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Review

Mycotoxin Contamination of Beverages Obtained from Tropical Crops

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Abstract: This review is mainly centered on beverages obtained from tropical crops, including tea, nut milk, coffee, cocoa, and those prepared from fruits. After considering the epidemiological data found on the matrices above, the focus was given to recent methodological approaches to assess the most relevant mycotoxins. Aspects such as singularities among the mycotoxin and the beverage in which their were found, and the economic effects and repercussions that the mycotoxin-tainted ingredients have on the beverage industry were pointed out. Finally, the burden of their consumption through beverages, including risk and health effects on humans, was addressed as well.

Keywords: Latin American beverages; mycotoxins; chromatographic methods; legislation; tropical beverages

1. Introduction

1.1. General Remarks for Mycotoxins

Mycotoxins are toxic secondary metabolites produced by aerobic, mycelial, microscopic fungi, especially from the genera *Aspergillus*, *Fusarium*, and *Penicillium* [1–3] (Figure 1A–F); these compounds may cause adverse health effects (e.g., hepatotoxicity, nephrotoxicity, neurotoxicity, and immunotoxicity) in humans and animals [4,5]. Toxic effects in plants related with mycotoxins and mechanisms governing endophytic plant colonization and disease have been described, as well [3]. Among multiple mycotoxin congeners characterized, aflatoxins (AFs), citrinin (CIT), patulin (PAT), penicillic acid (PA), tenuazonic acid (TEA), ochratoxin A (OTA), cytochalasins, deoxynivalenol (DON), fumonisins (FBs), fusarin C (FC), fusaric acid (FA), and zearalenone (ZEA) are considered the most common fungal contaminants in plant tissue (Figure 1) [3]. For humans, the majority of mycotoxicoses result from eating contaminated foods, and the symptoms depend on the type of mycotoxin, the amount and duration of the exposure, and certain inherent factors related to the patient such as age, sex, health and dietary status [4]. Under appropriate conditions (e.g., available genes in a strain, nature of the crop, moisture, and temperature), harvest and postharvest fungi colonization and mycotoxin production are feasible; this is particularly crucial for beverages made from tropical products such as different varieties of tea, coffee, cocoa, and fruits. Tropical products used as raw material for the production of drinks can be infected with toxigenic molds during crop growth, harvest, storage, or processing [6]. Mycotoxin accumulation in tropical crops may be relevant,

as these commodities are generally grown under relatively warm temperatures and high-humidity conditions [7]. Even though chemical (including biodetoxification) [8], biological [9], and physical (e.g., roasting, and shelling) detoxification or toxin reduction operations are possible, toxins can still reach the final consumer as they are relatively resistant to harsh processing conditions and are seldom spread homogenously across products. Hence, contamination of beverages (e.g., tea, coffee, and juices) is not uncommon. Adverse effects of these toxins on human and animal health have been documented, and particular interest has been drawn to the topic [10–17]. In general, beverages made from tropical products are widely consumed around the world and beverage overall quality depends on the raw materials used to prepare said drinks. However, especially in low-income countries, where toxin analysis may not be as widespread, or it is limited, unregulated raw materials may be processed. For example, traditionally prepared African beverages have recently been subject of study since grains from which these beverages are made were generally found to be heavily contaminated with mycotoxins [18]. Furthermore, mycotoxins may cause adverse economic effects, as they can hamper the international trading of contaminated products and eventually result in food waste [19,20]. In fact, some conservative estimates place the incidence of illness related to aflatoxins at 1%, in low and middle-income countries, which translate to ca. 2 billion USD in health expenditures [21].

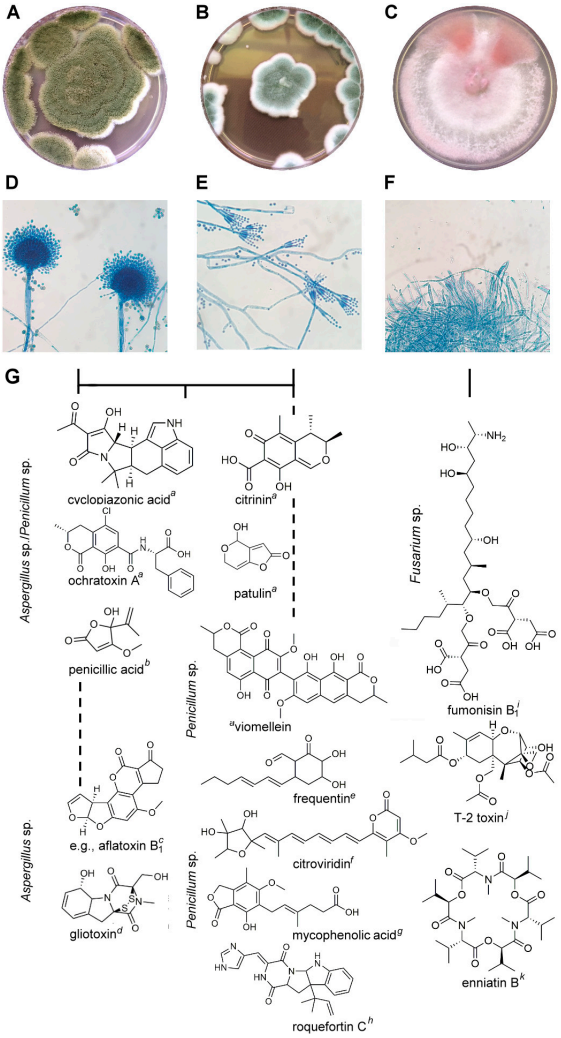

Figure 1. Colonies of mycotoxigenic fungi grown in Sabouraud Dextrose Agar and their respective microscopic images after staining with lactophenol cotton blue. (**A**,**D**) *Aspergillus flavus*; (**B**,**E**) *Penicillium* sp.; (**C**,**F**) *Fusarium solani*. (**G**). Structures of model mycotoxins produced by the aforementioned fungal species. [a] benzopyrans; [b] isopropylidene tetronic acid; [c] difuranocoumarin; [d] epipolythiodioxopiperazine;

[e] carbaldehyde; [f] pyranone; [g] meroterpenoid; [h] diketopiperazine; [i] monoterpene; [j] trichothecene/sesquiterpenoid; [k] cyclic depsipeptide.

1.2. Consumption Data for Tropical Beverages and Related Products

Substantial evidence has been found to suggest that there is a link between mycotoxin occurrence, exposure, and food consumption patterns [22]; therefore, this section deals with the production and consumption of the leading tropical beverages (Table 1). Outside Europe and the Americas, where coffee is preferred, tea is a favorite and relevant beverage. The uppermost tea consuming countries, including mate (a traditional caffeine-rich infused drink made from dry leaves from yerba mate, *Ilex paraguariensis* A. St.-Hill), are South American [23] (Table 1). However, other countries have high consumption patterns as well. In Iran, for example, a country with an estimated consumption of 1.83 kg per capita per year, black tea is preferred, and it is estimated that the average amount of tea consumed is 1 243 $\pm$ 530 mL day^{-1} [24]. In the United States, iced-tea drinks are preferred, and Europeans are more inclined toward drinking flavored fruit teas; "foamy" tea is primarily consumed in Taiwan, while herbal tea is favored in the United States, China, Japan, and Thailand, and ready-to-drink tea is consumed chiefly in Japan, USA, and Taiwan. On 2016, a consumption structure was reported to be 80.0, 69.2, 36.0, and 10.3% of black tea, 16.0, 7.1, 39.0, and 53.3% of green tea for the United States, the United Kingdom, Canada, and China, respectively [25]. Fruit or herbal teas are relevant in Canada and the United Kingdom with a 27.0 and 12.2% consumption rate, respectively [25].

On the other hand, coffee is considered one of the most important hot beverages. The 2016–2017 world coffee harvest amounted to a total of 157.43 million 60 kg bags. The foremost coffee consumers are Nordic countries (Table 1), while in Latin America, the primary consumers are Brazil (5.92) and Costa Rica (5.14 kg per capita per year) [26]. However, concerns about the adverse effects of caffeine on human health have had an impact on coffee-drinking frequency and quantity. Regarding chocolate, the consumption is also relatively high, ranging from 1.20 $\times$ 10^{-1} (China) to 8.98 $\times$ 10^{0} (Switzerland) kg per person per year [27,28] (Table 1). For example, Panamanian populations have been reported to reach consumption rates as high as ca. 8.87 $\times$ 10^{2} mL of cocoa-based beverages daily [28]. Though benefits of cocoa and chocolate consumption are numerous [29], mycotoxin-burdened products may counter these virtues.

Table 1. Top producing and consuming countries for tea, coffee, chocolate and cocoa, and dried and fresh fruit.

	Consumption		**Production Export Value**	
Tea (including mate) [23,25]				
Rank	Country	kg per capita per year	Country	Metric tons
1	Paraguay	12.22	China	2,414,802
2	Uruguay	9.66	India	1,252,174
3	Argentina	6.05	Kenya	473,000
4	Kenya	3.24	Sri Lanka	349,308
5	Gambia	3.22	Turkey	243,000
Coffee [26]				
Rank	Country	kg per capita per year	Country	Thousand 60 kg bags
1	Finland	12.2	Brazil	52,735
2	Sweden	10.1	Vietnam	29,500
3	Norway	8.9	Colombia	14,000
4	Austria	7.8	Indonesia	10,902
5	Switzerland	7.6	Ethiopia	7650
Chocolate [27,28]				
Rank	Country	kg per capita per year	Country	Thousand USD
1	Switzerland	8.98	Cote d'Ivoire	2,595,897
2	Germany	7.89	Ghana	1,090,910
3	Ireland	7.39	Indonesia	1,087,485
4	United Kingdom	7.39	Nigeria	599,000
5	Norway	6.62	Cameroon	540,281

Table 1. *Cont.*

	Consumption		Production Export Value	
Tropical beverage crops, fruits, and sugar [30]				
	Country	Million USD	Country	Million USD (percentage of the total agricultural products traded)
1	United States	2402	Brazil	16,466 (19.6)
2	Mexico	3071	Colombia	2951 (45.8)
3	Spain	3373	Ecuador	2782 (56.5)
4	China	3386	Vietnam	2678 (26.5)
5	Germany	4360	Guatemala	2403 (44.2)
6	Netherlands	4708	Ivory Coast	2276 (48.5)
7	United Kingdom	8602	Costa Rica	2015 (50.7)

Lastly, Central and South American countries are the principal producers of tropical beverage crops, fruits, and sugar [30] (Table 1). Additionally, consumer-wise, there has been a shift from nutrient-deficient, added-sugar and high fructose-containing beverages to more natural fruits and herbal infusions due to the prevalence of obesity [31,32]. Natural fruit juice drinking has changed the dynamics of the still drinks industry and consumption patterns. It is estimated that 2.35×10^1 million liters of juices and 1.70×10^1 million liters of nectar beverages were consumed globally in 2009; this represented an increase of 30.2% since 2003 [33]. Also, within the tropical juices category, the number of manufactured products and the consumption rates may vary among the crops. Pineapple juice is considered the most important fruit used for tropical juice production as the global business is estimated at ca. 2.50×10^5 tons per year. The consumption pattern for pineapple juice is mostly driven by the US and the European market, where people ingest more than 2.50×10^1 L per year [34].

1.3. Technological Processing and Mycotoxin Reduction

Good agricultural and manufacturing practices could be considered the best intervention to avoid mycotoxin contamination of beverages. Typically, the raw materials used for beverage production may be contaminated at the field (preharvest and harvest operations) or during storage. Selection of good quality material for processing is the first step to prevent further contamination; this is especially true in the case of fruit juices as ripened fruits are selected for processing [35]. For some beverages, the removal of rotten material or food parts with fungal contamination is a crucial step to reduce pollution in the final product [36]. During storage, the use of low temperatures and modified atmospheres is considered an essential control for fungal growth on the raw material. Storage facilities are considered the main factor to prevent contamination from the environment. Poor stowing conditions of the raw materials or even the finished product can have an incidence in the process downstream recontamination. Availability of facilities with proper infrastructure in tropical countries with developing economies may be limited, leading to the open storage of products intended for juice production [35]. On the other hand, processing techniques may reduce, to some extent, the mycotoxin level of contamination at the industry level (e.g., removing hulls from almonds), but unit operations seldom affect the former concentration. For example, the use of additives and thermal treatment may reduce the levels of discrete mycotoxins in beverages [35]. However, there is an inherited expense when additional stages (physical, chemical or otherwise) in food processing (including drinks) are used to reduce mycotoxin contamination. These procedures, however, are usually already part of the beverage-obtaining process, but they are not typically considered if they are not required to get the product. Available treatments and the consequences of introducing such steps have already been detailed elsewhere [37]. To obtain a beverage as a final product, usually, the raw material will have to undergo several operating units and technological steps; plant designs for both pineapple juice (Figure 2A) and cocoa (Figure 2B) are presented to help the reader relate to the topic.

