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Mycotoxins in fermented foods: A comprehensive review

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Abstract

While the super-swift escalation of antibiotic-resistant pathogenic microbial strains is a great matter of public health concern; however, mycotoxins are not to be forgotten as well. According to the statistical analyses, mycotoxins contaminate up to 25 % of the world's food supply and contribute to a substantial amount of food spoilage. These toxins, which are secondary metabolites of certain species of pathogenic fungi, are responsible for a variety of adverse health effects that range from acute food poisoning to long-term effects, such as cancer; pregnancy disruption, and immunodeficiency. Although fermented foods have been consumed since time immemorial, in the 21st century, they are gaining immense popularity owing to their numerous health benefits. However, it should be noted that mycotoxin-infested fermented food is not uncommon, due to the use of poor-quality starter microbial cultures for fermentation and/or other improper practices; thus making this mycotoxin-infested fermented food an important food safety issue. However, due to the recent developments in food processing and the advent of very sophisticated and precise techniques, such as immunoassay and chromatography analysis, which are used to detect these mycotoxins, detecting their presence has become easier. This review aimed to address several aspects pertaining to mycotoxins, including their predominant types and producer fungi; their harmful effects, methods of sampling and extraction from fermented foods, and their detection and analysis techniques, in addition to the methods used to mitigate those.

Keywords: Adverse health effects, Fermented foods, Food spoilage, Pathogenic fungi, Secondary metabolites

1. Introduction

According to conjecture, mycotoxins may have existed historically as far back as the time period represented by the Dead Sea Scrolls. There are several historical evidences of aflatoxins (AFs) being present frequently prior to their discovery in the early 1960s. Mycotoxins have been believed to be the products of grain storage and associated fungal growth, which produce these secondary metabolites that are harmful when ingested by humans and other animals (Richard, 2007). Later research has revealed that fungal inhabitants of crop plants in the field produce several types of mycotoxins, including aflatoxins (AFs) (Brown *et al*., 2021). While mycotoxins have likely existed for as long as there have been plant crops; however, their true chemical composition has only recently come to light (Richard, 2012). Mycotoxins are toxic secondary metabolites that are formed by certain fungal genera, including *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., and others (Marroquín-Cardona *et al*., 2014; Lee and Ryu, 2017). Mycotoxins are recognized to contaminate food, leading to the development of mycotoxicosis in animals and humans, who may have consumed the intoxicated food, and\ or may sometimes have been exposed to the same; either dermally or through inhalation (Capriotti *et al*., 2011). According to the World Health Organization (WHO), the adverse health effects induced by these mycotoxins are quite varied and range from acute food poisoning to chronic effects (WHO. 2018). A single previous study has documented that most of these symptoms appear after consumption of the mycotoxin-contaminated grains, and\ or products made from such grains; however, there are other portals of entry as well (Richard, 2007). The frequency of mycotoxins occurrence and\ or severity of the diseases they cause; particularly if they are known to be carcinogenic, can be used to determine which of these known mycotoxins are relevant (Richard, 2007). Because the symptoms of mycotoxicosis are similar to those of a number of other diseases and due to the fact that only a small amount of mycotoxin is known to be present in food samples; diagnosis of such mycotoxicosis has proven to be challenging (Singh and Mehta, 2020). Therefore, such diagnosis is highly dependent on adequate mycotoxins testing, which include sampling, sample preparation, and analyses (Singh and Mehta, 2020).

2. Contamination of fermented foods with mycotoxins

Originally, several fermentation techniques have been used to preserve food, because when food ingredients are fermented, they become microbiologically stable and potentially safe for consumption (Tian *et al*., 2022). In this way, the fermented food is believed to acquire protection from contamination by the mycotoxigenic fungi (Adams and Mitchell, 2002; Tian *et al*., 2022).

However, such contamination of fermented food with the mycotoxigenic fungi and the associated mycotoxins may sometimes occur through improperly-regulated and\ or spontaneous fermentations, which employ native microorganisms (Tian *et al*., 2022). The mycotoxigenic fungi may be present in or on the materials, tools, and containers used during the production of fermented foods (Tian *et al*., 2022). Despite the fact that many food fermentation techniques have been scaled up to the industrial levels; however, the bulk of fermented soybean products is still produced traditionally on a modest scale through using very basic processing facilities with varying degrees of hygienic conditions (Tian *et al*., 2022). Therefore, these fermentation products are susceptible to contamination by mycotoxigenic fungi, due to the lack of sterility and the use of spontaneous fermentation or inferior fermentation starters. If poorly pre-treated and\ or if the unsterilized soybean and other adjuncts are exposed to the environment for a long time; thus mycotoxin contamination almost becomes unavoidable (Tian *et al*., 2022). The environmental conditions used for storage and

processing of the fermented foods, including temperature and humidity, are other significant causes of infections of such food by the mycotoxigenic fungi. Although the majority of mycotoxins that exist in soybeans during the pre and post-harvest stages are typically within tolerable limits; however, poor storage conditions may lead to more serious contaminations (Tian *et al*., 2022).

3. Fungal species prevalent in fermented foods

Since ancient times, fermented products have played a significant role in the human diet in Asian countries, and they have recently gained popularity in the Western countries as well (Qin *et al*., 2022). Every day, millions of people worldwide consume such fermented dishes (Tian *et al*., 2022). Soy sauce is one of the most essential condiments in the Asian nations, where it is regularly used in most families; where Chinese adults are recorded to consume 8.2 g of soy sauce daily (Tian *et al*., 2022). Similar to the other fermented foods, the fermentation strain (monoculture fermentation) and\ or the interaction of microbial communities (multicultural fermentation) that are used as starter cultures combination; together with their complex metabolites, may determine the flavor; taste, texture, and functional qualities of the fermented soybean products (Singh *et al*., 2017).

Globally, the bacteria; mold fungi, and yeasts are the major groups of microorganisms associated with the fermentative production of several food items (Tian *et al*., 2022). In Asian countries, *Actinomucor* spp., *Aspergillus* spp., *Monascus* spp., *Mucor* spp., *Penicillium* spp., and *Rhizopus* spp. are the predominant molds used in the food fermentation processes (Tian *et al*., 2022). These molds produce several enzymes including α-amylase; amyloglucosidase, cellulase, β-galactosidase, hemicellulase, invertase, lipase, maltase, pectinase, and acid- and alkaline proteases (Tamang *et al*., 2016). These enzymes together with the degradative anti-nutritive factors employed during the fermentation process; improve the bioavailability of nutrients and minerals in the fermented food they produce (Tamang *et al*., 2016). The improved beneficial properties, including increased phenolic contents and enhanced free radical scavenging activities can also be observed in soybeans fermented by these molds (Ghanem *et al*., 2020).

4. Predominant types of mycotoxins in the fermented foods

During fermentation; perhaps some undesired or even harmful metabolites may be released into the food by some of the notorious fungal pathogens (Tian *et al*., 2022). Mycotoxins are such dangerous secondary metabolites produced by several fungal species of *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium*, where they can contaminate the food and may have substantial and immediate harmful effects on the human health (Marroquín-Cardona *et al*., 2014; Lee and Ryu, 2017). Among these mycotoxins, aflatoxins (AFs); deoxynivalenol (DON), fumonisin, ochratoxin (OCs), and zearalenone (ZEA) are the most frequently-encountered mycotoxins that contaminate food (Lee and Ryu, 2017).

