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Aflatoxin species: their health effects and determination methods in different foodstuffs

Review Article

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Abstract: Carcinogenic and mutagenic properties of aflatoxin species are known in literature. Their intake over a long time period might be health-dangerous for human even at trace levels. It is well known that different foodstuffs can be contaminated by aflatoxin species through growing and storage. Due to the serious health effects, sensitive determination of aflatoxin species in any matrices related with the human being is very crucial at trace levels. In literature, there are sensitive techniques to analyze the different samples for the contents of their aflatoxin species. Each technique has some advantages and disadvantages over the other techniques. This review aims to summarize the different health effects of aflatoxin species, development of analytical techniques and applications of developed techniques in a variety of matrices.

Keywords: *Aflatoxin • Separation • Determination • Health • HPLC*

1. Introduction

Scientific community has attempted to limit aflatoxin contamination in different matrices such as foods and feeds since the discovery of these mycotoxins in 1960 [1]. Aflatoxin is one type of the mycotoxin. The word “mycotoxin” is derived from 2 words; “mukes” refering to “fungi” (Greek) and “toxicum” referring to “poison” (Latin). The word “Aflatoxin” is the combination of 3 words; “a” for the *Aspergillus* genus and “fla” for the species *flavus*, and toxin meaning poison [2,3]. Aflatoxin species are known to be mutagenic, carcinogenic and teratogenic compounds. The main sources of aflatoxin species for human and animals are the ingestion through the dietary channel [4]. It has recently been discovered that the colonizing fungi produce low molecular-weight compounds generally known as “secondary metabolites” or “mycotoxins” which are relatively larger molecules and are not significantly volatile [2]. It was claimed that mycotoxin production by fungi might have evolved to be an effective anti-insect or an anti-rodent agent. Secondary metabolism of molds might be affected by the presence of inhibitory compounds like agricultural biocides [2,5]. *Aspergillus flavus* (*A. flavus*) and *Aspergillus Parasiticus*

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(*A. parasiticus*) through a polyketide pathway produce aflatoxin species as difuranocoumarin derivatives. In addition, it is known that aflatoxin species have polycyclic structure. G series aflatoxins are six-membered lactones and B series aflatoxins are pentanone derivatives [6,7].

Eighteen different types of aflatoxin species have been identified. Aflatoxin B1, B2, G1, G2 and M1 are the major species. AFB1 and AFB2 are produced by fungal species belonging to *Aspergillus flavus* while AFG1 and AFG2 as well as AFB1 and AFB2 are produced by *A. Parasiticus*. Categorization of the aflatoxin species can be done according to the characteristics of the aflatoxin species. For example, aflatoxins are divided into the sub-groups such as aflatoxin B_1 and B_2 (AFB_1, AFB_2) (blue color), and G_1 and G_2 (AFG_1, AFG_2) (green color) based upon their fluorescence colors [8-10].

2. Aflatoxin species and health

Among mycotoxins, aflatoxin has become the most serious species since it negatively affects not only the economy but also the human health [11-13]. Foodstuffs can be contaminated by aflatoxin while foods are growing, being harvested and finally stored [14]. Aflatoxin species are highly dangerous compounds for both human being and animals. They are highly toxic, carcinogenic, mutagenic and teratogenic compounds. Among types, AFB1 occurs mostly in food samples and it was found that it is the most effective natural carcinogen and toxic material [13,15-20]. In addition to several studies proving the fact that AFB1 is a potent carcinogen, International Agency for Research on Cancer (IARC) classified AFB1 as a Group 1 human carcinogen [13,21-23].

AFB1 is metabolically transformed to the hydroxylated form (also called called AFM1) which is found in milk and milk products of the animal that ingests aflatoxin contaminated feeds [24]. Hence, milk and milk products are the indirect source of aflatoxin [25]. Like AFB1, category of AFM1 was changed from Group 2B to Group 1 by IARC in 2002 in terms of toxic and carcinogenic effects [19,26]. However, AFB1 has more mutagenic potency than AFM1 [19,27].

Studies on animal have proved that exposure to aflatoxin can seriously affect growth and development [28]. Furthermore, experiments on poultry birds showed that acute or chronic aflatoxicosis causes lower meat/egg production, immunosupression and hepatotoxicosis [29-31]. When poultry birds are fed with foods contaminated with aflatoxin, residues of aflatoxin are found in their liver and muscle tissues [32]. Concentration of the aflatoxin residue depends on many factors such as dietary aflatoxin level, duration of exposure and age [33]. Among poultry birds, turkey is mostly affected by AFB1 [29,34]. In 1960s, many of the poultries died throughout Europe due to aflatoxicosis. This was called as “Turkey X Disease” [35].

Another study verified the adverse effects of aflatoxin. According to Edrington *et al.* AFB1 adversely affects the development and increases the mortality in chicken embryos [36]. Aflatoxicosis can cause various adverse effects depending on the exposure level for human being. High doses result in rapid death while hepatocellular carcinoma (HCC) develops as a chronic outcome. Synergic effect of AFB1 with hepatitis B or C viral infections can cause a HCC. HCC causes a liver cancer and it was found that the liver is the main target organ of AFB1 [37,38]. HCC is the most common cancer in many parts of sub-Saharan Africa, Southeast Asia and China. In these countries, up to 10% of adult male deaths are related with HCC [39]. Moreover, impaired growth of children can be the adverse health effects of aflatoxicosis. Slow growth of children was observed when foods with high amount of aflatoxin are taken by baby after breast milk nutrition [40]. Mother’s metabolism limits the transfer of dietary aflatoxins into the milk. Aflatoxin exposure of the babies can be measured by aflatoxin-albumin (AF-alb) adduct found in blood. Aflatoxin binds covalently to albumin and this adduct can be used as a biomarker. According to Wild *et al.* study; AF-alb adduct have been reported in cord blood samples of pregnant Gambian women. This is explained by the lipophilic nature of aflatoxins. They can easily cross the placental barrier [41]. One other adverse effect of aflatoxin is on immune system for human [42]. There are two studies proving the negative effects of aflatoxin on immune system [43,44]. According to these studies, aflatoxin species show immunosuppressive effects due to inhibition of DNA, RNA and protein synthesis through different mechanisms. In these studies, aflatoxin B1-albumin adducts in blood is used as a biomarker, Turner *et al.* conducted the study on Gambian children exposed to aflatoxin and investigated their antibody responses. They found that secretory immunoglobulin A (sIgA) levels in the saliva of children with aflatoxin exposure significantly reduced [43]. The role of sIgA is to bind to bacterial and viral surface antigens and forms the part of mucosal barrier in saliva, breast milk, tears and mucus of the bronchial, genitor-urinary and digestive tracts. Hence, decrease in the levels of sIgA causes lower intestinal resistance to bacteria. Occurrence of other types of mycotoxins such as ochratoxins and fumonisin can cause synergic affect with AFB1 on the immune system [43,45-48]. Unfortunately, there are not

too many studies on this topic and future research is needed. In conclusion, AFB1 affects not only the liver but also other organs and systems such as lung [49] and immune system [50] causing different health problems [49,51-54].

