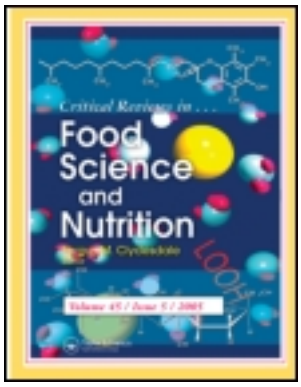

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Natural Occurrence, Analysis, and Prevention of Mycotoxins in Fruits and their Processed Products

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Mycotoxins are small toxic chemical products formed as the secondary metabolites by fungi that readily contaminate foods with toxins in the field or after harvest. The presence of mycotoxins, such as aflatoxins, ochratoxin A, and patulin, in fruits and their processed products is of high concern for human health due to their properties to induce severe acute and chronic toxicity at low-dose levels. Currently, a broad range of detection techniques used for practical analysis and detection of a wide spectrum of mycotoxins are available. Many analytical methods have been developed for the determination of each group of these mycotoxins in different food matrices, but new methods are still required to achieve higher sensitivity and address other challenges that are posed by these mycotoxins. Effective technologies are needed to reduce or even eliminate the presence of the mycotoxins in fruits and their processed products. Preventive measures aimed at the inhibition of mycotoxin formation in fruits and their processed products are the most effective approach. 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. This article reviewed the available information on the major mycotoxins found in foods and feeds, with an emphasis of fruits and their processed products, and the analytical methods used for their determination. Based on the current knowledge, the major strategies to prevent or even eliminate the presence of the mycotoxins in fruits and their processed products were proposed.

Keywords: Metabolites, fungus, control, contamination, detoxification, handling

INTRODCTION

Mycotoxins are toxic compounds produced by fungi, mostly by saprophytic moulds growing on a variety of foodstuffs (Logrieco, 2010) and they are a group of structurally diverse secondary metabolites (Figure 1) (Turner et al., 2009). However, the reason for the production of mycotoxins is not yet known as they are neither necessary for growth nor the development of the fungi (Fox and Howlett, 2008). Because mycotoxins weaken the receiving host, the fungus may use them as a strategy to better meet the environment for further fungal proliferation. Mycotoxin production can occur depending largely on the species of fungi. Fungi can infect agricultural crops during crop growth, harvest, storage, or processing. Some molds are capable of producing more than 1 mycotoxin and some mycotoxins are produced by more than 1 fungal species (Sakuda and Kimura, 2010). For example, ochratoxin (OTA) is produced by some species such as *Aspergillus ochraceus* mainly in tropical regions and by *Penicillium verrucosum* a common storage fungus in temperate areas, and in some cases, 1 species can form more than 1 mycotoxin. The mycotoxins include mainly aflatoxins, citrinin, cyclopiazonic acid, fumonisins, moniliformin, ochratoxins, patulin, sterigmatocystin, and trichothecenes. Aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids are the mycotoxins of the great agro-economic importance. Mycotoxins have adverse effects on humans, animals, and crops that result in illnesses and economic losses (Zain, 2010). The economic impact of mycotoxins include loss of human and animal life, increased health

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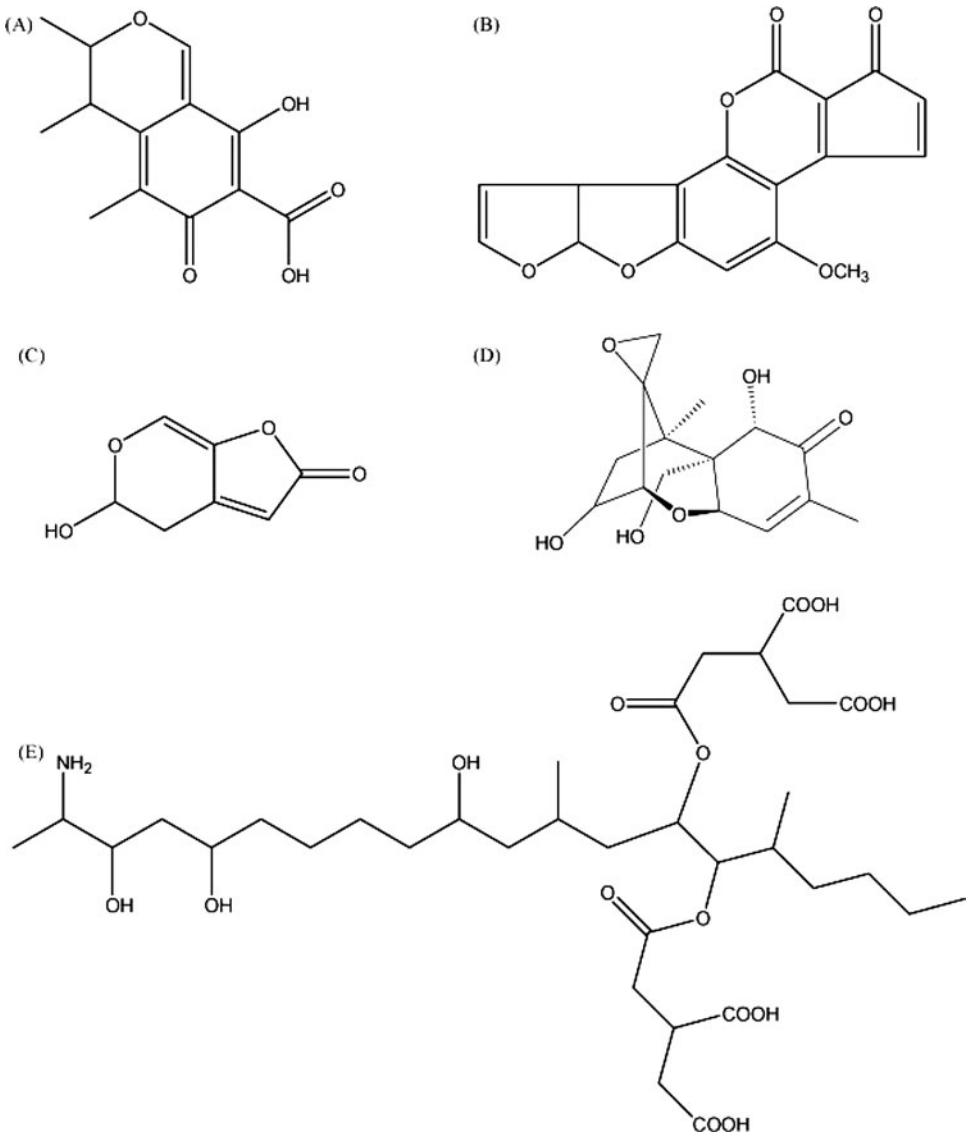

Figure 1 Examples of common mycotoxins demonstrating structural diversity of these compounds. A, citrinin; B, aflatoxin B1; C, patulin; D, deoxynivalenol; and E, fumonsin B1 (Turner et al., 2009).

care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce severity of the mycotoxin problem (Chassy, 2010).

The most commonly found in fruits and their processed products are aflatoxins, ochratoxin A, patulin, and *Alternaria* toxins (Drusch and Ragab, 2003; Barkai-Golan and Paster, 2008; Moss, 2008). These mycotoxins can contaminate food stuffs or human foods. The ingestion of these contaminated materials may be pathogenic in humans as they may lead to serious health problems, such as liver, kidney, or nervous system damage, immunosuppression, biphasic cellular response, and carcinogenesis (Bennett and Klich, 2003; Diesing et al., 2010; Maresca and Fantini, 2010).

Contaminations of mycotoxins were reported in a number of agricultural commodities, foods, feed stuffs as well as biological fluids at geographically diverse locations (Phillips et al., 1980; Gimeno and Martins, 1983; Heber et al., 2001; CAST, 2003; Marín et al., 2010; Meister, 2004; Soriano and Dragacci, 2004; Dashti and AL-Hamli, 2010; Yuan et al., 2010). Contamination can occur preharvest or postharvest, for example, deoxynivalenol and T-2 toxin produced by *Fusarium* (preharvest), or OTA by *Aspergillus* and *Penicillium* and aflatoxin (AFT) by *Aspergillus* (postharvest) (EMAN, 2003). A lot of mycotoxins have been identified in fruits and their processed products (Drusch and Aumann, 2005; Luttfullah and Hussain, 2010).