4.1. Aflatoxins (AFs)

Aflatoxins (AFs) are derivatives of difurano-coumarin (Abrar *et al*., 2013). They are low molecular weight heat stable compounds, and hence survive during the high cooking temperatures (Campagnollo *et al*., 2016; Atungulu *et al*., 2018).There are four main categories of AFs produced by *Aspergillus* spp.; namely, aflatoxin B1 (AFB1) (Fig. 1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG1). It is to be noted that AFB1, which is linked with hepatocellular cancer; is the highly toxic mycotoxin among all the AFs (Awuchi *et al*., 2021). All AFs isolated from *Aspergillus* spp. are not equally toxic (Ren *et al*., 2022). Warm temperatures and drought have been found to be conducive for AF contamination of the field crops, such as corn (Kerry *et al*., 2022). The asexual spores (conidia) of the responsible parasitic fungi; mainly, *A. flavus*, *A. parasiticus*, or *A. nomius* are dispersed within the crop fields through wind and/or insects (Luis, 2019).

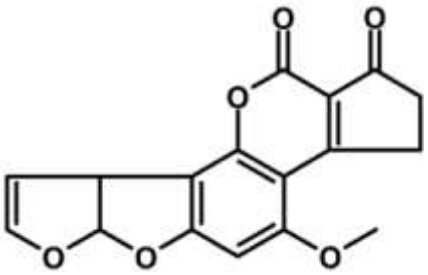

Fig. 1. Structure of AFB1, as a representative of the highly toxic AFs (Richard, 2007)

The yellow-green or gray-green masses of *Aspergillus* conidia may be observed at the locations of kernels damage, and\ or along the insect-feeding pathways in the crops that have been severely harmed (UGA Cooperative Extension. 2006). It has been reported that severely-infected individual maize kernels may contain as much as 400,000 µg\ kg of AFs (Ditta *et al*., 2019). By using the proper management techniques, such as keeping the stored corn grains dry, unharmed, and insect-free; AFs development can be avoided (Zaki, 2012).

Aflatoxins are one of the most potent known carcinogens, as well as mutagens of the hepatocellular chromosomes (Magnussen, 2013). The high incidence of liver cancer in some parts of the tropics has been attributed to the continual ingestion of low levels of AFs synergistically with the simultaneous infection by Hepatitis B virus (HBV) (National Cancer Institute. 2022). Upon metabolizing the harmful AFs in the liver cells, two toxic chemicals are produced; namely epoxide and its dihydroxy-derivative (Dhakal *et al*., 2022).

AFM1, a hydroxylated metabolite of AFB1; exists in human milk and in dairy products obtained from animals that have consumed contaminated feed (Schrenk *et al*., 2020). However, there is no direct evidence that feed grains are contaminated by AFM1 itself (Kang'ethe and Lang'a, 2009).

4.2. Deoxynivalenol (DON)

Deoxynivalenol (DON) (Fig. 2); commonly known as ‘vomitoxin’, is primarily produced by *Fusarium graminearum* and in some regions by *F. culmorum* (McLaughlin *et al*., 2015). It is a non-fluorescent mycotoxin that belongs to Type B trichothecenes (McLaughlin *et al*., 2015). This mycotoxin induces vomiting upon consumption, hence the name ‘vomitoxin’ (Flannery *et al*., 2012). Zearalenone (ZEA) is another mycotoxin produced by the same fungal species, and so may coexist with DON (Almeida *et al*., 2012).

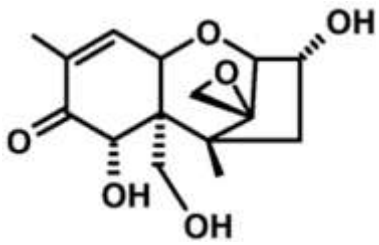

Fig. 2. Structure of Deoxynivalenol (DON) (Richard, 2007)

Fusarium spp. prefer to multiply rapidly under warm temperatures and humid conditions (Doohan *et al*., 2003). The main crops affected with *Fusarium* spp. are corn; wheat, oats, and barley (Cui *et al*., 2013). Most microorganisms can survive on crop residues that have been left in the agricultural fields from the previous season, which in turn act as sources of microbial inoculum for the following crop (Blandino *et al*., 2010).

4.3. Fumonisins

The fumonisins (Fig. 3) are non-fluorescent mycotoxins; with fumonisin B1 (FB1), fumonisin B2 (FB2), and fumonisin B3 (FB3) being the major types (Hosseini and Bagheri, 2012). Among the various species of *Aspergillus*, *Penicillium*, and *Fusarium*, it has been found that *F. verticillioides* and *F. proliferatum* are the most common genera that are responsible for fumonisin production (Marín *et al*., 2013; Kamle *et al*., 2019).

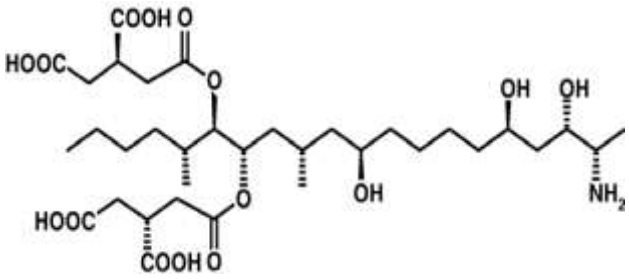

Fig. 3. Structure of Fumonisin B1 (FB1), as a representative of the fumonisins (Webster and Weber, 2012)

Fumonisins have been detected in a variety of crops, including sorghum; rice, corn, wheat, beans (white, adzuki, and mung), barley, soybean, asparagus spears, and figs (Kamle *et al*., 2019). Moreover, fumonisins have also been recognized in plants with edible parts, such as pea; asparagus, onion, and garlic (Kamle *et al*., 2019).

Although the precise origins of ear and kernel rot diseases are unknown; however, variations in the weather conditions, such as dry weather that is followed by warm and wet weather during flowering may be blamed (Kamle *et al*., 2019). Rainfall just before harvest may make corn more contaminated with fumonisins (Kamle *et al*., 2019). Using the insect-mediated damages as portals of entry, the natural strains of phytopathogens can enter the ear and kernels of the maize plant at the time of maturity. Sometimes, large concentrations of fumonisins may exist in the maize kernels that do not even have any disease symptoms (Kamle *et al*., 2019).

4.4. Ochratoxins (OCs)

Ochratoxins (OCs) are a group of closely related derivatives of isocoumarin that are linked to the amino acid L-β-phenylalanine, and are known to cause kidney damages (Fuchs and Peraica, 2005; Dickman and Grollman, 2010). *A. ochraceus* and *P. verrucosum* are known to produce this principal ochratoxin A (OCA) mycotoxin (Fig. 4), which is an inherently fluorescent compound (Zhihong *et al*., 2015; Gonzalez *et al*., 2020).

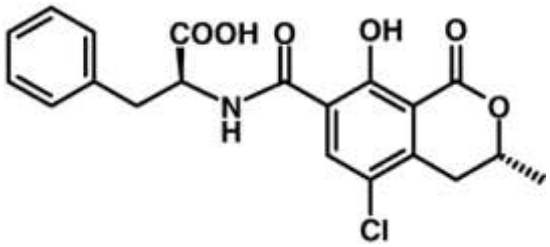

Fig. 4. Structure of Ochratoxin A (OC) (Richard, 2007)

There may be some products or geographical locations where other fungi, including those in the *A. niger* group, turn out to be significant OC producers (Tjamos *et al*., 2004). These fungi usually contaminate the grains, but are also detected in coffee, beans, and peanuts (Haschek *et al*., 2002). The predominant fungal species that produce OCs develop visible mycelial biomasses with a variety of colors, including yellowish-tan (*A. ochraceus*), blue-green (*P. verrucosum*), and black color (*A. niger*) (Richard, 2007).

4.5. Zearalenone (ZEA)

Zearalenone (ZEA) (Fig. 5); is a phenolic resorcylic acid lactone, which plays a significant role in regulating the sexual reproduction in the producer fungi, and behaves as an estrogenic substance in the animals consuming it; especially the pigs (Metzler *et al*., 2010).

