophobic forces. The hydrogen bonding interactions were observed between

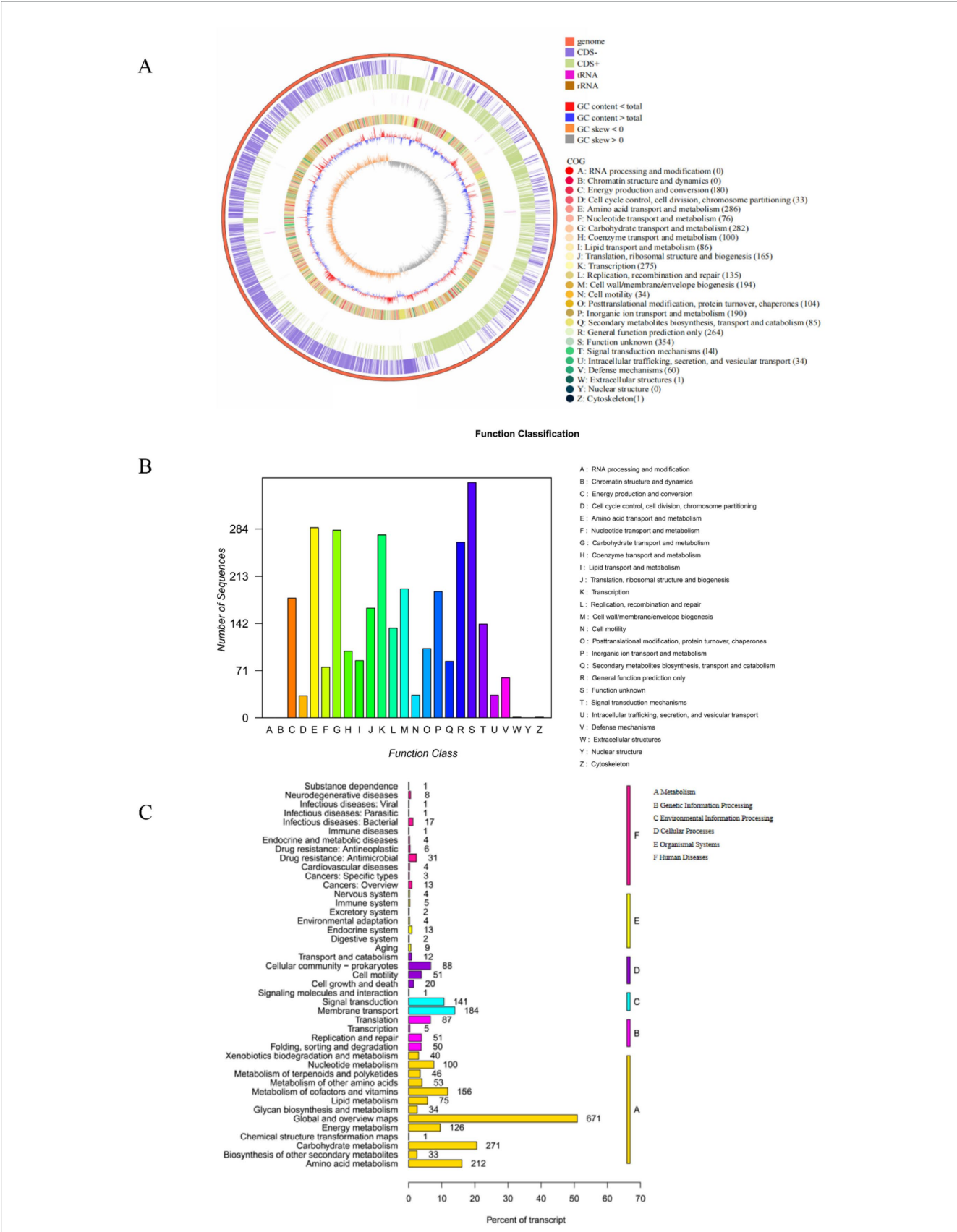


FIGURE 5
(A) *Bacillus mojavensis* L-4 sequencing gene loop diagram. (B,C) Functional annotations of the L-4 genome against COG and KEGG databases.

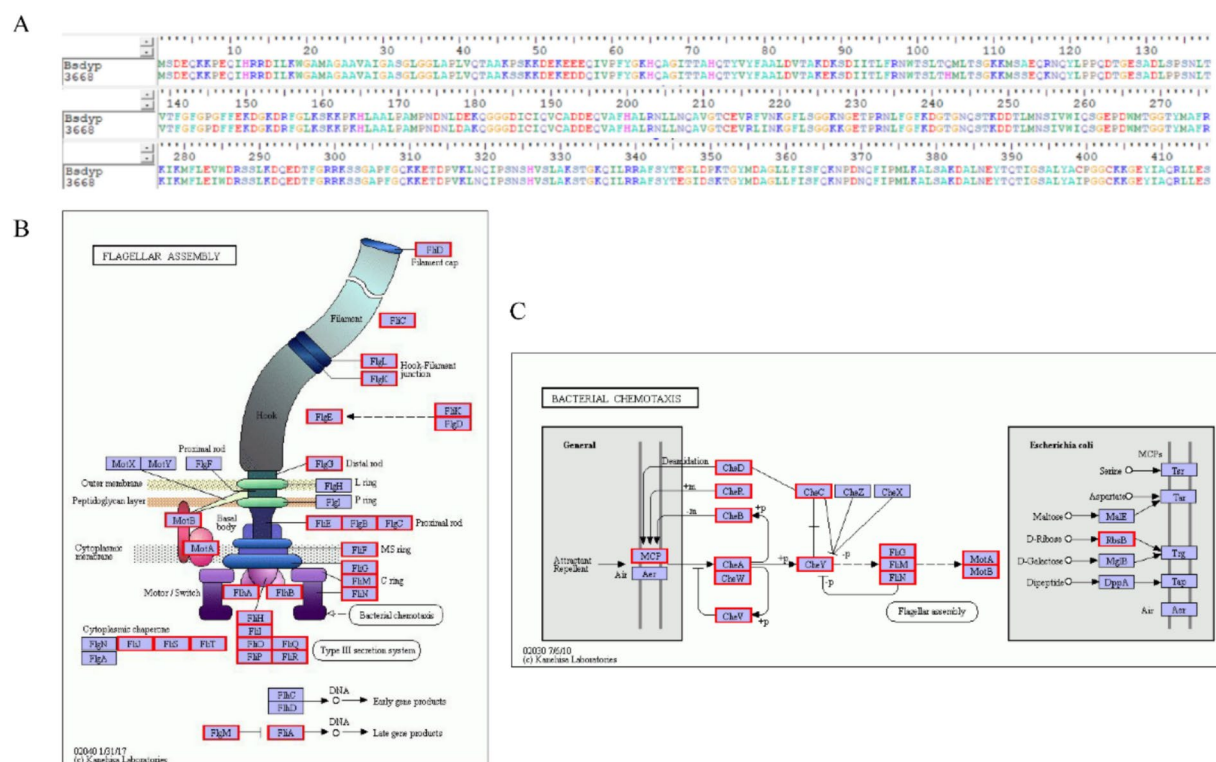


FIGURE 6

(A) Comparison of the protein sequence efeB 3,668 of *B. mojavensis* L-4 with the sequence of ZEN degradation protein BsDyP. (B) Flagellar assembly gene. (C) Annotation of bacterial chemotaxis genes in the L-4 genome.

ZEN and residues His326, Arg299, and Gly243 on efeB 3,668, while hydrophobic interactions were noted between ZEN and residues Ala330, Lys331, Val316, and Val327 on efeB 3,668. In addition, the Arg339 residue on the receptor exhibited electrostatic interactions with ZEN. These findings collectively suggested that ZEN serves as a suitable substrate for efeB 3,668.

3.6.5 Validation of the major ZEN degrading enzymes

When catalase was added to the supernatant, it was found that the degradation rate of ZEN was 49.84% at 72 h, which was lower than that of pure extracellular enzymes (as shown in Figure 7H). It is speculated that this is due to the reduction or absence of H_2O_2 , but this confirms our previous inference that peroxidase plays a major role in the degradation of ZEN in L-4 bacterial solutions.

4 Discussion

In the present study, we investigated numerous bacterial and fungal strains for their ZEN degradation capabilities and identified a bacterial strain L-4 with excellent ZEN-degrading ability. Then, we conducted comprehensive taxonomic identification of strain L-4 utilizing diverse methodologies. The initial morphological observations provided preliminary insights into the identity of strain L-4. Subsequently, molecular biological identification methods, particularly 16S rDNA sequencing analysis and whole-genome sequencing, which demonstrated high sensitivity and specificity as

well as remarkable advantages in species identification (Taş et al., 2021), confirmed the taxonomic affiliation of strain L-4 at the molecular level, ensuring the accuracy and reliability of our identification results. As shown in Figure 1, the morphological characteristics and sequencing results confirmed the classification of strain L-4 as *B. mojavensis*. Originally isolated from desert soil, *B. mojavensis* exhibited closest phylogenetic relationship with *B. amyloliquefaciens*, *B. atrophaeus*, and *B. subtilis* (Xu and Côté, 2003), suggesting that *B. mojavensis* could be considered as a subspecies of *B. subtilis*.

A variety of probiotics, including lactic acid bacteria, yeasts, and bacilli, have been observed to possess the ability to antagonize *Fusarium* and degrade ZEN. For instance, *S. cerevisiae* exhibited a 52% adsorption rate for 100.0 $\mu\text{g/mL}$ ZEN (Chlebicz and Śliżewska, 2020), while *L. plantarum* 3QB361, a strain isolated from Brazilian cheese, achieved a 70–80% detoxification rate for 2.0 $\mu\text{g/mL}$ ZEN within 15 min (Møller et al., 2021). Furthermore, *B. subtilis* YQ-1, isolated from soil, degraded 98.36% of ZEN in the fermentation broth (Zhou et al., 2024). However, unlike bacilli that can tolerate and survive in harsh environments and persist through processing and storage, while lactic acid bacteria are prone to deterioration during the processing, transportation and storage of food and feed, which limits their ZEN degradation efficiency. Moreover, yeasts are ineffective in detoxifying ZEN because they primarily adsorb ZEN onto their cell wall (Keller et al., 2015) and do not completely metabolize it. In the present study, the ZEN degradation ability of strain L-4 was studied under different conditions, and the results showed that strain L-4 exhibited excellent ZEN degradation ability in liquid medium and cornmeal. The

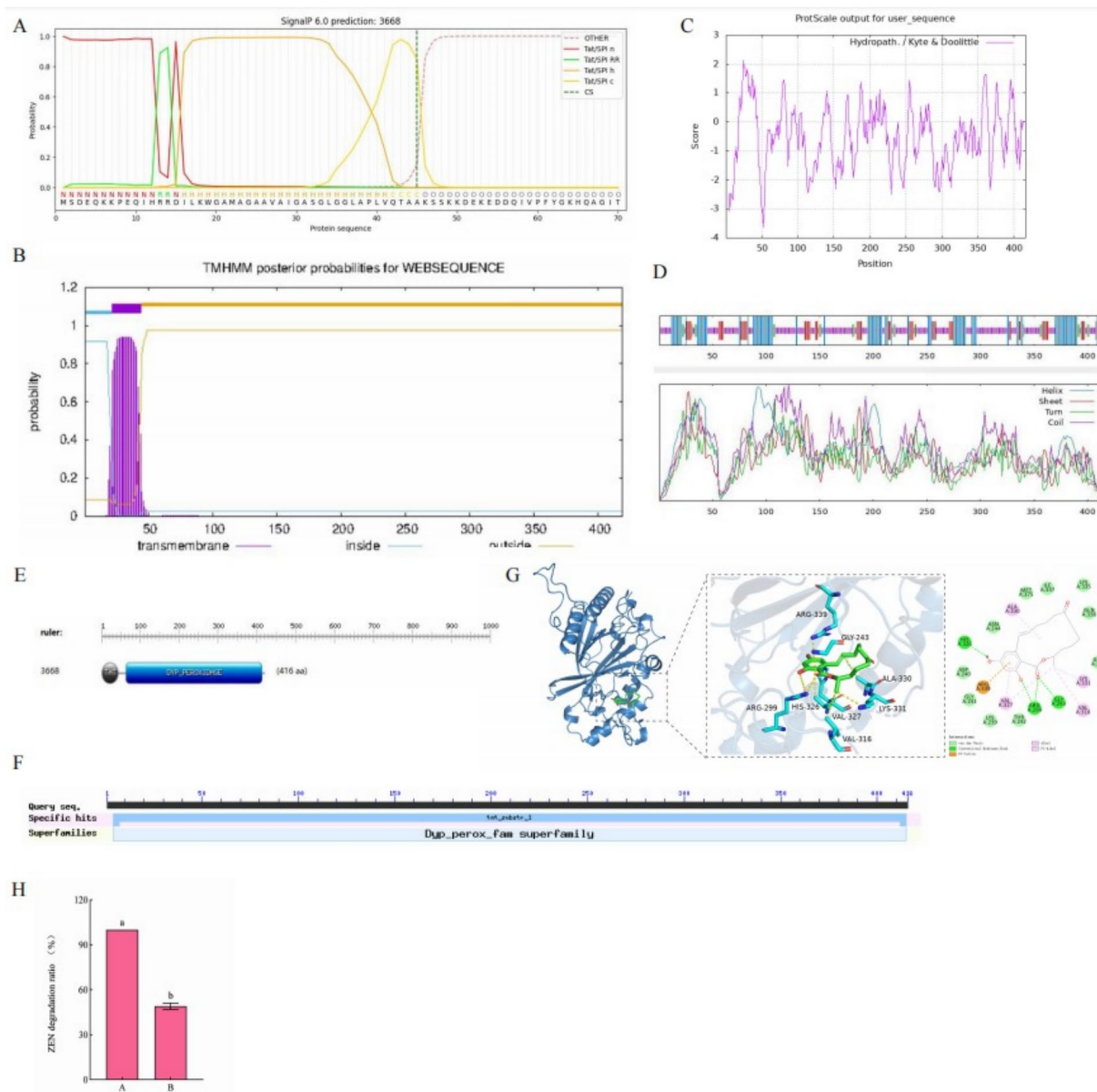


FIGURE 7

(A) Signal peptide, (B) transmembrane sequence, (C) hydrophobicity, (D) convoluted helical domain, (E) active center, and (F) molecular docking model characterizing the binding of ZEN to efbB 3,668. (G) Conserved structural domains of efbB 3,668. (H) Effect of catalase on ZEN degradation rate. A: no catalase was added to the supernatant; B: catalase was added to the supernatant.

degradation rate of 0.5 µg/mL ZEN by extracellular protein produced by strain L-4 in liquid medium reached 100%. Furthermore, strain L-4 showed remarkable adaptability to diverse conditions, wide pH and temperature range during fermentation. However, the ZEN degradation rate decreased as the pH of the medium and the culture temperature increased or decreased, which is consistent with the previous results on bacterial degradation of ZEN. Currently, there is a lack of research on the rapid and efficient degradation of concentrated ZEN in grains by semi-solid fermentation. We extended the incubation period to 72 h to ensure the complete removal of ZEN and take into account potential changes in bacterial activity under different experimental conditions. This method allowed us to confirm the stability and consistency of the degradation process over a longer

period of time. In the present study, *B. mojavensis* L-4 achieved a 49.41% degradation rate of ZEN in cornmeal, outperforming other probiotics. Given its ability to degrade ZEN across a broad spectrum of concentrations, temperatures, and pH values, *B. mojavensis* emerged as a promising candidate for agricultural and industrial applications.

Despite significant progress in the analysis of key enzymes, degradation products, and mechanisms for ZEN biodegradation, the potential unknown side effects or high levels of toxicity of ZEN and its derivatives continues to hinder the practical application of microbial ZEN degradation in industrial production (Tan et al., 2023). Although previous studies have demonstrated the effective degradation of ZEN by *B. licheniformis* CK1 (Fu et al., 2016), further

analysis of their degradation products is scarce. During the degradation of ZEN, many derivatives were formed, including α -zearalanol with high estrogenic toxicity, as well as zearalenone 14-glucoside and zearalenone 14-sulfate with lower estrogenic toxicity. In the present study, the degradation product L-4-1 was analyzed using an anionic mode at $m/z = 333.2000$, similar to the report by Guo et al. (2024) who employed an anionic mode ($m/z = 333.1331$ [M-H]⁻) and estimated a relative molecular mass of 333 for the ZEN degradation product. However, the present study detected additional daughter ions at m/z 315.3000, m/z 306.5000, m/z 293.1000, m/z 279.0000, m/z 275.2000, and m/z 201.5000, which were generated by the extracellular proteins of strain L-4 during ZEN degradation. Sun et al. (2024) and Hildebrand et al. (2012) reported that the fragment ion at m/z 201 was exclusive to 15-OH-ZEN, indicating that this degradation product was a C15-hydroxylated metabolite of ZEN with low estrogenic activity (Drzymala et al., 2015). This finding is in agreement with the peroxidase BsDyP mediated effect observed by Qin et al. (2021), thus implying a molecular formula of C₁₈H₂₃O₆ for the degradation product L-4-1.

The whole genome sequencing of strain L-4 provided valuable insights into the degradation mechanisms of ZEN and identification of related functional genes, and provides a theoretical basis for the study of bacterial enzyme-substrate interactions, metabolic pathways at the molecular level, and potential biological mechanisms. The genome circle plot of strain L-4 illustrated its genomic characteristics, encompassing gene distributions on both the forward and reverse strands, COG functional classification of genes, rRNA and tRNA distributions, as well as GC content, thereby facilitating a deeper understanding of its genetic information (Krzywinski et al., 2009). By utilizing Gene Ontology terminology (Ashburner et al., 2000), we annotated 3,956 genes within the L-4 genome. These genes were found to predominantly participate in a range of biological processes, including cellular metabolic processes, catalytic activity, binding capabilities, and transmembrane transport. In addition, in the cell component branch of strain L-4, the genes related to the overall composition of the cell membrane accounted for the largest proportion, followed by the organelles, reflecting the specific location of gene action. These data indicated that the protein functions of strain L-4 were primarily focused on cellular organization, catalysis, and metabolic processes, and had significant physiological effects on growth and ZEN biodegradation. The KEGG database is a comprehensive resource integrating genomic, chemical, and systems function information, and is the most extensively utilized database for metabolic pathways (Cao et al., 2019). In the KEGG analysis of the L-4 genome, a total of 2,637 genes were annotated, most of which are involved in metabolic processes. In a previous study by Qin et al. (2021) and Mwalilili et al. (2024) on *Bacillus* spp. using whole-genome sequencing, peroxidase and laccase enzymes were found to facilitate ZEN degradation.

We hypothesize that the degradation of ZEN by strain L-4 involves reduction and addition of relevant functional group bonds, as well as gene transcription and synthesis, which requires robust amino acid transport and metabolism. This highlights that strain L-4 requires vigorous metabolic activities and energy production, as evidenced by gene annotation (60 CDS) under the category “defense mechanism,” strain L-4 not only has vigorous growth and metabolic activities, but also has specific defense mechanisms and degradation capabilities.

These results indicated that strain L-4 had a strong metabolic transformation ability during ZEN degradation. It must be noted that bacterial chemotaxis enhances ZEN degradation and bioavailability, and strain L-4 typically responds to environmental pollutants by degrading them. By utilizing pollutants as nutrients or energy sources, strain L-4 exhibits active chemotaxis (Parales et al., 2015). Moreover, bacterial chemotaxis encompasses flagellar motility and signal transduction, and strain L-4 possesses well-developed flagellar assembly and chemotaxis-related genes, facilitating its orientation toward favorable conditions.

The genome scanning and sequencing analysis of strain L-4 can help to understand the functional genes related to degradation, and provide a theoretical basis for studying bacterial enzyme-substrate interactions, metabolic pathways at the molecular level, and underlying biological mechanisms. By comparing the sequence of the degrading enzymes, we predicted that the protein sequence efeB 3,668 was involved in ZEN degradation. In a previous study, Ding et al. (2024) cloned the PoDyP4 enzyme and identified its ability to convert to 15-OH-ZEN detoxification through oxidation and hydroxylation, similar to the degradation products observed in the present study. Subsequently, we predicted the subcellular localization, transmembrane regions, signal peptides, hydrophobicity, and secondary and tertiary structures of efeB 3,668, as well as performed molecular docking with ZEN, which revealed that efeB 3,668 was an effective ZEN-degrading enzyme. Moreover, the degradation of ZEN by *B. mojavensis* L-4 in liquid cultures and cornmeal was found to be significantly influenced by environmental conditions, including temperature, pH, inoculum size, and ZEN concentration. Thus, identification of optimal conditions and prediction of degradation proteins can provide valuable insights for developing effective strategies for the control of ZEN in corn.

In conclusion, this study successfully isolated a ZEN-degrading *B. mojavensis* L-4 strain from chicken intestine. Under optimal conditions of pH 7.0 and 37°C, strain L-4 completely converted 0.5–1.0 µg/mL ZEN to 15-OH-ZEN, a metabolite with reduced estrogenic toxicity, within a 24-h period. Furthermore, *B. mojavensis* L-4 achieved a notable degradation rate of 49.41% for up to 532.91 µg/kg ZEN in cornmeal. The predicted peroxidase secreted by strain L-4 (efeB 3,668) was noted to be primarily responsible for ZEN detoxification through hydrogen bonding and hydrophobic interactions. These findings indicated that *B. mojavensis* L-4 and its peroxidase had significant application potential for mitigating ZEN contamination in food and feed.

Data availability statement

The 16S rDNA sequence of strain L-4 was submitted to the National Center for Biotechnology Information (NCBI) with GenBank ID 649301.1.

Author contributions

JC: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. WD: Conceptualization, Data

curation, Formal analysis, Writing – review & editing, Validation. SG: Conceptualization, Data curation, Formal analysis, Writing – review & editing. LH: Conceptualization, Data curation, Formal analysis, Writing – review & editing. JD: Writing – review & editing. HQ: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. FC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

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References

- Almagro Armenteros, J. J., Tsirigos, K. D., Sønderby, C. K., Petersen, T. N., Winther, O., Brunak, S., et al. (2019). SignalP 5.0 improves signal peptide predictions using deep neural networks. *Nat. Biotechnol.* 37, 420–423. doi: 10.1038/s41587-019-0036-z
- Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., et al. (2000). Gene ontology: tool for the unification of biology. *Gene Ontol. Consortium. Nat Genet* 25, 25–29. doi: 10.1038/75556
- Cao, L., Zhang, J., Zhao, R., Deng, Y., Liu, J., Fu, W., et al. (2019). Genomic characterization, kinetics, and pathways of sulfamethazine biodegradation by *Paenarthrobacter* sp. A01. *Environ. Int.* 131:104961. doi: 10.1016/j.envint.2019.104961
- Chlebicz, A., and Śliżewska, K. (2020). In vitro detoxification of aflatoxin B (1), Deoxynivalenol, Fumonisin, T-2 toxin and Zearalenone by probiotic Bacteria from genus *Lactobacillus* and *Saccharomyces cerevisiae* yeast. *Probiotics Antimicrob Proteins* 12, 289–301. doi: 10.1007/s12602-018-9512-x
- Ding, S., Lin, C., Xiao, Q., Feng, F., Wang, J., Zhang, X., et al. (2024). Effective degradation of zearalenone by dye-decolorizing peroxidases from *Pleurotus ostreatus* and its metabolic pathway and toxicity analysis. *Sci. Total Environ.* 908:168500. doi: 10.1016/j.scitotenv.2023.168500
- Dong, W., Zhang, K., Wang, X., Li, J., Zou, H., Yuan, Y., et al. (2024). SIRT1 alleviates Cd nephrotoxicity through NF-κB/p65 deacetylation-mediated pyroptosis in rat renal tubular epithelial cells. *Sci. Total Environ.* 929:172392. doi: 10.1016/j.scitotenv.2024.172392
- Drzymala, S. S., Binder, J., Rosdehl, A., Penkert, M., Rosowski, M., Garbe, L. A., et al. (2015). Estrogenicity of novel phase I and phase II metabolites of zearalenone and cis-zearalenone. *Toxicol. Offic. J. Int. Society Toxicol.* 105, 10–12. doi: 10.1016/j.toxicol.2015.08.027
- El-Hachem, N., Haibe-Kains, B., Khalil, A., Kobeissy, F. H., and Nemer, G. (2017). AutoDock and AutoDockTools for protein-ligand docking: Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) as a case study. *Methods Mol. Biol.* 1598, 391–403. doi: 10.1007/978-1-4939-6952-4_20
- Fu, G., Ma, J., Wang, L., Yang, X., Liu, J., and Zhao, X. (2016). Effect of degradation of Zearalenone-contaminated feed by *Bacillus licheniformis* CK1 on Postweaning female piglets. *Toxins (Basel)* 8:300. doi: 10.3390/toxins8100300
- Gao, Z., Wu, H., Shi, L., Zhang, X., Sheng, R., Yin, F., et al. (2017). Study of *Bacillus subtilis* on growth performance, nutrition metabolism and intestinal microflora of 1 to 42 d broiler chickens. *Anim Nutr* 3, 109–113. doi: 10.1016/j.aninu.2017.02.002
- Gruber-Dorninger, C., Jenkins, T., and Schatzmayr, G. (2019). Global mycotoxin occurrence in feed: a ten-year survey. *Toxins (Basel)* 11:375. doi: 10.3390/toxins11070375
- Guo, Y., Tang, Y., Zhang, L., Liu, Y., Ma, Q., and Zhao, L. (2024). Enzymatic characterization and application of soybean hull peroxidase as an efficient and renewable biocatalyst for degradation of zearalenone. *Int. J. Biol. Macromol.* 260:129664. doi: 10.1016/j.ijbiomac.2024.129664
- Han, X., Huangfu, B., Xu, T., Xu, W., Asakiya, C., Huang, K., et al. (2022). Research Progress of safety of Zearalenone: a review. *Toxins (Basel)* 14:386. doi: 10.3390/toxins14060386
- Hildebrand, A. A., Pfeiffer, E., Damm, G., and Metzler, M. (2012). Combination of LC-MS2 and GC-MS as a tool to differentiate oxidative metabolites of Zearalenone with different chemical structures. *Int J Spectrosc.* 2012, 1–10. doi: 10.1155/2012/472031
- Ji, J., Yu, J., Xu, W., Zheng, Y., Zhang, Y., and Sun, X. (2022). Isolation and mechanistic characterization of a novel Zearalenone-degrading enzyme. *Foods (Basel, Switzerland)* 11:2908. doi: 10.3390/foods11182908
- Keller, L., Abrunhosa, L., Keller, K., Rosa, C. A., Cavaglieri, L., and Venâncio, A. (2015). Zearalenone and its derivatives α -Zearalenol and β -Zearalenol decontamination by *Saccharomyces cerevisiae* strains isolated from bovine forage. *Toxins (Basel)* 7, 3297–3308. doi: 10.3390/toxins7083297
- Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., et al. (2009). Circo: an information aesthetic for comparative genomics. *Genome Res.* 19, 1639–1645. doi: 10.1101/gr.092759.109
- Lau, W. Y. V., Hoad, G. R., Jin, V., Winsor, G. L., Madyan, A., Gray, K. L., et al. (2021). PSORTdb 4.0: expanded and redesigned bacterial and archaeal protein subcellular localization database incorporating new secondary localizations. *Nucleic Acids Res.* 49, D803–d808. doi: 10.1093/nar/gkaa1095
- Medeiros, S., Xie, J., Dyce, P. W., Cai, H. Y., DeLange, K., Zhang, H., et al. (2018). Isolation of bacteria from fermented food and grass carp intestine and their efficiencies in improving nutrient value of soybean meal in solid state fermentation. *J Anim Sci Biotechnol* 9:29. doi: 10.1186/s40104-018-0245-1
- Møller, C. O. A., Freire, L., Rosim, R. E., Margalho, L. P., Balthazar, C. F., Franco, L. T., et al. (2021). Effect of lactic acid Bacteria strains on the growth and aflatoxin production potential of *aspergillus parasiticus*, and their ability to bind aflatoxin B (1), Ochratoxin a, and Zearalenone in vitro. *Front. Microbiol.* 12:655386. doi: 10.3389/fmicb.2021.655386
- Murtaza, B., Li, X., Dong, L., Javed, M. T., Xu, L., Saleemi, M. K., et al. (2022). Microbial and enzymatic battle with food contaminant zearalenone (ZEN). *Appl. Microbiol. Biotechnol.* 106, 4353–4365. doi: 10.1007/s00253-022-12009-7
- Mwabulili, F., Xie, Y., Sun, S., Ma, W., Li, Q., Yang, Y., et al. (2024). Thermo-alkali-tolerant recombinant laccase from *Bacillus swazeyi* and its degradation potential against Zearalenone and aflatoxin B (1). *J. Agric. Food Chem.* 72, 13371–13381. doi: 10.1021/acs.jafc.4c01304
- Parales, R. E., Luu, R. A., Hughes, J. G., and Ditty, J. L. (2015). Bacterial chemotaxis to xenobiotic chemicals and naturally-occurring analogs. *Curr. Opin. Biotechnol.* 33, 318–326. doi: 10.1016/j.copbio.2015.03.017
- Qin, X., Su, X., Tu, T., Zhang, J., Wang, X., Wang, Y., et al. (2021). Enzymatic degradation of multiple major mycotoxins by dye-decolorizing peroxidase from *Bacillus subtilis*. *Toxins (Basel)* 13:429. doi: 10.3390/toxins13060429
- Shanakhat, H., Sorrentino, A., Raiola, A., Romano, A., Masi, P., and Cavella, S. (2018). Current methods for mycotoxins analysis and innovative strategies for their

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reduction in cereals: an overview. *J. Sci. Food Agric.* 98, 4003–4013. doi: 10.1002/jsfa.8933

She, R., Chu, J. S., Wang, K., Pei, J., and Chen, N. (2009). GenBlastA: enabling BLAST to identify homologous gene sequences. *Genome Res.* 19, 143–149. doi: 10.1101/gr.082081.108

Sun, Z., You, Y., Xu, H., You, Y., He, W., Wang, Z., et al. (2024). Food-grade expression of two laccases in *Pichia pastoris* and study on their enzymatic degradation characteristics for mycotoxins. *J. Agric. Food Chem.* doi: 10.1021/acs.jafc.4c00521

Tan, H., Zhou, H., Guo, T., Zhou, Y., Zhang, Y., Yuan, R., et al. (2023). Emerging Zein-bound Zearalenone in maize: thermal-induced mechanism of binding or releasing. *J. Agric. Food Chem.* 71, 20336–20347. doi: 10.1021/acs.jafc.3c05794

Taş, N., de Jong, A. E., Li, Y., Trubl, G., Xue, Y., and Dove, N. C. (2021). Metagenomic tools in microbial ecology research. *Curr. Opin. Biotechnol.* 67, 184–191. doi: 10.1016/j.copbio.2021.01.019

Wang, L., Wang, J., Wang, J., Zhu, L., Yang, L., and Yang, R. (2019). Distribution characteristics of antibiotic resistant bacteria and genes in fresh and composted manures of livestock farms. *Sci. Total Environ.* 695:133781. doi: 10.1016/j.scitotenv.2019.133781

Xu, D., and Côté, J. C. (2003). Phylogenetic relationships between *Bacillus* species and related genera inferred from comparison of 3' end 16S rDNA and 5' end 16S-23S ITS nucleotide sequences. *Int. J. Syst. Evol. Microbiol.* 53, 695–704. doi: 10.1099/ijs.0.02346-0

Xu, L., Sun, X., Wan, X., Li, H., Yan, F., Han, R., et al. (2020). Identification of a *Bacillus amyloliquefaciens* H6 Thioesterase involved in Zearalenone detoxification by

transcriptomic analysis. *J. Agric. Food Chem.* 68, 10071–10080. doi: 10.1021/acs.jafc.0c03954

Yang, K., Li, K., Pan, L., Luo, X., Xing, J., Wang, J., et al. (2020). Effect of ozone and Electron beam irradiation on degradation of Zearalenone and Ochratoxin a. *Toxins (Basel)* 12:138. doi: 10.3390/toxins12020138

Zhang, Z., Xu, W., Wu, H., Zhang, W., and Mu, W. (2020). Identification of a potent enzyme for the detoxification of Zearalenone. *J. Agric. Food Chem.* 68, 376–383. doi: 10.1021/acs.jafc.9b06223

Zhao, L., Zhang, L., Xu, Z., Liu, X., Chen, L., Dai, J., et al. (2021). Occurrence of aflatoxin B (1), deoxynivalenol and zearalenone in feeds in China during 2018–2020. *J. Anim Sci Biotechnol* 12:74. doi: 10.1186/s40104-021-00603-0

Zheng, W., Wang, B., Li, X., Wang, T., Zou, H., Gu, J., et al. (2018). Zearalenone promotes cell proliferation or causes cell death? *Toxins (Basel)* 10:184. doi: 10.3390/toxins10050184

Zhou, Y., Yang, J., Yu, Y., and Tang, Y. (2024). A novel glycosyltransferase from *Bacillus subtilis* achieves zearalenone detoxification by diglycosylation modification. *Food Funct.* 15, 6042–6053. doi: 10.1039/d4fo00872c

Zhu, F., Zhang, B., and Zhu, L. (2021). An up-converting phosphor technology-based lateral flow assay for rapid detection of major mycotoxins in feed: comparison with enzyme-linked immunosorbent assay and high-performance liquid chromatography-tandem mass spectrometry. *PLoS One* 16:e0250250. doi: 10.1371/journal.pone.0250250



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Analysis of fungal diversity in processed jujube products and the production of mycotoxins by typical toxigenic *Aspergillus* spp.

