

Aflatoxins: Contamination, Analysis and Control

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1. Introduction

Aflatoxins are toxic metabolites produced by different species of toxigenic fungi, called mycotoxins. Humans can be exposed to aflatoxins by the periodic consumption of contaminated food, contributing to an increase in nutritional deficiencies, immunosuppression and hepatocellular carcinoma (Wagacha & Muthomi, 2008).

Aflatoxins (AFs) have a wide occurrence in different kind of matrices, such as spices, cereals, oils, fruits, vegetables, milk, meat, etc. Among the 18 different types of aflatoxins identified, the major members are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1) and M2 (AFM2) which are produced by *Aspergillus flavus* and/or *Aspergillus parasiticus*. Strains of *A. flavus* can vary from non-toxic to highly toxigenic and are more likely to produce AFB1 than AFG1. Strains of *A. parasiticus* generally have less variation in toxigenicity and produce AFB1 and varying amounts of AFB2, AFG1 and AFG2 (Coppock & Christian, 2007).

Other fungi have been described in the literature as aflatoxins' producers such as *A. bombycis*, *A. ochraceoroseus* and *A. pseudotamari* (Klich et al, 2000; Mishra & Das, 2003). *A. flavus* and *A. fumigatus* have also been identified as pathogens for animals and humans (Zain, 2011).

The order of acute and chronic toxicity is AFB1 > AFG1 > AFB2 > AFG2, reflecting the role played by epoxidation of the 8,9-double bond and also the greater potency associated with the cyclopentenone ring of the B series, when compared with the six-membered lactone ring of the G series. AFM1 and AFM2 are hydroxylated forms of AFB1 and AFB2 (Mclean & Dutton, 1995; Wogan, 1966).

In the primary fungi metabolism a lot of interrelated reactions catalyzed by enzymes occur, with the objective of promoting energy and primary metabolites (synthetic intermediates and macromolecules), ensuring the growth and reproduction of fungi. Secondary

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metabolites are synthesized by a variety of routes from primary metabolites (Obrian et al., 2003; Ueno, 1986; Wild & Montesano, 2009). The biosynthesis of aflatoxins, as all secondary metabolites, is strongly dependent on growth conditions such as substrate composition or physical factors such as pH, water activity, temperature or modified atmospheres. Depending on the particular combination of external growth parameters the biosynthesis of aflatoxin can either be completely inhibited, albeit normal growth is still possible or the biosynthesis pathway can be fully activated. Knowledge about these relationships enables an assessment of which parameter combinations can control aflatoxin biosynthesis. The biochemical correlation between aflatoxin production and oxidative stress suggest that the latter is a prerequisite for aflatoxin synthesis (Ellis et al., 1993; Giorni et al., 2008; Luchese & Harrigan, 1993; Molina & Giannuzzi, 2002; Ribeiro et al., 2006; Schmidt-Heydt et al., 2009). The chapter gives a section on aflatoxin analysis, its occurrence in food and feed as well as its control, once aflatoxin is the major mycotoxin studied and thus is of great concern for human and animal's health due to its carcinogenic, mutagenic, teratogenic and immunosuppressive effects.

2. Factors promoting contamination in aflatoxins and occurrence

Fungi which grow and produce toxins in grains during storage are influenced by factors related to inadequate moisture and temperature, combined with long residence time in warehouses, which are stressful situations and originate toxigenic potential outbreak (Dilkin, 2002).

The most important factors that help predict the occurrence of aflatoxins in food include weather conditions (temperature and atmospheric humidity), agronomical practices (crop rotation and soil cultivation) and internal factors of the food chain (drying and storage conditions). A comprehensive approach is needed to identify and control risks related to food production system that could present a potential hazard to human health, being necessary to identify emerging risks which may include "newly" identified risks, not previously observed risks in human or animal food chain as well as known risks. The emerging risks need to be identified as early as possible in order to take appropriate preventive measures. Thus, the specific risk can be prevented from becoming a danger (Van der Fels-Klers et al., 2008).

Several groups of researchers from the European Union reached a consensus on the most important indicators, based on three stages in food production chain. For cultivation stage the selected indicators were: relative humidity, temperature, crop rotation, tillage practices and water activity of seeds. For transportation and storage the following factors were included: water activity, relative humidity, ventilation, temperature, storage capacity and logistics. For processing the indicators were: data quality, the fraction of grain used, the water activity of seeds, implanted traceability and system quality (Park & Bos, 2007; Van der Fels-Klers et al., 2010).

