Mycotoxins in Beverages

Subjects: Toxicology
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Mycotoxins are secondary metabolites of filamentous fungi that contaminate food products such as fruits, vegetables, cereals, beverages, and other agricultural commodities.

Keywords: contamination ; aflatoxins ; ochratoxin A ; patulin ; toxicity ; detoxification

1. Introduction

Mycotoxins are naturally occurring, poisonous compounds produced from filamentous fungi or molds that can be found in foods. Mycotoxins have a huge set of chemical compounds generated by diverse mycotoxigenic fungi species [1]. Over 400 toxic metabolites are produced by more than 100 fungi species [2]. Humans are exposed to mycotoxins through the consumption of contaminated foods [3]. They can pose negative health effects, ranging from acute toxicity to chronic symptoms, such as kidney damage, liver damage, immune deficiency, and cancer [4][5].

Cereal grains and fruits can be infected by molds at various stages of production, for example, during cultivation, harvesting, and storage [6]. The contamination of mycotoxins is a worldwide problem, but it is more serious in humid and warm environmental conditions that favor the growth of fungi and production of mycotoxins. As secondary metabolites, mycotoxins are very durable chemical components that can be transmitted from raw materials to processed products such as beverages, which can pose a serious health risk to consumers (Figure 1).

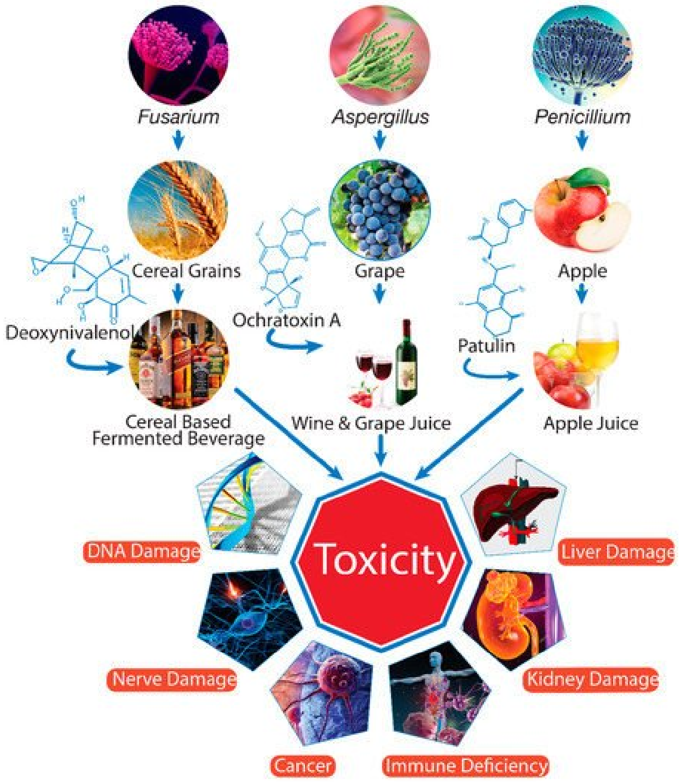

Figure 1. Mycotoxin contamination of beverages and adverse effects on health (drawn using Adobe Illustrator CC software).

Over the last few years, distinguishable progress in society has driven reforms in the world beverage market. Consumers are becoming more conscious about the effect of diet on their health. Beverages are not only responsible for providing energy and hydration but also for strengthening health and preventing nutrition-related defeciencies [7]. The application of effective measures to protect consumers from the toxic effects of mycotoxins and, subsequently, to defend against public health is very significant and crucial.

2. Major Mycotoxins in Beverages

2.1. Aflatoxins

Aflatoxins (AFs) are mainly produced by *Aspergillus spp*. In most of the cases, contamination with AFs takes place after harvesting and during storage. Inappropriate management during transportation and storage including exposure to conditions such as high humidity (˃65%) and temperatures rapidly increases the AF concentration in food.

2.2. Ochratoxin A

Ochratoxins (OTs) are group of mycotoxins that are mostly generated by *Aspergillus* and *Penicillium* species. The occurrence of OTA-producing fungi and the level of OTA may vary with the climatic conditions [8]. OTA is generally found in subtropical areas and in high-temperate climate regions and can be available in various food products in these areas, for example, beer, wine, and grape products [9]. Table 1 summarizes the major mycotoxins responsible for the contamination of beverages.