Even though processing may have an impact on mycotoxin stability, there are few studies related with beverages made from tropical crops. For example, the influence of industrial treatments on OTA content in cocoa bean processing has been investigated [38]. The authors concluded that roasting,

shelling and additive addition, significantly decreased OTA levels in cocoa finished products. On the other hand, Hao et al. [39] also observed that PAT content decreases in different apple juice-based products (including a mixture with pineapple) after processing with high-pressure technology; the authors noted a correlation between mycotoxin degradation with high pressure and the number of sulfhydryl groups in the juice.

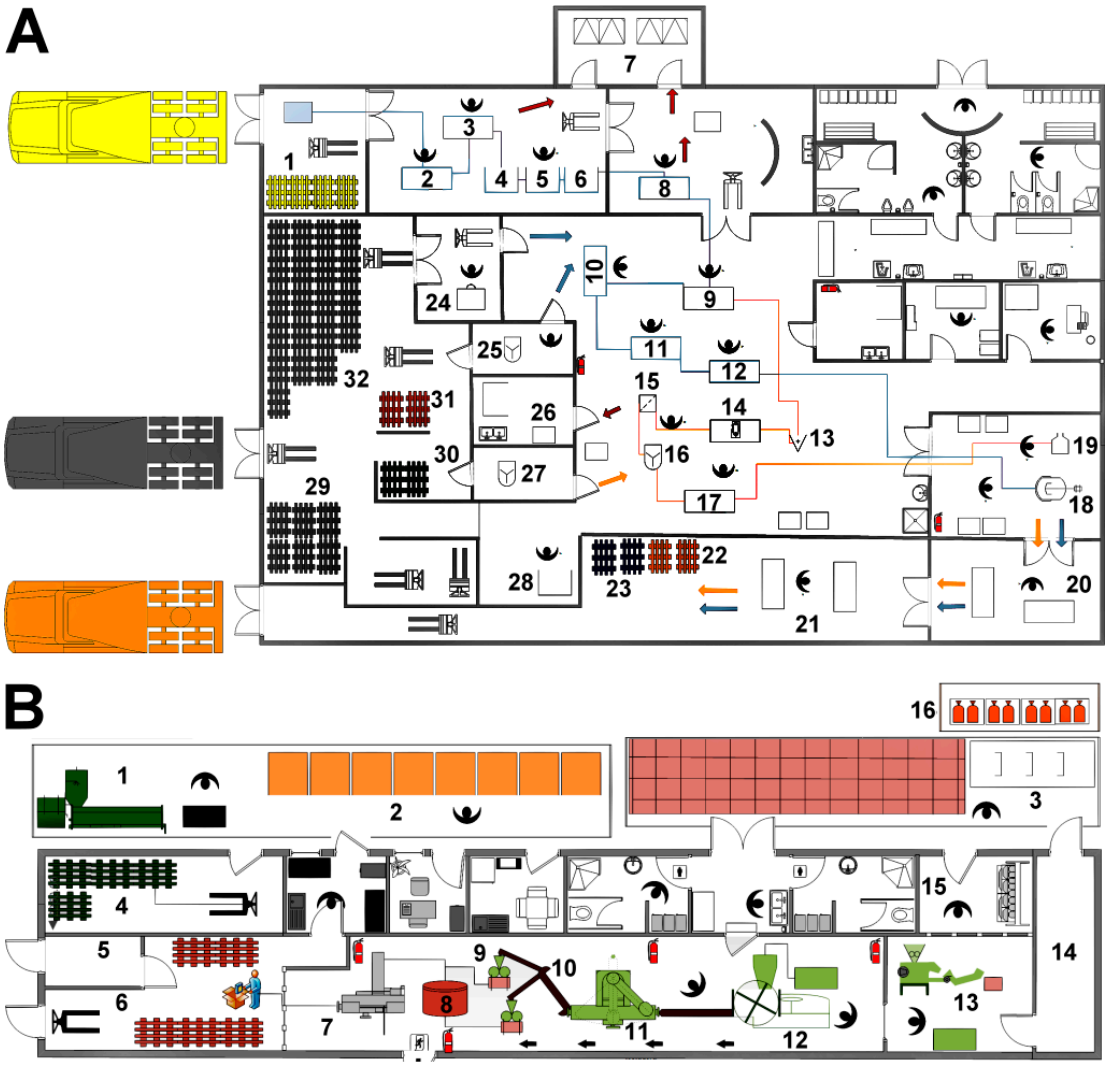

Figure 2. Existent Costa Rican factory layout, representing product lines for (**A**) Pineapple beverage and canned fruit facility: 1. Receipt 2. Fruit selection 3. Decrowning 4. Wash 5. Disinfection 6. Draining 7. Waste disposal 8. Peel and coring 9. Chopping 10. Canning 11. Juice addition 12. Can sealing 13. Crusher 14. Hydraulic press 15. Filter 16. Mixer 17. Bottling 18. Autoclave 19. Cooking pot 20. Cooling area 21. Labeling 22. Pineapple juice bottles 23. Canned apple chunks 24. Gas injection 25. Syrup formulation/mixer 26. Cleaning 27. Pineapple juice formulation 28. Bottle disinfection 29. Empty bottles 30. Citric acid 31. Sugar 32. Empty cans. Workflow for → Pineapple juice production → Canned pineapple chunks production → Waste disposal and treatment; and (**B**) Chocolate: **1**. Receipt and depulping **2**. Fermentation **3**. Drying (greenhouse) **4**. Raw material storage house **5**. Packaging material storage **6**. Packaging **7**. Baler **8**. Storage tank **9**. Rectification **10**. Elevator **11**. Mill **12**. Toaster **13**. Dehuller **14**. Fuel and maintenance material storage **15**. Cocoa dry bean storage **16**. Waste.

Other novel non-thermal techniques such as ozone decontamination or irradiation may have the potential to reduce mycotoxin levels in tropical juices [40,41]. As noted before, the availability of these technologies may be limited as tropical beverages are typically produced in countries with developing

economies. Finally, few articles have described the filamentous fungal contamination and its behavior from the raw material or ingredients to the finalized product. Some production lines have been investigated in their entirety including cocoa [42], tomato [43], beer [44], and dairy products [45,46]. Contaminants found in dairy products are relevant to the beverage industry not only because of milk consumption (in all its presentations, Latin America and the Caribbean dairy consumption is estimated at 7.84×10^1 kg hd^{-1} day^{-1}) [47,48], but because of the use of whey as an ingredient for novel functionalized ready-to-drink beverages that incorporate tropical ingredients [49–51]. These types of beverages have been developed worldwide, e.g., lemon and strawberry drinks in India and Brazil, respectively [52,53].

2. Common Drinks Produced in the Tropics Subjected to Mycotoxin Contamination

2.1. Tea (Including Mate)

Tea is among the most common drinks, and it is an essential product in the global market [54,55]. Tea consumption is a growing trend within the middle class and the urbanized population of many emerging and developing markets [56,57]. Nowadays, commercial presentations range from green to white to fruit-based infused teas. Herbs from which infused drinks are prepared enjoy popularity, as they are considered to function as both medicine and for food purposes [58].

2.1.1. Mycotoxin Contamination and Fungal Charge Found in Tea

In recent years, medicinal plants have gained interest as they may serve as a possible source of mycotoxin contamination [59]. AFB_1, AFB_2, AFG_1, AFG_2, OTA, ZEA, FB_1, CIT, DON, nivalenol (NIV), neosolaniol (NEO), fusarenone-X, and T-2 toxin have all been detected in medicinal herbs, including Latin American products [55]. Medicinal plants and herbs are noteworthy in this section, since they are used as materials for infused drinks [60]. A Chinese research group analyzed tea and found negligible risk regarding FB_1 and T-2 toxin, but a considerable number of samples did surpass established thresholds for AFB_1 and DON (Table 2) [61].

Processing-wise, tea is classified into six categories: green (unfermented), white (slightly fermented), yellow (partly fermented), Oolong (semi-fermented), black (fully fermented) and dark tea (post-fermented) [62]. Then, in the case of tea, processing has a profound effect on the initial fungal communities that can colonize herbs, and it will determine those strains that may persist during and after fermentation. At least one research group has focused on describing the changes in fungal communities during processing of Fu Brick, another post-fermented tea [63,64]. In one case, *Aspergillus* dominated the manufacturing process for this product, increasing its presence from 65.13 to 93.58% [63]. More recently, *Cyberlindnera* was found to also be a significant protagonist during processing [64]. Other Asian fermented (pickled) teas, produced from the leaves of *Camellia sinensis* var. *assamica* Pierre, and processed by anaerobic fermentation, include Miang (Thailand) [65], Laphet (Myanmar) [66], and Goichi-cha (Japan) [67]. Pu-Erh tea is another exciting example as it is also considered a "post-fermented" tea [54]. Leaves are fermented using mainly *Aspergillus niger* [54]. Hass and coworkers found no AF or FB contamination in the Pu-Erh tea samples, while fungal counts ranged from 1.0×10^1 to 2.6×10^6 CFU g^{-1} of tea. The most prevalent fungi included *Aspergillus*, *Penicillium*, and Mucorales [54]. On the other hand, Matei and coworkers found a high fungal charge in four types of tea: rosehip, sweet basil, black and nettle teas (ranging from 7.82×10^3 to 1.92×10^5 CFU g^{-1}), surpassing the 1.00×10^2 CFU g^{-1} legislative threshold; some of the specimens were identified as *Fusarium*. Considerable levels of AF contamination were found ($n = 7 > 10$ and $n = 3 > 1.00 \times 10^2$ μg kg^{-1}) and a St. John's wort tea sample greatly exceeded the limits imposed by European legislation [60]. In contrast, Siddique and coworkers have found low fungal counts (4×10^2 to 2×10^3 CFU g^{-1}) in several herbal samples and the authors associated this small microbial charge with the negligible concentrations of negligi found in some of the samples (i.e., $<9.00 \times 10^{-2}$ μg kg^{-1}) [68].

Research based in Costa Rica examined the prevalence of OTA and AFs in several varieties of supermarket-available chamomile and mint teas. Relatively low contamination was found in the examined herbs. The analysis determined residues of OTA produced by *Fusarium* and *Penicillium*. OTA was present in greater quantities in the mint tea compared to the chamomile tea. No relationship between fungal counts and OTA contamination was found [69]. In yet another example, Kong and coworkers found higher ZEA concentrations in Coix seeds used to prepare a very popular tea in Korea [70]. Finally, a Spanish study examined herbal samples and found that sage leaves, chamomile flower, valerian root, senna leaves, and rhubarb were among the most mycotoxin-contaminated herbs. Co-contamination with OTA, FBs, AFs, ZEA, T-2, DON, and CIT was also observed [71].

No clear regulation for mycotoxins in herbal products (or drinks derivate thereof, i.e., tea or infusions) has been established. However, the maximum concentrations in similar products tend to be low; the European Community Regulation EC 1881/2006 stipulates that the maximum limits for spices are 5.0×10^0 and 1.0×10^1 μg kg^{-1} for AFB_1 and total AFs (i.e., the sum of the four different aflatoxin fractions), respectively. Other safety limits have been set to 4.00×10^3, 1.00×10^3, 1.00×10^2 μg kg^{-1} for FB, DON, and T-2, respectively.

2.1.2. Mycotoxin Transference Rate from Herbs to Infusions

There is evidence that between 4.1 and 34.8% of OTA could be transferred to an already-prepared infusion [72]. Analogously, AF transfer from the herbs to the infusion has also been investigated. Considerable rates were found in five types of herbal medicines. Remarkably, despite being structurally related, a differential transference was found and calculated to be 7.26–115.36, 4.37–26.37 and 9.64–47.68% for AFG_2, AFB_1, and AFB_2, respectively [73].

