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Ozone: An Advanced Oxidation Technology to Enhance Sustainable Food Consumption through Mycotoxin Degradation

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ABSTRACT

Mycotoxins are health-threatening fungal metabolites that have been found in several foods around the world. Although agricultural, transportation, and storage management strategies have been employed to reduce the production of mycotoxins, they are not effective in eliminating mycotoxins. In this context, the application of ozone has emerged for the degradation of mycotoxins. Ozone has a strong oxidation rate and generates more free radicals, which can counter the functional groups of the mycotoxin by changing their molecular structures and forming products having lower molecular weight reduced number of double bonds, and reduced toxicity. Research indicates that ozone could be able to destroy mycotoxins without leaving any residues in the commodities. The ozone processing parameters, surface and nutritional properties of food, and fungal species are the primary determinants affecting the processing efficacy. Ozone can contribute to sustainable food consumption through mycotoxin degradation to achieve sustainable development goals (SDGs).

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Introduction

Critical aspects of food systems such as food production, availability, access, utilization, quality, and stability are challenged by climate change and the increased global population, affecting all the facets of food safety and nutritional security. Studies reported changes in global weather patterns, i.e., higher temperatures, lower precipitation rate, water scarcity, droughts, floods, and higher concentration of carbon dioxide in the atmosphere and consequent pest and disease incidence, consequently resulting in lower yields and higher mycotoxin contamination rates (Peter et al. 2020). In due course, it leads to a global concern since fungal colonization, and mycotoxin production on economically important agricultural commodities could globally affect food safety and food security (Magan, Medina, and Aldred 2011; Medina, González-Jartín, and Sainz 2017).

Mycotoxins are toxic fungal metabolites can infect multiple-food matrices showing carcinogenic, teratogenic, nephrotoxic, hepatotoxic, and genotoxic effects (Kim and Chan 2015). Marin et al. (2013) stated that mycotoxin classes of substantial agro-economic relevance are aflatoxins, ochratoxins, trichothecenes, fumonisins, fusarium mycotoxins, ergot alkaloids, enniatins, alternaria toxins, and patulin. Therefore, the allowable amounts of these toxic metabolites in food and feed have been restricted by legislation in several countries (Afsah-Hejri et al. 2011; Sarmast et al. 2021). Park and Troxell (2002) demonstrated that elements of mycotoxin management programs by the U.S. FDA are standardizing regulatory limits, sampling procedure, analytical methods, decontamination process, and converting to lower risk uses for the contaminated product. Also, eliminating such hazardous chemicals from foods can contribute to sustainable food consumption, among the sustainable development goals (SDGs).

Mycotoxin contamination of food is predisposed by diverse circumstances throughout production (Gavahian et al. 2021). It can occur in the food chain from diseased crops, which may be directly consumed by human beings or utilized as livestock feed, also

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present in animal products. Contamination of cereal and cereal-based products with mycotoxins implicated more significant concerns due to its adverse health effects on humans (Khaneghah et al. 2019; Mokhtarian et al. 2020). Mycotoxin occurrence in various crops can be seen in different food chain levels like pre-harvest, harvest, and post-harvest (Khaneghah et al. 2020). Henceforth, effective and unique technologies that can degrade mycotoxins are indispensable. To mitigate mycotoxins in food systems, strategies such as prevention, detoxification, and decontamination can be adopted at stages before and after harvest (Mahmood, Roya, and Amin, 2018; Mohammadi et al. 2020). About detoxification processes, they should detoxify mycotoxins without any toxic residues, assuring the food's nutritive value and with no alteration in the technological traits of the commodity to mitigate adverse pressures on health and economics in all the societies (Khaneghah, Rafael, and Hamid, 2017; Spadaro and Garibaldi 2017)

As the food industry grows increasingly competitive and dynamic, it tries to develop excellent quality, freshly prepared, safer ready-to-eat food products. The focus is now on ozone technology, a non-thermal, non-chemical, and residue-free process that can detoxify mycotoxin levels in food. Ozone is considered a powerful sanitizer that could be utilized in any food industry approved by the regulatory bodies and has consumer acceptance (Khadre, Yousef, and Kim 2001). As ozone has a higher oxidation rate and generates more free radicals when the ozone concentration is higher, it affects the functional groups found within the mycotoxin molecules. This can cause modifications in the molecular structures, thereby forming products with less molecular weight, the minimum number of double bonds, and reduced toxicity, leading to decontaminating and detoxifying mycotoxin in contaminated food or feed (Pandiselvam et al. 2017).

The ozone application in the food sector has expanded in the cereals, meat industry, dairy industry, and fresh agro-produces and their derivatives (Pandiselvam et al. 2019). As it comprises three singlet oxygen atoms, ozone is a potent oxidizing agent and has the powerful sanitizing property to maintain food surface hygiene, reuse wastewater, sanitation of food plant equipment, packaging materials disinfection, and decontamination of storage pest and diseases. The antimicrobial action of ozone is getting popular because of residue-free products (EEA 2018). Application of ozone in food or feed is made either in its gaseous state or aqueous form as ozonized water (De-felice et al. 2008). The ozone application in gaseous form was reported to have higher effectiveness and practical advantages than aqueous solutions (Vithu et al., 2015). Its treatment saves energy as thermal energy is not used (Khadre, Yousef, and Kim 2001). The U.S. FDA has approved of using ozone as a direct additive for food (FDA, 2001).

This review focuses on an overview of the various mycotoxins, their diversified structures, health implications on humans and animals, degradation or detoxifying properties of ozone technology to enhance food commodities' safety and quality.

Mycotoxins types, fungal genera producing mycotoxins, and their mycotoxicosis

Mycotoxicosis is caused due to exposure to mycotoxins which have an acute or chronic impact on human health, potentially causing death (e.g., aflatoxicosis, ergotism) (Marin et al. 2013; Ostry et al. 2017). Mycotoxins in food are caused by two types of fungi at the pre-harvest and post-harvest phases of the crops. The favorable conditions for producing mycotoxins are improper agronomic, harvesting, and post-harvest practices. *Aspergillus, Fusarium, Penicillium, Alternaria,* and *Clavicepsspp.* are the major mycotoxin-producing fungi (Akhila et al. 2021; Alassane-Kpembi et al. 2017; Bashiry et al. 2021) (Table 1). Khaneghah et al. (2021) collected and assessed the quantitative data concerning the occurrence and concentration of several food products and elucidated that the potent means of mycotoxin exposure to humans are contact, ingestion, and inhalation. Main mycotoxins are aflatoxins (AFs) (B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1)), ochratoxins (OTs) (ochratoxin A (OTA)), fumonisins (FBs) (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) (Bryden 2012; Farhadi et al. 2021; Khodaei, Javanmardi, and Khaneghah 2020).

Aflatoxins are furanocoumarins formed by *Aspergillus flavus, Aspergillus nomius,* and *Aspergillus parasiticus* in food/feeds and are contaminated chiefly insufficiency of farming or storage techniques (Javanmardi et al. 2020). The most studied aflatoxin out of 20 known types is aflatoxin AFB1, AFB2, AFG1, and AFG2, named per the blue and green fluorescence displayed in UV. The presence of hydroxylated metabolites like aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) are seen in animal meat and animal products, such as eggs and milk products (Bailey et al. 2006; de Souza, Khaneghah, and Oliveira 2021; Han et al. 2008;

Table 1. Major mycotoxins in food commodities.