Legislations for the safe maximum intake of AFB1 have tried to be established by considering the serious health problems caused by aflatoxin exposure. The United Nations-Food and Agriculture Organization (FAO) and World Health Organization (WHO) determined the upper aflatoxin exposure limit as 30 μg kg^{-1} in foodstuffs for human [23]. Later, in 2002 European Union established maximum safe level as 4 and 2 μg kg^{-1} for total aflatoxin and AFB1, respectively [55]. In addition, the Codex Alimentarius Commission, (joint FAO/WHO) decided the maximum limit of total aflatoxin in peanut as 15 μg kg^{-1} [56]. WHO fixed the limit at 5 μg kg^{-1} for different types of foods for AFB1 [4].

By considering these limits, most of the countries established their own legislations. In Table 1, maximum safe levels (MSL) of total aflatoxin and AFB1 are shown for some South America countries [57], Europe Union [58], Turkey [59], Korea [60], Japan, China [61] and Kenya where the aflatoxicosis is a big problem [20]. Total aflatoxin refers to the sum of AFB1, AFB2, AFG1 and AFG2.

In addition, safe limits were determined for AFM1 in milk and dairy products. These limits for some countries are given in Table 2. In developing countries such as Kenya, Mozambique, Democratic Republic of Congo (DRC) and India, aflatoxin problems are more serious than the developed countries. Climatic conditions (high temperature and humidity), insufficient transportation, marketing and storage practices are the main reasons [4,23,68]. According to *Stora*, DRC is found in the African countries list with high risk of liver cancer resulted from aflatoxicosis [69].

Unfortunately, severe aflatoxin-poisoning events were documented from different countries. First in India, 106 people died in 1974. This event was followed by 215 death in Kenya in 2004 and 100 deaths in Nigeria in 2005 [70,71]. In a study, it was observed that the exposure of 50 mg per day AFB1 *via* ingestion of contaminated maize resulted the aflatoxin-induced death in Kenya [72].

In order to reduce aflatoxin content in animal food, different methods have been applied. The ammoniation process is one of the methods used in literature. Ammoniation can be done with gaseous ammonia or ammonium hydroxide solution under suitable conditions. Reduction of aflatoxin process was successful after cottonseed meal was treated by ammoniation for 30 minutes at 48 psi and 118ºC; aflatoxin concentration decreased to 1% of the initial concentration [73]. Another method is the roasting of contaminated feeds such as corn. Using this method, aflatoxin content was reduced up to 66% at 165ºC [74].

3. Determination of aflatoxin species

3.1. Extraction of aflatoxin species from the matrix

In order to obtain more information about the toxicity of aflatoxin species in food stuffs, determination of different chemical forms should be performed in trace levels. Three steps namely extraction, separation and detection are crucial for the sensitive determination of each aflatoxin species. The extraction procedure is the most crucial step in the determination of aflatoxin species. In this step, all of the analytes of interest should be taken from matrix to the extraction solution without any alteration. The efficiency of extraction procedures depends on the sample matrix. There is a real need to develop the effective extraction methods to obtain higher extraction yields for all of the aflatoxin species in variety of matrices.

Different analytical approaches have been used in the literature for this purpose. Liquid–liquid extraction with different solvent mixtures, solid-phase extraction, solid-phase microextraction, pH-controlled solvent extraction, immunoaffinity column (for both clean-up and extraction) are some of the most commonly used methods in literature. Sheijooni-Fumani *et al*. used homogeneous liquid–liquid extraction for the extraction of aflatoxin B1 in the rice and grain samples. In the extraction method, 10 mL methanol/water (8:10) was used for 5 g of well-milled samples. Extraction was performed for 30 min at 400 rpm on a mechanical shaker. In addition, 6 mL of 3% aqueous potassium bromide was used to obtain a cloudy solution resulted in dispersion of fine droplets of extraction solvent in aqueous phase [75].

Prado et al. also used methanol-water mixture for the extraction of aflatoxin B1 from pepper (*Piper nigrum L*.) and oregano (*Origanum vulgare L*.). Recovery for aflatoxin B1 was higher than 72% with low RSD values (lower than 20%) [76].

Aguilera-Luiz *et al*. compared the efficiencies of different extraction procedures for the simultaneous determination of mycotoxins and pesticides in milk samples by ultra high-performance liquid chromatography-tandem mass spectrometry. In this study, they used different extraction methods including solid-phase extraction, “dilute-and-shoot” (liquid-liquid extraction-based procedures), and QuEChERS

(quick, easy, cheap, effective, rugged, and safe)-based methods. Recoveries were found in the range 60-120% with relative standard deviations <25% [77].

Santini *et al.* determined aflatoxins B1, B2, G1 and G2 and its metabolites, aflatoxins M1 and M2 in human serum by LC-MS/MS. In the extraction of the aflatoxins from human serum, subsequent pH-controlled solvent extractions were used. Recoveries in the range of 31 to 98% were obtained [78].

Grio *et al*. applied sonication extraction by using an ACN/water mixture (80:20 v/v) followed by a clean-up step utilizing C(18) as sorbent for the determination of aflatoxins B1, B2, G1, G2 and ochratoxin A in animal feed by ultra high-performance liquid chromatography-tandem mass spectrometry. In this study, recoveries for the analytes ranged from 84 to 113% with low RSD values (lower than 20%) [79].

Nonaka *et al*. used automated on-line in-tube solid-phase microextraction coupled with liquid chromatography-mass spectrometry for the determination of aflatoxins (B1, B2, G1, and G2) in nuts, cereals, dried fruits, and spices. Recoveries of aflatoxin species spiked into nuts and cereals were found to be higher than 80% with the RSD <11.2% [80].

Vega analyzed peanut butter samples for their aflatoxin (B1, B2, G1, and G2) contents. In the extraction, analytes were extracted by using 15% sodium chloride in methanol (7 + 3) solution followed by a second extraction with methanol. Recoveries of B1, B2, G1, and G2 for smooth peanut buffer were 95.2, 89.9, 94.1, and 62.4%, respectively, while recoveries for crunchy peanut butter were 92.4, 84.3, 85.5, and 53.7%, respectively [81].