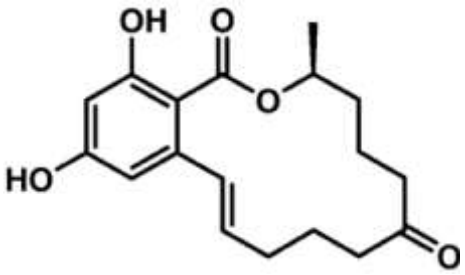

Fig. 5. Structure of zearalenone (ZEA) (Richard, 2007)

ZEA may coexist with DON; since both are produced by the toxigenic *F. graminearum* and *F. culmorum*, which thrive in the moist and chilly environments (Richard *et al*., 2003). Besides being found in corn, ZEA is also present in other important crops, such as rye; wheat, barley, and sorghum in various regions around the world (El-Desouky and Naguib, 2013). When the mycotoxigenic fungi infect such crops, a pink pigment is produced along with ZEA; which causes the infected crops to turn pink with age (Munkvold, 2016). As a protective strategy, the crops in general should be stored properly to prevent the growth of the fungal pathogens (Atanda *et al*., 2011). However, as ZEA can be formed at relatively cool temperatures; thus elevated levels of this mycotoxin can be detected in the crops even during their cold storage (Richard, 2007).

5. Harmful effects of the mycotoxins

Utilizing food and feed that are contaminated with mycotoxins can have short-term and\ or long-term impacts on the consumers, such as sterility; nephritis, cancer, teratogenicity, estrogenic effects, immunological suppression, and may even lead to death (Abd-Elsalam and Alghuthaymi, 2015). Some of the mycotoxins and the mycotoxicoses they cause are summarized in Table (1).

6. Sampling and extraction assays for analysis of mycotoxins in fermented foods

6.1. Sampling

The goal of any sampling procedure is to get a portion of the food sample that is contaminated with mycotoxins for the investigation and assessment of specific features for a particular batch (Shanakhat *et al*., 2018). The bulk wetness of cereals forms clumps that are particularly found in the moldy kernels. These tiny fractions of the extremely contaminated cereal clumps are dispersed at random across the food sample. Thus, the typical sampling techniques are insufficient for estimating the mycotoxins in the agricultural foods, due to the substantial variation in mycotoxins concentrations in these samples (Shanakhat *et al*., 2018).

6.2. Extraction

The method of extraction of a mycotoxin is chosen based on the physicochemical characteristics of the food sample material and the type of mycotoxin to be detected (Shanakhat *et al*., 2018). Typically, the extracting solvent is used to grind, homogenize, and filter the sample before moving on to the purification stage. The desired component that particularly contains the mycotoxins from the sample matrix is thus extracted for analysis, when the analyte moves in the extracting solvent during the extraction procedure. Since there isn't a single extracting solvent that can exclusively extract the desired mycotoxins, it is advisable to choose several extracting solvents that can extract most of the mycotoxins (Shanakhat *et al*., 2018).

6.2.1. Solid-liquid extraction

Most mycotoxins can be dissolved in polar solvents, but not in non-polar ones (Shanakhat et al., 2018). Water; acetonitrile, acetone, chloroform, methanol, and\ or mixtures of these solvents are examples of the polar solvents that have been frequently employed in various investigations to extract mycotoxins from cereals. Mycotoxins such as fumonisins can be dissolved in acidified solvents, which encourage the carboxylic group protonation and lead to a better separation from the matrix. On the other hand, greater mycotoxins recovery have been observed on using alkaline extracting solvents, such as 0.5 % KCl in 70 % methanol, which have been utilized to extract AFB1 from rice (Shanakhat *et al*., 2018).

6.2.2. A QuEChERS extraction method

A sample extraction technique known as QuEChERS (for Quick, Easy, Cheap, Effective, Rugged and Safe), has been extensively used to extract a number of analytes (Shanakhat *et al*., 2018). It can be used for effective extraction of target compounds,

Table 1. Summary of the different types of mycotoxins, their sources, their prevalence in certain foods, and the mycotoxicoses they cause

	Type of mycotoxin	Source of fungal species	Prevalence	Mycotoxicoses
1.	**Aflatoxin (AF)**	*Aspergillus* spp., including *Aspergillus flavus*, *A. parasiticus* and *A. nomius*; *Penicillium* spp. (Richard, 2007)	Fermented cereal gruels (Sivamaruthi *et al*., 2018), maize, wheat, rice, peanut, sorghum, pistachio, almond, ground nuts, tree nuts, figs, cottonseed, spices (Al Shannaq and Yu, 2017).	Aflatoxin B1 is the most potent aflatoxin (Richard, 2007). Aflatoxin poisoning or aflatoxicosis manifests in two ways. First is "acute intoxication", which is brought about by a brief exposure to a large amount of toxins, and is characterized by severe liver damage; jaundice, haemorrhage, oedema, and eventually death. Secondly, via "Chronic sub-lethal exposure", which causes immunosuppression, nutritional problems, and cancer (Marchese *et al*., 2018).
2.	**Deoxynivalenol (DON)**	*Fusarium* spp., including *F. graminearum* and *F. culmorum* (in some	Cereals, cereal products (Al Shannaq and Yu, 2017).	DON causes severe nausea; vomiting, diarrhoea, abdominal discomfort, headache, vertigo, and fever in humans, who consume DON-contaminated cereals (Pleadin *et al*., 2019). Acute DON

		geographical locations) (Atungulu *et al*., 2018).		exposure even causes anorexia and vomiting in animals (Pleadin *et al*., 2019).
3.	**Fumonisins**	*Fusarium verticillioides*, *F. proliferatum* and *F. culmorum* (Richard, 2007).	Maize, maize products, sorghum, asparagus (Al Shannaq and Yu, 2017).	The most poisonous type of fumonisins is fumonisin B1 (FB1), which has been proven to cause equine leukoencephalomalacia; a serious condition that affects horses, and results in softening of the white matter in their brains (Zain, 2011). It is also responsible for porcine pulmonary edema, and tumour progression in rats. Moreover, it is being linked to an increased risk of oesophageal cancer and heart failure in humans (Zain, 2011). Fumonisin B1 consumption has been shown to decrease the uptake of folate in several cell lines, and is thus believed to be linked to neural tube abnormalities in the newborn humans (Zain, 2011).
4.	**Ochratoxin (OC)**	*A. ochraceus*, and *P. verrucosum*; *A. niger* in some geographical	Cereals; cheese, figs, beef jerky, fruits and	OC is a teratogenic; neurotoxic, hepatotoxic and nephrotoxic, according to a previous research conducted on animals (Awuchi *et*

		locations (Tjamos *et al*., 2004; Richard, 2007).	wine (Al Shannaq and Yu, 2017).	*al*., 2021). The kidneys are mainly affected by acute toxicity of the OC. Following exposure to OC; the pigs displayed the highest sensitivity with the development of nephropathy (Awuchi *et al*., 2021). Likewise, urothelial tumours; chronic interstitial nephropathy, and Balkan endemic nephropathy have all been linked to OC exposure in humans as well (Pfohl-Leszkowicz *et al*., 2002). Moreover, epidemiological studies have demonstrated a link between OC exposure in adolescents and the development of testicular cancer (Awuchi *et al*., 2021).
5.	**Zearalenone (ZEA)**	*Fusarium* spp., including *F. graminearum* and *F. culmorum* (Richard *et al*., 2003; Richard, 2007)	Corn; barley, oats, wheat, maize, rice (Al Shannaq and Yu, 2017). The toxins may also travel up the food chain to the grain-fed meat;	Infertility problems have been detected in guinea pigs; rabbits, hamsters, rats, mice, and domestic animals; where all have been connected to ZEA toxicity (Awuchi *et al*., 2021). ZEA has also been found to be associated with hypo-estrogenic disorders in humans (Awuchi *et al*., 2021).

		eggs, dairy products, and even beer (Sivamaruthi *et al*., 2018).	

and recently it has also been reported for the extraction of mycotoxins as well. Magnesium sulfate ($MgSO_4$) is used in the extraction step to remove water from the food sample that is to be tested for mycotoxins contamination, and then a primary secondary amine (PSA) or octadecyl-bonded silica gel (C18) is employed as a sorbent material in the clean-up step; to preserve the sugars and fatty acids in the sample. Moreover, several samples of durum and soft wheat pasta have been extracted using the QuEChERS method for the simultaneous identification of 17 mycotoxins (Shanakhat *et al*., 2018). This extraction method produces acceptable results, because it is straightforward; consumes little solvent, repeatable (*i.e*., multiple runs of extraction can be carried out), has a good recovery, and allows for both inter- and intra-laboratory reproducibility (Shanakhat *et al*., 2018).