Mycotoxin	Structure type	Mycotoxin producing fungi	Food Commodity	Health-based guidance value (HBGV)	References
Zearalenone (ZEN)	6-(10-Hydroxy-6-oxo-trans-1-undecenyl)-β-resorcylic acidlactone	*Fusarium spp.*	Found mostly in all grains; highest levels in maize and wheat bran	PMTDI 0.5 µg/kg BW/day for ZEN, recommended that the total intake of ZEN and its metabolites should not exceed the PMTDI (JECFA 2000); TDI 0.25 µg/kg BW/d for ZEN (EFSA 2011b)	(JECFA 2000, European Food Safety Authority (EFSA) 2011b)
Aflatoxins FB1, FB2, FG1, FG2; metabolite AFM1 in milk	Difuranocoumarins	*Aspergillus parasiticus, A. flavus*	Beans, nuts, figs cottonseed, barley wheat, Peanut's cocoa, rice, copra, maize, dried fruits, spices, crude vegetable oils	Because of carcinogenicity, exposure should be kept as low as reasonably achievable. No official HBGV	(JECFA 2001a), (IARC 2012; EFSA 2007; JECFA 1999, (EFSA 2007; IARC 2012; JECFA 1999, 2001a)
Deoxynivalenol (DON) and its acetylated derivates (3- and 15-acetylDON)	-	*F. graminearum, F. culmorum*	Wheat, triticale, barley, maize, rye, oats, less often rice, and sorghum	TDI 1 µg/kg BW/day for DON (SCF 2002, EFSA 2004); group PMTDI 1 µg/kg BW/day; ARfD 8 µg/kg BW/day for DON and its acetylated derivatives	(EFSA 2004, 2011a; JECFA 2001a, 2011), (EFSA 2004; IARC 1993; JECFA 2001a; SCF 2002), (JECFA 2011)
Patulin (PAT)	Sesterterpene cyclic hexadepsipeptides, 3-hydroxycyclobut-3- ene-1,2-dione, 4-hydroxy-4Hfuro[3,2-c] pyran2(6 H)-one	*Byssochlamys spp., Penicillium spp., Aspergillus spp.*	Many fruits, strawberries, tomatoes, olives, and cereals	PMTDI 0.4 µg/kg BW/day (JECFA 1996)	(IARC 1986; JECFA 1996)
Other trichothecenes, e.g., T-2 toxin, HT-2 toxin, nivalenol (NIV)	Tetracyclic-12,13-epoxy trichothenes	*F. sporotrichioides, F. langsethiae), F. poae and F. cerealis, F. culmorum and F. graminearum*	Cereals	Group TDI 0.1 µg/kg BW/day (EFSA 2011a) and group PMTDI 0.06 µg/ kg BW/day (JECFA 2001a) for T-2 and HT-2 toxins combined. TDI 1.2 µg/kg BW/day for NIV (EFSA 2013)	(JECFA 2001a, (EFSA 2013), (European Food Safety Authority (EFSA) 2011a (IARC 1993)
Ergot alkaloids	Tetracyclic ergolines (tryptophan-derived alkaloids)	*Claviceps spp., in Europe mostly C. purpurea*	True grasses; most important on cereals (wheat, rye triticale, millet, barley, and oats)	Various EAs seem to have similar toxic potency; group ARfD 1 µg/kg BW/day and group TDI 0.6 µg/kg BW/ day; both apply to the sum of EAs (EFSA 2012)	(EFSA 2012, BfR 2004),
Fumonisins B1, B2, and B3 (FB1, FB2, FB3)	1, 2,3-propanetricar-boxylicacid	*Fusarium verticillioides, F. proliferatum, Aspergillus niger*	grapes (A. niger), Maize (Fusarium spp.)	Group PMTD and group TDI, 2 µg/kg BW/day for FB1, FB2, and FB3 alone or in combination	(EFSA 2005; JECFA 2001a, 2012), (EFSA 2005; JECFA 2001a; SCF 2003, IARC 2002), (IARC 2002), I (JECFA 2001a, 2012)
Ochratoxin A (OTA)	Polyketide-deriveddihydroisocoumarinsbound toL-β-phenylalanin byamid bond	*Aspergillus alutaceus, Aspergillus carbonarius, Penicillium verrucosum*	Peanuts, dried fruits, Grain, legumes, wine, grape juice, oleaginous seeds, cashews, coffee, spices, cocoa, meat products	PTWI 120 ng/kg BW/day and 100 ng/kg BW/day	(EFSA 2006), (JECFA 2001a; EFSA 2006), (IARC 1993)

PTWI provisional tolerable weekly intake, PMTDI provisional maximum tolerable daily intake, TDI tolerable daily intake, ARfD acute reference dose (for 1-day exposure)

Kumar et al. 2016; Richard 2007). Ochratoxin A (OTA) is a phenylalanyl derivative primarily produced by two groups of fungi, *Aspergillus ochraceus* and *Penicillium verrucosum*, which is categorized as carcinogenic to human (Group 2B) by IARC (Ostry et al. 2017).

The major fusarium toxins in food are fumonisins, trichothecenes, zearalenone, and emerging mycotoxins such as beauvericin and enniatins fusaproliferin, and moniliformin. In cereal-based foods, these generally occur either singly or in combinations with other mycotoxins, such as aflatoxins (Sadiq et al. 2019). The patulin is produced by fungi, such as *Penicillium, Aspergillus*, and *Byssochlamys* species. It is a usual contaminant in fruit and vegetable products, especially in apples. Damages to the vital organs, such as the kidney and liver, are seen in vitro toxicity assessments (Pal, Singh, and Ansari

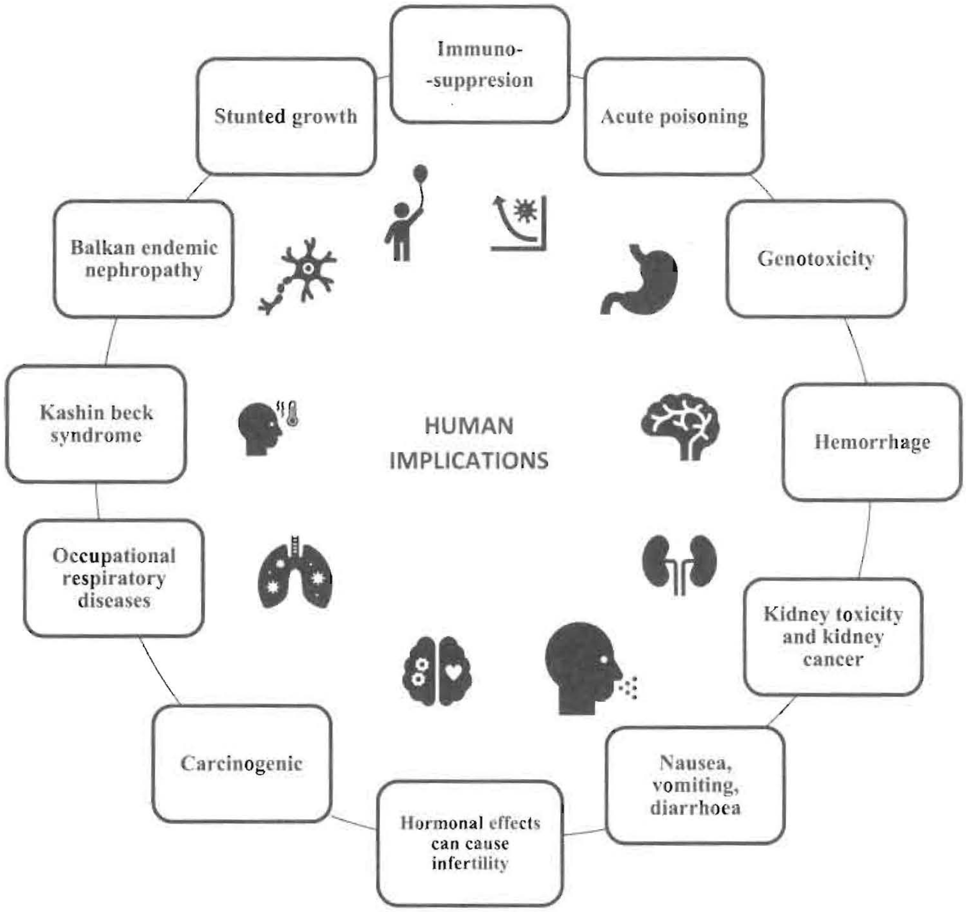

Figure 1. Human implications caused by mycotoxins.

