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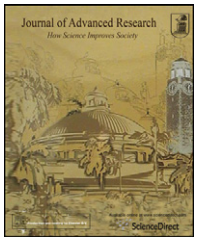

REVIEW

Mycotoxins in fruits and their processed products: Analysis, occurrence and health implications

María L. Fernández-Cruz, Marcia L. Mansilla, José L. Tadeo*

Environment Department, National Institute for Agrarian and Food Research and Technology, Madrid, Spain

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KEYWORDS

Aflatoxins;
Alternaria toxins;
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Abstract Mycotoxins are secondary metabolites of filamentous fungi that occur naturally in food and feed. The presence of these compounds in the food chain is of high concern for human health due to their properties to induce severe toxicity effects at low dose levels. The contamination of fruits with mycotoxins has not only caused health hazards but also resulted in economic losses, especially for exporting countries. The mycotoxins most commonly found in fruits and their processed products are aflatoxins, ochratoxin A, patulin and the *Alternaria* toxins alternariol, alternariol methyl ether and altenuene. The aim of this work is to review the toxicity of these major mycotoxins, their natural occurrence in fruits, dried fruits, juices, wines and other processed products, the analytical methods available for their determination and the strategies for their control.

Introduction

Mycotoxins are secondary metabolites of filamentous fungi and therefore occur naturally in food. They represent a very large group of different substances produced by different mycotoxigenic species. Moulds can infect agricultural crops during crop growth, harvest, storage or processing. The growth of fungi is not necessarily associated with the formation of mycotoxins and because of the stability of mycotoxins; they may be present in food when fungi are no longer present. Furthermore, a fungus may produce different mycotoxins, and a mycotoxin may be produced by several different fungi. The mycotoxigenic potential depends on species and strains of fungus, composition of matrix and environmental factors (temperature and moisture). Fruit contains natural acids (citric, malic and tartaric acids) that give the fruits tartness and slow down bacterial spoilage by lowering the pH. The pH of fruits varies from <2.5 to 5.0 and these values are tolerable for many fungal species but less for bacteria. Another factor that has a strong influence in the types of microorganisms which cause the spoilage of particular foods is the water activity (a_w) as a measure for water used by microorganisms and not the total amount of water. In general, the optimum a_w value for fungal growth is different from the optimum a_w value at which the maximum level of mycotoxin formation is observed [1,2].

The mycotoxins most commonly found in fruits and their processed products are aflatoxins, ochratoxin A, patulin and *Alternaria* toxins [2–4]. Mycotoxins are known for their toxicological properties and maximum levels (MLs) have been set for some of them in food and feed to protect animal and public health. On a worldwide basis, at least 99 countries had mycotoxin regulations for food and/or feed in 2003 [5]. European Union (EU) MLs set for mycotoxins in fruits are presented in Table 1 [6]. No regulation exists, in the EU or other countries, for the group of *Alternaria* mycotoxins. Regulatory limits require suitable validated analytical methods and rapid screening tests for cost-effective food control on a large scale.

The contamination of fruits with mycotoxins has not only caused health hazards but also resulted in economic losses, especially for exporting countries. The aim of this paper is to review the available

* Corresponding author. Tel.: +34 91 3476821; fax: +34 91 3474008.
E-mail address: tadeo@inia.es (J.L. Tadeo).

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information on the main mycotoxins found in fruits and their processed products, the analytical methods used for their determination and the human health implications of their occurrence.

Toxicity of mycotoxins

Contamination of crops by fungal action has been noted for over two millennia. Recently it has been suggested that the Biblical tenth plague could be attributed to trichothecene mycotoxins and that the thousands of deaths in Europe, in the Middle Ages, caused by the St Anthony's Fire (today recognized as ergotism) could be produced by the ingestion of the ergots of *Claviceps purpurea*, a fungus occurring on grains such as rye and wheat. During the Second World War, thousands of deaths in the former Soviet Union from the haemorrhagic syndrome known as alimentary toxic aleukia were caused by the T2 toxin produced by *Fusarium sporotrichioides*. The discovery in 1960 of aflatoxins focused attention on the adverse human health implications of the secondary metabolites of fungi [7,8].

The improvements in food safety in developed countries have eliminated acute human mycotoxicosis, however such outbreaks still occur in rural communities in the developing world where aflatoxins, fumonisins, deoxynivalenol, ochratoxin and zearalenone present in cereals have been involved in the deaths or acute diseases reported. Review of scientific literature on mycotoxin-related human diseases clearly reveals a linkage between ingesting mycotoxin-contaminated food and illness, especially hepatic, gastrointestinal, carcinogenic and teratogenic diseases [7,8]. Based on their known and suspected effects on human and animal health, aflatoxin, fumonisin, trichothecenes, ochratoxin, zearalenone and patulin are recognized as the most important agricultural mycotoxins.