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Processed jujube products are susceptible to contamination by fungi such as *Aspergillus* spp., which produces mycotoxins that could lead to health problems in consumers. In this study, 58 samples of processed jujube products (including 5 types such as dried jujubes) were collected from different markets in Shihezi (Xinjiang, China). The fungal diversity and the fungi isolated from processed jujube products were systematically analyzed through high-throughput sequencing and molecular biological identification (based on the ITS and/or *BenA* and *CaM* regions). In total, the 105 strains of fungi were isolated and identified as belonging to the dominant genera were *Aspergillus*, *Cladosporium*, *Alternaria*, and *Penicillium*. High-throughput sequencing indicated that *Alternaria*, *Didymella*, *Cladosporium*, and *Aspergillus* were the dominant fungi in processed jujube products. ELISA showed that *A. flavus* produced about 19.3862–21.7583 µg/L, 6.5309–11.0411 µg/L, 0–15.4407 µg/L, 0–5.6354 µg/L, and 0–6.0545 µg/L of AFT, AFB₁, AFB₂, AFM₁, and AFM₂, respectively. In addition, concentrations of OTA produced by *A. niger*, *A. tubingensis*, and *A. ochraceus* were found to range from 5.2019 to 18.5207 µg/L. Therefore, the separation of *Aspergillus* with good mycotoxin-producing abilities from processed jujube products poses a latent threat to consumer health.

KEYWORDS

processed jujube products, fungi, mycotoxins, aflatoxin, ochratoxin

1 Introduction

Jujube (*Zizyphus jujuba* Mill.), belongs to the Rhamnaceae family and has been cultivated for 2000 years in Xinjiang province and more than 4,000 years in China overall (Aafi et al., 2022; Yuan et al., 2019; Cheng et al., 2020). China is the largest producer of jujube and accounts for more than 90% of the world's jujube production (Liu et al., 2022). Due to their high water content, fresh jujubes are perishable and susceptible to fungal contamination during harvesting, processing, transportation, and storage (Zhang et al., 2022). The common fungal genera that contaminate jujubes are *Aspergillus*, *Alternaria*, *Penicillium*, and *Fusarium*, among others (Wu et al., 2017; Bertuzzi et al., 2015; Zhou et al., 2021). Xu et al. (2023) investigated that the main fungi present in decaying red jujubes were *Fusarium* and *Alternaria*. Meanwhile, Xin et al. (2023) reported that red jujubes were particularly susceptible to contamination with *A. flavus*, *A. ochraceus*, *A. niger*, and *Alternaria*. In addition to directly causing pathological

effects in plants, fungal contamination also leads to the production of mycotoxins (Kebede et al., 2020; James and Zikankuba, 2018). Furthermore, fungi and mycotoxins from the raw materials could enter the product (Schabo et al., 2021). Therefore, mycotoxin contamination is a major safety concern among consumers who purchase processed jujube products (Mukhtar et al., 2023).

Most of the jujube produced is processed by drying and frying to reduce its moisture content and prolong its shelf life (Bi and Chen, 2016). However, mycotoxin production is not easily degraded without special treatment, and processing could not completely remove mycotoxins (Drusch and Ragab, 2003). Given that processed jujube products are typically sold in a ready-to-eat form, they could lead to mycotoxin exposure if they contain mycotoxins, causing adverse effects on human health. Notably, mycotoxins are toxic secondary metabolites produced by fungi during their growth and reproduction (Yang et al., 2020; Janik et al., 2020). Common mycotoxins include aflatoxins, ochratoxins and *Alternaria* toxins. In particular, AFT (Aflatoxin) and ochratoxins are currently considered the most threatening of all mycotoxins (González-Curbelo and Kabak, 2023). Azaiez et al. (2015) showed that the 83% of all jujube samples were found to be contaminated with at least one mycotoxin, of which 23% contained aflatoxin and 22% ochratoxin A. Iqbal et al. (2014) found that the 31.6% of the jujube products were contaminated by aflatoxin, of which 16 samples exceeded the AFB₁ (aflatoxin B₁) content, and 20 samples exceeded the total aflatoxin content. Specifically, mycotoxins are known to have a negative impact on human health, exerting adverse effects such as hepatotoxicity, genotoxicity, and mutagenicity (Stoev, 2021; Cao et al., 2022; Mujahid et al., 2020; Puntischer et al., 2018).

In this context, the aim of the present study was to: (a) isolate representative toxin-producing fungi from samples of processed jujube products sold in the Shihezi (Xinjiang, China) wholesale markets; (b) identify and analyze the fungal species present in processed jujube products at the molecular level and to analyze the diversity of fungi in processed jujube products; and (c) analyze the metabolic features of typical toxin-producing *Aspergillus* strains (*A. flavus*, *A. niger*, *A. tubingensis*, and *A. ochraceus*) and identify typical secondary fungal metabolites that might be present in processed jujube products.

2 Materials and methods

2.1 Sample

From April to May 2023, a total of 58 processed jujube product samples [5 samples of dried jujube samples (ZG), 17 samples of jujube kernel cake samples (ZRG), 5 samples of crispy jujube samples (CZ), 19 samples of milk jujube samples (NZ), and 12 samples of jujube with walnut samples (ZJHT)] were collected from different markets in Shihezi City (Xinjiang, China). The average weight of each sample was more than 500 g. Among the processed jujube products, the ZG and the CZ were packed in bulk, and the ZRG, the ZJHT, and the NZ were vacuum packed. The sampling methods utilized ensured representative sampling of a wide range of markets in the region. The collected samples were sealed (relative humidity 39%) and stored in a refrigerator at −20°C to prevent fungal growth and metabolite production. After analysis and data evaluation, all samples were treated appropriately.

2.2 Chemicals and reagents

Potato dextrose water (PDW) medium, Bengal red agar medium, potato dextrose agar (PDA) medium, toxin-producing medium, Tris-HCl, ethylenediaminetetraacetic acid, and boric acid were purchased from Qingdao HaiBo Biotech Co., Ltd. (Shandong, China). The Biospin fungal genomic DNA extraction kit was purchased from Hangzhou Bori Technology Co., Ltd. (Zhejiang, China). The 6 × loading buffer, dNTPs, 10 × buffer, Tap DNA polymerase, DNA maker, and Gold View nucleic acid dyes were purchased from Tiangen Biochemical Technology Co., Ltd. (Beijing, China). Agarose was purchased from Biowest (Spain); *A. flavus*, *A. niger*, *A. tubingensis*, and *A. ochraceus* toxin-producing genes primers were purchased from Sangon Bioengineering Co., Ltd. (Shanghai, China). Enzyme-linked immunosorbent assays (ELISA) detection kits for the AFT, AFB₁, AFB₂ (aflatoxin B₂), AFM₁ (aflatoxin M₁), AFM₂ (aflatoxin M₂), and OTA (ochratoxin A) were purchased from Jiangsu Jingmei Biotechnology Co., Ltd. (Jiangsu, China).

2.3 Determination and analysis of fungal diversity

The 58 samples of processed jujube products were sent to Shanghai Meiji Biopharmaceutical Technology Co., Ltd. for high-throughput sequencing in order to analyze their fungal diversity, with each sample tested in six replicates. Sequencing yielded double-terminal paired-end (PE) reads. Firstly, the double-terminal PE reads were assembled into PE double-terminal sequences using FLASH version 1.2.11¹ while simultaneously ensuring quality control and filtering sequences that do not meet quality criteria. Based on a 97% threshold of sequence similarity, the sequences were clustered into operational taxonomic units (OTUs) using UPARSE (<http://www.drive5.com/uparse/>; dnt version 11). In addition, the tool may also be utilized for identifying and removing chimeric sequences. Each representative OTU sequence was taxonomically annotated based on the ITS database (<http://unite.ut.ee/index.php> dome Unite 8.0) using RDP Classifier (<https://sourceforge.net/projects/rdp-classifier/>, version 2.13) and a 0.7 confidence threshold. Taxonomic classifications at the phylum, class, order, family, genus, and species levels were analyzed and plotted with QIIME (<http://qiime.org/install/index.html>, version 1.9.1). R software (version 3.3.1) was used to run dilution curve analyses, Venn diagram analyses, and community composition analyses (bar and pie diagrams). This software was also used to plot a heat map of the relationship between dominant fungal genera and fruit quality (package pheatmap 1.0.8), as well as to conduct principal coordinate analysis (PCoA) and statistical analysis.

2.4 Determination of the total number of fungal colonies, water activity, and pH of processed jujube products

A total of 58 processed jujube product samples were collected. Each sample was placed in aseptic saline and homogenized to obtain a homogenized solution. This solution was subjected to serial

¹ <https://ccb.jhu.edu/software/FLASH/index.Shtml>

dilutions. Approximately 2–3 suitable gradients were selected. Then, the fungal solution was added to the PDA medium, mixed, and cultured (Ma, 2018). Three parallel experiments were performed for each dilution gradient.

Firstly, the sample from each category were obtained. Then, fresh distilled water was added to a beaker containing the samples and heated in a water bath at 50°C for 30 min. The samples were homogenized until the sample solution became uniform. After that, the pH value of the processed jujube products was subsequently measured (Shen and Hui, 1989). For water activity analysis, each sample was placed into a sample dish. After the instrument was fully balanced, the response value was recorded using a water activity meter (Institute HQaTSaI, 2016). Three parallel measurements were obtained for each type of product.

2.5 Fungal isolation

Each sample was randomly weighed (50 g, accounting for 10% of the total sample). The samples were cultured in a PDW medium at 28°C for 16 h, and the non-sample PDW medium was set as the control. About 1,000 µL of the enrichment solution and the blank control were pipetted onto the Bengal red agar medium, spread evenly with a sterile spreading rod, and incubated in a constant-temperature biochemical incubator at 28°C for 5 days. Colonies in each sample plate were observed, purified into a single colony using the plate marking method, and subsequently cultured (Guo and Ji, 2021).

2.6 Fungal identification

Fungal tissue from the isolated and purified single colonies was ground into fine powder in liquid nitrogen. According to the instructions, the Biospin fungal genomic DNA extraction kit was used to extract genomic DNA. Internal transcribed spacer region (ITS) and/or β -microtubulin (*BenA*) and calmodulin (*CaM*) gene sequences were used to analyze the phylogeny of *Aspergillus* (Susca et al., 2020; Deng et al., 2021; Guo et al., 2016). The PCR detection primers are indicated in Supplementary Table S1. Fungal characteristics and genera and species classifications were determined by PCR amplification and sequencing. The PCR reaction system (with a final volume of 25 µL) consisted of 12.5 µL PCR TaqMix (A solution composed of 6 × loading buffer, dNTPs, Tap DNA polymerase and other components), 1 µL primer 1, 1 µL primer 2, 0.5 µL genomic DNA, and 10 µL ddH₂O. The PCR amplification program was performed under the following conditions: pre-denaturation (94°C, 4 min); 30 cycles of denaturation (94°C, 30s), annealing (55°C, 1 min), and elongation (72°C, 1 min); and the final extension (72°C, 10 min) (Hu et al., 2022; Okayo et al., 2020). The PCR products were electrophoresed with 1% agarose gel in 1 × TBE electrophoresis buffer (A buffer solution composed of Tris-HCl, ethylenediaminetetraacetic acid, and boric acid). Latterly, the electrophoresis results were detected using the gel imaging system to determine whether the amplification was successful. Subsequently, the PCR products were subsequently sent to Youkang Bioengineering (Xinjiang) Co., Ltd. for sequencing. The consistency sequence was checked by the View Sequencer file (Trace Editor) from MEGA 7.0 software, and the Basic Local Alignment Search Tool (BLAST) was used for comparison and analysis. Phylogenetic analysis also was carried out using MEGA 7.0 software. After linked sequence alignment,

nucleotide gaps and missing data were deleted. Each phylogenetic tree was built using the neighbor connection method.

2.7 Detection of the toxin-producing genes of typical *Aspergillus* in processed jujube products

The four typical toxigenic *Aspergillus* (*A. flavus*, *A. niger*, *A. tubingensis*, and *A. ochraceus*) were isolated from processed jujube products. The aflatoxin-producing and ochratoxin A-producing genes from NCBI-GenBank were selected. The seven aflatoxin-producing genes (*aflD*, *aflP*, *alfQ*, *aflR*, *aflS*, *omtA*, *ver*) and seven ochratoxin A-producing genes (*AcLaeA*, *otaA*, *otaB*, *otaC*, *otaD*, *pks*, *nrps*) of the obtained isolates were detected using PCR (Akinola et al., 2019; Więckowska et al., 2023; Maor et al., 2021; Quaglia et al., 2020). The primers targeting the toxin-producing genes used for PCR analysis are shown in Supplementary Table S2. The *BenA* gene sequence served as a positive control for the genome and PCR conditions. Based on the amplification of the above mentioned toxin-producing genes, it was determined whether a strain produced a particular toxin. If the amplification results were negative, the strain was considered to not produce the toxin. If the amplification results were positive, it indicated the presence of toxin-producing genes in the strain, but it was still necessary to use the ELISA to further analyze whether these strains produce toxins.

2.8 Cultivation and toxin detection of typical toxigenic *Aspergillus*

The toxin-producing *A. flavus*, *A. niger*, *A. tubingensis*, and *A. ochraceus* strains were isolated. From each of above strain cultures, three mycelial plug (3 mm diameter) was taken to inoculate into 50 mL of toxin-producing medium. After 10 days of incubation in the dark, on an orbital shaker at 130 r/min and at 22 ± 2°C, each fungal culture was filtered through sterile filter paper to separate mycelia and culture filtrate. Subsequently, the culture filtrate was centrifuged at 8000 r/min, the supernatant was taken, and stored at −80°C (Okayo et al., 2020). Employing the ELISA method to measure the contents of AFT, AFB₁, AFB₂, AFM₁, AFM₂, and OTA in the supernatant from single-strain culture filtrate. The toxin content in each sample was measured in triplicate. The linear range of AFT is 0.1–25 µg/L, and the linear range of AFB₁, AFB₂, AFM₁, AFM₂, and OTA is 0.1–80 µg/L. The linear relationship is known to be well-characterized in this range ($R^2 > 0.997$, the average recovery rate: 96.36–108.08%).

2.9 Statistical data analysis

The obtained sequences were compared using BLAST in the NCBI database. Gene sequences with higher homology were selected, and MEGA 7.0 software was used to construct a phylogenetic tree using the neighbor-joining method. Mycotoxin content was analyzed by Microsoft Excel 2020 software. The sample group significance of water activity, pH value, and total colony count was analyzed by SPSS 20.

3 Results

3.1 Total number of fungal colonies, water activity, and pH of processed jujube products

The total number of fungal colonies, water activity, and pH value of the processed jujube products are displayed in [Tables 1, 2](#). The range of the logarithmic total colony counts for commercially available processed jujube products is from 1.26 ± 0.19 – 2.01 ± 0.053 log cfu/g. Particularly, the sample in ZJHT had the highest colony count (2.01 ± 0.053 log CFU/g). In general, the standard for the total number of fungal colonies in processed jujube products is not to exceed 150 CFU/g (The logarithmic value – 2.17) ([Association HFI, 2020](#)). The above results showed that all samples were contaminated with microorganisms, but all of them were below the standard requirements for date products mentioned above.

In the product samples, the pH value ranges from 4 to 6. This result indicates that the product samples are weakly acidic, which is suitable for the growth of most fungi. The water activity ranges from 0.30 to 0.65, with the CZ sample being the lowest (0.308 ± 0.0006) and the NZ sample being the highest (0.631 ± 0.0020). It is worth paying attention that although the ZG and CZ samples are bulk, they have lower water activity, resulting in fewer isolated and purified fungi (1.56 log CFU/g and 1.26 log CFU/g, respectively). In contrast, the ZRG and ZJHT samples have higher water activity and therefore contain more fungi (1.77 log CFU/g and 2.01 log CFU/g, respectively). From the above results, it could be concluded that high water activity is more favorable for fungal growth. The above findings are similar to the results obtained from the colony counts.

TABLE 1 The total number of fungal colonies in different processed jujube products.

Product name	Mean fungal colony counts (log CFU/g) $\pm$ S.D.	Range (CFU/g)
ZG	1.560 ± 0.076^d	26–46
ZRG	1.770 ± 0.064^b	36–78
CZ	1.260 ± 0.190^e	9–37
NZ	1.750 ± 0.098^c	30–83
ZJHT	2.010 ± 0.053^a	77–129

S.D., standard deviation; ZG represents dried jujube; ZRG represents jujube kernel cake; CZ represents crisp jujube; NZ represents milk date; ZJHT represents jujube with walnut; ^{abcde} represents different significance, $p < 0.05$.

TABLE 2 The water activity and pH value in different processed jujube products.

Product name	Water activity $\pm$ S.D.	Range	pH value $\pm$ S.D.	Range
ZG	0.3060 ± 0.0010^e	0.3020–0.3090	5.460 ± 0.025^b	5.430–5.520
ZRG	0.5350 ± 0.0015^c	0.5310–0.5390	5.230 ± 0.010^c	5.210–5.260
CZ	0.3080 ± 0.0006^d	0.3050–0.3100	5.620 ± 0.015^a	5.590–5.650
NZ	0.6310 ± 0.0020^a	0.6260–0.6360	5.270 ± 0.025^c	5.230–5.340
ZJHT	0.6130 ± 0.0012^b	0.6110–0.6160	4.480 ± 0.012^d	4.460–4.520

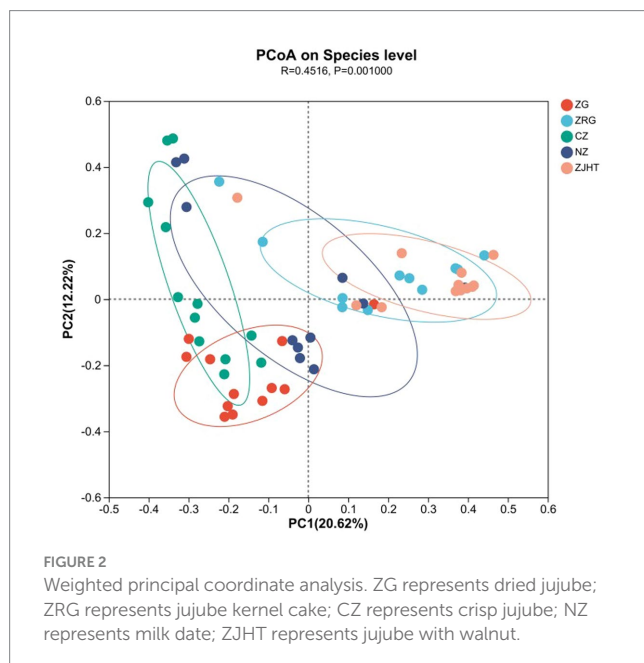
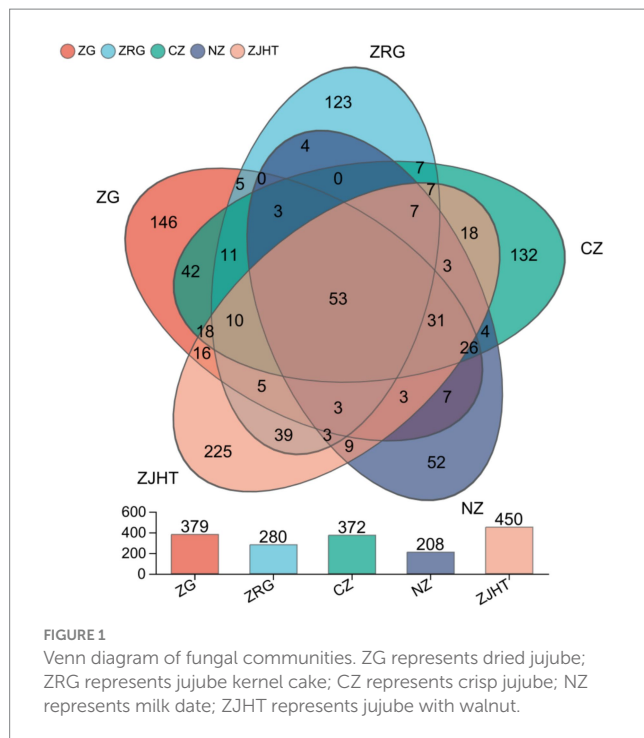
S.D., standard deviation; ZG represents dried jujube; ZRG represents jujube kernel cake; CZ represents crisp jujube; NZ represents milk date; ZJHT represents jujube with walnut; ^{abcde} represents different significance, $p < 0.05$.

3.2 Fungal diversity analysis of processed jujube products

High-throughput sequencing was used for the analysis of fungal diversity in processed jujube products samples. A total of 4,645,507 reads were obtained from the 58 product samples, and the effective fungal sequence length was found to be 211–300 bp. The optimized fungal sequences were clustered into OTUs based on a similarity threshold of 97%, and 1,012 OTUs in total were obtained. The number of OTUs in each sample was found to be between 208 and 450. The coverage rate of the samples was determined to be greater than 99%, indicating that the current sequencing volume provided an appropriate representation of the composition of the fungal communities present in processed jujube products.

In the samples of processed jujube products, all fungal OTUs could be divided into 11 phyla, 37 classes, 85 orders, 206 families, and 392 genera. The results are shown in [Figure 1](#). Among the 1,012 OTUs, 53 OTUs were found to be common across all samples and were considered the core fungal microorganisms of processed jujube product samples. The number of unique fungal OTUs for the ZG, the ZRG, the CZ, the NZ, and the ZJHT were 146, 123, 132, 52, and 225, respectively. Based on the results, it could be analyzed that the fungal community in the ZJHT exhibits greater diversity. Further analysis revealed that the 53 core fungal groups belonged to the *Ascomycota* and *Basidiomycota* phyla mainly comprising *Didymella* (20.59%), *Alternaria* (14.96%), *Cladosporium* (10.71%), and *Aspergillus* (7.39%) ([Supplementary Figure S3](#)).

Methods such as the weighted PCoA were employed to compare community structures across different jujube products. As illustrated in [Figure 2](#), based on the Bray-Curtis distances in PCoA, the sum of the horizontal and vertical coordinates was 32.84% (20.62% + 12.22%), reflecting a complex fungal community structure that would be difficult to characterize using two principal coordinates (PCs). There were extremely significant differences in the composition of fungal communities among the various samples ($p < 0.01$). It is noteworthy that there was a highly significant difference ($p < 0.01$) in the fungal community composition of the ZRG, CZ, and ZJHT. Similarly, there was a highly significant difference in fungal flora composition between ZG and ZJHT ($p < 0.01$). Meanwhile, significant differences in fungal flora composition were found between ZRG and ZJHT ($p < 0.01$). The CZ samples were observed to be distant in the PC2 direction, indicating the influence of PC2 on the fungal composition of this product. Based on the discrete distribution of sample points, it was also seen that the fungal composition of the CZ was quite different.



3.3 Analysis of fungal community structure

As depicted in Figure 3, taxonomical classification demonstrated the presence of two fungal phyla in all samples, namely, *Ascomycota* and *Basidiomycota*. *Ascomycota* was the most dominant phylum, accounting for 53.85 to 87.23% of the fungi in all samples. Among all samples, the ZJHT sample has the highest proportion of *Ascomycota* (87.23%). It is worth noting that the ZG sample has the lowest percentage of *Ascomycota* (53.85%), but its *Basidiomycota* proportion (45.50%) is the highest, roughly 4 times that of the ZRG (45.50/10.98) and the ZJHT (45.50/11.67). As reflected in Figure 4, the dominant

fungal genera present in product samples such as *Didymella*, *Alternaria*, *Cladosporium*, *Aspergillus*, and *Zygosaccharomyces*, all belonged to the *Ascomycota* phylum. Hence, the dominant fungal genera were consistent with the dominant fungal phyla.

As noted in Figure 5, *Didymella*, *Alternaria*, *Cladosporium*, and *Aspergillus* were detected in all samples with relatively high proportions, indicating they are the dominant fungal strains in the samples. Among all samples, the CZ sample had the highest relative abundance of *Didymella* and *Cladosporium*, the ZJHT sample had the highest relative abundance of *Alternaria*, and the ZRG sample had the highest relative abundance of *Aspergillus*. Additionally, it is important to note that *Fusarium* and *Penicillium* were primarily found in the ZJHT sample, *Candida* was mainly present in the CZ sample, and *Piskurozyma* was predominantly identified in the ZG sample. As shown in Figure 6, the fungal strains that were identified, such as *Cladosporium delicatulum*, *A. minisclerotigenes*, *Fusarium concentricum*, etc. *Alternaria* and *Aspergillus*, were only able to be identified at the genus level, and not species. It is notable that *Zygosaccharomyces* was detected exclusively in the NZ sample, while *Piskurozyma capsuligena* was found only in the ZG sample.

Integrating the aforementioned findings, it could be concluded that *Alternaria*, *Cladosporium*, and *Aspergillus* are the predominant fungal genera in the product samples. Additionally, fungi known to produce mycotoxins, such as *Alternaria* and *Aspergillus*, were detected in most samples, which could potentially affect the quality of processed jujube products.

3.4 Isolation and identification of fungi in processed jujube products

A total of 105 fungal strains were isolated from the processed jujube product samples. The molecular identification results of the 105 strains are noted in Supplementary Table S3, and the phylogenetic trees of *Aspergillus* and *Penicillium* are depicted in Supplementary Figures S1, S2.

The number and proportion of fungi across the different processed jujube products are displayed in Table 3. The comprehensive analysis in conjunction with Table 2 indicated that the ZJHT with higher water activity had a higher number of isolated strains (35, 33.33%). In contrast, the CZ with lower water activity also had a considerable number of isolated strains (13, 12.38%). Interestingly, despite the higher water activity of the NZ, the number of isolated strains was relatively low (17, 16.19%).

The species and quantity of fungi isolated from the processed jujube products are illustrated in Figure 7. In the study of processed jujube product samples, a total of 105 fungal strains were isolated and identified. As noted in Table 3, among all the isolated strains, there were 17 strains of the *Penicillium*. Notably, the ZRG had the highest isolation rate of *Penicillium* (5.71%, 6/105), followed by the ZJHT (4.76%, 5/105). Among these *Penicillium*, *P. olsonii*, *P. crustosum*, and *P. raistrickii* were the main species, along with other types such as *P. dierckxii*, *P. oxalicum*, *P. rubens*, *P. terrigenum*, *P. sajarovii*, *P. citrinum*, and *P. expansum*. Furthermore, the study also isolated and identified 66 strains of the *Aspergillus*. Among all the product samples, the proportion of *Aspergillus* isolated from ZRG was the highest (16.19%, 17/105), followed by the ZJHT (24.76%, 26/105). Among these *Aspergillus*, *A. fumigatus*, *A. flavus*, *A. oryzae*, *A. niger*, and *A. tubingensis* were the main species, along with other types such as

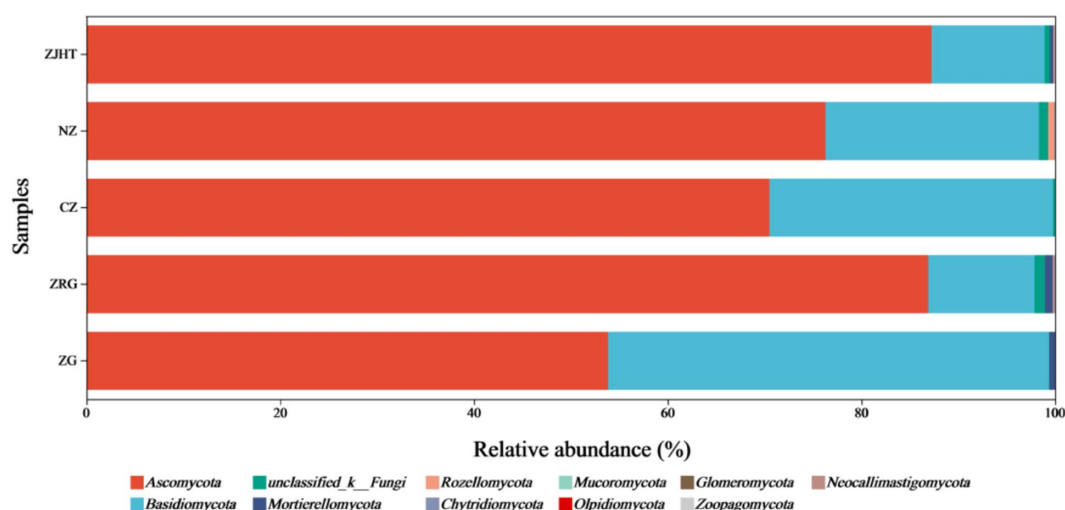


FIGURE 3

Relative abundance of fungi at the phylum level. ZG represents dried jujube; ZRG represents jujube kernel cake; CZ represents crisp jujube; NZ represents milk date; ZJHT represents jujube with walnut.