2017). Among more than 70 phytotoxins synthesized, the alternaria mycotoxins produced by *Alternaria* are chemically characterized and reported to cause mycotoxicosis (EFSA, 2011b). The groups like alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins (ATX-I and II), tenuazonic acid (TeA), and iso-tenuazonic acid (iso-TeA) belongs to Alternaria toxins (Marin et al. 2013). Myotoxicity is seen in carcinogenicity, mutagenicity, DNA strand breaks, photophosphorylation, and enzyme activity inactivation (Escriva et al., 2017). Ergot alkaloids (EAs) are toxic components that usually infect major grain cereals, and they are present in *Claviceps species*. It majorly affects the nervous system. Hodgson (2012) reported that ingestion of contaminated rye (*Secale cereale*) caused gangrenous and convulsive ergotism epidemics.

Mycotoxin contamination problems (Economic burdens and health effects)

The mycotoxins can cause mycotoxicosis in human, livestock, and domestic animals and therefore has public health significance. The extent of exposure, physiological and nutritional status, type of mycotoxins, pharmacogenetic variability, and synergistic effects with other chemicals are the probable factors (Figures 1 and 2) that determine the extent of adverse mycotoxin effects (Adaku et al., 2020; Gajecka et al., 2013).

Mycotoxin implicates both chronic and acute adverse effects on human health, such as reducing immunity, liver cancer, gangrene, convulsions, modifications in the protein metabolism, and respiratory disorders (Negedu et al. 2011). In this context, interventions to strengthen and imply legislations to control the extent of food mycotoxin contamination at the global level are very much essential (Freire and Da Rocha 2016).

Mycotoxins' economic consequences in society may be either by the direct market costs about the fewer revenues caused by contamination of various food commodities or the adverse health effects on humans. Mycotoxins cause lethal effects on humans and animals, causing higher health and veterinary costs, lower production efficacy, or making the food commodities unacceptable for domestic or global trade due to non-confirmation with the maximum

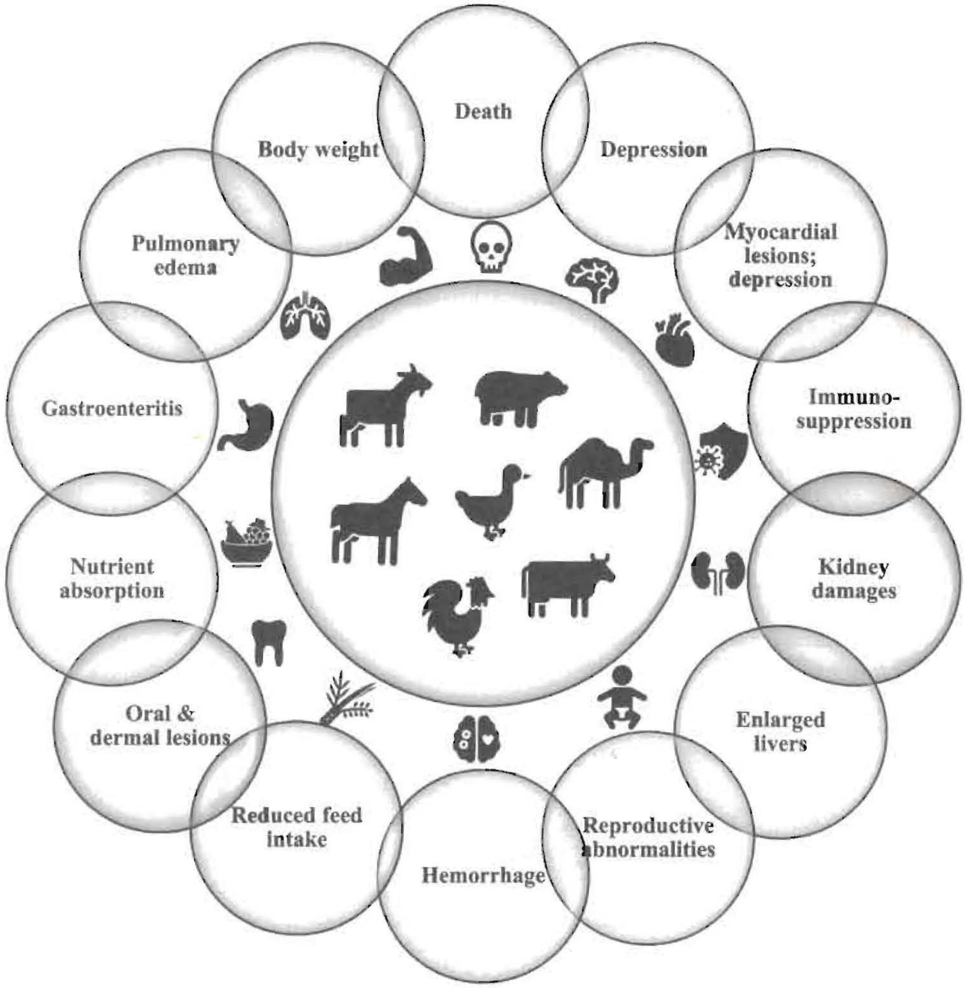

Figure 2. Health implications of mycotoxins in animal species.

tolerated levels mycotoxins contained in it (Pitt and Miller, 2017). The socio-economic impact of mycotoxins in foods is depicted in Figure 3.

Mycotoxin's occurrence in agricultural commodities affecting food quality

The toxins production is mainly confined to predisposing factors such as biological, chemical, physical, or environmental conditions (Figure 4). The mycotoxin production is majorly confined to the type of fungi present, agronomic practices, food composition, harvesting, handling, and storage conditions (Ashiq 2015; Bryden 2009). The quantity of the toxin production depends on conditions such as physical (moisture, temperature, relative humidity, and mechanical damage), chemical (oxygen, carbon dioxide, substrate composition, pesticide, and fungicides), and biological factors (plant variety and biotic and abiotic stress) (Lahouar et al. 2015). Mycotoxins can get into to food chain by direct consumption of contaminated food and plants and indirectly via animal food (milk, meat, and eggs) residues. The mycotoxin incidence in the food chain has been shown in Figure 5. The post-harvest strategies to limit mycotoxin contamination in food were assessed by Magan et al. (2010). The regulatory authorities should take stringent control strategies to mitigate this contamination issue for the country's safety and economy. Key critical control points in the grain processing chain for effective mycotoxin prevention are depicted in Figure 6.

Allowable mycotoxin level in food

There was a significant decline in the economy, nearly about 25%, owing to mycotoxins' presence in various food matrices (Marin et al. 2013). For limiting various mycotoxins, Food and Drug Administration (FDA) has set up various control programs that can remove these components that attack various food matrices and essential commodities in the food industry through risk

Figure 3. Socio-economic implications of mycotoxins.

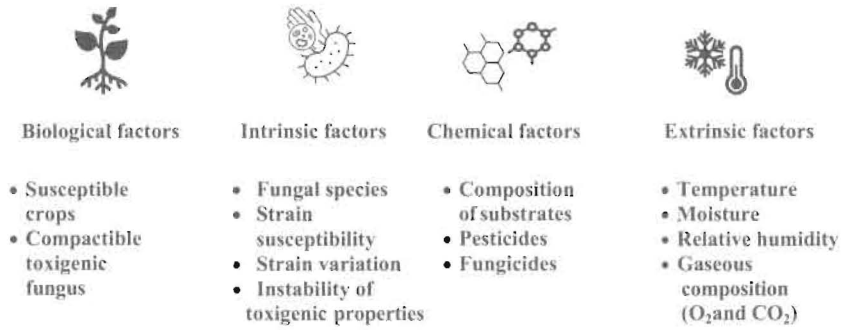

Figure 4. Pre-harvest factors affecting mycotoxins occurrence in food chain.

assessments. However, the requirement of specific extent limits of mycotoxins in food commodities depends on two major issues, that is, the impact on humans and animals' health by mycotoxin contamination and the occurrence of mycotoxins in human foods derived from animals fed with mycotoxin-contaminated feed (Park and Troxell 2002).