According to Park & Bos (2007) and Marvin et al. (2009a) to anticipate emerging risks models are developed to assess the risk from the indicators identified. The next step is to identify the sources of information for these indicators, such as climate change (changes in temperature and rainfall), market and consumer trends (crop demand, price and production) and market research (economics, as inflation and taxes) global trade (import and export data and trade barriers), transportation (strikes and transport company records), technology (covers of scientific journals), prevalence of pests, changes in legislation (registration of pesticides). The risk categories within each of the selected indicators should be defined for each specific food.

Among the models currently available in the literature to predict the occurrence of fungi and mycotoxins, are the meteorological indicators in combination with agricultural information. With respect to management strategies, monitoring and prevention are the main indicators derived from the food chain (Dekkers et al., 2008). It is important to stand out the potential interactions among indicators which should be taken into account, for example: between relative humidity and temperature during cultivation; among storage conditions and drying and finally, between crop rotation and management policies (Van der Fels-Klers, 2010; Marvin et al., 2009b).

Due to the great health concern in relation to mycotoxin contaminated food ingestion, studies are being conducted worldwide to verify the occurrence of aflatoxins. The main food products susceptible to fungal growth and consequently to mycotoxins' production, include peanuts (raw, roasted, sweet and infrosted), corn (popcorn, hominy and grains), wheat, rice, nut, walnuts, hazelnuts, cashews, almonds, dried fruits, spices, cotton seed, cassava, vegetable oils, cocoa and others that are normally used in the composition of foods and feeds. Thus, animals are also subjected to aflatoxin contamination, and when meat and milk from these animals are ingested, human contamination may also occur (Kwiatkowski & Alves, 2007). Mycotoxins importance relies on harm caused to human and animal health, besides economical losses in agriculture (Amaral et al., 2006).

Rubert et al. (2010) evaluated a total of 22 samples obtained from a local supermarket (10 samples of malt, 7 samples of coffee and 5 samples of instant-based cereal-breakfast beverage). Four samples of the total malt samples were positive for AFG2 and AFG1, and traces of AFB1 and AFB2 were detected. Khayoon et al. (2010) verified the occurrence of AFB1, B2, G1 and G2 in 42 animal feeds, comprising corn (16), soybean meal (8), mixed meal (13), sunflower, wheat, canola, palm kernel, copra meals (1 each). The results showed that eight samples (19%) were contaminated with aflatoxins, ranging from 6.5 to 101.9 ng g⁻¹. Ibáñez-Vea et al. (2011) evaluated AFG2, AFG1 and ZEA mycotoxins in 20 barley samples. All of the samples analyzed presented levels of AFB1 above its LOD, but only 5 (25%) presented quantifiable levels (>LOQ), with 0.173 µg kg⁻¹ and 0.185 µg kg⁻¹ being the mean of the positive values and the maximum level found, respectively. Reiter et al. (2010) evaluated eighty-one rice samples purchased from different markets. The results revealed that AFB1 (0.45 to 9.86 µg kg⁻¹) could be quantified in 15 samples and AFB2 (1.5 µg kg⁻¹) in one sample. Matumba et al. (2010) investigated aflatoxins in sorghum grain and malt samples, traditional opaque sweet beverage (*thobwa*) and beer prepared from sorghum malts. All malt and beer samples, 15% and 43% of the sorghum and *thobwa* samples, respectively, were contaminated. The sorghum malt prepared for beer brewing, had a significantly ($p < 0.01$) total aflatoxin content (average 408 ± 68 µg kg⁻¹) than any other type of sample. Dors et al. (2011) conducted a survey of mycotoxins in parboiled and whole rice. From the samples analyzed, 9% were contaminated with AFB1 in levels ranging from 11 to 74 µg kg⁻¹. Coelho et al. (1999) studied aflatoxin and ochratoxin A migration during rice parboiling process under different conditions of soaking, autoclaving and drying. It was noted that there was mycotoxin migration from the husk to the starchy endosperm in the following proportions: 32% AFB1, 44% AFB2, 36% AFG1 and 22% AFG2. Dors et al. (2009) assessed mycotoxin migration to the starchy endosperm during the parboiling process and the results showed a lower trend of migration from AFB1 in 6 h soaking and 30 min autoclaving.