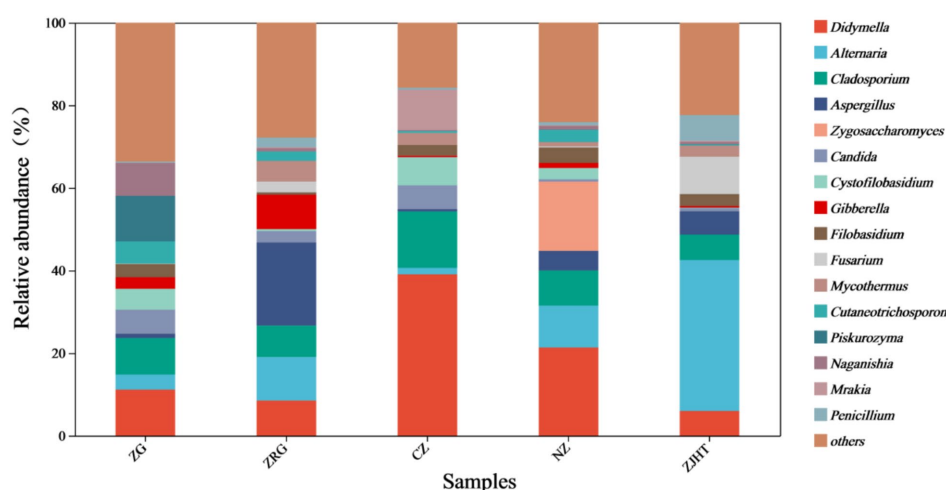


FIGURE 4

Relative abundance of fungi at the genus level. ZG represents dried jujube; ZRG represents jujube kernel cake; CZ represents crisp jujube; NZ represents milk date; ZJHT represents jujube with walnut.

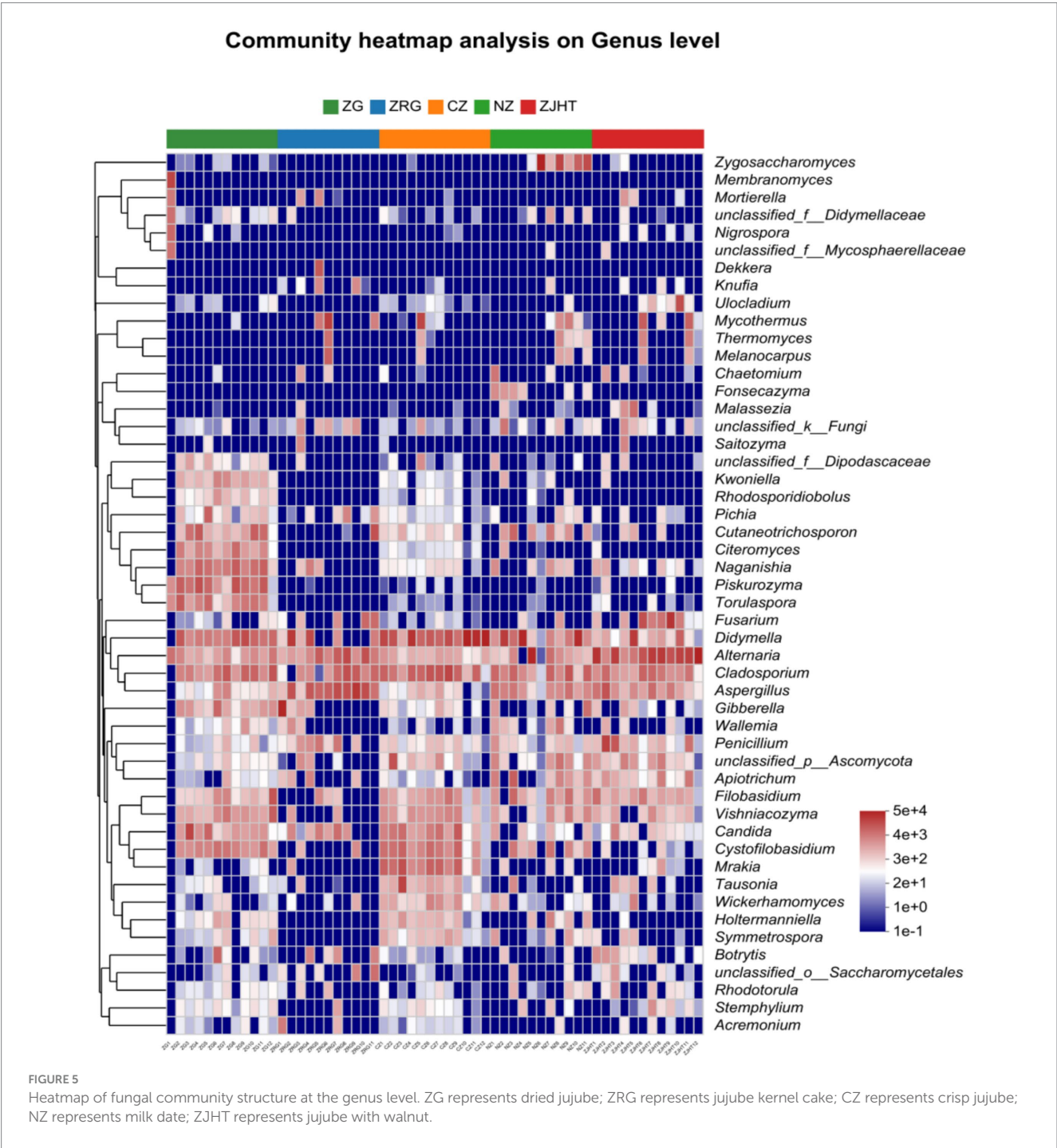
A. ochraceus, *A. sydowii*, and *A. westerdijkiae*. Furthermore, in the samples of NZ, ZRG, ZJHT, and CZ, the 5 strains of *Alternaria* were successfully isolated. At the same time, the 9 strains of *Talaromyces* were isolated from the CZ, the NZ, and the ZG, as well as the 8 strains of *Cladosporium* were primarily sourced from the CZ and the NZ.

3.5 Detection results of toxin-producing genes of typical *Aspergillus* spp. in processed jujube products

The results derived from the PCR amplification analysis of the aflatoxin-producing genes of *A. flavus* are shown in Table 4. The seven aflatoxin-producing genes of *A. flavus* tested were *alfQ* (57.14%), *alfP* (52.38%), *aflR* (47.62%), *aflD* (47.62%), *omtA* (42.86%), *ver* (38.10%),

and *alfS* (33.33%). In the product samples, a total of 21 *A. flavus* strains were isolated. Especially, five of these strains carried seven aflatoxin-producing genes, and nine strains carried four or more aflatoxin-producing genes. A total of 14 *A. flavus* strains were found to be positive for aflatoxin-producing genes, while the others were negative for the expression of these genes.

The results of the PCR amplification analysis of the ochratoxin A-producing genes of *A. niger*, *A. tubingensis* and *A. ochraceus* are indicated in Table 5. The seven ochratoxin A-producing genes of *A. niger*, *A. tubingensis*, and *A. ochraceus* tested were *pks* (61.90%), *nrps* (57.14%), *otaA* (52.38%), *otaD* (52.38%), *otaB* (47.62%), *AcLaeA* (28.57%), and *otaC* (28.57%). A total of 21 strains (7 *A. niger*, 13 *A. tubingensis*, and 1 *A. ochraceus*) were isolated from the samples. It is very noteworthy that three of these strains carried all 7 ochratoxin A-producing genes and 11 strains carried 4 or more ochratoxin



A-producing genes. In total, the 13 strains were positive for the expression of ochratoxin A-producing genes, while the others were negative for their expression. Detailed toxin production traits in these fungi need to be verified through further experiments.

3.6 Production of mycotoxins by typical *Aspergillus* spp. in processed jujube products

As shown in Table 6, a total of 21 strains of *A. flavus* were isolated from all product samples. It notably showed that 10 of these strains were

capable of producing aflatoxin. Among them, we found that only one strain produced AFB₁, but two strains produced both AFB₁ and AFB₂. Among these 10 strains, the levels of AFT, AFB₁, AFB₂, AFM₁, and AFM₂ were in the ranges of 19.3862–21.7583 µg/L, 6.5309–11.0411 µg/L, 0–15.4407 µg/L, 0–5.6354 µg/L, 0–6.0545 µg/L, with the average ranges of 20.1449 µg/L, 8.8021 µg/L, 9.8160 µg/L, 3.1780 µg/L and 3.5710 µg/L, respectively. It could be concluded that *A. flavus* strains in processed jujube products have the strongest ability to produce AFB₁ toxin. As shown in Table 7, the 7 strains of *A. niger*, 13 strains of *A. tubingensis*, and 1 strain of *A. ochraceus* were isolated from processed jujube products, of which 8 strains produced OTA. OTA content ranged from 5.2019 to 18.5207 µg/L, with a mean value of 8.3345 µg/L. From the

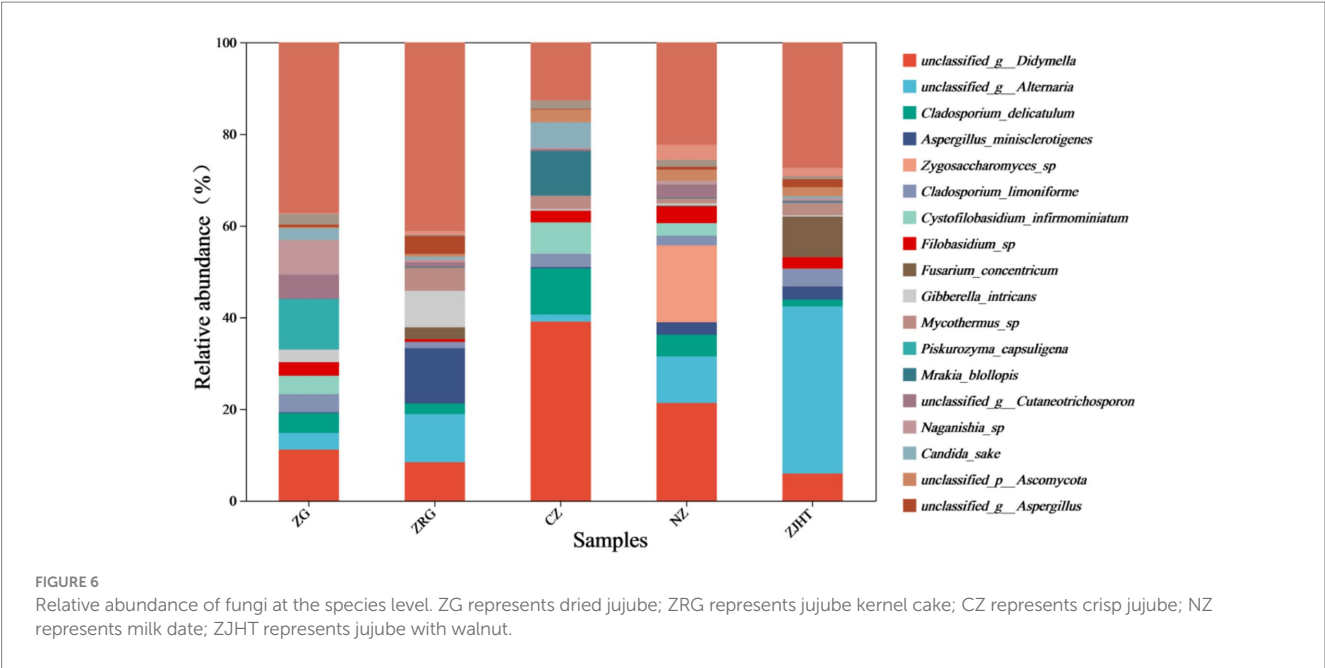


TABLE 3 The number and proportion of fungal genera in different processed jujube products.

Sample kind	Penicillium		Aspergillus		Alternaria		Talaromyces		Cladosporium		Total number of strains	Ratio (%)
	Number	Ratio (%)	Number	Ratio (%)	Number	Ratio (%)	Number	Ratio (%)	Number	Ratio (%)		
ZG	1	0.95	9	8.57	0	0.00	2	1.90	1	0.95	13	12.38
ZRG	6	5.71	17	16.19	1	0.95	0	0.00	1	0.95	25	23.81
CZ	2	1.90	6	5.71	1	0.95	4	3.81	2	1.90	15	14.29
NZ	3	2.86	8	7.62	1	0.95	2	1.90	3	2.86	17	16.19
ZJHT	5	4.76	26	24.76	2	1.90	1	0.95	1	0.95	35	33.33
Gross sample	17	16.19	66	62.86	5	4.76	9	8.57	8	7.62	105	100

The ratio columns refer to the ratio of the number of genus – level fungi in the processed red jujube product to the total number of isolated fungi; ZG represents dried jujube; ZRG represents jujube kernel cake; CZ represents crisp jujube; NZ represents milk date; ZJHT represents jujube with walnut.

aforementioned results, it could be inferred that the presence of *Aspergillus* with potent mycotoxin production capabilities can be isolated from product samples, which may pose a potential safety concern affecting the processed jujube products.

4 Discussion

4.1 Isolation and diversity of fungi from processed jujube products

The presence of fungi in jujube and processed jujube products has been frequently reported worldwide in recent years. *Aspergillus*, *Alternaria*, *Cladosporium*, and *Penicillium* were the most prevailing genera detected (Okayo et al., 2020; Sajidamu et al., 2018; Ghazi-Yaker et al., 2022; Tournas et al., 2015). This study also reached similar conclusions. All processed jujube product samples exhibited fungal contamination. At the phylum level, *Ascomycota* and *Basidiomycota* were dominant. The isolated strains were primarily from the

Aspergillus, followed by *Penicillium*. In particular, although the NZ sample has a high water activity, it has a lower total colony count. Mizzi et al. (2020) discovered that the addition of auxiliary materials during the processing could affect the growth of fungi. Due to the potential inhibitory effect of high sugar content on fungal growth, it could be the reason for the lower total number of colonies in the NZ. Noteworthily, the most frequently isolated strains within the *Aspergillus* were *A. flavus*, *A. niger*, and *A. tubingensis*. Many of these fungi are capable of producing mycotoxins, such as aflatoxins and ochratoxins (Fouad et al., 2019; Zhao et al., 2020). These mycotoxins could produced neurotoxic, carcinogenic, and other toxic effects, posing a threat to consumer health even at low levels (Xin et al., 2023; Samuel et al., 2021; Rocha et al., 2023).

Traditional culture techniques, limited by the cultivability of microorganisms (e.g., some fungi are difficult to grow or remain dormant on artificial culture media), fail to comprehensively analyze the true composition of fungal communities in processed jujube products, leading to insufficient analysis of fungal diversity within samples (Daniel, 2004). In contrast, high-throughput sequencing technology, based on

culture-independent metagenomics strategies, can deeply analyze the ITS sequence information of all fungi in samples, significantly improving the sensitivity of species detection (e.g., detecting low-abundance or hard-to-culture microbial communities). In this study, the application of this technology revealed variations in fungal composition among different product samples. In CZ samples, the dominant fungi were *Didymella* (39.07%) and *Cladosporium* (13.68%), with the highest relative abundance. This may be due to the fact that *Didymella* is a

fungus tolerant to high osmotic pressure, and *Cladosporium* spores have strong stress resistance and a strong ability to withstand desiccation (Correia et al., 2024; Duran et al., 2010). In ZJHT samples, *Alternaria* had the highest relative abundance (36.56%). This could be attributed to two reasons. On the one hand, the high water activity of jujube and walnut products, combined with *Alternaria*'s ability to adapt to adverse conditions, may promote its growth (Wang et al., 2024). On the other hand, the addition of walnuts during processing may contribute to the prevalence of *Alternaria*, as it is a dominant fungus in both jujube and walnut (Wei et al., 2020). Additionally, our results indicate significant differences in fungal composition among the samples ($p < 0.01$). On the one hand, the processing of jujube products (such as frying and drying) might alter the structure of the fungi. On the other hand, the addition of auxiliary materials during the product processing might affect the fungal communities present in the products (Isabel Galvan et al., 2022). The two reasons mentioned above might account for the differences in communities between all samples.

4.2 Analysis of toxin production in processed jujube products

In recent years, the contamination of mycotoxins in processed jujube products has garnered increasing global attention. A study on the fungal and mycotoxin contamination of dried dates showed that 2.9% detection rate of AFB₁ in dried dates, with levels below 700 µg/

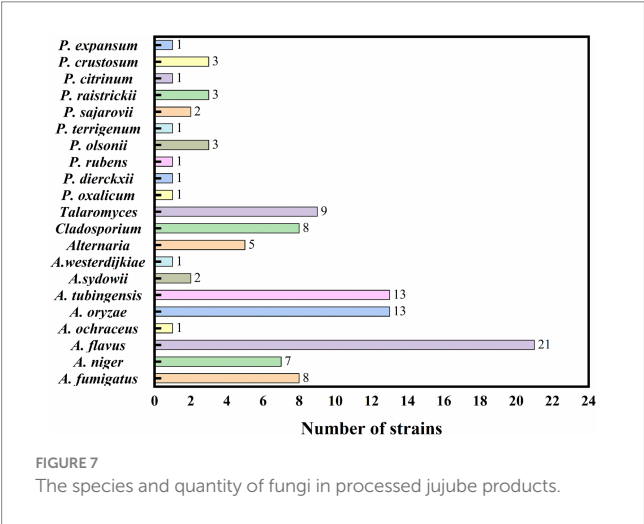


TABLE 4 Amplification results of aflatoxin-producing genes of *A. flavus*.

Serial number	Name of strain	Strain origin	Amplification results of aflatoxin-producing genes						
			<i>aflD</i>	<i>aflP</i>	<i>alfQ</i>	<i>aflR</i>	<i>aflS</i>	<i>omtA</i>	<i>ver</i>
1	<i>A. flavus</i>	ZG	+	+	–	+	–	–	–
2	<i>A. flavus</i>	ZRG	–	+	+	–	–	–	+
3	<i>A. flavus</i>	ZG	–	–	–	–	–	–	–
4	<i>A. flavus</i>	CZ	+	+	+	+	+	+	+
5	<i>A. flavus</i>	ZRG	+	+	+	+	+	+	+
6	<i>A. flavus</i>	ZJHT	–	–	–	–	–	–	–
7	<i>A. flavus</i>	NZ	+	+	+	+	+	+	+
8	<i>A. flavus</i>	ZG	+	+	+	+	+	+	+
9	<i>A. flavus</i>	CZ	–	+	–	–	–	–	–
10	<i>A. flavus</i>	ZRG	–	–	–	–	–	–	–
11	<i>A. flavus</i>	ZJHT	+	+	+	+	+	+	+
12	<i>A. flavus</i>	ZRG	–	–	–	–	–	–	–
13	<i>A. flavus</i>	ZJHT	–	–	+	–	+	–	+
14	<i>A. flavus</i>	ZJHT	–	–	–	–	–	–	–
15	<i>A. flavus</i>	NZ	–	–	–	–	–	–	–
16	<i>A. flavus</i>	ZJHT	+	–	+	+	–	+	–
17	<i>A. flavus</i>	ZJHT	+	+	+	+	–	–	+
18	<i>A. flavus</i>	ZJHT	–	–	–	–	–	–	–
19	<i>A. flavus</i>	ZRG	+	+	+	+	+	+	–
20	<i>A. flavus</i>	ZJHT	–	–	+	–	–	+	–
21	<i>A. flavus</i>	ZJHT	+	+	+	+	–	+	–

ZG represents dried jujube; ZRG represents jujube kernel cake; CZ represents crisp jujube; NZ represents milk date; ZJHT represents jujube with walnut.

TABLE 5 Amplification results of ochratoxin A – producing genes of *A. niger*, *A. tubingensis*, and *A. ochraceus*.

Serial number	Name of strain	Strain origin	Amplification results of ochratoxin A – producing genes						
			<i>AcLaeA</i>	<i>otaA</i>	<i>otaB</i>	<i>otaC</i>	<i>otaD</i>	<i>pks</i>	<i>nrps</i>
1	<i>A. niger</i>	ZRG	–	+	–	–	+	+	+
2	<i>A. niger</i>	CZ	–	–	–	–	–	–	–
3	<i>A. niger</i>	ZG	–	–	–	–	–	–	–
4	<i>A. niger</i>	ZRG	+	+	+	–	+	+	+
5	<i>A. niger</i>	NZ	+	–	+	–	–	+	+
6	<i>A. tubingensis</i>	ZG	+	+	–	–	+	+	+
7	<i>A. tubingensis</i>	ZRG	–	–	–	–	–	–	–
8	<i>A. niger</i>	ZG	–	–	–	–	–	–	–
9	<i>A. tubingensis</i>	ZJHT	+	+	+	+	+	+	+
10	<i>A. niger</i>	ZRG	–	–	–	–	–	–	–
11	<i>A. tubingensis</i>	ZJHT	–	+	+	–	+	+	+
12	<i>A. tubingensis</i>	ZJHT	–	–	–	–	+	+	+
13	<i>A. tubingensis</i>	ZJHT	–	+	+	–	+	+	+
14	<i>A. tubingensis</i>	ZRG	–	+	+	+	+	+	+
15	<i>A. tubingensis</i>	ZRG	+	+	+	+	+	+	+
16	<i>A. tubingensis</i>	ZJHT	–	+	+	+	+	+	+
17	<i>A. tubingensis</i>	CZ	–	–	–	–	–	–	–
18	<i>A. ochraceus</i>	ZJHT	+	+	+	+	+	+	+
19	<i>A. tubingensis</i>	ZRG	–	–	–	–	–	–	–
20	<i>A. tubingensis</i>	ZJHT	–	+	+	+	–	+	+
21	<i>A. tubingensis</i>	NZ	–	–	–	–	–	–	–

ZG represents dried jujube; ZRG represents jujube kernel cake; CZ represents crisp jujube; NZ represents milk date; ZJHT represents jujube with walnut.

TABLE 6 Aflatoxin content and types produced by 21 strains of *A. flavus*.

Name of strain	Strain origin	AFT (μg/L)	AFB ₁ (μg/L)	AFB ₂ (μg/L)	AFM ₁ (μg/L)	AFM ₂ (μg/L)
<i>A. flavus</i>	ZG	19.4271 ± 0.0053	6.5309 ± 0.0245	11.6912 ± 0.0166	2.9530 ± 0.0490	4.1815 ± 0.0180
<i>A. flavus</i>	ZRG	–	–	–	–	–
<i>A. flavus</i>	ZG	–	–	–	–	–
<i>A. flavus</i>	CZ	19.8077 ± 0.0059	8.3042 ± 0.0095	10.7021 ± 0.0122	5.6354 ± 0.0588	5.1991 ± 0.0488
<i>A. flavus</i>	ZRG	19.4390 ± 0.0048	8.5577 ± 0.0188	8.7941 ± 0.0238	–	–
<i>A. flavus</i>	ZJHT	–	–	–	–	–
<i>A. flavus</i>	NZ	21.7583 ± 0.0004	9.1858 ± 0.0086	13.7410 ± 0.0374	4.8912 ± 0.0137	5.4986 ± 0.0068
<i>A. flavus</i>	ZG	19.9410 ± 0.0058	10.1383 ± 0.0280	8.6137 ± 0.0335	–	–
<i>A. flavus</i>	CZ	–	–	–	–	–
<i>A. flavus</i>	ZRG	–	–	–	–	–
<i>A. flavus</i>	ZJHT	21.7329 ± 0.0008	10.9148 ± 0.0434	11.4122 ± 0.0432	3.6209 ± 0.0090	5.1143 ± 0.0105
<i>A. flavus</i>	ZRG	–	–	–	–	–
<i>A. flavus</i>	ZJHT	–	–	–	–	–
<i>A. flavus</i>	ZJHT	–	–	–	–	–
<i>A. flavus</i>	NZ	–	–	–	–	–
<i>A. flavus</i>	ZJHT	20.1880 ± 0.0031	8.7333 ± 0.0869	15.4407 ± 0.0267	4.4796 ± 0.0089	4.7947 ± 0.0193
<i>A. flavus</i>	ZJHT	19.3862 ± 0.0053	6.7583 ± 0.0238	10.0495 ± 0.0129	5.4882 ± 0.0272	4.8830 ± 0.0209
<i>A. flavus</i>	ZJHT	–	–	–	–	–
<i>A. flavus</i>	ZRG	20.0273 ± 0.0043	7.8566 ± 0.0323	7.7156 ± 0.0061	4.7098 ± 0.1056	6.0545 ± 0.0126
<i>A. flavus</i>	ZJHT	–	–	–	–	–
<i>A. flavus</i>	ZJHT	19.7410 ± 0.0020	11.0411 ± 0.0382	–	–	–

ZG represents dried jujube; ZRG represents jujube kernel cake; CZ represents crisp jujube; NZ represents milk date; ZJHT represents jujube with walnut.

TABLE 7 OTA content by 7 strains of *A. niger*, 13 strains of *A. tubingensis*, and 1 strain of *A. ochraceus*.

Name of strain	Strain origin	OTA (μg/L)
<i>A. niger</i>	ZRG	–
<i>A. niger</i>	CZ	9.2742 ± 0.0220
<i>A. niger</i>	ZG	–
<i>A. niger</i>	ZRG	–
<i>A. niger</i>	NZ	6.9835 ± 0.0639
<i>A. tubingensis</i>	ZG	–
<i>A. tubingensis</i>	ZRG	–
<i>A. niger</i>	ZG	–
<i>A. tubingensis</i>	ZJHT	–
<i>A. tubingensis</i>	ZRG	5.2019 ± 0.0242
<i>A. niger</i>	ZJHT	–
<i>A. tubingensis</i>	ZJHT	7.0344 ± 0.0206
<i>A. tubingensis</i>	ZJHT	–
<i>A. tubingensis</i>	ZRG	–
<i>A. tubingensis</i>	ZRG	–
<i>A. tubingensis</i>	ZJHT	–
<i>A. tubingensis</i>	CZ	5.8803 ± 0.0698
<i>A. tubingensis</i>	ZJHT	–
<i>A. tubingensis</i>	ZRG	7.0712 ± 0.0537
<i>A. ochraceus</i>	ZJHT	18.5207 ± 0.0487
<i>A. tubingensis</i>	NZ	6.7099 ± 0.0920

ZG represents dried jujube; ZRG represents jujube kernel cake; CZ represents crisp jujube; NZ represents milk date; ZJHT represents jujube with walnut.

kg (Wang et al., 2018). For instance, Han et al. (2016) assessed the presence of mycotoxins in dried fruits such as red jujube and detected OTA in 22.5% of jujube products. The detected levels of OTA ranged from 0.5 to 61.4 μg/L. In another investigation of ochratoxin A contamination in dried fruits such as jujubes collected from the United States, the results demonstrated that the OTA detection rate of dates was 2%, in which the OTA content was 0.39 μg/L on average (Palumbo et al., 2015). Similarly, in this study, strains producing aflatoxins and OTA were also detected.

In the present research, among the strains isolated from processed date products, 10 aflatoxin-producing strains and 8 ochratoxin A-producing strains were identified. Among the aflatoxins, the production level of AFB₁ was the highest, ranging from 6.5309 to 11.0411 μg/L. Additionally, the production levels of OTA ranged from 5.2019 to 18.5207 μg/L. Remarkably, aflatoxins are the most prevalent and toxic of nearly 400 mycotoxins identified in date samples (Deng et al., 2021). Additionally, ochratoxins are the most widely studied mycotoxins globally after aflatoxins (Agriopoulou et al., 2020). Therefore, in this study, the isolation of fungi with strong mycotoxin-producing capabilities from processed jujube products, along with the high toxicity of the fungal toxins, poses a significant potential threat to consumer health.

The European Union has established stringent standards for the levels of major mycotoxins in nuts, dried fruits, and their products manufactured for direct consumption. For these dried fruits intended for direct consumption, the maximum level of AFB₁ is set at 2 μg/kg, and the combined maximum level of AFB₁ + B₂ + G₁ + G₂ is set at

4 μg/kg. For processed nuts and dried fruits (e.g., those subjected to frying), the maximum permissible level of AFB₁ is 5 μg/kg, and the limit for AFT is 10 μg/kg (Li et al., 2017). Additionally, for products containing oilseeds, nuts, and/or dried fruits (excluding raisins and figs), the maximum level of OTA is set at 2.0 μg/kg (Commission Regulation (EU), 2022). There are no specific standards for aflatoxins and ochratoxins for processed jujube products. To ensure food safety, ready-to-eat jujube products should adhere to strict standards and testing procedures. The presence of toxigenic fungi in jujube products on the market could pose a threat to human health, as the toxins produced by these fungi are difficult to decompose and are not easily removed during the processing. In processed jujube products, contaminating fungi and mycotoxins are primarily introduced through the raw jujube materials. Furthermore, controlling conditions such as water activity during processing and storage is also crucial.

Controlling fungi and mycotoxins in jujube raw materials, biological control is a widely used method currently. This method involves the use of biological agents such as bacteria (*Bacillus*, *Pseudomonas*, etc.) and fungi (*Yeast*, *Trichoderma*, *Aspergillus*, etc.) and biodegradable compounds such as shell glycan and oligosaccharides to not only control the growth of fungi but also reduce mycotoxin production or degrade any produced mycotoxins (Shang et al., 2019; Lei et al., 2022; Liu et al., 2023). Jujubes are the raw materials for processed dates, and controlling the quality of jujubes is very important (Bukhari et al., 2023). Additionally, water activity is an important factor affecting fungal growth and mycotoxin production (Dong et al., 2023). Therefore, in addition to controlling the quality of the raw material for processed jujube products, it is also important to vacuum package these products to reduce their water activity.

5 Conclusion

In this study, the fungal diversity and mycotoxin production by typical *Aspergillus* strains in processed jujube products were analyzed. Traditional microbial isolation and sequencing of ITS and other regions identified 105 fungal strains, with dominant genera including *Aspergillus*, *Cladosporium*, and *Penicillium*. High-throughput sequencing revealed that the dominant phyla were *Ascomycota* and *Basidiomycota*, with *Alternaria*, *Didymella*, *Cladosporium*, and *Aspergillus* being the prevalent genera. The study found that the aflatoxin-producing strains isolated from processed jujube products had the highest capability to produce AFB₁, reaching a maximum of 11.0411 μg/L, and the maximum production of AFT was 21.7583 μg/L. The OTA content produced by *A. niger*, *A. tubingensis*, and *A. ochraceus* strains isolated from the product samples ranged from 5.2019 to 18.5207 μg/L. Potential mycotoxin-producing fungi were identified in the processed jujube products. Although the total fungal count was below the Chinese national standard limits, the toxigenic *Aspergillus* strains exhibited strong toxin production capabilities, posing a significant potential risk of mycotoxins and threatening consumer health. This research provides valuable insights into the fungal contamination in processed jujube products. To reduce fungal contamination and toxin risks, guidelines should be established, including the optimization of jujube raw material selection and storage conditions. This study was limited in sample size and region, future research should expand the sample range and delve into contamination factors to protect consumer health.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding authors.

Author contributions

TL: Conceptualization, Data curation, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. HJ: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. JS: Data curation, Project administration, Supervision, Writing – review & editing. YL: Conceptualization, Investigation, Writing – review & editing. YX: Data curation, Software, Writing – review & editing. WM: Investigation, Methodology, Writing – review & editing. HS: Conceptualization, Software, Writing – review & editing.