Continuous efforts of the Food Safety and Standards Authority of India (FSSAI), U.S. FDA, and European Union (EU) limits and levels (Table 2) conveys public health implications for combating mycotoxins in common foods milk residues, and animal feeds. Safety is a significant concern, and limits are imposed accordingly for public safety and animal health. Various health hazards and infections are caused if the levels are not standardized correctly in various food and feed. As mycotoxins are hazardous and crucial in the food industry, safety assurance must be followed at all stages of food processing (Lee and Ryu, 2017).

Table 2 depicts all the regulations established by the United States Food and Drug Administration (US FDA) and European Union (EU) on different mycotoxins by various fungal species on cereals (maize, wheat, rye, barley, and sorghum), legumes (peanuts), nuts (pistachio, almonds, tree nuts), vegetables (asparagus), fruits (apple, pears, grapes, apricot, olives, and other low acid fruits and juices), oilseeds, fermented beverages (wine and beer), fibers (cottonseed), spices, millets, milk and its products, and meat. While their (The International Agency for Research on Cancer) number given by the international agency of cancer research based on their severity and carcinogenicity. However, there are no

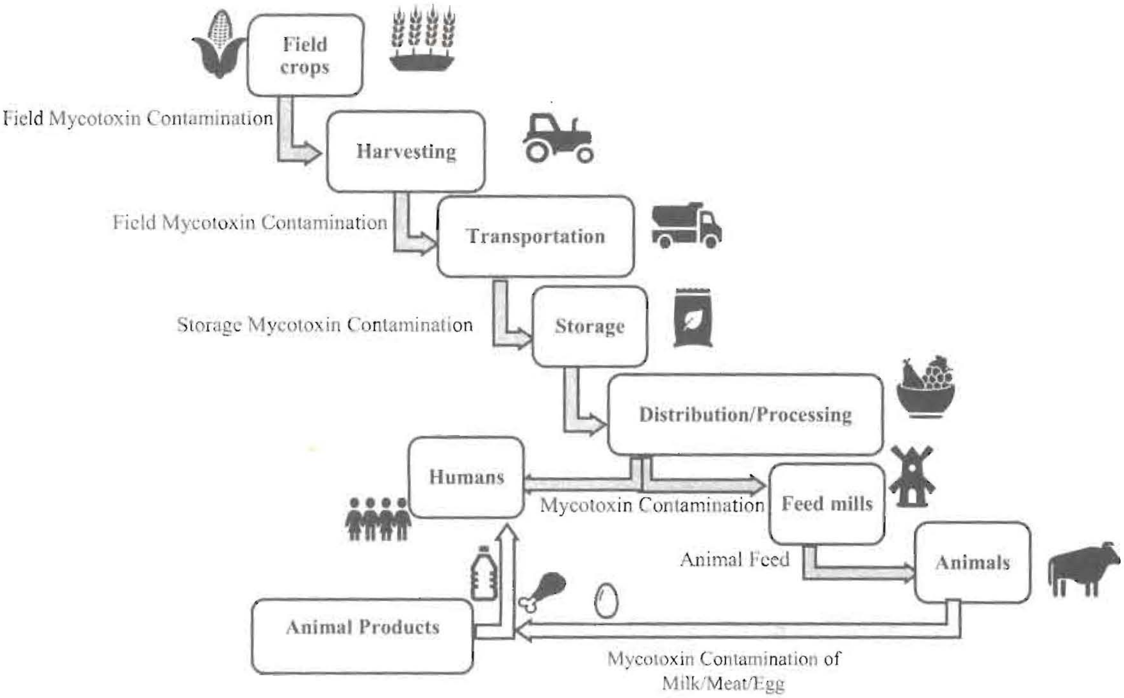

Figure 5. Post-harvest factors affecting mycotoxin occurrence in food chain.

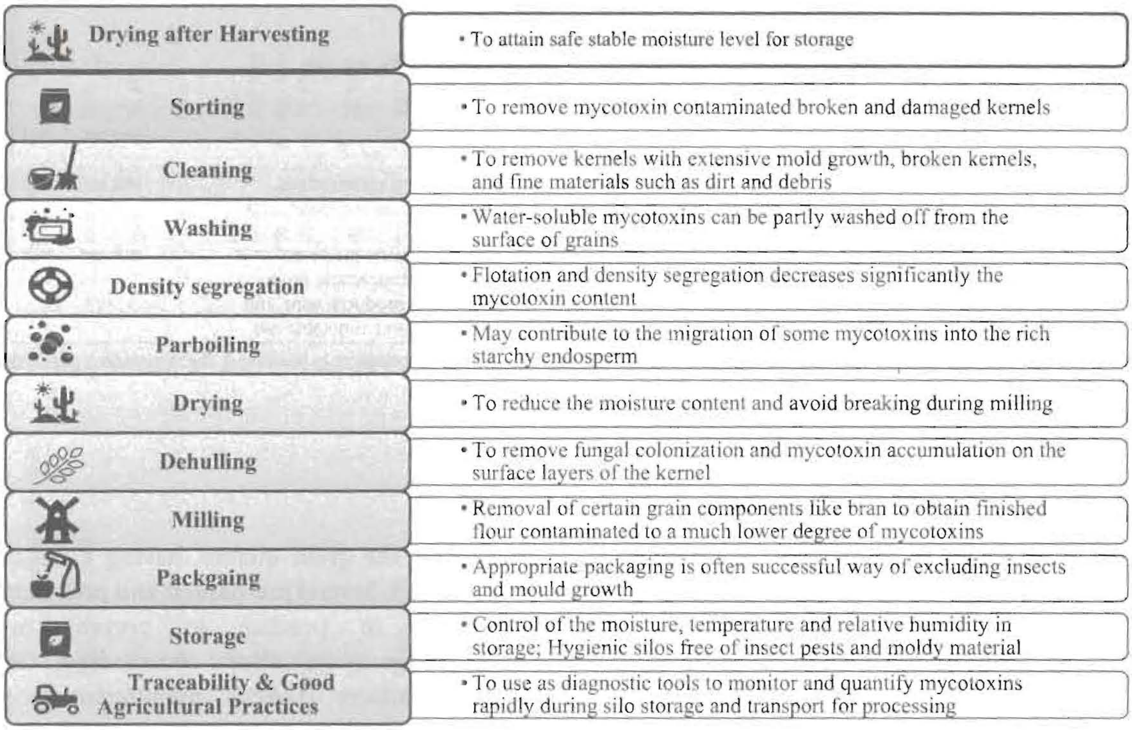

Figure 6. Key critical control points in the grain processing chain for effective mycotoxin prevention.

Table 2. Allowable mycotoxin levels in food in accordance with the U.S. FDA and EU.

Mycotoxin	IARC		Number *	Acronym	Fungal Species	Food Commodity
US FDA (μg/kg)	EU (μg/kg)					
Aflatoxins B1, B2, G1, G2	1*	AFB1	*Aspergillus flavus, Aspergillus parasiticus*	Maize, wheat, rice, peanut, sorghum, pistachio, almond, ground nuts, tree nuts, figs, cottonseed, spices	20 for total	2–12 for B1 4–15 for total
Aflatoxin M1	2B*	AFM1	*Metabolite of aflatoxin B1*	Milk, milk products, meat	0.5	0.05 in milk 0.025 in infant formulae and infant milk
Ochratoxin A	2B*	OTA	*Aspergillus ochraceus, Aspergillus carbonarius Penicillium verrucosum, Penicillium nordicum*	Cereals, dried vine fruit, wine, grapes, coffee, cocoa, cheese	Not set	2–10
Fumonisins B1, B2, B3	2B*	FB1 FB2 FB3	*Fusarium verticillioides, Fusarium proliferatum*	Maize, maize products, sorghum, asparagus	2000–4000	200–4000
Zearalenone	3*	ZEN	*Fusarium graminearum (F. roseum), Fusarium culmorum Fusarium equiseti, Fusarium cerealis, Fusarium verticillioides, Fusarium incarnatum*	Cereals, cereal products, maize, wheat, barley	Not set	20–100
Trichothecenes			deoxynivalenol)	3*	DON	*Fusarium*
(type B: *graminearum, Fusarium culmorum, Fusarium cerealis*	Cereals, cereal		products	1000	200–50	
Patulin	3*	PAT	*Penicillium expansumBysochlamisnivea, Aspergillus clavatus, Penicillium patulum Penicillium crustosum*	Apples, apple juice, and concentrate, pears, peaches, grapes, apricots, olives low acid fruit juices	50	10–50
Trichothecenes (type A: HT-2)	3*	HT2	*Fusarium langsethiae, Fusarium sporotrichioides*	Maize, wheat, barley, oat, rye	15	25–1000
Trichothecenes (type A: T-2 toxin)	3*	T-2	*Fusarium langsethiae, Fusarium sporotrichioides*	Maize, wheat, barley, oat, rye	15	25–1000
Enniatins		ENNs	*Fusarium tricinctum, Fusarium avenaceum*	Corn	Not set	Not set
Ergot alkaloids		EAs	*Claviceps purpurea, Claviceps fusiformis, Claviceps africana, Neotyphodiumspp*	Rye, rye-containing commodities, wheat, triticale, barley, millet, and oat	Not set	Not set
Alternariol		AOH	*Alternaria alternata*	Grain and grain-based products, vegetables and vegetable products, fruits and fruit products, wine and beer, oilseeds, and vegetable oils	Not set	Not set