7. Detection and analysis methods of mycotoxins in the fermented foods

7.1. Chromatographic techniques

In the 1960s, Thin-layer chromatography (TLC) was acknowledged for its significant contribution to the initial purification and identification of AFs (Zhang and Banerjee, 2020). However, this technique has been recently superseded by High performance liquid chromatography (HPLC). This is attributed to the fact that HPLC is more sensitive; as it has better sensitivity of the detectors and has greater advances in the design of the HPLC column and the column packing materials, which have increased its chromatographic efficiency (Shephard, 2016). Furthermore, a more recent advancement in this field of chromatography has been observed by the introduction of ultra-HPLC; with a uniform column packing material of particle size diameter around 1.5 µm. Previously, HPLC columns have been packed with a packing material of about 5 µm in diameter. This new advancement led to improved chromatographic resolution and efficiency; lower solvent consumption and significant increase in the sensitivity (Shephard, 2016).

The food sample that needs to be examined for mycotoxins contamination is prepared prior to TLC or HPLC analysis using solid-phase extraction techniques (SPE); as mycotoxins are present in the complex food matrixes at relatively low concentrations, often in a range of µg\ kg (Shephard, 2016). The analyte that contains the mycotoxin of interest needs to be purified from the several co-extracted contaminants, and then concentrated for reliable measurement, due to the low sensitivity and specificity of the traditional spectrometric detectors (Shephard, 2016). The SPE techniques have replaced the initial big open cleaning columns that have been packed with silica or another adsorbent. The new and improved chromatographic techniques have shown good sensitivity; increased resolution, shorter run times, and excellent reproducibility (Shephard, 2016). However, there are some shortcomings. The primary shortcomings of the HPLC method include its portability; with practical problems based on the sample type; matrix effect, sample preparation, and calibration selection (Singh and Mehta, 2020). Therefore, more analytical techniques are required (Singh and Mehta, 2020). Another significant drawback of this HPLC technique

is that it needs specialized equipment that costs a lot of money, and also it needs a dedicated operator to interpret the results (Yao *et al*., 2015).

Recently, mycotoxins in herbal remedies are analyzed using ultra-high-performance liquid chromatography (UPLC). This UPLC technique greatly improves the chromatographic resolution and sensitivity, compared with the conventional HPLC; in addition, it shortens the analysis cycle (Zhang *et al*., 2018).

7.2. Mass spectrometry (MS)

Mass spectrometry (MS) has been used as an ideal confirmatory technique for mycotoxins analysis due to the production of mass spectrum (Shephard, 2016). This technique has distinct advantages, such as chemical structural information; exquisite sensitivity, and specificity based on M/Z (mass-to-charge) ratio (Al Shannaq and Yu, 2017). It is an analysis method that divides the ionized objects such as atoms, molecules, and clusters based on variations in the ratios of their relative masses to their charges (m/z) (Murayama *et al*., 2009). In addition, a generated mass spectral profile provides an ideal confirmatory technique, as it helps in providing extra information about what kind of mycotoxins may be obtained based on calculations of the mass spectrometric data. Moreover, this technique helps in finding out the molecular weights of the mycotoxins under study (Al Shannaq and Yu, 2017). However, problems pertaining to a sample delivery into its ultra-low pressure environment have arisen, and there is a particular problem in coupling HPLC with its high solvent flow rates (Shephard, 2016). High Performance Liquid Chromatography (HPLC) coupled with Mass Spectrometry (MS) (HPLC-MS) or to tandem MS (HPLC-MS/MS) has become a flexible analytical tool, due to the development of Atmospheric Pressure Ionization (API) techniques such as electrospray ionization and atmospheric pressure chemical ionization, which are the most widely used ionization source for HPLC-MS/MS (Lee *et al*., 2015, Shephard, 2016). Typically, the ionization process severely fragments the sample molecule, and then a characteristic mass spectrum is produced for a large number of compounds (Li *et al*., 2015). However, under other circumstances; the sensitivity is decreased by fewer selective fragment ions and\ or excessive fragmentation. Using API techniques have helped in preserving the molecular ions and reducing the fragmentation. Because of the reduced fragmentation, the selection of the precursor ion in MS/MS no longer represents a compromise between sensitivity and selectivity (Li *et al*., 2015). Although the early applications of HPLC-MS and HPLC-MS/MS techniques have been restricted to a single mycotoxin analysis; however, later it has been realized that this technique can be efficiently used to detect multiple mycotoxins as well. Recently, these methods can detect more than 100 mycotoxins in a single run, which have enhanced the rates of mycotoxins determination and analysis (Al Shannaq and Yu, 2017).

7.3. Immunological methods

7.3.1. Radioimmunoassay (RIA)

Radioimmunoassay (RIA) is an analysis technique that is based on the competition between an unlabeled antigen (*i.e*., a mycotoxin) recovered from the food samples and a radiolabeled antigen in the assay system; for binding at specific sites on the anti-mycotoxin antibodies (Hui, 2019). The RIA procedure involves incubating the unknown sample or known standard in a phosphate buffer with a constant amount of labeled mycotoxin and a specific antibody at the same time (Hui, 2019). Once the bound and free mycotoxins are separated, the radioactivity of each fraction is measured. The obtained results are compared to a standard curve that is created by plotting the ratio of radio-activities in the bound and free fractions, versus log concentration of the unlabeled standard mycotoxin. This standard curve is used to calculate the toxin concentration in an unknown food sample (Waliyar *et al*., 2009). In general, RIA can detect as little as 0.25-0.5 ng of a purified mycotoxin in a standard preparation. The

lower limit of mycotoxins detection in food or feed samples is about 2 to 5 µg\ kg for samples that have not been subjected to an extensive clean-up treatment (Chu, 2004).

7.3.2. Enzyme-linked immunosorbent assay (ELISA)

Although Enzyme-linked immunosorbent assay (ELISA) can be carried out in number of ways, including a direct assay, a competitive direct assay, and a competitive indirect assay; however the competitive direct assay is the most often employed method (Al Shannaq and Yu, 2017). ELISA assay is based on the competitive interactions between antibodies that have been labeled with a toxin-enzyme conjugate and a mycotoxin, which operates as an antigen. The degree of color development depends on how much of the toxin-enzyme combination becomes bound to the antibody (Al Shannaq and Yu, 2017).

ELISA offers a quick, precise result, and is generally considered as a simple way to analyse the mycotoxins in food (Janik *et al*., 2021). This method is approximately 10-50 times more sensitive than RIA when purified mycotoxins are used; in addition, a mycotoxin concentration as low as 2.5 pg can occasionally be detected (Chu, 2004). The major advantages of ELISA are that it does not use radioactive substances, and avoids the use of expensive instrumentation and problems associated with disposal of the radioactive materials (Hui, 2019). However, ELISA has some drawbacks, such as the possibility of cross-reactivity and its dependence on a particular matrix (Al Shannaq and Yu, 2017). The relative advantages and disadvantages of the various techniques used for the detection and analysis of mycotoxins in fermented foods are summarized in Table (2).

