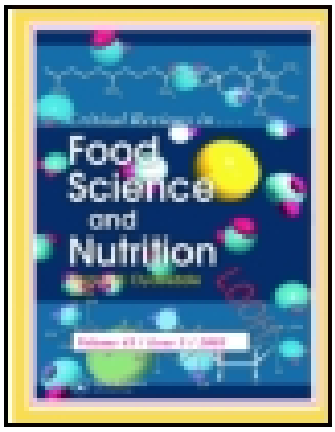

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Development of Methods for Determination of Aflatoxins

Lijuan Xie[ab], Min Chen[ab] & Yibin Ying[ab]
[a] College of Biosystems Engineering and Food Science, Zhejiang University, P. R. China
[b] Key Laboratory of Equipment and Informatization in Environment Controlled Agriculture, Ministry of Agriculture, P. R. China
Accepted author version posted online: 03 Apr 2015.

Development of Methods for Determination of Aflatoxins

Lijuan Xie[1,2], Min Chen[1,2], and Yibin Ying[1,2*]

1 College of Biosystems Engineering and Food Science, Zhejiang University, P. R. China

2 Key Laboratory of Equipment and Informatization in Environment Controlled Agriculture, Ministry of Agriculture, P. R. China

ABSTRACT. Aflatoxins can cause damage to the health of humans and animals. Several institutions around the world have established regulations to limit the levels of aflatoxins in food, and numerous analytical methods have been extensively developed for aflatoxin determination. This review covers the currently used analytical methods for the determination of aflatoxins in different food matrices, which includes sampling and sample preparation, sample pretreatment methods including extraction methods and purification methods of aflatoxin extracts, separation and determination methods. Validation for analysis of aflatoxins and safety considerations and precautions when doing the experiments are also discussed.

Keywords: aflatoxin, method, sample preparation, sample pretreatment, separation, determination

1. INTRODUCTION

Aflatoxins are a group of closely related mycotoxins that are mainly produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* through a polyketide pathway (Afsah-Hejri et al., 2011; Bakirdere et al., 2012; Turner et al., 2009). The term "aflatoxin" comes from three words: (i) the "a" that represents the *Aspergillus genus*; (ii) the "fla" that represents the species flavus; and (iii) the "toxin" that means poison (Bakirdere et al., 2012; Bhat et al., 2010). Until now, eighteen different types of aflatoxins have been identified (Bakirdere et al., 2012). Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) are the four major species based on their fluorescence under ultraviolet light (blue or green) and relative chromatographic mobility (Bennett and Klich, 2003; Schoental, 1967; Turner et al., 2009). Aflatoxins such as AFB1 are intrinsically fluorescent due to the presence of a chain of conjugated bonds and heteroatoms within the molecule, and their intrinsic fluorescence can be used for determination (Molina-García et al., 2012). AFB1 and AFB2 are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, while AFG1 and AFG2 only produced by *Aspergillus parasiticus* (Bakirdere et al., 2012; Shephard, 2009). In addition to these four types of aflatoxins, AFB1 and AFB2 ingested by mammals can produce respective aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2), which are two importance aflatoxin speices (Bakırdere et al., 2012; Dors et al., 2011; Fallah, 2010). Approximately 0.3-6.2% of AFB1 in animal feed is transformed into AFM1 in milk (Creppy, 2002; Tabari et al., 2011). The name 'M' indicates the original derivation from milk (Allcroft et al., 1966). It has been reported that AFM1 and AFM2

exhibited blue-violet and violet fluorescent on thin-layer chromatography (TLC) plates under ultraviolet light, respectively, and the fluorescence intensities of the AFM1 and AFM2 were three times stronger than those of the respective AFB1 and AFB2 (Dors et al., 2011). Chemical structures of these six types of aflatoxins incorporate dihydrofuran and tetrahydrofuran moieties coupled to a substituted coumarin (Santini et al., 2010). "B" analogues are pentanone derivatives, while "G" analogues are six-membered lactones (Afsah-Hejri et al., 2011; Bakirdere et al., 2012).

Aflatoxins tend to contaminate foodstuffs in the tropics and semi-tropics where high temperature and humidity are optimal for the growth of molds and production of toxins when food is growing, harvested and finally stored (Amate et al., 2010; Bakirdere et al., 2012; Bhat et al., 2010). The main food products susceptible to aflatoxin contamination are peanuts, maize, pistachio nut, cottonseed, copra and spices, among others (Shephard, 2009). Ingestion of these contaminated foodstuffs can cause not only adverse health effects to humans and animals (Zain, 2011) but also economic losses (Pearson et al., 2001).

Aflatoxins are highly toxic, mutagenic, carcinogenic, and teratogenic compounds (Aycicek et al., 2005; Bakirdere et al., 2012; Bhat et al., 2010). Endemic diseases in Asia, Africa and Europe, such as Kwarshiorkor and Reye's syndrome (damage to the liver and kidney), are caused by aflatoxins (Zöllner and Mayer-Helm, 2006). The toxicity order of aflatoxins is AFB1 > AFG1 > AFB2 > AFG2 (Agag, 2004; Espinosa-Calderón et al., 2011). AFB1 is the most toxic form, causing damage such as toxic hepatitis, hemorrhage, edema, immunosuppression and hepatic carcinoma (Nakai et al., 2008), and has been reported to be the most powerful natural carcinogen known in mammals (Fallah, 2010; Hussain et al., 2008). It usually comprises approximately 90%

of the aflatoxin residue observed on contaminated foodstuffs (Amate et al., 2010). The main target organ of AFB1 is the liver (Kamika and Takoy, 2011; Van Rensburg et al., 1985). Synergistic effects of AFB1 with viral infections of hepatitis B or C can cause liver cancer (Bakirdere et al., 2012). In 1993, the International Agency for Research on Cancer (IARC) (1993), a part of the World Health Organization (WHO), classified AFB1 as a Group 1 human carcinogen and AFB2, AFG1 and AFG2 as possible carcinogens to humans. Although AFM1 has less mutagenic potency than AFB1 (Bakirdere et al., 2012; Fallah, 2010; Hsieh et al., 1984), AFM1 can cause vitally important changes in liver parenchymal cells, dissociation of ribosomes from the rough endoplasmic reticulum, and proliferation of the smooth endoplasmic reticulum (van Egmond, 1983), which was initially classified by IARC as a Group 2B human carcinogen (International Agency for Research on Cancer, 1993) and has now move to Group 1 for its toxic and carcinogenic effects (International Agency for Research on Cancer, 2002).

2. LEGISLATION FOR AFLATOXINS

Because aflatoxins have a negative effect on human health noted above, regulations have been gradually developed for aflatoxins in food and feed by several institutions. The maximum levels of AFB1 and total aflatoxin levels (the sum of AFB1, AFB2, AFG1 and AFG2) are the parameters employed currently to limit aflatoxins in many foods (Hernández-Hierro et al., 2008). In more than 75 countries, the maximum limits for AFB1 and total aflatoxins in food are 5 and 10 μg kg^{-1}, respectively (Bennett and Klich, 2003). In the USA, the U.S. Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA) have established a level of 15 to 20 μg kg^{-1} for total aflatoxin concentrations in animal feed products (Espinosa-Calderón

et al., 2011). The European Union (EU) has established the maximum residue limits for aflatoxins in cereal and their derivatives as 2 μg kg^{-1} for AFB1, 4 μg kg^{-1} for total aflatoxins, and 0.1 μg kg^{-1} for AFB1 in processed cereal-based food and baby food for infants and young children (EC, 2006; Quinto et al., 2009). To ensure milk safety, the FDA and the EU have also established the maximum residue limits for AFM1. The U.S. FDA (1996) has established a maximum admissible level of 500 ng L^{-1} for AFM1 in milk. According to the EU, the maximum residue limit for AFM1 is 0.05 μg kg^{-1} in milk (EC, 2001; EC, 1998) and 0.025 μg kg^{-1} in baby food (EC, 2004). Following these legislations set by national and international organizations, most countries have established their own legislation. For instance, the maximum safe level for total aflatoxins in peanuts and corn is 20 μg kg^{-1} in Brazil (Abbas, 2005). In China, the maximum safe level for AFB1 in corn, peanut kernel, and peanut oil is 20 μg kg^{-1}, while for beans, fermented food and other grains, the maximum safe level is 5 μg kg^{-1} according to GB 2761-2011 (2011). In Korea, the maximum safe level for AFB1 in all food is 10 μg kg^{-1} (Abbas, 2005), while in Switzerland, the Netherlands and the USA, the AFB1 limits for nuts and dried fruits are 1 μg kg^{-1}, 5 μg kg^{-1} and 20 μg kg^{-1}, respectively (Bacaloni et al., 2008; Creppy, 2002). Additionally, the maximum acceptable levels of AFM1 in milk, cheese and butter are 0.05 μg kg^{-1}, 0.25 μg kg^{-1}, and 0.02 μg kg^{-1}, respectively, in Switzerl and Austria (Manetta et al., 2005). Table 1 lists the maximum limits for aflatoxins in different food and feeds from different institutions and countries mentioned above.

3. DETERMINATION OF AFLATOXINS

To meet food safety concerns and official legislated regulations, sensitive and accurate analytical methods for the qualitative and quantitative analysis of mycotoxins, including aflatoxins, in food, feed, and other biological matrices are required (Krska et al., 2008; Shephard, 2009). Measures have been set up by authorities in many countries to monitor and control mycotoxin levels, especially for agricultural import products from third world countries (Zöllner and Mayer-Helm, 2006). For aflatoxin determination, methods based on TLC for screening of samples contaminated with aflatoxins prior to analysis, high-performance liquid chromatography (HPLC) or enzyme-linked immunosorbent assay (ELISA) for rapid screening are commonly used in routine analysis (Tabari et al., 2011). The most frequently used method is HPLC coupled to fluorescence detection (FLD) (HPLC-FLD) after extraction and clean-up by immunoaffinity chromatography (IAC) (Amate et al., 2010). In addition, the liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) (LC-MS/MS) method has gained increasing importance due to its superior specificity (Amate et al., 2010; Chen et al., 2005). Other methods, such as immunoassays, dipsticks, and even newer methods such as biosensors and non-destructive techniques based on infrared spectroscopy have shown great potential for aflatoxin analysis (Krska et al., 2008). Current analytical methods usually include pretreatment steps consisting of an extraction step to extract analytes from matrices and a clean-up step to reduce or eliminate unwanted co-extracted matrix components, followed by a separation step with suitably specific detection ability. Because the distribution of mycotoxins in samples is not homogeneous (Campbell et al., 1986; Krska et al., 2008; van Egmond et al., 2007), it is necessary to take a relatively large number of particles cumulated from a number of sites in the lot and to then

properly prepare the sample before analysis to obtain suitably representative samples and a precise measurement (Campbell et al., 1986). In other words, sampling and sample preparation steps, which will be discussed below, are needed. In general, a mycotoxin test procedure consists of three steps: sampling step, sample preparation step, and analytical step. The sampling step consists of selecting a sample of a given size from a bulk lot. The sample preparation step is a two-step process that includes grinding the sample in a mill and taking a subsample of ground kernels from the comminuted sample. The analytical step consists of several steps where the mycotoxin is extracted from the subsample, the solvent is purified, and the mycotoxin in the solvent is quantified (Whitaker, 2003).

3.1. Sampling and Sample Preparation for Determination

Each step of the mycotoxin test procedure contributes to the overall variability of the test procedure, when measured by the variance, the total variability of the test procedure is the combination of sampling, sample preparation, and analytical variances (Whitaker, 2003). Sampling is the selection of a representative sample from the population for analysis, which plays a crucial part in the precision and determination of mycotoxin levels due to the heterogeneous distribution of the toxins in agricultural commodities and products (Krska et al., 2008; Pearson et al., 2001). As it is for peanuts, the number of aflatoxins contaminated kernels in a lot is usually very low, but the level of contamination within a single kernel can be very high (van Egmond et al., 2007; Whitaker et al., 1974). Sampling errors are therefore usually much larger than sample preparation errors and analytical errors (van Egmond et al., 2007; Whitaker, 2003). In fact, nearly 90% of the error associated with mycotoxin assays could be attributed to

how the original sample was collected (Turner et al., 2009). International organizations such as FAO/WHO have used scientific techniques to evaluate and design aflatoxin sampling plans for raw shelled peanuts traded in the export market (Whitaker, 2003). When a bulk lot is in a bin, truck, boxcar or similar container, samples should be taken by means of probes that can reach to the bottom of the container. When the lot is bagged, samples are best taken from the bags while they are being filled or emptied into containers (Campbell et al., 1986). It is easier to select a representative sample from a moving stream of product than from a static lot such as trucks or rail cars (Whitaker, 2003). When sampling from a moving stream such as a conveyor belt, small increments of the product should be taken from along the entire length of the moving stream, and the bulk sample is obtained by compositing all the increments. Furthermore, if the bulk sample is larger than required, then the bulk sample should be blended and subdivided to obtain the desired size sample for test (Whitaker, 2006). Because it is not practical to extract the mycotoxin from a large test sample, the test sample is comminuted in a mill and the mycotoxin is extracted from a small analytical subsample taken from the comminuted test sample (Whitaker, 2006). Increasing the fineness of grind and using larger subsamples can reduce sample preparation error (Whitaker, 2003). Automatic sampling equipment such as cross-cut samplers are commercially available with timers that automatically pass a diverter cup through the moving stream at predetermined and uniform intervals (Whitaker, 2006).

3.2. Sample Pretreatment (Extraction and Purification)

Only a few analytical techniques, such as non-destructive optical techniques based on infrared spectroscopy, are capable of detecting aflatoxin contamination directly in samples without the

necessity of further sample pretreatment steps (extraction and clean-up). For most analytical methods, the aflatoxins should be efficiently extracted from the tested samples and subsequently purified to reduce matrix effects before finally being analyzed. liquid-liquid extraction (LLE), liquid-solid extraction (LSE) with different solvent mixtures, ultrasound extraction, and modern sample clean-up techniques, such as solid-phase extraction (SPE), solid-phase microextraction (SPME), and IAC (for both extraction and purification), are some of the most commonly used methods in the literature to simplify protocols, improve selectivity, and improve performance characteristics (Bacaloni et al., 2008; Bakirdere et al., 2012). Other extraction methods such as supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), and quick, easy, cheap, effective, rugged, and safe (QuEChERS)-like or -based method, and other clean-up methods such as multifunctional column (MFC) (Akiyama et al., 2001; Khayoon et al., 2010; Krska et al., 2008) and solid-phase dispersion (MSPD) (Dors et al., 2011; Krska et al., 2008; Shephard, 2009) have also been applied.

3.2.1. Extraction of Aflatoxins from Matrices

Extraction of aflatoxins from various food matrices depends on the use of organic solvents, such as methanol, chloroform, and acetonitrile (Shephard, 2009; Turner et al., 2009). Although the solubility of many toxins in water is low, mixtures of some solvents with water have proven to be very effective because aqueous solvents can better penetrate into hydrophilic tissues than organic solvents alone (Sheibani and Ghaziaskar, 2009). Methanol/water and acetonitrile/water are commonly used to extract aflatoxins (Bankole et al., 2010; Dors et al., 2011; Sheibani and Ghaziaskar, 2009). The methanol/water system is also used in standard procedures for the

recovery of aflatoxins from real samples (Stroka and Anklam, 2000). Although chloroform is still used by some researchers to extracting aflatoxins when TLC method is applied (Shouman et al., 2012; Tripathi and Mishra, 2011; Var et al., 2007; Wang et al., 2012b), the use of it for extraction has been gradually reduced, as this solvent is an ecological hazard (Shephard, 2009; Turner et al., 2009).

3.2.1.1. LLE

LLE involves exploiting the differential solubility of the toxin in the aqueous phase and in the immiscible organic phase by extracting the compound into one solvent and leaving the rest of the matrix in another. The procedure is effective for several toxins and works well in small-scale preparations (Turner et al., 2009). LLE has been applied in the extraction of aflatoxins from non-alcoholic beer and the effects of solvent-types were studied. Among different solvents, ethyl acetate produced relatively high recoveries for all aflatoxins. The obtained recoveries were ranged from 85 to 96% with limit of detection (LOD) values between 0.001 and 0.003 ng mL^{-1}, R^2 value higher than 0.999, and repeatability and reproducibility precisions with relative standard deviation (RSD) lower than 5% (n = 5) achieved at 0.5 ng mL^{-1} concentration (Khan et al., 2013). However, the LLE method is time consuming and dependent on which matrix is being used and which compounds are being determined. Disadvantages also lie with the possible loss of sample by adsorption onto the glassware (Turner et al., 2009). Homogeneous LLE utilizes the phase separation phenomenon from a homogeneous solution, and the target solutes are extracted into a separated phase, accordingly, this procedure is simple and requires only the addition of a reagent without vigorous mechanical shaking (Rezaee et al., 2006). Sheijooni-Fumani et al. (2011)

applied homogeneous LLE method for the extraction and preconcentration of AFB1 in rice and grain prior to HPLC determination without the use of IAC clean-up. To obtain a high enrichment factor, the effect of different parameters such as the volume ratio of methanol to water, the type and volume of the extraction solvent and the salt concentration were optimized. With the optimal values, the calibration graph was linear in the concentration range of 0.01-1.0 ng g^{-1} with LOD of 0.003 ng g^{-1}. However, homogeneous LLE has some disadvantages. For instance, it requires the addition of reagent such as acid, salt, base, etc. which probably destroys some interested compounds and causes to the release of heat during extraction (Rezaee et al., 2006).

Dispersive liquid-liquid microextraction (DLLME) based on ternary component solvent system such as homogeneous LLE is a novel microextraction technique. In DLLME procedure, the appropriate mixture of extraction solvent and disperser solvent is injected into aqueous sample by syringe, rapidly. As a result of that, cloudy solution is formed. DLLME method is a high performance and powerful preconcentration method, which is also simple, rapid, and inexpensive (Rezaee et al., 2006). For example, DLLME coupled with HPLC-FLD has been applied for the determination of AFB1, AFB2, AFG1 and AFG2 in cereal products. Parameters affecting both extraction and DLLME procedures, such as extraction solvent, type and volume of DLLME extractant, volume of water and salt effect, were systematically investigated and optimized to achieve the best extraction efficiency. Under the optimal experimental conditions, the whole analytical method provided enrichment factors around 2.5 times and detection limits (0.01-0.17 μg kg^{-1}) below the maximum levels imposed by current regulation for aflatoxins in cereals and cereal products intended for direct human consumption. The proposed method was successfully

applied to the analysis of retail cereal products with quantitative results comparable to the IAC (Campone et al., 2011).