IARC number definitions: 1, the mycotoxin is carcinogenic to humans; 2B, the mycotoxin is possibly carcinogenic to humans; 3, the mycotoxin is not classifiable as to its carcinogenicity to humans

Source:(Agriopoulou,Stamatelopoulou, and Varzakas

findings on levels for some mycotoxins like Enniatins, Ergot alkaloids, and Alternariol as U.S. FDA and EU do not set them.

Mycotoxin degradation in food products using ozone

The agricultural industry has major ongoing concerns about pre-harvest and post-harvest contamination in developing nations. The factors such as high temperature, inadequate production technologies, and unsterile crop storage conditions contribute to mycotoxins' fungal growth and production. The presence of mycotoxins deteriorates the grain quality, having a negative economic impact. Several pre-harvest and post-harvest strategies are in practice to prevent mycotoxin contamination in agricultural commodities. Good agricultural practices (GAPs), manufacturing practices (GMPs), optimizing environmental factors, and storage practices are significant strategies for pre-harvest prevention. GAPs involve crop rotation using registered herbicides, insecticides, and fungicides to manage various pests, fungi, and weeds. The prolonged storage conditions, extreme temperature, and humidity after crop harvesting in cereals contribute to fungus development and mycotoxin contamination. Post-harvest approaches include sanitization of mycotoxins in

Table 3. Mycotoxin degradation using ozone in different food commodities.

Food Matrix	Target Mycotoxin	Ozone Concentration	Exposure time	State of ozone	% Reduction	References
Malting Barley	Deoxynivalenol (DON)	26 g m-3	120 Min	Gaseous	Zero (May be due to the less initial concentrations of DON)	Dodd et al. (2011)
Peanut kernels	Aflatoxin B1 (AFB1)	21 g m-3	96 h	Gaseous	25%	de Alencar et al. (2012)
	AFB1	6 g m-3	30 Min	Gaseous	66%	Proctor et al. (20044)
	AFB1	50 g m-3	60 h	Gaseous	89%	Diao, Hou, and Dong (2013)
Pistachio Kernels	AFB1	8 g m-3	2 h	Aqueous	48%	Bashiri et al. (2013)
	AFB1	9 g m-3	420 Min	Gaseous	23%	Akbas and Ozdemir (2006)
	AFT	9 g m-3	420 Min	Gaseous	24%	
De-hulled Dried Pistachio	AFB1	10 g m-3	15 Min	Aqueous	0%	Nooghi et al. (2016)
Wheat grains	AFB1	40 g m-3	20 Min	Gaseous		Mc kenzie et al. (1998)
	AF and DON	60 g m-3	300 Min	Gaseous	64%,48%	Trombete et al. (2016)
	DON	118 g m-3	180 Min	Gaseous		Savi et al. (2014a)
	AFB1, AFB2, AFG1, AFG2	118 g m-3	180 Min	Gaseous	95%	Savi, Piacentini, and Scussel (2015)
	Citrinin	118 g m-3	180 Min	Gaseous	75%	Savi, Piacentini, and Scussel (2015)
	AFB1	2.5 g m-3	180 Min	Aqueous	40%	
	AFG1 and AFG2	2 &1 g m-3	180 Min	Aqueous	100%	Kouchesfahani et al.(2015)
	DON	10 g m-3	30 s	Gaseous	94%	Li, Guan, and Bian (2015)
	DON	20 g m-3	130 Min	Gaseous	25%	Li, Guan, and Bian (2019)
	HT-2 toxin (HT-2), T-2 toxin (T-2) and Zearalenone (ZEA)	20 g m-3	40 and		130 Minutes	Gaseous
65%,62%,59%	Reinholds et al. (2016)					
Scabbed Wheat	DON	10 g m-3	30 s	Gaseous	94%	Reinholds et al. (2016)
Wheat Flour	DON	65 g m-3	180 Min	Aqueous	78%	Alexandre et al. (2017)
	DON	100 g m-3	1 h	Gaseous	78%	Sun et al. (2016)
	DON	75 g m-3	90 Min	Gaseous	54%	Wang et al. (2016)
Contaminated Wheat, Corn and Bran	DON	80 g m-3	10 Min	Aqueous	76%	Sun et al. (2016)
Wheat Bran from contaminated grains	DON		30 Min	Gaseous	45%	Alexandre et al. (2018)
	ZEA	62 g m-3	240 Min	Gaseous	61%	Alexandre et al. (2018)
Wheat grains, Semolina and Pasta	DON and DON-3-Glc	40 g m-3	6 h	Gaseous	29%,44%	Piemontese et al. (2018)

(Continued)

Table 3. (Continued).

Food Matrix	Target Mycotoxin	Ozone Concentration	Exposure time	State of ozone	% Reduction	References
Corn	AFB1	90 g m-3	40 Min	Gaseous	88%	El-Desouky et al. (2012)
	ZEA and OTA	100 g m-3	180 Min	Gaseous	91%,71%	Qi et al. (2016)
	ZEN	10 mg/L	2 s	Aqueous		Qi et al. (2016)
	OTA	10 mg/L	120 s	Aqueous	65.40%	Lijun et al. (2016)
Cornflour	AFB1. AFLATOXIN B2 (AFB2), AFLATOXIN G1 (AFG1) AND AFLATOXIN G2 (AFG2)	75 g m-3	60 Min	Gaseous	79%,71%,72%,75%	Luo et al. (2014a, 2014b)
	ZEN	50 g m-3	90 Min	Aqueous	95%	Xu et al. (2019)
	ZEA	52	60 Min	Gaseous	62%	Alexandre et al. (2019)
	ZEA	51.5 mg/L	60 Min	Gaseous	62.30%	Allana et al. (2019)
Corn Grits	AFB1, AFB2, AFG1, and AFG2	60 g m-3	480 Min	Gaseous	55%,57%,36%,30%	Porto et al. (2019)
Poultry feed composed of corn, barley, soybeanand sunflower meal	AFB1	5.3 g m-3	240 Min	Gaseous	86%	Torlak et al. (2016)
Apple Juice	Patulin	12 mg/L	15 Min	Gaseous	100%	Cataldo (2008)
Brazil Nut	AFT	14 mg/L	30 days	Gaseous	100%	Giordano et al. (2010)
Dried Figs	AFB1	13.8 mg/L	180 Min	Gaseous	95.20%	Zorlugenç et al. (2008)
	AFB1	1.71 mg/L	180 Min	Aqueous	88.60%	Zorlugenç et al. (2008)
Flaked Red Pepper	AFB1	66 Mg/L	60 Min	Gaseous	93%	Inan, Pala, and Doymaz (2007)

agricultural produce after harvest. The mycotoxin decontamination through chemical (oxidation, reduction, hydrolysis, alcoholysis, and absorption), physical (thermal insulation, radiation treatment, low-temperature plasma), and biological means are ineffective, time-consuming, expensive, and may cause nutrient loss.

