Mycotoxin

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Contributor: Theodoros Varzakas , Sofia Agriopoulou , Eygenia Stamatelopoulou

Mycotoxins are toxic substances that can infect many foods with carcinogenic, genotoxic, teratogenic, nephrotoxic, and hepatotoxic effects. Mycotoxin contamination of foodstuffs causes diseases worldwide. The major classes of mycotoxins that are of the greatest agroeconomic importance are aflatoxins, ochratoxins, fumonisins, trichothecenes, emerging *Fusarium* mycotoxins, enniatins, ergot alkaloids, *Alternaria* toxins, and patulin.

mycotoxins occurrence detoxification decontamination foodstuffs aflatoxins

1. Introduction

Mycotoxins belong to the category of toxic secondary metabolites, and they have a low molecular weight. They are produced by filamentous fungi belonging to the phylum Ascomycota or molds, and they have great importance in the health of humans and animals, being the cause of acute and chronic diseases [1][2][3][4]. Bennett defined that mycotoxins are natural products produced by fungi that induce a toxic response when introduced at a low concentration to higher vertebrates and other animals via natural route [5]. The Greek word “mykes” meaning “fungi” and the Latin word “toxicum” meaning “poison” are the origin of the word mycotoxin [6]. A variety of fungi such as *Aspergillus*, *Fusarium, Penicillium, Alternaria*, and *Claviceps* spp. colonize their host and produce mycotoxins [7]. Of the approximately 400 compounds identified as mycotoxins, 30 receive great attention, and they are considered a threat to human or animal health [8]. The most important mycotoxins are aflatoxins (AFs) (represented mainly by aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1)), ochratoxins (OTs) (represented mainly by ochratoxin A (OTA)), fumonisins (FBs) (represented mainly by fumonisins B1 (FB1), B2 (FB2), and B3 (FB3)), trichothecenes (TCs) (with type A represented by HT-2 toxin (HT2) and T-2 toxin (T2), and type B represented mainly by deoxynivalenol (DON)), zearalenone (ZEN), the emerging *Fusarium* mycotoxins (fusaproliferin (FP), moniliformin (MON), beauvericin (BEA), NX-2 toxin, and enniatins (ENNs)), ergot alkaloids (EAs), *Alternaria* toxins (ATs) (such as altenuene (ALT), alternariol (AOH), alternariol methyl ether (AME), altertoxin (ALTs), and tenuazonic acid (TeA)), and patulin (PAT). Mycotoxins cannot be detected by eye, but they can be seen under ultraviolet (UV) light; moreover, they have no characteristic odor and they do not alter the organoleptic characteristics of foods [9].

Certain mycotoxins are produced by more than one fungal species, while some fungi are capable of producing more than one mycotoxin. Moreover, more than one mycotoxin can be found on an infected substrate [10]. Favorable climatic conditions cause more fungal and mycotoxin contamination in developing and tropical countries than in developed and temperate ones [11].

2. Mycotoxin Control Strategies: Prevention and Decontamination/Detoxification in Foods

The reduction of mycotoxin contamination in agricultural commodities is a very important problem in many countries worldwide, which led to various preventive measures [11]. All pre-harvest strategies aim to avoid the development of toxigenic fungi and, hence, mycotoxins. However, once mycotoxins are produced, detoxification of foods should be based on post-harvest practices [12].

2.1. Pre-Harvest Strategies

Strategies for pre-harvest prevention include good agricultural practices (GAPs), good manufacturing practices (GMPs), appropriate environmental factors, and favorable storage practices [12]. GAPs include the implementation of a crop rotation program, use of registered insecticides, fungicides, and herbicides for control of insect damage, fungal infection, and weed eradication, proper treatment of the seed bed, soil analysis to determine the need to add fertilizers, and enhancement of genetic synthesis to suppress mycotoxin production [13][14]. Moreover, the use of biological control agents, such as antagonistic fungi, is an important pre-harvest strategy to prevent mycotoxin contamination in staple cereals, grapes, and apples [15][16]. At food processing plants, GMPs must be applied in conjunction with GAPs to act cooperatively with hazard analysis and critical control points (HACCP) [16]. Temperature and humidity exert the greatest influence on mycotoxigenic fungi for the production of mycotoxins, among the environmental factors. As it concerns favorable storage practices, temperature, moisture level, and humidity of warehouses are crucial factors for mold growth and mycotoxin production [12].