Aflatoxins (AF) are a group of closely related metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. They are difuranocoumarin derivatives and the main components of this group are aflatoxin B_1, B_2, G_1 and G_2 (Fig. 1), based on their fluorescence under UV light (blue or green) and their relative chromatographic mobility. They were first detected and characterized in the 1960s [9] and have been found in a variety of agricultural and food products, mainly cereals and oil seeds. Aflatoxins were discovered during an epidemic of disease that wiped out more than 100,000 turkeys in the 1960s. The disease was traced to turkey feed made of mouldy Brazilian peanuts. Some human fatalities have been reported in India and it has been estimated that ingestion of 2–6 mg/kg/day of AF over a month produce hepatitis [10]. However, a suicide attempt with 1.5 mg/kg of pure aflatoxin resulted only in nausea, headache and rash [11]. Repetitive incidents of deaths due to aflatoxin have occurred in Kenya during 1981, 2001, 2004 and 2005, with 125 and 32 deaths from 317 and 75 cases in 2004 and 2005, respectively [8,12]. The LD_{50} of AFB_1 ranges from 0.3 to 18 mg/kg depending on the animal species and routes of administration. Besides these reported acute effects, aflatoxins are of major concern with respect to public health, because of their potential as powerful hepatotoxins and carcinogens in humans and their proven toxicity to animals, birds and fish. AF B_1 is the most potent natural carcinogen known and is usually the major AF produced by toxigenic strains. Aflatoxins are classified by the International Agency for Research on Cancer (IARC) as being carcinogenic to humans (group 1) [7,8,13].

Alternaria fungi are commonly parasitic on plants and may cause spoilage of fruits and vegetables during transport and storage. *Alternaria alternata* produces a number of mycotoxins, including the dibenzo-α-pyrones alternariol (AOH), alternariol monomethyl ether (AME) and altenuene (ALT), altertoxin I and II (ATX-I and -II) and tenuazonic acid (TeA) a tetramic acid (Fig. 1). AOH and AME were first isolated in 1953. Of the mycotoxins isolated, altenuene and ATX-I are the most acutely toxic in mice with LD_{50} of 50 and 200 mg/kg, respectively. AOH and AME are not very acutely toxic to mice (LD_{50} 400 mg/kg), and TeA has been shown to be

Table 1 EU maximum levels (MLs) for mycotoxins in fruits and their processed products [6].

Commodities	MLs (μg/kg)	
Aflatoxins	B_1	$B_1 + B_2 + G_1 + G_2$
Dried fruit to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	5.0	10.0
Dried fruit and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs	2.0	4.0
Processed cereal-based foods and baby foods for infants and young children	0.10	–
Ochratoxin A		
Dried vine fruit (currants, raisins and sultanas)	10.0	
Wine (including sparkling wine, excluding liqueur wine and wine with an alcoholic strength of not less than 15 vol%) and fruit wine	2.0	
Aromatised wine, aromatised wine-based drinks and aromatised wine-product cocktails	2.0	
Grape juice, concentrated grape juice as reconstituted, grape nectar, grape must and concentrated grape must as reconstituted, intended for direct human consumption	2.0	
Processed cereal-based foods and baby foods for infants and young children	0.50	
Patulin		
Fruit juices, concentrated fruit juices as reconstituted and fruit nectars	50.0	
Spirit drinks, cider and other fermented drinks derived from apples or containing apple juice	50.0	
Solid apple products, including apple compote, apple puree intended for direct consumption	25.0	
Apple juice and solid apple products, including apple compote and apple puree, for infants and young children and labelled and sold as such	10.0	
Baby foods other than processed cereal-based foods for infants and young children	10.0	