Tabari *et al*. used an immunoaffinty column for the extraction and HPLC for quantification of AFM1 in yoghurt samples. Average recoveries on three different days were in the range of 72.57%-86.66% with 2.56%-8.41% of RSD while the interday and interlevel mean recovery value was 80% [82]. Roussi *et al*. also used immunoaffinity column extraction for the determination of AFM1 in raw and market milk commercialized in Greece [83].

3.2. Separation and detection of aflatoxins

3.2.1. High performance liquid chromatography (HPLC)

HPLC has been widely used in literature due to its high separation power, high sensitivity and reproducibility, ease to use, suitability for automation and ability to couple with different detectors [84-87]. Although, both normal phase and reversed phased chromatography can be applied for the determination of aflatoxins, reversed phase setup is far more applied in literature due to the general advantages of reversed phase chromatography

Table 1. Maximum safe levels of total aflatoxin and AFB1 for some countries.

Country	Food	MSL, $\mu g\ kg^{-1}$
Argentina	Peanut (AFB1, 5 $\mu g\ kg^{-1}$)	20
	Corn (AFB1, 5 $\mu g\ kg^{-1}$)	20
	Baby Food (AFB1, 0 $\mu g\ kg^{-1}$)	
	Soy Meal (AFB1, 30 $\mu g\ kg^{-1}$)	
Brazil	Peanut	20
	Corn	20
Colombia	Cereal	30
	Foods	20
Peru	All Foods	10
Uruguay	Peanut	30
	Dried Fruits	30
	Baby Food	3
Venezuela	Rice Flour	5
Kenya	All Foods	20
EU	Dried Fruit & nut (AFB1, 2 $\mu g\ kg^{-1}$)	4
Turkey	Red Pepper & nut (AFB1, 5 $\mu g\ kg^{-1}$)	10
Korea	All Food (AFB1, 10 $\mu g\ kg^{-1}$)	-
Japan	All Food (AFB1, ≤10 $\mu g\ kg^{-1}$)	-
China	Peanut (AFB1, 20 $\mu g\ kg^{-1}$)	-

[88-91]. Reversed phased separations mostly rely on C-18 columns and mobile phases consisting of water-acetonitrile-methanol in proper ratios. In one study, AFB1 content of peanut samples were measured by using C-18 columns but methanol was not added into acetonitrile-water mobile phase [61]. Tarin *et al*. was able to separate B1, B2, G1 and G2 aflatoxins on a 12 min chromatographic run by using an isocratic eluent consisting of water–acetonitrile–methanol (60:25:15, v/v) [92]. Khayoon *et al*. reported that use of mobile phase containing more than 70% (v/v) water decreases the sensitivity [93].

Different detectors are applied for the detection of the eluted species. Among them, fluorescent detectors are the most popular one [94-98]. Aflatoxins B2 and G2 are naturally strong fluorescent molecules due to the high conjugation of their oxygenated structures, whereas aflatoxins B1 and G1 are weak fluorescent molecules. As a result, most of the studies use a pre-post column derivatization to enhance the fluorescent intensity hence the sensitivity of their analytical system. Dall'asta *et al*. studied the effect of native and substituted cyclodextrin (CD) derivatives on the fluorescent intensity of aflatoxins B1, B2, G1 and G2 [99]. They showed that presence of succinyl-β-CD (at molar ratio AF: CD= 1.0×10^{6}) enhances the fluorescence intensity about 63 and 53.9 folds for aflatoxins B1 and G1, respectively. However, enhancement factors are not greater than 2.3 for highly fluorescent B2 and G2 molecules. Another widely used florescent enhancement reagent is trifluoroacetic acid (TFA). TFA can convert the B1 and G1 molecules to

Table 2. Safe limits of AFM1 in dairy foods for some countries [62-67].

Country	Milk, μg L^{-1}	Butter, μg kg^{-1}	Cheese, μg kg^{-1}
Sweden	0.050	-	-
Austria	0.050	-	0.250
Germany	0.050	-	-
Belgium	0.050	-	-
Netherlands	-	0.020	0.200
USA	0.500	-	-
Switzerland	0.050	-	0.250
Turkey	0.050	-	0.250
Iran	0.050	0.020	0.250
Brazil	0.500	-	2.500

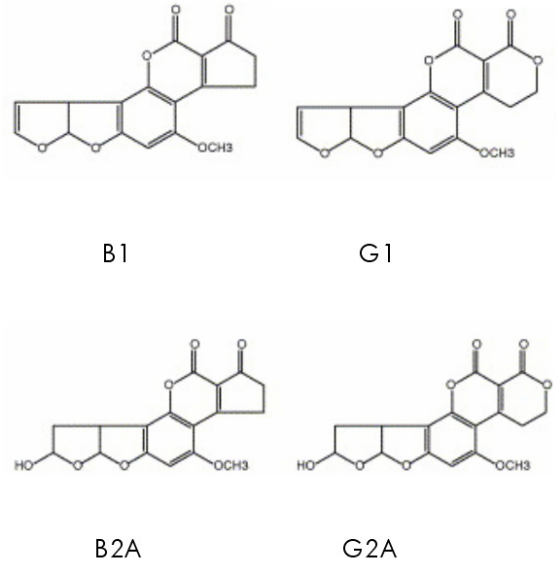

Figure 1. Structures of B1, G1, B2A and G2A.

their corresponding hemiacetal derivatives B2A and G2A whereas B2 and G2 are unaffected. Structures of B1, G1, B2A and G2A can be seen in Fig. 1.

TFA is a toxic and corrosive chemical and it may cause pre-derivatization. Hence, usage of TFA is not preferred in chemical analysis. However, Khayoon *et al*. used TFA solution mixed with n-hexane for pre-column derivatization in their study. Four types of aflatoxin species were determined after spiking procedure. The HPLC instrument which was used in this study had a fluorescence detector [93]. The chromatograms of one of the animal feed samples and sample with spiked aflatoxins are shown in Fig. 2.

Another alternative is the use of bromine or iodine as post column derivatization agents. Both of the reagents are good oxidizing agents. Bromine is simply generated by mixing of the column eluent with potassium bromide and nitric acid. Application of a small electrical current, usually in the order of 100-200 mA, leads to the generation of free bromine. Generated bromine reacts with target molecules in a reaction coil whose dimensions are optimized to yield best fluorescent signal [90,100-103]. This setup is known as Kobra cell. Iodine derivatization includes mixing of the column eluent with elemental iodine in methanol [104-108]. The resultant mixture is heated in a reaction coil to complete the reaction. A detailed study on the derivatization of B1 and G1 using iodine was conducted by Holcomb *et al.* using thermospray mass-spectrometry [108]. O'Riordan and Wilkinson compared the derivatization of aflatoxin extracts of chilli spice using Kobra cell and direct iodine reaction [109]. According to the study, bromine derivatization yields better sensitivity and recovery values with shorter reaction times.