2.2. Post-Harvest Strategies

Decontamination/detoxification of mycotoxins from various agricultural products is a global problem, both scientific and practical. It was shown that mycotoxins can be eliminated by natural means such as thermal insulation, radiation treatment, and low-temperature plasma, chemical methods, such as oxidation, reduction, hydrolysis, alcoholysis, and absorption, and biological methods with the use of biological agents [17]. Chemical and physical detoxification methods have many limitations; they cause nutrient loss, are time-consuming and ineffective, and need expensive equipment. In comparison, biological methods proved to be more effective, more specialized, and more environmentally friendly [18].

2.2.1. Physical Treatment

Various practices are used to remove mycotoxins naturally. Some of them are grading, sorting, and the removal of the obviously affected parts of a lot. Moreover, drying, washing, cleaning, segregation, milling, boiling, roasting, irradiation, extrusion, microwave heating, and peeling are used as physical treatments for mycotoxin decontamination. Implementing preventive post-harvest HACCP approaches can contribute to the problem of mycotoxin contamination [15][19].

Sorting

Undoubtedly, cleaning and sorting constitute the first step of natural disinfection. Techniques such as sorting might be regarded as superior methods since they pose no risk of producing degradable products [20]. Sorting and removal of decayed and poor-quality fruits can significantly reduce patulin levels in fruit products by up to 99% [12]. Total FBs decreased in percentage between 26% and 69% in maize after purification [20]. After sorting infected maize, a decrease of 27% to 93% FB was observed. Aflatoxin infection is usually heterogeneous; thus separation of damaged nuclei can effectively reduce infection. The use of ultraviolet radiation was also applied to reduce AFs in the sorting of cereals [21].

Processing

Processing techniques can reduce the concentration of mycotoxins, but they cannot completely destroy them [22]. The level of mycotoxin contamination can be reduced by softening, because the fungi accumulate on the surface of the granules. A study in Kenya showed a decrease in AFs in maize by peeling. The final flour was less contaminated, while mycotoxins DON and ZEN were detected on the surface of the granules at high levels. Temperature and time can affect the mycotoxin content of the final product. Although mycotoxins are thermally stable compounds, some conventional methods of preparing food (baking, frying) at temperatures above 100 °C may reduce certain mycotoxins. The processing temperature and moisture content of the granules affect the reduction of AFs by 50%–80% during the extrusion process [23]. Moreover, temperatures of 150–200 °C significantly reduced AFB1, causing 79% average reduction, being more effective at high humidity [24].

Storage

Storage conditions play an important role in controlling mycotoxins since they affect the overall growth of fungi. In particular, two main factors, temperature and high humidity, can promote both the fungal growth and the production of mycotoxins. Storage under controlled conditions, such as packaging practices, temperature control, ventilation, and appropriate air humidity, reduce the growth of fungi and the accumulation of mycotoxins [25]. Crop losses of 20% to 50% were recorded in developing countries due to inadequate storage practices [22].

Radiation

For many stored cereals, the use of natural detoxifying agents involves the use of radiation. Radiation is usually characterized as either ionizing radiation or non-ionizing radiation [23]. Radiation can reduce or eliminate pathogenic microorganisms, but it partially removes mycotoxins in foods. It can be applied on an industrial scale and is a technique that delivers energy and changes the molecular structure of food ingredients with a series of reactions [21].

Research showed that, in irradiated distilled water and fruit juices of orange, pineapple, and tomato infected with ZEA, ZEA toxicity was reduced and ZEA radiation was safe up to an irradiation of 10 kGy. A higher dose of radiation affected the quality of the fruit juices [26]. In a recent study by Luo et al. [19], after irradiation at 50 kGy with an electron beam in naturally infected corn to degrade ZEN and OTA, decreases of 71.1% and 67.9% were

recorded. In addition, reduction of AFB1 greater than 95% (at 6 kGy) was achieved when gamma irradiation was used for rice processing [25]. Irradiation in apple juice for 5 min caused a significant decrease in PAT (83%) [27].

While radiation was proposed as a promising approach to mycotoxin detoxification, its effectiveness remains questionable because it can cause physical, chemical, and biological effects following potential molecular reactions [19].

Cold Plasma

Cold plasma (CP) has strong antimicrobial effects [21] and it is used in food processing to eliminate pathogens [23]. The fourth state of matter is the alternative name of plasma, mainly consisting of photons, ions, and free radicals such as reactive oxygen and nitrogen species with unique physical and chemical properties [28][29]. Cold atmospheric pressure plasma (CAPP) technology is a different technique, which is promising, low-cost, and environmentally friendly for the decontamination of mycotoxins [21][29]. Low-pressure cold plasma was used for detoxification of up to 50% of alfatoxins on the surface of nuts [30]. This technique requires cautious use as no research on the possible formation of toxic compounds was performed [31].