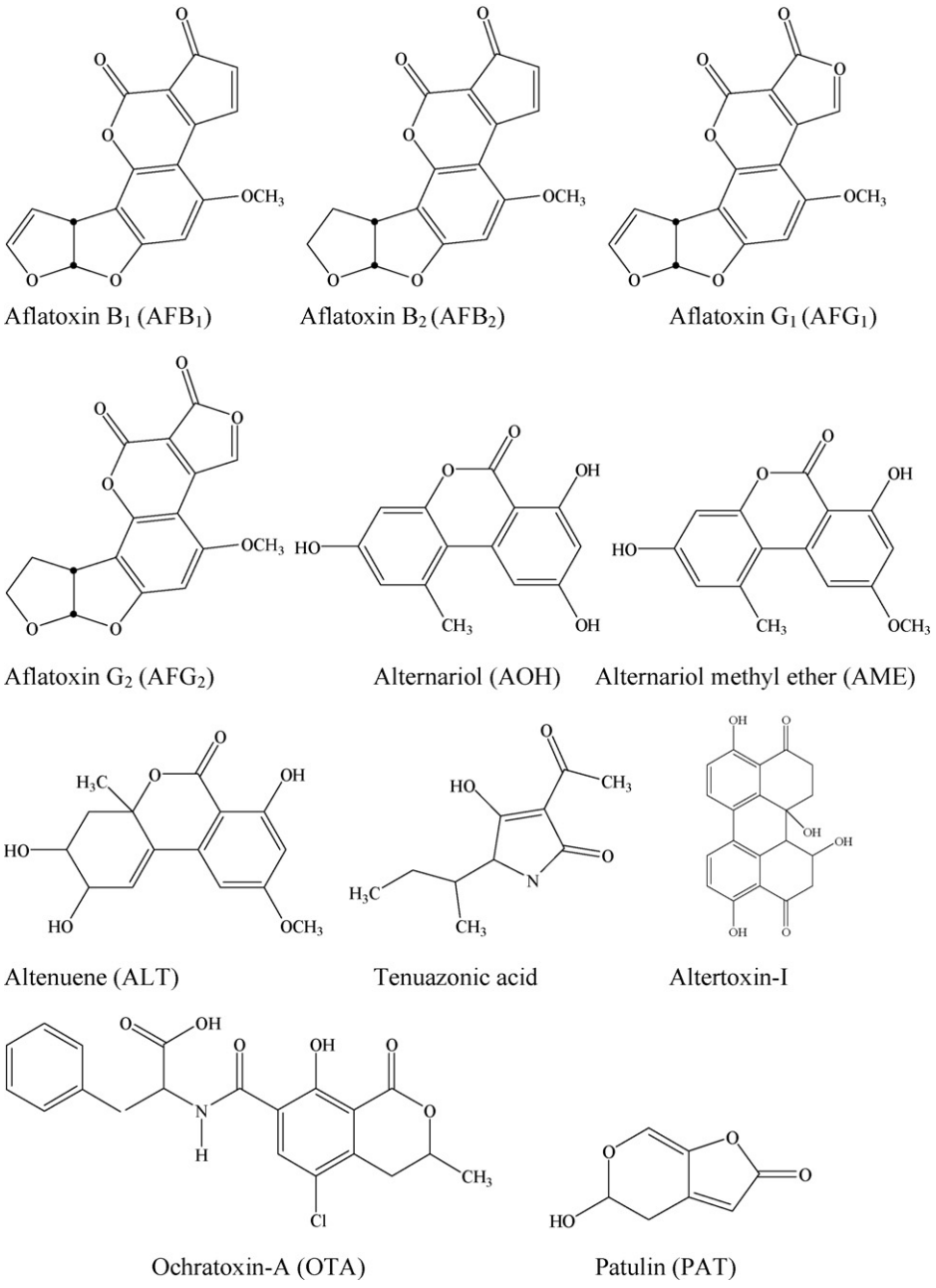

Figure 1 Chemical structures of mycotoxins.

sub-acutely toxic in mice (LD_{50} i.v. 115 mg/kg). Culture extracts of *A. alternata* are mutagenic in various microbial and cell systems and carcinogenic in rats. It has also been suggested that *A. alternata* might be one of the etiological factors for human oesophageal cancer in Lixian, China [14]. ATX-1, AOH and AME are mutagenic [14,15].

Ochratoxin A (OTA) was originally isolated from *Aspergillus ochraceus* in 1965. Several different ochratoxins exist, but ochratoxin A is the most common. The OTA molecule is a phenylalanine-dihydroisocoumarin derivative, which is very stable to both temperature and hydrolysis (Fig. 1). Other *Aspergillus* species are also capable of producing OTA. *Penicillium verrucosum* is the best known Penicillium species that is able to produce OTA. The contamination of foods with OTA in cool climates is usually caused by *P. verrucosum*, whereas the occurrence of OTA in foods in warmer and tropical climates is associated with *A. ochraceus*. Population studies in Tokyo and Canada have shown the presence of measurable concentrations of OTA in the blood plasma of many apparently healthy human subjects [16,17]. Higher plasma OTA concentrations were found in proportion to the severity of the disease in patients from Egypt with end-stage renal failure, nephrosis, and urothelial cancer [18]. It is widely believed, but not fully established, that OTA may be a prominent etiologic factor in the endemic disease Balkan nephropathy, a fatal renal disease [19,20]. The LD_{50} of OTA ranges from 0.5 mg/kg for dogs to over 50 mg/kg for mice. OTA is a potent kidney toxin and has been classified by the IARC as a 2B cancer compound, being possibly carcinogenic in humans. It is among the strongest carcinogenic compounds in rats and mice

and, its toxicological profile includes teratogenesis, nephrotoxicity and immunotoxicity [2,21]. Animal experiments have implicated cytochrome P450-related reactions and DNA adducts generation as possible mechanisms for the formation of renal tumours. Brain damage caused by OTA has also been demonstrated experimentally [2,7,8,21].

Patulin (PAT) is a toxic metabolite produced by several species of *Penicillium* and *Aspergillus*. The most important producer of PAT is the apple-rotting fungus *Penicillium expansum*. Chemically, PAT is an unsaturated heterocyclic lactone (Fig. 1). Various acute and chronic effects have been attributed to PAT [2,7,22]. The LD_{50} of patulin ranges from 15 to 25 mg/kg and varies with animal species and route of exposure. The acute symptoms in animals include lung and brain oedema, liver, spleen and kidney damage and toxicity to the immune system. For humans, nausea, gastrointestinal disturbances, and vomiting have been reported. The chronic symptoms include genotoxic, neurotoxic, immunotoxic, immunosuppressive and teratogenic effects. The IARC has classified PAT as category 3, not classifiable regarding its carcinogenicity to humans. At the cellular level, some examples of these effects are plasmatic membrane rupture, protein synthesis inhibition and DNA and RNA synthesis inhibition [2,7,22].

