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Article *in* Journal of Food Chemistry and Nanotechnology · January 2024

DOI: 10.17756/jfcn.2023-160

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Journal of
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https://doi.org/10.17756/jfcn.2023-160

Review Article

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Mycotoxins' Contamination in Food and Feeds: A Review

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Received: October 03, 2023
Accepted: November 03, 2023
Published: November 06, 2023

Citation: Morshedi A, Shakerardekani A, Karazhian R, Morshedi M, Valdes ME, et al. 2023. Mycotoxins' Contamination in Food and Feeds: A Review. *J Food Chem Nanotechnol* 9(3): 141-149.

Published by United Scientific Group

Abstract

Fungi are the most resistant microorganisms distributed worldwide in different foods and feedstuffs. They produce Mycotoxins as their secondary and toxic metabolites for humans and animals, and they are carcinogenic, mutagenic, teratogenic, and immunosuppressive. According to Mycotoxins structures, their risk for health is different. Therefore, aflatoxins, ochratoxins A, zearalenone, fumonisins, patulinand, and trichothecenes are recognized as the most hazardous for health. Climate changes during recent years have caused increasing mycotoxins contaminations. Since these secondary metabolites cause chronic and acute diseases, different standards exist to eliminate them in foodstuffs worldwide. The toxicity of mycotoxins and their maximum permitted in foods is different. Because of their toxicity feature, many scientists are studying to find better options for mycotoxin detection and reduction in foods and feeds. Up to now, HPLC (High performance liquid chromatography) coupled with fluorescence derivatization and MS/MS (Mass spectroscopy) is the most successful method for mycotoxin detection. There are different ways to reduce these contaminants, but in countries that have considerable rainfall besides temperature, which helps to grow molds and produce mycotoxins significantly.

Keywords

Aflatoxins, Ochratoxin, Patulin, Fumonisins, Zearalenone

Introduction

Nowadays, we face climate change all over the world. These changes have produced adverse effects on all aspects of food and nutrition security and safety, for instance: high temperature, CO_2 (carbon dioxide) increasing, rainfall amount and its distribution, disease and pest distribution which have led to increasing mycotoxins contaminations [1-8]. In the next 10 - 20 years, the environment will change markedly with atmospheric CO_2 concentration with an increasing rate of 1.5 μmol/year [9]. Not only increasing CO_2 but also greenhouse gases, causesglobal warming and are expected to increase the rate of air temperature by 0.03 °C per year. Paterson and Lima [10] suggest that if the temperature increases during future years, it will observe those aflatoxins and other mycotoxins production in crops will be increased consequently.

Another critical point that is rarely noticed is that most molds can fetch up several types of mycotoxins at the same time, which can show interaction too. Since food and feed can be substrates of several fungi simultaneously, humans

and animals face high contents of different types of mycotoxins [1, 11].

Among the emerging issue in food safety, is the increase inthe occurrence of toxigenic fungi (*Aspergillus, Fusarium,* and *Penicillium*) and their metabolism (aflatoxins, ochratoxins, patulin, zearalenone, fumonisin, and deoxynivalenol) is the primary concern because they are carcinogenic, mutagenic, teratogenic, and immunosuppressive. Finally, they are responsible for acute or chronic toxic effects with chronic disease in the central system of nervous, liver, and cardiovascular [12-19]. These contaminations always occur in poor socio-economic situations not only in pre-harvest but also in post-harvest stages, and consequently related health problems will occur with these products. The best point relating to these mycotoxins is that they can affect animals and their products by feeding too, and finally their adverse effects will be seen in humans who consume them [20, 21]. Mycotoxins are very stable, and this feature causes them to make pollution in different agro products, especially cereals, at different stages like pre-harvest, harvest, and post-harvest. Therefore, they are categorized as field and storage fungi [22-24]. Since these toxins production depends on climate and environmental conditions, product contaminations will vary in different countries [25, 26]. Different environmental factors, for instance, temperature, mold species, substrate, moisture percent, relative humidity, water activity, airflow, and ambient brightness, can affect toxin production. Besides some physical and chemical parameters like insect damages, fungicides application, storage conditions, poor operation during transportation and storage, and inadequate ventilation mycotoxins production can be different [25, 27].