The maximum levels have been set for some mycotoxins in food and feed to protect animal and public health, as described in detail by Zain (2010). The worldwide contamination of foods and feed with mycotoxins is a significant problem. Due to the widespread nature of fungi in the natural environment, mycotoxins are considered unavoidable contaminants in food

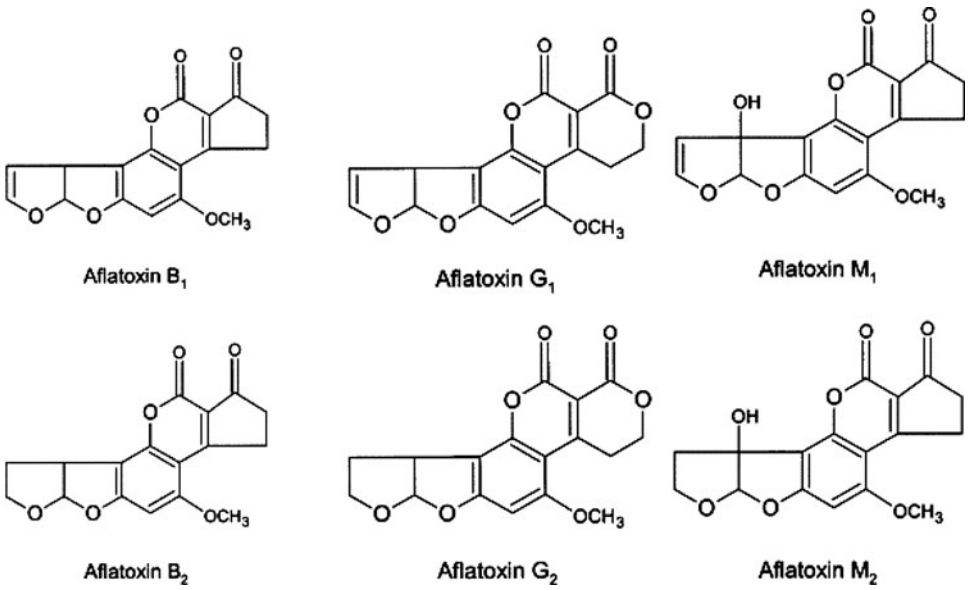

Figure 2 Chemical structure of aflatoxin B (AFB1 and AFB2), aflatoxin G (AFG1 and AFG2), and aflatoxin M (AFM1 and AFM2) (Zain, 2010).

and feed. On a worldwide basis, at least 99 countries established mycotoxin regulations for food and/or feed in 2003. Although efforts have continued internationally to set guidelines to control mycotoxins, practical measures have not been adequately implemented. Thus, mycotoxin management will meet an international challenge to ensure global food safety (López-García, 2010). It is important to develop rapid, sensitive, and reproducible assays to detect the presence of mycotoxins. At the same time, considerable efforts have been made to reduce or even eliminate the presence of the mycotoxins in foods and feeds. This article reviewed the available information on the major mycotoxins found in foods and feeds, with an emphasis of fruits and their processed products, and the analytical methods used for their determination. Based on the current knowledge, the major strategies to prevent or even eliminate the presence of the mycotoxins in fruits and their processed products were proposed.

TOXICITY OF MYCOTOXINS

Contamination of crops by fungal action has been noted for over 2 millennia. The improvements in food safety in developed countries have eliminated acute human mycotoxicosis, but such outbreaks still occur in rural communities in the developing world where aflatoxins, fumonisins, deoxynivalenol, ochratoxin, and zearalenone present in foods have been involved in the deaths or acute diseases reported (Shephard, 2008; Zain, 2010). The current literature on mycotoxin-related human diseases clearly reveals a linkage between ingesting mycotoxin-contaminated food and illness, especially hepatic, gastrointestinal, carcinogenic, and teratogenic diseases (Fung and Clark, 2004; Shephard, 2008). Based on the effects on human and animal health, aflatoxin, fumonisin, trichothecenes, ochratoxin, zearalenone, and patulin are recognized as the most important food mycotoxins. The most studied mycotoxins in fruits and their processed products have been AFT, OTA, and patulin. Detailed information on the toxicity of mycotoxins is given in 2 reports reviewed by Turner et al. (2009) and Fernández-Cruza et al. (2010).

MAJOR MYCOTOXINS

Aflatoxins 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 B1, B2, G1, and G2, based on their fluorescence under ultraviolet (UV) light (blue or green) and their relative chromatographic mobility (Figure 2) (Turner et al., 2009). They were first detected and characterized in the 1960s (Asao et al., 1965) and have been found in a variety of agricultural and food products. The LD_{50} of AFB1 ranges from 0.3 to 18 mg/kg depending on the animal species and routes of administration. Aflatoxins are classified by the International Agency for Research on Cancer as being carcinogenic to humans (Group 1) (World Health Organization, 1998; Fung and Clark, 2004; Shephard, 2008).

OTA is a phenylalanine-dihydroisocoumarin derivative (Figure 3) (Zain, 2010). The LD_{50} of OTA ranges from 0.5 mg/kg for dogs to over 50 mg/kg for mice and the toxicological profile of OTA includes teratogenesis, nephrotoxicity, and immunotoxicity (Soleas et al., 2001; Drusch and Ragab, 2003). Animal experiments have implicated cytochrome P450-related reactions and DNA adducts generation as possible mechanisms for the formation of renal tumours.

Patulin (PAT) is an unsaturated heterocyclic lactone. Various acute and chronic effects have been attributed to PAT (Drusch and Ragab, 2003; Fung and Clark, 2004; Sant'Ana et al., 2008).

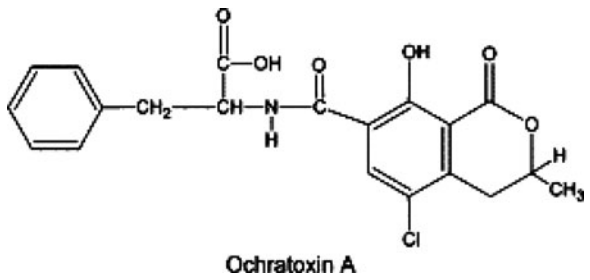

Figure 3 Chemical structure of ochratoxin A (Zain, 2010).

The LD_{50} of patulin ranges from 15 to 25 mg/kg and varies with animal species and route of exposure. The acute symptoms include lung and brain edema, liver, spleen, and kidney damage and toxicity to the immune system while the chronic symptoms include genotoxic, neurotoxic, immunotoxic, immunosuppressive, and teratogenic effects. At the cellular level, some examples of these effects by PAT are plasmatic membrane rupture and inhibitions of DNA, RNA, and protein syntheses (Drusch and Ragab, 2003; Fung and Clark, 2004; Sant'Ana et al., 2008; Zhou et al., 2010).

NATURAL OCCURRENCE OF MYCOTOXINS IN FRUITS AND THEIR PROCESSED PRODUCTS

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, alternariol monomethyl ether, and altenuene, altertoxin I and II, and tenuazonic acid—a tetramic acid. Culture extracts of *A. alternata* are mutagenic in various microbial and cell systems, and carcinogenic in rats. Ochratoxin A was originally isolated from *A. ochraceus* in 1965. Other *Aspergillus* species are also capable of producing OTA. *P. verrucosum* is the best known *Penicillium* species 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*. Several species of *Penicillium* and *Aspergillus* can also produce PAT. The most important producer of PAT is the apple-rotting fungus *Penicillium expansum*. However, a few literatures are available for the aflatoxins (except in dried figs) and the *Alternaria* toxins in fruits and their products, although the ubiquity and toxicity of the latter are well known. Very recently, some evidence for the presence of fumonisins B1 and B2 in fruits has been reported. Fumonisin B2 has been identified in visibly moldy dried figs (Senyuva and Gilbert, 2008). A high incidence of fumonisin B1 was found in dried figs in Turkey (Karbancioglu-Güler and Heperkan, 2008) while Logrieco et al. (2009) reported fumonisin B2 production by *Aspergillus* niger from grapes and natural occurrence in must. Table 1 gives the occurrence of mycotoxins in fruits and their processed products.