Natural occurrence of mycotoxins in fruits and their processed products

As it has been indicated above, the extent of fungal growth and subsequent possible mycotoxin contamination depends on endogenous and exogenous factors. Most mycotoxins are chemically stable during storage and processing, even when cooked at quite high temperatures [23]. This makes it important to avoid the conditions that lead to mycotoxin formation at all levels of production, harvesting, transport and storage, which is not always possible and not always achieved in practice. It has been demonstrated that environmental stress conditions such as insect infestation, drought, cultivar susceptibility, mechanical damage, nutritional deficiencies, and unseasonable temperature, rainfall or humidity can promote mycotoxin production in growing crops. In fact, changes in farming practices in the past few decades may result in increasing stress on plants and therefore enhance fungal invasion and mycotoxin contamination. The careful selection and proper storage of fruits are the most important factors in quality control [2,24].

The fate of patulin during apple juice production has been extensively studied and recently reviewed by Sant'Ana et al. [22]. They concluded that although the various stages of the manufacturing process of apple juice are capable of reducing the amount on the final product on a certain extent, the incidence of this mycotoxin throughout the world, confirms its stability. For the *Alternaria* toxins, little work has been published on their stability in food matrices. AOH and AME are stable on heating at 100 °C in sunflower flour [25]. Both mycotoxins are very stable in spiked apple juice at room temperature for up to five weeks and at 80 °C after 20 min. They are also stable in spiked white wine for almost 8 days at room temperature. ATX-1 added to apple juices is stable for up to 27 days at room temperature [26].

The most studied mycotoxins in fruits and their processed products have been patulin, mainly in apples and apple juice, and ochratoxin A in wines. However these mycotoxins have been found in other matrices. Less literature is available for the aflatoxins (except in dried figs) and the *Alternaria* toxins, although the ubiquity and toxicity of the latter are well known. Very recently, some evidence for the presence of fumonisins B_1 and B_2 in fruits has been reported. Fumonisin B_2 has been identified in visibly mouldy dried figs [27] and in must from southeastern Italy [28]. A high incidence of Fumonisin B_1 was found in dried figs, collected while the figs were drying, in Turkey [29]. These are the first reports on the presence of fumonisins in fruits. These mycotoxins are common contaminants of corn and maize and they have been associated with an increased risk of oesophageal carcinoma in humans in contaminated areas of China [30].

Aflatoxins

High temperatures (27–38 °C), a_w of 0.99 and high relative humidity (85%) favor the growth of *Aspergillus* in the field. Aflatoxins are produced under certain conditions that include temperature 13–40 °C (optimum 30 °C) and a_w of 0.95. AF B_1, B_2, G_1 and G_2 are generally found in fat containing food and feed like ground nuts and their processed products, almonds, pistachios, Brazil nuts, maize, rice, figs, cotton seed and spices.

Studies concerning aflatoxins on fruits are limited to fruits from regions with relatively high temperatures. Natural aflatoxin contamination has been reported in oranges, apples, and apple juices (Table 2) [2,31]. AFB_1 contamination of musts has been detected [32] with 40% of the musts samples containing the AF in a range from 0.01 to 0.46 μg/L. However, the most frequently reported occurrence of AF is in dried fig and raisins [33] (Table 2). AFB_1 has also been found in dried apricots, prunes and dates [33]. AFB_1 was detected in raisins in Brazil, Egypt, Greece, India and Morocco [33–35] in a range of maximum concentrations from 2 to 550 μg/kg. Dried figs have also been found to contain AF in numerous surveys [2,35–37]. Contamination of figs with AF begins during sun drying on the tree and continues during drying on the ground. Levels of AFB_1 can be up to 63 μg/kg (Table 2). More than one mycotoxin can occur in the same sample of dried figs: AFB_1 and OTA [38] and AF and PAT in Turkey [39].

Alternaria

The *Alternaria* toxins AOH and AME are produced over the temperature range of 5–30 °C and a_w range of 0.98–0.90, although at the marginal temperatures and 0.90 a_w little of any mycotoxin was produced. The minimum a_w allowing germination of *A. alternata* conidia is 0.85, whereas 0.88 a_w is necessary for growth on wheat extract agar at 25 °C. The limiting a_w for detectable mycotoxin production is thus slightly greater than that for growth, with optimum production occurring above 0.95 a_w [1]. Mycotoxins of *Alternaria* can be found in various fruits, in vegetables (tomatoes and olives) and also in grains, sunflower seeds, oilseed rape meal and pecans [15]. Results of natural occurrence in fruits and their processed products are presented in Table 2.

Alternariol (AOH) and alternariol monomethyl ether (AME) are among the main mycotoxins of *Alternaria* reported as naturally occurring in various infected fruits, including mandarins, oranges, lemons, melons, apples and different berries [2,15,40]. High levels of these toxins were found in infected apples, oranges and lemons [41] and mandarins from Italy [42]. The tenuazonic acid has also been found at high levels in these citrics [41,42], but only trace levels in apples and melons [41,43]. Magnani et al. [44] detected alternariol and alternariol monomethyl ether on tangerines from Brazil with and without symptoms of *Alternaria* spot disease; the levels of these mycotoxins on flavedo (epicarp or exocarp) varied from 0.90 to 17.40 μg/kg. On albedo tissues (mesocarp), neither

Table 2 Occurrence of mycotoxins in fruits and their processed products.