2.1.3. Yerba Mate

Few papers have focused on yerba mate infusion, a favorite drink from South America. However, there is evidence of the presence of some *Aspergillus* sections, including *Flavi*, *Nigri*, *Circumdati*, and *Fumigati* [74]. In another study, 2072 aspergilli (78.9% corresponding to section *Nigri*; 40% *Aspergillus japonicus* var. *japonicus*, 28% *A. japonicus* var. *aculeatus*, 16% *A. niger* var. *niger*, 12% *Aspergillus foetidus*, 2% *Aspergillus carbonarius*, 2% *A niger* var. *awamori*) strains were isolated from 41 samples of yerba mate. In vitro production of OTA was observed in 1% of the strains. Additionally, they demonstrated not only fungal presence and growth, but also fungal tolerance to pH variations and survival even after treatment with elevated temperatures (i.e., boiling water) [75].

2.1.4. The Relationship between Mycotoxins and Bioactive Compounds Found in Tea

Mycotoxin accumulation has been associated negatively with bioactive components (i.e., 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol), thus affecting the spice quality [76]. Dry ginger has been described as an adequate growth substrate for *A. niger*, *A. flavus*, *P. citrinum*, *P. verrucosum*, and *Fusarium moniliforme* [77]. Interestingly, epigallocatechin (a nutraceutical with antioxidant capacity widespread in plants, found in almond and present in relatively considerable quantities in green and black tea) has demonstrated a cytoprotective role against DON-induced damage in HT-29 cells (a human colon cancer cell line) [78]. A similar effect was observed in cytotoxicity induced by trichothecenes (TRC) [79].

2.2. Nutmilk and Similar Beverages

Several beverages are based on nuts and seeds; these ingredients may very well transfer mycotoxins to the final product; this is the case of the English-French origin almond-based orgeat syrup, which is used as a drink mixer. Nutmilk or "Horchata" is the name given to a series of aromatic traditional beverages based on several cereals, seeds or nuts, and spices. Diverse ingredients are used to prepare this type of beverage including tubers from *Cyperus esculentus* L. (tiger nut, ordinarily from West Africa, popular in Spain). Other variants include sesame seed (scented with vanilla and

cinnamon, popular in Puerto Rico and Venezuela), almond, rice (spiced with cinnamon or Súchil flower [*Plumeria rubra* L.], popular in Mexico), coconut and cantaloupe seeds (*Cucumis melo* L.). In several Central American countries, the drink is prepared using the dried fruit from *Crescentia cujete* L. Other ingredients may include ground cocoa, cinnamon, sesame seeds, nutmeg, tiger nuts, vanilla, ground peanuts, almonds, and cashews. On the other hand, an herbal infusion drink prepared in Ecuador called "Horchata lojana" is prepared with *Aerva sanguinolenta* (L.) Blume, *Aloysia triphylla* (L'Hér.) Britton, *Aloysia citriodora* (Lam.) Ortega ex Pers., *Melissa officinalis* L., mint, chamomile, and rose essence, among other things. Usually, this type of beverage is only found in artisanal food expenditures. Notwithstanding, horchata has become more popular, being subjected to commercialization, and it has grown relevant in several regions.

These ingredients and, thus, the beverages derived from them, are susceptible to mycotoxin contamination [80]. However, only a few papers have focused on the assessment of these products. For example, tiger nut-based soft drinks in Spanish and Belgian markets were assayed for AFB_1, finding $n = 1$ samples above the limit of detection of the method used by the researchers [81]. Similarly, Sebastiá and coworkers analyzed AFs in tiger nuts and tiger nut beverages. They examined the four different fractions of AFs, but total aflatoxin concentrations found ranged from 8.2 to 9.5 μg kg^{-1} and 4.6 to 6.4 μg kg^{-1} for tiger nuts and their beverages, respectively [82]. Peanuts, another primary ingredient in this type of nut-based drinks (and the popular among Andean people "chicha de maní" [83]), are continuously linked to fungal and toxin contamination [16]. Locally, we have demonstrated relatively high contamination for this crop (i.e., 21.8% prevalence) [84]. In regard to almonds, different authors have reported fungal contamination of this crop with *Aspergillus*, *Penicillium*, *Rhizopus*, *Alternaria* and *Fusarium* and, hence, its subsequent mycotoxin production, especially AF contamination [85]. Also, mycotoxigenic fungi—*A. flavus*, *A. niger*, and *A. ochraceus*—have been isolated from cinnamon [86,87]. Lamboni and coworkers isolated 150 strains of Aspergilli from cashews and assayed their mycotoxin production. Three mycotoxin groups were detected FBs, OTA and secalonic acids, indicating that these mycotoxins could occur in raw cashew nuts [88].

Table 2. Mycotoxin contamination found in herbal samples, nuts and nut-based beverages.

Matrix	Country	Analyzed Toxin	Positive Samples	Minimum, μg kg^{-1}	Maximum, μg kg^{-1}	Analysis Method	Reference
Mycotoxin Analysis in Tea							
Pu-Erh tea	China	OTA	$n = 4/36$	6.50×10^{-1}	9.47×10^{1}	HPLC-FLD	[54]
Medicinal plants	Rumania	AFs	$n = 7/10$	1.30×10^{1}	3.80×10^{2}	ELISA	[60]
		FBs	$n = 1/10$	4.60×10^{1}	2.19×10^{4}		
Pu-Erh tea	China	AFB_1	$n = 8/70$	2.10×10^{-2}	8.52×10^{0}	ELISA and HPLC	[61]
		DON	$n = 63/70$	3.57×10^{2}	3.11×10^{3}		
Medicinal plants	India	AFB_1	$n = 1/3$	4.00×10^{-2}	1.68×10^{0}	HPLC-MS/MS	[68]
		AFB_2	$n = 1/3$	5.00×10^{-2}	1.34×10^{0}		
Chamomile	Costa Rica	OTA	$n = 13/17$	1.40×10^{-1}	4.10×10^{-1}	HPLC-FLD	[69]
Mint			$n = 5/5$	3.20×10^{-1}	5.30×10^{-1}		
Job's tears	China	ZEA	$n = 5/8$	6.89×10^{1}	2.96×10^{2}	HPLC-FLD-MS/MS	[70]
Herbs	Spain	OTA	$n = 19/88$	8.00×10^{-1}	1.06×10^{1}	ELISA	[71]
		FBs	$n = 3/88$	1.40×10^{2}	2.37×10^{2}		
		AFs	$n = 30/88$	2.60×10^{0}	8.53×10^{2}		
		ZEA	$n = 29/88$	1.50×10^{0}	4.41×10^{1}		
		T-2	$n = 29/88$	6.00×10^{-1}	2.57×10^{2}		
		DON	$n = 22/88$	3.60×10^{1}	3.43×10^{2}		
		CIT	$n = 19/88$	1.49×10^{1}	3.55×10^{2}		
Mycotoxin Analysis Nut Milk and Related Beverages							
Soft drinks	Spain and Belgium	AFB_1	$n = 1/22$	2.00×10^{-2}	6.00×10^{-2}	HPLC-FLD	[81]
Tiger nuts	Spain	AFB_1	$n = 3/37$	7.00×10^{-1}	4.50×10^{0}	HPLC-FLD	[82]
Tiger nut beverages		AFB_1	$n = 3/25$	1.20×10^{0}	3.10×10^{0}		
Peanuts	Costa Rica	AFs	$n = 125/572$	4.80×10^{-1}	4.00×10^{2}	Fluorimetry	[84]
Almonds			$n = 3/65$	4.80×10^{-1}	8.90×10^{0}		
Almonds	Portugal	AFB_1	$n = 1/21$	4.60×10^{-1}	4.97×10^{0}	HPLC-FLD	[85]

Finally, soy and almond milk are also widely consumed, particularly in vegetarian diets. Even though Latin-American countries (e.g., Brazil, Argentina) are significant exporters of soybean [89], little research has been focused on the resulting concentration of toxins associated with this product. However, considering the nature of the starting material involved, there is a distinct probability of soy and almond milk being a route of exposure for consumers. For example, toxicologically relevant concentrations of OTA were found in every step of soy milk and bean curd processing [90]; however, these toxin levels were decreased compared to the original concentrations found in the soybean [90].

2.3. Fermented Beverages

Fermentation of milk, cereals and other substrates is used to produce traditional drinks, especially in regions such as Asia, Africa, Europe, the Middle East and South America [91]. Praised for their health-promoting benefits and functional properties, these drinks have gained popularity, and nowadays biotechnological advances have made it possible to develop them commercially [91]. However, these types of beverages are subject to contamination with mycotoxins, having a deleterious effect on any of the favorable properties mentioned above.

Ezekiel and coworkers gave special attention to traditional African fermented beverages as a source of mycotoxin contamination [18]. A recent example is "Namibian oshikundu", a traditional drink based on sorghum and pearl millet. Misihairabgwi and coworkers found AFs, cyclopiazonic acid, 3-nitropropionic acid, helvolic acid, gliotoxin, fumiquinazolines, cytochalasin E, PAT, FBs, curvularin, alternariol (AOH), dihydroergosine, and tryptoquivalines. Unusually high levels of 2.28×10^3 and 1.19×10^4 μg kg^{-1} for cyclopiazonic acid and 3-nitropropionic acid were found in sorghum malts, respectively. As with other beverages, fermented beverages such as oshikundu may harbor essential levels of mycotoxin contamination highly related to the quality of the raw ingredients [92].

South American fermented beverages based on cereals and vegetables such as "chichi" (based on corn, grains and fruit including corn beer or "chicha de jora" and the non-alcoholic chicha morada), "caxiri", and "cauim" or "manioc" beer (both based on *Manihot esculenta* L.), and "champús" (common in Peru, Ecuador and Colombia, mostly based on corn, honey, caramel, whole sugar cane, orange leaves, cloves, pineapple, "naranjilla" or "lulo" *Solanum quitoense* Lam. and cinnamon) can be cited [83,93]. Previously, toxigenic fungi have been described in corn and corn-based foods, feeds and vine fruits in South American countries [94,95]. However, fermented beverages based on lactic acid bacteria metabolism may suffer less from mycotoxin contamination as fungal colonization is considerably more difficult, since these bacteria can produce antimicrobial substances during the malting process [93,96]. Degradation of AFB_1 has already been described during alcoholic beverage fermentation [97]. In yet another example, fermented apple juice (produced from fruit previously inoculated with *Penicillium exapansum*) using *Saccharomyces cerevisiae* exhibited lower PAT concentrations when compared to the unfermented and the pasteurized juices [98].

As mentioned previously, whey, along with extracts from fruits and vegetables, have found widespread use in the manufacture of fermented beverages. Incorporation of these drinks into human diets can be beneficial, as they have good nutritional properties [99]. Fermented beverage manufacturing is practical for adding value to milk whey, a traditionally discarded by-product [99]. These types of beverages usually enjoy a high degree of acceptance due to their low acidity level, creamy consistency, and high commercial value at a relatively low cost [99]. Several tropical and subtropical fruit pulps are used for the production of fermented dairy beverages in Latin America, e.g., guava, soursop (*Annona muricata* L.), mango, umbu (*Spondias tuberosa* Arruda), strawberry and peach [99]. More examples of by-product repurposing include beverages prepared with passion fruit [100], i.e., fermented milk, and drinkable yogurts using peel [101–103], and peel and seed flour [104]. Later on, it will be evident that fruits can be a source of mycotoxin contamination which may very well reach final products [6]. Interestingly, allergic sensitization to whey protein has been described after exposure to DON [105].

2.4. Coffee

Coffee is the second-most consumed beverage worldwide and is prepared by infusion of roasted coffee (mainly *Coffea robusta* L. Linden, *Coffea arabica* L. and *Coffea liberica* W. Bull ex Hiern) seeds. During its cultivation and processing, different fungi can contaminate the beans and berries [15]. Among these fungi, *Aspergillus ochraceus*, *A. carbonarius*, *A. niger*, and *Penicillium verrucosum* have been described as OTA producers [15,106–108]. OTA has been associated with the Balkan Endemic Nephropathy, a chronic kidney disease, and the development of urinary tract tumors [5]; therefore, it has been classified as a possible carcinogenic compound (group 2B) for humans by the International Agency for Research on Cancer (IARC) [109]. Thus, due to its nephrotoxic, hepatotoxic, teratogenic and immunosuppressive effects [109], this mycotoxin contamination of coffee's raw materials and finished products has been extensively studied (Table 3).

Table 3. The occurrence of OTA in green, roasted and soluble (instant) coffee samples.