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References

- Aafi, E., Reza, M., and Mirabzadeh, M. (2022). Jujube (*Ziziphus jujuba* Mill. (Rhamnaceae)): a review on its pharmacological properties and phytochemistry. *Tradit. Med. Res.* 7, 38–40. doi: 10.53388/TMR20220905001
- Agriopoulou, S., Stamatiopoulou, E., and Varzakas, T. (2020). Advances in occurrence, importance, and mycotoxin control strategies: prevention and detoxification in foods. *Food Secur.* 9:137. doi: 10.3390/foods9020137
- Akinola, S. A., Ateba, C. N., and Mwanza, M. (2019). Polyphasic assessment of aflatoxin production potential in selected *aspergilli*. *Toxins* 11:692. doi: 10.3390/toxins11120692
- Association HFI. Products of Chinese jujube. (2020). Available online at: <http://down.foodmate.net/standard/sort/12/70607.html> (Accessed November, 2023).
- Azaiez, I., Font, G., Manes, J., and Fernandez-Franzon, M. (2015). Survey of mycotoxins in dates and dried fruits from Tunisia and Spanish markets. *Food Control* 51, 340–346. doi: 10.1016/j.foodcont.2014.11.033
- Bertuzzi, T., Rastelli, S., and Pietri, A. (2015). *Aspergillus* and *Penicillium* toxins in chestnuts and derived products produced in Italy. *Food Control* 50, 876–880. doi: 10.1016/j.foodcont.2014.10.047
- Bi, J., and Chen, Q. (2016). Chinese dates (jujubes): drying technology. United States: CRC Press, 221–256.
- Bukhari, S. H., Asghar, M. A., Ahmed, F., and Jabeen, S. (2023). The production of aflatoxin B₁ by *aspergillus parasiticus* in peanuts and walnuts under the influence of controlled temperature and water activity. *Int. J. Food Eng.* 19, 551–560. doi: 10.1515/ijfe-2023-0116
- Cao, W. Y., Yu, P., Yang, K. P., and Cao, D. L. (2022). Aflatoxin B₁: metabolism, toxicology, and its involvement in oxidative stress and cancer development. *Toxicol. Mech. Methods* 32, 395–419. doi: 10.1080/15376516.2021.2021339
- Cheng, S. B., Yu, Y., Guo, J. Y., Chen, G. G., and Guo, M. R. (2020). Effect of 1-methylcyclopropene and chitosan treatment on the storage quality of jujube fruit and its related enzyme activities. *Sci. Hortic.* 265:109281. doi: 10.1016/j.scienta.2020.109281
- Commission Regulation (EU) 2022/1370 of 5 August 2022 amending Regulation (EC) No 1881/2006 as regards maximum levels of ochratoxin A in certain foodstuffs. Off J Eur Union. (2022). Available online at: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32022R1370> (Accessed March, 2024).
- Correia, T. P., Francelino, M. R., Veloso, G. V., Michel, R. F., Schaefer, C. E., Fernandes Filho, E. I., et al. (2024). Resistance to adverse conditions and characterization of *Cladosporium* species from marine and terrestrial Antarctic samples. *An. Acad. Bras. Cienc.* 96:e20230743. doi: 10.1590/0001-3765202420230743
- Daniel, R. (2004). The soil metagenome—a rich resource for the discovery of novel natural products. *Curr. Opin. Biotechnol.* 15, 199–204. doi: 10.1016/j.copbio.2004.04.005
- Deng, Y. J., Wang, Y. L., Deng, Q., Sun, L. J., Wang, R. D., Ye, L., et al. (2021). Fungal diversity and mycotoxin contamination in dried fish products in Zhanjiang market, China. *Food Control* 121:107614. doi: 10.1016/j.foodcont.2020.107614
- Dong, T., Qiao, S., Xu, J., Shi, J., Qiu, J., and Ma, G. (2023). Effect of abiotic conditions on growth, mycotoxin production, and gene expression by *fusarium fujikuroi* species complex strains from maize. *Toxins* 15:260. doi: 10.3390/toxins15040260
- Drusch, S., and Ragab, W. (2003). Mycotoxins in fruits, fruit juices, and dried fruits. *J. Food Prot.* 66, 1514–1527. doi: 10.4315/0362-028X-66.8.1514
- Duran, R., Cary, J. W., and Calvo, A. M. (2010). Role of the osmotic stress regulatory pathway in morphogenesis and secondary metabolism in filamentous Fungi. *Toxins* 2, 367–381. doi: 10.3390/toxins2040367
- Fouad, A. M., Ruan, D., El-Senousey, H. K., Chen, W., Jiang, S. Q., and Zheng, C. T. (2019). Harmful effects and control strategies of aflatoxin B₁ produced by *aspergillus flavus* and *aspergillus parasiticus* strains on poultry: review. *Toxins* 11:176. doi: 10.3390/toxins11030176
- Ghazi-Yaker, A., Kraak, B., Houbraken, J., Houali, K., and Saadoun, N. (2022). Diversity of epiphytic and endophytic fungal communities associated with leaves of *Ziziphus lotus* (L.) Lam. from Algeria. *Pol. J. Ecol.* 70, 159–174. doi: 10.3161/15052249PJE2022.70.4.003
- González-Curbelo, M. A., and Kabak, B. (2023). Occurrence of mycotoxins in dried fruits worldwide, with a focus on aflatoxins and ochratoxin a: a review. *Toxins* 15:576. doi: 10.3390/toxins15090576
- Guo, Y., and Ji, H. (2021). Growth modeling kinetics of *aspergillus flavus* in dried jujube at different temperatures. *J. Pure Appl. Microbiol.* 15, 1873–1881. doi: 10.22207/JPAM.15.4.08
- Guo, K. F., Sun, J., Zhao, S. F., and He, L. (2016). Black spot disease of chinese jujube (*Ziziphus jujuba*) caused by *fusarium incarnatum* in China. *Plant Dis.* 100:529. doi: 10.1094/PDIS-06-15-0694-PDN

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2025.1499686/full#supplementary-material>

SUPPLEMENTARY FIGURE S1

The phylogenetic tree of *Penicillium* spp. Strains in processed jujube product.

SUPPLEMENTARY FIGURE S2

The phylogenetic tree *Aspergillus* spp. Strains in processed jujube product.

SUPPLEMENTARY FIGURE S3

Distribution of core fungal species in processed jujube products. ZG represents dried jujube; ZRG represents jujube kernel cake; CZ represents crisp jujube; NZ represents milk date; ZJHT represents jujube with walnut.

- Han, Z., Dong, M., Han, W., Shen, Y., Nie, D., Shi, W., et al. (2016). Occurrence and exposure assessment of multiple mycotoxins in dried fruits based on liquid chromatography-tandem mass spectrometry. *World Mycotoxin J.* 9, 465–474. doi: 10.3920/WMJ2015.1983
- Hu, D., Xiong, L., Xue, Y. W., Shan, C. H., Tang, F. X., Jiang, C. H., et al. (2022). Analysis of the fungal diversity in *Ziziphus jujuba* Mill. from Xinjiang using traditional culture method and high-throughput sequencing. *J. Food Saf. Qual.* 13, 8101–8108. doi: 10.19812/j.cnki.jfsq11-5956/ts.2022.24.041
- Institute HQaTSaI. National food safety standards determination of water activity in foods GB 5009.238-2016. (2016). Available online at: <http://down.foodmate.net/standard/sort/3/49405.html> (Accessed December, 2023).
- Iqbal, S. Z., Asi, M. R., and Jinap, S. (2014). Aflatoxins in dates and dates products. *Food Control* 43, 163–166. doi: 10.1016/j.foodcont.2014.03.010
- Isabel Galvan, A., de Guia, C. M., Rodriguez, A., Martin, A., Lopez-Corralles, M., Ruiz-Moyano, S., et al. (2022). Evaluation of fungal hazards associated with dried fig processing. *Int. J. Food Microbiol.* 365:109541. doi: 10.1016/j.ijfoodmicro.2022.109541
- James, A., and Zikankuba, V. L. (2018). Mycotoxins contamination in maize alarms food safety in sub-Saharan Africa. *Food Control* 90, 372–381. doi: 10.1016/j.foodcont.2018.03.018
- Janik, E., Niemcewicz, M., Ceremuga, M., Stela, M., Saluk-Bijak, J., Siadkowski, A., et al. (2020). Molecular aspects of mycotoxins—a serious problem for human health. *Int. J. Mol. Sci.* 21:21. doi: 10.3390/ijms21218187
- Kebede, H., Liu, X. M., Jin, J., and Xing, F. G. (2020). Current status of major mycotoxins contamination in food and feed in Africa. *Food Control* 110:106975. doi: 10.1016/j.foodcont.2019.106975
- Lei, X. M., Liu, Y., Guo, Y. X., Wang, W. J., Zhang, H. Y., Yi, L. H., et al. (2022). *Debaryomyces nepalensis* reduces fungal decay by affecting the postharvest microbiome during jujube storage. *Int. J. Food Microbiol.* 379:109866. doi: 10.1016/j.ijfoodmicro.2022.109866
- Li, Z. X., Nie, J. Y., Yan, Z., Zhang, X. N., Guan, D. K., Shen, Y. M., et al. (2017). Progress in research of detection, risk assessment and control of the mycotoxins in fruits and fruit products. *Sci. Agric. Sin.* 50, 332–347. doi: 10.3864/j.issn.0578-1752.2017.02.012
- Liu, M., Zhang, F., Liu, B. J., and Meng, X. H. (2023). Mechanisms and technology of marine oligosaccharides to control postharvest disease of fruits. *Food Chem.* 404:134664. doi: 10.1016/j.foodchem.2022.134664
- Liu, M. J., Zhao, Z. H., and Qi, Y. F. (2022). Future prospects of Chinese jujube industry and scientific and technological countermeasures. *V Int. Jujube Symp.* 1350, 1–6. doi: 10.17660/ActaHortic.2022.1350.1
- Ma, Q. F. (2018). Interpretation for GB 4789.15-2016-national food safety standard – food microbiological examination – enumeration of molds and yeasts. *China Health Stand. Manag.* 9:1. doi: 10.3969/j.issn.1674-9316.2018.05.001
- Maor, U., Barda, O., Sadhasivam, S., Bi, Y., Levin, E., Zakin, V., et al. (2021). Functional roles of Lae A, polyketide synthase, and glucose oxidase in the regulation of ochratoxin A biosynthesis and virulence in *aspergillus carbonarius*. *Mol. Plant Pathol.* 22, 117–129. doi: 10.1111/mp.13013
- Mizzi, L., Maniscalco, D., Gaspari, S., Chatzitzika, C., Gatt, R., and Valdramidis, V. P. (2020). Assessing the individual microbial inhibitory capacity of different sugars against pathogens commonly found in food systems. *Lett. Appl. Microbiol.* 71, 251–258. doi: 10.1111/lam.13306
- Mujahid, C., Savoy, M. C., Basle, Q., Woo, P. M., Ee, E. C. Y., Mottier, P., et al. (2020). Levels of *Alternaria* toxins in selected food commodities including green coffee. *Toxins* 12:595. doi: 10.3390/toxins12090595
- Mukhtar, K., Nabi, B. G., Ansar, S., Bhat, Z. F., Aadil, R. M., and Khaneghah, A. M. (2023). Mycotoxins and consumers' awareness: recent progress and future challenges. *Toxicon* 232:107227. doi: 10.1016/j.toxicon.2023.107227
- Okayo, R. O., Andika, D. O., Dida, M. M., K'Otuto, G. O., and Gichimu, B. M. (2020). Morphological and molecular characterization of toxigenic *aspergillus flavus* from groundnut gernels in Kenya. *Int. J. Microbiol.* 2020:8854718. doi: 10.1155/2020/8854718
- Palumbo, J. D., O'Keefe, T. L., Ho, Y. S., and Santillan, C. J. (2015). Occurrence of Ochratoxin A contamination and detection of ochratoxigenic *aspergillus* species in retail samples of dried fruits and nuts. *J. Food Prot.* 78, 836–842. doi: 10.4315/0362-028X.JFP-14-471
- Puntscher, H., Kuett, M. L., Skrinjar, P., Mikula, H., Podlech, J., Froehlich, J., et al. (2018). Tracking emerging mycotoxins in food: development of an LC-MS/MS method for free and modified *Alternaria* toxins. *Anal. Bioanal. Chem.* 410, 4481–4494. doi: 10.1007/s00216-018-1105-8
- Quaglia, M., Santinelli, M., Sulyok, M., Onofri, A., Covarelli, L., and Beccari, G. (2020). *Aspergillus*, *Penicillium* and *Cladosporium* species associated with dried date fruits collected in the Perugia (Umbria, Central Italy) market. *Int. J. Food Microbiol.* 322:108585. doi: 10.1016/j.ijfoodmicro.2020.108585
- Rocha, A. R., Cardoso, M. S., Souza Júnior, J. A., Gomes Júnior, E. A., Maciel, L. F., and Menezes-Filho, J. A. (2023). Occurrence of aflatoxins B₁, B₂, G₁, and G₂ in beers produced in Brazil and their carcinogenic risk evaluation. *Food Control* 166:110717. doi: 10.1016/j.foodcont.2024.110717
- Sajidamu, A., Mijiti, M., Qiang, Z. Z., Bai, X. T., and Yuan, G. Q. (2018). Identification of a pathogenic fungus infecting the leaves and fruits of red dates. *Xinjiang Agric. Sci.* 55, 1682–1688. doi: 10.6048/j.issn.1001-4330.2018.09.014
- Samuel, M. S., Jeyaram, K., Datta, S., Chandrasekar, N., Balaji, R., and Selvarajan, E. (2021). Detection, contamination, toxicity, and prevention methods of ochratoxins: an update review. *J. Agric. Food Chem.* 69, 13974–13989. doi: 10.1021/acs.jafc.1c05994
- Schabo, D. C., Freire, L., Sant'Ana, A. S., Schaffner, D. W., and Magnani, M. (2021). Mycotoxins in artisanal beers: an overview of relevant aspects of the raw material, manufacturing steps and regulatory issues involved. *Food Res. Int.* 141:110114. doi: 10.1016/j.foodres.2021.110114
- Shang, L. L., Bai, X. X., Chen, C., Liu, L. J., Li, M. Y., Xia, X. S., et al. (2019). Isolation and identification of a *Bacillus megaterium* strain with ochratoxin A removal ability and antifungal activity. *Food Control* 106:106743. doi: 10.1016/j.foodcont.2019.106743
- Shen, B., and Hui, J. Z. GB/T 10468–1989 fruit and vegetable products – determination of pH. (1989). Available online at: <http://down.foodmate.net/standard/sort/3/655.html> (Accessed December, 2023).
- Stoev, S. D. (2021). Follow up long term preliminary studies on carcinogenic and toxic effects of ochratoxin A in rats and the putative protection of phenylalanine. *Toxicon* 190, 41–49. doi: 10.1016/j.toxicon.2020.11.010
- Susca, A., Villani, A., Moretti, A., Stea, G., and Logrieco, A. (2020). Identification of toxigenic fungal species associated with maize ear rot: calmodulin as single informative gene. *Int. J. Food Microbiol.* 319:108491. doi: 10.1016/j.ijfoodmicro.2019.108491
- Tournas, V. H., Niaz, N. S., and Kohn, J. S. (2015). Fungal presence in selected tree nuts and dried fruits. *Microbiol. Insights* 8, 1–6. doi: 10.4137/MBIS24308
- Wang, Y. J., Nie, J. Y., Yan, Z., Li, Z. X., Cheng, Y., and Chang, W. X. (2018). Occurrence and co-occurrence of mycotoxins in nuts and dried fruits from China. *Food Control* 88, 181–189. doi: 10.1016/j.foodcont.2018.01.013
- Wang, D., Zhang, H. J., Meng, L. K., Tan, X. Y., Liu, R., Gao, Q. C., et al. (2024). Exogenous nitric oxide induces pathogenicity of *Alternaria alternata* on Huangguan pear fruit by regulating reactive oxygen species metabolism and Cell Wall modification. *J. Fungi* 10:726. doi: 10.3390/jof10100726
- Wang, G., Zhang, H. Y., Wang, Y. L., Liu, F., Li, E. F., Ma, J. N., et al. (2019). Requirement of Lae A, VeA, and VelB on asexual development, ochratoxin A biosynthesis, and fungal virulence in *aspergillus ochraceus*. *Front. Microbiol.* 10:2759. doi: 10.3389/fmicb.2019.02759
- Wei, L., Fu, H., Lin, M., Dang, H., Zhao, Y., Xu, Y., et al. (2020). Identification of dominant fungal contamination of walnut in northwestern China and effects of storage conditions on walnut kernels. *Sci. Hortic.* 264:109141. doi: 10.1016/j.scienta.2019.109141
- Więkowska, M., Szelenberger, R., Niemcewicz, M., Harmata, P., Poplawski, T., and Bijak, M. (2023). Ochratoxin A—the current knowledge concerning hepatotoxicity, mode of action and possible prevention. *Molecules* 28:6617. doi: 10.3390/molecules28186617
- Wu, L., Xiao, X. Y., Chen, K., Yin, W. M., Li, Q., Wang, P., et al. (2017). Ultrasensitive SERS detection of *Bacillus thuringiensis* special gene based on au@ag NRs and magnetic beads. *Biosens. Bioelectron.* 92, 321–327. doi: 10.1016/j.bios.2016.11.005
- Xin, X. Y., Nan, M. N., Bi, Y., Xue, H. L., Zhang, Y., Wang, J. J., et al. (2023). Effects of *Aspergillus niger* infection on the quality of jujube and Ochratoxin A cumulative effect. *Toxins* 15:15. doi: 10.3390/toxins15070406
- Xu, X., Guo, L. F., Yang, C. B., Teng, H. L., Shen, G. J., Wang, S., et al. (2023). Diversity and pathogenicity of fungi associated with fruit rot of winter jujube in Shandong province, China. *Plant Dis.* 107, 794–801. doi: 10.1094/PDIS-05-22-1254-RE
- Yang, Y., Li, G. L., Wu, D., Liu, J. H., Li, X. T., Luo, P. J., et al. (2020). Recent advances on toxicity and determination methods of mycotoxins in foodstuffs. *Trends Food Sci. Technol.* 96, 233–252. doi: 10.1016/j.tifs.2019.12.021
- Yuan, S. Z., Li, W. S., Li, Q. Q., Wang, L. M., Cao, J. K., and Jiang, W. B. (2019). Defense responses, induced by *p*-coumaric acid and methyl *p*-coumarate, of jujube (*Ziziphus jujuba* Mill.) fruit against black spot rot caused by *Alternaria alternata*. *J. Agric. Food Chem.* 67, 2801–2810. doi: 10.1021/acs.jafc.9b00087
- Zhang, H. Y., Wang, G., Yang, Q. L., Yang, X., Zheng, Y. Q., Liu, Y., et al. (2021). Effects of light on the Ochratoxigenic Fungi *aspergillus ochraceus* and *A. carbonarius*. *Toxins* 13:251. doi: 10.3390/toxins13040251
- Zhang, W. P., Yang, X. H., Mujumdar, A. S., Ju, H. Y., and Xiao, H. W. (2022). The influence mechanism and control strategy of relative humidity on hot air drying of fruits and vegetables: a review. *Dry. Technol.* 40, 2217–2234. doi: 10.1080/07373937.2021.1943669
- Zhao, M., Wang, X. Y., Xu, S. H., Yuan, G. Q., Shi, X. J., and Liang, Z. H. (2020). Degradation of ochratoxin A by supernatant and ochratoxinase of *Aspergillus niger* W-35 isolated from cereals. *World Mycotoxin J.* 13, 287–297. doi: 10.3920/WMJ2019.2446
- Zhou, J., Zhang, D. D., Chen, X. H., Guo, Y. B., Jin, M. C., and Zhao, Y. G. (2021). Investigation on the occurrence and contamination of multi-mycotoxin in chestnut and jujube (red date). *J. Chromatogr. A* 1659:462486. doi: 10.1016/j.chroma.2021.462486



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Xylitol promotes the antioxidant and biocontrol efficiency of the antagonistic yeast, *Meyerozyma guilliermondii*

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The biocontrol efficiency of the antagonist yeast *Meyerozyma guilliermondii* is significantly reduced under oxidative stress in adverse environments. However, effective strategies to improve *M. guilliermondii* under such abiotic stress remain limited. As an effective protectant of yeasts, xylitol has significant potential to improve the performance of *M. guilliermondii* under abiotic stress. We investigated xylitol's effects on the viability and efficiency of *M. guilliermondii* under oxidative stress. The results showed that 0.5 M and 1 M xylitol significantly enhanced yeast survival, antioxidant gene expression, and enzyme activity, including thioredoxin reductase (TrxR) and peroxidase (POD), while reducing intracellular reactive oxygen species levels as well as damage to mitochondrial membranes, and preserving the ATP content. Notably, xylitol-treated (XT) yeast exhibited higher intracellular xylitol levels and improved resistance to oxidative stress compared with the non-xylitol-treated cells. Additionally, XT yeast showed a greater biocontrol efficacy and lower postharvest fungal infection rate by gray mold and blue mold in apples. These results demonstrated that xylitol effectively boosts the resilience and biocontrol efficiency of *M. guilliermondii*, making it a promising candidate to improve postharvest disease management.

KEYWORDS

Meyerozyma guilliermondii, biocontrol efficiency, xylitol, antagonist, gray mold

1 Introduction

Postharvest fungal control using biocontrol yeasts offers both practical and economic advantages (Droby et al., 2016; Wisniewski et al., 2016; Kim et al., 2018). Over recent decades, a number of biocontrol yeast strains have been identified as effective antagonists against fungal diseases, such as gray mold (*Botrytis cinerea*) and blue mold (*Penicillium expansum*) (Romanazzi et al., 2016; Vilanova et al., 2017). Among the effective biocontrol yeasts, *Meyerozyma guilliermondii* shows high efficacy against various postharvest fungal pathogens of multiple fruits, including apples, pears, and kiwifruit during storage (Sui and Liu, 2014; Sadeghi et al., 2021).

Despite the demonstrated efficacy of antagonistic yeasts, their efficacy as biocontrol agents can be constrained by environmental factors, such as temperature, oxidative stress, and salinity, thereby limiting their effectiveness (Macarisin et al., 2010; Sui et al., 2015; Spadaro and Droby, 2016). The stress tolerance of antagonistic yeasts is closely linked to their survival and proliferation within host tissues, as well as their biocontrol efficacy against pathogens (Liu et al., 2013). Consequently, identifying effective and cost-efficient protectants is a strategic

approach to enhance the biocontrol capacity of these yeasts, offering both efficiency and rapid benefits (Liu et al., 2011; Ming et al., 2020).

Sugars or sugar alcohols have been shown to be effective protectants for biocontrol yeasts (Sui et al., 2012; Ming et al., 2020). Xylitol, a polyol derived from the hydrogenation of xylose, has demonstrated efficacy against ochratoxigenic fungus *Aspergillus carbonarius* and *Listeria monocytogenes* (Morón de Salim and Ramírez Mérida, 2013; Espinosa-Salgado et al., 2022). Xylitol's low cost and widespread use as a food additive, along with its inherent antioxidant properties, make it particularly well-suited for use as a protectant for biocontrol yeasts in both pre- and post-harvest applications. However, there is limited research on the application of these compounds as protectants for antagonistic yeasts under adverse environmental conditions.

Taking into account the above research, the present study was conducted to determine the effects of xylitol on the antioxidant response, stress tolerance, and biocontrol efficacy of the yeast *M. guilliermondii*. We determined: (1) The cell survival rate of xylitol-treated (XT) cells following exposure to oxidative stress induced by H₂O₂; (2) the impact of xylitol treatment on the expression of antioxidant genes, including those encoding thioredoxin reductase (TrxR) and peroxidase (POD), as well as their corresponding enzyme activities; (3) the accumulation of intracellular reactive oxygen species (ROS), mitochondrial membrane potential analysis, changes in ATP production and intracellular xylitol levels; and (4) the biocontrol efficacy of XT *M. guilliermondii* yeast cells against *B. cinerea* and *P. expansum* infections in apples.

2 Materials and methods

2.1 Antagonistic yeast

The yeast *M. guilliermondii*, known for its antagonistic properties, was first isolated from apple surfaces and identified through its general morphology and DNA sequencing of the ribosomal internal transcribed spacer (ITS) region (Leaw et al., 2006). This yeast was cultured in a yeast extract-peptone-dextrose (YPD) medium, which contained 10 g of yeast extract, 20 g of peptone, and 20 g of glucose dissolved in 1 liter of water. To cultivate the yeast, 40 mL of YPD medium was added to a 100 mL Erlenmeyer flask, followed by inoculation with *M. guilliermondii*. The initial yeast cell concentration was measured at 10⁵ cells/mL using a hemocytometer. The culture was then incubated at 25°C on a rotary shaker at 200 rpm for 16 h.

2.2 Fungal pathogens

The fungal pathogens *B. cinerea* and *P. expansum* were isolated from infected apples and pears and identified by DNA sequencing of the ITS region, and maintained on potato dextrose agar (PDA) at 4°C. To reactivate the culture and confirm its pathogenicity, each pathogen was inoculated into a wounded apple, and re-isolated onto PDA after infection was successfully confirmed. Spore suspensions of the fungal pathogens were prepared from 2-week PDA plates incubated at 25°C, with spore concentrations determined using a hemocytometer and adjusted to 10⁴ spores/mL with sterile distilled water before use.

2.3 Test fruit

Apples (*Malus × domestica* Borkh) were harvested when commercially ripe. Only fruits that were uniform in shape and size, and free from physical damage, were selected for the study. The apples were thoroughly inspected to ensure they were intact and free from decay. To sterilize the fruit surfaces, a 2% (v/v) sodium hypochlorite solution was applied for 2 min, followed by rinsing with tap water and air-drying. The sterilized apples were then used in biocontrol experiments.

2.4 Xylitol treatment of *Meyerozyma guilliermondii*

In order to investigate the effect of xylitol treatment (XT) on *M. guilliermondii*, yeast cultures were centrifuged at 8,000 × g for 3 min. The yeast cells were washed three times with sterile distilled water to remove residual growth medium (Liu et al., 2012b). The cleaned cells were then resuspended in 20 mL of fresh YPD medium, and xylitol was added to achieve final concentrations of 0.5 or 1 M. The cultures were incubated on a rotary shaker at 25°C, with shaking at 200 rpm for 3 h. The xylitol concentrations were selected based on preliminary experiments. Briefly, given that xylitol concentrations typically range from 0.3 to 3 M in market food applications, we initially confirmed the efficacy of a lower concentration (1 M) in pilot experiments. To assess the potential effectiveness of even lower concentrations, we halved the treatment concentration to 0.5 M for further analysis. A control group, referred to as the Non-xylitol (NX) group, underwent the same procedure without xylitol addition. After centrifugation and washing, the yeast cells from both XT (0.5 and 1 M) and NX groups were resuspended in water at a concentration of 1 × 10⁷ cells/mL for further analysis.

2.5 Effect of xylitol on the survival rate of *Meyerozyma guilliermondii*

Cell samples collected at 0, 10, 20, and 30 min after oxidative treatment were cultured on YPDA agar medium (YPD supplemented with 20 g of agar per liter). The medium was incubated at 25°C for 3 days, after which colony-forming units (CFUs) per medium were quantified. Cell viability was expressed as the percentage of CFUs following oxidative treatment, relative to the CFU count of total cells. Each treatment contained three replicates, and the experiment was repeated three times.

2.6 Intracellular ROS, mitochondrial membrane potential, and ATP determination

Intracellular ROS levels in yeast cells were determined using the oxidation-sensitive probe 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA; Invitrogen, Eugene, OR, USA), as previously described (Liu et al., 2011). Samples of NX and XT yeast cells were exposed to 30 mM H₂O₂ for 10, 20, or 30 min, with pre-exposure samples designated as time 0. The yeast cells were washed with

phosphate-buffered saline (PBS, pH 7.0) and resuspended in the same buffer with 25 μ M H₂DCFDA. The suspension was incubated in the dark at 30°C for 1 h, washed twice with PBS, and examined under an FV3000 confocal microscope (Olympus, Tokyo, Japan) using a 480 nm excitation and 520 nm emission filter. ROS-positive cells were quantified by randomly selecting 10 fields per slide (with a minimum of 200 cells per slide), and the percentage of fluorescent cells relative to the total count was determined. Each experiment included three biological replicates and was repeated three times.

Mitochondria damage and ATP levels analysis was according to previous studies (Liu et al., 2012a; Sui and Liu, 2014). Briefly, mitochondrial membrane potential was assessed using the Mitochondrial membrane potential assay kit with JC-1 (Beyotime, China), which contains the cationic dye JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-imidacarbocyanine iodide). This dye emits red fluorescence in the mitochondria of healthy cells. Upon collapse of the mitochondrial membrane potential, the cationic dye accumulates in the cytoplasm, emitting green fluorescence. Thus, the ratio of red to green fluorescence is higher in healthy cells and lower in damaged cells. In this study, all *M. guilliermondii* samples were collected and resuspended in the JC-1 reagent at a final concentration of 1×10^6 cells/ml and incubated at 37°C for 15 min. Cells were then centrifuged and resuspended in 1 mL of assay buffer provided by the kit, and the ratio of red (excitation at 550 nm, emission at 600 nm) to green (excitation at 485 nm, emission at 535 nm) fluorescence was immediately measured using a Laser Scanning Confocal Microscopy (Zeiss, Germany). Each treatment contained three replicates, and the experiment was repeated three times.

ATP levels analysis was performed according to Li et al. (2010). Briefly, ATP of approximately 20 mg of fresh weight cultured *M. guilliermondii* cells that exposed to 30 mM H₂O₂ for 0, 10, 20, 30, 60, and 120 min were extracted with 50 μ L of 2.5% trichloroacetic acid (TCA) and incubated at 4°C for 3 h. After centrifugation at $10,000 \times g$ for 15 min, 10 μ L supernatant was diluted with 115 μ L ATP-free water and 125 μ L ATP-free Tris-acetate buffer (40 mM, pH 8.0). ATP content was quantified using a luciferin/luciferase assay kit (Beyotime, China) according to the manufacturer's instructions. Luminescence emission was measured using a Laser Scanning Confocal Microscopy (Zeiss, Germany). Each treatment contained three replicates, and the experiment was repeated three times.

2.7 Intracellular xylitol concentration measurement

To analyze the intracellular xylitol levels, yeast cells were disrupted by physical methods. After centrifugation at 8000 rpm at -4°C for 5 min, the yeast cell samples were collected and resuspended in HPLC-grade water. An HPLC system was employed to determine the xylitol concentrations (Agilent Series 1260, Agilent Technologies, Santa Clara, CA, United States). The mobile phase consisted of acetonitrile-water (80:20, v/v) with a flow rate of 1 mL/min. Xylitol was quantified using a standard (Sigma-Aldrich, Shanghai, China) with a linear response range of 0.05–10 mg/mL (Krallish et al., 1997). Xylitol concentrations of yeast cells were expressed as mg/g. Each treatment contained three replicates, and the experiment was repeated three times.

2.8 RNA isolation and reverse transcription–quantitative real-time PCR analysis of gene expression

Total RNA from NX and XT cells was extracted, treated with DNase, and purified using the EasyPure® Plant RNA Kit (TransGen Biotech, Beijing, China). RNA quality was assessed by gel electrophoresis and spectrophotometry (Nanodrop, Thermo Fisher Scientific, Waltham, MA, United States). First-strand cDNA was synthesized using 1 μ g of total RNA, employing the TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix kit (TransGen Biotech). qPCR was performed on a Roche LightCycler® 480 (Roche, Basel, Switzerland) with the following program: 95°C for 5 min; 40 cycles of 95°C for 5 s and 60°C for 20 s; 95°C for 15 s; 60°C for 1 min, followed by a dissociation step at 95°C for 15 s. Gene expression of *TrxR* and *POD* was normalized to the reference gene *18S* rRNA using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Three independent biological replicates and three technical replicates were used, and the analysis was repeated three times.

2.9 Assay of enzyme activity

To measure antioxidant enzyme activity, XT and NX yeast cells were exposed to 30 mM H₂O₂ for 10, 20, or 30 min, with samples collected prior to oxidative stress exposure serving as time 0. Enzyme extracts were prepared according to the manufacturer's instructions. Cells (1×10^8) were frozen in liquid nitrogen, resuspended in chilled potassium phosphate buffer (0.1 M, pH 7.4), and centrifuged at $10,000 \times g$ for 20 min at 4°C. The supernatant was collected for enzyme activity analysis. Activities of TrxR and POD were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and expressed as U/mg protein. Protein content was determined using the Bradford assay, with bovine serum albumin as the standard (Bradford, 1976). One unit of TrxR activity was defined as the amount of enzyme that reduces 1 nmol of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) per minute at 25°C. One unit of POD activity was defined as the amount of enzyme that causes a 0.01 absorbance change per minute at 470 nm due to guaiacol oxidation. Each assay was performed with three biological replicates and repeated three times.