Up to 1985 FAO (Food and Agriculture Organization) assessed that 25% of global food crops contaminated by mycotoxins, but today the EU (European Union) estimated to be up to 60 - 80%. Of course, this high occurrence results from improved analytical methods and climate change during these years, by the way it is a global problem [28]. Approximately 25 - 50% of cereal and its products are mycotoxins contaminated worldwide, and 5 - 10% is not consumable and produces considerable economic loss [7, 29, 30].

There are many identified mycotoxins but the most population of them are aflatoxins, ochratoxins A, zearalenone, fumonisin, patulin, and trichothecenes [31]. Mycotoxins can be produced in the field, garden, or farm, and by polluting the raw materials can be conducted to processed food [24, 32]. While the first death reason in the world has been reported is a chronic disease, like cardiovascular, the second reason is respiratory diseases, and the third is all types of cancers; mycotoxin poisonings stand after them [33-37]. In table 1, different mycotoxins effects on humans are mentioned. Therefore, these secondary metabolites are responsible for major public health that governments should be aware of as much as air pollution. The FAO estimated that more than 25% of agro-foods worldwide are contaminated by mycotoxins and 4.5 - 5 billion people are at risk of chronic exposure to mycotoxins especially in poor countries [38, 39].

Different studies have demonstrated that the most mycotoxin contaminations belong to the parts with hot and humid climates, tropical, and Estevan regions. Mycotoxins are separated from a wide range of agro-products: cereals (wheat, rice, maize, barley, etc.); nuts (pistachio, peanut, almond, etc.); spices (pepper, cayenne, cinnamon, etc.); fruits and vegetables (apple, cherry, etc.); animal products (meat, milk, etc.) [40-46]. In figure 1, different factors for mycotoxin occurrence in food and feed are shown.

Mycotoxins consumption and intake lead to chronic illnesses: immune sickness [47], metabolic, biochemical, and allergic illnesses [48, 49], carcinogenicity, teratogenicity, mutagenicity, and death. Therefore, because of the high possibility of their intake of food and agricultural products, it is necessary for their content management in foods and even feeds [50-52]. Mycotoxins are entirely different in physicochemical properties and follow different pathways after intake. Since their body effect and toxicity is completely different, they categorized according to IARC (International Agency for Research on Cancer) to different groups (Table 2).

Types of Mycotoxins

Aflatoxins

These toxins are products of *Aspergillus* species through a polypeptide pathway [53]. They are mostly observed in nuts and cereals. In most cereal crops, these contaminations occur after harvest and during storage. Scientists believe high temperatures and relative humidity more than 65% are the profit situation for these toxins production [54].

Aflatoxin's structure

Six types of naturally existing aflatoxins are aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2, aflatoxin M_1, and aflatoxin M_2. Aflatoxins M_1 and M_2 are recognized in milk for the first time; therefore, the letter (M) comes from milk. Their origin is AFB_1 after animal body intake, restructure occurs and

Table 1: Mycotoxins effects on different humans' organs [39].

Mycotoxins	Humans' organs
Aflatoxins	Liver, small intestine, lymph nodes
Fumonisin	Brain, lymph nodes, stomach
Ochratoxin A	Kidney
Patulin	Lymph nodes
Sterigmatocystin	Lymph nodes, windpipe and throat

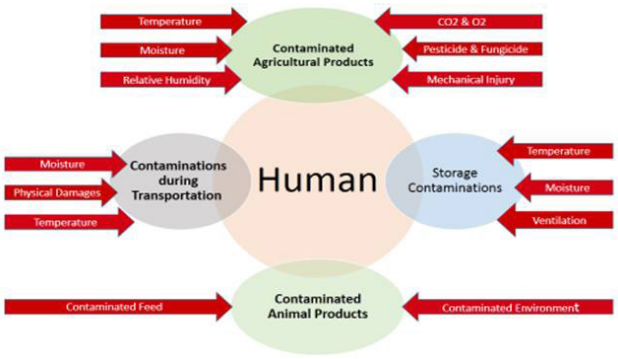

Figure 1: Different factors for mycotoxin occurrence in food and feed [29, 45, 46].