Country	Positive Samples	OTA Concentration (μg kg^{-1})		Analysis Method	Reference
		Mean	Max		
Decaffeinated coffee					
Spain	36/40 [e]	4.4×10^{0}	32.40	LC-MS/MS	[2]
Green coffee beans					
Denmark	7/18	1.7×10^{0}	2.8×10^{0}	UHPLC-MS/MS	[110]
Panamá	4/21 [a]	2.6×10^{1}	3.8×10^{1}	ELISA	[111]
Roasted coffee					
Spain	61/169	1.9×10^{0}	4.7×10^{0}	LC-MS/MS-QqQ-IT	[1]
Spain	7/52 [b]	4.1×10^{0}	1.1×10^{1}	LC-MS/MS	[2]
Chile	18/24	4.7×10^{-1}	8.5×10^{-1}	HPLC/fluorescence detector (FLD)	[15]
Costa Rica	54/57	3.5×10^{-1}	9.6×10^{-1}	ELISA (RIDASCREEN® Ochratoxin A)	[106]
Denmark	26/57 [c]	2.3×10^{0}	2.1×10^{1}	UHPLC-MS/MS	[110]
Brazil	23/34 [d]	9.0×10^{-1}	6.5×10^{0}	HPLC/FLD	[111]
Soluble coffee					
Italy	42/44	1.3×10^{0}	6.4×10^{0}	HPLC/FLD	[5]
Chile	37/39	1.8×10^{0}	7.3×10^{0}	HPLC/FLD	[15]
Denmark	14/25	4.5×10^{0}	8.3×10^{0}	UHPLC-MS/MS	[110]
Brazil	14/14	2.2×10^{0}	5.1×10^{0}	HPLC/FLD	[112]
Portugal	9/10 [f]	2.8×10^{0}	1.2×10^{1}	HPLC/FLD	[113]

[a] 3, [b] 2, [c] 5, [d] 5, [e] 3, [f] 1 samples exceeding the EU legislation (5 μg kg^{-1} for roasted coffee and 10 μg kg^{-1} for soluble coffee).

Santini and coworkers analyzed the effect of preparation methods on the concentration of OTA. They spiked samples of roasted Arabica coffee with 2.00 and 4.00 $\times$ 10^{0} μg OTA kg^{-1} and determined the OTA concentration of five coffee beverages: American, Moka, Neapolitan, Italian espresso and Turkish coffee. The coffee brewing process that resulted in the lowest amount of OTA was the Neapolitan coffee, with 46.5 and 40.7%, while the American coffee preparation retrieved the most significant amount of the toxin, with 81.0 and 70.0% at 2.00 and 4.00 $\times$ 10^{0} μg OTA kg^{-1}, respectively. Differences may be related to a longer contact time between the coffee powder and the hot water during American coffee preparation [107].

The European Community has established maximum regulatory thresholds for OTA in coffee at 5.00 $\times$ 10^{0} and 1.00 $\times$ 10^{1} μg kg^{-1} for roasted and soluble coffee, respectively (Regulation EC 1881/2006). Furthermore, in 2007, the Joint Expert Committee on Food Additives (JECFA) set a Provisional Tolerable Weekly Intake (PTWI) of 1.00 $\times$ 10^{-1} μg kg^{-1} bw week^{-1} [114], whereas the European Food Safety Authority (EFSA) Scientific Panel on Contaminants in the Food Chain has established a PTWI of 1.20 $\times$ 10^{-1} μg kg^{-1} bw week^{-1} [115]. On the other hand, OTA is not the only mycotoxin present in coffee. García-Moraleja and coworkers developed a method for the simultaneous determination of 21 mycotoxins (OTA, AFB_1, AFB_2, AFG_1, AFG_2, sterigmatocystin,

NIV, DON, 3-acetyldeoxynivalenol (3-aDON), 15-acetyldeoxynivalenol (15-aDON), diacetoxyscirpenol (DAS), NEO, T-2 toxin, HT-2 toxin, FB_1, FB_2, enniatin (EN) A, ENA_1, ENB, ENB_1 and beauvericin (BEA) in coffee beverages by liquid chromatography-tandem mass spectrometry. They analyzed six coffee samples (three of ground coffee and three of instant coffee), of which five were contaminated with mycotoxins, with the co-occurrence of at least six mycotoxins. OTA was present in two samples: 1.8×10^0 μg kg^{-1} (ground decaffeinated coffee) and 4.90×10^0 μg kg^{-1} (instant coffee with sugar and milk added). Only five (15-aDON, DAS, AFG_2, ENA, and FB_2) out of the 21 mycotoxins analyzed were not detected in any sample [116]. In another study, AFs were present in 53% (n = 90/169) of the analyzed coffee samples; however, there are no regulations for AFs in this product. It is worth noting that no sample exceeded 2.00×10^0 μg AFB_1 kg^{-1}, but 15% had a concentration of total AFs above 5.00×10^0 μg kg^{-1} due to AFG_2 contamination. The mycotoxins that presented the highest levels were ENA and ENA_1 [1].

2.5. Chocolate Beverage

Cocoa is the raw material from which chocolate is manufactured [42]. Cocoa trees (*Theobroma cacao* L.) grow best in humid, tropical climates; therefore, cocoa beans are mainly produced in West Africa (68.4% of world production), Asia and Oceania (account for 17.6%) and Central and South America (14.0%) [42,117,118]. Different microorganisms contaminate the cocoa beans from the outer surfaces of pods, workers' hands and tools, plant leaves, collection baskets, insects, and remaining mucilage in equipment [42]. Mishandled or improperly dried fermented beans are susceptible to filamentous fungi contamination [117]. Among the genera reported are: *Aspergillus* [42,117,119], *Fusarium*, *Geotrichum* [42], *Mucor*, *Penicillium* [42,114], *Rhizopus* and *Trichoderma* [42]. However, fungi not only spoil the cocoa beans, they are also capable of producing mycotoxins (e.g., OTA and AFs) which are thermally tolerant and can withstand the different stages of chocolate production [119,120]. For example, Copetti and coworkers isolated *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* from cocoa beans. These are fungi capable of producing AFs [121]. Regarding mycotoxin presence in cocoa and cocoa-derived goods, the majority of the studies have dealt with OTA and AF contamination (Table 4). It is worth noting that larger amounts of OTA have been detected in the early crop periods, related to the most humid months with the mildest temperatures [122].

Table 4. Mycotoxin contamination found in cocoa and cocoa-derived goods.

Country	Analyzed Toxin	Positive Samples	Mycotoxin Concentration (μg/kg)		Analysis Methods	References
			Mean	Max		
Cocoa beans						
Brazil	OTA	38/54	$1{,}0 \times 10^0$	>2	HPLC/FLD	[122]
Brazil	AFB_1	11/85 [a]	1.1×10^{-1}	6.7×10^0	HPLC/FLD	[121]
Cocoa powder						
Italy	OTA	40/40	5.1×10^{-1}	1.82×10^0	HPLC	[118]
Brazil	OTA	25/25	3.9×10^{-1}	9.2×10^{-1}	HPLC/FLD	[120]
Canada	OTA	n = 15	1.2×10^0	4.7×10^0	HPLC/FLD	[119]
Brazil	AF	24/25	5.3×10^{-1}	1.7×10^0	HPLC/FLD	[120]
Canada	AF	n = 15	1.2×10^0	3.52×10^0	HPLC/FLD	[119]
Chocolate						
Italy	OTA	139/260	1.4×10^{-1}	7.4×10^{-1}	HPLC	[118]
Brazil	OTA	98/100	2.0×10^{-1}	8.7×10^{-1}	HPLC/FLD	[120]
Canada	OTA	n = 30	2.9×10^{-1}	6.5×10^{-1}	HPLC/FLD	[119]
Brazil	AF	73/100	3.0×10^{-1}	9.1×10^{-1}	HPLC/FLD	[120]
Canada	AF	n = 30	1.9×10^{-1}	9.1×10^{-1}	HPLC/FLD	[119]
Baking chocolate						
Canada	OTA	n = 9	4.9×10^{-1}	9.1×10^{-1}	HPLC/FLD	[119]
Canada	AF	n = 9	2.7×10^{-1}	6.7×10^{-1}	HPLC/FLD	[119]

Table 4. *Cont.*

Country	Analyzed Toxin	Positive Samples	Mycotoxin Concentration (μg/kg)		Analysis Methods	References
			Mean	Max		
			Cocoa liquor			
Canada	OTA	$n = 5$	4.3×10^{-1}	5.6×10^{-1}	HPLC/FLD	[119]
Canada	AF	$n = 5$	5.1×10^{-1}	7.6×10^{-1}	HPLC/FLD	[119]

[a] Sun drying cocoa beans on platforms.

OTA regulation in cocoa and cocoa products has been set according to the Working Document of the Expert Committee on "Agricultural Contaminants" of the European Commission Scientific Committee on Food. Limits have been set as follows: 2×10^{0} μg kg^{-1} for raw material (e.g., cocoa beans, peeled beans, cocoa cake, nibs, and cocoa powder) and 1×10^{0} μg kg^{-1} for consumer's goods (chocolate powder, chocolate, and chocolate beverages) [122]. Finally, exposure to OTA through chocolate ingestion was determined by Brera and coworkers in an Italian population. They concluded that the teenage male group was the highest consumer of cocoa powder (1.48×10^{1} g per day), and that the highest OTA weekly intake belonged to the infant and children groups, with 2.42×10^{-3} and 1.71×10^{-3} μg kg^{-1} bw $week^{-1}$, respectively [118].

2.6. Fruits and Fruit Drinks

Generally speaking, fruit juices are among the most consumed beverages in the world. The popularity of fruit juices is mostly related to their levels of antioxidants, vitamins, and minerals [7]. The nutritious content of fruit juices is also implicated in health benefits such as prevention of cardiovascular disease, diabetes, and cancer [7]. However, the high content of nutrients in juices may represent a disadvantage regarding microbial spoilage, mostly from yeasts and molds. Fruits used as raw material for the production of beverages are also susceptible to fungal decay; the high sugar content and low pH typically associated with fruits (2.5–5.0) represents an environmental advantage for fungal growth [35]. As is the case for other tropical food products mentioned in this review, fungal growth in fruits may be accompanied by the accumulation of mycotoxins that can be transferred to derived products throughout the entire processing chain. This phenomenon may be important in those cases where poor-quality fruits are used for further processing [35]. Prevalence of mycotoxins such as PAT in apple juice, apple juice-based products, grape juice, and wine has been widely discussed in the literature [123]. Also, there is an extensive body of research describing the fungal growth and mycotoxin accumulation in the apples and grapes themselves [123]. On the other hand, scientific information about the presence of mycotoxins in juices derived from tropical fruits is scarce, and most of the reports are related to the presence of mycotoxigenic fungi in the raw products. Presence of pathogenic fungi and their metabolites in the raw product may give an idea of the potential for fruit juices to function as a source of contamination for consumers.

2.6.1. Mycotoxin in Pineapple and Pineapple Juice

Pineapple (*Ananas comosus* (L.) Merr.) is a tropical plant grown in warm and humid environments. Like many other tropical crops, pineapple is highly susceptible to fungal diseases, with fusariosis being the most severe [124]. The growth of different *Fusarium* species may be accompanied with varying levels of mycotoxin accumulation in the plant even in the case of asymptomatic infection [125]. Major secondary metabolites produced by *Fusarium* in pineapple are moniliformin (MON), BEA, FBs, fusaproliferin, FC and FA [124], regarded as mycotoxins with a high risk potential for animals and humans.