The extent of fungal growth and subsequent possible mycotoxin contamination depends largely on endogenous and exogenous factors (van der Westhuizen et al., 2010). 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 (Varga et al., 2010). 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. Various stages of the manufacturing process of apple juice are capable of reducing the amount on the final products on a certain extent, and the incidence of the mycotoxins throughout the world confirms their stabilities (Sant'Ana et al., 2008). The careful selection and proper storage of fruits are the most important implement reducing mycotoxins (Swanson, 1987; Drusch and Ragab, 2003).

THERMAL STABILITY OF MYCOTOXINS IN FRUITS AND THEIR PROCESSED PRODUCTS

Most mycotoxins are chemically stable during storage and processing, even when cooked at quite high temperatures of more than 90°C (Swanson, 1987). Dibenzo-α-pyrones alternariol (AOH) and alternariol monomethyl ether are stable on heating at 100°C in sunflower flour (Combina et al., 1999). Both mycotoxins are very stable in spiked apple juice at room temperature for up to 5 weeks and at 80°C after 20 min. Thus, mycotoxins are difficult to be degraded or decontaminated in fruits and their processed products.

EXTRACTION, SEPARAATION, AND/OR DETERMINATION OF MYCOTOXINS

The fact that most mycotoxins are toxic in very low concentrations requires sensitive and reliable methods for their detection. Due to the varied structures and chemical functions of these compounds, it is impossible to use one standard technique to detect all mycotoxins, as each will require a different method (Turner et al., 2009). Practical requirements for high-sensitivity detection and the need for a special laboratory setting create challenges for routine analysis. Depending on the physical and chemical properties of mycotoxins, more procedures have been developed around existing analytical techniques, which offer flexible and broad-based methods of detecting these compounds. The application of simpler, cheaper, and more effective solutions for the detection of mycotoxins is increasingly being required, due to their perceived importance, based around their toxicity and requirements of legislation for limits on amounts in foods. A successful detection method should be robust and sensitive, with a high degree of flexibility, over a wide range of compounds. All techniques should be reproducible to a high level, and the results gained must be relevant and easy to analyze. For fieldwork, the system should also be rapid and portable. Many

Table 1 Occurrence of mycotoxins in fruits and their processed products

Commodities	Toxins	Concentration range	Reference
Apple juice	AF-B_1 and AF-G_1	20–30 μg/l	Abdel Sater et al. (2001)
Dried raisins	AFT	Max. 2–550 μg/kg	Saxena and Mehrotra (1990), Trucksess and Scott (2008)
Dried figs	AFT	Max. 10–325 μg/kg	Juan et al. (1991), Drusch and Ragab (2003)
Rotten mandarins	AOH	1000–5200 μg/kg	Logrieco et al. (1990)
Tangerine flavedo	AOH	2.5–17.4 μg/kg	Magnani et al. (2007)
Apple juice	AOH	0.04–2.40 μg/l	Lau et al. (2003)
Red grape juices	AOH	0.03–0.46 μg/l	Scott et al. (2006)
Peaches	OTA	0.21 μg/kg	Engelhardt et al. (1999)
Cherries	OTA	2.71 μg/kg	Engelhardt et al. (1999)
Strawberry	OTA	1.44 μg/kg	Engelhardt et al. (1999)
Apple	OTA	0.41 μg/kg	Engelhardt et al. (1999)
Grape juice	OTA	0.010–5.3 μg/l	Battilani et al. (2006)
Vinegar	OTA	0.22–6.4 μg/l	Battilani et al. (2006)
Raisins	OTA	Max 26–250 μg/kg	Drusch and Ragab (2003), Battilani et al. (2006), Trucksess and Scott (2008)
Dried figs	OTA	<0.12–6900 μg/kg	Drusch and Ragab (2003), Karbancioglu-Güler and Heperkan (2008)
Apples rotten areas	PAT	2–11,3000 μg/kg	Drusch and Ragab (2003)
Apple juice	PAT	0.5–1150 μg/l	Leggott and Shephard (2001), Cheraghali et al. (2005), Murillo-Arbizu et al. (2009)
Apple juice conc	PAT	4.6–467.4 μg/l	Harris et al. (2009)
Apple puree	PAT	3–39 μg/kg	Funes and Resnik (2009)

of the techniques described below have been combined to form protocols, which are most used in laboratories today.

Sample Pretreatment Methods

Accurate determination of a mycotoxin relies on correct extraction and clean-up methods except perhaps enzyme-linked immunosorbent assay (ELISA) which may not require clean-up (Chu, 1992; Şenyuva and Gilbert, 2010). The extraction method used to remove the mycotoxin from a biological matrix is dependent largely on the structure of the mycotoxin. Polar metabolites, such as the fumonisins, require the presence of water, aided by the presence of organic solvents (Shephard, 1998). Hydrophobic mycotoxins such as AFT rely on use of organic solvents (Holcomb et al., 1992). These can be direct extractions, or may be partitioned with other solvents, such as *n*-hexane for partial clean-up, to remove excess components of the biological matrix. The choice of an appropriate extraction solvent is also dependent on the matrix from which the extraction is required, as the inherent components of the mixture can affect it (Wilkes and Sutherland, 1998). The clean-up procedure used in a protocol is the most important step, as the purity of the sample affects the resolution of separation and sensitivity of the results (Scott, 1995). Trace amounts of a target molecule may be masked by interfering compounds, found not only in the matrix but in solvents used in the technique. Glassware should also be free of contamination, such as alkaline detergents, which can form salts with the compounds and then interfere detection or result in false results.

Liquid–liquid Extraction

Liquid–liquid extraction involves exploiting the different solubility of the mycotoxins in 2 immiscible phases, to extract the target compounds into 1 solvent leaving the rest of the matrix in the other. For example, solvents such as hexane and cyclohexane are commonly used to remove nonpolar contaminants, for example, lipids and cholesterol. The procedure is effective for several mycotoxins and works well in small-scale preparations (Bauer and Gareis, 1987). Holcomb et al. (1996) reported supercritical fluid extraction (SFE) extraction of aflatoxins from corn. However, the method is time consuming and may cause loss of part of targets.

Solid Phase Extraction

The basic principle of solid phase extraction (SPE) technology is a variation of chromatographic techniques based on around small disposable cartridges packed with silica gel, or bonded phases which are called the stationary phase. The sample is loaded in one solvent, generally under reduced pressure, rinsed, where most of the contaminants are removed, and eluted in another solvent (EMAN, 2003; Kataoka et al., 2010). These cartridges have a high capacity for binding small molecules. These cartridges contain different bonding phases, ranging from silica gel, C-18 (octadecylsilane), florisil, phenyl, aminopropyl, ion-exchange materials, both anionic and cationic, to affinity materials such as immunoadsorbents and molecular imprinted polymers (Visconti and Pascale, 1998; Sharma and Marquez, 2001; Visconti et al., 2000; Zambonin et al., 2002; Muñoz et al., 2006; Giraudi et al., 2007; Katerere et al., 2008; Vatinno et al., 2008). Anion exchange bonded phases provide good clean-up of extracts (Sydenham et al., 1992). The SPE systems have many advantages, for example, they use considerably less solvent and are faster in operation. In addition to cleaning sample, they can also be used to preconcentrate the sample providing better detection results. SPE has found widespread use and is an integral part of many extraction and detection protocols.

Table 2 Examples of SPE methods used in the extraction and clean-up of mycotoxin samples

Toxin	SPE phase	Matrix	Reference
Alternariol	C-18-RP	Apple juice	Delgado et al. (1996)
Fumonisins	C-18-RP, SAX, Immunoaffinity	Various	Shephard (1998)
Patulin	Silica gel SPE column	Dried apple rings	Katerere et al. (2008)
Trichothecenes	C-18-RP	Grain	Radová et al. (1998)

Giraudi et al. (2007) described a preparation of a hexapeptide library by combinatorial synthesis and then identified a peptide which showed good affinity with OTA. In another study, a 2-dimensional extraction procedure employed SPE for the extraction of OTA (Maier et al., 2004). Furthermore, detection of OTA using solid phase micro extraction-coupled with liquid chromatography–mass spectrometry–mass spectrometry (SPME–LC–MS/MS) has been reported (Vatinno et al., 2008). High throughput was achieved by simultaneous preparation of up to 96 samples using multifiber SPME (solid phase micro extraction) device and multiwell plates. Katerere et al. (2008) reported the extraction of patulin using SPE and isocratic separation on high-performance liquid chromatography coupled with diode array detector (HPLC-DAD). Using this method, the accuracy and linearity at 10–50 ppb were obtained. Nowadays, SPE is by far the most popular technique used in routine analysis of mycotoxins. Table 2 presents some examples of SPE methods used in the extraction and clean-up of mycotoxin samples. Recently, solid-phase extraction using molecularly imprinted polymers for selective extraction of a mycotoxin has been developed and the method has exhibited a high selectivity and significant capacity of the molecularly imprinted polymers to extract OTA (Ali et al., 2010).