Table 2: Foods and different mycotoxins contaminations.

Group	Mycotoxin	Ref.
Cereals	Trichothecenes	[86]
	Aflatoxins	JECFA 1997, 1999, 2012, 2017; EFSA 2007, 2004a; IARC 2015, WHO 2000
	Fumonisin	IARC 2015; JECFA 2012, 2017; EFSA 2018a
	Deoxynivalenol	EFSA 2004b, 2011, 2017d; JECFA 2001, 2011
	Zearalenones	EFSA 2011, 2014, 2016, 2017c; JECFA 2000
	Ochratoxin	ESFA 2006; IARC 1983, 1993; JECFA 1996, 2001, 2007, 2008
Spices	Ochratoxin	ESFA 2006; IARC 1983, 1993; JECFA 1996, 2001, 2007, 2008
Nuts	Aflatoxins	JECFA 1997, 1999, 2012, 2015, 2017; IARC 2002, 2015; EFSA 2004a, 2007; WHO 2000
	Ochratoxins	ESFA 2006; IARC 1983, 1993; JECFA 1996, 2001, 2007, 2008
Fruits and vegetables	Patulin	-
	Ochratoxins	EFSA 2006; IARC 1983, 1993; JECFA 1996, 2001, 2007, 2008
Coffee and cacao	Ochratoxins	EFSA 2006; IARC 1983, 1993; JECFA 1996, 2001, 2007, 2008
Milk	Aflatoxins	JECFA 1997, 1999, 2017; IARC 2002; EFSA 2004a, 2007
	Fumonisins	IARC 2015; JECFA 2012, 2017
	Zearalenones	EFSA 2011, 2014, 2016, 2017c; IARC 1993; JECFA 2000; SCF 200a
	Ochratoxins	EFSA 2006; IARC 1983, 1993; JECFA 1996, 2001, 2007, 2008
Egg	Zearalenones	-
	Ochratoxins	EFSA 2006; IARC 1983, 1993; JECFA 1996, 2001, 2007, 2008

aflatoxin M_1 and aflatoxin M_2 will be formed [28]. The other four of these different types of aflatoxins are named because of their colors under ultraviolet shining during thin layer chromatography (TLC) analysis; B (Blue) and G (Green). On the thin layer, these mentioned aflatoxins have different retention factors. That's means toxins with the number (1) index have more relative mobility on TLC, number (2) index shows less mobility [28, 55].

Aflatoxins toxicity

Aflatoxins are the most important mycotoxins because of their genotoxic carcinogenic features; they are the most potent mutagenic and carcinogenic materials [28]. Toxicity rates are different in these types of aflatoxins: $B_1 > G_1 > M_1 > B_2 > G_2 > M_2$ [55]. The IARC recognized that AFB_1 is the most hazardous mycotoxin for human health [28, 56]. The severity of disease after aflatoxins consumption depends on the amount and duration of eating the toxin. Vomiting, hemorrhage, abdominal pain, icterus, cerebral edema, coma, convulsions, pulmonary and even death are the symptoms of aflatoxicosis which is aflatoxins poisoning disease. Besides the symptoms it is necessary to be mentioned that aflatoxicosis in chronic position can be observed as tumors and cancers especially liver cancer [28]. Because of these health problems produced by aflatoxins, United States Food and Drug Administration (USFDA) and EU, have set the maximum level for aflatoxins contamination in foods which are less than 20 µg/kg, and 0.1 - 2 µg/kg respectively for total aflatoxins [57]. The important point is that Sterigmatocystin is a genotoxic carcinogen. Its biosynthesis pathway is the same as aflatoxins, and finally both have the same target organ (liver) [58].

Fumonisin

These types of toxins are second metabolites of *Fusarium* species in fields, which produced in hot and dry or humid weather [59]. Up to now, more than 30 different types of fumonisins have been recognized while their number is growing [60]. Fumonisin were seen for the first time in cereal, especially maize (corn) [61, 62].