Amaral et al. (2006) examined 123 samples of food products based on corn and corn grain, of which 16 were positive with levels of 0.78 µg kg⁻¹. Ramos et al. (2008) detected the presence

of *Aspergillus* spp. and aflatoxin contamination grain samples (12) analyzed and this result was correlated with the greatest amount of rain during harvest. Levels of contamination ranged from "not detected" (nd) to 277.8 $\mu\text{g kg}^{-1}$, for AFB1; from 0.7 to 14 $\mu\text{g kg}^{-1}$ for AFB2; and from nd to 34.1 $\mu\text{g kg}^{-1}$ for AFG2. Oliveira et al. (2010) found aflatoxin contamination in 70% of maize samples from criollo varieties, which have not undergone genetic intervention, at levels ranging from 1 to 2.6 $\mu\text{g kg}^{-1}$.

Almeida et al. (2009) collected 80 samples of maize for poultry feed in two feed mills, from these samples 10% were contaminated with levels varying from 1 to 5 mg kg^{-1} . Marques (2007) analyzed 47 samples of corn grits for animal consumption and 46 were positive for aflatoxin with a maximum of 50 $\mu\text{g kg}^{-1}$. D'Angelo et al. (2007) reported injury in calves for veal production that had a corn-based diet. The toxicological analysis of corn-based feed revealed contamination in the following levels: 1400 $\mu\text{g kg}^{-1}$ AFB1, 120 $\mu\text{g kg}^{-1}$ AFB2, 80 $\mu\text{g kg}^{-1}$ AFG1 and 70 $\mu\text{g kg}^{-1}$ AFG2. In the liver of three animals were found levels of total aflatoxins of 0.1, 0.3 and 0.6 $\mu\text{g kg}^{-1}$. Velazquez et al. (2009) analyzed 40 samples of feed for dairy cattle and 92% of them were contaminated with aflatoxins at levels between 4.82 a 2.89 $\mu\text{g Kg}^{-1}$.

Most of AFB1 and AFB2 ingested by mammals is eliminated through urine and faeces, however a fraction is biotransformed in the liver and excreted together with milk in the form of aflatoxins AFM1 and AFM2, respectively. AFM1 could be detected in milk 12-24 h after the first AFB1 ingestion, reaching a high level after a few days. The ratio between AFB1 ingested and AFM1 excreted has been estimated to be 1-3%. One of the most used treatments for milk processing is heating, however, AFM1 is resistant to any thermal treatment (Carvajal et al., 2003; Park, 2002; Van Egmond, 1989).

Rahimi et al. (2010) analyzed 311 samples of raw milk from cow, water buffalo, camel, sheep, and goat. AFM1 was found in 42.1% of the samples by average concentration of $43.3 \pm 43.8 \text{ ng kg}^{-1}$. The incidence rates of AFM1 in raw cow, water buffalo, camel, sheep, and goat milks were, 78.7%, 38.7%, 12.5%, 37.3%, and 27.1%, respectively. Fallah (2010) investigated the occurrence of AFM1 in 225 commercial liquid milk samples composed of pasteurized milk (116 samples) and UHT milk (109 samples). AFM1 was detected in 151 (67.1%) samples, consisted of 83 (71.5%) pasteurized milk samples (mean: 52.8 ng L^{-1} ; range: 5.8–528.5 ng L^{-1}) and 68 (62.3%) UHT milk samples (mean: 46.4 ng L^{-1} ; range: 5.6–515.9 ng L^{-1}).

Heshmati and Milani (2010) verified the levels of AFM1 in UHT milk samples. Two hundred and ten UHT milk samples were obtained from supermarkets in Tehran, Iran. AFM1 was found in 116 (55.2%) of 210 UHT milk samples examined. The levels of AFM1 in 70 (33.3%) samples were higher than the maximum tolerance limit (0.05 $\mu\text{g L}^{-1}$) accepted by some European countries while none of the samples exceeded the prescribed limit of US regulations. The same authors also studied AFM1 levels of 61 milk samples delivered from small milking farms. The maximum mean concentrations of AFM1 recorded in winter-spring season were in the range of 35.8–58.6 ng L^{-1} and in summer-autumn season in the range of 11.6–14.9 ng L^{-1} .