Silica gel is a very popular material, frequently used for SPE. The surface of silica particles is heterogeneous, with a variety of silanol groups which can bind target compounds through multiple electrostatic interactions. The addition of various functional groups can widen the application of this material. Normally, this is achieved by reacting an organosilane with a long chain aliphatic derivative (e.g., C-18 phase). Silica gel can be used directly or after modification for mycotoxin detection. More frequently, however, it is a hydrophobic phase which is used in environmental and food analyses of these toxins, performed in both polar and nonpolar solvents. Leitner et al. (2002) demonstrated the use of C-18 reverse phase materials in the extraction of OTA. They have shown that the use of cartridges made of this material combined with mass spectroscopy offers good results (detection at sub-ppb level) which are comparable to clean-up with OTA-specific immunosorbents.

Ion-exchange materials are often used in SPE to isolate and preconcentrate mycotoxins found in aqueous solutions. They utilize electrostatic interactions formed between the target molecule and charged groups bonded to the silica material. The samples are set to a pH value where both groups are charged, and filtered through the SPE cartridge. The bound material is removed by the addition of a strong ionic solution or by altering pH. Several types exist in both anionic and cationic phases. Strong anion exchange (SAX) is the favored material for mycotoxin extraction. Pussemier et al. (2006) developed a sensitive protocol for OTA, citrinin (CIT), or deoxynivalenol (DON) detection and quantification using SAX columns in clean-up.

Mycosep^{TM} columns are made up of several absorbents specifically selected for recovery of individual mycotoxins, packed into a plastic tube, and used to remove the entire matrix leaving the desired compound in solution on top of the column (Akiyama et al., 2001; Mateo et al., 2002). This method is practical, portable, and quick with no additional rinsing steps required. However, columns are designed per analyte and, therefore, not useful in multitoxin analysis. A Mycosep^{TM} column specific for OTA has been developed, which works in tandem with other columns to ensure clean-up from a variety of matrices. With several companies working on development of newer columns for different mycotoxins, it is expected that Mycosep^{TM} columns would be used for more applications in future.

Antibody has been intensively investigated over the past few years, and has been shown to be of great value in the mycotoxin analysis (Zimmerli and Dick, 1995; Scudamore and MacDonald, 1998; Visconti and Pascale, 1998; Visconti et al., 2000; Mateo et al., 2002). They offer excellent recovery of analyte because of the specificity gained by using monoclonal or polyclonal antibodies. The main disadvantage in using these materials is high costs involved as each column can only be used once due to denaturation of antibodies. This has been taken into account with further development of sequential injection immunoassay (Garden and Strachan, 2001).

Separation and/or Determination Methods

Recent trends in sample separation include miniaturization, automation, high-throughput performance, and reduction in solvent consumption and operation time. There are several types of chromatographic methods available for mycotoxin analysis (Shephard, 1998; Valenta, 1998; Vaclavik et al., 2010), which will be introduced in more details in the following sections.

Thin Layer Chromatography

Traditionally, the most popular method used for mycotoxins analysis is thin-layer chromatography (TLC), which offers the ability to screen large numbers of samples economically. There are good reviews available on the applications of TLC for mycotoxin analysis (Betina, 1985, 1993; Scott, 1985, 1987;

Table 3 Examples of TLC protocols used for the detection of common mycotoxins

Toxin	Protocol	Mode of Detection	Matrix	Reference
Citrinin	Silica gel precoated G-25 HR TLC plates	Fluorescent	Apples and pears	Gimeno and Martins (1983), Martins et al. (2002)
Citrinin	Silica gel 60 TLC	Fluorescent	Fungi isolated from grapes	Abrunhosa et al. (2001)
Trichothecenes	Comparison with HPLC for detection	UV and fluorescent	Food	Tomlins et al. (1989)
OTA	Silica gel	Fluorescence densitometry	Rice	Abramson et al. (1999a)

Holcomb et al., 1992; Kok, 1994; Lin et al., 1998). The use of TLC analysis for mycotoxins is still popular for both quantitative and semiquantitative purposes (Table 3), due to its high throughput of samples, low operating cost, and ease of identification of target compounds with an aid of UV-V is spectral analysis. In this context, several methods have been developed to obtain the best results with each separate class of mycotoxin and both 1-dimensional and 2-dimensional analyses are used frequently (Gimeno, 1984; Eppley et al., 1986; Lin et al., 1998). However, one of the major requirements for TLC is that there is an inherent need for sample preparation, the type of clean-up protocol dependent on properties and the type of the mycotoxin in study. Silica gel layer seems to be one of the common layers used for TLC while octadecyl bonded ones seem to be the most widely used (Stahr et al., 1985; Edwards and Lillehoj, 1987; Frohlich et al., 1988). Phenyl nonpolar bonded (Bradburn et al., 1990), silanized (Tomlins et al., 1989), and impregnated with organic acid (Colinski and Rabarkiewicz-Szcesna, 1984) were also attempted to separate mycotoxins.

TLC has been reported to be used to assess mould spoilage of *C. sativa* nuts commercially sold in Canadian grocery stores (Overy et al., 2003). Three mycotoxigenic fungi dominating the samples were *P. crustosum*, *P. glabrum*/*spinulosum*, and *P. discolor*. A 1-dimensional TLC method, combined with immunoaffinity columns (IAC)-based clean-up, was used to determine AF in various food matrices (Stroka et al., 2000). Medina and Schwartz (1992) reported a TLC analysis for detection of estrone, estradiol, diethylstilbestrol, zearalanol, zearalanone, and zearalenol. TLC has been also used for the detection of diphenylindenone sulphonyl and esters of TRC mycotoxins (Turner et al., 2009). While TLC is still used continuously, improvement of sample preparation, further development of more sensitive analytical system and improved automation in terms of enhancing its performance could also make TLC more popular tool in future.

High-Performance Liquid Chromatography

Modern analysis of mycotoxins relies heavily on HPLC in 2 aspects: (1) interactions between the HPLC column (adsorbents) and the mycotoxins and (2) detection methods such as UV or fluorescence which relies largely on the presence of a chromophore in the molecules. A number of toxins already have natural fluorescence (e.g., OTA, AFT, and CIT) and can be detected directly by HPLC-fluorescence detector (FD) (Elizalde-Gonzalez et al., 1998). However, some mycotoxins, such as fumonisins produced by *Fusarium* genus, lack a suitable chromophore, so that their determination requires chemical derivatization (Shephard, 1998; EMAN, 2003). Some derivatizing agents, for example, *o*-phthaldialdehyde and 9-(fluorenylmethyl) chloroformate (Holcomb et al., 1993), can be employed in either pre- or postcolumn (Neely and Emerson, 1990; Kussak et al., 1995; Jimenez et al., 2000; Chiavaro et al., 2001). HPLC has been a standard for mycotoxin detection in food industry (Valenta, 1998). For example, reverse phase (RP)-HPLC coupled to sensitive FD was employed to analyze OTA formation (Hernández et al., 2006), which allowed estimation of OTA in 0.01 ng/ml concentration. In similar study, OTA contamination in dried figs, which was extracted by methanol and orthophosphoric acid followed by clean-up by an IAC, was investigated using HPLC-FD with the limits of detection (LOD) for OTA of 0.12 μg/kg (Karbancioglu-Güler and Heperkan, 2008). HPLC has also been employed for PAT detection in commercial pure apple juices and mixed apple juices marketed in Italy (Spadaro et al., 2007). There have been several other mycotoxins that have been determined using HPLC methods (Josephs et al., 1998; Ambrosino et al., 2004; Xu et al., 2006; Gonçalez et al., 2008). AFT and cyclopiazonic acid were also detected using HPLC method in Brazilian peanuts (Gonçalez et al., 2008). The main benefit of using HPLC along with the high quality of separation and low LOD provides the possibility to combine multiple detection systems (fluorescent, UV, and diode array) with this technology, allowing for multiple detections of compounds from one sample. For instance, an HPLC-FD has been used for simultaneous detection of *Fusarium* mycotoxins [zearalanone (ZON), nivalenol, and DON) in cereals (Tanaka et al., 1985). ZON in food has been determined using clean-up by either conventional SPE or IAC and detected by HPLC-MS with an atmospheric pressure chemical ionization interface (Tanaka et al., 1985; Rosenberg et al., 1998). Application of APCI-MS detection increased the sensitivity by a factor of ca. 50 in comparison with HPLC-FD. Due to the selectivity of MS detection, it also was possible to quantitatively determine ZON both in raw extracts without clean-up. Leitner et al. (2002) presented an excellent comparison of MS and FD for OTA analysis. Furthermore, a LC-MS/MS method for multimycotoxin determination was developed for 19 analytes and was applied for a semiquantitative screening of 87 moldy food samples from private households, including bread, fruits, vegetables, cheeses, nuts, and jam (Sulyok et al., 2010). Table 4 lists some examples of protocols using HPLC for common mycotoxin analyses. MS became the industry standard detection method allowing highly