Ozone plays a central role in controlling mycotoxigenic fungi in agricultural commodities and food products. Ozone has been shown to protect contaminated food and feed and to enhance food safety (Pandiselvam et al. 2019; Sivaranjani et al. 2021). Post-harvest decomposition problems are associated with molds, majorly to *Fusarium, Aspergillus*, and *Penicillium species*, at the time of storage (Filtenborg, Frisvad, and Thrane 1996; Rodrigues et al. 2009). Ozone is utilized in the detoxification of agricultural commodities, which comprises the organically labeled units (Gabler et al. 2010), and has given promising results in the food industry, such as mycotoxin and pesticide residues degradation. Table 3 represents the previously attempted mycotoxin inactivation in various grain and food products.

Ozone was used to decontaminate mycotoxins in barley, wheat, figs, and Brazil nuts (Wu, Doan, and Cuenca 2006; Zorlugenc et al., 2008), without affecting its nutritional and morphological characteristics. The improved storage traits of common cereals, namely wheat, corn, and rice (less fungal growth and mycotoxin), have been reported by ozone application (Ferreira et al. 2018). Additionally, ozone caused no modification in color and overall appearance of ozonated barley and wheat and rice (Wang et al. 2010). The study conducted by Kottapalli, Wolf-Hall, and Schwarz (2005) in wheat grain revealed that 15 min of ozone exposure at the dosage at 11 mg/g reduced *Fusarium* growth by 24–36%. Allen, Wu, and Doan (2003) investigated the fungicidal performance of gaseous ozone on the barley and confirmed that after 5 min of ozone exposure with a concentration of 49 mg ozone/minute (for barley seeds of 50 g), 96% of fungal spores were inactivated. However, the prolonged exposure at this rate decreased the germination of barley. This investigation confirmed a strong correlation between contact duration, gaseous ozone concentration, barley-water activity, and temperature. In general, in cereals, moderate ozone treatments remarkably improved the milling traits, swelling power of starch, and dough viscosity (Zhu 2018). The phytate, vitamin, and lipid content in wheat kernels were unaffected by moderate ozone treatments. However, excessive and prolonged ozone treatment denatured wheat proteins (gluten and glutenin), affecting dough rheology. In rice grains, excessive ozonation increased rice flour's viscosity due to enzyme inactivation, but rice flour's gelatinization properties were unaffected.

Zorlugenc et al. (2008) elucidated that exposure of figs to 13.8 g/m^3 gaseous ozone for 15 minutes inactivated all fungi. This investigation concludes that ozone in the aqueous phase is efficacious than ozone in the gaseous phase. Gabler et al. (2010) investigated the effect of gaseous ozone (up to 5 g/m^3) for 1 h on table grapes for controlling gray mold (*Botrytis cinerea*) and destruction of pesticide residues. The ozonation for an hour reduced gray mold by almost 50% for two-week storage at 15 C. The study concluded that, with an increased concentration of 10 g/m^3 for 1 hour, the residues of fenhexamid, cyprodinil, pyrimethanil, and pyraclostrobin were lowered by 68.5, 75.4, 83.7, and 100.0%, respectively, in table grapes.

The contamination of various food stuffs such as dairy items, edible oil, cereal, fruit-based products, nuts, and dry fruits with mycotoxin is continually a concern for the food industry. The fate of mycotoxins is majorly affected by the unit operations pertained in the processing line of the numerous food products. Mycotoxins remain active during food processing, including cooking, baking, and pasteurizing, resulting in food contamination (Mir et al. 2021; Khaneghah, Yadolah, and Anderson, 2018; Khaneghah et al., 2018 ;Khaneghah et al., 2019; Khaneghah et al., 2019).

Mycotoxin degradation using ozone eliminates the fungi producing mycotoxin with no effect on the sensory attributes of the foods. These days, ozone is replaced with chlorine to sanitize food and prevent occupational and environmental contamination (EPA, 1999). As ozone has a very high oxidation potential of 2.07 V, it makes it 1.5 and 1.3 times more potent than chlorine and H_2O_2, respectively (Khadre, Yousef, and Kim 2001). Ozone was approved as an antimicrobial factor in 2001 for detoxifying foods, tackling occupational and environmental problems using chlorine (Mahapatra, Muthukumarappan, and Julson 2005; Sharma 2005). Therefore, chlorine is replaced with ozone for microbial inactivation in food commodities, such as fruits and vegetables, juices, milk, and its products and poultry (Frietas-silva and Venancio, 2010). Besides, ozone is used in the food industry to decontaminate food-packaging material, food-contact surfaces and lower unwanted off-odors metabolites.

Application of ozonation in inactivation of mycotoxigenic fungi in various food commodities

Ozone has a great potential of up to a hundred percent reduction rates in inactivating several mycotoxins. This triatomic oxidizing powerful tool utilized in food industries as mentioned by various authors based on the evidence given below against major mycotoxins (Table 3). Several factors such as temperature, grain characteristics, organic matter presence, pathogens, insects, and moisture affect the diffusion of ozone in crops like maize (Wang et al., 2016) and similarly in other food commodities. The sensitivity of fungal conidia to ozone varies among genera and can be influenced by factors imposed by the environment. For example, the type and amount of organic material present diminish the effectiveness of ozone against *Aspergillus niger* conidia (Beuchat et al. 1999). On the other hand, ozonation reduced the fungal colonies of *Fusarium verticillioides* on maize at a concentration of 200–300 ppm (Mylona et al. 2014).

Wang et al. (2016) revealed results for detoxifying the DON in wheat by treating 75 mg/L ozone for a period of 30, 60, and 90 minutes to obtain the reduction percentages of 26.40%, 39.16%, and 53.48%, respectively. Usually, chemical degradation of DON takes place when O_3 molecules undergo a 1–3 dipolar reaction with a double bond. Besides detoxifying DON, the treatment could improve the flour quality (whiteness and tenacity). Therefore, it may be assumed that for eliminating DON, gaseous ozone drastically affected the pericarp of wheat grain than in the endosperm and ozonation before milling enhanced bran friability, thereby improved the flour quality.

Studies reported a 100% reduction percentage of AFG1 and AFG2 mycotoxins at even low ozone concentrations (2 and 1 g/m^3) for 180 minutes in wheat grains (Mohammadi et al. 2015). However, the potent aflatoxin, out of all the four, AFB1, is not entirely inhibited through more ozone concentration was supplied. Therefore, during the treatment, ozone in the liquid phase was a promising strategy for complete detoxification of fungal growth, thereby reducing the aflatoxin. Although negligible in the experiment process, the main factor for complete decontaminating the aflatoxin is the highest concentration, i.e., 2.5 mg/l. Besides, all these millings with uniform consistency, solubility, stability, and reactivity which are temperature-dependent ozone factors, resulted in proper control of mycotoxins.

Studies on peanut kernels depicted that AFB1 is hazardous among the other four aflatoxins and is carcinogenic among aflatoxins. IARC has classified mycotoxins into group I based on their carcinogenicity to humans (Agriopoulou, Eygenia, and Theodoros 2020). Elucidations by Agriopoulou et al. (2016) states that it is convenient to degrade aflatoxins (AFB1 and AFG1) as their structure showed C8-C9 double bonding.

A similar reduction percent, i.e., complete efficiency of ozone, was reported in deshelled brazil nut at 14 g/l ozone concentration for 30 days by Giordano et al. (2010) gaseous phase against AFT. However, in the shelled brazil nut, similar results were observed with less fungal growth due to the protection from the surface of the shell, and the destruction of mycotoxin depends on the factors like stages of growth and gas concentration applied.

The highest reduction percentages in Table 3 show ozone as a potential decontaminating agent for mycotoxin in various food and feed. However, in some studies conducted by Nooghi et al. (2016) in deshelled pistachio, there was no reduction of AFB1 when exposed to 10 gm^{-3} O_3 concentration for 15 minutes at room temperature. They revealed that the application of DHA had more anti-mycotoxigenic effects than ozone in the Iranian pistachios. However, this was controversial in the studies shown by Bashiri et al. (2013) as they depicted results from the 1 N choleric acids when combined with ozonated water to standardize the pH.