Cano-Sancho et al. (2010) found AFM1 occurrence in the main dairy products consumed, that is 94.4% (68/72) of whole UHT milk samples, in 2.8% (2/72) of yoghurt samples, but was not detected in cheese. The maximum level was detected in one yoghurt sample with 51.58 ng kg^{-1} . Martins & Martins (2004) determined the occurrence of AFM1 in 96 yoghurt samples, being 48 of them natural and 48 added by strawberry pieces. The results showed that 18.8% of the samples were contaminated with AFM1, being 2 samples of natural

yoghurt (0.043 and 0.045 $\mu\text{g L}^{-1}$) and 16 from fruit added yoghurt (0.019 and 0.098 $\mu\text{g L}^{-1}$). Khoury et al. (2011) investigated the presence and levels of AFM1 in 138 dairy samples (milk and yogurt). Results obtained showed that AFM1 was found in 40.62 % and 32.81 % of milk and yogurt samples respectively. Fallah et al. (2009) studied 210 cheese samples composed of white cheese (116 samples) and cream cheese (94 samples). AFM1 at measurable level (50 ng kg^{-1}) was detected in 161 (76.6%) samples, consisting of 93 (80.1%) white and 68 (72.3%) cream cheese samples.

Dashti et al. (2009) evaluated a total of 321 milk samples (177 fresh, 105 long-life, 27 powdered milk and 12 human milk), 40 cheese samples and 84 feed samples were analyzed for AFM1. Results showed that all fresh milk samples except one were contaminated with AFM1 ranging from 4.9 to 68.7 ng kg^{-1} , for the long-life milk samples were below the detection limit to (88.8 ng kg^{-1}) while in powdered milk samples ranged from 2.04 to 4.14 ng kg^{-1} . From human milk samples, only five were contaminated, with levels ranging from 8.83 to 15.2 ng kg^{-1} . The cheese samples recorded 80% contamination with AFM1 with a range of 23.8–452 ng kg^{-1} . Manetta et al. (2009) investigated samples of whey, curd and a typical hard and long maturing cheese such as Grana Padano produced with naturally contaminated milk in a range of 30–98 ng kg^{-1} . Experimental results showed that, in comparison to milk, AFM1 concentration levels increased both in curd (3-fold) and in long maturing cheese (4.5-fold), while AFM1 occurrence in whey decreased by 40%. Under review done by Montagna et al. (2008), there is an increase in aflatoxin M1 concentration as cheese ripening stage progresses, due to water loss and the consequent concentration of substances present.

Sassahara et al. (2005) collected 98 feed and 42 raw milk samples and the results showed that there was contamination by AFM1 in 26% commercial feed samples, besides 53% of feed samples prepared at the farm and in 100% of corn samples used in animal nutrition. As a result of this aflatoxin incidence in animal diet, milk showed 24% contamination in the collected samples. Romero et al. (2010) evaluated the presence of AFM1 in human urine samples from a specific Brazilian population, as well as in corn, peanut, and milk consumption measured by two types of food inquiry. A total of 69 samples were analyzed and 45 of them (65%) presented contaminations $\geq 1.8 \text{ pg mL}^{-1}$, which was the limit of quantification (LOQ). Seventy eight percent ($n = 54$) of the samples presented detectable concentrations of AFM1 ($>0.6 \text{ pg mL}^{-1}$). The AFM1 concentration among samples above LOQ ranged from 1.8 to 39.9 pg mL^{-1} . There were differences in food consumption profile among donors, although no association was found between food consumption and AFM1 concentration in urine. The high frequency of positive samples suggests exposure to aflatoxins by the studied population.

Aflatoxins are found in maize and peanuts, as well as in tree nuts and dried fruits (Zain, 2011). Nakai et al. (2008) evaluated the mycoflora and occurrence of aflatoxins in stored peanut samples (hulls and kernels). Analysis of hulls showed that 6.7% of the samples were contaminated with AFB1 and AFB2; in kernels, 33.3% of the samples were contaminated with AFB1 and 28.3% with AFB2. Analysis of the toxigenic potential revealed that 93.8% of the *A. flavus* strains isolated were producers of AFB1 and AFB2. Shenasi et al. (2002) detected aflatoxins in 12% of the samples at twenty-five varieties of dates (*Phoenix dactylifera*) although aflatoxigenic *Aspergillus* were detected in 40% of the varieties examined. Bircan (2009) tested aflatoxin contamination in 98 dried figs analyzed for OTA to determine the co-occurrence of both toxins. Seven samples were confirmed aflatoxin positive, in the range of 0.23–4.28 ng g^{-1} and only 2 samples contained both toxins, with a maximum concentration of 24.37 ng g^{-1} for OTA and 1.02 ng g^{-1} for AFB1.