Commodities	Positives/total	Toxins	Maximum concentration	Concentration range	Reference
Oranges	8/25	AFB1/AF	52/120 μg/kg		[2]
Apple rotten areas	30/30	AF	350 μg/kg		[2]
Apple remainders	0/30		–		
Apple juice	5/5	B_1, G_1		μg/L	[31]
Musts	19/47	AF B1		0.01–0.46 μg/L	[32]
Dried raisins		AF		Max. 2–550 μg/kg	[33–35]
Dried figs		AF		Max. 10–325 μg/kg	[2,35–37]
	7/8	AOH	59,000 μg/kg		[41]
	8/8	AME	2300 μg/kg		
Rotten apples	8/8	TEA	500 μg/kg		
Apples	1/22	AOH	160 μg/kg		[2]
	1/22	AME	250 μg/kg		
Rotten mandarins	2/2	AOH		1000–5200 μg/kg	[42]
		AME		500–1400 μg/kg	
		TEA		21,000–87,200 μg/kg	
Tangerine flavedo	6/8	AOH		2.5–17.4 μg/kg	[44]
		AME		0.9–3.5 μg/kg	
Apple juice concentrate	17/32	AOH		1.35–5.42 μg/L	[46]
	1/32	AME	1.71 μg/L		
Apple juice	11/11	AOH		0.04–2.40 μg/L	[45]
	10/11	AME		0.03–0.43 μg/L	
Red grape juices	5/10	AOH		0.03–0.46 μg/L	[40]
		AME		0.01–39.5 μg/L	
Red wine	20/25	AOH		0.03–7.41 μg/L	[40]
		AME		0.01–0.23 μg/L	
White wine	2/23	AOH		0.67–1.48 μg/L	[40]
		AME		0.02–0.06 μg/L	
Peaches	21/56	OTA		0.21 μg/kg	[48]
Cherries	6/6	OTA		2.71 μg/kg	[48]
Strawberry	4/10	OTA		1.44 μg/kg	[48]
Apple	2/4	OTA		0.41 μg/kg	[48]
Red wine	40–87%	OTA	Mean 0.30 μg/L	0.01–15.6 μg/kg	[52,53]
White wine	10%	OTA	Mean 0.18 μg/L	0.05–1.13 μg/L	[53]
Special wines	20–45%	OTA	Mean 4.47 μg/L	0.09–15.25 μg/L	[53,54]
Grape juice	29–85%	OTA	Mean 0.15–0.48 μg/L	0.010–5.3 μg/L	[50,55]
Vinegar	50–100%	OTA		0.22–6.4 μg/L	[50,55]
Raisins	60–98%	OTA	Mean 1.4–9.2 μg/kg	Max 26–250 μg/kg	[2,33,50]
Dried figs	3–100%	OTA	Median < 0.12 μg/kg	<0.12–6900 μg/kg	[2,56]
Apples rotten areas	30/30	PAT	1000 μg/kg	2–11,3000 μg/kg	[2]
Apples, remainders	30/30	PAT	300 μg/kg		[2]
Blueberries	1/12	PAT	21 μg/kg		[2]
Cherries	9/10	PAT	113 μg/kg		[2]
Strawberries	8/10	PAT	145 μg/kg		[2]
Raspberries	3/5	PAT	746 μg/kg		[2]
Apple juice	3–100%	PAT	Mean 1–140 μg/L	0.5–1150 μg/L	[57–60]
Apple juice conc	78–100%	PAT		7–376 μg/L	[57–60]
Cider mills	19%	PAT	36.9 μg/L	4.6–467.4 μg/L	[62]
Retail cider	28%	PAT	24.2 μg/L	15.3–35.2 μg/L	[62]
Apple puree	4/8	PAT	Mean 63.2 μg/kg	4–221 μg/kg	[63]
Apple marmalade	6/26	PAT	Mean 8.4 μg/kg	3–39 μg/kg	[63]
Pear marmalade	1/6	PAT	Mean 4.8 μg/kg	2–25 μg/kg	[63]

AOH nor AME were detected, suggesting that flavedo works as barrier to such substances.