3.2.1.2. LSE

The LSE method is one of the basic procedures for aflatoxin analysis in agricultural products such as grains and other solid materials. As an official method for aflatoxins analysis of pistachio samples recommended by the Association of Official Analytical Chemists (AOAC) (1988), classical LSE and LLE methods are used for the extraction of the aflatoxins and clean-up of the extract, respectively, and TLC/HPLC is used for separation and quantitation of the extracted aflatoxins (Sheibani and Ghaziaskar, 2009). **Figure 1** shows aflatoxin production by TLC and determination by HPLC (Wang et al., 2012b). In LSE procedure, extraction is achieved based on the solubility of aflatoxins in different organic solvents, a solvent or ideally a mixture of solvents should extract the analyte quantitatively with as little additional compounds as possible to avoid interferences in the final determination step (Sheibani and Ghaziaskar, 2009). The detailed procedure usually consists of weighing a mass of the homogenized sample and then adding the extraction solvent and agitating in a shaker. After these steps, filtration is carried out. In this procedure, different solvent types and different volumes are also employed (Dors et al., 2011). The choice of the extraction solvent depends on the chemical properties of the matrix and the toxin and is directly related to the extraction efficiency and the number of co-extractives (Dors et al., 2011; Sheibani and Ghaziaskar, 2009). Solvents composed mainly of methanol/water or acetonitrile/water and volumes ranging from 20 to 250 mL have always been used (Dors et al., 2011). To avoid chlorinated solvents and to use less hazardous solvents, the solution of 80% and

55% methanol/water was studied as an extraction solvent. The results showed that a solution of 55% methanol/water led to a lower extraction yield. Therefore, a solution of 80% methanol/water was selected as the extraction solvent (Lee et al., 2004; Sheibani and Ghaziaskar, 2009). This system has been selected as the best extraction solvent for nutmeg samples based on the values of toxin peak areas, the absence of interfering peaks in the HPLC-fluorescence chromatograms, and the higher toxicity of acetonitrile compared to methanol (Kong et al., 2013). Analysis of aflatoxins from Rhammus purshiana before SPE clean-up also used this system (Ventura et al., 2004). In addition, methanol appears to offer a more robust choice for subsequent clean-up by IAC because antibodies can tolerate higher amounts of this solvent (Shephard, 2009; Stroka et al., 1999). When using LSE method to analyze solid samples such as cereals or nuts, a reduction of the particle size prior to the extraction step is necessary to increase the surface area and to decrease the mean free path of the extraction solvent. Additionally, the volume of the waste from the extraction solvent generated in large routine laboratory preparations has been addressed in peanut butter analysis by reducing the sample size from 25 to 5 g and using a two-stage extraction procedure, first with a methanol-15% sodium chloride solution and then with pure methanol (Shephard, 2009; Vega, 2005). Although this approach may be useful in a processed product in which some homogenization has occurred, it should be used with caution in raw materials considering the highly inhomogeneous nature of aflatoxin contamination and the need for the analytical subsample to adequately represent the batch (Shephard, 2009). On the other hand, the extraction of dry sample material can lead to compositional changes in the extraction mixture due to water absorption by dry matrices such as infant formulae, animal feed, and paprika (Shephard, 2009; Stroka et al., 1999). Such water absorption effects vary greatly

between aqueous mixtures of methanol, acetone, and acetonitrile, with the last showing the greatest effect in which absorption of water by the dry matrix can lead to significant elevation in the measured aflatoxin level. This apparent elevation in aflatoxin level is dependent on the sample-to-extractant ratio, with an increasing effect as the ratio is changed from 10 g sample per 100 mL extractant to 40 g per 100 mL (Shephard, 2009).

3.2.1.3. Ultrasound Extraction or Ultrasonic Extraction

Ultrasound or ultrasonic extraction utilizes acoustic cavitation to cause molecular movement of solvent and sample, aggressively improving the transfer from the cell into the solvent with improved efficiency, reduced extraction time, low solvent consumption and a high level of automation compared to traditional extraction techniques (Dors et al., 2011; Kong et al., 2013). The ultrasonic extraction is carried out with an ultrasonic bath, and the duration of ultrasound application depends on the matrices (Amate et al., 2010; Bacaloni et al., 2008). For example, Bacaloni et al. (2008) tested and compared three different extraction techniques, that is, homogenization, ultrasound extraction, and MSPD, for extraction of aflatoxins from hazelnuts in terms of recovery, matrix effect, accuracy, and precision. In the ultrasound extraction procedure, extraction was carried out in an ultrasonic bath using the same sample size, solvent mixture and volume as used for homogenization. The duration of ultrasound application was also studied, and recoveries comparable to those obtained with the homogenization method were achieved with a sonication time of 10 min. The results also showed that using a longer recovery time could not improve the results significantly (Bacaloni et al., 2008). This auxiliary tool has also been used to simultaneously extract aflatoxins, dyes, and pesticides in spices by keeping the acetonitrile

extraction in an ultrasonic bath for 30 min without further clean-up before reversed-phase LC-MS/MS interfaced with electrospray ionization (ESI) detection (Amate et al., 2010).

3.2.1.4. SFE

SFE can minimize and eliminate the use of organic solvents by using supercritical carbon dioxide, which has been used to extract the flavors from spices while eliminating the need for large volumes of solvent and providing an extract closer in composition to the essential spice oil (Anklam et al., 1998; Shephard, 2009). Because the application of this technology to the extraction of polar aflatoxins has presented a number of problems with either low recoveries or high levels of co-extracted impurities such as lipids that interfere with subsequent clean-up and chromatography (Shephard, 2009), only a few instances in the literature of its use have been reported (Ehlers et al., 2006). For instance, SFE has been successfully utilized to extract aflatoxins contained in Zizyphi Fructus, a traditional Chinese medicine, and selectively reduced interferences from the matrices (Liau et al., 2007).

3.2.1.5. ASE

ASE (trade name Dionex), also known as pressurized liquid extraction (PLE) (Pallaroni and von Holst, 2004; Royer et al., 2004; Urraca et al., 2004) and pressurized fluid extraction (PFE) (Sheibani and Ghaziaskar, 2009), is a relatively new extraction technique that uses solvents at elevated pressures and temperatures to accelerate the kinetics of extraction, hence achieving complete extraction of analytes from solid and semi-solid samples with lower solvent volumes and shorter extraction times while automating the extraction process (Sheibani and Ghaziaskar,

2009; Urraca et al., 2004). The ASE method has been applied for the analysis of food, biological, and environmental samples (Breithaupt, 2004; Kot-Wasik and Wasik, 2005; Sheibani and Ghaziaskar, 2009). In particular, this technique was used as a multiresidue method to extract zearalenone and α-zearalenol from cereal samples and extract zearalenone, deoxynivalenol and fumonisin B1 from maize simultaneously (Royer et al., 2004). Compared with conventional extraction methods for aflatoxin, this method has the significant advantages of a higher recovery, lower limit of quantification (LOQ), and less solvent consumption. ASE using a modified SFE method apparatus was applied to extract AFB1 and AFB2 from spiked and naturally contaminated pistachio samples, and approximately 20% higher extraction yield or recovery in comparison with the AOAC method using a similar extraction solvent was obtained. This method has also been used as a powerful method to increase the recovery of aflatoxins from peanut samples (Sheibani and Ghaziaskar, 2009). Although the variables of temperature and pressure are less important in the ASE method, the SFE method is more difficult to operate and maintain the supercritical condition necessary for its use. Therefore, the developed ASE method is superior to the SFE method (David et al., 2000; David and Seiber, 1996; Sheibani and Ghaziaskar, 2009). However, the high cost of an ASE apparatus has limited the application of this technique in the field of mycotoxin analysis to a few laboratories (Krska et al., 2008).

3.2.1.6. QuEChERS-Like or -Based Method

Anastassiades et al. (2003) developed an original analytical methodology combining the extraction/isolation of pesticides from food matrices and clean-up of the extract. This technique involves micro-scale extraction using acetonitrile and purifying the extract using dispersive SPE.

They coined the acronym QuEChERS (quick, easy, cheap, effective, rugged, and safe) for it. Since the development and publication of the method, QuEChERS has been gaining significant popularity (Wilkowska and Biziuk, 2011). QuEChERS-based methodologies have been applied for the extraction of pesticides with a wide range of physico-chemical properties from different samples (Anastassiades et al., 2003; Asensio-Ramos et al., 2010; Cunha et al., 2007; Geis-Asteggiante et al., 2012; Lehotay et al., 2005; Romero-González et al., 2008; Wilkowska and Biziuk, 2011). In addition, it has been employed for other compounds such as bisphenol A and bisphenol B in canned seafood (Cunha et al., 2012) and mycotoxins from food matrixes (Desmarchelier et al., 2010; Romero-González et al., 2011).

This method was found to be easier to handle and allowed a higher sample throughput as compared to the ASE method with similar performances and precision for analysis of seventeen mycotoxins including AFB1, AFB2, AFG1, AFG2, zearalenone, fumonisin B1, fumonisin B2 and other toxins simutaneously in cereal-based commodities (Desmarchelier et al., 2010). Currently, acetate buffer-based QuEChERS method is the official method of the AOAC, whereas QuEChERS method using citrate buffer is the official method set by the European Norm (Aguilera-Luiz et al., 2011). However, the QuEChERS method using acetate and citrate buffers is not suitable for the extraction of aflatoxins at very low levels; thus, prior to this procedure, additional clean-up methods such as SPE should be used to pre-concentrate the analytes (Aguilera-Luiz et al., 2011). Acetate and citrate buffer-based QuEChERS methods have been tested and compared in the simultaneous determination of aflatoxins and pesticides in milk samples, and the best results were obtained with the citrate-based method using an additional clean-up step by SPE with Oasis® HLB to pre-concentrate AFM1 (Aguilera-Luiz et al., 2011). It

can be observed that the best results were obtained when QuEChERS procedure was used, allowing the extraction of more than 80 compounds with suitable recoveries (70-120%) and RSD lower than 20% (Romero-González et al., 2011).

3.2.2. Purification of Aflatoxin Extract

The sensitivity of an analysis, especially if the detection selectivity is low, depends strongly on the sample clean-up. A variety of different sample clean-up protocols have been established to sufficiently remove the food/feed matrix and/or to concentrate the target analytes (Zöllner and Mayer-Helm, 2006). Various clean-up methods have been applied for different food matrices prior to LC and HPLC analysis of aflatoxins. The extraction and purification of aflatoxins from cereals and their derivatives is usually performed by a methanol/water extraction followed by an IAC or SPE clean-up step (Huang et al., 2010; Reiter et al., 2010).

3.2.2.1. SPE

The principle of the SPE method for extracting toxins is that the toxins are first adsorbed on the SPE cartridge before being separated by elution with solvents of different polarities and then analyzed separately (Shephard, 2009). A wide variety of sorbent materials are available. Conventional SPE procedures use reverse-phase materials (e.g., C8, and C18), strong cation or anion exchangers (SCX, SAX) or polymeric materials with combined properties (Krska et al., 2008). The greatest advantage of SPE is that this method enables the extraction, pre-concentration and purification in one step without increasing the matrix content in the final extract compared to other extraction methods such as LLE and the QuEChERS method

(Aguilera-Luiz et al., 2011). Furthermore, the SPE technique can meet the need of monitoring several classes of substances in samples of different origins that are often present at trace levels (Capriotti et al., 2010). Although IAC is usually preferred compared to SPE because of the better performances of IAC in terms of yield and LOQ, the IA procedure is time and solvent consuming and requires a high expertise level and the use of expensive disposable cartridges (Quinto et al., 2009). Wang et al. (2012a) demonstrated that SPE clean-up with Oasis® HLB cartridges yielded comparable accuracy and precision characteristics for AFM1 in liquid milk and milk powder when compared to IAC clean-up when the LC-FLD methodology was used. In addition, the much cheaper SPE cartridge can be a promising alternative to the use of IAC in the clean-up of milk sample extracts.

3.2.2.2. SPME

SPE uses much less solvent than LLE, but can be relatively expensive (Rezaee et al., 2006). An efficient, economical, and miniaturized sample pretreatment method based on SPME have been developed (Arthur and Pawliszyn, 1990). SPME is a solvent free process that includes simultaneous extraction and preconcentration of analytes from aqueous samples or the headspace of the samples in single step (Rezaee et al., 2006), the time required for sample pretreatment is reduced (Es'haghi et al., 2011). In SPME procedure, analytes are partitioned between the sample matrix or sample extract and the phase on the fused silica fiber (Quinto et al., 2009). SPME technique is mainly coupled with gas chromatography (GC), but it is also easily interfaced with HPLC in order to extend its applicability to non-volatile compounds such as aflatoxins (Chen and Pawliszyn, 1995; Quinto et al., 2009). The first attempt for the analysis of aflatoxins in real

samples with this technique was proposed by Nonaka et al., who used a home-made "in-tube SPME" device coupled with LC coupled with mass spectrometry (MS) (LC-MS) for the determination of AFB1, AFB2, AFG1, and AFG2 in nuts, cereals, dried fruits, and spices. The recoveries of aflatoxins spiked into nuts and cereals were > 80%, and the relative standard deviations were < 11.2% (Nonaka et al., 2009). When using SPME, the optimization of the SPME parameters should allow to reach the highest value of the extraction ratio, a low desorption time, a high repeatability and reliability, and the increase of the fiber lifetime (Quinto et al., 2009). Quinto et al. (2009) investigated and optimized the SPME-HPLC method parameters (fiber polarity, temperature, pH, ionic strength, mobile phase, adsorption and desorption time) for the accurate determination of AFB1, AFB2, AFG1 and AFG2 in cereal flours. Before SPME procedure, methanol/PBS (pH 5.8) extraction was carried out. Due to methanol (as well as others with hydrophilic characteristics) can cause the swelling of the coating, with a consequent decrease of the fiber lifetime; therefore, the extraction solution was evaporated under a N_2 stream for 10 min to eliminate methonal prior to SPME. Final result showed that SPME technique was fast when compared with the complete analytical process using IAC. However, conventional SPME fibers have some draw-backs such as fiber breakage, stripping of coatings and memory effects, moreover, these fibers are relatively expensive and their useful lifetime is limited, which have limited its application (Es'haghi et al., 2011).

3.2.2.3. MSPD

MSPD is a sample preparation strategy which was developed in 1989 (Barker et al., 1989), and it has been widely applied to solid, semisolid or viscous samples, including animal tissues and

foods with a high lipidic content (Capriotti et al., 2010). The application of MSPD for the analysis of foods is based on the blending of a viscous, solid or semi-solid sample with an abrasive solid support material that has been derivatized to produce a bound organic phase on its surface (Barker, 2000a). This mixture is then packed in a glass column or cartridge and afterwards elution with a series of organic solvents is performed (Shephard, 2009).

Therefore, the MSPD can avoid many of the difficulties encountered by employing the classical SPE approach, such as the need of sample homogenization and tissue debris removal prior to column application, as well as incomplete cell disruption (Barker, 2000a; Barker, 2000b).

However, the selectivity of an MSPD procedure depends on the sorbent/solvent combination used. Most methods reported to date use reverse-phase materials, such as C8- and C18-bonded silica as the solid support, while silica, florisil and chemically-modified sorbents are used less frequently (Kristenson et al., 2006). Blesa et al. (2003) extracted AFB1, AFB2, AFG1 and AFG2 from peanuts using MSPD with recoveries of about 80% with RSD about 5% by dispersing 2 g of peanuts on 2 g of C18-bonded silica and elution with acetonitrile. This was the first attempt to apply the MSPD method for analysis of aflatoxins in foods. Cavaliere et al. (2007) has extracted these four aflatoxins from olive oil sample by means of MSPD, utilizing a low load C18 as dispersing material, with recoveries ranging 92-107% (RSD, below 13%). No further purification step was performed. Rubert et al. (2010) extracted the AFB1, AFB2, AFG1 and AFG2 from cereal using 1 g sample, 1 g C18 and 10 mL acetonitrile for the elution from the cartridges. Recoveries were reported to be between 64 and 91%, and LOQ_s of 1 $\mu g\ kg^{-1}$ were reached. Rubert et al. (2011) used MSPD method to extract aflatoxins and ochratoxin A on commercial

flour samples (with different cereals composition) followed by LC-MS/MS detection. Eight widely used solid phases were tested: C18, C8, celite, silica, florisil, phenyl, alumina (acidic, neutral and basic) and amine. C18 proved to be a good solid support for the extraction of aflatoxins. The possibility of achieving simultaneous extraction and clean-up using MSPD for analysis of aflatoxins in hot chilli products (Zheng et al., 2006), and high-pigment matrices such as chilli powder, green bean and black sesame (Hu et al., 2006), tigernuts and tigernut beverages (Sebastià et al., 2010) have also been reported.

3.2.2.4. IAC

IAC is a particular case of SPE, which contains immobilised antibodies that exclusively retain a certain mycotoxin or mycotoxin class. Due to their high specificity, IAC produce cleaner extracts with a minimum level of interfering matrix components and excellent signal-to-noise ratios compared to less selective SPE sorbent materials (Krska et al., 2008; Zöllner and Mayer-Helm, 2006). Moreover, IAC is also an automated sample clean-up method and reduces the amount of work per sample (Stroka and Anklam, 2000; Stroka and Anklam, 2002), IAC in combination with HPLC is increasingly used nowadays as reference methods and allows a sufficient elimination of matrix interferences, due to their high selectivity (Ariño et al., 2009; Beltrán et al., 2011; Dors et al., 2011; Karami-Osboo et al., 2012; Tabari et al., 2011). However, IAC procedure is time and solvent consuming and requires a high level of expertise and the use of expensive disposable cartridges (Krska et al., 2008). Moreover, IAC are generally designed for only one type of toxin, reducing the method multiresiduality, this drawback could be solved with the introduction of columns with specific antibodies. Multi-mytoxin IAC containing antibodies

specific to more than one mycotoxin have been developed (Krska et al., 2008; Shephard, 2009). For example, combined immunoaffinity materials that are specific to both aflatoxins and ochratoxin A has been used which reduces sample throughput and allows the simultaneous analysis of all these compounds (Beltrán et al., 2011; Janati et al., 2012; Pietri et al., 2010; Pietri et al., 2012).