Significant reduction of AFB1 and FB1 mycotoxins of up to 66% was achieved in maize by the use of CAPP, after just 10 min of treatment [29]. In addition, the use of cold atmospheric plasma caused a 93% reduction in AFs, 90% reduction in TCs, 100% reduction in ZEA, and 93% reduction in FUs after 8 min of exposure [32]. In addition, plasma treatments of only 5 s caused 100% degradation of AFB1, DON, and NIV [6].

Mycotoxin Binders

Mycotoxin binders inhibit the absorption of mycotoxins as they bind to mycotoxins and do not allow their entry into the bloodstream from the gut. Various absorbent materials are activated carbon, aluminosilicates, complex non-digestible carbohydrates, and cholesterol [33]. The use of binding mycotoxins is an alternative physical technique [34] to the microbial degradation of AFs. Cleavage of the lactone ring is a potential target for microbial enzymes, and its cleavage reduces the toxicity of AFs [34]. According to research, to remove patulin from naturally infected cider, as well as to remove aflatoxin in naturally infected milk, activated carbon was used. Mycotoxin level was reduced, but more studies are needed to ensure food safety [21].

2.2.2. Chemical Control

Bases (Ammonia, Hydrated Oxide)

Treatment of seeds with ammonia reduces a number of mycotoxins (AFs, FBs, OTs) to undetectable levels, while the growth of mycotoxigenic fungi is inhibited. Nevertheless, treatment with bases is forbidden in the EU for food intended for human consumption. The application of a mixture of glycerol and calcium hydroxide contributed significantly to mycotoxin detoxification [12]. Sodium hydroxide and potassium hydroxide are often used in the degradation of AFB1 in contaminated oil, although these chemicals can cause secondary contamination and have harmful effects on the nutritional value of the products [35].

Chitosan

Chitosan is a linear polysaccharide, second in abundance in nature after cellulose, inhibiting fungi, bacteria, and viruses. Biocompatibility and antimicrobial properties make chitosan very interesting for the preservation of foods [36][37]. The combined effects of chitosan and a_w for controlling the fungal growth and mycotoxin production of FBs and DON by the *Fusarium* species (*F. proliferatum*, *F. graminearum*, and *F. verticillioides*) on maize and wheat were reported, showing a decrease in DON and FB production in irradiated maize and wheat grains following the application of low-molecular-weight chitosan with deacetylation above 70%, and a dose of 0.5 mg/g [37]. In addition, the application of 1% chitosan enriched with 1% lemon essential oils in figs reduced the from marine brown algae *Ascophyllum nodosum* reduced the levels of DON in wheat grains [38].

Ozone Treatment

The use of ozone (O_3) in the degradation of several mycotoxins was reported in many papers [39][40][41][42][43][44]. Ozonation is an easy technology, which does not leave harmful residues after application. Ozone is used to disinfect cereals, vegetables, and fruits, or to detoxify mycotoxins [44].

Ozone gas was reported by Agriopoulou et al. [45] to be particularly successful in the degradation of aflatoxins, mainly AFB1 and AFG1, since there is a C8–C9 double bond in their structures. Specifically, AFG1 proved to be the most sensitive. Ozone treatment under optimum conditions (55 g $O_3 \cdot h^{-1}$ for 6 h) showed a significant decrease in DON (29%–32%) and its modified form DON-3-glucoside (DON-3-Glc) (44%). Moreover, significant microbial decline was observed in durum wheat, leaving chemical and rheological properties of semolina and pasta from ozonated wheat unaffected [39].

DON was transformed into 10 ozonized products ($C_{15}H_{18}O_7$, $C_{15}H_{18}O_9$, $C_{15}H_{22}O_9$, $C_{15}H_{20}O_{10}$, $C_{15}H_{18}O_8$, $C_{15}H_{20}O_9$, $C_{14}H_{18}O_7$, $C_{14}H_{16}O_6$, $C_{15}H_{20}O_7$, and $C_{15}H_{20}O_{10}$) after treatment with gaseous ozone [46]. DON degradation rate was positively correlated with ozone concentration and treatment time. Specifically, the rate of degradation of DON in solution reached 54.2%, for a treatment time of 30 s and an ozone concentration of 1 $mg \cdot L^{-1}$. DON degradation was significantly influenced by the moisture content of the granules. The degradation rate of DON was 57.3% when ozone concentrations of 60 $mg \cdot L^{-1}$ were applied for 12 h in wheat with a moisture content of 17.0% [44].