More recently, Herzallah et al. (2009) studied aflatoxin contamination in meat products collected in 5 different months. The AFB1, AFB2, AFG1 and AFG2 contents in the analysed food products ranged from 1.10 to 8.32 $\mu\text{g}\cdot\text{L}^{-1}$ and 0.15 to 6.36 $\mu\text{g}\cdot\text{L}^{-1}$ in imported and fresh meat samples collected during March, respectively.

Fruits and vegetables do not appear to be of major concern as possible sources of mycotoxin contamination in food and feeds because they were only listed as minor sources in a statement of the Institute of Food Science and Technology Trust Fund (2006). Major sources on the list included mold damaged foodstuffs, specifically cereals and oilseeds.

FAO has done a lot of work on mycotoxins in developing countries, although economic dimensions are rarely observed. In horticultural crops, mycotoxins are primarily associated with dried fruits (figs and prunes), certain processed products (apple and grape juice) and are probably in apples and grapes (Dombrink-Kurtzman, 2008).

Although a large number of different mycotoxins exist, there are only a few of them that are regularly found in foods. Most reports concerning aflatoxin formation on fruits refer to figs or citrus fruits (Drusch & Ragab, 2003). Aflatoxins constitute a problem that is already present in the orchard. Little contamination occurs when firm, ripe fruits are dried immediately (Steiner et al., 1988). From a practical point of view, the best approach for eliminating mycotoxins from foods is to prevent mold growth at all levels of production, including harvesting, transport, and storage (Boutrif, 1998).

Thus, the occurrence of fungi and mycotoxins can be controlled by applying a number of preventive measures both before and after harvest, including insect control, good harvesting, drying, and storage practices. If mycotoxin contamination has occurred, the levels of toxins can be reduced by physical, chemical or biological decontamination. Milling, food processing, and regulatory control of toxins to safety levels can also have a positive impact on food safety (Trucksess & Diaz-Amigo, 2011).

3. Sampling, measurement and analysis

3.1 Sample preparation

Since AFs are inhomogeneous distributed in food and feed, high-contaminated hotspots can occur. Thus, sampling is an important step in the analysis of contaminated food and feed (Reiter et al., 2009).

Relating to the sample preparation techniques used in the last years, liquid-solid extraction has been widely employed. Usually the procedure consists of weighing a mass of the homogenized sample, add the extractor solvent and agitate in a shaker. Commonly, after these steps, filtration is carried out. In these extractions different volumes and solvent kinds were employed. Solvent volumes ranging from 20 to 250 mL and composed mainly of methanol/water or acetonitrile/water have been used. The choice for the best extraction solvent is directly related to the extraction efficiency and the number of co-extractives that this solvent extracts. In the work developed by Capriotti et al. (2010) the authors compared the use of methanol, acetonitrile and acetone for mycotoxins' extraction from cereals, being observed the highest recovery for the analytes in the acetone solution.

Another tool that has been employed during extraction is the ultrasound assisted extraction (Amate et al., 2010; Bacaloni et al., 2008; Capriotti et al., 2010; Quinto et al., 2009). Ultrasound is a simple and versatile method because it aggressively agitates the solution system improving transfer from the cell into the solvent. Bacaloni et al. (2008) employed ultrasound extraction and compared the technique with matrix solid-phase dispersion (MSPD) and homogenization. Recoveries comparable to those obtained with the

homogenization method were achieved with a sonication time of 10 min. The authors concluded that the employment of ultrasound is time-saving because it is easy to handle and many samples can be treated at the same time. Besides, ultrasonic extraction may be an efficient, safe and reliable alternative to homogenization and MSPD extractions.

MSPD technique has been employed for aflatoxins' extraction in food samples (Cavaliere et al., 2007; Rubert et al., 2010; Sebastià et al., 2010). MSPD involves the homogenization of the sample together with a suitable sorbent (usually octadecylsilica) using a pestle and mortar. The solid mixture is transferred to a cartridge and after, the aflatoxins are eluted and determined. Rubert et al. (2010) extracted the aflatoxins 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 limits of quantification of $1 \mu\text{g kg}^{-1}$ were reached.