Stepien and coworkers [124] studied the diversity of *Fusarium* species present in samples of pineapple from different tropical countries. In their study, the authors identified ten different *Fusarium* species contaminating pineapple; most of the isolates originated from Costa Rica and Ecuador, both significant producers in the international market. *Fusarium proliferatum* was one of the most

common contaminants, and the species with the highest capacity to produce mycotoxins according to some in vitro experiments. In another study, Gorna and coworkers [125] studied the ability of some *F. proliferatum* isolates that originated from different crops (including pineapple) to produce fumonisin after exposure to host plant extracts. These authors demonstrated that pineapple extract was one of the most important promoters of fumonisin accumulation in culture media, and this correlated with a higher induction of genes involved in mycotoxin synthesis. Levels of FBs were as high as 3.97×10^5 μg kg^{-1} in culture media. Pineapple extract also induced fungal biomass growth of some of the tested strains. Mycotoxin contamination of pineapple in the field may increase the risk of disease from juice consumption. In their study, Stepien and coworkers [124] also observed contamination of pineapple juice samples with FBs and MON. Contamination as high as 2.37×10^4 μg kg^{-1} of FBs was found in some of the samples. On the other hand, some mycotoxins may be produced in the pineapple juice itself as some products could be contaminated with ascospores of some mycotoxigenic fungi (*Aspergillus*, *Penicillium*, *Fusarium*, *Byssochlamys*, and *Neosartorya*) [126]. Fungal ascospores can be resistant to physical and chemical treatment used during processing of fruit juices; these ascospores may germinate and spoil the final product while accumulating significant levels of dangerous mycotoxins. In an earlier study, Rice and coworkers [127] proved that *Byssochalmys fulva* could grow and produce PAT in pineapple juice, even though contamination levels (1.42×10^4 μg kg^{-1}) were lower compared with other juice matrices like blueberry and red raspberry. More recently, Zimmerman and coworkers [128] proved that ascospores of *Byssochlamys nivea* could germinate and grow in pineapple juice at water activity values as low as 0.90. *B. nivea* is a type of fungus with the capacity to produce PAT in pineapple juice while growing. As shown by Bevilacqua and coworkers [129], the survivability in pineapple juice of ascospores of mycotoxigenic species such as *F. oxysporum* can be reduced by the combination of processing treatments like sodium benzoate, citrus extracts, and High-Pressure Processing. Reports about the natural occurrence of mycotoxins in pineapple juice are scarce. In Greece, Mourkas and coworkers [130] reported that a sample of concentrated pineapple juice of unknown origin had low levels of PAT (7.70×10^0 μg kg^{-1}) after analysis with HPLC. Lee and coworkers [131] applied a new methodology to determine the levels of mycotoxins in different types of fruit juices. All the pineapple juice samples analyzed by Lee and coworkers were positive for 5-hydroxymethylfurfural with contamination levels up to 3.40×10^2 μg kg^{-1}; just 1 out of 6 samples was positive for PAT (3.31×10^1 μg kg^{-1}). Other studies have confirmed the absence of PAT [35,132] and OTA [133].

2.6.2. Citrus Fruit Juices

Citrus fruits (e.g., oranges, lemons, tangerines) are obtained from plants that grow in tropical and subtropical climates. As citrus fruits are grown in environments with high temperature and increased humidity, contamination with mycotoxigenic molds is not uncommon. Also, as they usually have pH values lower than 4, it is expected that most fungi will attack these fruits [7]. Literature reports have established the capacity of mycotoxigenic molds to proliferate and produce mycotoxins in artificially inoculated citrus fruits. In general, oranges seem to support higher levels of mold growth compared with other types of citrus fruits such as lemons, limes, and tangerines. Even though *Penicillium digitatum* and *Penicillium italicum* are the most severe infectious pathogens in oranges, other mold species can be observed as well [134,135]. Tournas and Katsoudas [136] demonstrated that several types of mycotoxigenic molds, including *Alternaria*, *Cladosporium*, *Penicillium*, and *Fusarium*, were able to proliferate in oranges, limes, lemons, and tangerines; higher growth was observed in oranges, with *Alternaria* being the most common type. In an early study, Alderman and Marth [137] studied the capacity of *Aspergillus parasiticus* to grow and produce aflatoxins in grapefruit, lemons, limes, and oranges; in this study, it was shown that the mold grew under conditions of low relative humidity (13–20%), and that it was able to produce aflatoxins up to 2.20×10^2 μg kg^{-1}. Differences were observed with regard to the type of fruit, with lower growth on limes compared with the other products.

Similarly, Stinson and coworkers [138] demonstrated that different *Alternaria* isolates were able to grow and produce mycotoxins in artificially inoculated whole oranges and lemons; mycotoxins produced included AOH, alternariol monomethyl ether (AME), and TEA. In oranges, some *Alternaria* isolates were able to synthesize up to 6.1×10^4 μg kg^{-1} of TEA and up to 4.1×10^4 μg kg^{-1} of AOH; mycotoxin production in lemons was significantly lower. *Alternaria* toxins have been documented as naturally occurring in tangerine [139]. Mold growth is also dependent on the substrate; in the case of grapefruits, mold growth is higher on the peel than in juices, and this may be associated with the concentration of nitrogenous compounds available for fungal metabolism [35].

Some studies have demonstrated the natural presence of mycotoxigenic molds and mycotoxins in citrus fruits and derived juices. Several species of mycotoxigenic molds such as *A. flavus*, *A. niger*, *F. oxysporium*, *Fusarium moniliforme*, and *Penicillium citrinum* can be isolated from fresh and rotten oranges [140]. As a consequence, natural presence of mycotoxins in citrus fruits is expected, as contamination with AOH, AME and TEA has been reported in oranges, lemons and tangerines [6,7]. AFB_1 at levels up to 5.20×10^1 μg kg^{-1} has also been observed in oranges [35]. Presence of AFs and other mycotoxins in citrus fruits is crucial, as they can be carried over to derived products such as purees and juices. Higher levels of contamination can be observed in the peel and pulp, but concentrations as high as 13% could be transferred to the juices [141]. Fungal contamination may also be transferred from the fruits to the juices and mycotoxins may be produced within the final products themselves. The study of Alderman and Marth [137] also demonstrated that, in grapefruit juice, some strains of *A. flavus* and *Aspergillus parasiticus* could produce up to 1.20×10^3 μg kg^{-1} of AFB_1 and 1.65×10^3 μg kg^{-1} of AFB_2, respectively. In orange juice, *A. parasiticus* was able to produce 5.5×10^3 μg kg^{-1} of AFB_1 after 14 days of incubation at 28 °C. Regardless of the type of juice, the levels of mycotoxins started to decline after prolonged storage.

Similarly, Varma and Verma [142] showed that some toxigenic *A. flavus* isolates could produce up to 6.5×10^3 μg kg^{-1} of AFB_1 in orange juice after 10 days of incubation at room temperature; these authors also observed a reduction in mycotoxins levels after some time. More recently, Marino and coworkers [143] demonstrated that another *Aspergillus* species, *A. westerdijkiae*, was able to grow in oranges and orange juice and produce OTA. After 20 days of incubation at 26 °C, OTA levels in the juice were above 2 μg kg^{-1} and decreased with longer incubation. Natural occurrence of mycotoxins in citrus fruits juices has been reported previously in different parts of the world. For example, in Greece, Mourkas and coworkers [130] reported PAT contamination of three orange juice samples sold in the local market, with an average amount of 6.80×10^0 μg kg^{-1} of the mycotoxin.

Similarly, Cho and coworkers [144] determined the levels of PAT contamination of various fruit juices sold in South Korea, including orange juice. Even though low levels of prevalence were reported (2 out of 24 positive samples), mycotoxin was quantified at concentrations up to 3.09×10^1 μg kg^{-1}, which surpasses the maximum daily recommendation for infants and young children, according to European and Korean authorities. In Argentina, Broggi and coworkers [145] reported positive orange and grapefruit juice samples for *Alternaria* mycotoxins even though the specific levels of contamination were not established. In China, Zhao and coworkers [146] reported TEA and AOH in 11.1% and 25% of citrus juices (type not specified). Levels of AOH ranged from 1.21×10^0 to 4.30×10^0 μg kg^{-1}, whereas the TEA was between 0.11 and 2.00×10^{-1} μg kg^{-1}. Regarding contamination with OTA, no contamination has been reported in the case of orange juices [147,148].

Mycotoxins in the final juice may present different levels of stability to common processing techniques. It was observed that pasteurization of orange juice can decrease the levels of aflatoxin in orange juice up to 20% of the single toxin. AFB_1 seems to be the most heat resistant mycotoxin [141]. Other control strategies are focused on the growth of pathogenic fungi on oranges, and some of those approaches try to get the advantage of the natural defenses of the citrus plants. For example, some compounds, like the phytoalexins, can be used to inhibit the growth of *A. parasiticus* and *F. verticillioides*, as well as the amount of AFs and FBs produced by some isolates in oranges [149]. The process of curing (treatment at 40 °C and subsequent storage at 5 and 20 °C for some days) has also been shown

to effectively control the levels of infection of oranges with *Penicillium* molds. It is estimated that this process of curing enhances the natural defenses already present in oranges [134]. Green mold sporulation in oranges, mostly from *Penicillium* species, could be prevented with the use of novel non-thermal approaches like disinfection with ozone. However, the use of ozone seems not to be applicable for fruits packaged in plastic bags and cardboards [150]. Other authors suggest that the best approach is to reduce rates of contamination through in situ monitoring of fungal infections of fruits during storage; this type of monitoring may include direct observation of rotten fruits or earlier detection of fungal metabolites [135].

2.6.3. Tomato Juice

Like in the case of citrus fruits, tomato plant (*Lycopersicum esculentum*) can grow in tropical and subtropical climates. Typically, tomato needs high temperature, high relative humidity, and at least 8 h of luminosity to develop properly. Many tropical regions offer the ideal climatic conditions for growing tomatoes, but this may increase the risk of contamination with mycotoxigenic molds. As tomatoes have a softer epidermis compared to other fruits, the fungal infestation is common for this product [151]. The soft tissue of tomato also increases the rate of mycotoxin penetration during fungal infection compared with other consistent fruits such as apples and pears [35]. Mycotoxigenic fungi isolated from tomatoes include several species of *Penicillium*, *Alternaria*, *Aspergillus* and *Fusarium* [151,152]. Hasan determined that *Alternaria alternata* was the most common fungi isolated from black lesions of rotten tomato followed by *A. niger* [36]. The pathogenic effect of *Alternaria* on tomato plants is higher when compared with other cultivars [153]. As *Alternaria* is the most common mycotoxigenic mold affecting the tomato plant, it is expected that an important fraction of tomatoes and derived products (including juice) will be contaminated with mycotoxins from this genus. Van de Perre and coworkers [151] determined the prevalence of *Alternaria* mycotoxins in tomatoes from different countries (Belgium, Spain, Brazil, India); levels of contamination were as high as 30 and 18.5% for AOH and AME, respectively. Important levels of contamination with TEA were also observed. Interestingly, Van de Perre and coworkers [151] observed lower levels of contamination in tomatoes from tropical regions compared with product from moderate climates. This situation may be attributable to a higher capacity of *Alternaria* to grow at lower temperatures. Pose and coworkers [154] determined that a temperature of 21 °C was the optimal condition for the production of AOH and TEA; this temperature may better reflect a condition of regions with subtropical climates. AOH and AME were also detected in tomato concentrates and tomato puree samples, but no contamination was observed for tomato juices. However, in another study, Ioi [155] reported contamination of tomato juice samples in Canada with AOH and AME. A lower contamination rate was observed for AME (17%) compared to AOH (50%), and the contamination levels were 1.64×10^1 and 2.34×10^1 μg kg^{-1}, respectively. Lopez and coworkers [156] observed that TEA was the most common mycotoxin (60%) in tomato-derived products analyzed in the Netherlands. Fourteen tomato juice samples were analyzed in this study, with 50% of them being positive for TEA (mean level of 7.70×10^1 μg kg^{-1}) and 28% for AOH (mean level of 3.3 μg kg^{-1}). No AME was detected in tomato juice samples, even though it was reported from other types of tomato-derived products (sauces, pastes). On the other hand, a study by Zhao and coworkers [146] showed that 100% of tomato juice samples (9/9) in China were positive for AOH, whereas just 77.8% of samples had contamination with TEA; levels of contamination were between 7.40×10^0–2.78×10^2 and 2.00×10^{-1}–5.8×10^0 μg kg^{-1} for AOH and TEA, respectively. Higher levels of contamination with TEA are not surprising, as previous in vitro studies [36] demonstrated that some *A. alternata* isolates could produce between 3.50×10^1 and 6.00×10^4 μg kg^{-1} of this mycotoxin in solid media. On the other hand, the same isolates produced just a small amount of AOH in the same experiment (3–7.5×10^3 μg kg^{-1}). Levels of contamination with *Alternaria* toxins seems to be lower for products made with whole tomato pieces, meaning that for products such as juices, pastes, and sauces, lower-quality raw material may be being used. This tendency was observed by Lopez and coworkers and Zhao and coworkers [146,156]; this latter study found no contamination of *Alternaria* mycotoxins in fresh tomato,

whereas derived products (juice, ketchup) were positive for this mycotoxin. As other mycotoxigenic molds different than *Alternaria* could be found in tomato samples, there is a risk for the presence of more mycotoxins as well. In Egypt, Hegazy [152] observed no contamination of fresh tomato samples with AFs and OTA. Also, Barros Mariutti and Valente Soares [157] studied the presence of AFs in different tomato-based products including ketchup, pulp paste, and puree; no contamination with aflatoxin was found, and even though no tomato juice samples were analyzed, these results may reflect a low risk of contamination for this product. Majerus and coworkers [147] found low levels of OTA in tomato juice samples from Germany but an important level of contamination was found in tomato ketchup. Presence of *Alternaria* mycotoxins in tomato is a concern, but few studies have focused on the effect of processing on the stability of these compounds. It seems that the use of high temperatures (121 °C) and high pressures (600 MPa) may cause a total reduction of 15.3 and 12.9% of AME in tomato juice. AME seems to be significantly more stable than AOH. Climate change and the rise of global temperature is another factor to consider, as it may modify the risk for contamination of tomato plants with *Alternaria*. According to Van de Perre and coworkers [43], the predicted rise in global temperature might decrease the levels of contamination in warmer countries (Spain) compared with more moderate climatic regions (Poland), where the temperature may increase to levels that are optimal for *Alternaria* growth (14.2–28.4 °C). As was demonstrated in earlier studies [36], optimal temperature conditions for mycotoxin production in *Alternaria* are between 14 and 28 °C, with a marked decrease at 35 °C. Hypothetically speaking, the increase in climate temperature may reduce the prevalence of *Alternaria* contamination in tomato from tropical regions, together with the accumulation of mycotoxins; however, more studies will be necessary to confirm this tendency. While fungal synthesis of AOH and TA is reduced at high temperatures, the production of AME may be favored under conditions of high temperature (35 °C) [154].