According to research by Li et al. [47], fresh noodles made from ozone-treated wheat flour retained more in relation to microbial growth.

2.2.3. Biological Control

In the last 20 years, many researches from groups with different backgrounds and research experience made great achievements in the search for biological agents for mycotoxin detoxification [48]. The use of microorganisms such as bacteria, yeast, and fungi in the degradation of mycotoxins in food and feed is widely reported [18][49][50]. Detoxification/degradation of mycotoxins by biological means offers an alternative approach to the control of

mycotoxins, since it can lead to the production of fewer or even no toxic intermediates and end products. The use of pure microbial strains greatly contributed to the disinfection of mycotoxins in vitro. Moreover, the effectiveness of fermentation in reducing and eliminating mycotoxins was also demonstrated [15].

Bacteria

Certain bacteria have the ability to bind mycotoxins in foods or liquids [49]. The only bacterium among the more than 1000 tested for possible degradation of AFs capable of irreversibly removing aflatoxin from solutions was *Flavobacterium aurantiacum* B-184. Detoxification of AFB1 through *Enterococcus faecium* is accomplished by binding to the cell-wall elements of the bacterium. Peptidoglycans and polysaccharides of bacterial cell walls were shown to be responsible for the binding of mycotoxins with the help of microorganisms [51].

Moreover, bacterial detoxification of mycotoxin DON evolved due to research efforts and advances. Aerobic oxidation and partitioning of this mycotoxin into C3 carbon carried by multiple species of *Devosia* provides solutions aimed at reducing DON contamination [48].

Lactic acid bacteria (*Lactobacillus* (L.) *casei* and *Lactobacillus reuteri*) proved effective in binding to AFs in aqueous solutions. In other in vitro tests, *Lactobacillus amylovorus* and *Lactobacillus rhamnosus* presented a binding efficiency of up to 60% AFB1, showing their potential to bind selected dietary contaminants [7]. Also, reductions 98% FB1 and 84% T-2 were demonstrated during the fermentation of whole-grain sorghum with *Lactobacillus fermentum* [52].

Yeast

The application of biological control agents (BCAs) is a promising strategy for the treatment of mycotoxin infection. The use of competing yeasts is of particular interest, since yeasts produce antimicrobial compounds with beneficial impacts on humans and animals; on the other hand they can develop rapidly on many substrates in bioreactors. In addition, unlike many filamentous fungi or bacterial antagonists, yeasts do not produce allergens or other secondary metabolites [53][54]. *Saccharomyces cerevisiae* is a probiotic yeast which can significantly degrade DON and reduce the rate of lactate dehydrogenase (LDH) release in DON-stimulated cells [55].

Moreover, low concentrations of mycotoxins AFB1 and OTA in chicken diets can be reduced with the addition *S. cerevisiae* yeast cell walls [56]. In addition, the effectiveness of reducing mycotoxin patulin by *S. cerevisiae* in fermented foods by increasing fermentation time and temperature was investigated. Yeast cells are capable of removing PAT via physical adsorption. In fact, the O-N/N-H protein and polysaccharide bonds of cell walls interact with PAT [57] *Kluyveromyces marxianus* were used to bind mycotoxins AFB1, OTA, or ZEA. The results showed that mycotoxins can bind to the cell membrane, especially to *C. utilis* [58]. In another study, the yeast *Yarrowia lipolytica* decreased the concentration of OTA to about half of the initial level introduced into the culture [59].

In addition, a yeast strain of *Rhodotorula mucilaginosa* (*R. mucilaginosa* JM19) was used to degrade PAT, and analysis was performed by HPLC-UV. The results showed that the degradation product of PAT was dexipitulic acid.

The temperature, cell density, and initial concentration of PAT contributed greatly to the degradation of PAT through *R. mucilaginosa* JM19. After 21 h at 35 °C and when the density of yeast cells was above 1×10^8 cells/L, a 90% decrease in PAT was observed. At an initial PAT concentration of 100 µg/mL, *R. mucilaginosa* JM19 was shown to be capable of causing more than 50% degradation, indicating its usefulness in the degradation of PAT in foods and raw materials [60].

Food Fermentation

The fermentation of foods improves their quality while granting them particularly desirable properties for consumers. Fermentation is a fairly inexpensive mycotoxin disinfection approach that can be used both to improve the ingredients in foods and to reduce and even eliminate mycotoxins. Fermentation can be an alternative and desirable technique to reduce mycotoxins compared to costly and impractical techniques. The nature of metabolites and the toxicity of products formed after fermentation should be carefully documented in order to produce safe foods [15].