A new derivatization approach is the use of photochemical reactors. In this approach, simply the outlet of the HPLC is connected to UV permeable PTFE tubing and it is wrapped over a high intensity UV lamp [101,103,110,111]. UV light irradiation generated hydroxyl radicals from water and these radicals react with B1 and G1 type aflatoxins to yield stable and highly fluorescent compounds [112]. It has several advantages over chemical derivatization procedures: it is simple, quick, and does not require external chemicals, pumps and heaters [112]. Just recently, Gnonlonfin *et al*. have used a photochemical reactor, too. In this study extracts were analyzed by reversed phase isocratic HPLC which has a fluorescence detector. Then, post column derivatization was performed by photochemical reactor for enhanced detection [113].

Tandem mass detectors (MS/MS) gained popularity in recent years [114-118]. This system provides the determination at ultra-trace levels as well as providing structural information. It is also possible to perform multi mycotoxin analysis with one experimental setup [119].

Several other detectors are also applied in aflatoxin quantification studies in literature. One class is the electrochemical detectors. Elizalde-González *et al*. developed an amperometric determination method for the determination of B1, B2, G1 and G2 [120]. Among

Table 3. Extraction procedures of some samples for determination of aflotoxin species.

Sample	Extraction Procedure	Aflatoxin Type	Reference
Rice and grain	Liquid- liquid extraction	AFB1	[75]
Pepper and oregano	Liquid- liquid extraction	AFB1	[76]
Milk	Solid phase extraction, Liquid liquid extraction, QuEChERS	Mycotoxin	[77]
Human Serum	pH-controlled solvent extraction	AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2	[78]
Animal feed	Sonication extraction	AFB1, AFB2, AFG1 and AFG2	[79]
Nut, cereals, dried fruits	Solid-phase microextraction	AFB1, AFB2, AFG1 and AFG2	[80]
Peanut butter	Liquid- liquid extraction	AFB1, AFB2, AFG1 and AFG2	[81]
Yoghurt	Immunoaffinty column extraction	AFM1	[82]
Milk	Immunoaffinty column extraction	AFM1	[83]

them, aflatoxin G1 was found to have the highest electroactivity. In another study, stripping voltammetry was successfully used for the determination of aflatoxins B1 and B2 in groundnut samples [121].

Cavaliere *et al*. investigated the suitability of electrospray ionization (ESI) and atmospheric pressure photoionization (APPI) techniques on the determination of aflatoxin M1 [119]. According to the results, APPI yield 2 times better sensitivity compared to ESI. This detector suffers from common ionization and matrix effects and reproducibility problems. Cervino *et al*. utilized isotope dilution method to overcome the pronounced difficulties [122].

3.2.2. Thin layer chromatography (TLC)

Although TLC has been largely replaced by HPLC and other techniques for the determination of aflatoxins due to lower sensitivity and reproducibility, TLC is still used by many scientists [6]. TLC is a fast, practical way of quantitative and semi-quantitative determination of aflatoxins in variety of matrices. Main advantages of TLC method are the fast analysis of several samples in a short period of time, low cost per sample analyzed and easy estimation of contamination levels [6,123]. There are several approaches applied in the quantification process, mainly separated spots are exposed to UV light and either fluorescence intensity [124,125] or absorbance [126] is measured. Another semi quantitative approach is the comparison of spot dimensions of extracted aflatoxins with standards [127-130]. Reliability of this method is strongly dependent on the operator's experience.

Application of over pressured thin layer chromatography (OPTLC or OPLC), also called as high pressure thin layer chromatography (HPTLC), combined with densitometric measurements increased the popularity of the method [13,131,132]. However, with the development of more reliable methods, use of TLC is largely replaced by HPLC and enzyme linked immunosorbent assay methods.

3.2.3. Enzyme linked immunosorbent assay (ELISA)

Chromatographic methods require expensive instrumentation and expertise on the field of chromatography. ELISA as the immunoassay based sensors is another practical alternative for the determination of aflatoxin species. ELISA is a rapid and sensitive technique for routine analysis of food products that demands minimum sample cleanup. This method is suitable for quick and sensitive analysis with high sample throughput and low sample volume requirement [133,134]. Although detection limits of ELISA is lower than instrumental techniques which are used for aflatoxin determination, matrix effect is a significant problem in immunoassay studies and affects the results [135]. Therefore, when it is compared with TLC and HPLC techniques, ELISA is less reliable if the sample has a complicated matrix. ELISA may produce false positives due to this matrix effects. In a study, the same matrix was analyzed by both ELISA and HPLC techniques and results from ELISA were found higher than that of HPLC [109]. This drawback of ELISA makes the usage of the method limited for some complex and invalidated matrixes [136].

Problem can be arisen from the chemical structure of target compound. Since target compounds in ELISA is aflatoxin species rather than antigens, aflatoxin compounds with similar chemical group would also interact with the antibodies by causing interference [137].

There are commercially available kits on the market for single use and many researchers use these kits for practical monitoring of aflatoxin levels [133,138-144]. These kits can include monoclonal or polyclonal antibodies for aflatoxins [145].

Table 4. Some examples for species determination of aflatoxins.

Method	Matrix	Analytes	Extraction	Chromatographic conditions	Analytical Figures	Recovery	References
LC-DAD	Pistachio	B1, B2, G1, G2	SEwith methanol/ water/hexane, (4/1/1.7) containing 0.75 g NaCl followed by C18 SPE cartridges	Column: ODS Mobile Phase: Linear gradient starting with methanol:water:acetonitrile 10:60:30 (v/v)	LOD (mg kg^{-1}) B1, 2.12 B2, 1.24 G1, 2.26 G2, 2.63	73-115%	[146]
HPLC-FD	artificially contaminated food	B1, B2, G1, G2	SE with methanol/water (70/30, v/v) followed by Aflatest-P affinity column	Column: C18 Mobile Phase: Water:methanol:acetonitrile 60:25:15 (v/v)	LOD ($\mu g\ kg^{-1}$) 1 for each	80-110%	[147]
HPLC-FD	Rainbow Trout Muscle and Liver	B1, B2, G1, G2, M1 and aflatoxicol	SE with acetonitrile/ water (9/1), purified on an immunoaffinity column	Column: ODS Mobile Phase: Water:methanol:acetonitrile 60:20:20 (v/v)	LOD ($\mu g\ kg^{-1}$) 0.002-0.012	71.4-93%	[148]
LC-MS/MS	Cassava Flour, Peanut Cake and Maize Samples	B1, B2	SE vith methanol/ethyl acetate/water (70:20:10, v/v/v)	Column: Symmetry RP-18 Mobile Phases: A: water:methanol: acetic acid, 94:5:1, v/v/v containing 5mM ammoniumacetate. B: methanol:water:acetic acid 97:2:1,v/v/v containing 5mM ammoniumacetate.	LOD ($\mu g\ kg^{-1}$) 10-346	72-120%	[149]
LC-MS/MS	Feeds	B1, B2, G1, G2, M1, M2	SE with acetonitrile/ water (84:16, v/v) followed by hexane liquid-liquid extraction		LOD ($\mu g\ kg^{-1}$) 0.1 - 0.8	59-107%	[150]
Two-dimensional TLC	milk and ice cream	M1	-	-	LOD ($\mu g\ L^{-1}$) 2	84.6-88%	[128]
Two-dimensional TLC	pasteurized milk, yoghurt, white cheese, butter, ice cream samples	M1	saturated sodium chloride and chloroform	Silica TLC Mobile Phase: diethyl ether:methanol:water 94:4.5:1.5, v/v/v and chloroform:acetone:methanol 87:10:3, v/v/v	LOD 0.012 ppb	81.4-89.5%	[129]
TLC	melon seed product	B1	methanol/water (80:20 v/v) plus 5 g of NaCl followed by SE with chloroform	Silica Jel TLC Mobile Phase: chloroform:acetone 88:12, v/v	LOD 5 ppb	78-86%	[151]