2.6.4. Mango Juice

Mango fruits are juicy stone fruits that comprise several different species of trees from South Asia. *Mangifera indica* L. is one of the most common varieties in different tropical regions. Even though mango trees can be cultivated in subtropical regions, most of the production comes from regions with warmer temperatures in the tropics [158]. As is the case with other tropical cultivars, mango can be affected with fungal diseases, among which, malformation disease seems to be the most relevant. Malformation disease in mango is mostly caused by *Fusarium* species, mostly *F. filiniforme*. The infection of the mango plant may be accompanied by accumulation of mycotoxins like T-2 toxin in the fruit tissue [159]. Some *Fusarium* isolates associated with mango may produce FBs and MON, as well. As shown by Waffa Haggag and coworkers [160], mango isolates such as *F. oxysporum* and *F. subglutinans* can produce 6.30×10^3 and 8.51×10^3 µg kg^{-1} of MON in liquid media, respectively; lower amounts of FBs are produced by these isolates. However, other fungi may be naturally present in mango as well. Other important species include *A. niger*, *A. flavus* and *F. oxysporus*; all three mycotoxigenic species [158]. Some reports in the literature establish a low level of mango contamination with mycotoxigenic fungi, regardless of symptoms of disease in the fruit [161]. Abdel-Sater and coworkers [162] determined the level of fungal contamination of canned fruit juices marketed in Egypt, including mango juice. The authors confirmed that mango juice samples were contaminated with several species of fungi including some mycotoxigenic varieties such as *Aspergillu*s and *Penicillium* species; however, no mycotoxin contamination was reported for any of the analyzed samples (0/5). Kataoka and coworkers [163] reported no contamination with PAT of mango juice samples marketed in Japan, and this was coincident with the report by Sylos and Rodriguez Amaya [132] in Brazil. Similarly, Filali and coworkers [133] determined that mango juice samples in Morocco were free of OTA contamination. In another study, Anwar and coworkers [164] determined that samples of mango juice in tetra pak containers were contaminated with different fungi with mycotoxigenic potential including *A. niger*, *A. flavus*, *A parasiticus*, and *Penicillium*. Fungal growth and mycotoxin concentration in mango may be decreased with the use of post-harvest treatments. Chathat and coworkers [165]

reported that γ irradiation (1.5 kGy for 2 h), UV light for 1 h, or hot water treatment at 55 °C could completely reduce the accumulation of aflatoxin in mango fruits. The fungal disease also could be prevented with the use of hot water (50 °C) or exposure to hot air (40 °C) for enough time [166].

2.6.5. Other Cases

Many other tropical fruits (banana, papaya, cantaloupe, guava, watermelon) may serve as raw materials for the production of juices. However, as was indicated at the beginning of this section, the available information in the literature about mycotoxin contamination in this type of commodity is scarce. In the case of banana (*Musa* spp.) fruits, it has been shown that some *Fusarium* isolates from this product have toxigenic potential. In vitro studies have shown that some isolates can produce important levels of FBs (2.90×10^6 μg kg^{-1}), MON (1.67×10^6 μg kg^{-1}) and ZEA (4.70×10^5 μg kg^{-1}) [167]. On the other hand, Li and coworker [168] determined that *F. oxysporum*, which is pathogenic to the banana plant, can produce BEA and FA that are toxic for the plants, and could have effects on human health. As banana is normally used as a complementary ingredient in fruit juices, the possibility of this commodity being an important source of mycotoxin contamination is limited. For guava juice, contamination with aflatoxins up to 1.20×10^1 μg kg^{-1} has been reported [162], and no PAT contamination was detected in guava (*Psidium guajava* L.) juice samples analyzed by Sylos and Rodriguez Amaya [131]. Chances of contamination of guava juice from the plant could be important, as this crop can be affected by important fungal pathogens with toxigenic potentials like *Fusarium* and *Alternaria* [161]. Some *Fusarium* isolates from guava fruits can produce FBs to levels up to 1.12×10^3 μg kg^{-1}. Other fruits like melons are also susceptible to fungal diseases from *Aspergillus* and *Penicillium* [123]; like in the case of tomato, melons are highly susceptible to fungal infection, as they have high water content and soft tissue [35]. *Fusarium* and *Aspergillus* are also common contaminants, causing disease in papaya fruits (*Carica papaya*) [169,170] that may lead to an accumulation of AFs and other metabolites in the fruit tissue. Other tropical crops such as sugar cane may also serve as sources of mycotoxin exposure for humans. As shown by Abdallah and coworkers [171], AFB_1 and AFG_1 could be detected in 48% of sugar cane grass samples and 58% of sugar cane juice, with a maximum concentration of 3.06×10^1 and 2.10×10^0 μg kg^{-1}, respectively. AFG_1 was also detected in 10% of grass samples (7.76×10^0 μg kg^{-1}) and 18% of juice samples. Finally, in an exploratory assay, chili pepper sauces were examined. PAT values varied from ($1.8 \times 10^2 \pm 5.0 \times 10$) to ($1.2 \times 10^3 \pm 3.1 \times 10^2$) μg kg^{-1} on dry basis. It is common to find cocktails with pepper sauce as an ingredient. Also, a variety of pepper-derived products, including drinks, are commonly found in other countries where chili pepper consumption is widespread (for example, Mexico). The maximum permitted limits for peppers is currently established at 5.00×10^1 μg kg^{-1} [172,173].

3. Methodological Aspects and Approaches for the Determination of Mycotoxins in Selected Matrices

Mycotoxin analysis and monitoring of both the raw materials from which beverages are made and the drinks themselves (as a final product usually reaching a large number of consumers) are paramount to performing risk analysis and helping industry stakeholders to take actions to reduce such health hazards. It is worth noting that accurate, sensitive, confirmatory and fit-for-purpose methods are available and can be performed in well-equipped and starting laboratories in both developing and high-income countries. Methods that target mycotoxins have been developed to assess multiple analytes in a single run. However, usually, this means that more sophisticated systems are needed and, as a result, are reserved for research and high-end quality-control laboratories. For example, recently, advances in LC/MS techniques to assess several analytes in herbal products (usually used in tea beverage preparation) have been detailed, including a section devoted to mycotoxins [174]. A thorough and specific source for advances in mycotoxin determination in herbs is also available [175].

3.1. Approaches in Sample Preparation

Wang and coworkers [176] used six different adsorbents (i.e., C_{18}, polymerically bonded secondary amines (PSA), hydrophilic balance (HLB), mixed-mode, strong cation-exchange (MCX), silica, and NH_2), and assayed 8 different mycotoxins, including AOH, AME, altenuene (ALT), tentoxin (TEN), TEA, OTA, PAT, and CIT. The combination of MCX and NH_2 was found to provide the most effective cleanup, removing the greatest number of matrix interferences and also allowing the quantification of all analyzed mycotoxins in fruits. La Barbera and coworkers used Fe_3O_4-graphitized carbon black (mGCB) composites, modified magnetic nanoparticles coated with 3-(trimethoxysilyl)-1-propanthiol, to extract 6 mycotoxins from cereals [177]. Other sample products have been pretreated using this approach (e.g., corn and rice [177], including AFM_1 and ZEA (mycoestrogen/F-2 toxin) analysis in milk [178,179]). Magnetized carbon nanotubes [180,181] have been used to extract type A TRC in coix seeds [182]. Considering that this is a relatively inexpensive treatment, modified versions of these approaches may very well, in the future, be suited as a food technology to remove toxins from beverages in bulk [183].

3.2. High-Throughput Multi-Analyte LC-Based Techniques

3.2.1. Coupled with MS Detectors

A significant amount of multi-toxin analysis is increasingly being based on HPLC-MS, HPLC-MS/MS and UPLC-MS/MS [184]. As shown in Table 5, electrospray ionization (ESI) interface is commonly used for mycotoxin analysis. These techniques provide highly sensitive, selective, rapid and reliable quantification and confirmation at the low concentration. For these methods, the most important and critical steps are still sample pretreatment and sample cleanup [185].

Table 5 summarizes the more relevant toxins in beverages and their raw ingredients and approaches used to assess them. Of note, for example, in Spain, García-Moraleja and coworkers developed an assay capable of the simultaneous determination of 21 mycotoxins in coffee beverages [116]. Also, Berthiller and coworkers have already reviewed aflatoxins, *Alternaria* toxins, ergot alkaloids, FB, OTA, PAT, TRC, and ZEA in botanicals and spices, with particular emphasis on sampling and including newly developed LC-MS-based multi-mycotoxin methods [186]. Nevertheless, regarding these products, a few noteworthy instances are presented herein (Tables 2 and 5). Lately, attention has been devoted to *Alternaria* toxins [187], with several multitoxin methods considering them during the survey [188,189]. On the other hand, derivatization has been used to enhance sensitivity during mycotoxin analysis, even when using MS detection. For example, as TEA is a carbonyl compound, derivatization using 2,4-dinitrophenylhydrazine has been proved to be successful [188]. This approach can also be used to enhance toxin analysis using variable wavelength, a photodiode array or fluorescence detectors.

3.2.2. Coupled with UV and FLD Detectors

Though there is a tendency to use tandem MS-based techniques, UV and FLD detector-based LC systems are still widespread in laboratories (see Tables 2–5) which perform routine mycotoxin analysis. As a relevant example, several researchers have used $ZnSO_4$ to detect *Alternaria* toxins by using UV wavelengths [189,190]. An interaction between the keto-enol moiety and the metal ion generate a chelate and, therefore, a structure with an absorption band in the region.

Table 5. Liquid chromatography-based methodological approaches for the determination of multiple toxins in beverages and raw materials.