The natural occurrence of *Alternaria* toxins in processed foods is of interest from the human health viewpoint. AOH has been detected in apple juice, wine, grape juice, cranberry juice, raspberry juice, and prune nectar. AME has been detected in apple juice, wine, grape juice and prune nectar. However these levels were very low (<1.5 μg/L) except in apple and grape juice and in red wines [2,40]. Lau et al. [45] reported natural occurrence of AOH and AME in apple juice, at levels ranging from 0.04 to 2.40 μg/L and from 0.03 to 0.43 μg/L, respectively. Other fruit juices such as grape juice had levels of 1.6 and 0.23 μg/L for AOH and AME, respectively, prune nectar 5.5 and 1.4 μg/L and cranberry nectar 5.6 and 0.7 μg/L. Low levels have also been detected in raspberry juice [40,45]. In apple juice concentrates from Spain both mycotoxins were found as natural contaminants in 50% of the samples analyzed. Levels of AOH were in the range of 1.35–5.42 μg/L. AME was present in most cases only at trace levels, and the highest amount detected was 1.71 μg/L in one sample [46]. The presence of these mycotoxins has also been reported in wines. AOH occurs very frequently

at low levels in red wine [40]. AOH was found in 13/17 Canadian red wines at levels of 0.03–5.02 μg/L and in 7/7 imported red wines at 0.27–19.4 μg/L, accompanied by lower concentrations of AME. White wines contained little AOH/AME (≤1.5 ng/mL). To our knowledge, there are no studies on co-occurrence of *Alternaria toxins* with other mycotoxins in fruits.

Ochratoxin A

The growth of *A. ochraceus* occurred over the temperature range 8–37 °C with an optimum of about 30 °C on barley grains. The highest amounts of OTA were obtained at 0.98 a_w. Both growth and OTA production increased with increasing a_w levels until 0.96–0.98 with 0.83–0.87 being the minimum a_w for OTA production [47]. OTA is common in cereals, beans and coffee and also in dried fruits and beverages such as beer, wine and grape juices. Engelhardt et al. [48] showed that damaged or moldy fruits can be contaminated with ochratoxin A to a certain degree, even after the removal of the rotten parts. They analyzed different fruits after removal of rotten tissue and found up to 2.71 μg/kg in cherries and up to 1.44 μg/kg in strawberries. Peaches and apples were also contaminated with OTA but in lesser degree (Table 2).

Wine is considered the second major source of human exposure to OTA after grain foods. Ochratoxin A was first detected in wines by Zimmerli and Dick [49]. Since then, the occurrence of OTA in different wines originating from various countries, mainly Mediterranean countries, has been reported. Red wines are frequently more contaminated than dessert, white or rosé wines. These differences have been explained by the wine-making techniques, the latitude of the production region (the lower the latitude, the occurrence is more frequent and the concentration greater), and by the weather conditions [50–53]. Southeast Spain, southeast France, southeast Italy and Greece were identified as of high risk. Wines with longer or double fermentation contain lower concentrations of OTA [53,54]. These authors analyzed 121 representative special wines from Europe. The wine groups with the highest OTA content and occurrence (>90%) were those where the must was fortified before fermentation (mean of 4.48 μg/L) and those made from grapes dried by means of sun exposure (mean of 2.77 μg/L). Fortified wines with long aging in wooden casks were about 50% contaminated, with OTA levels below 1.00 μg/L. Wines affected by noble rot, late harvest wines and ice wines did not contain OTA. Overall, 19.8% of the wines studied contained OTA levels above the MLs permitted in EU [54]. In a survey conducted by Soleas et al. [21] for detection of OTA in 942 wines, the mycotoxin was detected more frequently in red than in white wines, with the highest incidence in red wines from Spain and Argentina (Table 2).

Duarte et al. [55] reviewed the occurrence of OTA in different juices from Switzerland, Germany, Morocco and Brazil. They concluded that the most contaminated samples were grape juices. The pattern of contamination followed that of wine, i.e. red grape juices presented higher levels than white grape juices. The OTA incidence varied between 29% and 85% of the grape juice samples. The apple and orange juices were free of OTA, and black currant juices presented levels just above the limit of detection (LOD). These authors also reviewed works on OTA occurrence in vinegar. Vinegar, another grape derived product, was also found to be contaminated very frequently; 50–100% of the samples with maximum levels ranging from 0.22 to 6.4 μg/L. Balsamic vinegar was the most contaminated (Table 2). Similar results were reported previously by Battilani et al. [50] for grape juices from Spain and Germany and for vinegar from Italy and Germany.

OTA contamination of dried vine fruits has been examined in several countries and maximum levels ranging from 26 to 250 μg/kg have been reported. The incidence and median levels ranged between 60–98% and 1.4–9.2 μg/kg, respectively [2,33,50].

Dried figs have also been found in numerous surveys to contain OTA, however the incidence and levels reported varied considerably, from 3 to 100% and between <0.12 and 6900 μg/kg, respectively [2,56].

Patulin

The temperature range for *P. expansum* growth and patulin production is 0–24 °C. Minimum a_w for patulin production is 0.99 [47]. Patulin has mainly been found in apples and apple products and, occasionally in other fruits such as pears, apricots, peaches and grapes and it is mainly produced in rotten parts of the fruits [2,57]. High levels can also occur in different berries [2] (Table 2). Patulin has also been detected in visibly mouldy dried figs in Turkey [27,39].

Several surveys on levels of patulin contamination in apple juice and apple juice concentrates have been conducted worldwide [57–60] (Table 2). The high incidence of patulin observed indicates the need for improving production techniques by industry in order to reduce the incidence and level of patulin contamination in apple juices. The amount of PAT in the juices can be reduced after removal of the rotten or damaged fruit but cannot be eliminated completely as the mycotoxin diffuses into the healthy parts of the fruit. The largest amounts of PAT were found within 1 cm of the damaged area. No mycotoxin was detected at a distance of 2 cm from an area infected by *P. expansum* [22,61].