2.10 Biocontrol assay

The biocontrol efficacy was evaluated following the method outlined by Wang et al. (2018), with some adjustments. In brief, three wounds (4 mm deep $\times$ 3 mm wide) were created on the equator of each apple using a sterile nail. A 10 μ L suspension (1×10^7 cells/mL) of either XT or NX *M. guilliermondii* yeast cells was applied to each wound. One control group received sterile distilled water, while another control consisted of a 10 μ L suspension (1×10^7 cells/mL) of fresh yeast cells, which had not been exposed to sugar or oxidative stresses. The treated apples were divided into two groups. After the fruits were air-dried for 2 h, 10 μ L of a *B. cinerea* or *P. expansum* suspension (1×10^4 spores/mL) was introduced into each wound of the respective groups. The apples were then placed in covered plastic food trays, each sealed within a polyethylene bag, and stored at

25°C. After 4 days, the disease incidence and lesion diameter on each apple were measured. Incidence referred to the percentage of infected wounds, while lesion diameter was measured only for those wounds that showed infection. Each treatment was repeated three times with three replicates, each containing 10 apples. After 4 days treatments, uniformed apples of each treatment were photographed by a digital camera.

2.11 Data analysis

Statistical analyses were carried out using SPSS V26 (IBM Corp., Armonk, NY, United States). Comparisons between the NX and XT groups were made using Student's t-test, with a significance level of $p < 0.05$. The results presented are based on pooled data from three independent experimental replicates, as a one-way analysis of variance (ANOVA) showed no significant effects of the treatments or interactions between variables and experiments.

3 Results

3.1 The effects of xylitol on the survival rate of *Meyerozyma guilliermondii* under oxidative stress

Exposure to 30 mM H_2O_2 resulted in a decrease in cell viability as the treatment duration increased from 10 to 30 min (Figure 1). After 10 min of H_2O_2 exposure, the survival rates across all treatments were comparable, averaging around 86%. For yeast cells suspended in the NX group, survival rates dropped to 69 and 51% after 20 and 30 min of H_2O_2 exposure, respectively. Interestingly, survival rate of XT cells showed an increasing trend. Yeast cells treated with 1 M xylitol exhibited survival rates of 75 and 78% after 20 and 30 min of H_2O_2 exposure, respectively. Similarly, cells treated with 0.5 M xylitol exhibited over 65% viability under oxidative stress at both 20 and 30 min.

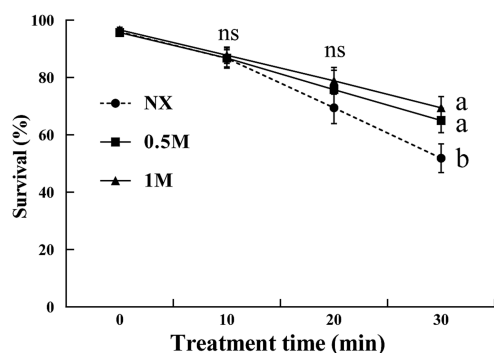


FIGURE 1
Percent viability of *Meyerozyma guilliermondii* cells under H_2O or xylitol subjected to a subsequent oxidative (30 mM H_2O_2) stress for 10, 20 or 30 min. Prior to exposure to the subsequent oxidative stress served as Time 0. Data represent the mean $\pm$ standard deviation of three independent experiments, where each experiment consisted of three biological replicates ($n = 9$). Columns with different letters are significantly different according to a Duncan's multiple range test at $p < 0.05$. ns, no significance.

3.2 Effect of xylitol on cell damage and ATP levels in *Meyerozyma guilliermondii* under oxidative stress

At the time 0 (immediately following a 30-min pre-treatment but before exposure to oxidative stress), about 5% of cells treated with 0.5 M and 1 M xylitol, as well as the NX cells, were ROS-positive (Figure 2A). This proportion increased after exposure to 30 mM H_2O_2 . At each time point, the cells treated with 0.5 M and 1 M xylitol showed significantly lower ROS levels compared to the NX cells. To investigate if the increase in ROS under oxidative stress was linked to mitochondrial dysfunction, $\Delta\Psi_m$ and ATP content were measured. Figure 2B showed that $\Delta\Psi_m$ was significantly changed between NX and XT cells under oxidative stress. And a decreasing trend of $\Delta\Psi_m$ was observed in the XT cells compared to the NX group. As higher damage was observed in NX cells, ATP levels, reached their lowest point at 120 min when exposed to 30 mM H_2O_2 stress. In contrast, the ATP decline in XT cells was notably slower, cells treated with 1 M xylitol maintaining ATP levels approximately 10 times higher than those in the NX group at the 120-min (Figure 2C).

3.3 Yeast cells uptake xylitol under oxidative stresses

Compared to the NX group, yeast cells under oxidative stress exhibited a significant increase in intracellular xylitol levels following xylitol treatment. The intracellular xylitol concentration showed a positive relation to the increasing external xylitol concentrations. After 10 min treatment, the xylitol concentration in 0.5 and 1 M XT cells was significantly increased by 35.5% and 45%, respectively. When treated to 30 min, the highest intracellular xylitol levels were observed in 1 M XT cells (Figure 3).

3.4 Effect of xylitol treatment on the antioxidant system of *Meyerozyma guilliermondii*

The activities of thioredoxin reductase (TrxR) and peroxidase (POD) were evaluated in pre-treated and NX samples in response to oxidative stress. The findings revealed that XT cells had significantly higher TrxR and POD activities compared to NX yeast cells (Figure 4). Under oxidative stress, TrxR activity in XT cells was significantly greater than in NX cells (Figure 4A). Similarly, POD activity in XT cells exposed to oxidative stress remained consistently higher than in NX cells across all time points (Figure 4B). In *M. guilliermondii* cells treated with xylitol, both the activities of these antioxidant enzymes and their corresponding gene expression levels (Figure 5) were significantly elevated at all-time points (from 0 to 30 min), both before and after exposure to oxidative stress, compared to untreated cells.

3.5 Biocontrol of postharvest diseases in apples using *Meyerozyma guilliermondii*

As shown, the antagonistic yeast *M. guilliermondii* significantly reduced the incidence and lesion diameter of blue mold and gray

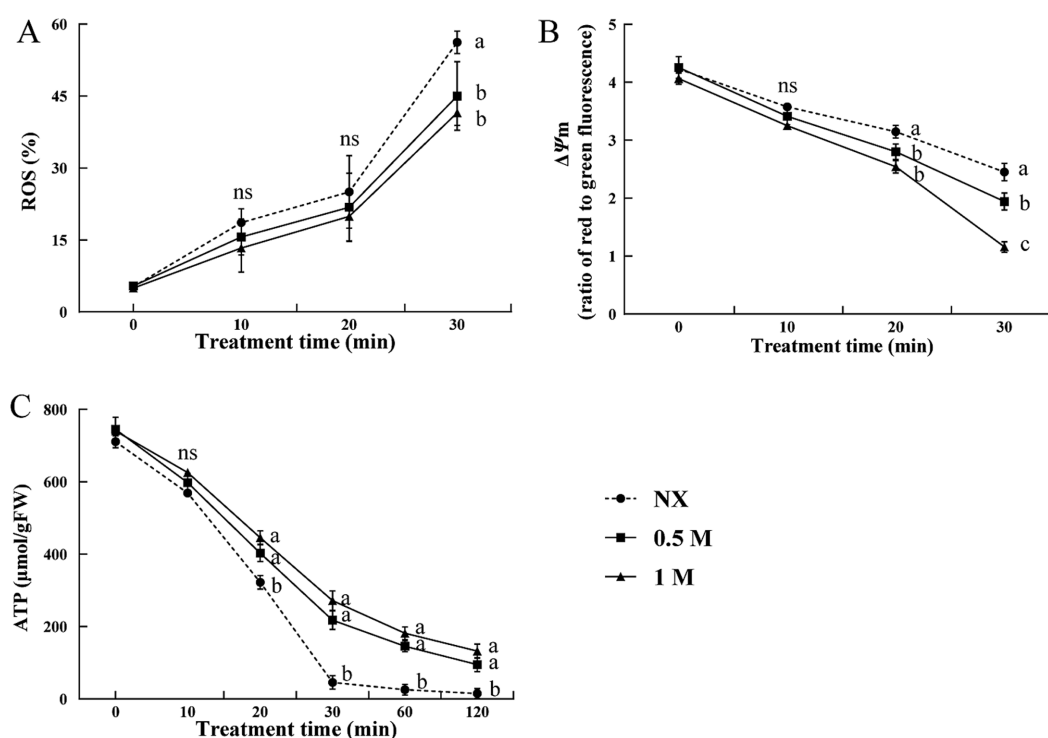


FIGURE 2

ROS accumulation (A), mitochondrial membrane potential analysis (B) and ATP levels (C) of *Meyerozyma guilliermondii* cells under H₂O or xylitol subjected to a subsequent oxidative (30 mM H₂O₂) stress for 10, 20 or 30 min, (60 and 120 min for ATP analysis). Prior to exposure to the subsequent oxidative stress served as Time 0. Data represent the mean ± standard deviation of three independent experiments, where each experiment consisted of three biological replicates ($n = 9$). Columns with different letters are significantly different according to a Duncan's multiple range test at $p < 0.05$. ns, no significance.

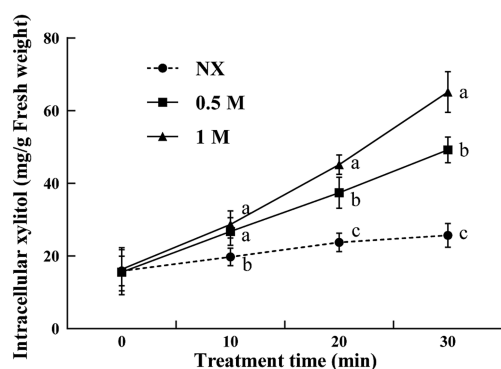


FIGURE 3

Intracellular xylitol concentrations of *Meyerozyma guilliermondii* cells under H₂O or xylitol subjected to a subsequent oxidative (30 mM H₂O₂) stress for 10, 20 or 30 min. Prior to exposure to the subsequent oxidative stress served as Time 0. Xylitol concentrations represent as milligram per gram of yeast cell fresh weight (mg/g FW). Data represent the mean ± standard deviation of three independent experiments, where each experiment consisted of three biological replicates ($n = 9$). Columns with different letters are significantly different according to a Duncan's multiple range test at $p < 0.05$. ns, no significance.

mold, caused by *Botrytis cinerea* and *Penicillium expansum*, respectively, in apples. Notably, the incidence of both molds in fruit treated with fresh yeast was approximately 50% lower than in the

controls, where disease incidence reached 100% for both pathogens. In contrast, the incidence of apple rot was significantly reduced in XT cells compared to the controls and H₂O₂ treatments (Figures 6A,C,E). Additionally, the lesion diameter on apples infected with *B. cinerea* and *P. expansum* was significantly smaller in fruit treated with XT cells than in the control and H₂O₂ groups (Figures 6B,D,F). These results demonstrate that *M. guilliermondii* effectively reduced the incidence and severity of blue mold and gray mold pathogens, with XT yeast cells further enhancing the level of control over the control treatments.

4 Discussion

Biocontrol agents used in postharvest disease management face challenges posed by various abiotic stresses in packing houses and postharvest environments, such as oxidative stress, elevated temperatures, nutrient deficiencies, and unfavorable pH levels. The ability of yeast to survive and remain active under these stresses is critical for improving their biocontrol efficacy (Wang et al., 2010; An et al., 2012). Since biocontrol yeasts are particularly vulnerable to oxidative conditions, their survival is significantly compromised under oxidative stress (Wang et al., 2018).

Xylitol, a sugar alcohol commonly used as a food additive, acts as an antioxidant, presenting a potential strategy to enhance the stress tolerance of yeasts (Kulikova-Borovikova et al., 2018; Wei et al., 2022). This study assessed the survival of yeast cultures treated with xylitol for 1 h under oxidative stress. The results demonstrate that xylitol

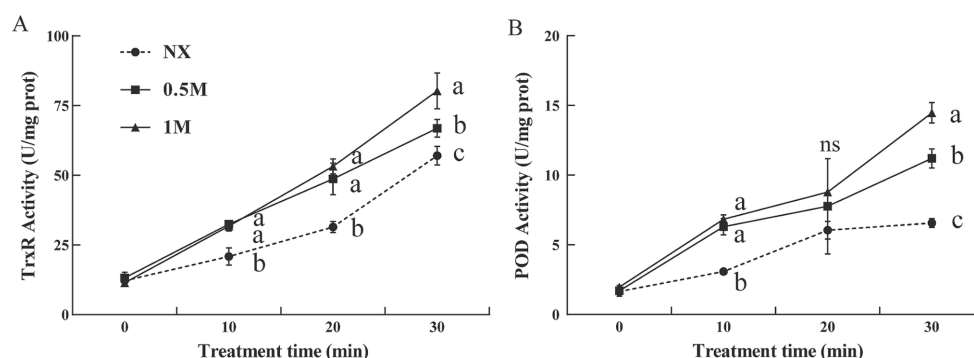


FIGURE 4

Thioredoxin reductase [(A), TrxR] and peroxidase [(B), POD] activity of *Meyerozyma guilliermondii* cells under H₂O or xylitol subjected to a subsequent oxidative (30 mM H₂O₂) stress for 10, 20, or 30 min. Prior to exposure to the subsequent oxidative stress served as Time 0. Data represent the mean $\pm$ standard deviation of three independent experiments, where each experiment consisted of three biological replicates ($n = 9$). Columns with different letters are significantly different according to a Duncan's multiple range test at $p < 0.05$. ns, no significance.

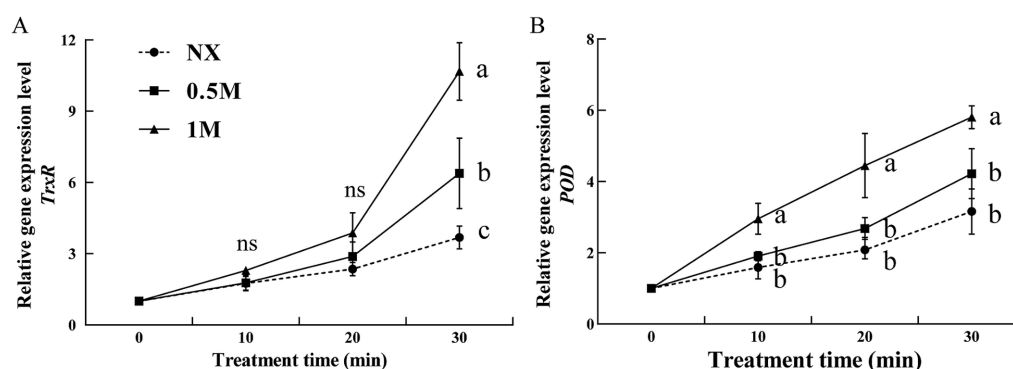


FIGURE 5

Two antioxidant genes [TrxR (A) and POD (B)] expression levels of *Meyerozyma guilliermondii* cells under H₂O or xylitol subjected to a subsequent oxidative (30 mM H₂O₂) stress for 10, 20, or 30 min. Prior to exposure to the subsequent oxidative stress served as Time 0. Data represent the mean $\pm$ standard deviation of three independent experiments, where each experiment consisted of three biological replicates ($n = 9$). Columns with different letters are significantly different according to a Duncan's multiple range test at $p < 0.05$. ns: no significance.

treatment showed a significant promoting effect on the survival rate of *M. guilliermondii* under oxidative stress, with higher xylitol concentrations leading to a more pronounced protective effect. The XT yeast cells exhibited higher survival rates, may be attributed to two potential mechanisms. Firstly, the xylitol treatment provides limited osmotic protection, which reduced oxidative stress-induced damage (Karlgrén et al., 2005). Secondly, the uptake of xylitol by yeast cells (Figure 3) may improve intracellular water utilization, thereby contributing to the resistance against oxidative stress (Krallish et al., 1997). It should be noted that the viability in this study was quantified as CFU, which may result in an underestimation of the actual level of viable cells. This is due to the fact that, following treatment, some yeast cells may have reduced vitality, preventing them from growing on the culture medium. However, these cells still exhibited detectable changes in enzyme activity and lower levels of ATP.

Previous research has demonstrated that exposure to oxidative stress can severely impair cell viability by increasing ROS production (Liu et al., 2011; Sui and Liu, 2014). The application of modified minimal mineral media, sugars, and sugar alcohols has been shown to mitigate the impact of high-temperature and oxidative stress in

biocontrol yeasts by reducing intracellular ROS levels and minimizing oxidative damage (Sui and Liu, 2014; Ming et al., 2020). In this study, yeast cells treated with xylitol (XT group) exhibited lower levels of ROS, mitochondria dysfunction and higher ATP levels compared to the control cells. The correlation between lower ROS production, mitochondrial membrane potential and improved cell viability under oxidative stress suggests that xylitol enhances the oxidative resistance of yeast used as biocontrol agents. Since xylitol is involved in the glycolysis and the pentose phosphate pathway (Bertels et al., 2021; Narisetty et al., 2022), the uptake of xylitol by the yeast may regulate sugar metabolism or act as energy source, thus maintaining ATP production (Figure 3).

Antioxidant gene expression in yeast is typically upregulated in response to various stressors, helping the cells cope with environmental challenges (Martínez-Pastor et al., 2010; Huang et al., 2021). It has been reported that POD and TrxR participate in the oxidative response and enzymatic detoxification of ROS (Wang et al., 2018; Sun et al., 2021). In this study, xylitol treatment resulted in increased expression of TrxR and POD in yeast cells exposed to oxidative stress, which may be effective in enhancing the antioxidative defense of yeasts. Moreover,

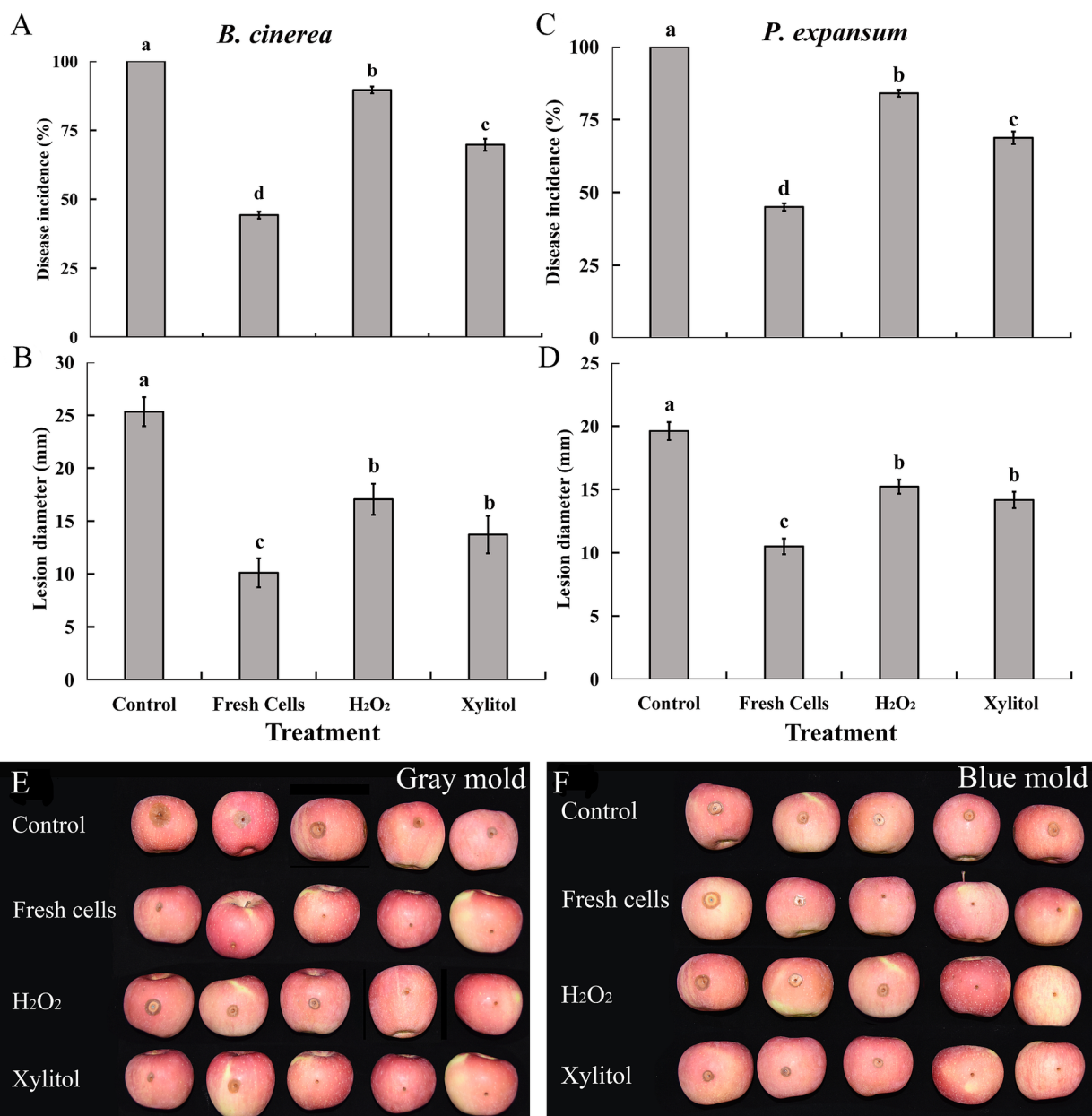


FIGURE 6 Biocontrol efficacy of *Meyerozyma guilliermondii* against gray mold caused by *Botrytis cinerea* (A,B), blue mold caused by *Penicillium expansum* (C,D) in apples. Panels (E,F) showing the efficacy of xylitol on *M. guilliermondii* against gray mold and blue mold. Data represent the mean $\pm$ standard deviation of three independent experiments, where each experiment consisted of three biological replicates ($n = 9$). Columns with different letters are significantly different according to a Duncan's multiple range test at $p < 0.05$.

the enzyme activities of TrxR and POD, both essential for ROS detoxification (Yan et al., 2018; Sun et al., 2021; Huang et al., 2022), were significantly higher in XT cells. TrxR catalyzes the reduction of thioredoxin, while POD protects cells from ROS damage, playing a key role in biocontrol yeast (Greetham and Grant, 2009; Gostimskaya and Grant, 2016; Sun et al., 2021). The enhanced activity of these enzymes, along with their increased gene expression, indicated improved ROS clearance and higher survival rates in the XT yeast cells. Oxidative stress has also been shown to cause mitochondrial damage (Sui and Liu, 2014; Ming et al., 2020). In this study, oxidative stress significantly decreased ATP levels in the control cells, whereas

xylitol treatment effectively mitigated this decline. The increase in survival, enhanced antioxidant enzyme activity, and reduction in ROS levels with xylitol treatment suggest a potential improvement in the biocontrol efficiency of yeast.

Xylitol, a widely utilized food additive, has been confirmed safe and healthy for consumption (Chen et al., 2010; Rice et al., 2020). Previous studies have indicated that under dehydration stress, yeasts such as *Saccharomyces cerevisiae* and *Pachysolen tannophilus* preferentially synthesize intracellular xylitol, which enhances their resistance to dehydration, with survival rates increased to 68% and 57%, respectively (Krallish et al., 1997). These findings underscore

the potential of xylitol in enhancing yeast stress tolerance. For instance, treatment with 1 M xylitol elevated the viability of dried *S. cerevisiae* cells to 70% under dehydration, while a 2% (approximately 0.13 M) xylitol treatment improved the survival rate of *Zygosaccharomyces rouxii* from 65% to 69% following a 20-min exposure to 40°C heat stress (Kulikova-Borovikova et al., 2018; Wei et al., 2022). In this study, we systematically examined the impact of xylitol treatment on biocontrol yeasts under oxidative stress. Given xylitol's involvement in the xylose metabolic pathway (Kumar et al., 2022), its absorption by yeast may enhance metabolic activity, thereby improving cell survival. Under oxidative stress induced by 30 mM H₂O₂, XT cells exhibited at least a 6% higher viability compared to previous reports. Furthermore, we demonstrated for the first time that xylitol treatment significantly mitigated oxidative damage in *M. guilliermondii*, with TrxR and POD activities increasing by at least 18 and 40%, respectively, leading to a 25% increase in ATP production. Notably, XT cells showed enhanced efficacy in reducing apple infection by *B. cinerea* and *P. expansum*.

High survival and growth of biocontrol yeasts on wounds and fruit surfaces provide them with a competitive edge in acquiring nutrients and space (Liu et al., 2013). XT yeast cells exhibited improved survival and biocontrol efficiency compared to untreated cells. Previous studies have also shown that pretreatment with protectants such as glucose and sorbitol can enhance the biocontrol efficacy of yeast (Sui et al., 2012; Sui and Liu, 2014; Ming et al., 2020). Lesion diameters were measured at their largest point, providing an indicator of the maximum extent of infection. Additionally, XT cells were more effective in reducing the incidence of gray mold and blue mold than the control, consistent with previous research, which demonstrated that pretreatments can improve stress tolerance and activate antioxidant defenses, leading to enhanced biocontrol efficiency (Liu et al., 2011; Chi et al., 2015).

5 Conclusion

This study demonstrated that xylitol treatment significantly enhances the tolerance and efficacy of *M. guilliermondii* under oxidative stress conditions. Xylitol treatment notably improved yeast survival rates, antioxidant enzyme activities and intracellular xylitol levels, thereby reducing the intracellular accumulation of ROS, mitochondrial dysfunction and preserving ATP levels during oxidative stress. The enhanced oxidative stress tolerance conferred by xylitol also contributed to improved biocontrol performance, as evidenced by reduced disease incidence and lesion diameter in apples subjected to pathogenic fungal infections. These findings underscore the potential of xylitol as an effective treatment to enhance the efficiency of biocontrol yeasts.

References

- An, B., Li, B., Qin, G., and Tian, S. (2012). Exogenous calcium improves viability of biocontrol yeasts under heat stress by reducing ROS accumulation and oxidative damage of cellular protein. *Curr. Microbiol.* 65, 122–127. doi: 10.1007/s00284-012-0133-4
- Bertels, L.-K., Fernández Murillo, L., and Heinisch, J. J. (2021). The pentose phosphate pathway in yeasts—more than a poor cousin of glycolysis. *Biomol. Ther.* 11:725. doi: 10.3390/biom11050725
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254. doi: 10.1016/0003-2697(76)90527-3
- Chen, X., Jiang, Z.-H., Chen, S., and Qin, W. (2010). Microbial and bioconversion production of D-xylitol and its detection and application. *Int. J. Biol. Sci.* 6, 834–844. doi: 10.7150/ijbs.6.834
- Chi, M., Li, G., Liu, Y., Liu, G., Li, M., Zhang, X., et al. (2015). Increase in antioxidant enzyme activity, stress tolerance and biocontrol efficacy of *Pichia kudriavzevii* with the transition from a yeast-like to biofilm morphology. *Biol. Control* 90, 113–119. doi: 10.1016/j.biocontrol.2015.06.006
- Droby, S., Wisniewski, M., Teixidó, N., Spadaro, D., and Jijakli, M. H. (2016). The science, development, and commercialization of postharvest biocontrol products. *Postharvest Biol. Technol.* 122, 22–29. doi: 10.1016/j.postharvbio.2016.04.006

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

WZ: Data curation, Formal analysis, Investigation, Software, Visualization, Writing – original draft. SZ: Conceptualization, Funding acquisition, Resources, Supervision, Validation, Writing – review & editing. JG: Data curation, Investigation, Software, Visualization, Writing – review & editing.