8. Control methods of mycotoxins contamination

8.1. Using lactic acid bacteria (LAB)

The lactic acid bacteria (LAB) have been widely used to eliminate mycotoxins from fermented foods (Sivamaruthi *et al*., 2018). The most widely used strains for eradicating mycotoxins are *Lactobacillus* spp.; *Lactococcus* spp., *Bifidobacterium* spp., *Leuconostoc* spp., *Pediococcus* spp., *Streptococcus* spp., and *Propionibacterium* spp.; owing mainly to their abilities to bind antagonistically to these toxins (Sivamaruthi *et al*., 2018). LAB strains have been recently discovered to eradicate AF; the most potent mycotoxin that is abundant in fermented foods (Sivamaruthi *et al*., 2018). The *Lactobacillus* strains, including *L. fermentum* RS2, *L. fermentum* OYB, *L. plantarum* MW, *L. plantarum* YO, *Lactococcus* spp. RS3, and *L. brevis* WS3, which are isolated from fermented cereal gruels have been recorded to have anti-aflatoxin activity. In vitro assays revealed that *L. plantarum* YO prevented the growth of food-contaminating AF-producing *Aspergillus* spp. (Sivamaruthi *et al*., 2018). *L. casei* has been experimentally recorded to reduce AFM1 contamination in kefir samples by 88.17 %, where the starter cultures of kefir and *L. casei* work together to reduce AFM1 levels in the kefir samples. The levels of spiked FB1 and ZEA in fermented maize meal have been decreased through the use of LAB (*i.e*., *Streptococcus lactis* and *L. delbrueckii*)-mediated fermentation procedures (Sivamaruthi *et al*., 2018). When the cytotoxicity is tested in vitro against the SNO [spindle-shaped N-cadherin(+) CD45(-) osteoblast] cell line, the recorded results have revealed that the level of mycotoxins is significantly lower in the LAB-mediated fermentation processes, as compared with the control fermentations without incorporating LAB strains (Mokoena *et al*., 2005). *L. plantarum* strains that are isolated from several fermented foods have been screened *in vitro* for ZEA-degradation ability. *L. plantarum* 4, *L. plantarum* 22, and *L. plantarum* 39 strains have shown about 39 %, 47 % and 38 % of ZEA degradation, respectively (Sivamaruthi *et al*., 2018). Therefore, the utilization of these *L. plantarum* strains has contributed to a reduction in the ZEA contamination of fermented foods. All these outcomes have indicated that using

Table 2. Relative advantages and disadvantages of the various techniques used for the detection and analysis of mycotoxins in fermented foods

Techniques	Advantages	Disadvantages
i) Chromatographic techniques	Techniques like TLC and HPLC are very precise, reliable, and highly accurate analytical tools, which are used for mycotoxins detection (Yao *et al*., 2015).	Extremely expensive equipment is required for HPLC, and must be handled with care and expertise (Shanakhat *et al*., 2018).
ii) Mass spectrometry	Multiple mycotoxins can be detected at the same time, and is able to produce structural information about the analytes (Shanakhat *et al*., 2018).	Costly equipment and hydrocarbons that produce similar ions cannot be identified (Shanakhat *et al*., 2018).
iii) Radioimmunoassay (RIA)	RIA is very specific and sensitive (Batra, 2019).	Radiation poses a risk; thus particular precautions must be handled for storing the radioactive material; in addition, the waste disposal is expensive (Batra, 2019).
iv) Enzyme-linked Immunosorbent Assay (ELISA)	Rapid detection kits are widely available; colorimetric analysis is simple; also, no special skills or expensive equipment are required (Yao *et al*., 2015).	Less specific or sensitive than mass spectrometry (MS), and the various chromatographic methods such as HPLC. Moreover, the cross-reactivity

		with related mycotoxins can pose a problem (Yao *et al*., 2015).

Lactobacillus spp. that are hostile to reduce and\ or prevent mycotoxins production in the fermented food products, is beneficial (Sivamaruthi *et al*., 2018).

8.2. Using inhibitors of mycotoxin biosynthesis

The antifungal medicines frequently have broad-spectrum activities against a variety of fungi. Thus, if applied during food fermentation; they may alter the fungal communities (Tian *et al*., 2022). In order to limit mycotoxins production during food fermentation without upsetting the natural microbial communities; using natural agents that solely inhibit mycotoxins biosynthesis without impacting the growth of the other essential fermenting fungi may be very helpful (Tian *et al*., 2022). The plant-based natural compounds; mainly 5-O-demethyl nobiletin, alisol A, meso-dihydroguaiaretic acid, 20(S)-protopanaxatriol, syringaresinol, and kaempferol have strongly inhibited AFB1 production (> 85%), without inhibiting the fungal growth in the culture medium (Tian *et al*., 2022). Caffeic acid has reportedly inhibited AFs biosynthesis by *A. flavus* in a fat-based growth medium by > 95 %; with no inhibitory effects on the growth of the fermenting fungi. Similarly, fennel essential oil has suppressed OCA production by *A. carbonarius* by 89 %, and has reduced the growth of the desired fungal species by 14 % only (Tian *et al*., 2022).

9. Preventive measures against mycotoxins contamination

A variety of control methods have been employed to lessen mycotoxins contamination of foods (Agriopoulou *et al*., 2020).

9.1. Pre-harvest mycotoxins prevention

The synthetic fungicides can be effectively used to inhibit and decrease mycotoxins contamination at the pre-harvest level (Stracquadanio *et al*., 2021). Because these classes of chemical fungicides have a high efficiency and a broad antifungal spectrum against a wide variety of pathogens including the mycotoxigenic fungi, some of them have been used successfully to reduce the levels of mycotoxins contamination in food crops (Tian *et al*., 2022). Currently, azoles and strobilurins are the most widely used synthetic fungicides in agriculture (Tian *et al*., 2022). For many years, both fungicides have dominated the chemical treatment markets for eradicating the fungal infections in soybeans (Juliatti and Azevedo, 2017). However, the development of fungicide-resistant strains has emerged as a problem facing the control of mycotoxin-producing fungi. Another promising biocontrol strategy against mycotoxins contamination involves the use of natural antifungals and anti-mycotoxigenic compounds. Many natural secondary metabolites such as thiols, polyphenols and terpenoids, in addition to the essential oils, can be used effectively to control mycotoxins contamination in foods (Redondo-Blanco *et al*., 2020).

9.2. Prevention of mycotoxins contamination during post-harvest and storage conditions

The storage conditions play an important role in influencing the mycotoxigenic fungal growth, mycotoxins accumulation, and discoloration (Mannaa and Kim, 2017). Several environmental conditions such as temperature, water activity (aw), and gases can potentially affect the fungal growth and the production of mycotoxins in food (Mannaa and Kim, 2017). Lowering the temperature and moisture levels of the agricultural products during storage can inhibit the fungal growth and metabolism (Tian *et al*., 2022). A temperature below 150C or aw below 0.87 is unsuitable for AFs production in soybeans (Tian *et al*., 2022). Other factors that can inhibit fungal development and mycotoxins biosynthesis are the

relatively low O_2 and high CO_2 levels (Kumar *et al*., 2021). *A. flavus* and *P. roqueforti* are unable to grow in an environment containing 40 % or more of CO_2 and less than 0.5 % of O_2 balanced with N_2 (Taniwaki *et al*., 2009). On the larger scale; however, controlling the storage atmosphere is quite expensive (Tian *et al*., 2022).

9.3. Prevention of mycotoxins production before fermentation

Adjustment of the food fermentation conditions, such as temperature, pH, and salt concentration affects the mycotoxigenic fungal growth (Awuchi *et al*., 2022). An important point is to set the fermentation conditions in such a way that they favor the growth of the fermenting fungi only, and help them to become the dominant fungi over the harmful ones. Another important strategy is to use starter cultures that can multiply rapidly and out-compete the mycotoxin-producing fungi, thereby eradicating their existence to a large extent (Tian *et al*., 2022). Such a used starter culture includes *A. oryzae*, which has been used in the production of sake, soybean paste (*i.e*., doenjang, miso), and soy sauce (Tian *et al*., 2022).