Table 4 Examples of protocols using HPLC for common mycotoxins

Toxin	Protocol	Detection	Matrix	Reference
Aflatoxins	UV-HPLC	Spectrofluorimetric	Brazilian peanut kernels	Gonçalez et al. (2008), Afsah-Hejri et al. (2010)
Aflatoxin B1 and OTA	RP- HPLC	Fluorescent	Gapes	Khoury et al. (2008)
Aflatoxin B1, citrinin, and OTA	Normal HPLC	Fluorescent	Various samples	Nguyen et al. (2007), Brera et al. (2010)
Aflatoxins, the aflatoxin metabolite M1, ochratoxin a	Ultra HPLC-MS	Orthogonal Z-spray-electrospray interface.	Food	Beltrán et al. (2010)
Beauvericin	HPLC followed by confirmation by LC-MS	Diode array detector	Maize	Ambrosino et al., (2004)
Citrinin	Normal-HPLC	Fluorescent	Corn	Abramson et al. (1999b)
Citrinin	Normal-HPLC	Fluorescent	Cereals	Meister (2004)
Citrinin	Many chromatographic techniques compared	Various detection methods	Various samples	Xu et al. (2006)
Ergosterol	HPLC compared with ELISA and TLC	Various detection methods	Various samples	Janardhana et al. (1999)
Fuminosin	(HPLC-ELSD)	Evaporative laser scattering detection	Corn samples	Wang et al. (2008)
Patulin	Microemulsion electrokinetic chromatography for quantification	UV and fluorescent	Commercial apple juice	Murillo-Arbizu et al. (2008)
Patulin	Normal HPLC	UV diode array detector	Fruit juice	Spadaro et al. (2007), Moukas et al. (2008)
Penitrem A, chaetoglobosin A and C, emodin, and OTA	Normal HPLC	Diode array detection	Extracts prepared from naturally infected nut tissue	Overy et al. (2003)
Trichothecene	HPLC analysis after SFE	Diode array detection or GC with electron capture detection	Wheat	Josephs et al. (1998)
OTA	Normal HPLC	Fluorescent	Dried figs	Karbancioglu-Güler and Heperkan, (2008)
OTA	Reversed-phase coupled to HPLC	Fluorescent	Grape	Hernández et al. (2006)
Zearalenone	Atmospheric-pressure chemical ionization mass spectrometry	Fluorescent	Food	Rosenberg et al. (1998)
Deoxynivalenol, T-2	Direct analysis without derivatization	GC-MS	Cereals	Onji et al. (1998)
Deoxynivalenol and OTA	GC-MS combined with electronic nose	GC-MS	Barley grains	Olsson et al. (2002)
Trichothecenes	Trichothecenes in fungal cultures using GC-tandem MS	GC-MS	Fungal cultures	Nielsen and Thrane (2001)
Sterigmatocystin	GC-MS along with other methods for comparison such as LC-MS, LC-MS/MS, and LC-UV methods for STE determination	UV detection	Food samples	Tanaka et al. (2007)

accurate and specific detection of mycotoxins. However, the use of MS as an analytical tool will also add the high cost of equipment, complex laboratory requirements, and limitations in the type of the solvents used in extraction and separation.

Gas Chromatography

Gas chromatography (GC) is regularly used to identify and quantify the presence of mycotoxins in food samples, by which many protocols have been developed for these samples. Normally, the system is linked to MS, flame ionization detector or Fourier transform infrared spectroscopy detection techniques (Onji et al., 1998). However, most mycotoxins are not volatile and, therefore, have to be chemically derivatized prior to GC analysis (Scott, 1995). So far, several techniques have been developed for the derivatization of mycotoxins. For example, chemical reactions such as silylation or polyfluoroacylation are employed in order to obtain a volatile material (Scott, 1995). Several examples of OTA detection utilizing derivatization have been reported (Jiao et al., 1992). This approach gave a LOD of 0.1 μg/kg. In general, the use of GC detection is not expected for commercial protocols due to existence of cheaper and faster alternatives such as HPLC.

Capillary Electrophoresis

The individual detection of closely related mycotoxins requires sophisticated separation technique. The effective separation of components can be based upon charge- and mass-dependent migration in an electrical field. The fast separations

can be accomplished by capillary electrophoresis in aqueous buffer solutions, excluding the need for organic solvents. The combination of CE with sensitive fluorescence-based detection methods have been described for AFT (Pena et al., 2002) and fuminosin (Wilkes and Sutherland, 1998). Maragos and Appell (2007) and Murillo-Arbizu et al. (2010) reported the determinations of zearalenone in maize and patulin in apple juice using capillary electrophoresis, respectively, with the high selectivity, satisfactory recovery, and low-limit detection.

OTHER DETERMINATION METHODS

Colorimetric Technique

Some mycotoxins such as citrinin have a conjugated, planar structure that gives natural fluorescence, which makes it feasible for qualitative and quantitative determination by using a fluorometer. Rapid semiquantitative fluorimetric assay was used for citrinin testing in peanuts (Trantham and Wilson, 1984). Under an acidic environment condition, the weak native fluorescence of citrinin could be remarkably enhanced. Due to diverse influencing factors of the colorimetric technique, relatively low sensitivity restricted the commercial analysis of citrinin, but this analysis procedure is rapid and bears a low cost for analyzing a large number of samples. In addition, this technique allows the high-throughput analysis of a large number of industrial samples. Yang et al. (2010) reported an aptasensor for OTA using unmodified gold nanoparticlesindicator. The method exhibited a linear range for a wide OTA concentration from 20 to 625 nM, with the detection limit of 20 nM. However, the colorimetric detection method requires a tedious sample preparation of extraction and clean-up. Besides, there is loss of mycotoxins during sample treatment, or due to instable chromatographic behavior or relatively low sensitivity and recovery.

Bioassay Technique

Bioassays have become increasingly useful for mycotoxin detection (Watson and Lindsay, 1982; Yates, 1986) as an alternative of chemical analysis. Bioassay provided a rapid means for screening samples and allowed the analyst to make an informed decision. Cheli et al. (2008) reported the detection of mycotoxin-producing fungi and mycotoxin toxicity cell-based bioassays as rapid tools for evaluating food and feed mycotoxin contamination. Mitterbauer et al. (2003) reported the bioassay of strain YZRM7 allowed qualitative detection and quantification of total estrogenic activity in cereal extracts without requiring additional clean-up steps. Its high sensitivity makes this assay suitable for low-cost monitoring of contamination of maize and small grain cereals with estrogenic *Fusarium* mycotxins.