Patulin was also detected in 18.7% of cider mill samples, with 11 samples (2.2%) having patulin concentrations higher than 50 μg/L. Among retail grocery store samples, 28% of cider samples contained detectable patulin but lower than 50 μg/L [62] (Table 2). One study in Argentina in apple and pear products showed a high incidence of positive samples, mainly in apple puree (50%) with a mean concentration of 63.2 μg/kg [63] (Table 2).

Analytical methods

The fact that most mycotoxins are toxic at very low concentrations makes it necessary to have sensitive and reliable methods for their detection. Recent reviews on analytical methods for the determination of mycotoxins are available in the literature [15,64–68]. A number of different analytical methods have been applied to mycotoxin analysis due to their varied structures. These include widely applicable liquid chromatography (LC) methods with UV or fluorimetric detection (FLD), which are extensively used in research and for legal enforcement of food safety legislation and regulations in international agricultural trade. Other chromatographic methods, such as thin layer chromatography (TLC) and gas chromatography (GC), are also employed for the determination of mycotoxins, whereas recent advances in analytical instrumentation have highlighted the potential of LC–mass spectrometric (MS) methods, especially for multi-toxin determination and for confirmation purposes. Because different mycotoxins can be present in the same matrix, analytical methods for the simultaneous determination of different mycotoxins have been developed recently. Various *Fusarium* mycotoxins, OTA and aflatoxins can be analyzed on cellulose filters and in fungal cultures [69], in corn feeds and peanut butter [70] and in spelt, rice and barley grains [71]. Monbaliu et al.

[72] developed a multi-mycotoxin LC/tandem MS method for the determination of these mycotoxins and also *Alternaria* toxins (in total 23 mycotoxins) in sweet pepper. However to our knowledge, there are no multi-mycotoxin methods available for the determination of different groups of mycotoxins in fruits or in processed fruit products.

Conventional chromatographic methods are generally time consuming and capital intensive, and hence a range of methods, mostly based on immunological principles, have been developed and commercialised for rapid analysis. These methods include, among others, enzyme-linked immunosorbent assay (ELISA), direct fluorimetry, fluorescence polarization, and various biosensors and strip methods. Direct and indirect ELISA methods have been developed for the detection of aflatoxins and *Fusarium* toxins in cereals and also for OTA and PAT in wines and food samples. A description of these studies can be found in the reviews cited above [66,68].

The sampling stage is one of the most critical steps in any analysis and this is particularly the case with mycotoxins, where the contamination is known to be extremely heterogeneous. No sufficient sampling plans have been developed to cover completely the range of matrices and mycotoxins. Moreover little work has been done in validating the procedures of grinding, mincing or homogenizing samples [73,74].

Aflatoxins

The detection of AF in extremely low quantities in food and feed is important and requires sophisticated sampling, sample preparation, extraction and analytical techniques. The analysis of AF can be carried out using different strategies. In the sample clean up of AF inmunoaffinity columns (IACs) have nearly replaced other methods such as liquid–liquid partitioning and solid-phase extraction (SPE). Comparing the clean-up methods, IACs show the highest selectivity. The chromatographic method of choice for aflatoxin detection is LC-FLD; however, aflatoxins have a weak native fluorescence which can be enhanced by pre- or post-column derivatization. Immunobased techniques such as ELISA have many advantages since no clean up is required. However, drawbacks of the ELISA are cross reactivities of the antibodies, which can lead to false positive results [67].

Alternaria toxins

Alternaria mycotoxins, mainly AOH and AME, have been determined by TLC, GC and LC, mainly with ultraviolet detection, although fluorescence and electrochemical detectors have also been used. Methods of analysis have recently been reviewed [15]. Two ionization techniques, namely atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) were investigated for the LC–MS detection of AOH and AME in different fruit beverages. Both techniques offer much higher sensitivity and specificity than the conventional UV detection procedure. A combination of ESI with negative ion detection and tandem mass spectrometry (MS/MS) is the procedure of choice. A detection limit of sub-μg/L amounts of AOH and AME in fruit juice samples can be easily obtained. The clean-up of the juices was on C18 and aminopropyl SPE columns [45]. Similar methods have been developed for the determination of AOH and AME in wines [40] and tangerines [44].

Ochratoxin A

OTA is a colourless crystalline compound with blue fluorescence under UV light and weakly acidic character. The most widely used technique for the determination of OTA in fruits and their processed products is LC with FLD following a clean-up method involving SPE with an IAC [75,76]. Despite the fact that immunoextraction increases yields and eases the analytical protocol, it suffers from several drawbacks. Thus, over the last few years, many efforts have been made to substitute antibodies with combinatorial peptides, low mass synthetic ligands, aptamers and molecularly imprinted polymers. Among these different approaches, well-designed combinatorial peptides have great potential as capturing agents and allow good recoveries (>95%) at limits of quantification of 2 μg/L [77]. Confirmation of the presence of OTA in various matrices has frequently been achieved by LC–MS [78–80]. To routinely assay the concentrations of OTA in wines and beers, SPE on a C18 cartridge followed by LC with a photodiode array detector has been proposed and shown to have good recoveries at a limit of quantification (LOQ) of 0.10 μg/L [21].