Fumonisin structure

Fumonisins have polyketide non-fluorescent structures. Fumonisin considering their chemical structures is divided into four categories: A, B, C, and P [63]. In fumonisin B group (fumonisin B_1, fumonisin B_2, fumonisin B_3, and fumonisin B_4), fumonisin is the most prevalent and toxic one and is observed 70 - 80% more than the others. Fumonisin B_2 and fumonisin B_3 co-occur with fumonisin B_1, but among the fumonisin Bs group, fumonisin B_1 is considered in Group 2B possibly carcinogenic to humans by the IARC [56, 58].

Fumonisin toxicity

The reported symptoms are disruption of sphingolipids metabolism and effecting on neural tube defects in the brain and spinal, which are very similar to sphingosine and produce some neurological manifestations such as nervousness, ataxia, aimless circling, lameness, facial paralysis, abnormal movements and finally inability to drink or eat. However, the target organ for fumonisin B in laboratory animals is the liver. Fumonisin B_1 is categorized in IARC 2B which means it is possibly carcinogenic to humans [64]. According to FDA standards, the maximum advisory level of fumonisin Bs is 2 - 4 mg/kg for human consumption.

Ochratoxins

Ochratoxins are another mycotoxins group that can be produced by *Aspergillus ochraceous*, *Apergillus carbonarius*, and *Penicillium verrucosum* species mostly [65]. It is separated from different foods (e.g., cereals, coffee and cacao beans, nuts, spices, milk, and egg) [66].

Ochratoxin structure

There are different functional groups in this toxin main structure therefore, according to that, ochratoxins can be exported into three groups: A, B, and C. The phenylalanyl derivative of isocoumarin substituted is named ochratoxin A which is recognized as the second most important mycotoxin [67]. The pathway of ochratoxins A is not known completely against aflatoxins and fumonisin Bs, but it recognized isocoumarin group is originated from acetate and molanate via a polyketide synthesis pathway [68].

Ochratoxin toxicity

When ochratoxins A enters the body through foods, it binds to plasma protein through gastrointestinal and sediment in the kidney with a very long half-life (about 35 days) [28]. Besides, ochratoxins A can corrival with phenylalanine hydroxide in both kidney and liver to stop certain protein synthesis as well as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) synthesis. Ochratoxins A has been recognized as a carcinogen factor in animal studies therefore, it categorized in 2B group possibly carcinogenic to humans by the IARC [56]. The FDA has not set regulations on ochratoxins A content in foods, while the EU has established ochratoxins A limitations of 3 and 5 mg/kg for food products and raw, respectively.

Deoxynivalenol

Deoxynivalenols are mainly produced by *Fusarium graminearum* and *Fusarium culmorum* in the field or during post-harvest storage mainly in cereals [28].

Deoxynivalenol structure

More than 200 different trichothecenes have been recognized up to know that is subdivided into four main groups: A, B, C, and D. Type A of them, is known as the most toxic group as for example T-2 and HT-2 are in this category [5]. Chemically, deoxynivalenol is considered a type B of trichothecene with a characteristic ketone at C8, and three hydroxyl groups at C3, C7, and C15. Another derives of deoxynivalenol are its acetylated, 3-acetyldeoxynivalenol (3ADON), and nivalenol (NIV) which is type B trichothecenes and deoxynivalenol-3-glucoside [25, 69]. According to some research, deoxynivalenols have an amphipathic nature; therefore, they can cross the cell membrane and interact with endoplasmic reticulum, mitochondria [25], and chloroplast [25, 70].