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- Espinosa-Salgado, R., Tamayo-Galván, V., Perraud-Gaime, I., Rodríguez-Serrano, G. M., González-Robles, R. O., Durand, N., et al. (2022). Polyols induce the production of antifungal compounds by *Lactobacillus plantarum*. *Curr. Microbiol.* 79:99. doi: 10.1007/s00284-022-02761-4
- Gostimskaia, I., and Grant, C. M. (2016). Yeast mitochondrial glutathione is an essential antioxidant with mitochondrial thioredoxin providing a back-up system. *Free Radic. Biol. Med.* 94, 55–65. doi: 10.1016/j.freeradbiomed.2016.02.015
- Greetham, D., and Grant, C. M. (2009). Antioxidant activity of the yeast mitochondrial one-cys peroxiredoxin is dependent on thioredoxin reductase and glutathione in vivo. *Mol. Cell. Biol.* 29, 3229–3240. doi: 10.1128/MCB.01918-08
- Huang, Y., Cai, Y., and Yu, T. (2022). Sodium glutamate as a booster: inducing *Rhodospiridium paludigenum* to enhance the inhibition of *Penicillium expansum* on pears. *J. Appl. Microbiol.* 132, 1239–1249. doi: 10.1111/jam.15212
- Huang, K., Sui, Y., Miao, C., Chang, C., Wang, L., Cao, S., et al. (2021). Melatonin enhances the resistance of ginger rhizomes to postharvest fungal decay. *Postharvest Biol. Technol.* 182:111706. doi: 10.1016/j.postharvbio.2021.111706
- Karlgrén, S., Pettersson, N., Nordlander, B., Mathai, J. C., Brodsky, J. L., Zeidel, M. L., et al. (2005). Conditional osmotic stress in yeast: A system to study transport through aquaglyceroporins and osmotic stress signaling. *J. Biol. Chem.* 280, 7186–7193. doi: 10.1074/jbc.M413210200
- Kim, S. M., Lee, S. M., Seo, J.-A., and Kim, Y.-S. (2018). Changes in volatile compounds emitted by fungal pathogen spoilage of apples during decay. *Postharvest Biol. Technol.* 146, 51–59. doi: 10.1016/j.postharvbio.2018.08.003
- Krallish, I., Jeppsson, H., Rapoport, A., and Hahn-Hägerdal, B. (1997). Effect of xylitol and trehalose on dry resistance of yeasts. *Appl. Microbiol. Biotechnol.* 47, 447–451. doi: 10.1007/s002530050954
- Kulikova-Borovikova, D., Lisi, S., Daus, E., Alamae, T., Buzzini, P., Hallsworth, J. E., et al. (2018). Activity of the α -glucoside transporter Agt1 in *Saccharomyces cerevisiae* cells during dehydration-rehydration events. *Fungal Biol.* 122, 613–620. doi: 10.1016/j.funbio.2018.03.006
- Kumar, K., Singh, E., and Shrivastava, S. (2022). Microbial xylitol production. *Appl. Microbiol. Biotechnol.* 106, 971–979. doi: 10.1007/s00253-022-11793-6
- Leaw, S. N., Chang, H. C., Sun, H. F., Barton, R., Bouchara, J.-P., and Chang, T. C. (2006). Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. *J. Clin. Microbiol.* 44, 693–699. doi: 10.1128/jcm.44.3.693-699.2006
- Li, B., Lai, T., Qin, G., and Tian, S. (2010). Ambient pH stress inhibits spore germination of *Penicillium expansum* by impairing protein synthesis and folding: A proteomic-based study. *J. Proteome Res.* 9, 298–307. doi: 10.1021/pr900622j
- Liu, J., Sui, Y., Wisniewski, M., Droby, S., and Liu, Y. (2013). Review: utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. *Int. J. Food Microbiol.* 167, 153–160. doi: 10.1016/j.ijfoodmicro.2013.09.004
- Liu, J., Sui, Y., Wisniewski, M., Droby, S., Tian, S., Norelli, J., et al. (2012a). Effect of heat treatment on inhibition of *Monilinia fructicola* and induction of disease resistance in peach fruit. *Postharvest Biol. Technol.* 65, 61–68. doi: 10.1016/j.postharvbio.2011.11.002
- Liu, J., Wisniewski, M., Droby, S., Norelli, J., Hershkovitz, V., Tian, S., et al. (2012b). Increase in antioxidant gene transcripts, stress tolerance and biocontrol efficacy of *Candida oleophila* following sublethal oxidative stress exposure. *FEMS Microbiol. Ecol.* 80, 578–590. doi: 10.1111/j.1574-6941.2012.01324.x
- Liu, J., Wisniewski, M., Droby, S., Vero, S., Tian, S., and Hershkovitz, V. (2011). Glycine betaine improves oxidative stress tolerance and biocontrol efficacy of the antagonistic yeast *Cystofilobasidium infirmominatum*. *Int. J. Food Microbiol.* 146, 76–83. doi: 10.1016/j.ijfoodmicro.2011.02.007
- Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262
- Macarasin, D., Droby, S., Bauman, G., and Wisniewski, M. (2010). Superoxide anion and hydrogen peroxide in the yeast antagonist–fruit interaction: A new role for reactive oxygen species in postharvest biocontrol? *Postharvest Biol. Technol.* 58, 194–202. doi: 10.1016/j.postharvbio.2010.07.008
- Martínez-Pastor, M., Profit, M., and Pascual-Ahuir, A. (2010). Adaptive changes of the yeast mitochondrial proteome in response to salt stress. *OMICS* 14, 541–552. doi: 10.1089/omi.2010.0020
- Ming, X., Wang, Y., and Sui, Y. (2020). Pretreatment of the antagonistic yeast, *Debaryomyces hansenii*, with mannitol and sorbitol improves stress tolerance and biocontrol efficacy. *Front. Microbiol.* 11:601. doi: 10.3389/fmicb.2020.00601
- Morón de Salim, A. R., and Ramírez Mérida, L. G. (2013). Bacteriostatic effect and/or xylitol bactericide of crops on *Listeria monocytogenes*. *Arch. Latinoam. Nutr.* 63, 173–179
- Narisetty, V., Cox, R., Bommarreddy, R., Agrawal, D., Ahmad, E., Pant, K. K., et al. (2022). Valorisation of xylose to renewable fuels and chemicals, an essential step in augmenting the commercial viability of lignocellulosic biorefineries. *Sustain. Energy Fuels* 6, 29–65. doi: 10.1039/D1SE00927C
- Rice, T., Zannini, E., Arendt, K., and Coffey, A. (2020). A review of polyols—biotechnological production, food applications, regulation, labeling and health effects. *Crit. Rev. Food Sci. Nutr.* 60, 2034–2051. doi: 10.1080/10408398.2019.1625859
- Romanazzi, G., Smilanick, J. L., Feliziani, E., and Droby, S. (2016). Integrated management of postharvest gray mold on fruit crops. *Postharvest Biol. Technol.* 113, 69–76. doi: 10.1016/j.postharvbio.2015.11.003
- Sadeghi, R., Aminian, H., Remize, F., Sheikh, M., and Ebrahimi, L. (2021). Integrated control of blue and gray molds of apples with antagonistic yeasts combined with carbon dioxide or ozone. *J. Plant Pathol.* 103, 943–953. doi: 10.1007/s42161-021-00891-5
- Spadaro, D., and Droby, S. (2016). Development of biocontrol products for postharvest diseases of fruit: the importance of elucidating the mechanisms of action of yeast antagonists. *Trends Food Sci. Tech.* 47, 39–49. doi: 10.1016/j.tifs.2015.11.003
- Sui, Y., and Liu, J. (2014). Effect of glucose on thermotolerance and biocontrol efficacy of the antagonistic yeast *Pichia guilliermondii*. *Biol. Control* 74, 59–64. doi: 10.1016/j.biocontrol.2014.04.003
- Sui, Y., Liu, J., Wisniewski, M., Droby, S., Norelli, J., and Hershkovitz, V. (2012). Pretreatment of the yeast antagonist, *Candida oleophila*, with glycine betaine increases oxidative stress tolerance in the microenvironment of apple wounds. *Int. J. Food Microbiol.* 157, 45–51. doi: 10.1016/j.ijfoodmicro.2012.04.010
- Sui, Y., Wisniewski, M., Droby, S., and Liu, J. (2015). Responses of yeast biocontrol agents to environmental stress. *Appl. Environ. Microbiol.* 81, 2968–2975. doi: 10.1128/AEM.04203-14
- Sun, K., Wang, Z., Zhang, X., Wei, Z., Zhang, X., Li, L., et al. (2021). Enhancement of biocontrol efficacy of *Pichia kudriavzevii* induced by ca ascorbate against *Botrytis cinerea* in cherry tomato fruit and the possible mechanisms of action. *Microbiol. Spectr.* 9, e01507–e01521. doi: 10.1128/spectrum.01507-21
- Vilanova, L., Vall-Iaura, N., Torres, R., Usall, J., Teixidó, N., Larrigaudière, C., et al. (2017). *Penicillium expansum* (compatible) and *Penicillium digitatum* (non-host) pathogen infection differentially alter ethylene biosynthesis in apple fruit. *Plant Physiol. Biochem.* 120, 132–143. doi: 10.1016/j.plaphy.2017.09.024
- Wang, Y., Luo, Y., Sui, Y., Xie, Z., Liu, Y., Jiang, M., et al. (2018). Exposure of *Candida oleophila* to sublethal salt stress induces an antioxidant response and improves biocontrol efficacy. *Biol. Control* 127, 109–115. doi: 10.1016/j.biocontrol.2018.09.002
- Wang, Y., Wang, P., Xia, J., Yu, T., Lou, B., Wang, J., et al. (2010). Effect of water activity on stress tolerance and biocontrol activity in antagonistic yeast *Rhodospiridium paludigenum*. *Int. J. Food Microbiol.* 143, 103–108. doi: 10.1016/j.ijfoodmicro.2010.07.035
- Wei, Y., Yan, Z., Liu, M., Chen, D., Chen, X., and Li, X. (2022). Metabolic characteristics of intracellular trehalose enrichment in salt-tolerant *Zygosaccharomyces rouxii*. *Front. Microbiol.* 13:935756. doi: 10.3389/fmicb.2022.935756
- Wisniewski, M., Droby, S., Norelli, J., Liu, J., and Schena, L. (2016). Alternative management technologies for postharvest disease control: the journey from simplicity to complexity. *Postharvest Biol. Technol.* 122, 3–10. doi: 10.1016/j.postharvbio.2016.05.012
- Yan, Y., Zhang, X., Zheng, X., Apaliya, M. T., Yang, Q., Zhao, L., et al. (2018). Control of postharvest blue mold decay in pears by *Meyerozyma guilliermondii* and its effects on the protein expression profile of pears. *Postharvest Biol. Technol.* 136, 124–131. doi: 10.1016/j.postharvbio.2017.10.016



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Innovative probiotic fermentation approach for zearalenone detoxification in dried distiller's grains

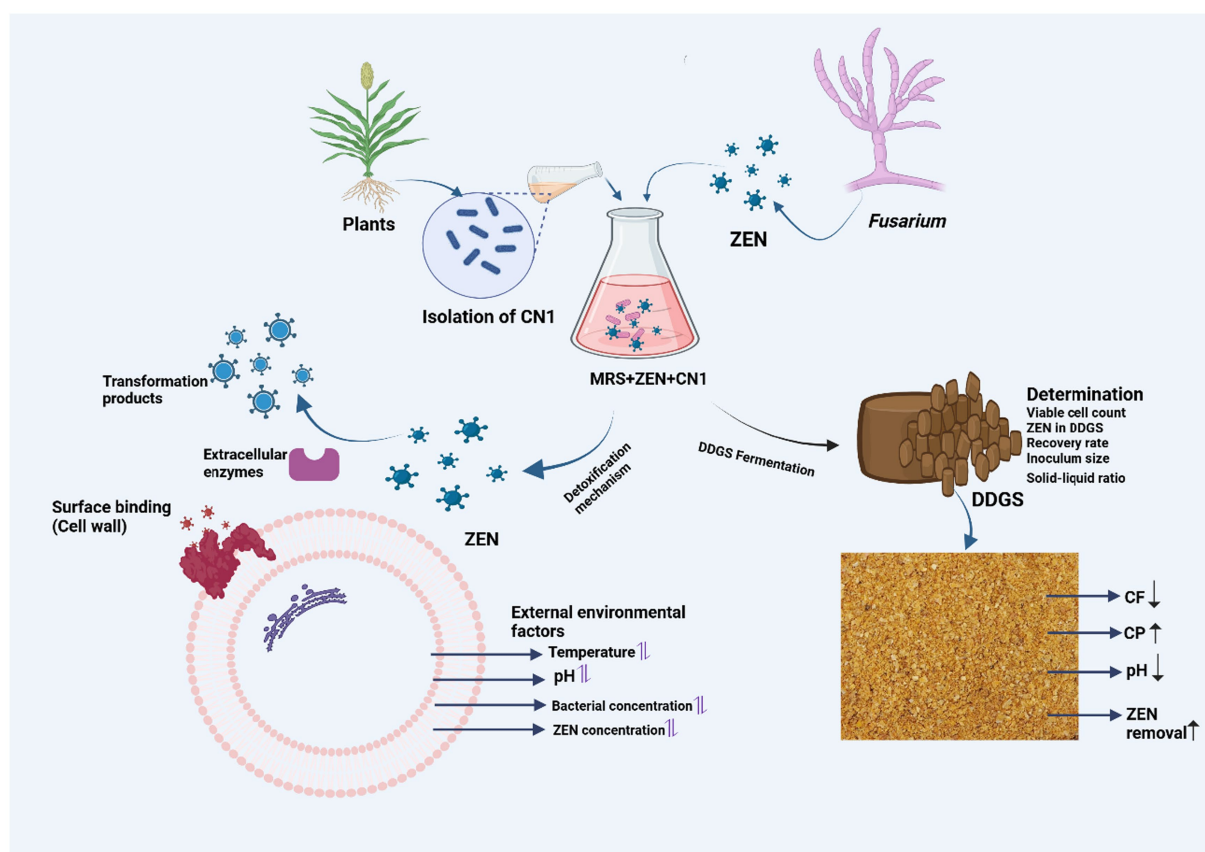
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Zearalenone (ZEN) contamination in dried distiller's grains and solubles (DDGS) poses serious health risks and economic losses in animal farming. This study aimed to evaluate the effectiveness of probiotic fermentation using *Lactobacillus plantarum* CN1 in detoxifying ZEN and optimizing fermentation conditions for maximum efficiency. *L. plantarum* CN1, identified with 99% genetic homology, was used for DDGS fermentation. The detoxification mechanism was analyzed through adsorption assays, post-heat treatment effects, and scanning electron microscopy (SEM). ZEN removal was assessed over 72 h under various conditions, including bacterial concentration, temperature, and pH optimization. The results showed that CN1 achieved a maximum ZEN removal rate of 69% within 72 h, with an optimized efficiency of 75.6% at 4×10^9 CFU/mL. Over 60% of ZEN was adsorbed by the bacterial cell wall, while removal in the fermentation supernatant and intracellular fluid remained below 5%. Scanning electron microscopy (SEM) analysis highlighted structural changes in the bacterial cells, particularly elongation and thinning, with more pronounced cell damage observed following heat and ZEN treatment. These modifications may explain the varying adsorption efficiencies observed. Heat treatment, particularly autoclaving, significantly enhanced adsorption efficiency to 82.9%, whereas acid and alkali treatments reduced it. Fermentation also improved the nutritional quality of DDGS, increasing crude protein by 7.16%, reducing crude fiber by 0.65%, and lowering pH to 4.3. These findings demonstrate that probiotic fermentation with CN1 offers a promising, cost-effective strategy for mitigating ZEN contamination while enhancing DDGS quality. Future studies should explore large-scale applications and the potential of CN1 in multi-mycotoxin detoxification to further improve feed safety.

KEYWORDS

feed safety, zearalenone mitigation, probiotic fermentation, DDGS, fungal toxin



GRAPHICAL ABSTRACT

1 Introduction

Distiller's dried grains with solubles (DDGS) is a high-quality protein feed derived from the fuel ethanol industry, which has grown significantly in recent years (Pont et al., 2023). The production of corn DDGS has increased, but it often contains high levels of mycotoxins, posing serious health risks. Mycotoxins are toxic secondary metabolites produced by fungi such as *Aspergillus*, *Penicillium*, and *Fusarium* under specific environmental conditions (Mohammadi Shad et al., 2021). Approximately 700 mycotoxins have been identified, with around 30 posing significant health threats to humans and animals, including carcinogenicity, immunotoxicity, and intestinal toxicity (Murtaza et al., 2024d; Sulyok et al., 2024).

Zearalenone (ZEN) is primarily produced by several species of *Fusarium*, which are known to contaminate cereals and other agricultural products (Murtaza et al., 2025). ZEN has a structure similar to 17- β -estradiol, giving it estrogenic properties that cause reproductive issues. ZEN binds to cytosolic estrogenic receptors in the uterus and mammary glands, leading to adverse effects such as pig ovarian atrophy, infertility, and abortion (Yang et al., 2021; Murtaza et al., 2022). Additionally, ZEN causes reproductive and cellular deformities, including atypical ovaries, abnormally shaped rat sperm, hypertrophied ovaries in sows, faulty embryos in zebrafish, and atrophy of seminiferous tubules (Murtaza et al., 2024a; Zhu et al., 2021). Because of these possible risks, ZEN contamination in food and animal feed must be addressed (Cai et al., 2022).

As a result of the danger that ZEN pollution poses, a great deal of study has gone into developing chemical, physical, and biological detoxification procedures. There are several physical and chemical techniques for removing ZEN, some of which include extrusion, grinding, washing, the adsorption of ozone, and hydrogen peroxide treatments (Wang et al., 2022; Ding et al., 2024). But there are certain drawbacks to these methods as well, such as significant nutrient losses and costly equipment and maintenance costs (Wang Y. et al., 2024). Because of its greater efficacy, selectivity, and little environmental impact, the biological detoxification strategy is strongly advised. Diverse bacterial and fungal species, such as *Bacillus* species (Wang et al., 2018b; Shi et al., 2024), *Lactobacillus plantarum* strain (Adunphatcharaphon et al., 2021; Murtaza et al., 2022), and *Rhizopus arrhizus* (Ahmed et al., 2024) have exhibited the capability for ZEN degradation. However, it is imperative to acknowledge that ZEN degradation may not culminate in complete detoxification, potentially giving rise to more estrogenic derivatives, including α -zearalenol (α -ZAL) and β -zearalenol (β -ZAL; Murtaza et al., 2023b, 2024b). Therefore, achieving comprehensive ZEN detoxification requires a multifaceted process involving multiple enzymes and new strains.

Thus, *Lactobacillus*, which is renowned for its resilience, was evaluated for its potential to lower ZEN levels in order to get a thorough understanding of ZEN elimination in different *Lactobacillus* species. This study aimed to address the critical gap in identifying effective probiotic strains capable of detoxifying ZEN in DDGS, a

challenge that poses significant health risks and economic losses in animal feed production. Current methods for ZEN detoxification are often inefficient or costly, highlighting the need for innovative biological approaches. To bridge this gap, we focused on isolating and characterizing a new ZEN-removing probiotic strain, *Lactobacillus plantarum* CN1. Our *in vitro* research assessed the detoxification capacity of this strain under various conditions, including different ZEN concentrations, incubation pH, temperatures, and the influence of the bacterial cell wall. We specifically aimed to evaluate the adsorption mechanisms of CN1, optimize the fermentation medium, and explore the combined use of probiotics to enhance the nutritional value of DDGS while reducing its mycotoxin content.

2 Materials and methods

2.1 Chemicals and culture media

Zearalenone (ZEN) standard and De Man, Rogosa, and Sharpe agar (MRS) from Merck (Darmstadt, Germany) were used to culture the plant-derived bacteria. Chemicals such as NaCl, KCl, Na_2HPO_4 , and KH_2PO_4 were supplied by Carlo Erba Reagents (Bangkok, Thailand). ZEN (99%) was obtained from TRC (Canada). ZEN detection kits were provided by Heilongjiang Wanlird Biotechnology Co., Ltd., and Qingdao Pribolab Biological Engineering Co., Ltd. DDGS was purchased from Heilongjiang Wanli Runda Biotechnology Co., Ltd. Other chemicals used included hydrochloric acid from Beijing Chemical Plant and sodium hydroxide from Shanghai McLean Biochemical Technology Co., Ltd.

MRS medium: MRS medium was prepared by dissolving the appropriate amount of MRS powder in distilled water, as per the manufacturer's instructions. The mixture was heated and stirred until fully dissolved. The pH was adjusted to 6.2–6.6 using a pH meter, and the medium was sterilized by autoclaving at 121°C for 15 min. After sterilization, the medium was allowed to cool to room temperature before use.

Inorganic salt medium (MM): Inorganic salt medium (MM) was prepared by dissolving 1 g of $(\text{NH}_4)_2\text{SO}_4$, 0.2 g of MgSO_4 , 0.1 g of CaCl_2 , 0.5 g of NaH_2PO_4 , and 0.5 g of KH_2PO_4 in distilled water to a final volume of 1,000 mL. The pH was adjusted to 7.0, and the medium was autoclaved at 121°C for 25 min. For solid culture, 2% agar was added before sterilization. The preparation of this medium was based on the protocols described by Wang et al. (2018a).

2.2 Screening of anaerobic strains

Dissolve 1 g of each of the 18 purchased lactic acid bacteria products in 4 mL of PBS buffer and vortex for 1 min. Inoculate 1 mL of this suspension into 10 mL of MRS liquid culture medium and incubate anaerobically at 37°C for 24 h. Following incubation, dilute the culture 100-fold with PBS and spread it onto MRS medium plates. Place the plates in an anaerobic bag and incubate at 37°C for 24 h. Select single colonies of different shapes from the plates and streak them onto fresh MRS solid medium plates for further culturing. After multiple purification steps, when the colonies exhibit consistent growth patterns, select single colonies for preservation. For

enrichment, inoculate the selected single colonies into 10 mL of MRS liquid medium and incubate anaerobically at 37°C for 24 h. Centrifuge the culture at 4°C at $7168 \times g$ for 10 min, discard the supernatant, and resuspend the bacterial cells in MM liquid medium, adjusting the volume to 5 mL. Inoculate 0.5 mL (10% of the inoculum volume) into 0.5 mL of rescreening medium and incubate on a shaker at 37°C and $160 \times g$ for 72 h. Adjust the volume to 1 mL with MM liquid culture medium, centrifuge at $9775 \times g$ for 10 min, and filter the supernatant through a 0.22 μm filter membrane. Prepare samples for HPLC detection from the filtered supernatant.

2.3 Morphological observation and 16S rDNA sequencing

After the purification process, streaks of the chosen bacterial strains were applied onto MRS solid culture media plates. These plates underwent static incubation in a 37°C constant-temperature incubator for 12 and 24 h, respectively. Throughout the incubation period, close observation was carried out to record the characteristics of bacterial colonies, encompassing morphology, color, size, and texture. Single colonies demonstrating unique features were carefully chosen for subsequent analysis. Gram staining techniques were then utilized to categorize the bacterial strains according to their cell wall composition, followed by microscopic examination to evaluate cell morphology and arrangement.

Bacterial DNA was isolated using a simplified boiling method. Briefly, 1 mL of bacterial suspension, with concentrations ranging from 10^8 to 10^9 CFU/mL, was subjected to centrifugation at $8000 \times g$ for 2 min to pellet the cells. The supernatant was discarded, and the pellet was washed by resuspension in sterile deionized water, followed by a second centrifugation step under the same conditions. The resulting pellet was then resuspended in 100 μL of sterile deionized water and incubated at 100°C for 10 min to facilitate cell lysis. After heat treatment, the suspension was centrifuged again at $8000 \times g$ for 2 min, and the resulting supernatant, containing crude genomic DNA, was used as the template for subsequent analysis (Wang et al., 2020). The targeted gene amplified via PCR is the 16S rRNA gene, utilizing the universal primer pair: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTACGACTT-3'). The PCR amplification program comprises a pre-denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 90 s. Finally, a single extension step is carried out at 72°C for 10 min. Subsequently, the resultant PCR products are sent to Shanghai, Sangon, for sequencing. The sequencing outcomes are then compared through Blast in NCBI to analyze their homology. Using MEGA 11 software, the identified homologous sequences are aligned with the target sequences, employing the neighbor-joining method for sequence alignment and homology analysis. Finally, a phylogenetic tree is constructed to elucidate the evolutionary relationships among the examined bacterial strains.

2.4 High-performance liquid chromatography and determination of adsorption percentage (ADS%)

The HPLC analysis of ZEN-containing materials was conducted following the method described by Kim and Vujanovic (2017). The

centrifuged supernatant (0.5 mL) was diluted with HPLC-grade methanol, processed for 20 min, and filtered through a 0.22 µm organic membrane filter before analysis. ZEN levels were quantified using an Agilent 1,260 Infinity II HPLC system (Agilent Technologies, China) equipped with a fluorescence detector (FLD) and an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 250 mm, 5 µm). The operational column temperature was 30°C, with a mobile phase composition of acetonitrile/water (40:60, v/v) and a flow rate of 1.0 mL/min. The sample injection volume was 15 µL, and detection was carried out at an excitation wavelength of 236 nm and an emission wavelength of 450 nm. The limit of detection (LOD) and limit of quantification (LOQ) were determined as 0.02 ppm and 0.06 ppm, respectively. Calibration was performed using a standard ZEN solution prepared in methanol, with a five-point calibration curve ($R^2 > 0.999$) ensuring accuracy. The adsorption amount was determined by comparing the peak areas of ZEN in the post-adsorption supernatant to those in the control solution. The ZEN adsorption efficiency (ADS%) was calculated using Equation 1 (Wei et al., 2019).

As expressed in the following formula:

$$ADS = 100 - AUC1 / AUC2 \times 100. \quad (1)$$

Where:

ADS represents the adsorption percentage.

AUC2 represents the area where the ZEN peaks in the supernatant.

AUC1 is used to describe the region that contains the ZEN peak in the control positive.

2.5 ZEN removal and adsorption by CN1 strain

The CN1 bacteria were initially cultured on MRS agar for 48 h at 37°C. Individual colonies were then transferred into 5 mL of MRS culture medium containing ZEN at a concentration of 10 µg/mL and incubated for 72 h at 37°C. The ZEN-degrading strain was subsequently preserved in glycerol (25%) and stored at -80°C until further use. For the experiment, the strain was inoculated into 30 mL of MRS broth and incubated in a shaker incubator (ZWY-211B, LABWIT Scientific Pty. Ltd., Australia) at 37°C and 150 × g for 48 h. Following incubation, the cells and supernatants were separated via centrifugation at 7168 × g for 20 min at 4°C. The supernatants were then analyzed for residual ZEN content. Additionally, isolated cells were disrupted using an ultrasonic disruptor after resuspension in PBS. The disrupted cells were centrifuged, the supernatant was discarded, and the resulting broken residue (bacterial cell wall) was preserved. Subsequently, the inactivated bacteria solution and broken residue were incubated with 10 µg/mL ZEN for 72 h at 37°C and 150 × g, and ZEN levels were detected using HPLC.

2.6 ZEN removal by CN1 strain post-acid and heat treatments

After culturing CN1 cells at 37°C, the cells were extracted and mixed with 2 M HCl and phosphate-buffered saline (PBS, pH 7.0, 1 M) for 1 h. The final pH of the acid-treated suspension reached approximately pH 1.5, while the alkali-treated samples reached approximately pH 12.5. Subsequently, each pellet was autoclaved at

121°C for 15 min. The cells were then centrifuged at 5488 × g for 5 min to remove them. To achieve the desired concentration, bacteria at 4×10^9 (CFU/mL) were suspended in 5 mL of PBS (pH 7.0). A 1 mL sample was taken from this suspension and centrifuged at 5488 × g for 8 min at 4°C before being analyzed using HPLC.

2.7 ZEN removal by CN1 under varying conditions

After cultivating CN1 cells, a concentration of 4×10^9 CFU/mL of bacterial cells was added to 5 mL of MRS broth with a pH of 7.0, along with varying concentrations of ZEN (2.5, 5, 8.5, and 200 µg/mL). This mixture was then incubated at 37°C for 72 h with agitation at 150 × g. After incubation, 1 mL samples were taken and centrifuged at 5488 × g for 10 min at 4°C. CN1 cells were also introduced into 5 mL of MRS broth with varying pH levels (3.0, 6.0, 7.0, and 8.0) containing 10 µg/mL ZEN to reach a concentration of 4×10^9 CFU/mL. These samples were incubated for 72 h at 37°C with agitation at 150 × g. ZEN levels were measured using HPLC after centrifuging 1 mL samples at 5488 × g for 10 min at 4°C. Before testing, the MRS broth was brought to temperatures of 4, 25, 37, and 42°C. CN1 cell density reached 4×10^9 CFU/mL in 5 mL of LB broth containing 10 µg/mL ZEN. Each sample was agitated for 72 h at 4, 25, 37, and 42°C with agitation at 150 × g. After incubation, toxin concentrations were determined using HPLC following centrifugation of 1 mL samples at 5488 × g for 10 min at 4°C. After 72 h of cultivation, CN1 cells were introduced into 10 mL of MRS broth with a pH of 7.0 containing 10 µg/mL of ZEN and different bacterial concentrations (4×10^9 , 2×10^9 , 4×10^8 , and 2×10^8 CFU/mL). These mixtures were continuously agitated for 3 d at 150 × g and 37°C. Following centrifugation of the samples at 5488 × g for 10 min at 4°C, the concentrations of ZEN were assessed using HPLC. In a shaker operating at 37°C and 150 × g for 48 h, CN1 cells were added to 30 mL of MRS broth. After incubation, the bacterial cells were separated from the culture supernatant by centrifugation at 5488 × g for 8 min at 4°C. The isolated cells in PBS were then disrupted using ultrasonic waves, and the resulting mixture was centrifuged again to separate the bacterial cell wall residue. The ZEN concentration in the culture supernatant, disrupted supernatant, and residual cell wall was maintained at 10 µg/mL. ZEN levels were measured using HPLC after 72 h in the shaker at 37°C and 150 × g.

2.8 Scanning electron microscopy analysis

The Scanning Electron Microscope (SEM) analysis was conducted to investigate the morphological changes in bacterial samples, specifically strain CN1 treated with ZEN and heat. The analysis was performed using a NOVA NANOSEM-450 scanning electron microscope (FEI, United States). The bacterial pellets, subjected to different treatments, were fixed using a modified Karnofsky's solution containing 1.6% glutaraldehyde and 2.6% paraformaldehyde in a 0.1 M sodium phosphate buffer (pH 7.2). After a 24-h incubation at 4°C, the pellets were dehydrated through a series of ethyl alcohol and acetone solutions with 10-min intervals between each concentration. The ethyl alcohol concentrations used were 30, 50, 70, 90, and 100%, with 10-min breaks between each stage. Subsequently, the samples were dried, gold-coated using an ion sputter JFC-1100 (JEOL, Japan), and secured to

stubs using carbon tape. SEM images were acquired to examine bacterial morphology and elemental composition (Ge et al., 2017).

2.9 Determination of strain tolerance to ZEN

The CN1 strain was cultured and enriched, adjusting bacterial concentrations to 1×10^9 CFU/mL before inoculating them into MRS culture media with a 1% inoculation volume and a 1 mL culture system. Each reaction space in a centrifuge tube was 1.5 mL, and various ZEN concentrations from 0 to 100 µg/mL were prepared. After thorough mixing, 200 µL of each sample was dispensed into a 96-well plate and incubated in a microplate reader at 37°C with shaking for 18 h. Measurements were taken every hour at a wavelength of 600 nm, allowing for the continuous monitoring of bacterial growth and activity in response to different ZEN concentrations.

2.10 Effects of different treatment methods on ZEN adsorption by CN1 strain

To assess the potential of CN1 cells for ZEN removal via cell wall adsorption, the cells were deactivated using various methods. Following deactivation, the cells were collected through centrifugation, washed twice with PBS, and adjusted to a bacterial concentration of 1×10^{10} CFU/mL. The washed cells were divided into five groups for different treatments: A. Autoclaving: Cells were autoclaved at 121°C for 20 min. B. Water bath: Cells were subjected to a 100°C water bath for 40 min. C. Acid treatment: Cells were treated with 2 mL of 1 M HCl at 37°C with shaking at $160 \times g$ for 1 h. D. Alkali treatment: Cells were treated with 2 mL of 1 M NaOH at 37°C with shaking at $160 \times g$ for 1 h. E. Control group: No treatment was administered to this group. The treated bacterial cells from each group were centrifuged at $5488 \times g$ and 4°C for 10 min, followed by resuspension in 2 mL of MM liquid culture medium. ZEN was introduced into each suspension to achieve a concentration of 20 µg/mL. These suspensions were then placed on a shaker at 37°C with agitation at $160 \times g$ for 72 h. After the incubation period, the suspensions underwent centrifugation at $5488 \times g$ for 10 min at 4°C, and the resulting supernatant was filtered through a 0.22 µm filter to prepare the samples. HPLC analysis was employed to detect the concentration of ZEN in the samples. A control group with a ZEN solution of the same concentration but without added bacteria was included for comparison, and each group underwent triplicate testing to ensure statistical validity.

2.11 Adsorption stability of CN1 strain to ZEN

After the activation of CN1 cells, they were centrifuged at 4°C, $5488 \times g$ for 10 min to gather them. Following that, the cells underwent two washes with PBS buffer to eliminate any potential interference from the culture medium. Next, the cells were suspended in an MM culture medium with a final concentration of 20 µg/mL ZEN. This suspension was then placed in a shaker at $160 \times g$ and incubated at 37°C for 24 h. Following the incubation period, the

suspension underwent centrifugation at $5488 \times g$ for 10 min, and the resulting supernatant was sampled for testing. The pellets were resuspended with 1 mL of MM culture medium and divided into three groups for further treatment. Group A underwent incubation at 80°C for 10 min, group B was vortexed for 5 min, and group C was treated with 1 mL of 95% ethanol and incubated at 37°C for 10 min. Post-treatment, each group underwent centrifugation at $5488 \times g$ for 10 min, and the supernatant of each group was collected. These collected supernatants were then passed through a 0.22 µm filter membrane and subjected to HPLC analysis to determine the desorption rate. The desorption rate (M) was calculated using the formula: $M = (B/B_0) \times 100\%$, where B_0 represents the adsorption amount of CN1 bacteria and B is the ZEN content in the supernatant after treatment.