Pressurized fluid extraction (PFE), with trade name of accelerated solvent extraction (ASE) has also been employed for aflatoxins' extraction (Sheibani & Ghaziaskar, 2009; Desmarchelier et al., 2010). This technique employs solvents at elevated pressures and temperatures to achieve complete extraction of analytes from solid and semi-solid samples with lower solvent volumes and shorter extraction times (Sheibani & Ghaziaskar, 2009).

The accelerated extraction solvent was compared to QuEChERS procedure (acronym name for Quick, Easy, Cheap, Effective, Rugged, and Safe) for extraction of mycotoxins including aflatoxins from food samples in the study developed by Desmarchelier et al. (2010). Both methods showed high extraction efficiency in a broad range of cereal-based products and with a comparable sensitivity. Nevertheless, the easiness-to-handle of these extraction methods was definitely in favor of the QuEChERS-like procedure, avoiding any tedious preparation of extraction cells, requiring less reagents and glassware and involving less intermediate steps. Consequently, a higher sample throughput was possible, with up to 40 individual samples extracted over one working day as compared to the 24 individual samples processed over one and a half working days by the ASE procedure. On a routine basis, the QuEChERS-like method constitutes undeniably the best option.

Solid-phase extractions have been used for mycotoxins' extraction from different kinds of samples. Solid-phase microextraction (SPME) was used by Nonaka et al. (2009). The authors optimized the on-line in-tube SPME-LC-MS and concluded that using this approach it's possible to continuously extract aflatoxins from samples extracts with no requirement of any other pretreatments, which can then be analyzed by LC-MS. This method is automatic, simple, rapid, selective, and sensitive, and may be easily applied to the analysis of various food samples.

Solid-phase extraction (SPE) has also been applied for many years to mycotoxins analysis, once this technique enables the extraction, preconcentration and purification in one step (Alcaide-Molina et al., 2009).

3.2 Clean-up

Due to the large number of co-extractives that are present in the sample extracts, most matrices are unsuitable for direct chromatographic analysis, needing a clean-up step.

Some studies, according to the detection technique that will be employed only uses the dilution approach to reduce the matrix interferences, as we could observe in the work developed by Acharya & Dhar (2008). The authors describe a simple approach for performing broad-specific noncompetitive immunoassays for the determination of total aflatoxins (AFB1 + AFB2 + AFG1 + AFG2). Twenty grams sample were extracted with 100 mL MeOH:H₂O (70:30, v/v) and stirred for 0.5 h. Extracts were filtered through a filter paper.

The matrix interferences were eliminated by diluting the sample 10-fold with the assay buffer.

The most employed clean-up methods in some laboratories are the solid-phase extraction, multifunctional columns or immunoaffinity columns (IACs) (Bacaloni et al., 2008; Huang et al., 2010; Piermarini et al., 2009; Reiter et al., 2010). IACs in combination with HPLC are increasingly used nowadays as reference methods and allow a sufficient elimination of matrix interferences, due to their high selectivity. The immunoaffinity is based on the binding of the immobilized specific antibodies on the surface of a column (Shepard, 2009). Clean-up only with solvents is rarely found nowadays (Sheibani & Ghaziaskar, 2009). The advantages of IACs are the effective and specific extract purification provided, the economic use of organic solvents and the improved chromatographic performance achieved with cleaner samples (Shepard, 2009).

The clean-up step has an important role in the quantification techniques, avoiding false positives, allowing better recoveries and helping with the time-life of the equipments.

3.3 Separation and detection

Different techniques have been found for the determination of aflatoxins in the last years. Techniques based on ELISA detection (Li et al., 2009), electrochemical sensor (Tan et al., 2009), immunoassays (Saha et al., 2007), Liquid Chromatography tandem Mass Spectrometry (LC-MS) (Kokkonen & Jestoi, 2009; Rubert et al., 2010), Liquid Chromatography with Fluorescence Detection (LC-FLD) (Ibáñez-Vea et al., 2011), Liquid chromatography with ultraviolet detection (Fu et al., 2008) and adsorptive stripping voltammetry (Hajian & Ensafi, 2009) are found in the literature.

Aflatoxins separation has been performed for many years by HPLC, using mainly reversed-phase columns, with mobile phases composed of water, methanol and acetonitrile mixtures. Chromatographic performance has improved with column technology, particularly with reduced size of the column packing material (Shepard, 2009). Researches employing the Ultra-Performance Liquid Chromatography (UPLC) have brought lower run times and better peak shapes. Huang et al. (2010) employed the UHPLC-MS/MS for the separation and detection of aflatoxins after an extraction with acetonitrile and water and a clean-up with SPE, reaching limits of quantification between 0.012 and 0.073 $\mu\text{g kg}^{-1}$. The total run time for the separation of AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2 was less than 9 min.