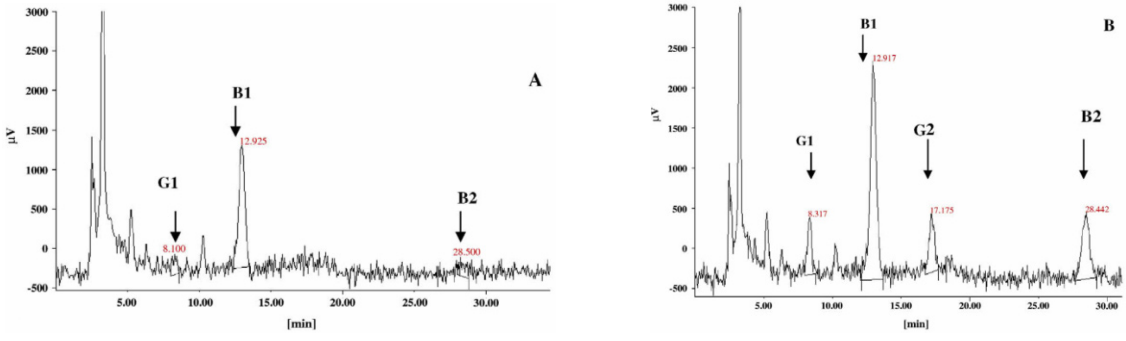

Figure 2. Chromatograms of animal feed sample (A) and sample with spiked aflatoxins (B).

The working principle is simple: a range of antibodies are arranged on a specifically designed plate or column. When the plate or column 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 [6].

The quality and stability of antibodies are the important parameters for the best performance of immunoassay. For the long-term storage, stability of the reagents has to be ensured. In order to do this, protein structure of the enzymes is modified. Modifications can be done by direct site mutagenesis, immobilization to solid-phase, applying chemical modifications or addition of stabilizing agent. For ELISA kits horseradish peroxidase (HRP) is the most widely used enzyme. Environment condition is the one factor that affects enzyme stability [135].

Some of the examples where the common separation and detection techniques are used for the determination of aflatoxins can be seen in Table 4. According to this table, when HPLC-FD is used, it is possible to determine

AFB1, AFB2, AFG1 and AFG2 in the below parts of the μg kg^{-1}. Although recovery values were between 71.4 and 93% after solid-phase extraction with acetonitrile:water (9:1), purified on an immunoaffinity column, HPLC-FD technique that uses ODS column had higher sensitivity compared to C-18 column. In addition, HPLC-FD technique whether with the ODS or C-18 column has significantly lower detection limit compared to LC-DAD technique. While LOD values of HPLC-FD techniques were in μg kg^{-1} level that of the LC-DAD was in mg kg^{-1} level. Tandem MS systems have high sensitivity and they have been used mostly recently. When the sensitivity of LC-MS/MS was compared by considering the similar recovery values, HPLC-FD technique was better.

Lastly, for the determination of AFM1 specie, two-dimensional TLC technique has high sensitivity despite extraction recovery which was 89.5% at maximum.

4. Conclusion

The health effect of aflatoxin species, their regulations in different countries, sample pretreatment and recent advances in the development of separation and detection methods have been summarized and discussed in this review. Aflatoxin species as mycotoxins of major significance are the natural contaminants for a variety of foodstuffs throughout the world and these compounds affect the both human and animal health. Due to the high toxicity of these compounds, there have been significant studies for practical detection methods. It is still impossible to apply a single technique for the detection of all aflatoxin species due to the big differences in the structures of aflatoxins. It is clear from the extent of the literature that interest in the seperation and detection technologies for aflatoxin species is likely to continue while many separation and detection methods have been used for aflatoxins in different matrices. Extraction of aflatoxin species having the different physical characteristics is one of the main challenges remaining for scientist in detection and determination. It is apparent that there is a real need for the development of extraction methods to obtain higher extraction yields for all of the aflatoxin species in variety of matrices.

References

[1] M. Reverberi, A. Ricelli, S. Zjalic, A.A. Fabbri, C. Fanelli, Appl. Microbiol. Biot. 87, 899 (2010)
[2] R. Bhat, R.V. Rai, A.A. Karim, Compr. Rev. Food Sci. F. 9, 57 (2010)
[3] W.O. Ellis, J.P. Smith, B.K. Simpson, J.H. Oldham, Crit. Rev. Food Sci. 30, 403 (1991)
[4] E. Papp, K. H-Otta, G. Zaray, E. Mincsovics, Microchem. J. 73, 39 (2002)
[5] D.T. Wicklow, P.F. Dowd, J.B. Gloer, Genus Aspergillus: from Taxonomy and Genetics to Industrial Application (Plenum Press, New York, 1994)
[6] N.W. Turner, S. Subrahmanyam, S.A. Piletsky, Anal. Chim. Acta 632, 168 (2009)
[7] L. Afsah-Hejri, S. Jinap, S. Arzandeh, H. Mirhosseini, Food Control 22, 381 (2011)
[8] R.R. Dalvi, Vet. Res. Commun. 10, 429 (1986)
[9] R.D. Hartley, J. Okelly, F. Nesbitt, Nature 198, 1056 (1963)
[10] J.W. Bennett, M. Klich, Clin. Microbiol. Rev. 16, 497 (2003)
[11] H. Njapau, E.M. Muzungaile, R.C. Changa, J. Sci. Food Agr. 76, 450 (1998)
[12] R.V. Bhat, S. Vashanti, Agricultural Development 23, 50 (1999)
[13] I. Kamika, L.L. Takoy, Food Control 22, 1760 (2011)
[14] S.A. Bankole, A. Adebanjo, Afr. J. Biotechnol. 2, 254 (2003)
[15] H. Aycicek, A. Aksoy, S. Saygi, Food Control 16, 263 (2005)
[16] S.H. Henry, F.X. Bosch, T.C. Troxell, P.M. Bolger, Science 286, 2453 (1999)
[17] I. Hussain, J. Anwar, M.A. Munawar, M.R. Asi, Food Control 19, 1126 (2008)
[18] M.J. Sweeney, A.D.W. Dobson, Int. J. Food Microbiol. 43, 141 (1998)
[19] A.A. Fallah, Food Control 21, 1478 (2010)
[20] L. Lewis, M. Onsongo, H. Njapau, H. Schurz-Rogers, G. Luber, S. Kieszak, J. Nyamongo, L. Backer, A.M. Dahiye, A. Misore, K. DeCock, C. Rubin, I. Kenya Aflatoxicosis, Environ. Health Persp. 113, 1763 (2005)
[21] H.S. Hussein, J.M. Brasel, Toxicology 167, 101 (2001)
[22] IARC, Monograph on the Evaluation of Carcinogenic Risks to Humans, World Health Organization (IARC Press, Lyon, France, 1993)