2.12 Determination of nutritional components of fermented DDGS

2.12.1 Fermentation culture process and crude protein determination

Under sterile conditions, transfer the fermentation product into a zip-lock bag with a one-way air valve and inoculate with 5% CN1. Seal the bag using a heating plastic sealing machine and incubate anaerobically for 14 d. Based on the findings in Section 2.7, maintain the fermentation temperature at 37°C and the initial pH at 6.5 for both strains. The determination of crude protein was performed following the guidelines outlined in the GB/T 6432–2018 standard, utilizing a Kjeldahl nitrogen analyzer. In this method, a 1.0 g sample was digested with a mixture of sulfuric acid, potassium sulfate, and copper sulfate. Subsequently, the nitrogen compounds were distilled, converting them to ammonium sulfate, which was then released as ammonia and absorbed in boric acid. The ammonia was titrated with hydrochloric acid using methyl red and bromocresol blue indicators. The crude protein content was calculated by multiplying the nitrogen content by 6.25. The digestion process involved boiling at 150°C for 30 min, followed by heating at 280°C for another 30 min, and finally at 380°C for 2.5 h. Each experiment was conducted in triplicate, including a blank control group for comparison.

2.12.2 Crude fiber and pH determination

The determination of crude fiber followed the GB/T 6434–2006 standard protocol. Initially, the sample was weighed and subjected to digestion with acid and alkali solutions. Ether and acetone were then used to eliminate ether-soluble components. High-temperature burning was employed to remove minerals, leaving behind the crude fiber content for measurement. This analytical procedure was conducted at the Animal Nutrition and Feed Research Institute in Changchun. Specifically, 10 g of the sample were mixed with 100 mL of ultrapure water, stirred for 10 min with a magnetic stirrer, and filtered through gauze. The pH of the filtrate was measured using a pH meter as part of the process.

2.12.3 ZEN determination by ELISA assay

The indirect competitive ELISA method involves pre-coating a ZEN antigen on an enzyme-labeled microplate. ELISA was selected as a practical and validated method suitable for rapid, high-throughput screening in complex feed matrices. The primary goal was to compare

ZEN levels between treated and untreated DDGS samples, rather than to obtain absolute quantification. For sample preparation, 5 g of fermented DDGS are weighed into a 50 mL centrifuge tube and mixed with ultrapure water. After vortexing for 10 min, the sample is sonicated for 30 min and then centrifuged at $1792 \times g$. A 100 μ L portion of the clear liquid is taken, mixed with 400 μ L of ultrapure water, and prepared as the sample to be tested. To verify the recovery rate, the pre-fermentation sample is weighed and then spiked with standard ZEN solutions to achieve concentrations of 1 μ g/mL, 5 μ g/mL, and 10 μ g/mL in the fermentation medium. Three replicates are done for each concentration without adding microbial agents. The same sample preparation steps are followed as in the fermentation process, and statistical methods are used to calculate the ZEN content and recovery rate in the samples.

2.13 Statistical analysis

The experiments were conducted with three technical replicates each. The mean standard error ($X \pm SE$) for all statistical analyses was calculated using GraphPad Prism. Group mean differences were assessed using the one-way analysis of variance (ANOVA), with significance denoted by $p < 0.05$. In the DDGS with ZEN study, data processing and analysis were performed using Origin2023.

3 Results and discussion

3.1 Screening of anaerobic strains

The ZEN removal ability of 10 strains of lactic acid bacteria from different probiotic products was studied using a re-screening method. It was found that 5 strains had a significant effect on ZEN removal, as shown in Figure 1A. The strain with the highest removal ability, LP (CN1), achieved a 69% removal rate, while the strain with the lowest, LP4, had a removal rate of only 9.1%. Strains LP2, LP3, and LP5, with removal rates greater than 40%, were selected for further research. During the initial screening, none of the 16 lactic acid bacteria strains could grow on inorganic salt medium plates with ZEN as the sole carbon source. However, 5 strains demonstrated removal effects in MM medium liquid with ZEN as the sole carbon source. Current research indicates that most lactic acid bacteria cannot use ZEN for growth, and toxin removal is primarily due to the physical adsorption by the cell wall, reducing toxin concentration. The specific degradation mechanisms of ZEN by each strain will be studied in subsequent experiments.

3.2 Morphological and molecular characteristics of strains

The identification of the CN1 strain through colony characteristics, Gram staining (Figure 1C), and 16S rDNA sequencing is a standard approach that aligns with methods used in previous studies for microbial identification. The description of the CN1 strain colonies, with a milky white center, translucent edges, and smooth, moist surfaces with ridges, is consistent with typical descriptions of *Lactobacillus plantarum* colonies

(Figure 1B). Ricciardi et al. (2015) described similar morphological characteristics in their identification of *Lactobacillus plantarum* strains isolated from fermented foods. The amplification of the 16S rDNA conserved sequence and subsequent phylogenetic analysis using tools like MEGA11 further validate the identification process. Kim and Vujanovic (2017) utilized 16S rDNA sequencing to accurately identify and classify *Lactobacillus* strains from various environments. The phylogenetic tree constructed to identify the LP strain as *Lactobacillus plantarum* CN1 provides robust support for its classification (Figure 1D). This approach is corroborated by the study of Seddik et al. (2017), who also employed phylogenetic analysis to distinguish between closely related *Lactobacillus* species, emphasizing the reliability of this method. According to the Ministry of Agriculture of the People's Republic of China's Announcement No. 2045 in the "Feed Additive Catalog (2013)," *Lactobacillus plantarum* CN1 is approved for use as a feed additive. This regulatory context ensures that the strain meets the safety and efficacy standards required for inclusion in animal feed.

3.3 ZEN degradation and adsorption capacity of CN1 strain

This research is the first to investigate the mycotoxin-reducing potential of vegetative plant-derived *Lactobacillus* strains, which are utilized in a variety of fermented foods traditionally prepared throughout Asia and Southeast Asia. The ability of the CN1 strain to remove ZEN within 72 h was evaluated. Initially, the removal rate of ZEN by CN1 exceeded 60% within the first 12 h. This rate remained relatively stable between 12 and 72 h, ultimately reaching a maximum removal rate of 69% at 72 h (Figure 2b). Microorganisms employ processes such as ring cleavage, acetylation, deamination, and hydrolysis to biodegrade or transform mycotoxins (Hathout and Aly, 2014; Li et al., 2021; Murtaza et al., 2024c). The adsorption mechanism is likely governed by non-covalent interactions between ZEN hydrophobic backbone and functional groups present on the bacterial cell wall, such as peptidoglycan, surface-layer proteins, and teichoic acids. These components offer binding sites that facilitate hydrogen bonding and hydrophobic interactions, consistent with previous findings on lactic acid bacteria mycotoxin interactions (Wang X. et al., 2024). Bentonite and other clay-based adsorbents have shown 97% ZEA removal *in vitro* but often face limitations in specificity and nutrient binding (Ahn et al., 2022). Similarly, yeast cell walls, especially those enriched in β -glucans and mannoproteins, offer ZEA adsorption rates of around 68%, depending on the strain and processing conditions (Joannis-Cassan et al., 2011). In contrast, CN1 achieved a removal efficiency of 69% under optimal conditions in our study, which is comparable to or better than many of these agents. Previous research indicates that lactic acid bacteria, such as *Lactobacillus plantarum*, primarily degrade toxins through physical adsorption mechanisms (Vega et al., 2017). During the adsorption process, physical binding between the bacterial cell wall and the toxin can be somewhat unstable, leading to fluctuations in the adsorption rate. This instability might account for the observed variations in the removal efficiency over time. Pourmohammadi et al. (2022) demonstrated that the adsorption of mycotoxins by lactic acid bacteria could be influenced by environmental factors such as pH and

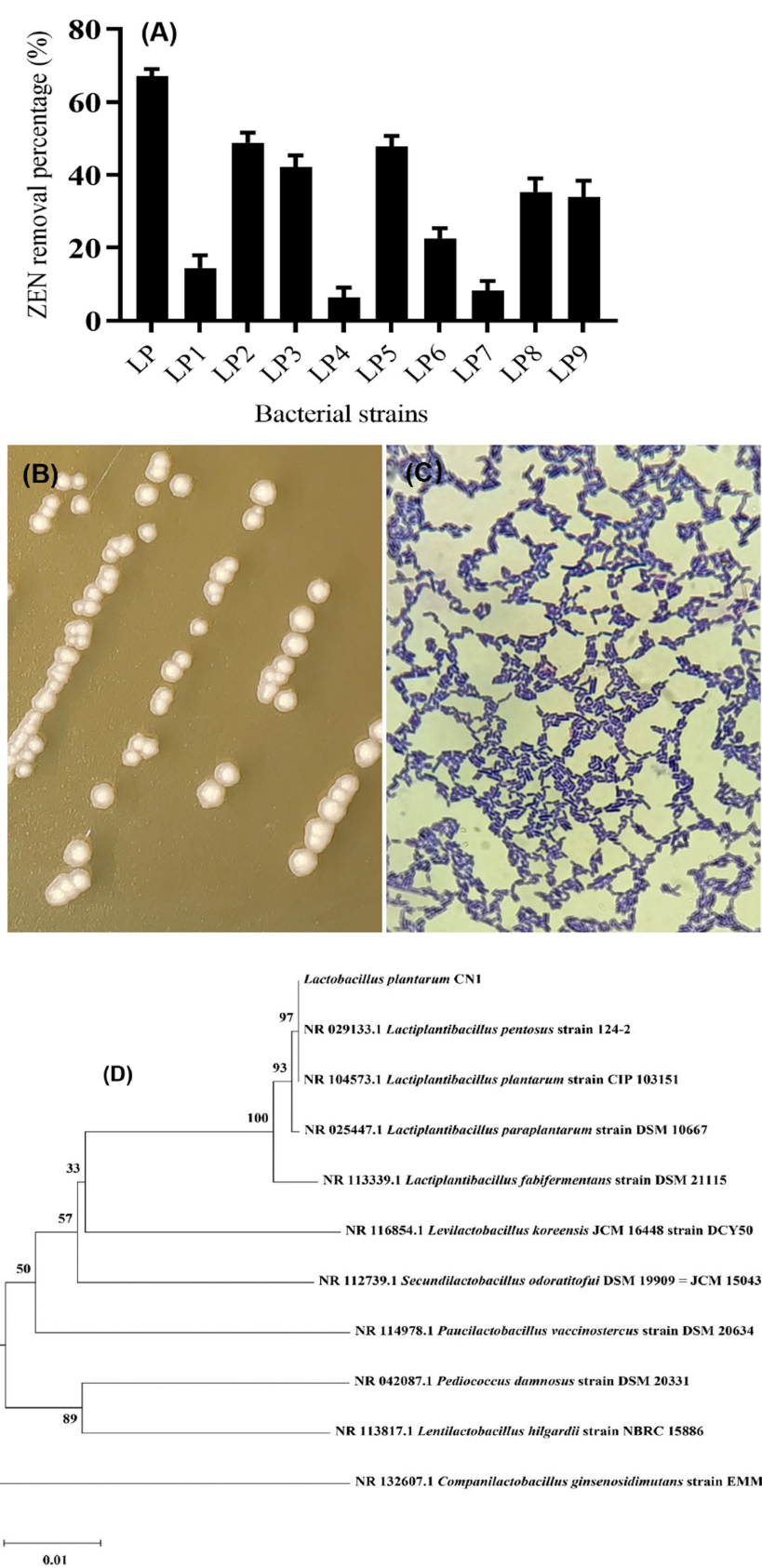


FIGURE 1
(A) Screening results of ZEN removal strains; (B) Colony morphology of CN1 strain; (C) Gram staining; (D) Phylogenetic tree constructed by gene sequence of strain CN1 16S rDNA.

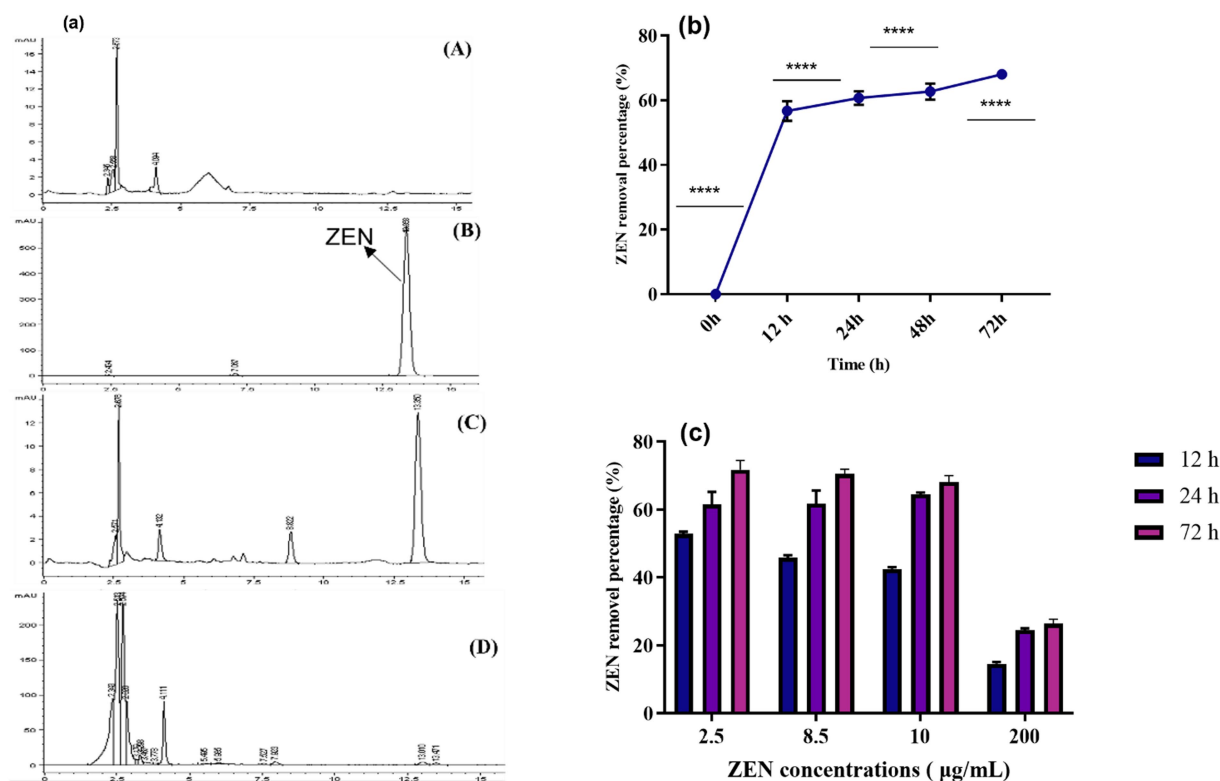


FIGURE 2

(a) HPLC of ZEN removal. ZEN (10 μg/mL) was added to CN1 and cultured in the MRS medium for 72 h, and the removal rate was then calculated using HPLC. (A) MRS medium; (B) Control group (ZEN toxin); (C) MRS medium containing ZEN toxin. (D) (ZEN in MRS medium + CN1) MRS + ZEN with CN1 strain; (b) ZEN removal (%) was calculated; (c) ZEN removal ability of CN1 under acid and heat conditions. At 37°C for 72 h, CN1 cells (4×10^9 CFU/mL) and MRS broth with various ZEN concentrations (2.5, 8.5, 10, and 200 μg/mL) were incubated. One-way ANOVA was used to analyze significant differences ($p < 0.05$).

temperature, which affect the stability of the adsorption process. The decline in the strain degradation ability over time might also be attributed to the natural lifecycle of the bacteria, where metabolic activity diminishes as the cells enter the stationary or decline phases (Rasmussen et al., 2015).

3.4 HPLC analysis of ZEN removal

In Figure 2a, the HPLC peaks illustrate the comparative analysis of groups A (MRS medium), B (control group), C (ZEN + MRS medium), and D (ZEN in MRS medium + CN1). The control group displayed a distinct peak at 13.3 min. In the ZEN + MRS medium group, two peaks were observed, indicating the presence of ZEN in the medium. Notably, the peak area was significantly reduced in the ZEN in the MRS medium + CN1 group, suggesting a decrease in ZEN concentration. These results demonstrate that after 72 h of incubation in MRS medium at 37°C, the CN1 strain effectively removed 69% of ZEN at a concentration of 10 μg/mL. This reduction is consistent with findings from other studies that highlight the capability of *Lactobacillus plantarum* strains to adsorb and degrade mycotoxins such as ZEN. El-Nezami et al. (2002) have documented similar adsorption and degradation capacities in lactic acid bacteria, emphasizing their potential use in detoxifying contaminated feeds.

3.5 ZEN removal by CN1 strain under acid and heat treatments

The influence of acid and heat treatment on the ZEN removal rate by CN1 (4×10^9 CFU/mL) in MRS broth with ZEN concentrations ranging from 2.5 to 200 μg/mL was studied. CN1 achieved a removal rate of over 40% for ZEN at concentrations of 2.5 and 8.5 μg/mL after 12 h. Specifically, at ZEN concentrations of 10 and 200 μg/mL, CN1 removed 42.5 and 14.77% of ZEN, respectively. After 72 h, CN1 demonstrated higher ZEN removal efficiency at 2.5 and 8.5 μg/mL. Overall, CN1 significantly removed more ZEN at higher concentrations (2.5, 8.5, and 10 μg/mL) after 72 h (Figure 2c). Our results on CN1 resilience to HCl and heat treatment for ZEN removal align with findings on bacterial tolerance in acidic and thermal environments, highlighting potential challenges and adaptations. The application of strong acid and alkali treatments may alter the structural integrity of bacterial cell wall components, particularly peptidoglycan and surface proteins, potentially affecting ZEN adsorption behavior. These treatments were used to explore the contribution of surface components under extreme conditions, but may not reflect physiological interactions *in vivo*. Studies show that acidic environments, like gastric HCl, can impair bacterial cell membranes and enzymatic function, thus reducing ZEN degradation (Guan and Liu, 2020). Additionally, while moderate heat may stimulate some microbial activity, excessive heat can denature proteins critical for

biodegradation (Laskowska et al., 1996). Consequently, to maintain CN1 efficacy in feed, future applications may consider delivery methods that shield the bacteria until they reach the intestines. These findings align with previous studies that underscore the effectiveness of lactic acid bacteria in mycotoxin removal. Previous studies demonstrated the ability of various *Lactobacillus* species to reduce aflatoxin levels through adsorption mechanisms (Li et al., 2021; Murtaza et al., 2024b). Additionally, Liu et al. (2019) highlighted that lactic acid bacteria can remove mycotoxins like ZEN through cell wall adsorption, similar to the findings in this study.

3.6 ZEN removal by CN1 strain at varying concentrations, pH levels, and temperatures

The ZEN removal rate of CN1 (4×10^9 CFU/mL) in MRS was tested with ZEN concentrations ranging from 2.5 to 200 $\mu\text{g/mL}$ (Figure 3A). At 2.5 and 8.5 $\mu\text{g/mL}$, CN1 removed more than 50% of ZEN after 12 h. For higher concentrations (10 and 200 $\mu\text{g/mL}$), CN1 quickly removed 39.5 and 8.5% of ZEN, respectively. After 72 h of incubation, CN1 cells continued to remove more ZEN at 2.5, 8.5, and 10 $\mu\text{g/mL}$. We hypothesized that the greater the ZEN concentration and the longer the incubation period, the more likely ZEN would connect to the remaining binding sites on the outermost layer of CN1. We discovered that ZEN removal by CN1 is a quick response since

CN1 cleared 56 and 53.5% of ZEN soon after contact with ZEN at levels of 2.5 and 8.5 $\mu\text{g/mL}$, respectively. At high beginning levels of ZEN (10 and 200 $\mu\text{g/mL}$), lower quantities of ZEN were removed by CN1 (39.5 and 8.5%, respectively) shortly after the inclusion of ZEN. Nonetheless, after incubating CN1 for 24 and 72 h with ZEN (10 $\mu\text{g/mL}$), the quantity of ZEN removed by the CN1 rose to roughly 43 and 69%, respectively. These findings are consistent with recent studies on the mycotoxin removal capabilities of lactic acid bacteria. Similarly, a study demonstrated that *Lactobacillus plantarum* exhibited significant efficacy in removing aflatoxin B1 and ZEN through adsorption mechanisms (Huang et al., 2018).

Moreover, a study investigated the factors influencing mycotoxin binding by lactic acid bacteria and highlighted those environmental conditions, such as pH and temperature, play a crucial role in optimizing mycotoxin removal (Nasrollahzadeh et al., 2022). After being consumed by mouth, the feed digesta passes through the animal's gastrointestinal system in various pH states. The pH range for the pig gastrointestinal system is 6.1–6.7 for the small intestine, 4.4 for the stomach, 6.1–6.6 for the colon, and 6.0–6.4 for the caecum (AlTabbaa and Ankrah, 2016). Therefore, the efficacy of a mycotoxin-degrading agent was evaluated under different pH settings. In their experiments, optimal mycotoxin binding was observed under specific pH conditions, similar to the results observed in the current study, where CN1 showed effective ZEN removal at 2.5, 8.5, and 10 $\mu\text{g/mL}$ concentrations after 24 h. The effects of pH on CN1 ability to remove ZEN were tested after 12, 24, and 72 h of treatment with ZEN at pH 3.0, 6.0, 7.0, and 8.0. The

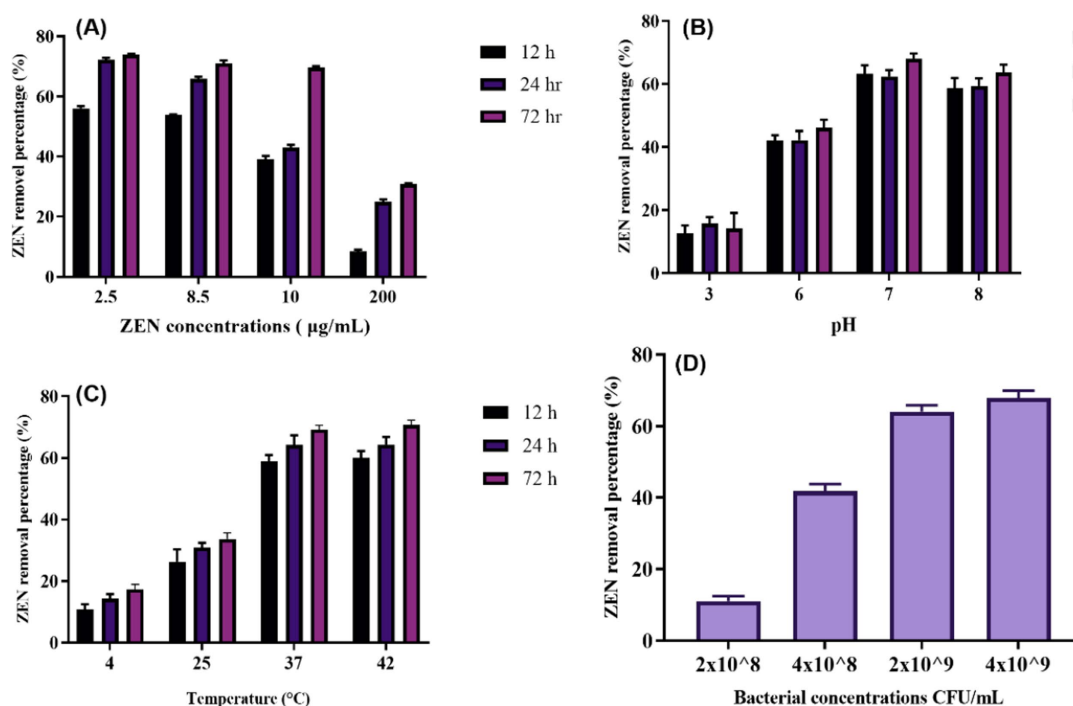


FIGURE 3

ZEN removing ability of CN1 under different ZEN-concentrations, pH, temperature and bacterial concentrations: (A) The 72-h incubation at 37°C with CN1 cells (4×10^9 CFU/mL) in MRS broth with various concentrations of ZEN (2.5, 8.5, 10, and 200 $\mu\text{g/mL}$); (B) A final concentration of 4×10^9 CFU/mL of bacterium cells were achieved by adding 5 mL of MRS broth (pH 3.0, 6.0, 7.0, and 8.0) with 10 $\mu\text{g/mL}$ of ZEN to CN1 cells throughout a 72-h period at 37°C ; (C) 5 mL of MRS broth containing 10 $\mu\text{g/mL}$ of ZEN was added to the CN1 cells to create a final concentration of 4×10^9 CFU/mL of bacterium cells. It was discovered how different temperatures (4, 25, 37, and 42°C) affected the ability of CN1 to remove substances; (D) CN1 cells in MRS broth with different bacterial quantities (4×10^8 , 2×10^8 , 4×10^8 , and 2×10^9 CFU/mL; ZEN 10 $\mu\text{g/mL}$, pH 7.0). One-way ANOVA was used to analyze significant differences ($p < 0.05$).

levels of ZEN eliminated by CN1 at pH 3.0 and 6.0 (10 and 17%, respectively) were much lower than those at pH 7.0 and 8.0 (39.5 and 36%, respectively). After 24 and 72 h at pH 7.0 and 8.0, the quantity of ZEN removed by CN1 rose considerably (Figure 3B).

The effects of temperature on CN1 ability to degrade ZEN found that CN1 removed over 50% of ZEN immediately at 37°C and achieved the highest removal rate of 69.01% after 72 h at this temperature (Figure 3C). The degradation rates were significantly lower at 4 and 25°C but significantly higher at 42°C. The results show that although the removal rate at 42°C was numerically higher than at 37°C, the difference was not statistically significant ($p > 0.05$), as determined by one-way ANOVA. This finding suggests that while both temperatures support effective ZEN degradation by CN1, 37°C remains the optimal condition considering both efficiency and potential thermal stress on the bacterial cells. These findings are the same as those of Hsu et al. (2018), which reported that the CK1 strain removed more ZEN at 42°C after 24 h compared to 4, 25, and 37°C. A more recent study demonstrated that another microbial strain, B73, showed optimal ZEN degradation at 30°C, with efficiency dropping significantly at higher and lower temperatures (Liu et al., 2023). Similarly, a study found that ZEN degradation by strain XZ-2 peaked at 35°C but decreased at temperatures above 40°C (Wu et al., 2024). These studies collectively suggest that optimal degradation temperatures vary significantly between microbial strains, underscoring the importance of selecting the appropriate strain and maintaining optimal environmental conditions for effective ZEN management.

3.7 ZEN removal by CN1 strain at different bacterial concentrations

The capacity of various concentrations of CN1 (4×10^9 , 2×10^9 , 4×10^8 , and 2×10^8 CFU/mL) to remove ZEN in MRS broth with a ZEN concentration of 10 µg/mL over 72 h (Figure 3D). The results indicated that CN1 at higher concentrations of 4×10^9 and 2×10^9 CFU/mL removed 69 and 65.6% of ZEN, respectively. In contrast, lower bacterial concentrations of 4×10^8 and 2×10^8 CFU/mL resulted in significantly lower ZEN removal rates of 43.88 and 10.21%, respectively. These findings suggest that a critical threshold of CN1 biomass is required to achieve effective ZEN removal, as lower concentrations showed a marked reduction in detoxification efficiency. While the increase from 2×10^9 to 4×10^9 CFU/mL did not yield a significant difference, concentrations below 109 CFU/mL resulted in a sharp decline in performance, indicating a potential saturation point for adsorption capacity. Comparatively, a recent study investigated the impact of different bacterial concentrations on mycotoxin degradation and found a similar trend, where higher concentrations of the tested strain significantly improved the removal rates of aflatoxin B1 (Li et al., 2023). Additionally, Park et al. (2022) reported that the efficiency of ochratoxin A degradation by *Lactobacillus plantarum* increased with bacterial density, emphasizing that higher cell concentrations provide more active sites for toxin binding and enzymatic degradation.

3.8 ZEN removal by culture supernatant, cell walls, and bacterial contents

Figure 4A shows the results of ZEN removal using the CN1 strain cell wall, culture supernatant, and intracellular contents. The cell wall

effectively removes ZEN, achieving a removal rate of over 60%. In contrast, the removal rates of ZEN in the fermentation supernatant and intracellular fluid are both below 5%. This suggests that the removal of ZEN by CN1 primarily relies on the adsorption capacity of the bacterial cell wall. It can be demonstrated that this strain eliminates ZEN through enzyme secretion and cell wall action. Previous research found that the breakdown rate of *Escherichia coli* CG1061 supernatant from culture was 61.8%, but the biological degradation of intracellular isolates was only 17.6%, indicating that the active component was continuously discharged into the extracellular area. The breakdown rate was reduced to 37.5% when the cultured supernatant was treated with 1 mg/mL proteinase K but remained at 51.3% after 20 min at 100°C (Wang et al., 2019). Furthermore, research revealed that certain mycotoxins were inactivated or absorbed through the cell wall (Yiannikouris et al., 2021).

3.9 Characterization of ZEN adsorption mechanism

After exposing the bacterial strain to ZEN, an SEM examination was conducted to investigate their shape and fundamental makeup. The alterations in bacterial cells following ZEN treatment are depicted in Figure 4D. When compared to the control sample, SEM pictures acquired at levels of magnification of 20,000x and 40,000x demonstrated visible damage to the treated cells (a). The strains exhibited elongation and thinning when cultured in an MRS medium, warranting further investigation. Moreover, SEM examination after the incubation of the two treatments in an MRS medium with ZEN revealed minimal differences in bacterial morphology (b). However, after heat and ZEN treatment, increased cell damage was observed (c). These observed modifications may elucidate the diverse adsorption percentages achieved after ZEN adsorption and suggest an alternative cellular wall structure. Additionally, SEM examination revealed intercellular connections among bacterial strains throughout the adsorption process. Furthermore, SEM revealed elusive variations in bacterial morphology after incubation in LAPTg and MRS medium, consistent with our study's findings. Specifically, *Bacillus* strains exhibited a more elongated and thin morphology when cultivated in LAPTg compared to MRS (Adunphatcharaphon et al., 2021; Murtaza et al., 2023a). This intriguing characteristic of the strain warrants further investigation in future studies. Importantly, the results suggest that these treatments had no appreciable effect on the ratio of chemical components on the bacterial cell surfaces. The glycopolymers and proteins that make up the cell wall of typical *Lactobacillus* are intricately arranged. Proteins, polysaccharides, and teichoic acids are used to adorn the thick peptidoglycans that encase the cytoplasmic membranes (Chapot-Chartier and Kulakauskas, 2014). The pattern and structure of bacterial cell surfaces were changed as a result of alterations in the sugars and amino acids found in glycopolymer or protein structures (Stefanović et al., 2021). This shows that the *Lactobacillus* cell wall's distinctive structure and content are key to the organism's ability to detoxify ZEN.