The AFs are named due to their properties under UV-irradiation, where AFB1 and AFB2 emit blue fluorescence (350 nm), AFG1 and AFG2 green fluorescence (350 nm). These important features can be used for rapid identification and detection (Reiter et al., 2009). So, although aflatoxins are naturally strongly fluorescent compounds, making them ideal subjects for fluorescence detection, various analogues exhibit solvent-dependent quenching in HPLC solvent systems. In the aqueous mixtures used for reversed-phase chromatography, the fluorescence of AFB1 and AFG1 are significantly quenched (Shepard, 2009). This is generally overcome by some derivatization procedure. In the last years works employing post-column derivatization have been found. Ariño et al. (2009) determined AFB1, AFB2, AFG1 and AFG2 with liquid chromatography using post-column photochemical derivatization for improved sensitivity and selectivity. This technique allowed a fluorescence enhancement about 30 times for aflatoxin B1 and G1. Results showed that post-column photochemical derivatization of aflatoxins increased detectability and selectivity of responses for the LC-FLD system. The average recovery was between 84 and 91%, and LOQ was 0.1 $\mu\text{g kg}^{-1}$.

The coupling of HPLC to mass spectrometry is the more commonly employed detection technique in the last years. The ionization sources employed based on atmospheric pressure ionization techniques such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) has resulted in a range of new methods (Beltrán et al., 2011; Cavaliere et al., 2007; Kokkonen & Jestoi, 2009; Sulyok et al., 2007). The advantages of LC-MS techniques lie in the improved detection limits, the confirmation provided by mass spectral fragmentation and the ability to filter out by mass any impurities that interfere in spectrophotometric detectors. For the determination of 32 mycotoxins, including aflatoxins, in beer, Zachariasova et al. (2010), developed a study with the aim of optimize a simple and high-throughput method. For determination of analytes, ultra-high-performance liquid chromatography hyphenated with high-resolution mass spectrometry utilizing an orbitrap (U-HPLC-orbitrapMS) or time-of-flight (TOFMS) technology was used. Because of significantly better detection capabilities of the orbitrap technology, the U-HPLC-orbitrapMS method was chosen. The U-HPLC-orbitrapMS technology represents a progressive alternative equivalent to MS/MS. The U-HPLC-orbitrapMS system used within this study operates in APCI mode enabled rapid determination of trace levels of multiple mycotoxins potentially occurring in beer samples.

Relating to the source of ionization, for aflatoxin determination we have found more studies employing the ESI as source of ionization. Atmospheric pressure photoionization (APPI) is the latest interface introduced in the field of soft ionization techniques, and it was employed in the study developed by Capriotti et al. (2010). Using APPI, detection limits for the investigated compounds were lower than by using ESI, due to a much lower noise and matrix effect. For aflatoxins, LOQs between 0.1 and 0.5 $\mu\text{g kg}^{-1}$ were reached.

The application of aflatoxin-specific antibodies has produced a range of immunoassay analytical methods (Acharya & Dhar, 2008; Li et al., 2009; Saha et al., 2007). A number of commercial enzyme-linked immunosorbent assays (ELISAs) are well established and available. The essential principle of these assays is the immobilization on a suitable surface of antibody or antigen and the establishment of a competitive process involving this resource and components of the analytical solution (Shepard, 2009). Piermarini et al. (2009) developed a method, called ELIME-array (Enzyme-Linked-Immuno-Magnetic-Electrochemical-array) for the determination of AFB1 in corn samples. In order to determine AFB1 at a level of regulatory relevance, a sample treatment that employs extraction, clean-up and concentration steps, was selected. The recovery of the ELIME-array was calculated by analyzing replicates of four certificate reference materials (CRMs). The method showed recoveries between 95 and 114% with a LOQ of 1.5 ng mL^{-1} .

3.3.1 Matrix effect

Another special issue about the determination of contaminants, such as aflatoxins in a variety of samples is the matrix effect. Mainly related to the mass spectrometric techniques, the matrix effect is known as the change of ionization efficiency for the studied analytes in the presence of other compounds (Kruve et al., 2008).