Table 1. Major mycotoxins involved in the contamination of beverages.

Mycotoxins	Products Contaminated	Producing Microorganisms	References
Aflatoxins B1, B2, G1, G2	**Orange, apple juice, grape juice, grapefruit peel**	***Aspergillus chevallieri*, *A. flavus*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. repens*, *A. ruber*, *A. tamarii*, and *A. wentii***	[10][11]
Ochratoxin A (OTA)	**Grape juice, coffee, beer, and wine**	***A. ochraceus*, *A. carbonarius*, *A. niger*, *A. tubingensis*, and *Penicillium expansum***	[10][12]
Patulin (PAT)	**Fruit juices**	***Penicillium expansum*, *P. patulum*, *Aspergillus clavatus*, *Byssochlamys fulva*, and *B. nivea***	[13][14]
Fumonisins (FBs)	**Beer**	***Fusariumproliferatum*, *F. verticillioides*, and *F. nygamai***	[15][16][17]
Trichothecenes (type B: Deoxynivalenol (DON))	**Plant-based beverages, beer**	***F. graminearum*, *F. cerealis*, and *F. culmorum***	[16][18][19][20]
Trichothecenes (type A: HT-2)	**Functional vegetable milks, beer**	***F. sporotrichioides*,and *F. langsethiae***	[20][21]
Trichothecenes (type A: T-2 toxin)	**Plant-based milks, beer**	***F. sporotrichioides*, and *F. langsethiae***	[19][21]
Zearalenone (ZEN)	**Beer, wine**	***F. graminearum*, *F. culmorum*, *F. equiseti*, *F. cerealis*, *F. verticillioides*, and *F. incarnatum***	[16][22]
Alternaria toxins (TeA, AOH, AME)	**Fruit juices, wine, beer**	***Alternaria alternate*, *A. tenuissima*, and *A. arborescens***	[23][24]

2.3. Patulin

Patulin (PAT) is predominantly generated from various *Penicillium*, *Aspergillus*, and *Byssochlamys* species and possesses various hazardous features such as toxicity, carcinogenicity, and mutagenicity. *P. expansum*, *B. fulva*, and *B. nivea* are significant PAT-producing microorganisms. Patulin has been identified in many foods, particularly in fruits and beverages [25].

2.4. Fumonisin

Fumonisin (FB) mycotoxins are secondary metabolites of *Fusarium spp*, mostly *Fusarium verticillioides* and *F. proliferatum*. It was found to be a contaminant of wheat, corn, and barley.

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2.5. Trichothecenes

Trichothecenes (TCs) belong to a large group of structurally related toxins, mainly produced by fungal species of the Fusarium genus [26]. T-2 and HT-2 toxins have been detected in barley, oat, maize, wheat, rice, beer, and plant-based milks, especially in oat- and soy-based milks and beverages [19][20][27][28]. Deoxynivalenol (DON) is synthesized by different species of the Fusarium genus, mainly by *Fusarium culmorum* and *Fusarium graminearum* in cereals [29]. It also contaminates cereal-based food products, for instance, pasta, bread, and beer.

2.6. Zearalenone

Zearalenone (ZEA) is produced by various species of *Fusarium*, mainly *F. graminearum*, *F. culmorum*, and *F. sporotrichioides*. It infects corn, wheat, barley, oat, and rye, mainly in areas with temperate climates [30].

2.7. Alternaria Toxins

The main Alternaria mycotoxins are Tenuazonic acid (TeA), Alternariol (AOH), and alternariol monomethyl ether (AME). *Alternaria spp.*, mainly *Alternaria alternata*, *A. tenuissima*, and *A. arborescens* produce Alternariols and are found in a large range of foods including berries, prune nectar, carrot juice, apple juice concentrate, grape juice, raspberry juice, cranberry juice, beer, and red wine [31][32].