3.2.2.5. MFC

MFC is designed to retain particular groups of compounds that may interfere in the analytical method but at the same time allows analytes of interest to pass through (Khayoon et al., 2010). The proprietary packing material in MFC contains both lipophilic (non-polar) and charged (polar) active sites. Lipophilic sites are largely responsible for the removal of fats and other non-polar components such as xanthophyll pigments. Charged sites consist of both dipolar and anion-exchange sites that remove proteinaceous compounds, carbohydrate and other polar interfering compounds. Contrary to SPE, the MFC procedure does not require washing or elution procedures (Khayoon et al., 2010). Cleaning-up of the extracts using the MFC is effective in removing unwanted interfering components, thus can be recommended as alternative clean-up sorbent to the more expensive IAC (Khayoon et al., 2010). Another advantage of the MFC procedure is the elimination of the irreversible adsorption or premature elution of analyte from the sorbent column (Aycicek et al., 2005; Khayoon et al., 2010). Multifunctional MycoSep® columns containing a mixture of charcoal, ion-exchange resins and other materials are suitable for aflatoxins, trichothecenes, ochratoxins, zearalenone, moniliformin and patulin (Krska et al., 2008). However, Akiyama et al. (2001) compared MycoSep® #226, #228 column (MycoSep®

type), MultiSep® #228 column (cartridge type) and Isolute Multimode® (cartridge type), which indicated that both the MultiSep® #228 column (cartridge type) and MycoSep® #228 column (MycoSep® type) showed better results than the others in terms of the recoveries of aflatoxins from red pepper. Multimode® Columns have been applied to the clean-up of AFB1, AFB2, AFG1, and AFG2 in animal feeds, comprising corn, soya bean meal, mixed meal, sunflower, wheat, canola, palm kernel, and copra meals, when analysed by HPLC-FLD. By adopting an isocratic chromatographic system using a mobile phase comprising acetonitrile/methanol/water, the separation of the four aflatoxins was possible within 30 min. Recoveries for AFB1, AFB2, AFG1, and AFG2 were 98 ± 0.7%, 95 ± 1.0%, 94 ± 3.6% and 97 ± 4.3%, respectively (Khayoon et al., 2010).

3.2.2.6. Other Clean-up Methods

Other clean-up methods that have been applied to other mycotoxins that may show future promise for the cleanup of aflatoxin extracts are molecularly imprinted polymers (MIPs) based on the chemical generation of a simulated binding site for the mycotoxin in a polymeric lattice (Shephard, 2009; Urraca et al., 2006), and DNA aptamers based on the synthesis of single-stranded oligonucleotides (Cruz-Aguado and Penner, 2008). MIPs are also considered as a potential and cheaper alternative for clean-up, which, contrary to IAC, does not suffer from the storage limitations and stability problems that originate from the use of organic solvents and have been developed to clean-up many mycotoxins such as deoxynivalenol, zearalenone, ochratoxin A, and moniliformin (Krska et al., 2008).

3.3. Separation and Detection of Aflatoxins

The monitoring of aflatoxins in food at regulatory limits depends directly on precise and reliable analytical methods for their determination. Different techniques have been found for this purpose, including chromatographic methods such as TLC and HPLC coupled with different detectors, capillary electrophoresis (CE), immunological methods such as the ELISA, lateral-flow immunoassay (LFIA), immunosensors and non-destructive optical techniques like spectroscopy and imaging technology.

3.3.1. Chromatographic Separation Coupled to a Suitable Detection System

Chromatography coupled to different detection systems is used to confirm unambiguously positive findings and enables exact quantification when a maximum tolerable level or a relevant guideline level has been exceeded (Zöllner and Mayer-Helm, 2006). Because aflatoxins are low molecular mass polar compounds that possess significant ultraviolet absorbance and fluorescence properties, liquid separation techniques have predominated in their analysis, which was initially dominated by the use of TLC but subsequently by HPLC (Shephard, 2009).

3.3.1.1. TLC

The TLC method is the earliest method was developed to measure aflatoxins (Măruţoiu et al., 2004) and has been recommended and officially recognized by the AOAC for the analysis of aflatoxins in pistachio samples (AOAC, 1988; Sheibani and Ghaziaskar, 2009). Although it was replaced in developed countries in the early 1980s due to technical progress in HPLC and later in

ELISA and fluorometric techniques, it is still used commonly in developing countries owing to its simplicity and practicability (Stroka and Anklam, 2002).

The TLC method is a fast, practical method for qualitative (Shouman et al., 2012; Tripathi and Mishra, 2011), quantitative, and semi-quantitative determination of aflatoxins (Bakirdere et al., 2012). The qualitative determination or confirmation of aflatoixn can be done through retention factor (R_f) value and the fluorescence color, after comparison with external standard (Shouman et al., 2012). For example, the identity of AFM1 was easily confirmed directly on a two-dimentional TLC plate by reacting AFM1 with trifluoroacetic acid. Trifluoroacetic acid was superimposed on the AFM1 spot, then the plate was kept in the dark 3 min, heated to 75°C for 5 min, and developed with chloroform/methanol/acetic acid/water. The R_f value of the blue-fluorescent derivative was compared with that for the AFM1 standard. This method was used successfully on extracts of milk, cheese, and liver, and AFM1 quantitied on the plate as low as 0.5 ng could be confirmed by this method. The method was also suitable for simultaneous confirmation of AFB1 (van Egmond et al., 1978). Semi-quantification is achieved by comparison of the sample with authentic standards using visual estimation of fluorescence of the separated spots under long wavelength ultraviolet light (Bakirdere et al., 2012; Fallah, 2010; Fallah et al., 2011; Shephard, 2009). In this approach, the precision and reliability of the results depends directly on skilled and experienced persons (Stroka and Anklam, 2000). In the quantification process, mainly separated spots on the TLC plate are exposed to ultraviolet light, and either fluorescence intensity (Bankole et al., 2010; Shouman et al., 2012; Stroka and Anklam, 2000; Tripathi and Mishra, 2011; Var et al., 2007) or absorbance (Kamkar, 2006) is measured. Modified and unmodified silica gel layers have both been commonly used for TLC (Fallah,

2010; Turner et al., 2009; Var et al., 2007). *tert*-butyl methyl ether/methanol/water (Stroka and Anklam, 2000; Stroka et al., 2000), toluene/ethylacetate/chloroform/formic acid (Caldas and Silva, 2007), toluene/acetic acid/formic acid (Shouman et al., 2012), chloroform/acetone (Bankole et al., 2010; Sheibani and Ghaziaskar, 2009; Wang et al., 2012b), and toluene/isoamyl-alcohol/methanol (Tripathi and Mishra, 2011) have been utilized as a developing solution for the separation of aflatoxins. Moreover, anhydrous/diethyl/ether and chloroform/acetone/methanol were once used as the first and second developing solution, respectively, to separate AFB1 in TLC plates (Var et al., 2007).

TLC can been applied both in one- and two-dimensional format (Shephard et al., 2013; Stroka and Anklam, 2000). For example, a one-dimensional TLC with IAC clean-up method to determine AFB1, AFB2, AFG1, and AFG2 in various food matrices was developed by Stroka and Anklam (2000). In this TLC procedure, exactly 100 mL of the re-dissolved aflatoxin solution was spotted on a silica gel 60 TLC plate with microlitre syringe, dried, pre-concentrated with methanol, and finally separated with a mobile phase of *tert*-butyl methyl ether/methanol/water, the method allowed the visual quantification of aflatoxins at concentrations of less than 1 ng g^{-1} (Stroka et al., 2000). The two-dimentional TLC procedure for confirmation of AFM1 has been detailed above (van Egmond et al., 1978). Another survey was undertaken by Atanda et al. to determine the AFM1 contamination of milk and some dairy products by two-dimensional TLC, and the LOD of this method was 2 μg L^{-1} (Atanda et al., 2007). Fallah (2010) also examined the AFM1 contamination in pasteurized milk, yoghurt, white cheese, butter and ice cream using modified two-dimentional TLC. In their study, thin layer plate of silica gel was marked with a pencil before spotting. The plate was developed in the first direction with diethyl

ether/methanol/water and dried. The plate was turned 90° and developed in chloroform/acetone/methanol as the second direction. Then, the plate was dried and concentration of AFM1 in the sample was estimated by visual comparison of sample extract fluorescent spots with that of standard AFM1 spots exposed to the ultraviolet light (364 nm). The identity of AFM1 was confirmed by the formation on the plate of a derivative using trifluoroacetic acid and comparison of the thin layer chromatographic properties of the derivatives of the sample spot and the standard. The lowest LOD of the method is 0.012 μg kg^{-1}. Two-dimentional TLC method was also been used to analyse AFB1 in helva. The re-dissolved eluates and AFB1 standards were spotted on TLC plate, then TLC plates were developed in first and second mobile phase consisting of anhydrous diethyl ether and chloroform/acetone/methanol, respectively. After development, the TLC plates were dried in the dark and exposed to long wave- ultraviolet (365 nm) for visual estimation and comparison of sample spots to AFB1 standard in terms of R_f and intensity (Var et al., 2007). Main advantages of TLC are the fast analysis of several samples in short period of time, low cost per sample analyzed, and easy estimation of contamination levels (Bakirdere et al., 2012; Shephard, 2009; Turner et al., 2009). However, TLC has been largely replaced by HPLC and other techniques for the determination of aflatoxins due to lower sensitivity and reproducibility (Bakirdere et al., 2012). For instance, the presence of different mycotoxins, including aflatoxin, fumonisin B1, zearalenone, deoxynivalenol, and ochratoxin A in dried food samples including maize, peanut, bean, soybean, rice, cassava flakes and flour and pumpkin seeds was confirmed by TLC using different mobile phases. However, samples that had low mycotoxin levels (less than 2 μg kg^{-1} for aflatoxin and ochratoxin A, 200 μg kg^{-1} for fumonisin B1, and 50 μg kg^{-1} for zearalenone and deoxynivalenol

as obtained on HPLC gave negative results via TLC (Njobeh et al., 2010). Application of over pressured TLC (OPTLC or OPLC), also called as high pressure TLC (HPTLC), combining with densitometric measurements increased the popularity of TLC (Bakirdere et al., 2012). This method integrates the benefits of HPLC and TLC. Linear OPLC is a forced flow technique, using external pressure on a chromatoplate sealed on the edges and a pump system for the admission of mobile phase into the stationary phase. Compared with HPLC, it requires less mobile phase using an off-line method and allows faster examination with the possibility of parallel analysis. OPLC is more efficient than the conventional TLC method, providing better resolution and more compact spots (Móricz et al., 2007). A conventional TLC tank was compared with a specially designed continuous linear development chamber for the ether development phase of the quantitation of aflatoxins by bi-directional HPTLC. The ability of different concentrations of aqueous acetone, aqueous methanol, and aqueous acetone/methanol to extract aflatoxin from naturally contaminated maize was also assessed. No significant difference was detected in the precision of the method. However, the continuous linear development chamber produced more compact and intense spots, and with greater separation between the aflatoxins. In addition, the ether development required only half of the development time (15 rain) needed for the conventional TLC tank (30 rain), and used a much smaller volume of solvent. Furthermore, 80% aqueous acetone has been found to extract more aflatoxin from maize than other concentrations of aqueous acetone, aqueous methanol, and aqueous acetone/methanol (1:1) (Bradburn et al., 1990). OPLC coupled with fluorescence densitometry as a quantification step could achieve LOD as low as 0.5 μg kg^{-1} (Shephard, 2009). OPLC methods were developed for the measurement of AFB1, AFB2, AFG1, and AFG2 contamination in various foodstuffs (e.g.,

maize, wheat, peanut, fish meat, rice, and sunflower seeds) (Pappa et al., 2002) and for the investigation of the toxic effect of the above mentioned AFB1, AFB2, AFG1, and AFG2 using the BioArena system (Móricz et al., 2003). HPTLC method was also developed for the determination of AFB1 in cereals. The samples were extracted first using methanol/water (80:20, v/v) and were purified further by SPE. Separation and quantification were achieved by HPTLC using a bifarious mobile phase of acetone/chloroform (8:92, v/v) on precoated silica gel glass plates. Densitometric analysis of AFB1 was carried out in the fluorescence mode at 366 nm. The calibration curves were linear in the range of 0.8-4.8 ng (Gao et al., 2011).

3.3.1.2. HPLC

TLC method requires large quantities of solvent, intensive laboratory procedures and is characterised by a lack of automation (Turner et al., 2009). LC is another separative method which offers good sensitivity, high dynamic range, versatility, and soft ionization conditions that permits access to the molecular mass of intact biological molecules, LC is similar to TLC in many aspects, including analyte application, stationary phase, and mobile phase (Awad et al., 2012). HPLC is one case of LC, subsequent advances have come in the areas of purification techniques and separation science with the development of HPLC and associated detectors (Shephard, 2008). The most frequently used method for the determination of aflatoxins is HPLC coupled to FLD after IAC extraction and clean-up (Amate et al., 2010). In 2009, the newest official methods, as validated by the AOAC or Comité Européen de Normalisation, rely on IAC clean-up of conventional extracts followed by HPLC separation of the analogues with detection based on natural fluorescence or the fluorescence generated by various derivatization methods

(Shephard, 2009). HPLC has been widely used in the literature due to its high separation power, high sensitivity, good reproducibility, ease of use, suitability for automation and ability to couple with different detectors (Bakirdere et al., 2012). Both normal-phase and reverse-phase columns have been used, but reverse-phase HPLC separations of aflatoxins are more widely used (Bakirdere et al., 2012; Es'haghi et al., 2011; Shephard, 2009). Reverse-phase HPLC has the easier manipulation as well as the smaller toxicity of watery mobile phases (Jaimez et al., 2000). The vast majority of separations are performed on reverse-phase systems and mostly rely on C18 columns (Bakirdere et al., 2012) and mobile phases composed of water, methanol and acetonitrile mixtures in the proper ratios (Bakirdere et al., 2012; Shephard, 2009). Apart from fluorescence detectors, ultraviolet detector is sometimes used in the HPLC method for analyzing aflatoxins, while the fluorescence detector is more widely used for its higher sensitivity. HPLC coupled with fluorescence stage uses the fluorescence properties of aflatoxins to quantify them. For the fluorescence intensity of AFB1, AFB2, AFG1, and AFG2, AFB2 and AFG2 are naturally strongly fluorescent molecules due to the high conjugation of their oxygenated structures, while AFB1 and AFG1 are weakly fluorescent molecules (Bakirdere et al., 2012). Consequently, some useful measures should be taken to improve the fluorescence properties of AFB1 and AFG1, as a result of that, better sensitivity can be obtained for aflatoxin detection. The most common measures to improve the fluorescence properties are pre- or post-column derivatization methods. In pre-column approach, trifluoroacetic acid is applied for the formation of the corresponding hemiacetals which has stronger fluorescence, while in the post-column approach, either bromination by an electrochemical cell (Kobra Cell) or the addition of bromide, the addition of pyridinium hydrobromide perbromide and the formation of an iodine derivative are frequently

used. Trifluoroacetic acid can convert the AFB1 and AFG1 molecules to their corresponding hemiacetal derivatives aflatoxin B2A (AFB2A) and aflatoxin G2A (AFG2A), whereas AFB2 and AFG2 are unaffected (Bakirdere et al., 2012; Shephard, 2009). However, trifluoroacetic acid is a toxic and corrosive chemical, hence, the use of trifluoroacetic acid is not preferred in chemical analysis (Bakirdere et al., 2012; Var et al., 2007). In addition, the relative instabilities of these derivatives in the pre-column method and the advantages of automation offered by post-column derivatization methods led to the adoption of this latter technique (Shephard, 2009). The iodine post-column derivatization method was originally optimized in the 1980s and provided good performance (Spanjer et al., 2008). However, the iodination method needs a separate pump and a heated reaction coil, which can cause peak broadening and the possible crystallization of iodine in incorrectly operated systems. Bromine has greater analyte response than achieved with iodine (Shephard, 2009). Another post-column derivatizing agent is pyridinium hydrobromide perbromide. For example, Manetta applied HPLC-FLD to detect AFM1 in milk using pyridinium hydrobromide perbromide as a post-column derivatizing agent of this toxin for the first time. The maximum detectability was achieved using 50 mg L^{-1} pyridinium hydrobromide perbromide at a flow rate of 0.3 mL min^{-1}, which resulted in a three-fold increased signal of AFM1 after derivatization. Both derivatization methods mentioned above produce molecules that are more fluorescent than their precursors, but, on the other hand, they present several disadvantages. The post-column derivatization using iodine or bromine needs extra pumps and chemical reactors on the HPLC system and a long time to prepare the mobile phase (Kong et al., 2013). A new derivatization approach is the use of post-column photochemical reactors (Es'haghi et al., 2011; Quinto et al., 2009). Photochemical derivatization is an alternative and more economical post-

column derivatization method (Shephard, 2009). In this approach, the outlet of the HPLC is simply connected to ultraviolet permeable polytetrafluoroethylene tubing and wrapped over a high intensity ultraviolet lamp. Ultraviolet light irradiation generates hydroxyl radicals from water that react with AFB1 and AFG1 type aflatoxins to yield the stable and highly fluorescent compounds, AFB2A and AFG2A, respectively (Bakirdere et al., 2012). The photochemical derivatization technique exhibits a number of advantages, such as simplicity, linearity of response, reproducibility, and the absence of chemical reagents, additional pumps or electrochemical cells (Hurst et al., 1991; Kong et al., 2013). This derivatization method coupled with IAC/HPLC has been used to analyze aflatoxins in bulk and pre-packed pistachios. For this application, AFB1 and AFG1 were derivatized photochemically prior to detection by irradiation with ultraviolet light at 254 nm with post-column photochemical derivatization with a fluorescence enhancement of approximately 30 times for AFB1 and AFG1 (Ariño et al., 2009). For another application, Campone et al. (2011) determined and evaluated AFB1, AFB2, AFG1, and AFG2 in maize, rice and wheat products using HPLC-FLD with photochemical derivation based on DLLME. The main advantages of this method were the simplicity of operation, the rapidity in achieving a very high sample throughput and the low cost. Earlier, Papadopoulou-Bouraoui et al.(2002) compared two post-column derivatization (PCD) techniques, ultraviolet irradiation (PCDUV), and electrochemical bromination (PCDEC), coupled with LC-FLD for the determination of AFB1, AFB2, AFG1, and AFG2 in corn, pistachio paste, peanut butter, fig paste, and animal feed samples. Photochemical fluorescence enhancement was obtained with two different commercially available systems (PCDUV1 and PCDUV2). An electrochemical bromination apparatus were used for bromination. The response ratios PCDUV/PCDEC for

AFB1, AFB2, AFG1, and AFG2 were 0.86, 0.96, 0.70, and 0.96, respectively, for PCDUV1 and 0.82, 0.95, 0.60, and 0.90, respectively, for PCDUV2. Enhancement of the fluorescence of aflatoxins can also be achieved without chemical derivatization by the incorporation of specific cyclodextrins into the mobile phase (Shephard, 2009).