3.10 Bacterial tolerance to ZEN

Since ZEN is toxic to cells, its presence can impact the growth of microbial strains. Therefore, high tolerance to ZEN is essential for practical applications. Our results indicate that the growth of CN1 was

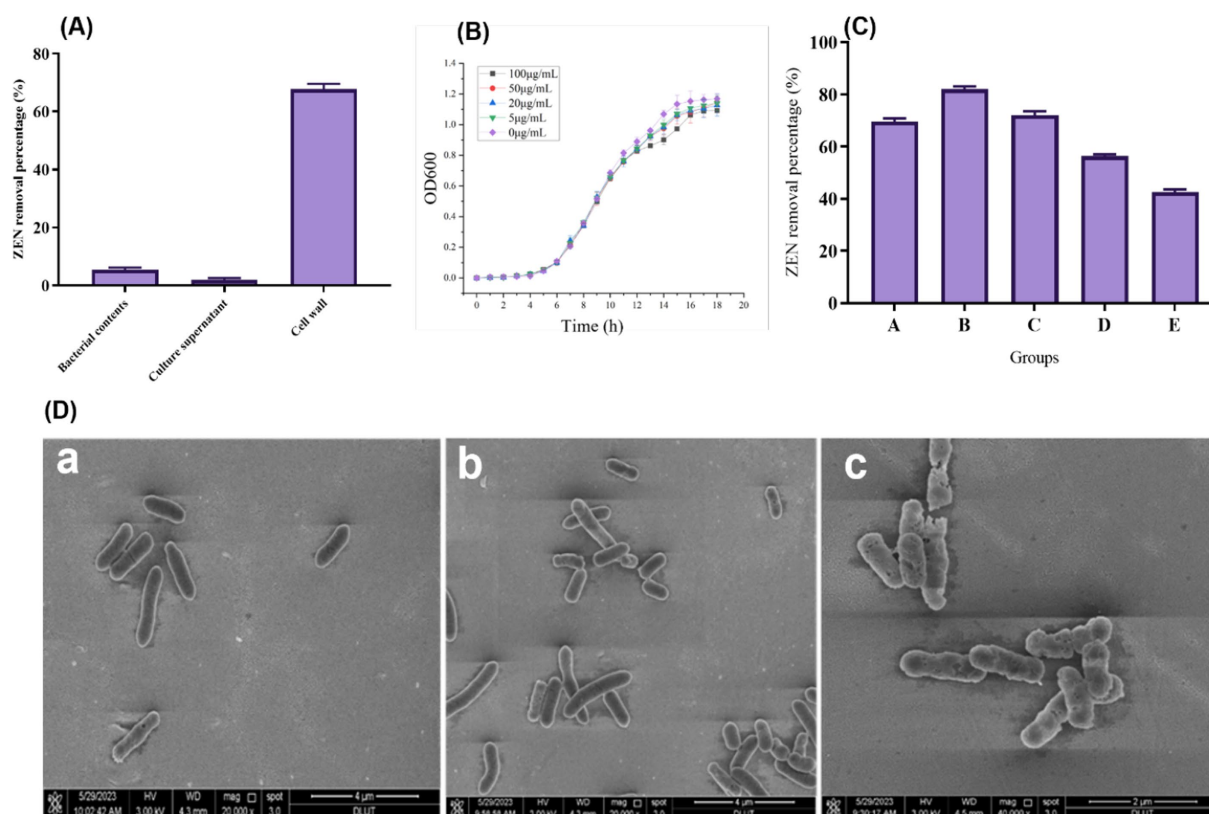


FIGURE 4

(A) Degradation effect of fermentation supernatant, cell wall and intracellular fluid of strain on ZEN; (B) Growth status of CN1 bacteria at different ZEN concentrations; (C) Effect of different ways to treat CN1 bacteria on ZEN removal; (D) Scanning Electron Microscope (SEM) images of bacterial cells at magnifications of 20,000x and 40,000x (a) Bacterial cells with no treatment; (b) Cells subjected to ZEN treatment; (c) Cells treated with both ZEN and heat. One-way ANOVA was used to analyze significant differences ($p < 0.05$).

only slightly affected at a ZEN concentration of 100 $\mu\text{g/mL}$, as shown in Figure 4B. This suggests that CN1 exhibits strong tolerance to ZEN. These findings are consistent with previous studies that have highlighted the resilience of certain lactic acid bacteria to mycotoxins. For instance, a study demonstrated that some *Lactobacillus* strains could tolerate and even reduce ZEN concentrations in contaminated media through adsorption mechanisms (Topcu et al., 2010). Similarly, research found that *Lactobacillus rhamnosus* strains were capable of binding and sequestering ZEN, thereby reducing its bioavailability and toxicity (Fuchs et al., 2002). The ability of CN1 to withstand high levels of ZEN and maintain growth is particularly advantageous for applications in food and feed safety. Moreover, the strong tolerance of CN1 to ZEN supports its potential use in fermentation processes aimed at detoxifying contaminated feedstocks, thereby improving their safety and nutritional quality.

3.11 Effect of different treatments on ZEN removal by CN1 strain

To investigate the adsorption effect of CN1, various treatments were applied to the bacteria, as illustrated in Figure 4C. The untreated group (Group A) demonstrated an adsorption rate of over 65%. Heat treatments significantly enhanced adsorption capabilities: Group B, subjected to autoclaving, achieved an adsorption rate of 82.9%, while

Group C, treated in a 100°C water bath, exhibited a rate of 73.3%. In contrast, the adsorption efficiency decreased following acid and alkali treatments. Specifically, Group D, treated with acid, showed an adsorption rate of 56.2%, and Group E, treated with alkali, had a rate of 43.5%. These findings align with previous research that has shown the impact of different treatments on the adsorption abilities of lactic acid bacteria. For instance, studies have demonstrated that heat treatment can expose more binding sites on the bacterial cell wall, enhancing the adsorption of toxins such as ZEN. Our study reported similar improvements in adsorption rates following heat treatment of *Lactobacillus* strains, suggesting that thermal processes can denature cell wall proteins, thereby increasing their binding capacity for mycotoxins (Murtaza et al., 2023a). Conversely, the reduction in adsorption following acid and alkali treatments can be attributed to the alteration or degradation of cell wall components, which play a crucial role in toxin binding. Research found that extreme pH conditions could negatively affect the structural integrity of bacterial cell walls, thereby diminishing their adsorption efficiency (Niderkorn et al., 2006).

3.12 Stability of ZEN adsorption by CN1 strain

In this study, CN1 removed ZEN through physical adsorption by its cell wall. However, under various external conditions, ZEN

adsorbed on the cell wall may desorb back into the system. Therefore, the stability of the ZEN-cell wall conjugate was investigated. After treating the ZEN-adsorbed bacteria with heating, shaking, and organic solvents (Figure 5A), some ZEN was detected in the system. The desorption effect was most pronounced with organic solvent washing, which had a desorption rate as high as 65%. Heat treatment resulted in the separation of 43.4% of ZEN from the complex, while the shaking method had the least effect, with a desorption rate of only 22.1%.

These results indicate that the adsorption of ZEN by the microbial cell wall is reversible. Consequently, caution is required when using this method in production, and attention must be paid to the storage conditions of subsequent products to prevent the re-release of ZEN. This reversibility aligns with findings from other studies. A study found that the binding of mycotoxins to yeast cell walls could be influenced by environmental conditions, potentially leading to desorption under certain circumstances (Kihal et al., 2022). Additionally, Johnson (1990) demonstrated that the stability of toxin adsorption could be affected by pH and temperature changes, reinforcing the need for careful consideration of storage and handling conditions. In practical applications, these findings underscore the importance of optimizing both the adsorption process and subsequent storage conditions to maintain the efficacy of mycotoxin removal.

3.13 Determination of nutritional components after fermentation

As illustrated in Figure 5B, fermentation significantly altered the nutrient profile of DDGS. Post-fermentation analysis revealed a 0.79% decrease in crude fiber content, enhancing digestibility for livestock and poultry. High cellulose levels in DDGS typically exceed feed standards, so reducing cellulose content is beneficial for increasing its use in the breeding industry. This reduction aligns with findings that lower fiber content improves nutrient absorption and feed efficiency in livestock (Jha and Berrocoso, 2015).

Conversely, crude protein content increased by 7.65% after fermentation. This rise is primarily due to the proliferation of CN1 during fermentation, which produces bacterial and exocrine proteins while consuming energy. Increased microbial protein synthesis during fermentation is well documented and improves the nutritional quality of feed (Olukomaiya et al., 2020). Additionally, the pH of DDGS dropped significantly from 6.5 to 4.2 post-fermentation. This reduction is attributed to the anaerobic fermentation of CN1, creating an acidic environment that inhibits the growth of harmful bacteria and molds, thereby extending shelf life. Notably, after 180 days, no mold growth was observed under high moisture conditions, underscoring the effectiveness of fermentation in prolonging feed stability. Finally, the fermented DDGS exhibited a more pronounced lactic acid taste, potentially enhancing palatability and food intake for animals. This improvement in taste could lead to higher feed consumption, although further verification is needed. Enhanced palatability through fermentation has been supported by other research, suggesting that lactic acid bacteria can improve feed flavor and acceptance (Bintsis, 2018).

3.14 ZEN determination after fermentation

As shown in Table 1, ZEN in the sample was extracted and tested with a ZEN ELISA detection kit. The recovery rate of ZEN reached 94.7%, confirming the feasibility of the extraction and detection method. For DDGS feed products fermented by CN1, the ZEN content was measured post-fermentation using the same kit, revealing a removal rate of 75.7%. This indicates that the CN1 fermentation method is highly effective at removing ZEN, surpassing the capabilities of other strains. Additionally, the CN1 fermentation process enhances the degradation effect, optimizing the reduction of ZEN in DDGS feed products. Previous studies have extensively explored methods for removing mycotoxins, including ZEN, from agricultural products and feed. Many of these studies have focused on utilizing microorganisms like lactic acid bacteria (LAB) due to their ability to degrade

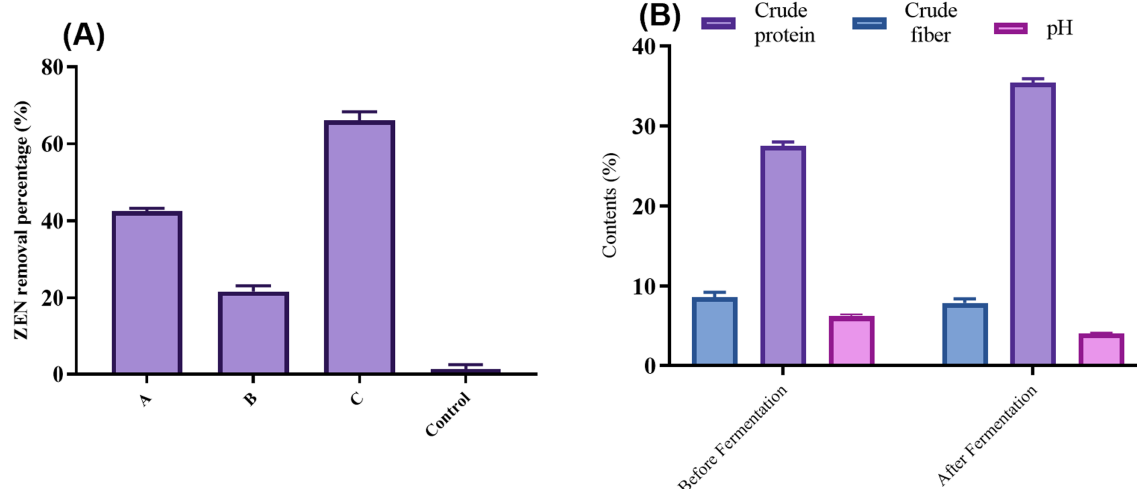


FIGURE 5

(A) Desorption rate of cell wall ZEN complex after different treatments; (B) Changes of crude fibers, crude protein, and pH content before and after DDGS fermentation. One-way ANOVA was used to analyze significant differences ($p < 0.05$).

TABLE 1 ZEN recovery rate verification results.

ZEN concentration (μg/mg)	Detected concentration (μg/mg)	Recovery rate (%)
2	0.85	85
5	4.70	94.50
10	9.50	95.50

mycotoxins through various mechanisms. For instance, the study by Li et al. (2020) demonstrated the efficient removal of ZEN from contaminated grains using LAB strains, with removal rates ranging from 40% to over 90% depending on the strain and conditions. Similarly, the work highlighted the potential of LAB in reducing ZEN levels in feed materials, achieving removal rates exceeding in some cases (Chen et al., 2018). These findings align with the current study's results, which show a remarkable removal rate of 75.7% using CN1 fermentation on DDGS feed. Furthermore, studies investigating the stability and reversibility of ZEN adsorption on microbial cell walls have emphasized the importance of understanding desorption processes. Research explored the desorption behavior of ZEN from LAB cell walls under different conditions, corroborating the current study findings regarding the reversibility of ZEN adsorption. The high recovery rate (94.7%) of ZEN extraction using the method in this study aligns with previous research demonstrating the reliability and accuracy of ELISA-based detection kits for mycotoxins in agricultural samples.

4 Conclusion

This study presents an innovative approach to mitigating ZEN contamination in DDGS through probiotic fermentation using CN1. The results demonstrate that CN1 can significantly reduce ZEN levels by up to 75.6%, primarily through adsorption to the bacterial cell wall, with heat treatments further enhancing adsorption efficiency to 82.9%. This method not only surpasses the effectiveness of traditional physical adsorption techniques, which often compromise feed quality, but also improves the nutritional profile of DDGS by increasing crude protein content by 7.16% and reducing crude fiber by 0.65%. Scanning electron microscopy revealed structural changes in CN1 cells under stress conditions, contributing to their enhanced adsorption capacity. The dual benefit of detoxification and nutritional enhancement offers a novel, practical solution for improving feed safety and economic value. This approach holds significant potential for large-scale industrial application, particularly in regions with high mycotoxin contamination, and could set a new standard in sustainable agricultural practices. Future research may explore its application to other mycotoxins or feed types, optimizing the process for diverse conditions and combining it with other strategies for even greater efficacy.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

BM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. L-LG: Conceptualization, Funding acquisition, Resources, Writing – review & editing, Visualization. LW: Conceptualization, Funding acquisition, Supervision, Writing – review & editing, Resources. XL: Funding acquisition, Writing – review & editing, Conceptualization, Data curation, Supervision, Visualization. LZ: Writing – review & editing, Funding acquisition, Validation, Supervision. BJ: Writing – review & editing, Conceptualization, Formal analysis, Methodology, Software. J-bL: Resources, Supervision, Validation, Visualization, Writing – review & editing. YX: Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

BM, J-bL, and YX were employed by the company Dalian SEM Bioengineering Technology Co., Ltd.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

- Adunphatcharaphon, S., Petchkongkaew, A., and Visessanguan, W. (2021). In vitro mechanism assessment of zearalenone removal by plant-derived *Lactobacillus plantarum* bcc 47723. *Toxins (Basel)*. 13:286. doi: 10.3390/TOXINS13040286
- Ahmed, S., Abdelbaqey, A., and Hassan, M. (2024). Detection and analysis of mycoflora associated corn and wheat flours and their toxins. *Benha J. Appl. Sci.* 9, 7–15. doi: 10.21608/bjas.2024.283888.1409
- Ahn, J. Y., Kim, J., Cheong, D. H., Hong, H., Jeong, J. Y., and Kim, B. G. (2022). An in vitro study on the efficacy of mycotoxin sequestering agents for aflatoxin B1, Deoxynivalenol, and Zearalenone. *Animals* 12, 1–7. doi: 10.3390/ani12030333
- AlTabbaa, O., and Ankrah, S. (2016). Assessment of gastrointestinal pH, fluid and lymphoid tissue in the guinea pig, rabbit and pig, and implications for their use in drug development. *Technol. Forecast. Soc. Chang.* 104, 1–15.
- Bintsis, T. (2018). Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. *AIMS Microbiol.* 4, 665–684. doi: 10.3934/microbiol.2018.4.665
- Cai, G., Zhong, F., Cao, Q., Bai, Y., Zou, H., Gu, J., et al. (2022). ZEA and DON inhibited inflammation after *L. monocytogenes* infection and induced ribosomal hyperfunction. *Ecotoxicol. Environ. Saf.* 236:113470. doi: 10.1016/j.ecoenv.2022.113470
- Chapot-Chartier, M. P., and Kulakauskas, S. (2014). Cell wall structure and function in lactic acid bacteria. *Microb. Cell Factories* 13:S9. doi: 10.1186/1475-2859-13-S1-S9
- Chen, S. W., Hsu, J. T., Chou, Y. A., and Wang, H. T. (2018). The application of digestive tract lactic acid bacteria with high esterase activity for zearalenone detoxification. *J. Sci. Food Agric.* 98, 3870–3879. doi: 10.1002/jsfa.8904
- Ding, S., Lin, C., Xiao, Q., Feng, F., Wang, J., Zhang, X., et al. (2024). Effective degradation of zearalenone by dye-decolorizing peroxidases from *Pleurotus ostreatus* and its metabolic pathway and toxicity analysis. *Sci. Total Environ.* 908:168500. doi: 10.1016/j.scitotenv.2023.168500
- El-Nezami, H., Polychronaki, N., Salminen, S., and Mykkänen, H. (2002). Binding rather than metabolism may explain the interaction of two food-grade *Lactobacillus* strains with zearalenone and its derivative α -zearalenol. *Appl. Environ. Microbiol.* 68, 3545–3549. doi: 10.1128/AEM.68.7.3545-3549.2002
- Fuchs, E., Binder, E. M., Heidler, D., and Krška, R. (2002). Structural characterization of metabolites after the microbial degradation of type A trichothecenes by the bacterial strain BBSH 797. *Food Addit. Contam.* 19, 379–386. doi: 10.1080/02652030110091154
- Ge, N., Xu, J., Peng, B., and Pan, S. (2017). Adsorption mechanism of tenuazonic acid using inactivated lactic acid bacteria. *Food Control* 82, 274–282. doi: 10.1016/j.foodcont.2017.07.009
- Guan, N., and Liu, L. (2020). Microbial response to acid stress: mechanisms and applications. *Appl. Microbiol. Biotechnol.* 104, 51–65. doi: 10.1007/s00253-019-10226-1
- Hathout, A. S., and Aly, S. E. (2014). Biological detoxification of mycotoxins: A review. *Ann. Microbiol.* 64, 905–919. doi: 10.1007/s13213-014-0899-7
- Hsu, T. C., Yi, P. J., Lee, T. Y., and Liu, J. R. (2018). Probiotic characteristics and zearalenone-removal ability of a *Bacillus licheniformis* strain. *PLoS One* 13, e0194866–e0194818. doi: 10.1371/journal.pone.0194866
- Huang, W., Chang, J., Wang, P., Liu, C., Yin, Q., Zhu, Q., et al. (2018). Effect of the combined compound probiotics with mycotoxin-degradation enzyme on detoxifying aflatoxin B1 and zearalenone. *J. Toxicol. Sci.* 43, 377–385. doi: 10.2131/jts.43.377
- Jha, R., and Berrocoso, J. D. (2015). Review: dietary fiber utilization and its effects on physiological functions and gut health of swine. *Animal* 9, 1441–1452. doi: 10.1017/S1751731115000919
- Joannis-Cassan, C., Tozlovanu, M., Hadjeba-Medjdoub, K., Ballet, N., and Pfohl-Leszkowicz, A. (2011). Binding of zearalenone, aflatoxin b1, and ochratoxin a by yeast-based products: A method for quantification of adsorption performance. *J. Food Prot.* 74, 1175–1185. doi: 10.4315/0362-028X.JFP-11-023
- Johnson, B. B. (1990). Effect of pH, temperature, and concentration on the adsorption of cadmium on goethite. *Environ. Sci. Technol.* 24, 112–118.
- Kihal, A., Rodríguez-Prado, M., and Calsamiglia, S. (2022). The efficacy of mycotoxin binders to control mycotoxins in feeds and the potential risk of interactions with nutrient: A review. *J. Anim. Sci.* 100, 1–14. doi: 10.1093/jas/skac328
- Kim, S. H., and Vujanovic, V. (2017). Biodegradation and biotransformation of fusarium mycotoxins by *Sphaerodes mycoparasitica*. *sAMB Express* 7, 145–149. doi: 10.1186/s13568-017-0446-6
- Laskowska, E., Kuczyńska-Wisnik, D., Skórko-Glonek, J., and Taylor, A. (1996). Degradation by proteases Lon, Clp and HtrA, of *Escherichia coli* proteins aggregated in vivo by heat shock; HtrA protease action in vivo and in vitro. *Mol. Microbiol.* 22, 555–571.
- Li, G., Li, X., Dong, L., Li, C., Zou, P., Saleemi, M. K., et al. (2021). Isolation, identification and characterization of *Paenibacillus pabuli* E1 to explore its aflatoxin B1 degradation potential. *Curr. Microbiol.* 78, 3686–3695. doi: 10.1007/s00284-021-02624-4
- Li, W., Li, W., Zhang, C., Xu, N., Fu, C., Wang, C., et al. (2023). Study on the mechanism of aflatoxin B1 degradation by *Tetragenococcus halophilus*. *Lwt* 180:114662. doi: 10.1016/j.lwt.2023.114662
- Li, P., Su, R., Yin, R., Lai, D., Wang, M., Liu, Y., et al. (2020). Detoxification of mycotoxins through biotransformation. *Toxins (Basel)*. 12:121. doi: 10.3390/toxins12020121
- Liu, F., Malaphan, W., Xing, F., and Yu, B. (2019). Biotransformation of fungal mycotoxins zearalenone by engineered probiotic bacterium *Lactobacillus reuteri* with surface-displayed lactonohydrolase. *Appl. Microbiol. Biotechnol.* 103, 8813–8824. doi: 10.1007/s00253-019-10153-1
- Liu, X., Wu, N., Zhang, M., Xue, F., and Xu, Q. (2023). Isolation and characterization of the Zearalenone-degrading strain, *Bacillus spizizenii* B73, inspired by esterase activity. *Toxins (Basel)*. 15:488. doi: 10.3390/toxins15080488
- Mohammadi Shad, Z., Venkatasamy, C., and Wen, Z. (2021). Corn distillers dried grains with solubles: production, properties, and potential uses. *Cereal Chem.* 98, 999–1019. doi: 10.1002/cche.10445
- Murtaza, B., Jin, B., Wang, L., Li, X., Kashif, M., Majeed, S., et al. (2023a). Mitigation of zearalenone in vitro using probiotic strains. *LWT* 186:115265. doi: 10.1016/j.lwt.2023.115265
- Murtaza, B., Li, X., Dong, L., Javed, M. T., Xu, L., Saleemi, M. K., et al. (2022). Microbial and enzymatic battle with food contaminant zearalenone (ZEN). *Appl. Microbiol. Biotechnol.* 106, 4353–4365. doi: 10.1007/s00253-022-12009-7
- Murtaza, B., Li, X., Dong, L., Saleemi, M. K., Iqbal, M., Majeed, S., et al. (2023b). New trends for the efficient bio-degradation of food contaminant zearalenone using a plant rhizobacterial strain: an in-vitro study. *World Mycotoxin J.* 16, 251–260. doi: 10.3920/wmj2022.2798
- Murtaza, B., Li, X., Nawaz, M. Y., Saleemi, M. K., Li, G., Jin, B., et al. (2024a). Toxicodynamic of combined mycotoxins: MicroRNAs and acute-phase proteins as diagnostic biomarkers. *Compr. Rev. Food Sci. Food Saf.* 23, 1–27. doi: 10.1111/1541-4337.13338
- Murtaza, B., Ling-ling, G., Wang, L., Li, X., Ali, A., Saleemi, M. K., et al. (2025). Mycotoxin detection in corn and distillers dried grains for food security. *Renew. Sust. Energ. Rev.* 210:115194. doi: 10.1016/j.rser.2024.115194
- Murtaza, B., Wang, L., Li, X., Ali, A., Ul, S., Ji-bin, L., et al. (2024b). Novel lactobacillus consortium for effective zearalenone adsorption and biodegradation. *Int. Biodeterior. Biodegradation* 194:105889. doi: 10.1016/j.ibiod.2024.105889
- Murtaza, B., Wang, L., Li, X., Nawaz, M. Y., Saleemi, M. K., Khatoon, A., et al. (2024c). Recalling the reported toxicity assessment of deoxynivalenol, mitigating strategies and its toxicity mechanisms: comprehensive review. *Chem. Biol. Interact.* 387:110799. doi: 10.1016/j.cbi.2023.110799
- Murtaza, B., Wang, L., Li, X., Saleemi, M. K., Nawaz, M. Y., Wang, L., et al. (2024d). Cold plasma: A success road to mycotoxins mitigation and food value edition. *Food Chem.* 445:138378. doi: 10.1016/j.foodchem.2024.138378
- Nasrollahzadeh, A., Mokhtari, S., Khomeiri, M., and Saris, P. (2022). Mycotoxin detoxification of food by lactic acid bacteria. *Int. J. Food Contam.* 9, 1–9. doi: 10.1186/s40550-021-00087-w
- Niderkorn, V., Boudra, H., and Morgavi, D. P. (2006). Binding of fusarium mycotoxins by fermentative bacteria in vitro. *J. Appl. Microbiol.* 101, 849–856. doi: 10.1111/j.1365-2672.2006.02958.x
- Olukomaiya, O. O., Fernando, W. C., Mereddy, R., Li, X., and Sultanbawa, Y. (2020). Solid-state fermentation of canola meal with *Aspergillus sojae*, *Aspergillus ficum* and their co-cultures: effects on physicochemical, microbiological and functional properties. *Lwt* 127:109362. doi: 10.1016/j.lwt.2020.109362
- Park, S., Koo, J., Kim, B., Pushparaj, K., Malaisamy, A., Liu, W. C., et al. (2022). Evaluation of the safety and Ochratoxin A degradation capacity of *Pediococcus pentosaceus* as a dietary probiotic with molecular docking approach and pharmacokinetic toxicity assessment. *Int. J. Mol. Sci.* 23:9062. doi: 10.3390/ijms23169062
- Pont, G. C. D., Lee, A., Bortoluzzi, C., Rohloff Junior, N., Farnell, Y. Z., Pilla, R., et al. (2023). Distillers dried grains with soluble and enzyme inclusion in the diet effects broilers performance, intestinal health, and microbiota composition. *Poult. Sci.* 102:102981. doi: 10.1016/j.psj.2023.102981
- Pourmohammadi, K., Sayadi, M., Abedi, E., and Mousavifard, M. (2022). Determining the adsorption capacity and stability of aflatoxin B1, Ochratoxin A, and Zearalenon on single and co-culture *L. acidophilus* and *L. rhamnosus* surfaces. *J. Food Compos. Anal.* 110:104517. doi: 10.1016/j.jfca.2022.104517
- Rasmussen, S., Allentoft, M. E., Nielsen, K., Orlando, L., Sikora, M., Sjögren, K. G., et al. (2015). Early divergent strains of *Yersinia pestis* in Eurasia 5,000 years ago. *Cell* 163, 571–582. doi: 10.1016/j.cell.2015.10.009
- Ricciardi, L., Demartini, B., Fotopoulou, A., and Edwards, M. J. (2015). Alexithymia in neurological disease: a review. *The Journal of neuropsychiatry and clinical neurosciences*, 27, 179–187.
- Seddik, H. A., Bendali, F., Gancel, F., Fliss, I., Spano, G., and Drider, D. (2017). *Lactobacillus plantarum* and its probiotic and food potentialities. *Probiotics Antimicrob. Proteins* 9, 111–122. doi: 10.1007/s12602-017-9264-z
- Shi, J., Mwambuli, F., Xie, Y., Yang, Y., Sun, S., Li, Q., et al. (2024). Characterization, structural analysis, and thermal stability mutation of a new Zearalenone-degrading enzyme mined from *Bacillus subtilis*. *J. Agric. Food Chem.* 72, 3025–3035. doi: 10.1021/acs.jafc.3c06767
- Stefanović, C., Hager, F. F., and Schäffer, C. (2021). Lytr-cpsa-psr glycopolymer transferases: essential bricks in gram-positive bacterial cell wall assembly. *Int. J. Mol. Sci.* 22, 1–34. doi: 10.3390/ijms22020908

- Sulyok, M., Suman, M., and Krska, R. (2024). Quantification of 700 mycotoxins and other secondary metabolites of fungi and plants in grain products. *Npj Sci Food* 8, 1–8. doi: 10.1038/s41538-024-00294-7
- Topcu, A., Bulat, T., and Wishah, R. (2010). Detoxification of aflatoxin B1 and patulin by *Enterococcus faecium* strains. *Int. J. Food Microbiol.* 139, 202–205. doi: 10.1016/j.ijfoodmicro.2010.03.006
- Vega, M. F., Dieguez, S. N., Riccio, B., Aranguren, S., Giordano, A., Denzoin, L., et al. (2017). Zearalenone adsorption capacity of lactic acid bacteria isolated from pigs. *Braz. J. Microbiol.* 48, 715–723. doi: 10.1016/j.bjm.2017.05.001
- Wang, X., Han, Y., Niu, H., Zhang, L. H., Xiang, Q., and Zong, W. (2022). Alternaria mycotoxin degradation and quality evaluation of jujube juice by cold plasma treatment. *Food Control* 137, 108926–108911. doi: 10.1016/j.foodcont.2022.108926
- Wang, Y., Ke, Y., Liu, W., Sun, Y., and Ding, X. (2020). A one-pot toolbox based on Cas12a/crRNA enables rapid foodborne pathogen detection at Attomolar level. *ACS Sensors* 5, 1427–1435. doi: 10.1021/acssensors.0c00320
- Wang, X., Li, S., Han, Y., Hou, M., and Gao, Z. (2024). Novel strategies for efficiently detoxifying mycotoxins in plant-derived foods inspired by non-thermal technologies and biological resources: a review. *Crit. Rev. Food Sci. Nutr.* 1, 1–27. doi: 10.1080/10408398.2024.2434723
- Wang, N., Li, P., Pan, J., Wang, M., Long, M., Zang, J., et al. (2018a). *Bacillus velezensis* A2 fermentation exerts a protective effect on renal injury induced by Zearalenone in mice. *Sci. Rep.* 8, 1–14. doi: 10.1038/s41598-018-32006-z
- Wang, N., Li, P., Wang, M., Chen, S., Huang, S., Long, M., et al. (2018b). The protective role of *Bacillus velezensis* A2 on the biochemical and hepatic toxicity of zearalenone in mice. *Toxins (Basel)*. 10:449. doi: 10.3390/toxins10110449
- Wang, L., Wu, J., Liu, Z., Shi, Y., Liu, J., Xu, X., et al. (2019). Aflatoxin B1 degradation and detoxification by *Escherichia coli* CG1061 isolated from chicken cecum. *Front. Pharmacol.* 9, 1–9. doi: 10.3389/fphar.2018.01548
- Wang, Y., Zhou, A., Yu, B., and Sun, X. (2024). Recent advances in non-contact food decontamination Technologies for Removing Mycotoxins and Fungal Contaminants. *Food Secur.* 13:2244. doi: 10.3390/foods13142244
- Wei, T., Ren, P., Huang, L., Ouyang, Z., Wang, Z., Kong, X., et al. (2019). Simultaneous detection of aflatoxin B1, ochratoxin A, zearalenone and deoxynivalenol in corn and wheat using surface plasmon resonance. *Food Chem.* 300:125176. doi: 10.1016/j.foodchem.2019.125176
- Wu, J., Wang, Z., An, W., Gao, B., Li, C., Han, B., et al. (2024). *Bacillus subtilis* simultaneously detoxified aflatoxin B1 and Zearalenone. *Appl. Sci.* 14:1589. doi: 10.3390/app14041589
- Yang, S. B., Zheng, H. C., Xu, J. Y., Zhao, X. Y., Shu, W. J., Li, X. M., et al. (2021). New biotransformation mode of Zearalenone identified in *Bacillus subtilis* Y816 revealing a novel ZEN conjugate. *J. Agric. Food Chem.* 69, 7409–7419. doi: 10.1021/acs.jafc.1c01817
- Yiannikouris, A., Apajalahti, J., Kettunen, H., Ojanperä, S., Bell, A. N. W., Keegan, J. D., et al. (2021). Efficient aflatoxin B1 sequestration by yeast Cell Wall extract and hydrated sodium calcium Aluminosilicate evaluated using a multimodal in-vitro and ex-vivo methodology. *Toxins (Basel)*. 13:24. doi: 10.3390/TOXINS13010024
- Zhu, Y., Wang, H., Wang, J., Han, S., Zhang, Y., Ma, M., et al. (2021). Zearalenone induces apoptosis and Cytoprotective autophagy in chicken granulosa cells by PI3K-AKT-mTOR and MAPK signaling pathways. *Toxins (Basel)*. 13:199. doi: 10.3390/TOXINS13030199

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