Immunoassay Technique

Immunochemical methods provided a convenient and sensitive alternative for detecting many mycotoxins due to their relatively low cost and easy application (Morgan, 1989; Chu, 1991; Goryacheva et al., 2007; Guan et al., 2011). Commercially available ELISA kits for detection of mycotoxins are normally based on a competitive assay format that uses either a primary antibody specific for the target molecule or a conjugate of an enzyme and the required target (Morgan, 1989; Pestka et al., 1995; Stanker et al., 2008). The simultaneous detection of multiple mycotoxins (aflatoxins B1, zearalenone, and HT-2) by the giant magnetoresistive-based immunoassay was also developed, with a detection limit of 50 pg/ml (Mak et al., 2010). A commercial ELISA kit was used to determine OTA concentrations in commonly consumed food items in Sri Lanka (Wanigasuriya et al., 2008). Table 5 presents some examples of protocols and detection limits using ELISA for the most common mycotoxins. An indirect competitive ELISA using rabbit antisera was firstly performed to detect citrinin in buffer solutions at 1–13 ng/ml (0.05–0.65 ng per assay) (Abramson et al., 1995). A direct competitive ELISA was established also, with citrinin coupled to horseradish peroxidase as the labeled antigen (Abramson et al., 1999a). More recently, a competitive ELISA for determination of citrinin concentration in dietary supplements was developed (Heber et al., 2001). Direct ELISA is quick since only 1 antibody is used and cross reactivity of secondary antibody in some cases is completely eliminated, but they suffer from the fact that immunoreactivity of the primary antibody may be reduced as a result of labeling and signal amplification becomes difficult. Conversely, indirect ELISA has several advantages, in the sense that a wide range of labeled secondary antibodies are commercially available. Indirect ELISA is generally more sensitive because each primary antibody contains several epitopes that can be bound by the labeled secondary antibody thereby enhancing signal amplification. However, the cross-reactivity may occur with the secondary antibody, resulting in nonspecific signal. Thus, the results of ELISA require to be confirmed further by other analytical methods such as sensitive HPLC. The antibodies developed for ELISA were also used for self-made immunoaffinity columns for HPLC extract clean-up (Vrabcheva et al., 1999). In addition, based on specific mycotoxin-monoclonal as well as polyclonal antibodies, a strip tests for mycotoxin screening of food and feed was developed (Cvak et al., 2010; Xu et al., 2010).

Biosensor Technique

While highly specific and sensitive, traditional immunochemical assays such as enzyme-linked immunosorbent assays are adopted, the rapid and easy use of biosensors has been developed. Paniel et al. (2010) reported the development of an electrochemical immunosensor for the detection of aflatoxin M_1 while a screen-printed carbon electrode is chosen as the transducer. Actis et al. (2010) used the ion nanogating technology as a label-free biosensor capable of identifying DNA and proteins and the sensor exhibited the ultrasensitive detection of HT-2 toxin with a detection limit of 100 fg/ml, which can compare the

Table 5 Examples of protocols and detection limits using ELISA for the most common mycotoxins

Toxin	Protocol	Matrix	Reference
Aflatoxin	Diverse microplate-based hybridoma screening methods for the generation of hapten-(aflatoxin-)specific McAbs	Commercial mycotoxin samples	Cervino et al. (2008)
Aflatoxins	Sequential injection immunoassay	Spiked food	Betina (1984), Ye et al. (2010)
Aflatoxins	Noncommercial ELISA	Peanuts and maize	Smart et al. (1990)
Deoxynivalenol	Noncommercial ELISA	Kenyan wheat samples	Molloy et al. (2003)
Deoxynivalenol	A commercial ELISA Kit, sol-gel immunoaffinity chromatography	Wheat samples Food samples	Torres et al. (1998), Klinglmayr et al. (2010)
Deoxynivalenon and Zearalenone	Noncommercial ELISA	Field contaminated maize	Cavaliere et al. (2005)
Dihydroergosine	Competitive ELISA	Grains and mixed animal feed	Kiihn et al. (1995)
Ergosterol	Monoclonal antibody based indirect competitive antibody based ELISA	Maize grains	Janardhana et al. (1999)
OTA	Commercial ELISA 96 well format	Various products	Matrella et al. (2006)
OTA	CE with laser-induced fluorescence	Food samples	Corneli and Maragos (1998)
OTA	Comparison of CE and ELISA	Food samples	Köller et al. (2006)
OTA	Noncommercial ELISA	Food samples	Pronyk et al. (2006)

conventional assay techniques. Furthermore, Khan et al. (2010) demonstrated mycotoxin detection using antibody immobilized conducting polymer supported electrochemically polymerized acacia gum while Yang et al. (2010) reported the aptamer-based colorimetric biosensor to detect Ochratoxin A using unmodified gold nanoparticles indicator. In addition, horseradish peroxidase has been successfully immobilized in a polypyrrole matrix onto disposable screen-printed carbon electrodes for the selective detection of ochratoxin A, with the capability of 0.1 ng/ml (Alonso-Lomillo et al., 2010). Thus, the biosensor technique exhibits great potential for rapid and simple monitoring of food contamination.

Molecular Technique

Unprecedented biotechnological advances in the past decade have delivered powerful transcriptomics methods that provide new technique for mycotoxin detection. Interestingly, the real-time PCR-based system has been developed for the detection and quantification of the most common *Fusarium* species with the similar profiles of mycotoxins they can potentially produce, and such a system enables simultaneous identification of different mycotoxin producer groups in samples (Stakheev et al., 2010). Furthermore, the fingerprinting method based on metabolomics, with respect to the identification of emerging contaminants at all stages of the food-production chain, can be developed for risk assessment (Lancova et al., 2010).

PREVENTION OR DECONTAMINATION OF MYCOTOXINS

Prevention or decontamination of mycotoxins from fungi in food is an important issue. Although spoiled products must be removed, inoculum sources such as agricultural residues or dirty farming materials have to be minimized to reduced or avoid contamination (FAO/WHO/UNEP, 1999a; Codex Alimentarius Commission, 2003; Amézqueta et al., 2009). The use of chemical compounds is a very effective strategy to prevent mycotoxin production (FAO/WHO/UNEP, 1999b). For example, in the case of grapes, some fungicides have been found to be effective against fungal colonization and OTA production (Varga and Kozakiewicz, 2006). However, fungicides must be applied with special care since some of them, such as carbendazim, have been found to reduce fungal flora but to stimulate OTA production (Medina et al., 2007b). Application of other fungicide azoxystrobin can inhibited mycotoxin production in a carbendazim-resistant strain of *Fusarium sporotrichioides* (D'Mello et al., 2001). Table 6 lists some examples to prevent mycotoxins produced by fungi.

Table 6 Examples of prevention from mycotoxins

Toxin	Protocol	Matrix	Reference
OTA	Gamma radiation	Black pepper	Jalili et al. (2010)
Aflatoxins	Gamma radiation	Black pepper	Jalili et al. (2010)
Aflatoxins	Ozone	Agricultural products	Denvir et al. (1999)
Ochratoxin A	Temperature and relative humidity	Grapes	Bellí et al. (2007b)
Ochratoxin A	Preharvest fungicides	Dehydrating grapes	Valero et al. (2007b)
Ochratoxin A	Adsorbent	Food stuff	Daković et al. (2003)
Citrinin	High hydrostatic pressure	Black table olives	Tokuşoğlu et al. (2010)
OTA	*A. nigri*	Dehydrating grapes	Valero et al. (2007b)
Patulin	*R. glutinis* strain LS11	Apple	Castoria et al. (2005)

Preharvest Strategies

Cultural practices including crop rotation, tillage, planting date, and management of irrigation and fertilization have limited effects on infection and subsequent mycotoxins accumulation, whereas environmental conditions greatly affect crops, and fungi and their mycotoxins (Pose et al., 2010; Tirado et al., 2010). Kokkonen et al. (2010) investigated the influence of environmental conditions on the toxin production by *Fusarium langsethiae*, *Fusarium avenaceum,* and *Fusarium tricinctum*. To avoid fungal invasion, plant moisture, and drought stress must be reduced. Some of the strategies involved are appropriate irrigation, mineral nutrition and fertilization (FAO/WHO/UNEP, 1999b). Other good agricultural practices (GAP) are crop rotation, culture, and harvest in the appropriate seasons and conditions (space each plant for optimal growth, avoid extreme drought periods) and land deep plough (Codex Alimentarius Commission, 2003; Elmholt, 2003; Aldred and Magan, 2004). At present, new and promising preharvest biotechnology strategies are being explored (FAO/WHO/UNEP, 1999b). These new approaches include the design and production of new plants that reduce the incidence of fungal infection, restrict the growth of toxigenic fungi, prevent toxin accumulation, or are resistant to the action of insects.