Apart from the above mentioned HPLC-FLD, another novel approach is HPLC coupled with diode array detection (DAD) (HPLC-DAD), which has been successfully applied to the determination of AFB1, AFB2, AFG1, and AFG2 in pistachio nuts (Liau et al., 2007), and AFB1 and AFB2 in rice, peanut and wheat samples (Es'haghi et al., 2011). Although TLC and HPLC-DAD are sufficiently selective for the determination of fungal extracts or single target analysis in food stuffs, these methods are incapable of dealing with a large number of analytes in complicated samples (Cavaliere et al., 2006; Takino et al., 2004). Although the HPLC method has been widely used for aflatoxin analysis, this method is expensive and time-consuming. The recent introduction of ultra-high pressure LC (UHPLC) has allowed faster and more efficient chromatographic separations, reducing run times, and narrower peaks are obtained with this method, which results in increased sensitivity and improved peak resolution (Beltrán et al., 2011). These advantages are based on the use of columns filled with particles smaller than 2 µm and instruments with high pressure fluidic modules (Frenich et al., 2009). UHPLC-FLD method has been developed and validated with satisfactory results for the simultaneous determination of AFB1, AFG1, AFB2, and AFG2, ochratoxin A and zearalenone in barley. LOD_s were 340 ng kg^{-1} for zearalenone, 13 ng kg^{-1} for ochratoxin A and varied from 0.5 to 15 ng kg^{-1} for aflatoxins. Recovery percentages were between 78.2% and 109.2% (Ibáñez-Vea et al., 2011). Some applications of this technique in the mycotoxin analysis field have been recently reported in

combination with tandem MS (Beltrán et al., 2011; Frenich et al., 2009). For example, UHPLC with tandem mass spectrometry (UHPLC-MS/MS) was developed to determine 12 mycotoxins (deoxynivalenol, AFB1, AFB2, AFG1, AFG2 and AFM1, fumonisin B1 and fumonisin B2, ochratoxin A, HT-2 and T-2 toxin and zearalenone) simultaneously in maize, walnuts, biscuits and breakfast cereals. The selectivity of the MS/MS detection allowed the elimination of further clean up steps. Extraction, chromatographic and detection conditions were optimised in order to increase sample throughput and sensitivity. Matrix-matched calibration was used for quantification and recoveries of the extraction process ranged from 70.0% and 108.4%, with relative standard deviations lower than 25% in all the cases, when samples were fortified at 5 and 50 μg kg^{-1}. LOD_s ranged from 0.01 to 2.1 μg kg^{-1} and LOQ_s ranged from 0.03 to 6.30 μg kg^{-1} (Frenich et al., 2009). In addition, UHPLC coupled to a triple quadrupole mass spectrometer in the SRM mode is one of the most selective and sensitive techniques for the quantification and confirmation of organic contaminants and residues in food due to the fast high-resolution of UHPLC and the enhanced selectivity obtained with the triple quadrupole mass analyzer in SRM mode. Using this method, the chromatographic separation of AFG1, AFG2, AFB1, AFB2, AFM1, and ochratoxin A in baby food commodities and milk can achieved in only 4 min in one study (Beltrán et al., 2011). The quadrupole analyzer allows the minimization of sample treatment, facilitating the determination of all analytes without the need to apply an SPE cleanup that might restrict the multiresiduality of the method (Beltrán et al., 2009). UHPLC-MS/MS with the triple quadrupole analyzer has also been used to simultaneously analyze eleven regulated mycotoxins including aflatoxins in different food commodities with minimal sample treatment in less than 5 min, and satisfactory recoveries were obtained (70-110%) (Beltrán et al., 2009).

However, difficulties have been found in reaching the sensitivity required for AFB1 and AFM1 in baby food due to the low concentration levels permitted by the EC (Beltrán et al., 2011; Beltrán et al., 2009).

3.3.1.3. LC-MS/MS

Because a single agricultural commodity can be contaminated with different fungal species or a single fungal specie can produce different toxins resulting in the co-occurrence of a number of different mycotoxins, the current trend for determination of mycotoxins in food is focused on the simultaneous detection of multiple mycotoxins via LC-MS/MS (Shephard et al., 2013). The main advantages of this technique include its general applicability to a broad range of compounds, its high sensitivity and its outstanding selectivity. Most importantly, in principle, it enables multi-analyte determination in one run without dedicated sample clean-up (Shephard et al., 2013; Spanjer et al., 2008). Many authors avoid purification steps and inject the crude extract to increase the multi-analyte capacity of the method (Beltrán et al., 2009; Frenich et al., 2009; Herebian et al., 2009; Spanjer et al., 2008; Sulyok et al., 2010). Methods using LC-MS were rarely employed before 1996 (Zöllner and Mayer-Helm, 2006). In addition, thermospray (TSP), particle beam (PB), and fast atom bombardment (FAB) interfaces were predominantly used for the trace analysis at the level of trichothecenes, fumonisins, zearalenone, and aflatoxins (Hurst et al., 1991). This situation changed rapidly from the mid-1990s onward when the benefits of atmospheric pressure ionization (API) interfaces were realized when compared to LC-TSP/MS and GC/MS techniques, as API interfaces overcome the traditional drawbacks of GC/MS regarding volatility and thermal stability. LC-atmospheric pressure chemical ionization (APCI)

(LC-APCI/MS) and LC-ESI/MS have become widely used in environment and food analysis, owing to their robustness, easy handling, high sensitivity, accuracy, analyte selectivity and their compatibility with almost the whole range of compound polarities (Zöllner and Mayer-Helm, 2006). The coupling of HPLC to MS via API techniques such as ESI (Amate et al., 2010; Bandeira et al., 2012; Cavaliere et al., 2007; Desmarchelier et al., 2010; Warth et al., 2012), APCI (Liau et al., 2007) or atmospheric pressure photoionization (APPI) (Cavaliere et al., 2006; Takino et al., 2004) has resulted in a range of new methods for single mycotoxins, single mycotoxin groups (such as aflatoxins) or for true multitoxin analyses. LC-APCI-MS/MS and LC-ESI-MS/MS on a quadrupole ion trap instrument in the selected reaction monitoring (SRM) mode were tested and compared when determining aflatoxin levels in Zizyphi Fructus. The LOD_s for AFB1, AFB2, AFG1, and AFG2 were between 1.3 and 2.6 ng mL^{-1} with the ESI technique but varied between 0.5 and 1.1 ng mL^{-1} with the APCI technique. Consequently, the latter was adopted as the ionization method in the subsequent study. In particular, use of the SRM mode allowed unambiguous identification as well as concurrent quantification of AFB1, AFB2, AFG1, and AFG2, even if their concentrations were as low as 1 ng g^{-1} (Liau et al., 2007). Due to the use of the mass analyzer to overcome the problem of co-eluting impurities and the high sensitivity and specificity of the techniques, analysis of aflatoxins using LC-MS/MS methods, where "dilute and shoot" methods have been developed, does not need a purification step (Shephard, 2009). When using ESI, the matrix complexity can give rise to an unpredictable matrix effect; therefore, although very selective, LC-MS/MS also requires time and labor-intensive sample preparation steps (Bacaloni et al., 2008). In particular, multi-mycotoxin IACs for aflatoxins, ochratoxin A, and fusarium toxins have been used for the clean-up step in multiple

toxin analyses using LC-MS/MS (Shephard, 2009). Nonaka et al. (2009) also used automated on-line in-tube SPME coupled with LC-MS for the determination of AFB1, AFB2, AFG1, and AFG2 in nuts, cereals, dried fruits, and spices. This method was applied successfully to analyze food samples without interference peaks. The recovery values of aflatoxins spiked into nuts and cereals were > 80% with relative standard deviations of < 11.2%. Aflatoxins were detected at < 10 ng g^{-1} in several commercial food samples.

3.3.2. CE

The effective separation of components, particularly when individually detecting closely related toxins can be based upon charge and mass dependent migration in an electrical field. The fast separations can be accomplished by CE in aqueous buffer solutions, excluding the need for organic solvents (Turner et al., 2009). Furthermore, CE can offer rapid analysis with high column efficiency and simplicity. These advantages have led CE to become a potentially more convenient method for the determination of mycotoxins (Peña et al., 2002). CE methods with laser-induced fluorescence were applied to detect AFB1 in corn. The limit of detection by CE was 0.5 μg kg^{-1} with a useful range of 1-100 μg kg^{-1} of AFB1 in spiked corn. Recovery of AFB1 averaged 85% over the range of 1-50 μg kg^{-1} (89% by HPLC). Forty naturally contaminated corn samples examined using CE showed good agreement with each other (R^2 = 0.969) (Maragos and Greer, 1997). In another example, a flow system coupled to CE for the quantification of AFB1, AFB2, AFG1, AFG2, and ochratoxin A, OIB has been developed. This flow system allowed the total determination of several compounds using a fluorometric screening system. The micellar electrokinetic capillary chromatography (MECC) used in this research enabled the separation of

the six mycotoxins within 50 min with high reproducibility, as shown by RSD between 7.45 and 13.06% and LOD_s between 0.02 and 0.06 mg L^{-1} for all of the mycotoxins (Peña et al., 2002). The MECC technique is a highly efficient liquid phase separation technique that has been applied to the determination of AFB1, AFB2, AFG1, and AFG2 in earlier years (Cole et al., 1992; Holland and Sepaniak, 1993; Janini et al., 1996).

3.3.3. Immunological Methods

The chromatographic methods mentioned above require expensive instrumentation and expertise in the field of chromatography (Bakirdere et al., 2012). With advances in biotechnology, highly specific antibody tests based on the affinities of monoclonal or polyclonal antibodies for aflatoxins are now commercially available. Immunochemical methods based on ELISA can be used for screening procedures due to their speed, ease of operation, sensitivity, and high sample throughput without any clean-up or analyte enrichment steps (Goryacheva et al., 2007).

3.3.3.1. ELISA

ELISA is a sort of immunoassay technique that appeared in the 1960s. The working principle of this technique is simple: a range of antibodies is arranged on a specifically designed plate or column. When the plate or column is exposed to aflatoxins, epitopes of aflatoxins in the sample are recognized by the antibodies on the plate or column to form a complex. This complex then interacts with a chromogenic substrate and creates a signal in the form of electricity, light or other measurable parameters (Bakirdere et al., 2012; Turner et al., 2009). **Figure 2** shows its detection principle. ELISA has many advantages, including low LOD_s, low sample volume

requirement, high sample throughput, high speed, high sensitivity, ease of application, and minimum sample clean-up, which raise its popularity over other methods. ELISA is suitable for quick and sensitive analysis with high sample throughput and low sample volume (Pei et al., 2009). Furthermore, ELISA methods have become routinely used tools for the rapid monitoring of most mycotoxins, especially for the screening of raw materials (Krska et al., 2008). ELISA test kits are commercially available for most of the major mycotoxins (Krska et al., 2008). Horseradish peroxidase (HRP) is the most widely used enzyme in commercially available ELISA kits (Bakirdere et al., 2012). The ELISA methods employed are all commercial test kits in a competitive format (Shephard, 2009). There are two common types of competitive formats of the ELISA method for aflatoxin analysis: the direct competitive ELISA (Chun et al., 2007; Karami-Osboo et al., 2012) and indirect competitive ELISA (Rossi et al., 2012). The principle of the direct competitive ELISA method is that AFB1, AFB2, AFG1, and AFG2 standards of varying concentrations or samples will compete with AFB1-HRP conjugates to bind the *anti*-AFB1 antibody coated onto the polystyrene microtiter plate wells (Chun et al., 2007). In the indirect competitive method, AFB1 standards or feed extracts compete with AFB1-bovine serum albumin in PBS coated on the wells to react with anti-AFB1 monoclonal antibody, then HRP-labeled goat anti-mouse IgG is added to react with the AFB1 monoclonal antibody. In both methods, the absorbance is measured at 450 nm after binding in an ELISA reader. The average absorbance is calculated from the individual absorbance obtained from triplicate wells (Rossi et al., 2012). The strong interest in this technique is furthermore reflected in the increasing number of commercially available test kits for field use, based mostly on direct competitive assays (Krska et al., 2008). The ELISA technique has been widely used as a screening method for AFM1 in

recent years (Shephard et al., 2013). For example, a green indirect competitive ELISA method with a surrogate calibrator curve was developed by Guan et al. to measure AFM1 in milk. In this assay, a polyclonal anti-idiotype (anti-Id) antibody, used as an AFM1 surrogate, was generated by immunizing rabbits with fragments from an *anti*-AFM1 monoclonal antibody. The surrogate calibrator curve and the AFM1 standard curve had a similar shape, and a correlation between the two standard materials was observed (r = 0.9997). The within and between assay variations were below 10.8%, which demonstrated that the anti-Id antibody can be used in place of AFM1. In addition, the AFM1 milk sample data quantified with the surrogate calibrator curve showed a good correlation (r = 0.9922) when compared with a reference HPLC method (Guan et al., 2011). Kanungo et al. (2011) developed a novel sandwich ELISA to detect AFM1 in milk with different fat percentages after pretreatment. The assay involved the immobilization of a rat monoclonal antibody of AFM1 in a 384-well microtiter plate to capture the AFM1 antigen. The linear range of AFM1 detection achieved in 3% fat milk was 6.25-250 pg mL^{-1}, and the miniaturized assay (10 μL) enabled ultra-trace analysis of AFM1 in milk with a much improved lower limit of detection of 0.005 pg mL^{-1}. The total assay time to detect AFM1 by sandwich ELISA was approximately 4-5 h. Moreover, the hybrid system created by coupling the 1° Ab immobilized to an magnetic nanoparticle column with the microwell plate assay enabled simultaneous measurement of low (0.5 pg mL^{-1}) and high AFM1 contamination (200 pg mL^{-1}). Although ELISA can be used for screening procedures due to its speed, ease of operation, sensitivity and high sample throughput, the ELISA assay can provide pseudo-positive results and, sometimes, unacceptable quantification accuracy (Beltrán et al., 2011). When compared with the TLC and

HPLC techniques, ELISA is less reliable if the sample has a complicated matrix (Bakirdere et al., 2012). Therefore, ELISA requires additional confirmatory analysis (Beltrán et al., 2011).

3.3.3.2. LFIA

LFIA, also named the immunochromatographic assay or immune-gold colloid immunoassay, has attracted scientific and industrial interest for its attractive property of enabling very rapid, one-step, in situ analysis (Anfossi et al., 2013). LFIA has been applied for the qualitative and semi-quantitative detection of aflatoxins in food and feed. The first LFIA aimed at measuring aflatoxins was reported in 2002, which was also one of the first reported lateral flow assays for food contaminants. The absolute limit of detection in this test was 18 ng of AFB1, and the test could be completed in a total of 12 minutes, including sample preparation (Ho and Wauchope, 2002). From this pioneering approach, several papers have been published that describe devices mainly aimed at measuring AFB1. The simplest LFD is a dipstick, which is dipped directly into the sample solution. Labelled antibodies can be added to the sample as a concentrated suspension or provided in a lyophilized form to be re-suspended by the sample itself (Anfossi et al., 2013). In 2005, Delmulle et al. (2005) reported the development of a dipstick to detect AFB1 in pig feed. The visual LOD was set at 5 μg kg^{-1} and the analysis could be completed in 10 minutes. Visual LFIA for detecting AFB1 in rice, barley, and feed was also described by Shim et al., in this work, visual LOD for AFB1 of 5-10 μg L^{-1} (rice, barley) and 10-20 μg L^{-1} (feed) were obtained (Shim et al., 2008). Shim et al. (2009) also developed a *multi*-analyte device aimed at contemporary measuring AFB1 and OTA in feed. The described method in their study allowed the simultaneous detection of the two toxins in 15 minutes and showed a visual LOD of 10 μg kg^{-1}

for AFB1. Method validation by means of ELISA and HPLC confirmatory analyses was also reported in this study. More recently, Anforssi et al. (2011) developed a quantitative LFIA for total aflatoxin determination in corn samples. The assay could be completed in 10 minutes, showed a LOD of 10 μg L^{-1} and was validated through comparison with HPLC on twenty-five samples. In addition, an aqueous extracting medium was also optimized and proven to allow reliable quantification of total aflatoxin. Due to the extreme sensitivity required by legislations, the development of LFIAs for AFM1 is one of the most challenging goals in this research field. Very few papers have been published for AFM1 using LFIAs. Wang et al. (2011) first described the development of a later-flow device for the detection of AFM1, the whole analytical procedure could be completed in 10 minutes, as no sample treatment was required, and the cut-off level was 0.5-1 μg L^{-1}. The obtained visual LOD remains far away from the detectability demand imposed by the maximum residue limits according to EU for AFM1 contaminant.