9.4. Prevention of mycotoxins production during fermentation

When plant extracts such as *Nelumbo nucifera*, clover, and *Ginkgo biloba* have been added during the fermentative production of Meju, they reduced the growth of the harmful fungal microflora, which produce AFs in Meju. Hence, the fermentation process involving such plant extracts has improved the quality and reduced the toxicity in Meju (Sivamaruthi *et al*., 2018). The traditional preparations, such as fermentation; draining of water, washing, milling, and sieving of Ogi (*i.e*., a complementary diet made from maize) have reduced the mycotoxin levels in Ogi by up to 90 %. Soaking of maize, sorghum, and millet grains in water for 48 h has accelerated mycotoxins elimination from Ogi, thus has assisted in the production of a relatively safe Ogi diet (Sivamaruthi *et al*., 2018).

Conclusion

Many people consume fermented foods on a regular basis, and mycotoxins are one of the major contaminants of these fermented foods; as they significantly deteriorate them. Most of the raw materials used for making fermented foods are the major sources of these mycotoxins. For instance, cereals (i.e., wheat, oats, and barley) contain several mycotoxins, including DON, zearalenone, and AFs. There are numerous documented foodborne outbreaks that have been attributed to food poisoning caused by mycotoxins; in addition to the manifestation of one of the most infamous diseases known to mankind, cancer. Recent studies have shown that the use of particular starter cultures such as LAB, optimum fermentation conditions, and standardized pre- or post-processing treatments, effectively prevent and/or reduce these hazardous components in the fermented foods. Recent developments in food processing boost the safety of foods. According to several recent studies; just adhering to the conventional procedures of food preparation (*i.e*., long-term soaking and frequent washing) and monitoring of the post-harvest conditions can help to stop the production of the harmful mycotoxins in food. Although the quality control measurements have been enhanced by modern and effective detection techniques; however, further advancements are needed to speculate and prevent mycotoxicity in the fermented foods.

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Conflict of interest

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10. References

Abd-Elsalam, K.A. and Alghuthaymi, M.A. (2015). Mycotoxins as agroterrorism nanoweapon. Journal of Nanotechnology and Materials Science. 2(2): 47-49. https://doi.org/10.15436/2377-1372.15.020

Abrar, M.; Anjum, F.M.; Butt, M.S.; Pasha, I.; Randhawa, M.A.; Saeed, F. et al. (2013). Aflatoxins: Biosynthesis, occurrence, toxicity, and remedies. Critical Reviews in Food Science and Nutrition. 53(8): 862-874. https://doi.org/10.1080/10408398.2011.563154

Adams, M. and Mitchell, R. (2002). Fermentation and pathogen control: a risk assessment approach. International Journal of Food Microbiology. 79(1-2): 75–83. https://doi.org/10.1016/s0168-1605(02)00181-2

Agriopoulou, S.; Stamatelopoulou, E. and Varzakas, T. (2020). Advances in occurrence, importance, and mycotoxin control strategies: prevention and detoxification in foods. Foods. 9(2): 137. https://doi.org/10.3390/foods9020137

Almeida, M.I.; Almeida, N.G.; Carvalho, K.L.; Gonçalves, G.A.A.; Silva, C.N.; Santos, E.A. et al. (2012). Co-occurrence of aflatoxins B1, B2, G1 and G2, ochratoxin A, zearalenone, deoxynivalenol, and citreoviridin in rice in Brazil. Food Additives & Contaminants: Part A. 29(4): 694-703. https://doi.org/10.1080/19440049.2011.651750

Al Shannaq, A. and Yu, J.H. (2017). Occurrence, toxicity, and analysis of major mycotoxins in food. International Journal of Environmental Research and Public Health. 14(6): 632. https://doi.org/10.3390/ijerph14060632

Atanda, S.A.; Pessu, P.O.; Agoda, S; Isong, I.U. and Ikotun, I. (2011). The concepts and problems of post–harvest food losses in perishable crops. African Journal of Food Science. 5(11): 603-613. https://academicjournals.org/journal/AJFS/article-full-text-pdf/30EFBA310005.pdf

Atungulu, G.G.; Mohammadi-Shad, Z. and Wilson, S. (2018). Mycotoxin issues in pet food. Food and Feed Safety Systems and Analysis. 2: 25-44. https://doi.org/10.1016/b978-0-12-811835-1.00002-6

Awuchi, C.G.; NyakundiOndari, E.; Eseoghene, I.J.; Twinomuhwezi, H.; Amagwula, I.O. and Morya, S. (2022). Fungal growth and mycotoxins production: Types, toxicities, control strategies, and detoxification. Fungal Reproduction and Growth. 1-21. https://doi.org/10.5772/intechopen.100207

Awuchi, C.G.; Ondari, E.N.; Ogbonna, C.U.; Upadhyay, A.K.; Baran, K.R. Okpala, C.O. et al. (2021). Mycotoxins affecting animals, foods, humans, and plants: Types, occurrence, toxicities, action mechanisms, prevention, and detoxification strategies—A revisit. Foods. 10(6): 1279. https://doi.org/10.3390/foods10061279

Batra, S. (2019). Radioimmunoassay (RIA) - Principle, procedure, advantages, applications. Serology test. https://serologytest.com/radioimmunoassay-ria-principle-procedure-advantages-disadvantages-applications/

Blandino, M.; Pilati, A.; Reyneri, A. and Scudellari, D. (2010). Effect of maize crop residue density on *Fusarium* head blight and on deoxynivalenol contamination of common wheat grains. Cereal Research Communications. 38(4): 550–559. https://doi.org/10.1556/crc.38.2010.4.12

Brown, R.; Priest, E.; Naglik, J.R. and Richardson, J.P. (2021). Fungal toxins and host immune responses.

Frontiers in Microbiology. 12: 643639. https://doi.org/10.3389/fmicb.2021.643639

Campagnollo, F.B.; Ganev, K.C.; Khaneghah, A.M.; Portela, J.B.; Cruz, A.G.; Granato, D. et al. (2016). The occurrence and effect of unit operations for dairy products processing on the fate of aflatoxin M1: A review. Food Control. 68: 310–329. https://doi.org/10.1016/j.foodcont.2016.04.007

Capriotti, A.L.; Caruso, G.; Cavaliere, C.; Foglia, P.; Samperi, R. and Laganà, A. (2011). Multiclass mycotoxin analysis in food, environmental and biological matrices with chromatography/mass spectrometry. Mass Spectrometry Reviews. 31(4): 466–503. https://doi.org/10.1002/mas.20351

Chu, F.S. (2004). Immunochemical methods for mycotoxin analysis: from radioimmunoassay to biosensors. Mycotoxins. 54(1): 1-14. https://doi.org/10.2520/myco.54.1

Cui, L.; Selvaraj, J.N.; Xing, F.; Zhao, Y.; Zhou, L. and Liu, Y. (2013). A minor survey of deoxynivalenol in *Fusarium* infected wheat from Yangtze–Huaihe River Basin region in China. Food Control. 30(2): 469-473. https://doi.org/10.1016/j.foodcont.2012.08.011

Dhakal, A.; Hashmi, M.F. and Sbar, E. (2022). Aflatoxin Toxicity - StatPearls - NCBI Bookshelf. PMID: 32491713. https://www.ncbi.nlm.nih.gov/books/NBK557781/

Dickman, K. and Grollman, A. (2010). Nephrotoxicity of natural products: aristolochic acid and fungal toxins. Comprehensive Toxicology (2nd Edition). 7: 433-458. https://doi.org/10.1016/b978-0-08-046884-6.00823-x

Ditta, Y.A.; Mahad, S. and Bacha, U. (2019). Aflatoxins: Their toxic effect on poultry and recent advances in their treatment. In: Njobeh, P.B. and Stepman, F. (Editors). Mycotoxins - Impact and Management Strategies. 7: 125-147. https://doi.org/10.5772/intechopen.80363

Doohan, F.; Brennan, J. and Cooke, B. (2003). Influence of climatic factors on *Fusarium* species pathogenic to cereals. Journal of Plant Pathology. 109(7): 755-768. https://doi.org/10.1023/A:1026090626994

El-Desouky, T.A. and Naguib, K. (2013). Occurrence of zearalenone contamination in some cereals in Egypt. Journal of Agroalimentary Processes and Technologies. 19(4): 445-450.