Powerful triatomic ozone can detoxify to a maximum level in very little time reported in some studies were shown in this review. The 10 gm^{-3} gaseous ozone applications only for 30 seconds resulted in a 94% reduction in scabbed wheat grains (Reinholds et al. 2016). This has experimented in view that more prolonged exposure to ozone leads to a reduction in germination rates. Reinholds et al. (2016) supported that fumigation is the key to the substantial elimination of mycotoxins in wheat. However, high moisture levels in the wheat decrease the efficacy of ozonation due to its decomposition. Consequently, the exposure time was prolonged. Biochemical and physical attributes shown that no possible effects on grain characteristics after treatment.

Ozone application studies focused on various fruits, and their feasibility of using them as a decontamination tool for juice processing was reported in several studies. For example, Catlado (2008) experimented in diluted apple juice against major mycotoxin Patulin with 12 mg/L O_3 concentration for 15 minutes, resulting in a 100% reduction rate because of 2 conjugated ethylene double bonds and with keto groups of lactose moiety, which was sensitive to ozone attack. The factors influencing here are due to the crystal clear of juice when diluted and

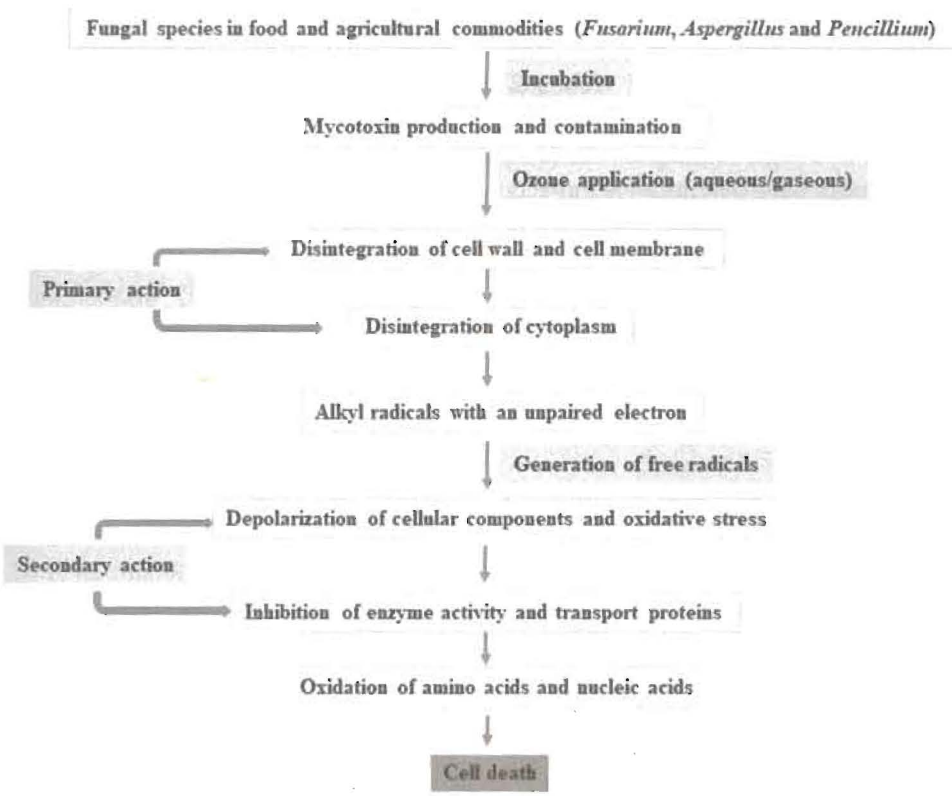

Figure 7. The effect of ozone on mycotoxigenic fungi.

bubbled, and after elution of sugars in the juice, patulin was detoxified effectively with lesser ozone application into the juice; however, there was a significant observation in the study. Therefore, medium treatment of O_3 leads to reducing patulin toxin in various juices such as pears and apples with a superior industrial application.

Dehydrated fruits such as dried figs contaminated with mycotoxins like AFB1 were decontaminated at 13.8 mg/L for 180 min, reducing 95.2%. The dried figs had good efficacy of ozone due to low moisture and sun-dried figs. In addition, several spices like flaked red pepper are also a critical food matrix that was contaminated with few mycotoxins. Therefore, reducing mycotoxins characteristics like color decline was an essential attribute for marketable acceptance by consumers that may affect the market demand. Contrastingly investigations showed by Inan, Pala, and Doymaz (2007) interestingly shown 93% in reduction rate without a change in the color values even after the ozone application, and more reduction in levels of mycotoxins were recorded below the levels of Turkish Food Codex as this was a significant focus of the study in that region.

Major determinants affecting the processing efficacy

The ozone technology in mycotoxin degradation can be well explored to develop a detoxification method to ensure food quality and safety. O_3 inhibits fungal growth, sporulation, and germination. To degrade the mycotoxins by ozonation, it follows a pseudo-first-order rate (Aguilar et al., 2018). Ozonation efficiency increases with higher temperature and more prolonged exposure. The efficiency of the ozonolysis process depends on the concentration of ozone, exposure time, and temperature. Ozone decomposes quickly at temperatures higher than 50°C (Diao, Hou, and Dong 2013). The efficacy of ozone is determined by factors, such as treatment method (Aqueous or gaseous phase), dosage rate, and exposure time of its application, fungal population or contaminants, and the kind of food or feed. Due to its short half-life of 20–30 min at ambient temperature and 7.0 pH (Khadre, Yousef, and Kim 2001), its permanence in the aqueous phase is influenced by the pH and exposure time. The greater the pH, the poorer is the stability of O_3 (i.e., shorter half-life). For fungal and pathogenic microbes, minimal exposure time and dose are needed to attain the required deactivation (Cullen et al. 2009; Gujer and von Gunten 2003; Karaca and Velioglu, 2007). The ozone application should not surpass a certain threshold level as a high ozone concentration and long exposure times can have deteriorative impacts on the food quality traits. The processing efficiency is dependent on fungal species, the age of culture, the population density, the existence of compounds having demand for O_3, modes of O_3 application (aqueous/gaseous), precision of procedures, and method to assess the antimicrobial efficiency (Guzel-Seydim, Greene, and Seydim 2004).

The mechanism of mycotoxin detoxification using ozone

Mycotoxins have structural differences that significantly affect the response to ozone. The mechanism of decontamination is unknown for some mycotoxins. Understanding the mechanism(s) of mycotoxin degradation by ozone will help design the best mycotoxin degradation method to improve food safety and quality. Previous studies report that ozone reacts with the functional groups in the mycotoxin molecules, particularly constituents of the cell membrane (enzymes, proteins, unsaturated fatty acids), cell walls (peptidoglycans), cytoplasm (enzymes, nucleic acids), fungal coats, and virus capsids (proteins and peptidoglycan) (Guzel-Seydim, Greene, and Seydim 2004a; Greene, Guzel-Seydim, and Seydim 2012; Pirani 2010). There are primarily two modes of mycotoxin inactivation by ozone. Firstly, sulfhydryl groups, amino acids of enzymes, peptides, and proteins oxidize to produce smaller peptides. Secondly, polyunsaturated fatty acids oxidize to produce acid peroxides.

This interaction results in the loss of double bonds and modification of molecular structures (Wang et al., 2016), causing leakage of their contents, and ultimately, cell death. Also, ozone causes extensive oxidation of integral cellular proteins affecting growth and rapid cell lysis. The first step in mycotoxin detoxification is the breakdown of the cell wall (Figure 7). Following the initial stage and a cycle of chemical reactions, the lipids are entirely peroxidized by free radicals. The H_2 molecule is removed from other unsaturated fatty acids, resulting in the "free alkyl radical with an unpaired electron in a carbon atom." The peroxidation products alter the cell membranes' physical attributes, such as depolarization and inhibition of transport proteins and enzyme activities. Further, the weakened cell membrane causes oxidation and destruction of amino acid, protein, and nucleic acid, causing cell lysis (Greene, Guzel-Seydim, and Seydim 2012; Margalit et al. 2001; Pirani 2010). The research depicted that UPLC Q-TOF MS was a valuable analytic means for conforming and detecting a sequence of unknown by-products after ozonolysis of mycotoxins (Luo et al., 2014c).