3.3.3.3 Immunosensor Methods

Alternatives to ELISA for aflatoxin analysis include a number of electrical and electrochemical immunosensors. These immunosensors consist of a pair of electrodes (measuring and reference) implemented using the screen-printing technique. The measuring electrode is coated with specific antibodies that will retain aflatoxins of interest in the sample, whereas the other electrode (reference) is commonly made of a combination of Ag/AgCl (Espinosa-Calderón et al., 2011). Electrochemical immunosensors with surface-adsorbed antibodies on screen-printed carbon electrodes have been fabricated for the detection of AFM1 in milk (Micheli et al., 2005), and in an array configuration, for the detection of AFB1 (Pemberton et al., 2006). However, the

electrodes developed by Tan et al. were coated with conjugate aflatoxins instead of being coated with specific antibody, whereas the sample was mixed with the antibody. In this manner, some antibodies were captured by free aflatoxins in the sample and some others by aflatoxins attached to the electrode (Tan et al., 2009). An electrochemical immunosensor for AFB1 was developed by the electrode position of graphene oxide and then gold nanoparticles onto a gold electrode. The developed immunosensor was tested with food samples (peanut, rice, milk, flour, and soybean) and showed recoveries of 93.3-101.2% (Linting et al., 2012). In a further label-free immunochemical approach for the detection of AFB1 and ochratoxin A, optical waveguide lightmode spectroscopy was used with integrated optical waveguide sensor chips that measure the resonance incoupling angle of polarized light, thus determining the surface coverage (Adányi et al., 2007). Developments in the area of electrochemical immunosensors for AFM1 have already been described by Shephard et al. (2012). Additionally, Bacher et al. (2012) developed a sensitive label-free silver wire-based impedimetric immunosensor for the detection of AFM1 at levels down to 6.25 ng L^{-1}, which is quite low in view of current regulatory limits for AFM1 in milk and infant food around the world.

3.3.3.4. Other Biosensor Methods

Array biosensors have been developed using competitive-based immunoassays for the simultaneous detection of multiple mycotoxins, including ochratoxin A, fumonisin, AFB1, and deoxynivalenol, on a single waveguide surface by imaging the fluorescent pattern onto a charge-coupled device (CCD) camera (Sapsford et al., 2006). Affinity-based surface plasmon resonance sensors have the advantage of not requiring any labeling of the target mycotoxin (Tüdös et al.,

2003) and may become an alternative method for rapid screening and may also enable the simultaneous detection of multiple mycotoxins using serially connected flow cells (van der Gaag et al., 2003). Photosensitive biosensors employing metallic nanoparticles have been considered as a promising candidate for overcoming some of the drawbacks of conventional fluorescence-based methods (Ahn et al., 2010). A photosensitive biosensor that overcomes some of the drawbacks of conventional biosensor fluorescence methods for AFB1 has been demonstrated in principle (Park et al., 2011). The concept involves a competitive antibody-based immunoassay performed on a photodiode sensing area. Due to the competitive nature of the assay, low levels of AFB1 would achieve the darkest response. Electronic circuitry for sensitive detection was described, and the range of AFB1 determination is claimed to be 0-15 μg L^{-1}. A novel technique worth mentioning is the DNA biosensor developed by Dinckaya et al. (2011). This study described the preparation and application of a thiol-modified single-stranded DNA probe that specifically binds AFM1. The probe was immobilized onto gold electrodes with the help of cysteamide and gold nanoparticles. The differences before and after binding of AFM1 to the probe were analyzed by cyclic voltammetric electrochemical impedance spectroscopy. An AFM1 calibration curve was prepared by considering the differences in electron transfer resistances before and after AFM1 binding. In-house repetitive testing with milk spiked with AFM1 at 0.4 μg L^{-1} showed a recovery of 107% and an RSD of 4.25% (Benedetti et al., 2005). Xu et al. (2013) developed a one-step and label-free optical biosensor based on competitive dispersion of gold nanorods for AFB1 analysis. The designed biosensing system could detect AFB1 in a linear range from 0.5-20 ng mL^{-1} with a good correlation coefficient (r = 0.99), and the LOD was 0.16 ng mL^{-1}.

3.3.4. Non-destructive Techniques

Traditional chemical and immunological methods require the use of toxic chemicals and are complex, time-consuming, require expertise, are expensive and are not suitable for real-time control measures (Firrao et al., 2010; Teena et al., 2013; Tripathi and Mishra, 2009). In addition, aflatoxin contamination is highly heterogeneous, and contaminated foodstuffs are often unevenly distributed (Kalkan et al., 2011; Schatzki and Pan, 1996). Therefore, it is more appropriate to pinpoint and remove these contaminated foodstuffs by simple, rapid, and non-destructive methods to avoid the loss of an entire lot (Kalkan et al., 2011). Several methods have been developed to measure the fungal or toxin contamination non-invasively (Kalkan et al., 2011).

3.3.4.1. Optical or Spectroscopy Methods

Optical or spectroscopy methods, such as near-infrared spectroscopy (NIRS) or Fourier transform NIRS (FT-NIRS) are promising techniques for the fast and non-destructive detection of mycotoxins and fungal contaminants in foodstuffs, including the studies of deoxynivalenol content in wheat kernels (Pettersson and Aberg, 2003), fusarium verticillioide contamination in maize (Berardo et al., 2005), and identification and characterization of filamentous fungi and yeasts (Santos et al., 2010). The NIR region of the spectrum is dominated by relatively weak and broad overtone and combination bands of fundamental vibrations involving mainly C-H, N-H, and O-H functional groups (Tripathi and Mishra, 2009). NIRS technique is a fast, nondestructive, environmentally friendly, and highly accurate method that requires little expert training (Dachoupakan Sirisomboon et al., 2013). FT-NIRS, by use of an interferometer, further improves the spectral reproducibility and wave number precision compared with NIR dispersion

instruments (Rodriguez-Saona et al., 2004; Tripathi and Mishra, 2009). A comparison between different NIRS instruments has confirmed the quality of spectral data when using FT-NIRS compared with NIRS dispersive instruments. In one study, the calibration models developed on FT-NIR spectral data allowed for the obtainment of better spectral information without mathematical pretreatment (Fernández-Ibañez et al., 2009).

Both transmittance spectra and reflectance modes coupled with NIRS can be used to detect aflatoxins in food. Pearson et al. (2001) utilized transmittance (500-950 nm) NIRS with a silicon photo diode-array fiber-optic spectrometer and reflectance (550-1700 nm) NIRS with a diode-array NIR spectrometer to distinguish aflatoxin contamination in single whole corn kernels in which corn kernels were manually placed on probes. Spectra were analyzed using discriminant analysis and partial least squares regression. More than 95% of the kernels were correctly classified as containing either high (> 100 μg kg^{-1}) or low (< 10 μg kg^{-1}) levels of aflatoxin, and the transmittance mode yielded slightly better results than the reflectance mode. However, the classification accuracy was reduced to 25% in the range between 10 and 100 μg kg^{-1}. For paprika determination, the reflectance mode was usually used. For instance, the potential of the NIRS technique for the analysis of red paprika for AFB1, ochratoxin A, and total aflatoxins was also explored with a remote reflectance fiber-optic probe applied directly onto the samples of paprika with no pretreatment or manipulation of samples. The prediction capacity of the model was assessed using the ratio performance deviation parameter. The ratio performance deviation values obtained with the samples used for AFB1, ochratoxin A, and total aflatoxins were of 5.2, 2.8 and 4.4, respectively, which indicated that the NIRS technique, using a fiber-optic probe, offered an alternative for the determination of AFB1, ochratoxin A and total aflatoxins in paprika,

with an advantageously lower cost and higher speed compared to the chemical method (Hernández-Hierro et al., 2008). In another study about AFB1 analysis in red chili powder, diffuse reflectance FT-NIRS with a sphere macro sample integrating sphere measurement channel was used. To gain high reproducibility and to remove inhomogeneity in the sample, spiked chili powder was densely packed into a sample cup and placed in a sample holder in a rotating sample wheel accessory. Each sample was collected three times after rotating the sample cup by 360°, with the mean of three spectra of the same sample used in further spectral analysis steps. There were no statistically significant differences in the means of the FT-NIRS method and the reference chemical methods for AFB1 detection, but the use of FT-NIRS methods was really time-saving, with acquisition requiring only 10 s (Tripathi and Mishra, 2009). Recently, the application of NIRS for detection of aflatoxin and aflatoxigenic fungi in rice was reported for the first time by Dachoupakan Sirisomboon et al. (Dachoupakan Sirisomboon et al., 2013). NIRS (950-1650 nm) in reflectance mode was successfully applied for detection of total fungal infection (%) and yellow-green Aspergillus infection (%) in rice samples. For yellow-green Aspergillus infection, the most accurate predictive statistical model was developed using an NIR spectra pretreated by maximum normalization (r = 0.437, SEP = 18.723% and bias = 4.613%). Additionally, the NIR spectra showed that the moisture and starch content in rice could affect the overall extent of fungal infection. Consequently, these parameters should be considered to improve the accuracy of the model (Dachoupakan Sirisomboon et al., 2013). Although NIRS is well established as a non-destructive tool for aflatoxin contaminant analysis of food materials, spectrometers only detect a small portion of the samples and are sometimes not representative of the whole sample, especially when the ingredients are not evenly distributed (Feng and Sun,

2012). In addition, spectroscopic assessments with relatively small point-source measurements do not contain spatial information. Other methods should be taken into consideration.

3.3.4.2. Machine Vision or Computer Vision

To obtain spatial information that spectroscopy methods cannot offer, another technology, machine vision (also known as computer vision) based on hyperspectral imaging (HSI) and multispectral imaging (MSI) techniques is available. HIS and MSI systems combine spatial and spectral parameters by using imaging spectrographs or filters and are widely used in food applications. The main advantage of the HSI system is its aptitude to incorporate both spectroscopy and imaging techniques not only to make a direct assessment of different components simultaneously but also to locate the spatial distribution of such components in the tested products (Elmasry et al., 2012). HSI techniques include near-infrared HSI, fluorescence HSI, and Raman HSI or their combinations (Feng and Sun, 2012). Most of the hyperspectral reflectance imaging research has been carried out in the a visible-NIR (400-1000 nm) or NIR (1000-1700 nm) range and has been used to detect defects, contaminants and quality parameters of food products (fruits, vegetables, meat products) (Del Fiore et al., 2010). For example, visible-NIR reflectance HSI has been used to study spectral responses of *Aspergillus flavus* growth. Statistical analysis indicated that the day-to-day surface reflectance of *Aspergillus flavus* differed significantly in specific regions of the wavelength spectrum and that external disturbances due to environmental changes had an impact on the normal *Aspergillus flavus* growth and consequently altered the reflectance patterns of *Aspergillus flavus* (DiCrispino et al., 2005). The potential of NIR (1000-1600 nm) reflectance HSI for the detection of fungal infected wheat kernels was

described by Singh et al. (2007). In addition, visible-NIR (400-1000 nm) reflectance HIS method was successfully tested by Jin et al. to classify toxigenic and atoxigenic strains of *Aspergillus flavus* (Jin et al., 2009). HSI at the visible-NIR spectral range of 400-1000 nm was also used to detect fungal contamination and the extent of damage in maize kernels (Del Fiore et al., 2010). Delwiche et al. (2009) used visible-NIR (418-918 nm) reflectance HSI to differentiate sound wheat kernels from those with the fungal condition called black point or black tip. Later, Singh et al. (2012) used a short-wave NIR spectra in the range of 700-1100 nm to detect storage fungi in wheat. Aflatoxin emits fluorescence when excited with ultraviolet light (Carnaghan et al., 1963; Goryacheva et al., 2008). In addition, *Aspergillus flavus* infected grains also emit bright greenish yellow fluorescence (BGYF) under ultraviolet (365 nm) excitation. BGYF is produced by the reaction of kojic acid formed by the fungus and a peroxidase enzyme from living corn or nuts (Bothast and Hesseltine, 1975; Wicklow, 1999; Yao et al., 2010a). The BGYF phenomenon has been widely used in presumptive tests for the presence of aflatoxins in corn (Bothast and Hesseltine, 1975; Maupin et al., 2003). and pistachio nuts (Hadavi, 2005). The BGYF approach is only used as a presumptive test rather than for quantitative or even qualitative analysis as it encounters both false-positive and false-negative detection problems (Bothast and Hesseltine, 1975; Yao et al., 2006). Because aflatoxin contaminated corn kernels do not always exhibit BGYF due to the insufficient amount of peroxidase in kernels. Moreover, other types of fungi that do not produce aflatoxin may yield kojic acid in foods and may be classified as aflatoxin-contaminated by the BGYF test (Kalkan et al., 2011).

Fluorescence HSI technology has been developed to enable the acquisition of fluorescence image data with both high spectral and spatial resolution (Kim et al., 2001). This method has been

utilized to study corn contaminated with aflatoxin and atoxigenic fungal strains by Yao et al. (Hadavi, 2005; Yao et al., 2010a; Yao et al., 2013; Yao et al., 2008; Yao et al., 2010b), and to detect aflatoxins in red chili pepper by Atas et al. (Ataş et al., 2010; Ataş et al., 2011; Ataş et al., 2012). However, the rich information in HSI results in difficulties in data processing, which makes it hard for industrial online applications. To overcome this problem, MSI is available. Like HSI, the MSI technique integrates conventional imaging and spectroscopy to attain both spatial and spectral information from an object, but these two methods are still slightly different. For HSI, there are normally more than 100 bands, while for MSI, it is usually less than 10 (Elmasry et al., 2012; Feng and Sun, 2012; Kalkan et al., 2011). MSI has been used to detect aflatoxin-contaminated hazelnut kernels and red chili peppers. Classification accuracies of 92.3% and 80% were achieved for aflatoxin-contaminated and uncontaminated hazelnuts and red chili peppers, respectively, without the use of any chemicals or sample preparation (Kalkan et al., 2011). This is also the first time that MSI was applied for analysis of aflatoxin contamination in food, to the best of our knowledge. In the future, MSI will be widely applied.

4. VALIDATION FOR ANALYSIS OF AFLATOXINS

The EC has laid down certain requirements for sampling and performance criteria for the official control of levels of various mycotoxins in foodstuffs such as cereal, groundnuts and nuts, spices, dried fruits, fruit juices, must and wine, milk and coffee, as well as food for infants and young children (Krska et al., 2008). Therefore, the whole analytical method, including sampling, sample preparation, clean-up and final determination used by enforcement laboratories for the implementation and control of legislation and regulatory limits must be subject to a validation

procedure to show that the method produces reliable results and meets the set performance criteria (Josephs et al., 2004; Krska et al., 2008). Typical performance characteristics to be evaluated for the validation of a quantitative method are the LOD and the LOQ, linearity, precision (repeatability and reproducibility), selectivity (interference of other compounds and/or matrix components), robustness/ruggedness, working range and trueness/bias. Several protocols and guidelines for method validation have been published (Ataş et al., 2010; Ataş et al., 2011; Ataş et al., 2012). There is a multitude of analytical methods available that have been validated and accepted by official authorities, such as the European Committee for Standardization, the AOAC International, and the International Organisation for Standardization (ISO) (Gilbert and Anklam, 2002). Each laboratory should implement quality assurance measures such as frequently checking the accuracy and precision of their methods (Josephs et al., 2004).

5. SAFETY CONSIDERARTIONS AND PRECAUTIONS

Aflatoxins are carcinogenic, mutagenic, teratogenic, and toxic compounds (Bakirdere et al., 2012). Consequently, the handling or preparation of standards, working solutions, and extracts must be performed in a fume hood with a laboratory coat, mask, and gloves. Glassware used for standards or samples should be soaked in 5% aqueous sodium hypochlorite to destroy residual aflatoxins before cleaning and reuse (Desmarchelier et al., 2010). Aflatoxins undergo light degradation; thus, the standard solutions should be kept in amber vials (Bacaloni et al., 2008; Nakai et al., 2008).

6. SUMMARY AND OUTLOOK

In conclusion, a broad range of detection techniques used for practical analysis and detection of aflatoxins are available. It is worthy of note there are substantial variations in results since usually contamination is not uniformly distributed. It is crucial to prepare sample in a way that ensures that the analytical sample is truly representative. Given that aflatoxin contamination of food and feed will still be an issue in the foreseeable future, methods for its analysis will continue to be developed and improved, although remarkable achievements have been attained. There are still substantial development challenges and opportunities: (i) Methods for rapid or non-destructive detection of aflatoxins have recently become of increased interest. It is needed to explain the recognition mechanism and improve the detection accuracy and sensitivity. The combination of optical, immunchemical, and immunsensor is a good choice; (ii) Methods for the simultaneous detection and quantification of aflatoxins from different kinds of samples should be further developed; (iii) The use of new materials on crops seems to be an alternative against toxins and replace substances that are harmful for human health; and (iv) It is important to establish a routine monitoring program from production until consumption in terms of aflatoxin levels and a more stringent food safety system to control the aflatoxins at the lowest possible level and realize minimizing the public health risk.

ACKNOWLEDGMENTS

We would like to gratefully acknowledge the financial support provided by the National Natural Science Foundation of China (No. 31000668).

REFERENCES

International Agency for Research on Cancer. (1993). Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 56. IARC, Lyon, France.

International Agency for Research on Cancer. (2002). Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 82. IARC, Lyon, France.

Abbas, H. K. (2005). Aflatoxin and food safety, CRC Press.

Adányi, N., Levkovets, I. A., Rodriguez-Gil, S., Ronald, A., Váradi, M., and Szendrő, I. (2007). Development of immunosensor based on OWLS technique for determining Aflatoxin B1 and Ochratoxin A. *Biosens. Bioelectron* **22**:797-802.

Afsah-Hejri, L., Jinap, S., Arzandeh, S., and Mirhosseini, H. (2011). Optimization of HPLC conditions for quantitative analysis of aflatoxins in contaminated peanut. *Food Control* **22**:381-388.

Agag, B. (2004). Mycotoxins in foods and feeds: 1-aflatoxins. *Ass. Univ. Bull. Environ. Res.* **7**:176-206.

Aguilera-Luiz, M. M., Plaza-Bolaños, P., Romero-González, R., Martínez Vidal, J. L., and Frenich, A. G. (2011). Comparison of the efficiency of different extraction methods for the simultaneous determination of mycotoxins and pesticides in milk samples by ultra high-performance liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **399**:2863-2875.

Ahn, C.-G., Ah, C. S., Kim, T.-Y., Park, C. W., Yang, J.-H., Kim, A., and Yong Sung, G. (2010). Photosensitive biosensor array system using optical addressing without an addressing circuit on array biochips. *Appl. Phys. Lett.* **97**:103703-103703-3.