Matrix	Target Toxins	Extraction Method	Column, Detection Method	Concentrations Found, μg kg^{-1} or μg L^{-1} (Total of Samples Assayed, *n*)	Reference
Ochratoxin Determination					
Liquorice	OTA	80:20 MeOH:H_2O, NERCB-Solid phase extraction (SPE)	Xbridge™ C_{18} 150 × 2.1 mm, 3.5 μm, LC-MS/MS QTRAP®, ESI^+	12.99–39.03 (26)	[191]
Fermented beverages	OTA, T-2 toxin	1-octanol, 80:20 methanol (MeOH):H_2O, hollow fiber liquid phase microextraction	UPLC-MS/MS, ESI^+	<0.02–1.1 (9)	[192]
Malt beverages	OTA	Dispersive liquid-liquid acetone/$CHCl_3$ (73:27)	LC-FLD λ_{ex} 330 nm, λ_{em} 460 nm Chromolith, RP_{18} HPLC column (100 × 4.6 mm)	<0.5–4 (Validation data)	[193]
Alternaria Toxins					
Tomato and tomato juice	ALT, AOH, TEN, TEA, AME, and CIT	MeOH, cleanup Strata-XL SPE cartridges (200 mg, 6 mL, 100 μm)	Ascentis Express C-18 (100 × 2.1 mm, 2.7 μm) TEA derivatized using 2,4-dinitrophenylhydrazine TSQ Quantum Ultra triple quadrupole MS detector, ESI−	2–50 (Validation data)	[188]
Tomato products (pulp, paste, ketchup)	AOH and AME	MeOH, 10 g/100 mL NH_4SO_4, liquid-liquid extraction $CHCl_3$	C_{18} 300 × 2.9 mm, 10 μm. 300 mg $ZnSO_4$/L in mobile phase. UV at 250 nm	Ketchup: AOH 0.42–1.16	[189]
Peppers	AOH, AME, TEA	1. EtOAc, 1 mL/100 mL HCOOH and 2. Liquid-liquid 20 g/ 100 mL NH_4SO_4, $CHCl_3$, HCl	1. Kinetex 100 × 2.1 mm, 2.6 μm. UPLC-DAD/QTOF mass spectrometer ESI 2. Phenomenex Jupiter 250 × 4.6 mm, 5 μm C_{18}. 300 mg $ZnSO_4$/L in the mobile phase. UV 258 nm for AOH and AME, and 280 nm for TEA	TEA: 8–11 422 AOH: 3–98 AME: 7–262	[190]
Tomato	AOH, AME, TEA	Liquid-liquid extraction MeOH/EtOAc	Spherisorb, ODS2 250 × 4.6 mm, 5.0 μm. UV 254 nm	UV absorption match (Qualitative analysis)	[194]
Cereals and cereal products	AOH, AME, ALT, TEA, TEN, altertoxin-I, and conjugated (sulfates and glucosides) of AOH and AME	Acetonitrile (ACN)/H_2O/CH_3COOH (79/19.5/1.5), combined with a hexane defatting step	UPLC-$ESI^{\pm}$-MS/MS AcquityUPLC HSS T3 (1.8 μm, 2.1 mm × 100 mm)	Rice: 71% (*n* = 22/31), 35% (*n* = 11/31), 19% (6/31) contaminated with TEA, ranges (1.90 ± 0.12–113 ± 12), TEN (3.6 ± 0.7–15.6 ± 2.9), and AOH (1.83 ± 0.14–2.97 ± 0.23). Oats flakes: 31% (*n* = 5/16) contaminated with TEA (2.13 ± 0.18–39 ± 5)	[195]
Aflatoxin Determination					
Ginger	AFB_1, AFB_2, AFG_1, AFG_2, OTA	Immunoaffinity column, MeOH	Agilent Poroshell 120 ECC_{18} column (50 × 4.6 mm, 2.7 μm) UFLC 5500 QTRAP® hybrid triple quadrupole/near ion trap mass spectrometer equipped, ESI^+	<0.25–13.98 AFB_1 <0.10–3 045.37 OTA (3 inoculated ginger powders with *A. flavus* and *A. carbonarius*)	[71]
Medicinal herbs	AFB_1, AFB_2, AFG_1, AFG_2	70:30 MeOH:H_2O, Immunoafinity column	Agilent XDB C18-column 4.6 × 50 mm, 1.8 μm, MS/MS ESI^+	<0.14–290.80 (174)	[196]
Edible and medicinal herbs	AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, AFM_2, ZEA, zearalanone, α/β-zeralanol, and α/β-zearalenol	Immunoaffinity column, *N*-hydroxysuccinimide-activated Sepharose 4B Fast Flow gel with two group-specific monoclonal antibodies	Acquity HSS T3 2.1 mm × 100 mm; 1.8 μm and Acquity BEH C_{18} 2.1 mm × 100 mm; 1.7 μm, MS/MS $ESI^{\pm}$	AFB1 < 0.03–0.15 AFB2 < 0.03–0.54 ZEA < 0.05–2.78 ZAN < 0.06–10.5 (15)	[197]

Table 5. *Cont.*

Matrix	Target Toxins	Extraction Method	Column, Detection Method	Concentrations Found, μg kg^{-1} or μg L^{-1} (Total of Samples Assayed, *n*)	Reference
Multi-Toxin Analysis					
Coffee beverages	OTA, AFB_1, AFB_2, AFG_1, AFG_2, STG, NIV, DON, 3-aDON, 15-aDON, DAS, NEO, HT-2, T-2, FB1, FB 2, ENA, ENA_1, ENB, ENB_1, and BEA	Ethyl acetate/formic acid (95:5). Reconstitution H_2O/MeOH (50:50)	LC-MS/MS-TI, C_{18} column (150 mm × 2 mm, 3 μm, 110 Å), MRM; ESI^+	OTA < 0.24–4.93 AFB_1 < 0.05–3.66 AFB_2 < 0.04–5.64 AFG_1 < 0.04–6.65 STG < 1.00–36.54 NIV < 0.02–24.46 DON <8.23–18.34 3-aDON < 1.00–5.17 NEO < 1.22–30.24 T2 < 0.21–3.57 HT2 < 5.41–14.39 FB_1 < 2.78–5.18 ENA_1 < 0.02–4.82 ENB < 0.14–36.14 ENB_1 < 0.15– 5.33 BEA < 0.03–0.37 (6)	[116]
Fresh tomatoes, bell peppers, onions, and soft red fruits	AOH, AME, OTA, FB_1, FB_2, and FB_3	ACN/EtAOc/HCOOH (60:39:1)	Agilent Zorbax SB-C_8, LC-TOF-MS, $ESI^{\pm}$	Tomato: TEA 0.7–4.8; Overall < 1.3–90.0 (319)	[151]
Fruits (i.e., apple, orange, sweet cherry, and tomato fruits)	AOH, AME, ALT, TEN, TEA, OTA, PAT, CIT	ACN, NaCl. SPE ACN/H_2O (3:7) containing 5 mmol L^{-1} ammonium acetate	Acquity Cortecs UPLC C_{18} column (100 × 2.1 mm, 1.6 μm), UPLC–MS/MS, $ESI^{\pm}$	<1–50 (Validation data)	[176]
Dry ginger	AFG_1, AFG_2, AFB_1, AFB_2, OTA, CIT	ACN/H_2O (80:20)	Agilent Poroshell 120 EC C_{18}, 100 × 2.1 mm, LC-MS/MS	Ginger AFB_1 *n* = 16/28, OTA *n* = 20/28, CIT *n* = 16/28	[76]
Cereals (corn and wheat meal)	AFB_1, AFB_2, AFG_1, AFG_2, OTA, and ZEA	mSPE, ACN/H_2O/HCOOH (80:19.8:0.2). Elution form mGCB CH_2Cl_2/MeOH 80:20 containing 0.2 mL/100 mL	Thermo Fisher Hypersil Gold C_{18} column 50 × 2.1 mm, 1.9 μm, UHPLC/$ESI^{\pm}$ MS/MS (TSQ, triple stage quadrupole)	OTA < 0.10–1.3 (10) ZEA 1.0 <–72.9 (10)	[177]
Medicinal plants	ZEA, α-ZON	MeOH/H_2O (80:20), NaCl. VICAM® ZearalaTest column, elution MeOH.	LC-FLD λ_{ex} 274 nm, λ_{em} 440 nm, Ultimate XB-C_{18} column 250 × 4.6, 5 μm LC-MS/MS, ESI^-, Gemini C18 20 mm × 2 mm, 3 μm	ZEA < 4–295.8 α-ZON < 2.5 (100)	[70]
Tea, Herbal Infusions and the Derived Drinkable Products (from *Camellia sinensis*)	Nivalenol (NIV), DON, fusarenon-X (FSX), neosolaniol (NEO), 3a-DON, 15a-DON, AFG_1, AFG_2, AFB_1, AFB_2, sterigmatocystin (STE), OTA, FB_1, FB_2, FB_3, AOH, AME, ALT, HT-2, T-2 toxin, diacetoxyscirpenol (DAS), ZEA	Raw material: Ethyl acetate (EtOAc)/HCOOH (99:1) NH_2 SPE Drinkable products: C_{18} SPE, elution methanol	Acquity UPLC BEH C_{18}, 100 × 2.1 mm, 1.7 μm, UPLC-MS/MS, ESI^+	FB_1 Ceylon mélange < 37–76 (91)	[198]
Tea Beverages	AFB_1, AFB_2, AFG_1, AFG_2, 3aDON, 15aDON, NIV, HT-2, T-2, ZEA, OTA, ENN, BEA	Dispersion liquid-liquid: NaCl, ACN/EtOAc (60:40), MeOH/$CHCl_3$ (60:40)	LC-MS/MS Turbo ion spray (TI), ESI^+, Gemini NX C_{18} 150 × 4.6 mm, 5 μm	Black, red, green and green/teas (44). Green/mint tea: *n* = 6/8 AFB_2 14.4–32.2, *n* = 2/8 15aDON 60.5–61, *n* = 4/8 AFG_2 1.9–2.6	[199]
Soy, oat and rice plant-based Beverages	AFB_1, AFB_2, AFG_1, AFG_2, OTA, DON, ZEA, T-2, HT-2, FB_1, and FB_2	ACN with 1 mL/100 mL HCOOH, $MgSO_4$, and NaCl	Cortecs UHPLC C_{18} column (100 × 2.1 mm, 1.6 μm), Triple Quadrupole MS/MS, ESI^+	Oat: 0.1 AFG_1, 0.4 AFB_2, AFB_2 0.2–0.3, T-2 0.4–1.3, OTA 0.2 Rice: DON 15–19 (9)	[200]

Table 5. *Cont.*

Matrix	Target Toxins	Extraction Method	Column, Detection Method	Concentrations Found, μg kg^{-1} or μg L^{-1} (Total of Samples Assayed, *n*)	Reference
Green Coffee Bean Extracts	OTA, OTB, AFB_1, AFB_2, AFG_1, AFG_2, TRC A, and B, *Alternaria* toxins, FB_1, FB_2, FB_3, enniatins (enniatin A, A_1, B, B_1), BEA, CIT, cyclopiazonic, mycophenolic, and, PA, penitrem A, roquefortine C, gliotoxin, STE.	H_2O with 2 mL/100 mL HCOOH and ACN (50:50). $MgSO_4$, NaCl (QuEChERS)	UFLC-QTRAP® TurboIon electrospray, ESI and UPLC quadrupole-orbitrap HESI-II ESI	OTA, OTB, FB_1 and mycophenolic acid prevalence: 36%, 32%, 10%, and 16%, respectively. OTA < 1.0−136.9, OTB < 1.0−20.2, FB_1 < 50.0−415.0, mycophenolic acid, < 5.0−395.0	[201]
Berry by-products Jam/Juice (i.e., strawberries, blackberries, blueberries, cranberries, and raspberries) and mixed in minor percentage with grape, pomegranate, cherry, apple and plum juice.	AFB_1, AFB_2, AFG_1, AFG_2, OTA, AOH, AME, TEN	ACN sodium citrate, $MgSO_4$, and NaCl (QuEChERS)	Phenomenex Gemini-NX C_{18} (150 × 2.0 mm, 3 μm) LC-MS/MS QTRAP®, ESI^+ (Turbo-V™)	Jams: 1.6 AFG_1 Juices: 0.9 AFB_2 0.4 AFG_1, AFG_2 0.7–79, *Alternaria* toxins (AOH 2.5–85 and AME 267–308) 47% prevalence (52)	[202]
Evaporated cow milk	AFM_1, AFB_1, AFB_2, AFG_1, AFG_2; OTA, OTB, FB_1, FB_2, FB_3, HT-2, T-2, NIV, DON, deepoxy-DON, 3 and 15a-DON, DAS, FSX, NEO, STE, and ZEA	ACN acidified with HCOOH	Ascentis Express C_{18} column (150 × 2.1 mm, 2.7 μm), Triple Quad (QqQ) LC-MS/MS	OTA: 4/30 < 0.2 (30)	[203]

3.3. Non-Chromatographic Multiple Mycotoxin Analysis

Constant surveillance of beverages for the presence of mycotoxins would be prudent to reduce risks to human health. Alternative non-chromatographic analytical methods include bioassays and various immunochemical methods, surface plasmon resonance, fluorescence polarization [204], microarray chips, lateral flow [205–207], and nanoparticle-based biosensors [208]. Several new non-chromatographic techniques have been developed to assay mycotoxins in alcoholic beverages (e.g., nanostructured imaging surface plasmon resonance [209], multi-toxin immunoassay [210]), and could be easily implemented to assess contaminants in the matrices mentioned above.