Strategies during Harvest

The correct performance and cleanliness of farming equipment is recommended (Codex Alimentarius Commission, 2003). Mature fruits must be collected in sacks or containers free of contamination. Those overripe, damaged, or fallen onto the soil must be discarded and eliminated even off the field, as they are likely to have high OTA levels or to harbor OTA-producing molds which could be rapidly propagated (Bucheli and Taniwaki, 2002; Paulino de Moraes and Luchese, 2003; Pérez de Obanos et al., 2005; Belli et al., 2007a). Blend with poor quality products or with other commodities is also unadvisable (López-Garcia et al., 2008).

Postharvest Handling Strategies

Prevention through preharvest management is the effective method for controlling mycotoxin contamination. In the postharvest phase, the major topic is to prevent contamination from mycotoxins (FAO/WHO/UNEP, 1999c). Postharvest handling strategies to prevent mycotoxins from fungi include physical, chemical, or microbiological treatments, storage, and processing by destroying, modifying or absorbing these mycotoxins (FAO/WHO/UNEP, 1999a; Morales et al., 2010). The ideal methods would be easy to use and economical, and would not generate toxic compounds or alter other fruit quality parameters such as nutrient content (EMAN, 2003).

Physical Methods

A freezing (-20°C) process, and UV and gamma treatments are able to diminish the production of mold conidia. Nevertheless, only gamma radiation can destroy the mycotoxin (Deberghes et al., 1993, 1995; Aziz et al., 2004; Bhat et al., 2010), but the gamma rays even at 60 kGy were not effective in completely destroying of ochratoxin and aflatoxins present in black pepper (Jalili et al., 2010). Application of combined infrared radiation heating with ultraviolet irradiation reduced surface decontamination of fig fruit (Hamanaka et al., 2010). However, thermal treatments do not completely eliminate OTA (Boudra et al., 1995). Another proposed method is ozonization. The development of electrochemical techniques has allowed the ozone use for OTA removal up to undetectable levels in foodstuffs such as grains, nuts, or vegetables (McKenzie et al., 1997; Denvir et al., 1999).

Chemical Methods

Postharvest treatment with fungicides can reduce production of mycotoxins by fungi. In grapes, it has been demonstrated that some fungicides, pesticides, and insecticides have positive effects on mycotoxin detoxification (Varga and Kozakiewicz, 2006; Reddy et al., 2007; Bellí et al., 2007b; Valero et al., 2007b; Lehoczki-Krsjak et al., 2010). Some fungicides such as itraconazole and amphotericin B have been shown to effectively control the aflatoxin-producing *Aspergillus* species (Ni and Streett, 2005). Application of alternative fungicide azoxystrobin can inhibit mycotoxin production in a carbendazim-resistant strain of *F. sporotrichioides* (D'Mello et al., 2001). Overall, use of fungicides is being discouraged due to economic reasons and growing concern for environment- and food-safety issues. Other chemicals such as ethanol inhibited food spoilage fungi and, thus, reduced mycotoxin production (Dao and Dantigny, 2010).

For OTA detoxification with chemical compounds, many adsorbent materials such as activated charcoal, cholestyramine, sodium and calcium aluminum silicates (mainly zeolites), bentonite, or wood fragments have been tested (Bauer, 1994; Leong et al., 2006; Varga and Kozakiewicz, 2006; Péteri et al., 2007; Ringot et al., 2007; Savino et al., 2007). However, their activities in vivo were not as high as expected except for the activated charcoal (Scott, 1996; Huwig et al., 2001). A new insoluble vegetal fiber has been developed in order to adsorb the OTA present in liquid food products (Tangni et al., 2005). Currently, the most promising adsorbent materials are modified zeolites as they have shown good results in foodstuff decontamination (Schall et al., 2002; Daković et al., 2003; Tomasevic-Canovic et al., 2003; Daković et al., 2005). It is noted that there is a risk that nonspecific adsorption agents using enterosorbents may prevent uptake of micronutrients from the food.

As OTA is known to cause cell membrane damage through increased lipid peroxidation, the protective properties of antioxidant substances are being investigated. These properties of these

compounds can act as superoxide anion scavengers, thereby protecting cell membrane from the mycotoxin-induced damage (Atroshi et al., 2000; Atroshi et al., 2002; Ruiz, 2010). A new strategy under study is the use of antioxidants such as vanillic acid or 4-hydroxybenzoic acid (Palumbo et al., 2007) and essential oils extracted from plants such as *Thymus vulgaris*, *Aframomum danielli*, *Ocimum gratissimum* L., or *Trachyspermum ammi* (L.) Sprague ex Turrill (Aroyeun and Adegoke, 2007; Nguefack et al., 2007; Prakash et al., 2010; Velazhahan et al., 2010), which can inhibit mold growth and OTA synthesis. Furthermore, the degradation of mycotoxins can be reduced with an ethanolic solution and intermittent ultrasounds (Lindner, 1992). Alkaline hydrogen peroxide, sodium hydroxide, and monomethylamine or ammonium with calcium hydroxide treatments are also effective methods against OTA in this matrix.

Biological Methods

Significant efforts have been made in establishing various biocontrol strategies such as development of atoxigenic biocontrol fungi that can out-compete their closely related and toxigenic strains in field environments, thus reducing the levels of mycotoxins in the crops. Bejaoui et al. (2004) reported ochratoxin A removal in synthetic and natural grape juices by selected oenological *Saccharomyces* strains while Castoria et al. (2005) evaluated the effect of a yeast *Rhodotorula glutinis* strain LS11 on patulin accumulation in stored apples. In the case of products affected by *A. carbonarius*, natamycin appears to be an effective substance for controlling fungal growth and OTA production (Medina et al., 2007a). Application of nontoxigenic biocompetitive agents as nontoxigenic *A. niger* strains is also a potentially useful strategy (Valero et al., 2007a). Carboxypeptidase A is an enzyme capable of destroying OTA (Deberghes et al., 1995) and the use of atoxigenic *A. niger* strains as carboxypeptidase sources has been suggested (Varga et al., 2000). Other enzymes that can be obtained from *A. niger* strains and can efficiently degrade OTA are lipases and metalloenzyme (Stander et al., 2000; Abrunhosa et al., 2006; Abrunhosa and Venancio, 2007). A carboxypeptidase present in *Phaffia rhodozyma* can also degrade OTA up to 90% (Péteri et al., 2007). Moreover, certain bacteria belonging to *Streptococus*, *Bifidobacterium*, *Lactobacillus*, *Butyribrio*, *Phenylobacterium*, *Pleurotus*, *Saccharomyces*, *Bacillus,* and *Acinetobacter* genera (Varga et al., 2000; Varga et al., 2005; Fuchs et al., 2008) and certain fungi belonging to *Aspergillus* (*A. fumigatus*, *A. niger*, *A. carbonarius*, *A. japonicus*, *A. versicolor*, *A. wentii,* and *A. ochraceus*), *Alternaria*, *Botrytis*, *Cladosporium*, *Phaffia*, *Penicillum,* and *Rhizopus* (*R. stolonifer* and *R. oryzae*) genera (Abrunhosa et al., 2002; Varga et al., 2005; Péteri et al., 2007) are able to degrade OTA in vitro up to more than 95%. Recently, Heinl et al. (2010) have reported the degradation of fumonisin B1 by the consecutive action of 2 bacterial enzymes from *Sphingopyxis* sp. MTA144. Moreover, some of them have shown detoxifying properties in vivo assays (Fuchs et al., 2008). For example, detoxification of aflatoxin B1 and patulin by *Enterococcus faecium* strains was reported by Topcu et al. (2010). In addition, the established the *Monascus* genetic system showed high efficiency and specificity, which provides us a potential approach to manipulate and improve industrial *Monascus* strains to eliminate the citrinin production (Jia et al., 2010).