Akiyama, H., Goda, Y., Tanaka, T., and Toyoda, M. (2001). Determination of aflatoxins B1, B2, G1 and G2 in spices using a multifunctional column clean-up. *J. Chromatogr. A* **932**:153-157.

Allcroft, R., Rogers, H., Lewis, G., Nabney, J., and Best, P. E. (1966). Metabolism of Aflatoxin in Sheep-Excretion of Milk Toxin. *Nature* **209**:154-155.

Amate, C. F., Unterluggauer, H., Fischer, R., Fernández-Alba, A., and Masselter, S. (2010). Development and validation of a LC-MS/MS method for the simultaneous determination of aflatoxins, dyes and pesticides in spices. *Anal. Bioanal. Chem.* **397**:93-107.

Anastassiades, M., Lehotay, S. J., Stajnbaher, D., and Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *J. AOAC Int.* **86**:412-431.

Anfossi, L., Baggiani, C., Giovannoli, C., D'Arco, G., and Giraudi, G. (2013). Lateral-flow immunoassays for mycotoxins and phycotoxins: a review. *Anal. Bioanal. Chem.* **405**:467-480.

Anfossi, L., D'Arco, G., Calderara, M., Baggiani, C., Giovannoli, C., and Giraudi, G. (2011). Development of a quantitative lateral flow immunoassay for the detection of aflatoxins in maize. *Food Addit. Contam.* **28**:226-234.

Anklam, E., Berg, H., Mathiasson, L., Sharman, M., and Ulberth, F. (1998). Supercritical fluid extraction (SFE) in food analysis: A review. *Food Addit. Contam.* **15**:729-750.

Association of Official Analytical Chemists International. (1988). Official Method No. 969.22. Official methods of Analysis of the AOAC International.

Ariño, A., Herrera, M., Estopañan, G., Rota, M. C., Carramiñana, J. J., Juan, T., and Herrera, A. (2009). Aflatoxins in bulk and pre-packed pistachios sold in Spain and effect of roasting. *Food Control* **20**:811-814.

Arthur, C. L., and Pawliszyn, J. (1990). Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **62**:2145-2148.

Asensio-Ramos, M., Hernández-Borges, J., Ravelo-Pérez, L. M., and Rodríguez-Delgado, M. A. (2010). Evaluation of a modified QuEChERS method for the extraction of pesticides from agricultural, ornamental and forestal soils. *Anal. Bioanal. Chem.* **396**:2307-2319.

Atanda, O., Oguntubo, A., Adejumo, O., Ikeorah, J., and Akpan, I. (2007). Aflatoxin M_1 contamination of milk and ice cream in Abeokuta and Odeda local governments of Ogun State, Nigeria. *Chemosphere* **68**:1455-1458.

Ataş, M., Temizel, A., and Yardimci, Y. (2010). Classification of aflatoxin contaminated chili pepper using hyperspectral imaging and artificial neural networks. **In**: Signal Processing and Communications Applications Conference (SIU), 2010 IEEE 18th, IEEE: 9-12.

Ataş, M., Yardimci, Y., and Temizel, A. (2011). Aflatoxin contaminated chili pepper detection by hyperspectral imaging and machine learning. *Proc. of SPIE* **8027**:8027F.

Ataş, M., Yardimci, Y., and Temizel, A. (2012). A new approach to aflatoxin detection in chili pepper by machine vision. *Comput. Electron. Agric.* **87**:129-141.

Awad, W. A., Ghareeb, K., and Böhm, J. (2012). Occurrence, health risks and methods of analysis for Aflatoxins and Ochratoxin A. *J. Vet. Anim. Sci.* **2**:1-10.

Aycicek, H., Aksoy, A., and Saygi, S. (2005). Determination of aflatoxin levels in some dairy and food products which consumed in Ankara, Turkey. *Food Control* **16**:263-266.

Bacaloni, A., Cavaliere, C., Cucci, F., Foglia, P., Samperi, R., and Laganà, A. (2008). Determination of aflatoxins in hazelnuts by various sample preparation methods and liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* **1179**:182-189.

Bacher, G., Pal, S., Kanungo, L., and Bhand, S. (2012). A label-free silver wire based impedimetric immunosensor for detection of aflatoxin M1 in milk. *Sensor. Actuat. B: Chem.* **168**:223-230.

Bakirdere, S., Bora, S., Bakirdere, E. G., Aydin, F., Arslan, Y., Komesli, O. T., Aydin, I., and Yildirim, E. (2012). Aflatoxin species: their health effects and determination methods in different foodstuffs. *Cent. Eur. J. Chem.* **10**:675-685.

Bakırdere, S., Yaroğlu, T., Tırık, N., Demiröz, M., and Karaca, A. (2012). Determination of trace aflatoxin M1 levels in milk and milk products consumed in Turkey by using enzyme-linked immunosorbent assay. *Food Agr. Immunol.* (ahead-of-print):1-9.

Bandeira, R. D. C. C., Uekane, T. M., Da Cunha, C. P., Geaquinto, L. R. O., Cunha, V. S., Caixeiro, J. M. R., Godoy, R. L. O., and De la Cruz, M. H. C. (2012). Development and validation of a method for the analysis of Ochratoxin A in roasted coffee by liquid chromatography/electrospray-mass spectrometry in Tandem (LC/ESI-MS/MS). *Quim. Nova.* **35**:66-71.

Bankole, S. A., Adenusi, A. A., Lawal, O., and Adesanya, O. (2010). Occurrence of aflatoxin B1 in food products derivable from 'egusi'melon seeds consumed in southwestern Nigeria. *Food Control* **21**:974-976.

Barker, S. A. (2000a). Applications of matrix solid-phase dispersion in food analysis. *J. Chromatogr. A* **880**:63-68.

Barker, S. A. (2000b). Matrix solid-phase dispersion. *J. Chromatogr. A* **885**:115-127.

Barker, S. A., Long, A. R., and Short, C. R. (1989). Isolation of drug residues from tissues by solid phase dispersion. *J. Chromatogr. A* **475**:353-361.

Beltrán, E., Ibáñez, M., Sancho, J. V., Cortés, M. Á., Yusà, V., and Hernández, F. (2011). UHPLC-MS/MS highly sensitive determination of aflatoxins, the aflatoxin metabolite M1 and ochratoxin A in baby food and milk. *Food Chem.* **126**:737-744.

Beltrán, E., Ibáñez, M., Sancho, J. V., and Hernández, F. (2009). Determination of mycotoxins in different food commodities by ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry. *Rapid Commun. Mass Sp.* **23**:1801-1809.

Benedetti, S., Iametti, S., Bonomi, F., and Mannino, S. (2005). Head space sensor array for the detection of aflatoxin M1 in raw ewe's milk. *J. Food Protect.* **68**:1089-1092.

Bennett, J. W., and Klich, M. (2003). Mycotoxins. *Clin Microbiol Rev* **16**:497-516.

Berardo, N., Pisacane, V., Battilani, P., Scandolara, A., Pietri, A., and Marocco, A. (2005). Rapid detection of kernel rots and mycotoxins in maize by near-infrared reflectance spectroscopy. *J. Agr. Food Chem.* **53**:8128-8134.

Bhat, R., Rai, R. V., and Karim, A. A. (2010). Mycotoxins in Food and Feed: Present Status and Future Concerns. *Compr. Rev. Food Sci. F.* **9**:57-81.

Blesa, J., Soriano, J. M., Moltó, J. C., Marín, R., and Mañes, J. (2003). Determination of aflatoxins in peanuts by matrix solid-phase dispersion and liquid chromatography. *J. Chromatogr. A* **1011**:49-54.

Bothast, R. J., and Hesseltine, C. W. (1975). Bright greenish-yellow fluorescence and aflatoxin in agricultural commodities. *Appl. Environ. Microbiol.* **30**:337-338.

Bradburn, N., Coker, R. D., Jewers, K., and Tomlins, K. I. (1990). Evaluation of the ability of different concentrations of aqueous acetone, aqueous methanol and aqueous acetone: Methanol (1:1) to extract aflatoxin from naturally contaminated maize. *Chromatographia* **29**:435-440.

Breithaupt, D. E. (2004). Simultaneous HPLC determination of carotenoids used as food coloring additives: applicability of accelerated solvent extraction. *Food Chem.* **86**:449-456.

Caldas, E. D., and Silva, A. C. (2007). Mycotoxins in corn-based food products consumed in Brazil: an exposure assessment for fumonisins. *J. Agr. Food Chem.* **55**:7974-7980.

Campbell, A. D., Whitaker, T. B., Pohland, A. E., Dickens, J. W., and Park, D. L. (1986). Sampling, sample preparation, and sampling plans for foodstuffs for mycotoxin analysis. *Pure Appl. Chem.* **58**:305-314.

Campone, L., Piccinelli, A. L., Celano, R., and Rastrelli, L. (2011). Application of dispersive liquid-liquid microextraction for the determination of aflatoxins B1, B2, G1 and G2 in cereal products. *J. Chromatogr. A* **1218**:7648-7654.

Capriotti, A. L., Cavaliere, C., Giansanti, P., Gubbiotti, R., Samperi, R., and Laganà, A. (2010). Recent developments in matrix solid-phase dispersion extraction. *J. Chromatogr. A* **1217**:2521-2532.

Carnaghan, R. B. A., Hartley, R. D., and O'Kelly, J. (1963). Toxicity and fluorescence properties of the aflatoxins. *Nature* **200**:1101-1101.

Cavaliere, C., Foglia, P., Guarino, C., Nazzari, M., Samperi, R., and Laganà, A. (2007). Determination of aflatoxins in olive oil by liquid chromatography–tandem mass spectrometry. *Anal. Chim. Acta* **596**:141-148.

Cavaliere, C., Foglia, P., Pastorini, E., Samperi, R., and Laganà, A. (2006). Liquid chromatography/tandem mass spectrometric confirmatory method for determining aflatoxin M1 in cow milk: comparison between electrospray and atmospheric pressure photoionization sources. *J. Chromatogr. A* **1101**:69-78.

Chen, C.-Y., Li, W.-J., and Peng, K.-Y. (2005). Determination of aflatoxin M1 in milk and milk powder using high-flow solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J. Agr. Food Chem.* **53**:8474-8480.

Chen, J., and Pawliszyn, J. B. (1995). Solid phase microextraction coupled to high-performance liquid chromatography. *Anal. Chem.* **67**:2530-2533.

Chun, H. S., Kim, H. J., Ok, H. E., Hwang, J.-B., and Chung, D.-H. (2007). Determination of aflatoxin levels in nuts and their products consumed in South Korea. *Food Chem.* **102**:385-391.

Cole, R. O., Holland, R. D., and Sepaniak, M. J. (1992). Factors influencing performance in the rapid separation of aflatoxins by micellar electrokinetic capillary chromatography. *Talanta* **39**:1139-1147.

Creppy, E. E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol. Lett.* **127**:19-28.

Cruz-Aguado, J. A., and Penner, G. (2008). Determination of ochratoxin A with a DNA aptamer. *J. Agr. Food Chem.* **56**:10456-10461.

Cunha, S. C., Cunha, C., Ferreira, A. R., and Fernandes, J. O. (2012). Determination of bisphenol A and bisphenol B in canned seafood combining QuEChERS extraction with dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry. *Anal. Bioanal. Chem.* **404**:2453-2463.

Cunha, S. C., Lehotay, S. J., Mastovska, K., Fernandes, J. O., Beatriz, M., and Oliveira, P. P. (2007). Evaluation of the QuEChERS sample preparation approach for the analysis of pesticide residues in olives. *JSS* **30**:620-632.

Dachoupakan Sirisomboon, C., Putthang, R., and Sirisomboon, P. (2013). Application of near infrared spectroscopy to detect aflatoxigenic fungal contamination in rice. *Food Control* **33**:207-214.

David, M. D., Campbell, S., and Li, Q. X. (2000). Pressurized fluid extraction of nonpolar pesticides and polar herbicides using in situ derivatization. *Anal. Chem.* **72**:3665-3670.

David, M. D., and Seiber, J. N. (1996). Comparison of extraction techniques, including supercritical fluid, high-pressure solvent, and Soxhlet, for organophosphorus hydraulic fluids from soil. *Anal. Chem.* **68**:3038-3044.

Del Fiore, A., Reverberi, M., Ricelli, A., Pinzari, F., Serranti, S., Fabbri, A. A., Bonifazi, G., and Fanelli, C. (2010). Early detection of toxigenic fungi on maize by hyperspectral imaging analysis. *Int. J. Food Microbiol.* **144**:64-71.

Delmulle, B. S., De Saeger, S. M., Sibanda, L., Barna-Vetro, I., and Van Peteghem, C. H. (2005). Development of an immunoassay-based lateral flow dipstick for the rapid detection of aflatoxin B1 in pig feed. *J. Agr. Food Chem.* **53**:3364-3368.

Delwiche, S. R., Yang, I.-C., and Kim, M. S. (2009). Hyperspectral imaging for detection of black tip damage in wheat kernels. *Proc. of SPIE* **7315**:73150K.

Desmarchelier, A., Oberson, J.-M., Tella, P., Gremaud, E., Seefelder, W., and Mottier, P. (2010). Development and comparison of two multiresidue methods for the analysis of 17 mycotoxins in cereals by liquid chromatography electrospray ionization tandem mass spectrometry. *J. Agr. Food Chem.* **58**:7510-7519.

DiCrispino, K., Yao, H., Hruska, Z., Brabham, K., Lewis, D., Beach, J., Brown, R. L., and Cleveland, T. E. (2005). Hyperspectral imagery for observing spectral signature change in *Aspergillus flavus*. *Proc. of SPIE* **5996**:599606.

Dinckaya, E., Kınık, Ö., Sezgintürk, M. K., Altuğ, Ç., and Akkoca, A. (2011). Development of an impedimetric Aflatoxin M1 biosensor based on a DNA probe and gold nanoparticles. *Biosens. Bioelectron.* **26**:3806-3811.

Dors, G. C., Caldas, S. S., Feddern, V., Bemvenuti, R. H., dos Santos Hackbart, H. C., de Souza, M. M., dos Santos Oliveira, M., Garda-Buffon, J., Primel, E. G., and Badiale-Furlong, E. (2011). Aflatoxins: Contamination, Analysis and Control. **In**: Aflatoxins-Biochemistry cand Molcecular Biology, pp. 415-438. Guevara-Gonzalez, R. G., Eds., InTech, Rijeka.

European Commission. (2001). Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities* L **77**:1-13.

European Commission. (2004). Commission Regulation (EC) No 683/2004 of 13 April 2004 amending Regulation (EC) No 466/2001 as regards aflatoxins and ochratoxin A in foods for infants and young children. *Official Journal of the European Union* L **106**:3-5.

European Commission. (1998). Commission Regulation (EC) No 1525/98 of 16 July 1998, amending Regulation (EC) No 194/97 of 31 January 1997 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities* L **201**:43-46.

European Commission. (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union* L **364**:5-24.

Ehlers, D., Czech, E., Quirin, K. W., and Weber, R. (2006). Distribution of aflatoxins between extract and extraction residue of paprika using supercritical carbon dioxide. *Phytochem. Analysis* **17**:114-120.

Elmasry, G., Kamruzzaman, M., Sun, D.-W., and Allen, P. (2012). Principles and applications of hyperspectral imaging in quality evaluation of agro-food products: a review. *Crit. Rev. Food Sci. Nutr.* **52**:999-1023.

Es'haghi, Z., Sorayaei, H., Samadi, F., Masrournia, M., and Bakherad, Z. (2011). Fabrication of a novel nanocomposite based on sol–gel process for hollow fiber-solid phase microextraction of aflatoxins: B_1 and B_2, in cereals combined with high performane liquid chromatography-diode array detection. *J. Chromatogr. B* **879**:3034-3040.

Espinosa-Calderón, A., Contreras-Medina, L. M., Muñoz-Huerta, R. F., Millán-Almaraz, J. R., González, R. G. G., and Torres-Pacheco, I. (2011). Methods for Detection and Quantification of Aflatoxins. **In:** Detection, Measurement and Control, pp. 109-128. Torres-Pacheco, I., Eds., InTech, Rijeka.

Fallah, A. A. (2010). Aflatoxin M_1 contamination in dairy products marketed in Iran during winter and summer. *Food Control* **21**:1478-1481.

Fallah, A. A., Rahnama, M., Jafari, T., and Saei-Dehkordi, S. S. (2011). Seasonal variation of aflatoxin M_1 contamination in industrial and traditional Iranian dairy products. *Food Control* **22**:1653-1656.

U.S. Food and Drug Administration. (1996). Sec. 527. 400 Whole milk, low fat milk, skim milk-aflatoxin M1 (*CPG* 7106.210). FDA compliance policy guides. FDA, Washington, DC, 219.

Feng, Y.-Z., and Sun, D.-W. (2012). Application of hyperspectral imaging in food safety inspection and control: a review. *Crit. Rev. Food. Sci. Nutr.* **52**:1039-1058.

Fernández-Ibañez, V., Soldado, A., Martínez-Fernández, A., and de la Roza-Delgado, B. (2009). Application of near infrared spectroscopy for rapid detection of aflatoxin B1 in maize and barley as analytical quality assessment. *Food Chem.* **113**:629-634.

Firrao, G., Torelli, E., Gobbi, E., Raranciuc, S., Bianchi, G., and Locci, R. (2010). Prediction of milled maize fumonisin contamination by multispectral image analysis. *J. Cereal Sci.* **52**:327-330.

Frenich, A. G., Vidal, J. L. M., Romero-González, R., and Aguilera-Luiz, M. d. M. (2009). Simple and high-throughput method for the multimycotoxin analysis in cereals and related foods by ultra-high performance liquid chromatography/tandem mass spectrometry. *Food Chem.* **117**:705-712.

Gao, H., Chen, L., Pan, G., and Tu, C. (2011). Development and Validation of an HPTLC Method for Determination of Aflatoxin B1. *LC GC North America* **29**:71-74.

National Criterion of China. (2011). National Food Safety Standard-Limitation Standard for mycotoxins in foods. GB 2761–2011.

Geis-Asteggiante, L., Lehotay, S. J., and Heinzen, H. (2012). Effects of Temperature and Purity of Magnesium Sulfate During Extraction of Pesticide Residues Using the QuEChERS Method. *J. AOAC Int.* **95**:1311-1318.