[23] M.O. Moss, Mycotoxic fungi (Chapman & Hall, London, 1996)
[24] H. Boudra, J. Barnouin, S. Dragacci, D.P. Morgavi, J. Dairy Sci. 90, 3197 (2007)
[25] M.E. Zain, Journal of Saudi Chemical Society 15, 129 (2011)
[26] IARC, Aflatoxins, Monograph on the Evaluation of Carcinogenic Risk to Humans, World Health Organization (IARC Press, Lyon, France, 2002)
[27] D.P.H. Hsieh, J.M. Cullen, B.H. Ruebner, Food Chem. Toxicol. 22, 1027 (1984)
[28] Y. Dersjant-Li, M.W.A. Verstegen, W.J.J. Gerrits, Nutr. Res. Rev. 16, 223 (2003).
[29] A.S. Arafa, R.J. Bloomer, H.R. Wilson, C.F. Simpson, R.H. Harms, Brit. Poultry Sci. 22, 431 (1981)
[30] J. Verma, T.S. Johri, B.K. Swain, S. Ameena, Brit. Poultry Sci. 45, 512 (2004)
[31] W.A. Khan, M.Z. Khan, A. Khan, I. Hussain, Pakistan Veterinary Journal 30, 155 (2010)
[32] A. Bintvihok, S. Thiengnin, K. Doi, S. Kumagai, J. Vet. Med. Sci. 64, 1037 (2002)
[33] Z. Hussain, M.Z. Khan, A. Khan, I. Javed, M.K. Saleemi, S. Mahmood, M.R. Asi, Food Chem. Toxicol. 48, 3304 (2010)
[34] S. Rawal, J.E. Kim, R. Coulombe, Res. Vet. Sci. 89, 325 (2010)
[35] K.M. Smith, Vet. Rec. 72 (1960)
[36] T.S. Edrington, R. B. Harvey, L.F. Kubena, B.Environ. Contam. Tox. 54, 325 (1995)
[37] R.G. Hendrickse, Bull. Soc. Pathol. Exot. Filiales 76, 559 (1983)
[38] S.J. Van Rensburg, P. Cook-Mozaffari, D.J. Van Schalkwyk, J.J. Van der Watt, Brit. J. Cancer 51, 712 (1985)
[39] Y.Y. Gong, P.C. Turner, A.J. Hall, C.P. Wild, In: J.F. Leslie, R. Bandyopadhyay, A. Visconti (Eds.), Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade (Cromwell Press, Trowbridge, 2008)
[40] J.F. Leslie, R. Bandyopadhyay, A. Visconti (Eds.), Mycotoxins, Detection Methods, Management, Public Health and Agricultural Trade (Cromwell Press, Trowbridge, 2008)
[41] C.P. Wild, F.N. Rasheed, M.F.B. Jawla, A.J. Hall, L.A.M. Jansen, R. Montesano, Lancet 337, 1602 (1991)
[42] T.W. Kensler, B.D. Roebuck, G.N. Wogan, J.D. Groopman, Toxicol. Sci., 120, S28 (2011)
[43] P.C. Turner, S.E. Moore, A.J. Hall, A.M. Prentice, C.P. Wild, Environ. Health Persp. 111, 217 (2003)
[44] Y. Jiang, P.E. Jolly, W.O. Ellis, J.S. Wang, T.D. Phillips, J.H. Williams, Int. Immunol. 17, 807 (2005)
[45] F.Q. Li, T. Yoshizawa, O. Kawamura, X.Y. Luo, Y.W. Li, J. Agr. Food Chem. 49, 4122 (2001).
[46] C. McKean, L. Tang, M. Tang, M. Billam, Z. Wang, C.W. Theodorakis, Food Chem. Toxicol. 44, 868 (2006)
[47] D.M. Parkin, E. Laara, C.S. Muir, Int. J. Cancer 41, 184 (1988)
[48] P.C. Turner, M. Mendy, H. Whittle, M. Fortuin, A.J. Hall, C.P. Wild, Trop. Med. Int. Health 5, 837 (2000)
[49] I. Dvorackova, C. Stora, N. Ayraud, J. Cancer Res. Clin. 100, 221 (1981)
[50] M.A. Qureshi, J. Brake, P.B. Hamilton, W.M. Hagler, S. Nesheim, Poultry Sci. 77, 812 (1998)
[51] A. Ciegler, W. Bennett, BioScience, 30, 512 (1980)
[52] R. Coulombe, The Toxicology of aflatoxins: Human health, veterinary, and agricultural significance (Academic Press, San Diego, 1994)
[53] F. Fung, R.F. Clark, Journal of Toxicology-Clinical Toxicology 42, 217 (2004)
[54] Y.Y. Gong, K. Cardwell, A. Hounsa, S. Egal, P.C. Turner, A.J. Hall, C.P. Wild, British Med. J. 325, 20 (2002)
[55] M.O. Moss, Int. Biodet. Biodeg. 50, 137 (2002)
[56] X.X. Ding, P.W. Li, Y.Z. Bai, H.Y. Zhou, Food Control 23, 143 (2012)
[57] H.K. Abbas, Aflatoxin and Food Safety (Taylor & Francis Group, Boca Raton, 2005)
[58] C. Juan, A. Zinedine, J.C. Molto, L. Idrissi, J. Manes, Food Control 19, 849 (2008)
[59] E. Set, O. Erkmen, Food Chem. Toxicol. 48, 2532 (2010)
[60] H.E. Ok, H.J. Kim, W.B. Shim, H.M. Lee, D.H. Bae, D.H. Chung, H.S. Chuni, J. Food Protec. 70, 2824 (2007)
[61] X.X. Ding, P.W. Li, Y.Z. Bai, H.Y. Zhou, Food Control 23, 143 (2012)
[62] E.E. Creppy, Toxicol. Lett. 127, 19 (2002)
[63] National Standard No. 5925, MaximumTolerated Limits of Mycotoxins in Foods and Feeds (ISIRI, Institute of Standard and Industrial Research of Iran, Iran, 2002)
[64] E. Commission, Official Journal of the European Communities, L77 (2001) 1
[65] U. FDA, FDA compliance policy guides (FDA, Washington, DC, 1996)
[66] H. Sakuma, Y. Kamata, Y. Sugita-Konishi, H. Kawakami, Food Hygiene and Safety Science 52, 220 (2011)
[67] M.H. Iha, C.B. Barbosa, I.A. Okada, M.W. Trucksess, Food Control 22, 1971 (2011)
[68] V.K. Nakai, L.D. Rocha, E. Goncalez, H. Fonseca, E.M.M. Ortega, B. Correa, Food Chem. 106, 285