Fungi

The use of non-toxic strains of *A. flavus* and *A. parasiticus* on maize, cotton, pistachio, and peanuts yielded remarkable success in reducing aflatoxin contamination. Regarding the fungi and their detoxification, it was reported that fungi capable of producing aflatoxins could also break them down. This is because these fungi are often able to degrade and possibly convert and use degradation products as a source of energy under starvation conditions [7]. Fungi such as *Aspergillus*, *Rhizopus, Trichoderma, Clonostachys,* and *Penicillium* spp. show efficient abilities in the detoxification of mycotoxins [14]. In both west and east Africa, the biocontrol of AFs in maize with non-toxigenic microbial strains is based on competition. Specifically, large amounts of non-toxic inoculants of *A. flavus* and *A. parasiticus* enter the soil around the crops and compete with toxigenic strains [16].

2.2.4. Enzymatic Detoxification

The enzymatic detoxification of mycotoxins combines the characteristics of chemical and biological processing. It has high performance and specialization, with application under mild conditions, and it does not cause toxicity to organisms. In addition, enzymes as catalysts are involved in non-stoichiometric ratios of mycotoxins [17]. Some *Aspergillus* species can produce an enzyme that is naturally capable of detoxifying fumonisins, including those produced by *Fusarium* [61]. The activity of enzymes such as β-1,3-glucanase and chitinase against pathogens may vary depending on the characteristics of the microorganism. The delay and decrease in growth of fruit spoilage fungi are affected by the application of β-1,3-glucanases and chitinases [62]. Inhibition of *Penicillium simplicissimum*, *A. Niger* complex, *Penicillium nalgiovense*, and *A. flavus* growth on salami surface samples was induced by spraying β-glucanase at 50% and chitinase at 50% and 40% concentrations. Therefore, β-glucanase and chitinase may be a safe alternative for the fermented sausage industry to control fungal spoilage [62]. Moreover, microbial manganese peroxide, oxidase enzymes, catalase, and laccase enzymes were used for the enzymatic detoxification of AFB1 [16][23]. However, enzymes have an unexplored profile when detoxifying food

contaminants due to their favorable toxicology and specialization. In the EU, no enzyme is approved for the removal of mycotoxin contamination from foodstuffs [23].

2.2.5. Novel Detoxification Strategies

Nanoparticles

Many papers proposed the removal of mycotoxins using the promising adsorbents of nanoparticles. Magnetic carbon nanocomposites were used for AFB1 detoxification, chitosan-coated Fe_3O_4 nanoparticles were reported for PAT decontamination, and silver nanoparticles were reported for degradation of *Fusarium* spp. and their main associated mycotoxins [12][63]. According to a recent study, a new photocatalyst nanoparticle UCNP@TiO_2 (upconversion nanoparticle) was synthesized and used to degrade DON. The results showed a decrease of DON in cereal products below the permissible limits (1 ppm) after 90 min and total degradation after 120 min of illumination. The UCNP@TiO_2 composite material was efficient and green, and the degradation products were only slightly toxic or even non-toxic. Therefore, this degradation technology can be used for mycotoxin detoxification [64]. González-Jartín et al. [65] reported elimination of up to 87% of mycotoxins from nanocomposites composed of mixtures of activated carbon, bentonite, and aluminum oxide.

Plant Extracts

Different essential oils (EOs) and their main bioactive compounds were used for the antifungal and antimycotoxigenic properties [66][67][68], and they were demonstrated to inhibit the production of some mycotoxins [39]. The use of botanicals is usually preferable in the removal of toxigenic fungi and mycotoxins compared to chemical treatments because they are considered safe to humans and environmentally friendly. Several researchers reported that the oil of clove and its major ingredient, eugenol, as well as the turmeric essential oil, inhibit *Aspergillus* growth and AFB1 production. The growth of *A. flavus* and *P. citrinum* and their toxins were inhibited by the application of whole clove in culture media and rice grains [12][69].

A recent scientific study demonstrated the effect of the Spanish paprika smoker “Pimentón de la Vera” on the development of *A. parasiticus* and *P. nordicum* and the production of AFB1, AFG1, and OTA. The addition 2%–3% Spanish paprika smoker in meat products such as fillets or sausage preparations helped minimize the development and production of mycotoxins AF and OTA [70]. Moreover, capsaicin, a natural compound, inhibited OTA production in grapes by *Aspergillus* section *Nigri* strains from 28.9% to 78.1%, and by *A. carbonarius* of 61.5% [71].

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