Gilbert, J., and Anklam, E. (2002). Validation of analytical methods for determining mycotoxins in foodstuffs. *TrAC-Trend. Anal. Chem.* **21**:468-486.

Goryacheva, I. Y., De Saeger, S., Eremin, S. A., and Van Peteghem, C. (2007). Immunochemical methods for rapid mycotoxin detection: Evolution from single to multiple analyte screening: A review. *Food Addit. Contam.* **24**:1169-1183.

Goryacheva, I. Y., Rusanova, T. Y., and Pankin, K. (2008). Fluorescent properties of aflatoxins in organized media based on surfactants, cyclodextrins, and calixresorcinarenes. *J. Anal. Chem+.* **63**:751-755.

Guan, D., Li, P., Cui, Y., Zhang, Q., and Zhang, W. (2011). A competitive immunoassay with a surrogate calibrator curve for aflatoxin M_1 in milk. *Anal. Chim. Acta* **703**:64-69.

Hadavi, E. (2005). Several physical properties of aflatoxin-contaminated pistachio nuts: Application of BGY fluorescence for separation of aflatoxin-contaminated nuts. *Food Addit. Contam.* **22**:1144-1153.

Herebian, D., Zühlke, S., Lamshöft, M., and Spiteller, M. (2009). Multi-mycotoxin analysis in complex biological matrices using LC-ESI/MS: Experimental study using triple stage quadrupole and LTQ-Orbitrap. *JSS* **32**:939-948.

Hernández-Hierro, J. M., García-Villanova, R. J., and González-Martín, I. (2008). Potential of near infrared spectroscopy for the analysis of mycotoxins applied to naturally contaminated red paprika found in the Spanish market. *Anal. Chim. Acta* **622**:189-194.

Ho, J.-A., and Wauchope, R. (2002). A strip liposome immunoassay for aflatoxin B1. *Anal. Chem.* **74**:1493-1496.

Holland, R. D., and Sepaniak, M. J. (1993). Qualitative analysis for mycotoxins using micellar electrokinetic capillary chromatography. *Anal. Chem.* **65**:1140-1146.

Hsieh, D. P. H., Cullen, J. M., and Ruebner, B. H. (1984). Comparative hepatocarcinogenicity of aflatoxins B_1 and M_1 in the rat. *Food Chem. Toxicol.* **22**:1027-1028.

Hu, Y.-Y., Zheng, P., Zhang, Z.-X., and He, Y.-Z. (2006). Determination of aflatoxins in high-pigment content samples by matrix solid-phase dispersion and high-performance liquid chromatography. *J. Agr. Food Chem.* **54**:4126-4130.

Huang, B., Han, Z., Cai, Z., Wu, Y., and Ren, Y. (2010). Simultaneous determination of aflatoxins B1, B2, G1, G2, M1 and M2 in peanuts and their derivative products by ultra-high-performance liquid chromatography–tandem mass spectrometry. *Anal. Chim. Acta* **662**:62-68.

Hurst, W. J., Martin, Jr R. A., and Vestal, C. (1991). The use of HPLC/thermospray MS for the confirmation of aflatoxins in peanuts. *J. Liq. chromatogr.* **14**:2541-2550.

Hussain, I., Anwar, J., Munawar, M. A., and Asi, M. R. (2008). Variation of levels of aflatoxin M_1 in raw milk from different localities in the central areas of Punjab, Pakistan. *Food Control* **19**:1126-1129.

Ibáñez-Vea, M., Corcuera, L. A., Remiro, R., Murillo-Arbizu, M. T., González-Peñas, E., and Lizarraga, E. (2011). Validation of a UHPLC-FLD method for the simultaneous quantification of aflatoxins, ochratoxin A and zearalenone in barley. *Food Chem.* **127**:351-358.

Jaimez, J., Fente, C. A., Vazquez, B. I., Franco, C. M., Cepeda, A., Mahuzier, G., and Prognon, P. (2000). Application of the assay of aflatoxins by liquid chromatography with fluorescence detection in food analysis. *J. Chromatogr. A* **882**:1-10.

Janati, S. S. F., Beheshti, H. R., Asadi, M., Mihanparast, S., and Feizy, J. (2012). Preliminary survey of aflatoxins and ochratoxin A in dried fruits from Iran. *Bull. Environ. Contam. Toxicol.* **88**:391-395.

Janini, G. M., Muschik, G. M., and Issaq, H. J. (1996). Electrokinetic chromatography in suppressed electroosmotic flow environment: Use of a charged cyclodextrin for the separation of enantiomers and geometric isomers. *Electrophoresis* **17**:1575-1583.

Jin, J., Tang, L., Hruska, Z., and Yao, H. (2009). Classification of toxigenic and atoxigenic strains of *Aspergillus flavus* with hyperspectral imaging. *Comput. Electron. Agric.* **69**:158-164.

Josephs, R. D., Derbyshire, M., Stroka, J., Emons, H., and Anklam, E. (2004). Trichothecenes: reference materials and method validation. *Toxicol. Lett.* **153**:123-132.

Kalkan, H., Beriat, P., Yardimci, Y., and Pearson, T. C. (2011). Detection of contaminated hazelnuts and ground red chili pepper flakes by multispectral imaging. *Comput. Electron. Agric.* **77**:28-34.

Kamika, I., and Takoy, L. L. (2011). Natural occurrence of Aflatoxin B1 in peanut collected from Kinshasa, Democratic Republic of Congo. *Food Control* **22**:1760-1764.

Kamkar, A. (2006). A study on the occurrence of aflatoxin M_1 in Iranian Feta cheese. *Food Control* **17**:768-775.

Kanungo, L., Pal, S., and Bhand, S. (2011). Miniaturised hybrid immunoassay for high sensitivity analysis of aflatoxin M1 in milk. *Biosens. Bioelectron.* **26**:2601-2606.

Karami-Osboo, R., Mirabolfathy, M., Kamran, R., Shetab-Boushehri, M., and Sarkari, S. (2012). Aflatoxin B1 in maize harvested over 3 years in Iran. *Food Control* **23**:271-274.

Khan, M. R., Alothman, Z. A., Ghfar, A. A., and Wabaidur, S. M. (2013). Analysis of aflatoxins in nonalcoholic beer using liquid-liquid extraction and ultraperformance LC-MS/MS. *J. Sep. Sci.* **36**:572-577.

Khayoon, W. S., Saad, B., Yan, C. B., Hashim, N. H., Ali, A. S. M., Salleh, M. I., and Salleh, B. (2010). Determination of aflatoxins in animal feeds by HPLC with multifunctional column clean-up. *Food Chem.* **118**:882-886.

Kim, M. S., Chen, Y. R., and Mehl, P. M. (2001). Hyperspectral reflectance and fluorescence imaging system for food quality and safety. *Trans. ASAE* **44**:721-730.

Kong, W.-J., Liu, S.-Y., Qiu, F., Xiao, X.-H., and Yang, M.-H. (2013). Simultaneous multi-mycotoxin determination in nutmeg by ultrasound-assisted solid-liquid extraction and immunoaffinity column clean-up coupled with liquid chromatography and on-line post-column photochemical derivatization-fluorescence detection. *Analyst* **138**:2729-2739.

Kot-Wasik, A., and Wasik, A. (2005). Determination of robenidine in animal feeds by liquid chromatography coupled with diode-array detection and mass spectrometry after accelerated solvent extraction. *Anal. Chim. Acta* **543**:46-51.

Kristenson, E. M., Brinkman, U. A. T., and Ramos, L. (2006). Recent advances in matrix solid-phase dispersion. *TrAC-Trend. Anal. Chem.* **25**:96-111.

Krska, R., Schubert-Ullrich, P., Molinelli, A., Sulyok, M., MacDonald, S., and Crews, C. (2008). Mycotoxin analysis: An update. *Food Addit. Contam. A* **25**:152-163.

Lee, N. A., Wang, S., Allan, R. D., and Kennedy, I. R. (2004). A rapid aflatoxin B1 ELISA: development and validation with reduced matrix effects for peanuts, corn, pistachio, and soybeans. *J. Agr. Food Chem.* **52**:2746-2755.

Lehotay, S. J., Mastovska, K., and Lightfield, A. R. (2005). Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. *J. AOAC Int.* **88**:615-629.

Liau, B.-C., Jong, T.-T., Lee, M.-R., and Chang, C.-M. J. (2007). Supercritical fluid extraction and quantification of aflatoxins in Zizyphi Fructus by liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. *Rapid Commun. Mass Sp.* **21**:667-673.

Linting, Z., Ruiyi, L., Zaijun, L., Qianfang, X., Yinjun, F., and Junkang, L. (2012). An immunosensor for ultrasensitive detection of aflatoxin B1 with an enhanced electrochemical performance based on graphene/conducting polymer/gold nanoparticles/the ionic liquid composite film on modified gold electrode with electrodeposition. *Sensor. Actuat. B: Chem.* **174**:359-365.

Móricz, Á., H.-Otta, K., Ott, P., and Tyihák, E. (2003). Separation and detection of aflatoxins using overpressured-layer chromatography and bioautography. *JPC-J. Planar Chromat.* **16**:417-420.

Móricz, Á. M., Fatér, Z., Otta, K. H., Tyihák, E., and Mincsovics, E. (2007). Overpressured layer chromatographic determination of aflatoxin B1, B2, G1 and G2 in red paprika. *Microchem. J* **85**:140-144.

Manetta, A. C., Di Giuseppe, L., Giammarco, M., Fusaro, I., Simonella, A., Gramenzi, A., and Formigoni, A. (2005). High-performance liquid chromatography with post-column derivatisation and fluorescence detection for sensitive determination of aflatoxin M_1 in milk and cheese. *J. Chromatogr. A* **1083**:219-222.

Maragos, C. M., and Greer, J. I. (1997). Analysis of aflatoxin B1 in corn using capillary electrophoresis with laser-induced fluorescence detection. *J. Agr. Food Chem.* **45**:4337-4341.

Măruţoiu, C., Puiu, S., Moise, M. I. Soran, L., Măruţoiu, O. F., and Boboş, L. (2004). Optimization of the separation of some aflatoxins by thin-layer chromatography. *JPC-J. Planar Chromat.* **17**:372-374.

Maupin, L. M., Clements, M. J., and White, D. G. (2003). Evaluation of the MI82 corn line as a source of resistance to aflatoxin in grain and use of BGYF as a selection tool. *Plant Dis.* **87**:1059-1066.

Micheli, L., Grecco, R., Badea, M., Moscone, D., and Palleschi, G. (2005). An electrochemical immunosensor for aflatoxin M1 determination in milk using screen-printed electrodes. *Biosens. Bioelectron.* **21**:588-596.

Molina-García, L., Córdova, M. L. F.-d., and Ruiz-Medina, A. (2012). Indirect determination of aflatoxin B1 in beer via a multi-commuted optical sensor. *Food Addit. Contam. A* **29**:392-402.

Nakai, V. K., de Oliveira Rocha, L., Gonçalez, E., Fonseca, H., Ortega, E. M. M., and Corrêa, B. (2008). Distribution of fungi and aflatoxins in a stored peanut variety. *Food Chem.* **106**:285-290.

Njobeh, P. B., Dutton, M. F., Koch, S. H., Chuturgoon, A. A., Stoev, S. D., and Mosonik, J. S. (2010). Simultaneous occurrence of mycotoxins in human food commodities from Cameroon. *Mycotoxin research* **26**:47-57.

Nonaka, Y., Saito, K., Hanioka, N., Narimatsu, S., and Kataoka, H. (2009). Determination of aflatoxins in food samples by automated on-line in-tube solid-phase microextraction coupled with liquid chromatography–mass spectrometry. *J. Chromatogr. A* **1216**:4416-4422.

Pallaroni, L., and von Holst, C. (2004). Development of an extraction method for the determination of zearalenone in corn using less organic solvents. *J. Chromatogr. A* **1055**:247-249.

Papadopoulou-Bouraoui, A., Stroka, J., and Anklam, E. (2002). Comparison of Two Post-Column Derivatization Systems, Ultraviolet Irradiation and Electrochemical Determination, for the Liquid Chromatographic Determination of Aflatoxins in Food. *J. AOAC Int.* **85**:411-416(416).

Pappa, E., H-Otta, K., Záraya, G., and Mincsovics, E. (2002). Liquid chromatographic determination of aflatoxins. *Microchem. J* **73**:39-46.

Park, C. W., Yang, J.-H., Ah, C. S., Ahn, C.-G., Choi, Y. H., Chung, K. H., Kim, W.-J., and Sung, G. Y. (2011). Toxin detection by Si photosensitive biosensors with a new measurement scheme. *Biosens. Bioelectron.* **29**:219-223.

Peña, R., Alcaraz, M., Arce, L., Rıos, A., and Valcarcel, M. (2002). Screening of aflatoxins in feed samples using a flow system coupled to capillary electrophoresis. *J. Chromatogr. A* **967**:303-314.

Pearson, T. C., Wicklow, D. T., Maghirang, E. B., Xie, F., and Dowell, F. E. (2001). Detecting aflatoxin in single corn kernels by transmittance and reflectance spectroscopy. *T. ASAE* **44**:1247-1254.

Pei, S. C., Zhang, Y. Y., Eremin, S. A., and Lee, W. J. (2009). Detection of aflatoxin M1 in milk products from China by ELISA using monoclonal antibodies. *Food Control* **20**:1080-1085.

Pemberton, R. M., Pittson, R., Biddle, N., Drago, G. A., and Hart, J. P. (2006). Studies Towards the Development of a Screen-Printed Carbon Electrochemical Immunosensor Array for Mycotoxins: A Sensor for Aflatoxin B1. *Anal. Lett.* **39**:1573-1586.

Pettersson, H., and Aberg, L. (2003). Near infrared spectroscopy for determination of mycotoxins in cereals. *Food control* **14**:229-232.

Pietri, A., Rastelli, S., and Bertuzzi, T. (2010). Ochratoxin A and aflatoxins in liquorice products. *Toxins* **2**:758-770.

Pietri, A., Rastelli, S., Mulazzi, A., and Bertuzzi, T. (2012). Aflatoxins and ochratoxin A in dried chestnuts and chestnut flour produced in Italy. *Food Control* **25**:601-606.

Quinto, M., Spadaccino, G., Palermo, C., and Centonze, D. (2009). Determination of aflatoxins in cereal flours by solid-phase microextraction coupled with liquid chromatography and post-column photochemical derivatization-fluorescence detection. *J. Chromatogr. A* **1216**:8636-8641.

Reiter, E. V., Vouk, F., Böhm, J., and Razzazi-Fazeli, E. (2010). Aflatoxins in rice-A limited survey of products marketed in Austria. *Food Control* **21**:988-991.

Rezaee, M., Assadi, Y., Milani Hosseini, M.-R., Aghaee, E., Ahmadi, F., and Berijani, S. (2006). Determination of organic compounds in water using dispersive liquid–liquid microextraction. *J. Chromatogr. A* **1116**:1-9.

Rodriguez-Saona, L. E., Khambaty, F. M., Fry, F. S., Dubois, J., and Calvey, E. M. (2004). Detection and identification of bacteria in a juice matrix with Fourier transform-near infrared spectroscopy and multivariate analysis. *J. Food Protect.* **67**:2555-2559(2555).

Romero-González, R., Frenich, A. G., and Vidal, J. L. M. (2008). Multiresidue method for fast determination of pesticides in fruit juices by ultra performance liquid chromatography coupled to tandem mass spectrometry. *Talanta* **76**:211-225.

Romero-González, R., Garrido Frenich, A., Martínez Vidal, J. L., Prestes, O. D., and Grio, S. L. (2011). Simultaneous determination of pesticides, biopesticides and mycotoxins in organic products applying a quick, easy, cheap, effective, rugged and safe extraction procedure and ultra-high performance liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* **1218**:1477-1485.

Rossi, C. N., Takabayashi, C. R., Ono, M. A., Saito, G. H., Itano, E. N., Kawamura, O., Hirooka, E. Y., and Ono, E. Y. S. (2012). Immunoassay based on monoclonal antibody for aflatoxin detection in poultry feed. *Food Chem.* **132**:2211-2216.

Royer, D., Humpf, H. U., and Guy, P. A. (2004). Quantitative analysis of Fusarium mycotoxins in maize using accelerated solvent extraction before liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. *Food Addit. Contam.* **21**:678-692.

Rubert, J., Soler, C., and Mañes, J. (2011). Evaluation of matrix solid-phase dispersion (MSPD) extraction for multi-mycotoxin determination in different flours using LC–MS/MS. *Talanta* **85**:206-215.

Rubert, J., Soler, C., and Mañes, J. (2010). Optimization of matrix solid-phase dispersion method for simultaneous extraction of aflatoxins and OTA in cereals and its application to commercial samples. *Talanta* **82**:567-574.

Santini, A., Ferracane, R., Meca, G., and Ritieni, A. (2010). Comparison and improvement of the existing methods for the determination of aflatoxins in human serum by LC-MS/MS. *Anal Methods-Uk* **2**:884-889.

Santos, C., Fraga, M. E., Kozakiewicz, Z., and Lima, N. (2010). Fourier transform infrared as a powerful technique for the identification and characterization of filamentous fungi and yeasts. *Res Microbiol.* **161**:168-175.

Sapsford, K. E., Ngundi, M. M., Moore, M. H., Lassman, M. E., Shriver-Lake, L. C., Taitt, C. R., and Ligler, F. S. (2006). Rapid detection of foodborne contaminants using an Array Biosensor. *Sensor. Actuat. B: Chem.* **113**:599-607.

Schatzki, T. F., and Pan, J. L. (1996). Distribution of aflatoxin in pistachios. 3. Distribution in pistachio process streams. *J. Agr. Food Chem.* **44**:1076-1084.

Schoental, R. (1967). Aflatoxins. *Annu. Rev. Pharmacol.* **7**:343-356.

Sebastià, N., Soler, C., Soriano, J. M., and Mañes, J. (2010). Occurrence of Aflatoxins in Tigernuts and Their Beverages Commercialized in Spain. *J. Agr. Food Chem.* **58**:2609-2612.

Sheibani, A., and Ghaziaskar, H. S. (2009). Pressurized fluid extraction for quantitative recovery of aflatoxins B_1 and B_2 from pistachio. *Food Control* **20**:124-128.