(2008)
[69] C. Stora, Journal of Environmental Pathology, Toxicology and Oncology 10, 129 (1990)
[70] J.M. Wagacha, J.W. Muthomi, Int. J. Food Microbiol. 124, 1 (2008)
[71] S.Z.B. Elshafie, A. ElMubarak, S.A.F. El-Nagerabi, A.E. Elshafie, Mycopathologia 171, 435 (2011)
[72] C. Probst, H. Njapau, P.J. Cotty, Appl. Environ. Microbiol. 73, 2762 (2007)
[73] H. K. Gardner, S.P. Koltun, F.G. Dollear, E.T. Rayner, J. Am. Oil Chem. Soc. 48, 70 (1971)
[74] H.F. Conway, R.A. Anderson, E.B. Bagley, Cereal Chem. 55, 115 (1978)
[75] N. Sheijooni-Fumani, J. Hassan, S.R. Yousefi, Journal of Separation Science 34, 1333 (2011)
[76] G. Prado, M.S. de Oliveira, A.P.A. Moreira, A.D. Lima, R.D. Souza, M.D. Alves, Quimica Nova, 31, 514 (2008)
[77] M.M. Aguilera-Luiz, P. Plaza-Bolanos, R. Romero-Gonzalez, J.L.M. Vidal, A.G. Frenich, Anal. Bioanal. Chem. 399, 2863 (2011)
[78] A. Santini, R. Ferracane, G. Meca, A. Ritieni, Analytical Methods 2, 884 (2010)
[79] S.J.L. Grio, A.G. Frenich, J.L.M. Vidal, R. Romero-Gonzalez, J. Sep. Sci. 33, 502 (2010)
[80] Y. Nonaka, K. Saito, N. Hanioka, S. Narimatsu, H. Kataoka, J. Chromatogr. A, 1216, 4416 (2009)
[81] V.A. Vega, J. Aoac Int. 88, 1383 (2005)
[82] M. Tabari, G. Karim, M. Ghavami, M. Chamani, Toxicol. Ind. Health 27, 629 (2011)
[83] V. Roussi, A. Govais, A. Varagouli, N.A. Botsoglou, Food Addit. Contam. A. 19, 863 (2002)
[84] S. Lindsay, D. Kealey, High performance liquid chromatography (John Wiley, Chichester, 1987)
[85] N.J. Wu, R. Thompson, J. Liquid Chromatogr. R. T. 29, 949 (2006)
[86] C.G.A. da Silva, C.H. Collins, Quim. Nova 34, 665 (2011)
[87] R.I. Boysen, M.T.W. Hearn, In: M. Lew, L. Hung-Wen (Eds.), Comprehensive Natural Products II (Elsevier, Oxford, 2010) 5
[88] R. Ghali, I. Belouaer, S. Hdiri, H. Ghorbel, K. Maaroufi, A. Hedilli, J. Food Compos. Anal. 22, 751 (2009)
[89] L. Afsah-Hejri, S. Jinap, S. Arzandeh, H. Mirhosseini, Food Control, 22 381 (2011)
[90] W.T. Kok, J. Chromatogr. B. 659, 127 (1994)
[91] R.D. Wei, S.C. Chang, S.S. Lee, Journal of the Association of Official Analytical Chemists 63, 1269 (1980)
[92] A. Tarín, M.G. Rosell, X. Guardino, J. Chromatogr. A. 1047, 235 (2004)
[93] W.S. Khayoon, B. Saad, C.B. Yan, N.H. Hashim, A.S.M. Ali, M.I. Salleh, B. Salleh, Food Chem. 118, 882 (2010)
[94] E. Calleri, G. Marrubini, G. Brusotti, G. Massolini, G. Caccialanza, J. Pharmaceut. Biomed. 44, 396 (2007)
[95] A.C. Manetta, L. Di Giuseppe, M. Giammarco, I. Fusaro, A. Simonella, A. Gramenzi, A. Formigoni, J. Chromatogr. A. 1083, 219 (2005)
[96] G. Tavcar-Kalcher, K. Vrtac, U. Pestevsek, A. Vengust, Food Control 18, 333 (2007)
[97] A. Gürbay, S.A. Sabuncuoglu, G. Girgin, G. Sahin, S. Yigit, M. Yurdakök, G. Tekinalp, Food Chem. Toxicol. 48, 314 (2010)
[98] D. Heperkan, S. Somuncuoglu, F. Karbancioglu-Güler, N. Mecik, Food Control 23, 82 (2012)
[99] C. Dall'asta, G. Ingletto, R. Corradini, G. Galaverna, R. Marchelli, J. Inc. Phenom. Macro. 45, 257 (2003)
[100] W.T. Kok, T.C.H. van Neer, W.A. Traag, L.G.T. Tuinstra, J. Chromatogr. A. 367, 231 (1986)
[101] A. Ariño, M. Herrera, G. Estopañan, M.C. Rota, J.J. Carramiñana, T. Juan, A. Herrera, Food Control 20, 811 (2009)
[102] A. Sheibani, H.S. Ghaziaskar, Food Control 20, 124 (2009)
[103] M. Ibáñez-Vea, L.A. Corcuera, R. Remiro, M.T. Murillo-Arbizu, E. González-Peñas, E. Lizarraga, Food Chem. 127, 352 (2011)
[104] J. Feizy, H.R. Beheshti, N.K. Fahim, S.S.F. Janati, G. Davari, Food Addit. Contam. B. 3, 263 (2010)
[105] A.E. Waltking, D. Wilson, D. Chan, E. Dunn, J. Humphries, H. Kandler, E. Sizoo, J. Aoac Int. 89, 678 (2006)
[106] W. Tassaneeyakul, E. Razzazi-Fazeli, S. Porasuphatana, J. Bohm, Mycopathologia 158, 239 (2004)
[107] O.G. Roch, G. Blunden, R.D. Coker, S. Nawaz, Food Chem. 52, 93 (1995)
[108] M. Holcomb, W.A. Korfmacher, H.C. Thompson, J. Anal. Toxicol. 15, 289 (1991)
[109] M.J. O' Riordan, M.G. Wilkinson, Food Control 20, 700 (2009)
[110] M. Quinto, G. Spadaccino, C. Palermo, D. Centonze, J. Chromatogr. A. 1216, 8636 (2009)
[111] L. Campone, A.L. Piccinelli, R. Celano, L. Rastrelli, J. Chromatogr. A 1218, 7648 (2011)
[112] H. Joshua, J. Chromatogr. A. 654, 247 (1993)
[113] G.J.B. Gnonlonfin, C.S.Y. Adjovi, D.R. Katerere, G.S. Shephard, A. Sanni, L. Brimer, Food Control 23, 333 (2012)