Rapid and on-site screening methods are the first level of screening for food contaminants. Rapid methods have been developed for the detection of such contaminants in foods and beverages. These approaches can be performed by non-specialized personnel, are usually cheap, and some of them can even be applied in situ within fields, plants, and industries (e.g., raw materials, finished product, equipment, work surfaces). Among the rapid methods available, immunochromatographic or lateral flow immunoassay technology has increased scientific and industrial interest in the past few years, and its exploitation has rapidly spread, particularly for mycotoxin detection.

The selection of commercially available tests includes RIDA® QUICK Aflatoxin RQS ECO (AFs in corn), Neogen® Reveal Q^+ lateral flow (several mycotoxins in grain products) and ProGnosis Biotech, which has an application with an extraction buffer with no organic solvent intervention (Symmetric Total ES Green, AFs in grain products). However, in beverages, these methods have largely been disseminated for alcoholic drink (e.g., wine and grape must [211]), milk [212,213] and cereal analysis [214]. Recent reports have described the use of these techniques to assess *Alternaria* toxins in fruit [215] and fruit juice [216].

Multiplex lateral flow tests (e.g., AFB_1, ZEA, and DON) and biotin/streptavidin-based ELISA (AFB_1, OTA, ZEA) have been developed so that range, versatility, and analyte diversity have been improved [216,217]. The main attraction of these rapid tests is that no laboratory equipment needs

to be applied. The downside is that sensitivity is usually lacking, and in some cases, one can only assess whether the toxin is present above a certain threshold [218]. Despite any shortcomings, it was calculated that the food and beverage industry invested 95 million USD in these types of tests in 2015 alone [219]. Recently, R-Biopharm® has developed a portable application (RIDA® SMART APP) that can even evaluate the results of a lateral flow assay.

Nowadays, food analysis using microchip is also common [220]. At least one research group has used a microarray immunochip and fluorescence detection to identify OTA in a coffee beverage with a 7 μg kg^{-1} limit of detection [221]. For example, Randox Food Diagnostics has developed a Biochip Array Technology, a multiplex system designed to enable the simultaneous detection of up to 10 of the world's most prevalent mycotoxins. Additionally, an excellent review of biosensor-based methods applied to beverages can be found elsewhere [222]. Aptamer-based technologies are an example of a recent approach [223]. As an example of a novel non-chromatographic analytical method, an aptamer-based technology for the detection of PAT has been described [224]. A comprehensive review of other techniques applied to the mycotoxin analysis in fruits can be found elsewhere [7].

Along the previous sections, we have cited several examples of ELISA applications. Several ELISA kits are commercially available, including Neogen® Veratox® (OTA in green coffee), Astori Tecnic (OTA in coffee, cocoa, cocoa butter), RenekaBio (OTA in coffee, cocoa, and spices), Eurofins Technologies I'screen (OTA in cocoa and green coffee). Even though they are usually considered a screening tool, some ELISA tests can be extremely sensitive. For example, in Iran, non-alcoholic beverages have been evaluated for mycotoxins using ELISA, and higher levels were found in local samples (5.00×10^{-4}–5.54×10^{-1} μg L^{-1}) than those that were imported (9.00×10^{-4}–2.29×10^{-1} μg L^{-1}) [225]. Most of the immunology-based research for mycotoxin detection is based on the preparation of monoclonal antibodies against the metabolite; these antibodies can be further applied in ELISA techniques and immunoaffinity columns for sample preparation before fluorimetry of HPLC [224]. For example, monoclonal antibodies against OTB were prepared to assay coffee samples [226]. Nanobodies (known as VHH antibodies) have recently been developed for the sole purpose of mycotoxin analysis, as they are easily engineered and have superior stability [227].

4. Mycotoxin Risk Assessment and Integrated Management Approaches

4.1. Masked and Hidden Mycotoxins

Food is a well-established vehicle for toxin contamination; as such, strategies to estimate mycotoxin exposure must be set [228]. Identification, management, and prevention of emerging food safety risks are paramount as a device for preventing health-threatening incidents [229–231]. The presence of mycotoxins in crops and, more recently, the presence of masked and modified [232,233] mycotoxins, have been considered emerging risks. In this regard, for example, masked and modified forms of fumonisins, FB_1, FB_2, and FB_3, were assessed to evaluate them as a potential risk for food-producing animals [234].

4.2. Mycotoxin Risk and Climate Change

Risk assessment applied to mycotoxins research has demonstrated the need for inclusion of another variable: the effect of climate change [235–238]. As was mentioned previously, some models suggest that increasing temperatures will have a profound effect on *Alternaria* spp. growth in tomatoes [239,240]. As a consequence, AOH and AME exposure may increase, as more fungi-colonized tomatoes enter the production line (e.g., tomatoes are processed to crop juices). Hence, pre-harvest preventive measures are vital to circumvent mycotoxin contamination. The estimated mean exposure for AOH and AME were calculated between 0.004 and 0.008 μg kg^{-1} bw day^{-1}, respectively [239]. Extreme weather conditions can lead to more damage to crops and, hence, more vulnerability to fungi colonization [239]. For example, for groundnut, drought and areas with high humidity favor pre-harvest and post-harvest contamination, respectively [240].

4.3. Multidisciplinary Approaches to Reduce Mycotoxin Contamination

Food safety issues regarding mycotoxins have been attacked from several perspectives. Pre- and post-harvest prevention practices, decontamination methods [241] and novel physical and chemical approaches for the decontamination of aflatoxin in foods have been recently described [242]. Biological control agents and improved packaging materials have also been considered [243,244]. Genetic transformation, proteomics, RNAi technology, and marker-assisted selection have demonstrated potential in minimizing pre-harvest mycotoxin contamination in crops [245]. Nowadays, plant varietal selection based on the availability of resistant genotypes to fungi could be an option to reduce fungal infestation in cultivars. For example, corn and peanut varietals tolerant to *A. flavus* infection have been described [246,247]. Also, competitive exclusion using non-toxigenic fungal strains has been defined as a routine measure to combat contamination [248]. A GRAS line of attack against mycotoxins for fruits and vegetables (post-harvest) has been suggested [249]. Integrated practices including soil preparation (deep tillage), crop in the rotation and the selection of cultivars have been proposed to control *Fusarium* head blight and DON in wheat [250]. These approaches, in turn, reduce the risk of exposure to mycotoxins. There is a complex relationship between fungi, the crop, the environment, and mycotoxin generation. Understanding such interactions is indispensable when designing or applying measures for fungal colonization and mycotoxin prevention and control.

4.4. Mycotoxin Contamination and the Food Chain

Contamination with pathogens can occur at any point within the production chain, and transdisciplinary approaches are deemed necessary to tackle the issue [251]. Beverage (and raw material) fungal spoilage during processing [252,253] or storage [254] are considered the main causes for production loss [255]. Risk assessment analysis specific to mycotoxins has been documented in grains from developing countries (e.g., corn and peanut, [256,257]) and the most sensitive populations have been considered [258]. Although multidimensional data analysis, harmonization, and interpretation are available for an already-multifaceted issue such as mycotoxins, this issue still represents a challenge for researchers [259]. Good agricultural and manufacturing practices, hazard analysis and critical control points (established within beverage industries) will serve their purpose as long as fungal and mycotoxin contamination analysis (a biological and chemical hazard, respectively) are considered part of quality control [99,260–263]. At least one report has described how to apply a risk management standard directly (i.e., AS/NZS 4360) to the beverage industry [264]. Physical (foreign material, e.g., insect fragments), chemical (water content, total solids, soluble solids, pH and acidity), microbiological (i.e., mycelial filament count) [190] and biochemical (H_2O_2 production, cellular damage and death) [194] properties have been described for one fruit that was associated with *Alternaria* toxins.

4.5. Selected Health Issues Related to Mycotoxin Exposure

Mycotoxin risk assessment has, thus far, focused on mutagenic, genotoxic and carcinogenic potential. However, recently, research has integrated gastrointestinal epithelial cell damage into consideration [265]. Significant cellular structural changes and exacerbated immune system activity have been reported after TRC (especially DON) and PAT exposure [265]. Furthermore, chronic intestinal inflammatory diseases and allergic reactions to food, particularly in children and immunocompromised patients, have been linked to mycotoxin exposure [101,266]. As yet another health consequence of mycotoxins, there may be an inherent risk of invasive fungal infections resulting from ingestion or inhalation of food, beverages, or dietary supplements [267]. Vieira and coworkers had previously determined that yerba mate infusion had the potential for carrying potentially pathogenic fungi which were able to survive extreme variations in pH and temperature, posing a potential health risk [268]. Additionally, case studies for asthma, bronchitis, skin diseases, and other health disorders related to living in moldy humid places have been described [269]. Risk of health effects are increased

as exposure to multiple toxins is increased; an exposure model has been developed specifically for Latin-American countries [270].

5. Concluding Remarks and Future Perspectives

The tropical nations possess a broad diversity of flora and fauna. Throughout the years, many types of vegetable products have been repeatedly exploited for the production of different hot and cold beverages. However, the raw materials from which they are made are prone to fungal contamination and the resulting mycotoxin accumulation. Many tropical countries that provide raw material for beverages are developing economies, where the conditions for cultivation and processing may not be adequate. Also, there may be limited resources to regulate the presence of mycotoxins in some cultivars or to provide appropriate analytical techniques for monitoring levels of contamination. Within the context of a globalized economy, this situation represents a threat to consumers at both the local and global levels; this is evident from the consumption patterns established above. The major consumption trend of beverages made from tropical crops is driven by countries that are not the producers of these cultivars. This means that collaborative efforts should be made between regulatory and scientific organizations of different countries in order to invest in high-quality research to understand the dynamics of mycotoxin contamination for tropical products.

Mycotoxigenic fungi and their metabolites are frequently present in tropical cultivars and their derived products, including different types of beverages (industrial and traditional). According to this review, there seems not to be a specific tendency in terms of lower or higher risk related with some specific products. It seems that all the products reviewed in this document are prone to fungal contamination, and that the most important differences are basically the type of mycotoxins that are present. Even though in some cases there is an extensive body of literature regarding levels of contamination (coffee, tomato), this does not mean that for other products the risk is lower; this is just a reminder that more scientific research is necessary to properly characterize that risk. However, one conclusion that can be made is that products like juices or other beverages may harbor important levels of mycotoxin contamination as they may be processed from low quality raw material. The level of exposure from such products could be significant taking into consideration that some beverages are produced in high quantities using (in some instances) raw material from different sources.

Aspects that are necessary to scientifically address mycotoxin contamination in beverages and their raw materials include the study of the type of fungal contamination of tropical products (with special attention to other genus different than *Aspergillus* and *Penicillium*) and the factors affecting it (with special attention to the role of aerial contamination in tropical environments), the fate of mycotoxins during manufacturing of beverages, and the levels of contamination in the beverages already available for consumers; the latter is strictly necessary to establish a baseline for future work. This information could be combined with more recent consumption data, clinical studies related with the pathogenic potential of these toxins and the biology of each fungus to assist in the risk characterization process. For example, metagenomic studies of soil and plant fungal populations could also be conducted to understand mycotoxin production and its role in nature. In terms of methodological approaches, it is clear that various options are suitable for the analysis of mycotoxins in the products described in this review; this is an advantage, as some of them could be considered as inexpensive approaches that could be applied in countries with limited financial resources. This is true in terms of lower cost for some of the techniques (rapid tests, ELISA) or the possibility to adapt one single approach for the analysis of multiple mycotoxins in multiple matrices (MS detectors). However, scientific studies are necessary to validate the application of these methodologies to different beverages.

Considering that beverage production is a manufacturing process that involves several steps of complex food chains, the industry should invest in risk management programs for their crops, as contaminated raw material or finalized products may hinder international trade as a result of strict regulation in high-value markets. Additionally, within the beverage industry, constant surveillance programs should be implemented, especially for the monitoring of raw materials, e.g., dry herbs (i.e.,

aromatic and medicinal plants), coffee cherries, cocoa beans (fruits and fresh/dried seeds), nuts and fresh fruits and pulps. Such surveillance should be performed by versatile, specific, sensible and accurate methods, taking into consideration the most common contaminants present in such matrices, but also considering emerging contaminants (e.g., *Fusarium* and *Alternaria* toxins).

Finally, even though mycotoxins are a Public Health issue, since they can be hepatotoxic, nephrotoxic, neurotoxic and teratogenic, there is a lack of international and local legislation that establishes maximum permitted levels of these compounds in tropical commodities.

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