Sheijooni-Fumani, N., Hassan, J., and Yousefi, S. R. (2011). Determination of aflatoxin B1 in cereals by homogeneous liquid–liquid extraction coupled to high performance liquid chromatography-fluorescence detection. *JSS* **34**:1333-1337.

Shephard, G. S. (2009). Aflatoxin analysis at the beginning of the twenty-first century. *Anal. Bioanal. Chem.* **395**:1215-1224.

Shephard, G. S. (2008). Determination of mycotoxins in human foods. *Chem. Soc. Rev.* **37**:2468-2477.

Shephard, G. S., Berthiller, F., Burdaspal, P. A., Crews, C., Jonker, M. A., Krska, R., Lattanzio, V. M. T., MacDonald, S., Malone, R. J., Maragos, C., Sabino, M., Solfrizzo, M., van Egmond, H. P., and Whitaker, T. B. (2013). Developments in mycotoxin analysis: an update for 2011-2012. *World Mycotoxin Journal* **6**:3-30.

Shephard, G. S., Berthiller, F., Burdaspal, P. A., Crews, C., Jonker, M. A., Krska, R., MacDonald, S., Malone, R. J., Maragos, C., Sabino, M., Solfrizzo, M., Egmond, H. P. V., and Whitaker, T. B. (2012). Developments in mycotoxin analysis: an update for 2010-2011. *World Mycotox. J.* **5**:3-30.

Shim, W.-B., Kim, J. S., Kim, J.-Y., Choi, J.-G., Je, J.-H., Kuzmina, N. S., Eremin, S. A., and Chung, D.-H. (2008). Determination of aflatoxin B1 in rice, barley, and feed by non-instrumental immunochromatographic strip-test and high sensitive ELISA. *Food Sci. Biotechnol.* **17**:623-630.

Shim, W. B., Kim, G., Ryu, H. J., Nam, M., and Chung, D. H. (2009). *Food Sci. Biotechnol.* **18**:641-648.

Shouman, B. O., El Morsi, D., Shabaan, S., Abdel-Hamid, A.-H., and Mehrim, A. (2012). Aflatoxin B1 Level in Relation to Child's Feeding and Growth. *Indian J. Pediatr.* **79**:56-61.

Singh, C. B., Jayas, D. S., Paliwal, J., and White, N. D. G. (2012). Fungal damage detection in wheat using short-wave near-infrared hyperspectral and digital colour imaging. *Int. J. Food Prop.* **15**:11-24.

Singh, C. B., Jayas, D. S., Paliwal, J., and White, N. D. G. (2007). Fungal detection in wheat using near-infrared hyperspectral imaging. *T. ASABE* **50**:2171-2176.

Spanjer, M. C., Rensen, P. M., and Scholten, J. M. (2008). LC–MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. *Food Addit. Contam. A* **25**:472-489.

Stroka, J., and Anklam, E. (2000). Development of a simplified densitometer for the determination of aflatoxins by thin-layer chromatography. *J. Chromatogr. A* **904**:263-268.

Stroka, J., and Anklam, E. (2002). New strategies for the screening and determination of aflatoxins and the detection of aflatoxin-producing moulds in food and feed. *TrAC-Trend. Anal. Chem.* **21**:90-95.

Stroka, J., Petz, M., Joerissen, U., and Anklam, E. (1999). Investigation of various extractants for the analysis of aflatoxin B1 in different food and feed matrices. *Food Addit. Contam.* **16**:331-338.

Stroka, J., van Otterdijk, R., and Anklam, E. (2000). Immunoaffinity column clean-up prior to thin-layer chromatography for the determination of aflatoxins in various food matrices. *J. Chromatogr. A* **904**:251-256.

Sulyok, M., Krska, R., and Schuhmacher, R. (2010). Application of an LC–MS/MS based multi-mycotoxin method for the semi-quantitative determination of mycotoxins occurring in different types of food infected by moulds. *Food Chem.* **119**:408-416.

Tüdös, A. J., Lucas-Van Den Bos, E. R., and Stigter, E. C. (2003). Rapid surface plasmon resonance-based inhibition assay of deoxynivalenol. *J. Agr. Food Chem.* **51**:5843-5848.

Tabari, M., Karim, G., Ghavami, M., and Chamani, M. (2011). Method validation for aflatoxin M1 determination in yoghurt using immunoaffinity column clean-up prior to high-performance liquid chromatography. *Toxicol Ind. Health* **27**:629-635.

Takino, M., Tanaka, T., Yamaguchi, K., and Nakahara, T. (2004). Atmospheric pressure photo-ionization liquid chromatography/mass spectrometric determination of aflatoxins in food. *Food Addit. Contam.* **21**:76-84.

Tan, Y., Chu, X., Shen, G.-L., and Yu, R.-Q. (2009). A signal-amplified electrochemical immunosensor for aflatoxin B_1 determination in rice. *Anal, Biochem,* **387**:82-86.

Teena, M., Manickavasagan, A., Mothershaw, A., El Hadi, S., and Jayas, D. (2013). Potential of Machine Vision Techniques for Detecting Fecal and Microbial Contamination of Food Products: A Review. *Food Bioprocess Tech.*:1-14.

Tripathi, S., and Mishra, H. N. (2011). Modeling and optimization of enzymatic degradation of aflatoxin B (AFB) in red chili powder using response surface methodology. *Food Bioprocess Tech.* **4**:770-780.

Tripathi, S., and Mishra, H. N. (2009). A rapid FT-NIR method for estimation of aflatoxin B_1 in red chili powder. *Food Control* **20**:840-846.

Turner, N. W., Subrahmanyam, S., and Piletsky, S. A. (2009). Analytical methods for determination of mycotoxins: A review. *Anal. Chim. Acta* **632**:168-180.

Urraca, J. L., Marazuela, M. D., and Moreno-Bondi, M. C. (2004). Analysis for zearalenone and α-zearalenol in cereals and swine feed using accelerated solvent extraction and liquid chromatography with fluorescence detection. *Anal. Chim. Acta* **524**:175-183.

Urraca, J. L., Marazuela, M. D., and Moreno-Bondi, M. C. (2006). Molecularly imprinted polymers applied to the clean-up of zearalenone and α-zearalenol from cereal and swine feed sample extracts. *Anal. Bioanal. Chem.* **385**:1155-1161.

van der Gaag, B., Spath, S., Dietrich, H., Stigter, E., Boonzaaijer, G., van Osenbruggen, T., and Koopal, K. (2003). Biosensors and multiple mycotoxin analysis. *Food Control* **14**:251-254.

van Egmond, H. P. (1983). Mycotoxins in dairy products. *Food Chemistry* **11**:289-307.

van Egmond, H. P., Paulsch, W. E., and Schuller, P. L. (1978). Confirmatory test for aflatoxin M1 on a thin layer plate. *J. AOAC* **61**:809-812.

van Egmond, H. P., Schothorst, R. C., and Jonker, M. A. (2007). Regulations relating to mycotoxins in food. *Anal. Bioanal. Chem.* **389**:147-157.

Van Rensburg, S. J., Cook-Mozaffari, P., Van Schalkwyk, D. J., Van der Watt, J. J., Vincent, T. J., and Purchase, I. F. (1985). Hepatocellular carcinoma and dietary aflatoxin in Mozambique and Transkei. *Br. J. Cancer.* **51**:713-726.

Var, I., Kabak, B., and Gök, F. (2007). Survey of aflatoxin B_1 in helva, a traditional Turkish food, by TLC. *Food Control* **18**:59-62.

Vega, V. A. (2005). Rapid extraction of aflatoxin from creamy and crunchy peanut butter. *J. AOAC Int.* **88**:1383-1386.

Ventura, M., Gomez, A., Anaya, I., Díaz, J., Broto, F., Agut, M., and Comellas, L. (2004). Determination of aflatoxins B1, G1, B2 and G2 in medicinal herbs by liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* **1048**:25-29.

Wang, J.-J., Liu, B.-H., Hsu, Y.-T., and Yu, F.-Y. (2011). Sensitive competitive direct enzyme-linked immunosorbent assay and gold nanoparticle immunochromatographic strip for detecting aflatoxin M1 in milk. *Food Control* **22**:964-969.

Wang, Y., Liu, X., Xiao, C., Wang, Z., Wang, J., Xiao, H., Cui, L., Xiang, Q., and Yue, T. (2012a). HPLC determination of aflatoxin M_1 in liquid milk and milk powder using solid phase extraction on OASIS HLB. *Food Control* **28**:131-134.

Wang, Z., Yan, S., Liu, C., Chen, F., and Wang, T. (2012b). Proteomic analysis reveals an aflatoxin-triggered immune response in cotyledons of Arachis hypogaea infected with Aspergillus flavus. *J. Proteom. Res.* **11**:2739-2753.

Warth, B., Sulyok, M., Fruhmann, P., Mikula, H., Berthiller, F., Schuhmacher, R., Hametner, C., Abia, W. A., Adam, G., and Fröhlich, J. (2012). Development and validation of a rapid multi-biomarker liquid chromatography/tandem mass spectrometry method to assess human exposure to mycotoxins. *Rapid Commun. Mass Sp.* **26**:1533-1540.

Whitaker, T. B. (2006). Sampling Foods for Mycotoxins. *Food Addit. Contam.* **23**:50-61.

Whitaker, T. B. (2003). Standardisation of mycotoxin sampling procedures: an urgent necessity. *Food Control* **14**:233-237.

Whitaker, T. B., Dickens J. W., and Monroe, R. J. (1974). Variability of aflatoxin test results. *J. AOCS* **51**:214-218.

Wicklow, D. T. (1999). Influence of Aspergillus flavus strains on aflatoxin and bright greenish yellow fluorescence of corn kernels. *Plant Dis.* **83**:1146-1148.

Wilkowska, A., and Biziuk, M. (2011). Determination of pesticide residues in food matrices using the QuEChERS methodology. *Food Chem.* **125**:803-812.

Xu, X., Liu, X., Li, Y., and Ying, Y. (2013). A Simple and Rapid Optical Biosensor for Detection of Aflatoxin B1 Based on Competitive Dispersion of Gold Nanorods. *Biosens. Bioelectron.* **47**:361-367.

Yao, H., Hruska, Z., Brown, R. L., and Cleveland, T. E. (2006). Hyperspectral bright greenish-yellow fluorescence (BGYF) imaging of aflatoxin contaminated corn kernels. *Proc. of SPIE* **6381**:63810B.

Yao, H., Hruska, Z., Kincaid, R., Brown, R., Cleveland, T., and Bhatnagar, D. (2010a). Correlation and classification of single kernel fluorescence hyperspectral data with aflatoxin concentration in corn kernels inoculated with Aspergillus flavus spores. *Food Addit. Contam.* **27**:701-709.

Yao, H., Hruska, Z., Kincaid, R., Brown, R. L., Bhatnagar, D., and Cleveland, T. E. (2013). Detecting maize inoculated with toxigenic and atoxigenic fungal strains with fluorescence hyperspectral imagery. *Biosyst. Eng.* **115**:125-135.

Yao, H., Hruska, Z., Kincaid, R., Brown, R. L., and Cleveland, T. E. (2008). Differentiation of toxigenic fungi using hyperspectral imagery. *Sens. & Instrumen. Food Qual.* **2**:215-224.

Yao, H., Hruska, Z., Kincaid, R., Ononye, A., Brown, R. L., and Cleveland, T. E. (2010b). Single aflatoxin contaminated corn kernel analysis with fluorescence hyperspectral image. *Proc. of SPIE* **7676**:76760D.

Zöllner, P., and Mayer-Helm, B. (2006). Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography–atmospheric pressure ionisation mass spectrometry. *J. Chromatogr. A* **1136**:123-169.

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *J. Saudi. Chem. Soc.* **15**:129-144.

Zheng, P., Sheng, X., Yu, X., and Hu, Y. (2006). Determination of Aflatoxins in Hot Chilli Products by Matrix Solid-Phase Dispersion and Liquid Chromatography. *Chinese J. Chromatogr.*:62-64.

Figure Legends

Figure 1. Fungal growth and aflatoxin production after infection of Arachis hypogaea cotyledons with toxigenic (As 3.2890) or atoxigenic (AF051) Aspergillus flavus strains. (A) Phenotypes of A. hypogaea cotyledons and A. flavus strains at days 1-6 after the infection. (B) Aflatoxin production after infection with the 2 A. flavus strains by thin-layer chromatography (TLC). (C) High-performance liquid chromatography (HPLC) determination of aflatoxins after infection with toxigenic strain. AFB1-AFG2, aflatoxin B1-G2. Adapted from ref. Wand et al, (2012b).

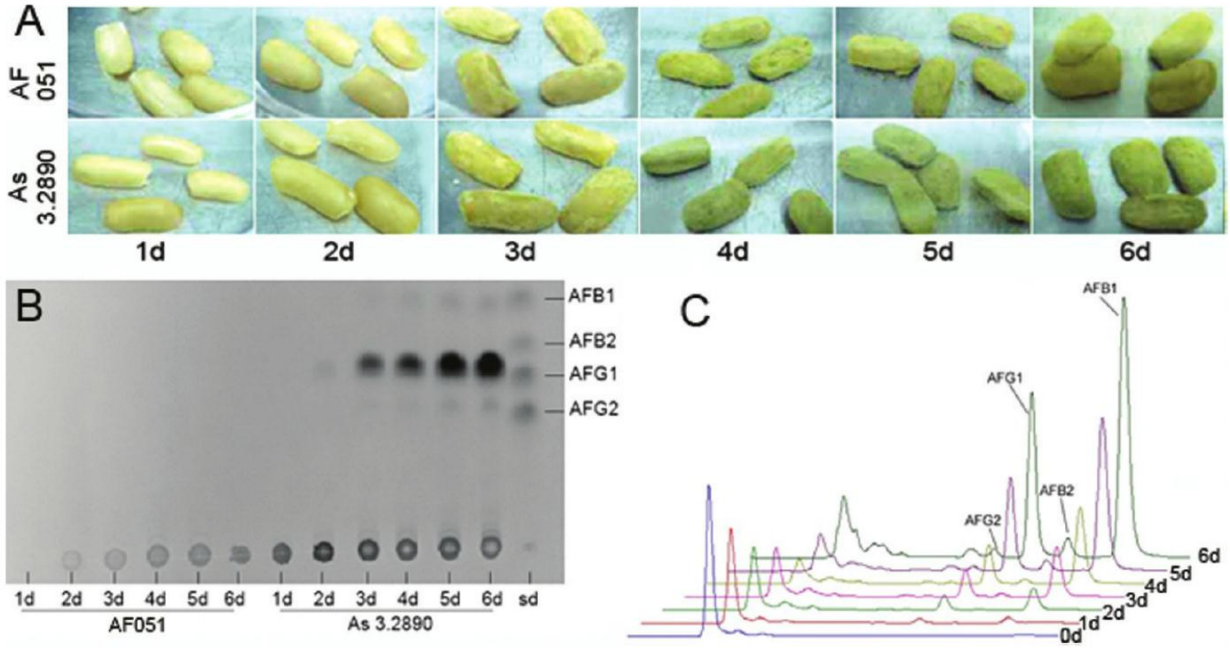

Figure 2. Schematic diagram of the principle of the ELISA method.

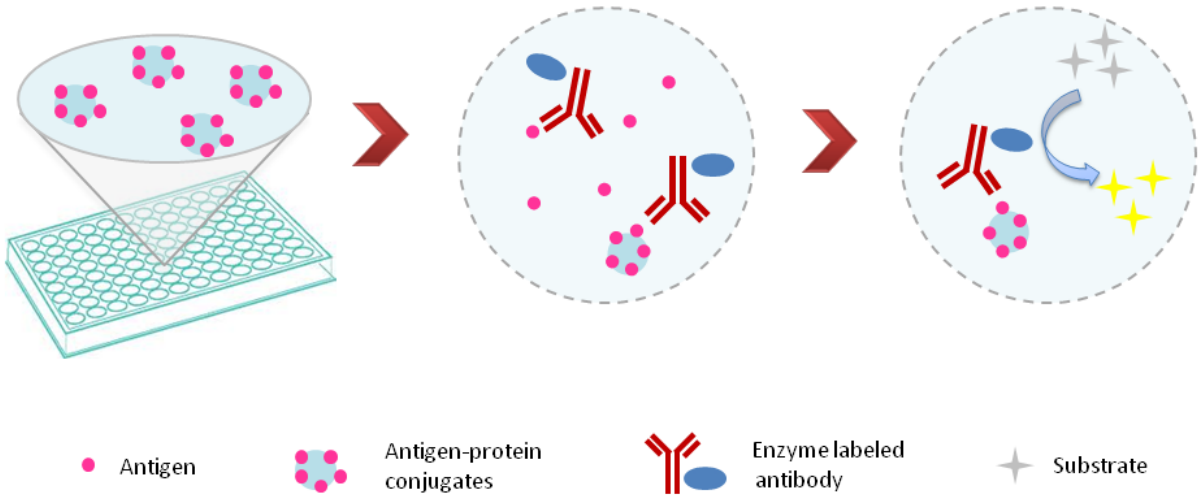

Table 1. Maximum limits for aflatoxins in different food and feeds from different institutions and countries.

Institutions/Countries	Aflatoxins	Food and Feeds	Maximum limits ($\mu g\ kg^{-1}$)	References
USDA and FDA	Total aflatoxins	animal feed products	15-20	Espinosa-Calderón et al., 2011
EU	AFB1	cereal	2	EC, 2006; Quinto et al., 2009
		processed cereal-based food and baby food	0.1	
	Total aflatoxins	cereal	4	
	AFM1	milk	0.05	EC, 2001; EC, 1998
		baby food	0.025	EC, 2004
Brazil	Total aflatoxins	peanuts and corn	20	Abbas, 2005
China	AFB1	corn, peanut kernel, and peanut oil	20	National Criterion of China. (2011)
		beans, fermented food, and other grains	5	
Korea	AFB1	all food	10	Abbas, 2005
Switzerland,	AFB1	nuts and dried fruits	1	Bacaloni et al., 2008; Creppy, 2002
Netherlands	AFB1	nuts and dried fruits	5	
USA	AFB1	nuts and dried fruits	20	
Switzerland and Austria	AFM1	milk	0.05	Manetta et al., 2005
		cheese	0.25	
		butter	0.02	