[114] E. Beltrán, M. Ibáñez, J.V. Sancho, M.Á. Cortés, V. Yusà, F. Hernández, Food Chem. 126, 737 (2011)
[115] M. Ventura, A. Gómez, I. Anaya, J. Díaz, F. Broto, M. Agut, L. Comellas, J. Chromatogr. A. 1048, 25 (2004)
[116] C. Cavaliere, P. Foglia, C. Guarino, M. Nazzari, R. Samperi, A. Laganà, Anal. Chim. Acta 596, 141 (2007)
[117] B. Huang, Z. Han, Z. Cai, Y. Wu, Y. Ren, Anal. Chim. Acta 662, 62 (2010)
[118] A. Bacaloni, C. Cavaliere, F. Cucci, P. Foglia, R. Samperi, A. Laganà, J. Chromatogr. A. 1179, 182 (2008)
[119] C. Cavaliere, P. Foglia, E. Pastorini, R. Samperi, A. Laganà, J. Chromatogr. A. 110, 69 (2006)
[120] M.P. Elizalde-González, J. Mattusch, R. Wennrich, J. Chromatogr. A. 828, 439 (1998)
[121] R. Hajian, A.A. Ensafi, Food Chem. 115, 1034 (2009)
[122] C. Cervino, S. Asam, D. Knopp, M. Rychlik, R. Niessner, J. Agr. Food Chem. 56, 1873 (2008)
[123] G.S. Shephard, Anal. Bioanal. Chem. 395, 1215 (2009)
[124] J. Stroka, E. Anklam, J. Chromatogr. A. 904, 263 (2000)
[125] Y.L. Liang, M.E. Baker, B.T. Yeager, M.B. Denton, Anal. Chem. 68, 3885 (1996)
[126] A. Kamkar, Food Control 17, 768 (2006)
[127] I. Var, B. Kabak, F. Gök, Food Control 18, 59 (2007)
[128] O. Atanda, A. Oguntubo, O. Adejumo, J. Ikeorah, I. Akpan, Chemosphere 68, 1455 (2007)
[129] A.A. Fallah, Food Control 21, 1478 (2010)
[130] A.A. Fallah, M. Rahnama, T. Jafari, S.S. Saei-Dehkordi, Food Control 22, 1653 (2011)
[131] H.G. Gao, L. Chen, G.S. Pan, C.Y. Tu, Lc Gc North America, 29, 348 (2011)
[132] Á.M. Móricz, Z. Fatér, K.H. Otta, E. Tyihák, E. Mincsovics, Microchem. J. 85, 140 (2007)
[133] S.C. Pei, Y.Y. Zhang, S.A. Eremin, W.J. Lee, Food Control 20, 1080 (2009)
[134] N.A. Lee, S. Wang, R.D. Allan, I.R. Kennedy, J. Agr. Food Chem. 52, 2746 (2004)
[135] A.Y. Kolosova, W.B. Shim, Z.Y. Yang, S.A. Eremin, D.H. Chung, Anal. Bioanal. Chem. 384, 286 (2006)
[136] J. Gilbert, E. Anklam, Trac-Trend Anal. Chem. 21, 468 (2002)
[137] Z.M. Zheng, C.W. Humphrey, R.S. King, J.L. Richard, Mycopathologia 159, 255 (2005)
[138] B. Sarlmehmetoglu, O. Kuplulu, T. Haluk Celik, Food Control 15, 45 (2004)
[139] M. Ardic, Y. Karakaya, M. Atasever, H. Durmaz, Food Chem. Toxicol. 46, 1596 (2008)
[140] K.K. Tekinsen, G. Uçar, Food Control 19, 27 (2008)
[141] K. Kav, R. Col, K. Kaan Tekinsen, Food Control 22, 1883 (2011)
[142] A. Radoi, M. Targa, B. Prieto-Simon, J.L. Marty, Talanta 77, 138 (2008)
[143] D. Bhattacharya, R. Bhattacharya, T.K. Dhar, J. Immunol. Methods 230, 71 (1999)
[144] N. Nuryono, A. Agus, S. Wedhastri, Y.B. Maryudani, F.M.C. Sigit Setyabudi, J. Böhm, E. Razzazi-Fazeli, Food Control 20, 721 (2009)
[145] J.K. Gathumbi, E. Usleber, E. Martlbauer, Lett. Appl. Microbiol. 32, 349 (2001)
[146] M. Vosough, A. Salemi, Food Chem. 127, 827 (2011)
[147] F.K. Saalia, R.D. Phillips, LWT - Food Sci. Technol. 44, 1496 (2011)
[148] M. Ogiso, A. Kimura, M. Chikasou, M. Saito, M. Takemoto, M. Watai, Food Hygiene and Safety Science 52, 161 (2011)
[149] E.N. Ediage, J.D. Di Mavungu, S. Monbaliu, C. Van Peteghem, S. De Saeger, J. Agr. Food Chem. 59, 5173 (2011)
[150] Y.F. Ying, C.Y. Zhu, M.J. Wei, H.H. Chen, J.A. Qu, C.B. Lu, X.J. Lin, C.J. Luo, Chinese J. Anal. Chem. 38, 1759 (2010)
[151] S.A. Bankole, A.A. Adenusi, O.S. Lawal, O.O. Adesanya, Food Control 21, 974 (2010)