3. Detection and Quantification of Mycotoxins in Beverages

In most cases, mycotoxin levels in contaminated food and beverages can be very low, and this necessitates the development of a suitable, rapid, and sensitive detection method. Various analytical testing procedures have been developed for mycotoxin detection and quantification due to their diverse forms [33]. Normal chromatographic procedures are usually time consuming and cost intensive; therefore, a range of methods, mostly based on immunological principles, have been developed and commercialized for quick determination [34]. Some common mycotoxin detection methods in beverages as well as beverage-producing crops are summarized in Table 2.

Table 2. Overview of common detection methods for mycotoxins in beverages as well as beverage-producing crops.

Analytical Methods	Detection Method	Toxin	Applicability	LOD	References	Advantages	Disadvantages
TLC	CCD	Patulin	Apple Juice	14 μg/L	[35]	Time saving, specific fluorescence spot on UV light	Limited plate length and environmental effects on measurement
HPLC	FD	OTA	Wheat	23 pg	[36]	Fast, high resolution data, accurate and easily reproducible. Less training required	Expensive and method development could be challenging
	MS/MS	OTA	Wine	0.005 ng/ml	[37]		
			Wine	0.09 μg/L	[38]		
	FD	AFs	Food items	1.6-5.2 ng/Kg	[39]		
	UV and FD	AFs	Milk	0.13–0.16 mg/L	[40]		
		OTA	Wine	0.07 ng/ml	[41]		
LC	FD	ZEN	Barley, Maize, Wheat	100 μg/Kg	[42]	Several mycotoxin detections, high sensitivity, provides confirmation	Expensive, required expertise In case of MS, sensitivity depends on ionization
		AFB1	Corn	2–5 ng/g	[43]		
	MS/MS	Trichothecenes	Wheat and maize	0.2–3.3 μg/Kg	[44]		

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Analytical Methods	Detection Method	Toxin	Applicability	LOD	References	Advantages	Disadvantages
Automated microarray chip reader	Chemiluminescence	OTA	Coffee	0.3 µg/L	[45]	High throughput, multiplexed, parallel processing method	Not so common to their variability and reproducibility, require intensive labor
Electropolymerization onto surface	SPR	ZEN	Corn	0.3 ng/ml	[46]	Suitable for cereals sample, simplicity, portability, and ease to use, can be used in field	Optimization and validation not reported for this method
Immunochromatographic strip	Highly Luminescent Quantum Dot Beads	AFB1	Maize	0.42 pg/ml	[47]	A simple method for rapid screening, superior performance	Required expertise
	SPR	OTA	Beverages	0.042 µg/L	[48]	Rapid, cost effective, and sensitive	
Direct, competitive magneto-immunoassay	Electrochemical	FB	Maize	0.33 µg/L	[49][50]		
Lateral flow immunoassay	Colorimetric			199 µg/Kg	[51]	Fast, one-step assay, no washing step, low cost and simple	Qualitative or semi quantitative results, sample volume governs precision
Photonics immobilization technique	Quartz-crystal microbalance (QCM)	Patulin	Apple puree	56 ng/ml	[52]	Specific, higher sensitivity, generality, response (only requires a few minutes), flexibility, and portability	The decrease of the signal in the presence of high analyte concentrations, in situ analysis
Surface-enhanced Raman scattering (SERS)-based immunoassay	Silica-encapsulated hollow gold nanoparticles	AFB1	Food	0.1 ng/ml	[53]	Enhanced ELISA method	Hard to synthesize and expensive
		AFM1	Milk	4–25 ng/L	[54]		
ELISA	UV absorbance	ZEN	Maize	0.02 µg/L	[55]	Fast, simple, economical, high sensitivity, simultaneous analysis of multiple samples, easy to screen	Lack of precision at low concentrations, matrix interference problems, possible false-positive/negative results
		AFB1 and AFM1	Food items	0.13–0.16 mg/L	[40]		
	Electrochemical	FB	Corn	5 µg/L	[56]		

Thin layer chromatography = TLC, High performance liquid chromatography = HPLC, Liquid chromatography = LC, Enzyme-linked immunosorbent assay = ELISA, FD = Fluorescence detection, Ultraviolet = UV, Charge-coupled device = CCD, Surface plasmon resonance = SPR, Mass spectrometry = MS.