Deoxynivalenol toxicity

The epoxide ring of trichothecenes has toxicity action that its solution is not only stable against PH but also against heat [71]. According to [72, 73] studies, deoxynivalenols effects can inhibit cell dividing, synthesis of RNA and DNA, membrane disruption, and mitochondrial functions. Deoxynivalenols prevent initial peptide bonds. Meanwhile, although deoxynivalenols inhibit DNA and protein synthesis, there is no report about its mutagenic or carcinogenic effect in humans. They affect immunity by protein synthesis inhibition [67]; decrease antibody and immunoglobulin levels in humans [74]; easily absorbed through skin and cause rapid irritation. In animals, weight loss and anorexia are the most common chronic effects. It confirmed that deoxynivalenol toxicity is less than type 'A' trichothecene. As well, these types of mycotoxins consumption have produced fatal and chronic toxicoses not only in humans but also in animals. They can affect plant regeneration and mammals' reproduction [75]. Deoxynivalenol poisonous symptoms are vomiting, diarrhea, headache, dizziness, abdominal pain, and fever [76]. These family mycotoxins are recognized as phytotoxic for parsnip, wheat, and maize. The flowering period is considered a critical stage for plants for *Fusarium* species. Contaminants that can make progress in warm and moist conditions. T-2 and HT-2 chronic consumption symptoms are hematotoxicity and immunotoxicity and in acute situations vomiting in animals [77, 78]. The IARC categorized deoxynivalenol and T-2 toxin in group 3 which are not carcinogenic to humans. According to FDA standards, the maximum content of deoxynivalenol should be 1 mg/kg in final food products [56].

Zearalenone

Zearalenone is a common contamination in maize and cereal grains and products. A field fungus that grows in cool and moist conditions during different stages: pre-harvest, post-harvest, and storage. However, toxin production usually happens after harvesting and during storage and it is observed in cereals [79]. The zearalenone name was F-2 toxin previously [80] and is achieved from polyketide pathway by *Fusarium* species [81]. Another interesting point is that cereal contamination by zearalenone and deoxynivalenol usually coincide simultaneously because the same fungus produces both [82].

Zearalenone structure

The structure of zearalenone is (3, 4, 5, 6, 9, 10-hexahydro-14, 16-dihydroxy-3-methyl-1H-2 benzoxacyclotetradecin-1, 7(8H)-dione. Therefore, it is a macro cyclic β-resorcylic acid lactone [67]. Because of zearalenone complicated chemical structure, it is a heat resistant mycotoxin. To degrade it, should use alkaline solutions combine a temperature higher than 150 °C. However, children who consume cereal-based foods might receive this toxin more than others [25, 67].

Zearalenone toxicity

The IARC data shows that zearalenone is classified as group 3 and is not a carcinogen for humans [56]. The maximum zearalenone level in human foods depends on the food category and should be 75 - 350 µg/kg, according to EU standards. The FDA defines no limitation [68, 83].

Patulin

Patulin is the second metabolite of 60 different species of *Penicillium* which is considered a serious hazard for fruits, especially during the post-harvest period [5, 29]. The mold first grows on the surface of the fruit, like apples, cherries, figs, etc., and then contaminates all the fruit and fruit juice [29, 84, 85].

The reported data has shown that approximately 50% of apple juice is highly contaminated by patulin worldwide [86].

Patulin structure

Structurally patulin is a heterocyclic lactone (4-hidroxi-4H-furo(3, 2-c)piran-2(6H)-ona) [5]. It is a water-soluble and colorless molecule.

Patulin toxicity

It is supposed to cause patulin toxicity in its reaction with thiol groups (cysteine, glutathione, etc.) in the cytoplasm [87]. It affects enzymes that usually are digestion, metabolism, and energy production responsible [87]. Therefore, patulin chronic toxicity symptoms are nausea, vomiting, gastrointestinal, kidney and liver damage, and immunosuppression in animals [29]. It is not clear that patulin is a carcinogen for humans [5]. Both EU and FDA have set an upper limit of 50 µg/L or µg/kg patulin in apple and fruit juices, while for solid apple products like compote or puree, it is 25 µg/kg [5, 40, 88].

Mycotoxins Analysis

All the analysis methods have different risks that can be classified into five categories: health, safety, environment, energy, and waste based on toxicity [89].

For mycotoxin analysis, the method should be quick, cheap, and effective besides accurate detection. Aflatoxins, ochratoxins, fumonisins, and zearalenones have similar chemical structures with the same physicochemical properties [68, 90-92]. Due to the use of hazardous and toxic chemical materials, the analysis should be done according to set burdens. Nowadays, green analysis is developed by different research societies: Analytical Eco-Scales [93], National Environmental Methods Index, and Green Analytical Procedure Index [89]. All these methods are planned to reduce environmental damage and take into rapid and efficient analysis [94]. According to different researcher's publications, there are routine and different stages for mycotoxin analysis:

Sample collection

The first step of every analytical procedure is sample gathering. There are four ways for this step: in-line, on-line, at-line, and off-line sampling [89] of these models, the greenest one is in-line sampling, online and at-line are categorized in medium-green approach and off-line sampling should be avoided [95].