Flannery, K.; Frank, J. and Kato, M. (2012). School disciplinary responses to truancy: Current practice and future directions. Journal of School Violence 11. https://doi.org/10.1080/15388220.2011.653433

Fuchs, R. and Peraica, M. (2005). Ochratoxin A in human kidney diseases. Food Additives & Contaminants. 22(sup1): 53-57. https://doi.org/10.1080/02652030500309368

Ghanem, K.Z.; Mahran, M.Z.; Ramadan, M.M.; Ghanem, H.Z.; Fadel, M. and Mahmoud, M.H. (2020). A comparative study on flavour components and therapeutic properties of unfermented and fermented defatted soybean meal extract. Scientific Reports. 10(1): 5998. https://doi.org/10.1038/s41598-020-62907-x

Gonzalez, A.L.; Lozano, V.A.; Escandar, G.M. and Bravo, M.A. (2020). Determination of ochratoxin A in coffee and tea samples by coupling second-order multivariate calibration and fluorescence spectroscopy. Talanta. 219: 121288. https://doi.org/10.1016/j.talanta.2020.121288

Haschek, W.; Voss, K. and Beasley, V. (2002). Selected mycotoxins affecting animal and human health. In: Haschek, W.M.; Rousseaux, C.G. and Wallig, M.A. (Editors). Handbook of Toxicologic Pathology (2nd Edition). 1: 645-699. https://doi.org/10.1016/b978-012330215-1/50026-0

Hosseini, S. and Bagheri, R. (2012). Some major mycotoxin and their mycotoxicoses in nuts and dried

fruits. Acta Horticulturae. 963: 251–257. https://doi.org/10.17660/actahortic.2012.963.41

Hui, Y.H. (2019). Foodborne Disease Handbook, 2nd Edition, Volume III: Plant Pathogens. CRC Press. 687-688. https://doi.org/10.1201/9781351072090

Janik, E.; Niemcewicz, M.; Podogrocki, M.; Ceremuga, M.; Gorniak, L.; Stela, M. et al. (2021). The existing methods and novel approaches in mycotoxins' detection. Molecules. 26(13): 3981. https://doi.org/10.3390/molecules26133981

Juliatti, F.C. and Azevedo, L.A.S.D. (2017). Strategies of chemical protection for controlling soybean rust. Soybean - the Basis of Yield, Biomass and Productivity. 3: 35-62. https://doi.org/10.5772/67454

Kamle, M.; Mahato, D.K.; Devi, S.; Lee, K.E.; Kang, S.G. and Kumar, P. (2019). Fumonisins: Impact on agriculture, food, and human health and their management strategies. Toxins. 11(6): 328. https://doi.org/10.3390/toxins11060328

Kang'ethe, E.K. and Lang'a, K.A. (2009). Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centers in Kenya. African Health Sciences. 9(4): 218-226. https://pubmed.ncbi.nlm.nih.gov/21503172/

Kerry, R.; Ingram, B.; Ortiz, B.V. and Salvacion, A. (2022). Using soil, plant, topographic and remotely sensed data to determine the best method for defining aflatoxin contamination risk zones within fields for precision management. Agronomy. 12(10): 2524. https://doi.org/10.3390/agronomy12102524

Kumar, A.; Pathak, H.; Bhadauria, S. and Sudan, J. (2021). Aflatoxin contamination in food crops: causes, detection, and management: A review. Food Production, Processing and Nutrition. 3(17): 1-9. https://doi.org/10.1186/s43014-021-00064-y

Lee, H.J. and Ryu, D. (2017). Worldwide occurrence of mycotoxins in cereals and cereal-derived food products: public health perspectives of their co-occurrence. Journal of Agricultural and Food Chemistry. 65(33): 7034–7051. https://doi.org/10.1021/acs.jafc.6b04847

Lee, H.R.; Kochhar, S. and Shim, S.M. (2015). Comparison of electrospray ionization and atmospheric chemical ionization coupled with the liquid chromatography-tandem mass spectrometry for the analysis of cholesteryl esters. International Journal of Analytical Chemistry. 2015: 1-6. https://doi.org/10.1155/2015/650927

Li, D.X.; Gan, L.; Bronja, A. and Schmitz, O.J. (2015). Gas chromatography coupled to atmospheric pressure ionization mass spectrometry (GC-API-MS): Review. Analytica Chimica Acta. 891: 43-61. https://doi.org/10.1016/j.aca.2015.08.002

Luis, J.M.S.O. (2019). Sexual Reproduction and Enhancing the Efficacy of Biocontrol of Aflatoxin Contamination by *Aspergillus flavus*. North Carolina State University ProQuest Dissertations Publishing: 27700923. https://search.proquest.com/openview/06e98e9074daf04d31b7534746830b8a/1?pq-origsite=gscholar&cbl=51922&diss=y

Magnussen, A. (2013). Aflatoxins, hepatocellular carcinoma and public health. World Journal of Gastroenterology. 19(10): 1508-1512. https://doi.org/10.3748/wjg.v19.i10.1508

Mannaa, M. and Kim, K.D. (2017). Influence of temperature and water activity on deleterious fungi and mycotoxin production during grain storage. Mycobiology. 45(4): 240-254. https://doi.org/10.5941/myco.2017.45.4.240

Marchese, S.; Polo, A.; Ariano, A.; Velotto, S.; Costantini, S. and Severino, L. (2018). Aflatoxin B1 and M1: Biological properties and their involvement in cancer development. Toxins. 10(6): 214. https://doi.org/10.3390/toxins10060214

Marín, P.; de Ory, A.; Cruz, A.; Magan, N. and González-Jaén, M.T. (2013). Potential effects of environmental conditions on the efficiency of the

antifungal tebuconazole controlling *Fusarium verticillioides* and *Fusarium proliferatum* growth rate and fumonisin biosynthesis. International Journal of Food Microbiology. 165(3): 251-258. https://doi.org/10.1016/j.ijfoodmicro.2013.05.022

Marroquín-Cardona, A.; Johnson, N.; Phillips, T. and Hayes, A. (2014). Mycotoxins in a changing global environment – A review. Food and Chemical Toxicology. 69: 220-230. https://doi.org/10.1016/j.fct.2014.04.025

McLaughlin, J.E.; Bin-Umer, M.A.; Widiez, T.; Finn, D.; McCormick, S. and Tumer, N.E. (2015). A lipid transfer protein increases the glutathione content and enhances *Arabidopsis* resistance to a trichothecene mycotoxin. PLoS ONE. 10(6): 1-20. https://doi.org/10.1371/journal.pone.0130204

Metzler, M.; Pfeiffer, E. and Hildebrand, A. (2010). Zearalenone and its metabolites as endocrine disrupting chemicals. World Mycotoxin Journal. 3(4): 385–401. https://doi.org/10.3920/wmj2010.1244

Mokoena, M.P.; Chelule, P.K. and Gqaleni, N. (2005). Reduction of fumonisin B1 and zearalenone by lactic acid bacteria in fermented maize meal. Journal of Food Protection. 68(10): 2095-2099. https://doi.org/10.4315/0362-028x-68.10.2095