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4. Mitigation Policies of Mycotoxin Contamination in Beverages

Nearly all mycotoxins are thermally resistant and cannot be simply degraded by normal heat treatment methods during food processing or household cooking methods [17]. Normally, mycotoxin contamination in beverages can be controlled by preventing the contamination of agricultural raw materials used for the production of beverages [57][58]. Implementation of good manufacturing practices will help to ensure safe beverage production without mycotoxin residues. Good manufacturing practices (GMPs) include the use of proper sorting, processing, drying, cooling, and storage conditions for agricultural raw materials. Complete reduction in the number of mycotoxins, or at least a number not higher than the maximum allowable limits, can be achieved by different pre- and postharvest treatments [59] (Figure 2).

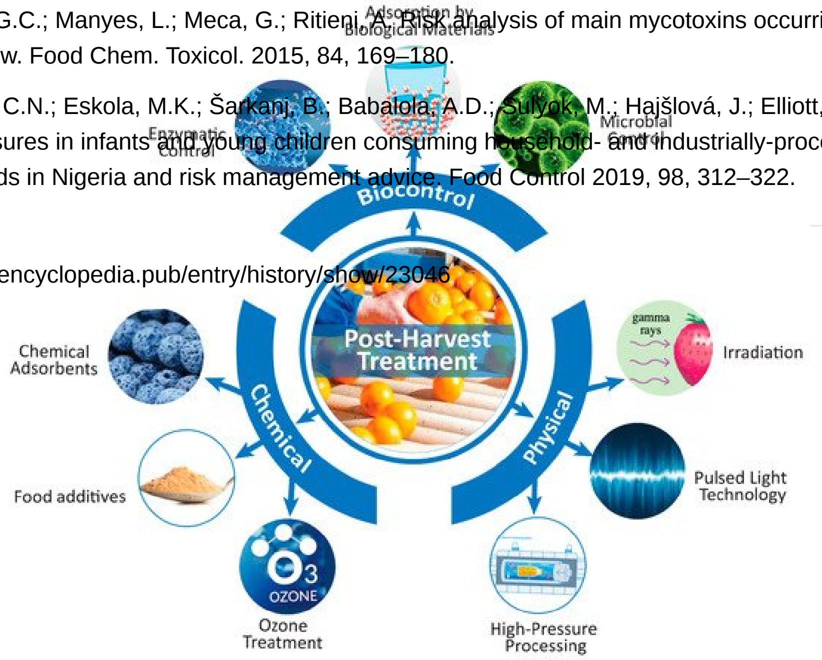

Figure 2. Scheme for reducing the mycotoxin concentration in beverages using postharvest treatments (drawn using Adobe Illustrator CC software).

5. Critical Challenges of Mycotoxins in Beverages

Mycotoxins possess very stable chemical structures that remain unchanged after pasteurization treatment. It has been reported that proper selection, adequate cleaning and washing, and careful sorting of fruits are very crucial factors for the mitigation of mycotoxin contamination during the manufacturing of beverages [60]. As children drink more juices than wine as compared to adults, therefore, the incidence of mycotoxins in fruit juices is a matter of serious concern [61][62].

Physical methods can be applied at large and small scales for a wider range of food, but some physical methods including irradiation have negative effects on the nutritional, antioxidant, and sensorial properties of food. Chemical methods are easy to use and comparatively cheap, but their main limitation is the toxicity of residues and secondary products. Additionally, the toxicity of the mycotoxin-degraded products needs to be measured. Although the adsorption of mycotoxins by chemical adsorbents is one of the most inexpensive detoxification methods, the safety of absorbent materials and the removal of the adsorbent–mycotoxin complex from foods is still challenging. In addition, the overall sensorial quality and final quality parameters (color, clarity, brix, titratable acidity, pH, and TSS) can be adversely affected by chemical treatments. Biological control methods are healthy and environmental friendly. However, microbial approaches may deteriorate the food quality by absorbing nutrients and releasing metabolites into the food matrices. Additionally, biological control methods are more expensive than physical and chemical control measures. Another critical challenge is the commercialization of biological control methods by overcoming the limitations in translation from laboratory trials to commercial applications.

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