Fruit Storage and Processing

Storage environmental conditions greatly affect fungi and their mycotoxins (van der Westhuizen et al., 2010). Proper storage is necessary to prevent biological activity through adequate drying to less than 10% moisture, elimination of insect activity that can increase moisture content through condensation of moisture resulting from respiration, low temperatures, and inert atmospheres. A storage temperature of 6°C or below could be considered as safe for tomato fruits and high moisture enhanced production of *Alternaria* toxins (Pose et al., 2010). Application of the controlled atmospheres containing 80% carbon dioxide and 20% oxygen reduced significantly the growth and mycotoxin production by *Mucor plumbeus*, *Fusarium oxysporum*, *Byssochlamys fulva*, *Byssochlamys nivea*, *Penicillium commune*, *Penicillium roqueforti*, *A. flavus*, *Eurotium chevalieri,* and *Xeromyces bisporus* (Taniwaki et al., 2010). Furthermore, the modified atmosphere packaging conditions inhibited growth and reduced aflatoxin production of *A. flavus* (Ellis et al., 1993). In addition, the sealed or vacuum conditions can prevent mycotoxin occurrence of red chili pepper during storage (Duman, 2010). Thus, fruit storage is needed to optimize the conditions of temperatures, humidity, gas atmosphere, and packaging.

During vine fruits production, efficient drying and turning over are required because, in these stages, moisture and sugar content stimulates *Aspergillus* and OTA synthesis (Magan and Aldred, 2005). It has also been proposed to eliminate the products colonized by molds from the manufacturing process. For example, high hydrostatic pressure as a nonthermal technology is an effective tool for microbiologically safe and can reduce the citrinin production by filamentous fungi such as *Penicillium citrinum*, *Penicillium*, *Aspergillus*, and *Monascus* (Tokuşoğlu et al., 2010). For grape case, fruit washing, cooking, and pressing can also promote the OTA content drop (Delage et al., 2003; Ratola et al., 2005).

HACCP System

Hazard Analysis and Critical Control Point (HACCP) is a quality system that identifies, evaluates, and controls hazards which are significant for food safety. It is a structured, systematic approach for the control of food safety throughout the commodity system, from the plough to the plate (Martínez-Rodríguez and Carrascosa, 2009). It requires a good understanding of the relationship between cause and effect and it is a key element in total quality management. HACCP builds on the foundations

of well-established quality management systems such as good manufacturing practice, good hygienic practice, GAP, and good storage practice (FAO, 2001). By applying the HACCP concept to the management of the likelihood of an adverse health effect as a result of exposure to mycotoxins, an adequate, wholesome, and safe food supply would be maintained. In order to design and develop effective HACCP-based integrated mycotoxin management programs, a given country would have to consider factors such as climate, farming systems, preharvest and postharvest technologies, public health significance of the contaminant, producer/processor compliance, the availability of analytical resources, and even the economy. In an ideal integrated management system that incorporates the HACCP concept, mycotoxins would be minimized at every stage of production, harvesting, processing, and distribution (FAO/WHO/UNEP, 1999c).

CONCLUSION AND PROSPECTS

The presence of mycotoxins such as aflatoxins, 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 (Garcia et al., 2009; Chassy, 2010). 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, that is, apple for patulin, grape for ochratoxin, and fig for aflatoxins (Sakuda and Kimura, 2010). Particularly, 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 cooccurrence of these different mycotoxins in the same matrix is another point that requires more studies from a toxicological and occurrence point of view.

A broad range of detection techniques used for practical analysis and detection of a wide spectrum of mycotoxins are available (Turner et al., 2009). Many analytical methods have been developed for the determination of each group of these mycotoxins in different matrices. Though there have been several recent successes in detection of mycotoxins, new methods are still required to achieve higher sensitivity and address other challenges that are posed by these mycotoxins. The application of MS in conjunction with other tools for decreasing limits of detection has been of increased interest in the recent times. Future trends would focus on rapid assays and tools that would measure multiple toxins from a single matrix and the development of rapid screening methods is also advisable in order to increase the number of monitored samples.

Apart from the regulatory controls, some major strategies have been adopted to decrease or even eliminate the presence of the mycotoxins in foods: prevention of mycotoxin contamination during the preharvest and postharvest 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, appropriate culture methods, use of insecticides, fungicides and biological control, irrigation, and cultivar selection ensure plants less vulnerable to fungal infection. Postharvest contamination can be avoided by controlling moisture, temperature, and microbiological, insect and animal pests. However, 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, but further studies are needed. In addition, efforts have been done to study the critical points of the mycotoxin presence in the manufacturing chain of affected commodities. To effectively eliminate mycotoxins, an integrated management system should be adopted at every stage of production, harvesting, processing, and distribution.

As fungus-derived mycotoxins are unavoidable, new approaches should developed using biotechnology. Potential biochemical and genetic resistance markers have been identified in crops in different parts of the world which are being utilized as selectable markers in breeding for resistance to aflatoxin contamination. Prototypes of genetically engineered crops have been developed which contain genes for resistance to the phytotoxic effects of certain trichothecenes, thereby helping reduce fungal virulence or contain genes encoding fungal growth inhibitors for reducing fungal infection. Breeding approaches allowed in the past the creation of new varieties with lower toxin levels (Lehoczki-Krsjak et al., 2010). With the rapid development of molecular biology, gene technology could further improve crop varieties to better prevent contamination of mycotoxins by fungi in fruits and their processed products.

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ABBREVIATIONS

AFB-1	= Aflatoxin B1;
AFG-1	= Aflatoxin G1;
AFT	= Aflatoxin;
AOH	= Dibenzo-α-pyrones alternariol;
ALT	= Altenuene;

APCI = Atmospheric pressure chemical ionization;
ATX-I = Altertoxin I;
ATX-II = Altertoxin II;
C-18 = Octadecylsilane polymer resin;
CE = Capillary electrophoresis;
CI-ELISA = Competitive indirect enzyme-linked immunosorbent assay;
CIT = Citrinin;
CPZ = Cyclopiazonic acid;
DAD = Diode array detection;
DNA = Deoxyribonucleic acid;
DON = Deoxynivalenol;
ECD = Electron capture detector;
ELISA = Enzyme-linked immunosorbent assay;
FB1 = Fuminosin B1;
FB2 = Fuminosin B2;
FI = Flame ionization;
FID = Flame ionization detector;
FD = Fluorescence detector;
FTIR = Fourier transform infrared spectroscopy;
GC = Gas chromatography;
GC-ECD = Gas chromatography coupled with electron capture detector;
GC-MS = Gas chromatography–mass spectrometry;
HACCP = Hazard analysis and critical control point;
HPLC = High-performance liquid chromatography;
HPLC-DAD = High-performance liquid chromatography coupled with diode array detector; HPLC-ELSD, HPLC coupled with an evaporative laser scattering detector;
HPLC-FD = High-performance liquid chromatography coupled with fluorescence detector; HT-2, HT-2 toxin (15-Acetoxy-3α,4β-dihydroxy-8α-(3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene);
IAC = Immuno affinity columns;
IARC = International Agency for Research on Cancer;
LC-MS = Liquid chromatography–mass spectrometry;
LLC = Liquid–liquid chromatography;
LLE = Liquid–liquid extraction;
LOD = Limits of detection;
LOQ = Limits of quantification;
MIP = Molecularly imprinted polymers;
MS = Mass spectrometry;
MW = Molecular weight;
NICI = Negative ion chemical ionization;
NIV = Nivalenol;
OPA = *o*-phthaldialdehyde;
OTA = Ochratoxin;
PAT = Patulin; ppb, parts per billion; ppm, parts per million;
RNA = Ribonucleic acid;
RP = Reverse phase;
RP-HPLC-ESI-MS = Reversed-phase HPLC-electrospray ionization-mass spectrometry;
SAX = Strong anion exchange;
SFE = Supercritical fluid extraction;
SIIA = Sequential injection immunoassay;
SPE = Solid phase extraction;
SPME = Solid phase microextraction;
SPME–LC–MS/MS = Solid phase microextraction coupled with liquid chromatography–mass spectrometry–mass spectrometry;
SPR = Surface plasmon resonance;
TLC = Thin-layer chromatography;
TRC = Trichothecenes;
WHO = World Health Organization;
ZEA = Zearalone;
ZON = Zearalanone

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