When studied on the AFB1 degradation products treated with aqueous ozone, it seems complex in species and minimal for the lower concentrations of each product. UPLC Q-TOF-MS derived most of the critical degradation products, and their toxicity was evaluated based on structural activity relationship. The double bond in the terminal furan ring of AFB1 got destructed there by the toxicity had been significantly minimized. It was found that aqueous ozone treatment was a potent approach for degrading AFB1 (Luo et al. 2013).

Studies illustrated that when corn with different moisture content (13.47% and 20.37%) was exposed to ozone at three various concentrations (40, 65, and 90 mg/L) for varied ozonation time (0, 5, 10, 20, and 40 min) under conditions of 25°C and 75% R.H it was found that ozone can efficiently degrade AFB1. Here, the degradation rate enhances with the rise in ozone dosage and exposure time. In addition, the moisture content in the corn helped in having more contact area between the commodity and ozone. Therefore, it enhanced the ozone's capacity to degrade AFB1 (Luo et al. 2014a).

A study on ozonolysis of aflatoxin B1 (400 μg/mL) in acetonitrile solution was performed with an ozone dose of 6.28 mg/L at the flow rate of 60 mL/min for varied times. The results demonstrated that ozone was an efficient decontamination agent due to its potent oxidative function. To confirm and identify the by-products of ozonolysis of AFB1, thin-layer chromatography and liquid chromatography-quadrupole time-of-flight mass spectra were utilized and helped in knowing the AFB1 ozonolysis pathway. The toxicity of AFB1 was substantially lowered due to the loss of "the double bond on the terminal furan ring or the lactone moiety on the benzene ring" (Diao et al. 2012)

Role of ozone in other mycotoxin decontamination technologies

As described in the previous sections, ozone has been used to decontaminate mycotoxins. Simultaneously, some other mycotoxin degradation technologies, such as ammonification (Chelkowski et al. 1981), cold plasma, owe their efficiency to ozone. Indeed, cold plasma without ozone might not be as effective as expected in the elimination of mycotoxins (Gavahian and Cullen 2020). It was previously explained how similar the mechanisms are involved in decontamination with ozone and cold plasma (Gavahian, Sarangapani, and Misra 2021). In this sense, future research on cold plasma without ozone can be interesting to better understand the ozone percentages contributing to plasma-induced decontamination.

Challenges associated with ozone technology

Ozonation has considerable and specific demerits. Ozone can also cause alterations in food or feed, such as alterations in sensory attributes, color loss, lipid oxidation, degradation of phenolic compounds, and vitamins (Zhu 2018). Therefore, it is crucial to research this method's effect on overall product quality (Alexandre et al. 2017; Li, Guan, and Bian 2015). Besides, end-degradation products are undefined, which is a crucial obstacle. Moreover, decomposition is faster in water than in air. Due to its unstable nature, ozone should be generated near its point of application and should be kept constant along the process (Pankaj, Shi, and Keener 2018). It is highly corrosive due to its high-level oxidation or reduction capability (Garud et al., 2019; Pandiselvam et al. 2019). Therefore, special considerations should be taken not to use metals in ozone fumigation systems. The ozone technology for the industries in high-scale treatment of various food matrices and feed, involving input from multiple disciplines. The generation of high concentration ozone at a larger scale is a challenging task (Pandiselvam et al. 2020).

The oxidative effect of ozone is due to oxygen and highly reactive hydroxyl radicals when it dissolves in water. The reactive oxygen species (ROS) reacts with organic substances, including the human body. Ozone gas negatively affects the respiratory tract of humans. Therefore, special care must be taken to reduce exposure and dosage of ozone (Zhu 2018). The existence of reactive elements in water affects the effectiveness of ozone in the aqueous phase (Smilanick, 2003), and therefore before ozonation, water should be pre-conditioned to remove organic compounds through a continuous filtration process. During long-term storage of products, gaseous ozone reacts with atmospheric water, reducing the relative humidity of air (Raila et al. 2006). Hence, in vivo and in vitro toxicological examinations must be performed to monitor the impacts of degradation products on humans' and animals' health.

The sensitivity of vitamins to the application of ozone based on food and its constituents, pH, and moisture affects the efficacy of the ozone (Pankaj, Shi, and Keener 2018). The decontamination of mycotoxins from various fruits and vegetables hydrophobic surfaces requires a higher level of ozonation. Ozone application may cause loss of nutrients and affect sensory qualities, microbial, and undesirable compound degradation. Therefore, the optimization of ozone processing parameters with a minimum physicochemical effect on food products is significant.

The ozone application for any food products or post-harvest grain storage should be validated on a pilot scale before commercial application. To improve the ozonation processes, overall efficiency is vital to carry out further research to strengthen more robust and compact systems that can handle severe environmental conditions.

Conclusion and future research directions

The intensifying demand for novel technologies to limit toxin accumulation, contamination, and spoilage in food commodities, with zero residues seems ambiguous. O_3 treatment may inhibit fungi' proliferation, but its impacts are highly based on fungal species, growth stage, ozone concentration, and exposure time. O_3 does not considerably increase the food processing temperature. It offers in consequential nutrient loss or organoleptic attributes of food. As a strong oxidizer, the electrophilic attack mechanism and reactivity against unsaturated chemical bonds leads to antimicrobial action and mycotoxin degradation, respectively. The ozone application in fluid food-processing has remarkable benefits as the FDA approves it as a safe antimicrobial agent. It has advantages as an environment-friendly decontaminating agent, recycling, and reusing water by reducing its microbial load in food processing plants and fresh food-like safe and nutritious food. Moreover, the limitations comprise its instability due to quick decomposition, health effects on exposure, corrosiveness, dependency on the dose, time, and temperature.

Several investigations showed that ozone could decrease mycotoxin levels in food matrices with significant exposure and dose time variations. Generally, greater concentrations of O_3 are required to reduce mycotoxins than those required for mycotoxigenic fungi growth suppression. The factors to be considered while choosing ozone treatment are the physical state of food, the extent of fungal contamination, and the nutritional components in food. Other treatments in combination with ozonation such as protective layer coating for fruits, irradiation treatment for peanut, grains, and apple juice using UV, organic acids for pH adjustments for cleaning fruits, hydrostatic pressure treatment for cereal flours, and high-temperature treatment for dry fruits further be utilized to prevent post ozone treatment contamination, to minimize the concentration of ozone or time of exposure and to minimize the adverse effects of ozone on the physicochemical attributes of the ozonated product. Despite all these challenges, ozonation seems to be an advanced oxidation technology that may be utilized as safe and eco-friendly to degrade mycotoxin and food preservation. Much more research is required to address the challenges associated with ozone technology.

Future studies should focus on the reduction of toxicological risk associated with mycotoxins contaminated processed commodities. Standardization of effective ozonation technology with refinement in the application conditions, safe delivery, cost-efficiency, and prevention of recontamination during storage are needed. To screen the adverse health effects, in vivo and in vitro toxicological examinations must be performed. Ozone technology usage in the food processing units, storage facilities, and pack houses demands alterations in design and equipment, modification of processes, and training. Food engineering research is needed to resolve problems such as the less penetration power of ozone in granulated food matrixes. Before commercial application, pilot testing must be conducted since every ozone application is unique. There is a need to establish further stringent regulatory controls to implement future insights where safety and risk assessment practices perform an avital role in improving controls and advisories for mycotoxins. Moreover, there is an utmost necessity of awareness and education to the public and farmers on the pervasiveness and risk of mycotoxins, alongside detoxification strategies that can diminish toxin absorption into food products.

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