Munkvold, G.P. (2016). *Fusarium* species and their associated mycotoxins. Methods in Molecular Biology -Mycotoxigenic Fungi. 1542: 51-106. https://doi.org/10.1007/978-1-4939-6707-0_4

Murayama, C.; Kimura, Y. and Setou, M. (2009). Imaging mass spectrometry: principle and application. Biophysical Reviews. 1(3): 131-139. https://doi.org/10.1007/s12551-009-0015-6

National Cancer Institute. (2022). Liver Cancer Causes, Risk Factors, and Prevention. https://www.cancer.gov/types/liver/what-is-liver-cancer/causes-risk-factors

Pfohl-Leszkowicz, A.; Petkova-Bocharova, T.; Chernozemsky, I.N. and Castegnaro, M. (2002). Balkan endemic nephropathy and associated urinary tract tumours: A review on aetiological causes and the potential role of mycotoxins. Food Additives and Contaminants. 19(3): 282-302. https://doi.org/10.1080/02652030110079815

Pleadin, J.; Frece, J. and Markov, K. (2019). Mycotoxins in food and feed. Advances in Food and Nutrition Research. 89: 297-345. https://doi.org/10.1016/bs.afnr.2019.02.007

Qin, P.; Wang, T. and Luo, Y. (2022). A review on plant-based proteins from soybean: Health benefits and soy product development. Journal of Agriculture and Food Research. 7: 100265. https://doi.org/10.1016/j.jafr.2021.100265

Redondo-Blanco, S.; Fernández, J.; López-Ibáñez, S.; Miguélez, E.M.; Villar, C.J. and Lombó, F. (2020). Plant phytochemicals in food preservation: Antifungal bioactivity: A review. Journal of Food Protection. 83(1): 163-171. https://doi.org/10.4315/0362-028x.jfp-19-163

Ren, X.; Branà, M.T.; Haidukowski, M.; Gallo, A.; Zhang, Q.; Logrieco, A.F. et al. (2022). Potential of *Trichoderma* spp. for biocontrol of aflatoxin-producing *Aspergillus flavus*. Toxins. 14(2): 86. https://doi.org/10.3390/toxins14020086

Richard, J.L. (2012). Mycotoxins-an overview. Romer Labs' Guide To Mycotoxins- 4th Edition. 1: 1-48.

Richard, J.L. (2007). Some major mycotoxins and their mycotoxicoses-An overview. International Journal of Food Microbiology. 119(1-2): 3-10. https://doi.org/10.1016/j.ijfoodmicro.2007.07.019

Richard, J.; Payne, G.; Desjardins, A.; Maragos, C.; Norred, W. and Pestka, J. (2003). Mycotoxins: Risks in plant, animal, and human systems. Council for Agricultural Science and Technology. 101-103.

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 Journal. 18(3): 6040. https://doi.org/10.2903/j.efsa.2020.6040

Shanakhat, H.; Sorrentino, A.; Raiola, A.; Romano, A.; Masi, P. and Cavella, S. (2018). Current methods for mycotoxins analysis and innovative strategies for their reduction in cereals: An overview. Journal of the Science of Food and Agriculture. 98(11): 4003-4013. https://doi.org/10.1002/jsfa.8933

Shephard, G.S. (2016). Current status of mycotoxin analysis: A critical review. Journal of AOAC International. 99(4): 842-848. https://doi.org/10.5740/jaoacint.16-0111

Singh, J. and Mehta, A. (2020). Rapid and sensitive detection of mycotoxins by advanced and emerging analytical methods: A review. Food Science & Nutrition. 8(5): 2183-2204. https://doi.org/10.1002/fsn3.1474

Singh, D.; Lee, S. and Lee, C.H. (2017). Metabolomics for empirical delineation of the traditional Korean fermented foods and beverages. Trends in Food Science & Technology. 61: 103-115. https://doi.org/10.1016/j.tifs.2017.01.001

Sivamaruthi, B.; Kesika, P. and Chaiyasut, C. (2018). Toxins in fermented foods: prevalence and preventions-A mini review. Toxins. 11(1): 4. https://doi.org/10.3390/toxins11010004

Stracquadanio, C.; Luz, C.; La Spada, F.; Meca, G. and Cacciola, S.O. (2021). Inhibition of mycotoxigenic fungi in different vegetable matrices by extracts of *trichoderma* species. Journal of Fungi. 7(6): 445. https://doi.org/10.3390/jof7060445

Tamang, J.P.; Watanabe, K. and Holzapfel, W.H. (2016). Review: diversity of microorganisms in global fermented foods and beverages. Frontiers in Microbiology. 7: 377. https://doi.org/10.3389/fmicb.2016.00377

Taniwaki, M.; Hocking, A.; Pitt, J. and Fleet, G. (2009). Growth and mycotoxin production by food spoilage fungi under high carbon dioxide and low oxygen atmospheres. International Journal of Food Microbiology. 132(2-3): 100-108. https://doi.org/10.1016/j.ijfoodmicro.2009.04.005

Tian, F.; Woo, S.Y.; Lee, S.Y.; Park, S.B.; Im, J.H. and Chun, H.S. (2022). Mycotoxins in soybean-based foods fermented with filamentous fungi: Occurrence and preventive strategies. Comprehensive Reviews in Food Science and Food Safety. 21(6): 5131-5152. https://doi.org/10.1111/1541-4337.13032

Tjamos, S.E.; Antoniou, P.P.; Kazantzidou, A.; Antonopoulos, D.F.; Papageorgiou, I. and Tjamos, E.C. (2004). *Aspergillus niger* and *Aspergillus carbonarius* in Corinth raisin and wine-producing vineyards in Greece: Population composition, ochratoxin a production and chemical control. Journal of Phytopathology. 152(4): 250-255. https://doi.org/10.1111/j.1439-0434.2004.00838.x

UGA Cooperative Extension. (2006). Reducing aflatoxin in corn during harvest and storage. https://extension.uga.edu/publications/detail.html?number=B1231&title=reducing-aflatoxin-in-corn-during-harvest-and-storage.

Waliyar, F.; Reddy, S.V. and Lava-Kumar, P. (2009). Review of immunological methods for the quantification of aflatoxins in peanut and other foods. Peanut Science. 36(1): 54-59. https://doi.org/10.3146/at07-007.1

Webster, J. and Weber, R. (2012). Introduction to Fungi- 3rd Edition. Kindle Edition. Cambridge Core. https://doi.org/10.1017/CBO9780511809026

WHO. (World Health Organization). (2018). Mycotoxins. https://www.who.int/news-room/fact-sheets/detail/mycotoxins

Yao, H.; Hruska, Z. and Di Mavungu, J.D. (2015). Developments in detection and determination of aflatoxins. World Mycotoxin Journal. 8(2): 181-191. https://doi.org/10.3920/wmj2014.1797

Zain, M.E. (2011). Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society.

15(2): 129-144. https://doi.org/10.1016/j.jscs.2010.06.006

Zaki, M. (2012). Mycotoxins in animals: Occurrence, effects, prevention and management. Journal of Toxicology and Environmental Health Sciences. 4(1): 13-28. https://doi.org/10.5897/jtehs11.072

Zhang, K. and Banerjee, K. (2020). A review: sample preparation and chromatographic technologies for detection of aflatoxins in foods. Toxins. 12(9): 539. https://doi.org/10.3390/toxins12090539

Zhang, L.; Dou, X.W.; Zhang, C.; Logrieco, A. and Yang, M.H. (2018). A review of current methods for analysis of mycotoxins in herbal medicines. Toxins. 10(2): 65. https://doi.org/10.3390/toxins10020065

Zhihong, L.; Kunlun, H. and Yunbo, L. (2015). Ochratoxin A and ochratoxin-producing fungi on cereal grain in China: A review. Food Additives & Contaminants: Part A. 32(4): 461-470. https://doi.org/10.1080/19440049.2014.996787