Patulin

Liquid–liquid extraction (LLE) has been the traditional method of sample preparation for patulin analysis in food samples. LLE with ethyl acetate has been successfully validated through a collaborative study for patulin determination in clear and cloudy apple juices and apple puree and has been adopted by AOAC International as an official method. However, LLE is considerably expensive and time consuming. SPE and matrix solid-phase dispersion (MSPD) have been used by other analysts [81]. Wu et al. [82] showed that for apple juice concentrates and apple samples, a MSPD method was the most suitable for extracting patulin among the three extraction methods assayed. However for apple juices, SPE gave the best recovery rates. Several analytical methods have been proposed mainly using GC and LC [81]. MS has been coupled with both analytical LC and GC; MS–MS methods provide additional selectivity and increased sensitivity. LC–MS methods are more robust and reproducible than the corresponding GC–MS methods, although in many cases less sensitive. Sewram et al. [83] used an LC/APCI-MS/MS method with an ion trap analyzer (negative ion mode) for patulin analysis in apple juices, with a LOD of 4 μg/L and a LOQ of 10 μg/L. Takino et al. [84] carried out a comparative study between APCI and an atmospheric pressure photo ionization (APPI) technique for the determination of patulin in apple juice. APPI detection provided higher selectivity and a lower matrix effect than APCI. Quantitative GC–MS determinations of patulin are based on previous derivatization, such as trimethylsilyl or acetyl derivatives, and require isotopically labelled patulin as internal standard, which has not been commercially available until very recently. This recent commercialization increases the possibility of an exact quantification of this mycotoxin in complex matrices. A recent method [85] based on extraction of patulin with ethyl acetate–hexane, alkalinisation and silylation, and determination by GC–MS using $^{13}C_{5-7}$ patulin as internal standard has been developed. The method was successfully applied to the determination of patulin in apple fruit and apple products including juice, cider and baby food and also in quince fruit and quince jam.

Conclusions and future trends

The presence of aflatoxins, *Alternaria* toxins, ochratoxin A and patulin in fruits and their processed products such as juices, wines or cider is of high concern for human health due to their properties to induce severe acute and chronic toxicity at low dose levels.

There are increasing reports on different and less obvious sources of alimentary exposure, in addition to the conventional studied and worldwide consumed fruit matrices for these mycotoxins, i.e. apple for patulin, grape for ochratoxin and fig for aflatoxins. Because these mycotoxins are very stable even to heat processes and because they can diffuse from the rotten parts to healthy parts of the fruits, their presence, especially in processed products, is unavoidable. The occurrence of mycotoxins in juices is of high concern because children are one of the main consumers and because juice consumption is greater than that of wine. Consequently, improved monitoring programs should be encouraged. The co-occurrence of these different mycotoxins in the same matrix is another point that requires more studies from a toxicological and occurrence point of view.

Many analytical methods have been developed for the determination of each group of these mycotoxins in different matrices. However there are no analytical methods for their simultaneous determination in fruits and their processed products. The development of rapid screening methods is also advisable in order to increase the number of monitored samples.

Recently considerable efforts have been made to set maximum levels in many countries for the most important mycotoxins and in the most frequent commodities where they occur. However not all the possibilities are regulated and no regulation exists for the *Alternaria* toxins. The observed occurrence of the latter toxins on numerous fruits and the high toxicity of these toxins suggest that they may pose a hazard comparable to that from more widely studied mycotoxins.

Apart from the regulatory controls, three main strategies have been adopted to decrease or even eliminate the presence of the mycotoxins in foods [24,86]: prevention of mycotoxin contamination during the pre-harvest and post-harvest periods, detoxification of mycotoxins present in foods and inhibition of mycotoxin absorption in the gastrointestinal tract. Preventive measures aimed at the inhibition of mycotoxin formation in agricultural products are the most effective approach for avoiding consumer exposure. Good farm management, methods of culture to improve plant vigour, use of insecticides, fungicides and biological control, irrigation and cultivar selection ensure plants less vulnerable to stress. Post-harvest contamination can be avoided by controlling moisture, temperature and microbiological, insect and animal pests. Detoxification of mycotoxins by different physical, chemical and biological methods are less effective and sometimes restricted because of concerns of safety, possible losses in nutritional quality of the treated commodities and cost implications. Some of the most promising interventions studied to date involve the use of microorganisms to reduce absorption of mycotoxins from consumed foods in the gastrointestinal tract. Experimentally, clear evidence exists regarding the ability of probiotic bacteria to decrease the potential bioavailability of certain mycotoxins in humans but further studies are needed.

Exposure to mycotoxins is a serious risk to human health especially in the developing world where the application of modern agricultural practices and the presence of a legislatively regulated food processing and marketing system are less developed.

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