How many should be a sample size? It completely depends on the bulk weight and food type [96]. For example, if we have 50,000 kg of cereals, we should gather at least 100 incremental samples and the final weight should be 10 kg. There is a rule for sampling according to 'lot' and 'sublot' weight.

The next important step related to sample gathering is sample protection until analysis. There are three methods of preservation: chemical, physical, and physicochemical. Choosing each method is wholly dependent on the sample nature [89].

Extraction procedure

This procedure is the most crucial stage in every analytical experiment. In this step, considering the physicochemical properties of the sample (food matrix) and the mycotoxin that should be analyzed, the extraction method is different. However, grinding, homogenization with organic solvents or strong acids, and finally, filtration are its steps [89, 96].

The routine methods of these steps are liquid-liquid solvent extraction, immuno-affinity column extraction, solid-phase extraction, and the newest one is pressurized hot water extraction [97]. During extraction, the solvent will be removed analyte from the food matrix. Certainly, solvent choosing has a key role in this procedure and the point is that there is no specific solvent for an analyte or food. The best solvent is the solvent that removes as much analyte as possible from the food matrix [96].

Most of the mycotoxins are soluble in polar solvents. Therefore, solvents, used in the extraction process, up to now, are water, acetone, acetonitrile, methanol, chloroform, potassium chloride, or a combination of these [96]. Solvent and ground food should mix completely by mechanical shaking, ultrasound, supercritical fluid extraction, accelerated solvent extraction, and microwave-assisted extraction. Finally, the eluate will evaporate under the nitrogen stream. The following steps are re-dissolution and filtration [90-92, 94, 96].

Purification (clean up) procedure

The aim of purification is to separate target molecules or molecules. Each sample extract contains co-extracted materials that interfere with the analyte during detection with analytical instruments.

Solid phase extraction (SPE) is based on molecularly imprinted polymers and used for this purpose. These columns are different and produced commercially. According to matrix, analyte, and interference, it is necessary to choose the column. The newest and best purification method is using immune-affinity column (IAC). The point is that these columns act completely specific and for each type of mycotoxin, it is needed to use specified columns because they contain antibodies [94, 96]. In some of the analyses, it recommended to use both SPE and IAC for purification [96].

Detection and quantification

Analytical instrument selection is the main factor in the success of an analytical method. There are official mycotoxins methods approved by regulatory authorities such as the USFDA, The Association of Official Analytical Chemists, and the European Commission. The base of all official methods is the chromatography method. Commonly, Liquid Chromatography (HPLC or UHPLC) coupled to the tandem mass spectrometer (MS/MS) is recognized as the industry standard for mycotoxins analysis.

Among chromatographic techniques, HPLC coupled with fluorescence derivatives and MS/MS is the most popular method for cereal mycotoxin detection [98]. Some scientists are studying new methods focusing on multi-mycotoxin extraction, which not only have lower costs but are also categorized as green methods (like LC-MS/MS), but also is not an official method yet [97].

Conclusion

Mycotoxins are harmful second metabolites of fungi with different toxicity. The most harmful toxicity observed in aflatoxins fungi contaminants start from the field and continue during transportation, storage, and manufacturing; finally, they will transport to food and feed. During this way, many direct factors like crops genetics, instability of toxigenic properties, fungal species are important. Other factors like climate changes (CO_2 and O_2 concentration, temperature, and relative humidity), moisture, mechanical injuries, pesticides, and fungicides are important as well. Mycotoxins can be transported to humans directly and indirectly. When a person eats mycotoxin contaminated food, they directly consume it, but indirectly he/she can intake it by animal products e.g., milk, egg, meat, etc. Therefore, the best way for prevention consuming mycotoxins is molds growing control in different part of food and feed production chain.

Acknowledgements

None.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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