Relating to this topic some procedures could be done to guarantee the trueness of the results, avoiding false positives. For aflatoxins' determination the approaches observed were: dilution, matrix-matched calibration, standard addition and use of internal standard. Some studies employ the AFM1 as I.S, and in others the use of a deuterated one ($^{13}\text{C}_{17}$ -AFB1) was observed. The sample clean-up, many times is enough to avoid the matrix effects, but in other cases not.

3.4 Analytical criteria

Some performance criteria are important for obtaining reliable results for aflatoxins' determination. Table 1 shows a summary of some manuscripts published after 2007, showing which aflatoxins were determined, kind of sample, sample preparation, clean-up, matrix effect, detection, limit of quantification and recoveries.

Aflatoxins	Matrix	Sample preparation (sample mass, type and volume of extractor solvent)	Clean-up	Matrix Effect	Detection	LOQ	R%	Reference
total aflatoxins AFB1, AFB2, AFG1, AFG2	corn	20 g 100 mL MeOH:H ₂ O (70:30, v/v)	-	Dilution 10-fold to eliminate the matrix interferents	broad-specific noncompetitive immunoassay	5 µg kg ⁻¹ (LOD)	86-100	Acharya & Dhar, 2008
AFB1, AFB2 AFG1, AFG2	Olive leaves and drupes	Automatic SPE 5 g 25 mL MeOH:H ₂ O (70:30, v/v)	Automatic SPE	Matrix-matched calibration	LC-ESI-MS/MS	0.03 - 0.11 µg kg ⁻¹	96-102	Alcaide-Molina et al., 2009
AFB1, AFB2 AFG1, FG2	spices	1 g 10 mL ACN ultrasonic bath (30 min)	-	Matrix-matched calibration	LC-ESI-MS/MS	1-20 µg kg ⁻¹	100-139	Amate et al., 2010
AFB1, AFB2 AFG1, AFG2	pistachios	10 g 1 g NaCl 40 mL MeOH:H ₂ O (8:2, v/v) 20 mL hexane	Immunoaffinity column		LC-FLD post-column photochemical derivatization	0.1 µg kg ⁻¹	84-91	Ariño et al., 2009
AFB1, AFB2 AFG1, AFG2 AFM1 (IS)	hazelnuts	1 g 20 mL ACN: H ₂ O (80:20, v/v). Ultrasonic bath (10 min)	SPE (Carbograph-4)	Matrix-matched calibration and internal standard	LC-ESI-MS/MS	0.04 - 0.07 µg kg ⁻¹	91-102	Bacaloni et al., 2008
AFB1, AFB2 AFG1, AFG2 AFM1	Baby food and milk	Cereals infant formula - 5 g 20 mL ACN:H ₂ O (80:20, v/v) Liquid samples - 8 g 32 mL ACN	immunoaffinity column	Cleanup eliminated the matrix effect	UHPLC-ESI-MS/MS	0.003 - 0.025 µg kg ⁻¹	79 - 112	Beltrán et al., 2011
AFB1, AFB2 AFG1, AFG2	Baby food and paprika	Baby food - 50 g 5 g NaCl 250 mL MeOH:H ₂ O (80:20, v/v). Paprika - 25 g 2.5 g NaCl 100 mL MeOH:H ₂ O (80:20, v/v)	Immunoaffinity column	-	HPLC-FLD	0.02 - 0.2 µg kg ⁻¹	86-96	Brera et al., 2011
AFB1, AFB2 AFG1, AFG2 AFM1	Cereals Wheat and maize samples	1 g 6 mL CH ₃ COCH ₃ :H ₂ O:CH ₃ COOH (80:19:1, v/v/v) ultrasonic bath (20 min)	-		LC-APPI-MS/MS	0.1 - 0.5 µg kg ⁻¹	86-104	Capriotti et al., 2010
AFB1, AFB2 AFG1, AFG2 AFM1 (LS)	Olive oil	MSPD (C18) 0.32 g 6 mL MeOH:H ₂ O (80:20, v/v)	-	Matrix-matched calibration	LC-ESI-MS/MS	0.04 - 0.12 µg kg ⁻¹	92-107%	Cavaliere et al., 2007
AFB1, AFB2 AFG1, AFG2	Curry Red pepper paste Ginger product Red pepper flour Black pepper Cinnamon powder	25 g 100 mL MeOH:H ₂ O (70:30, v/v) 1% NaCl	Immunoaffinity column		HPLC-FLD	0.03-0.45 µg kg ⁻¹	68.1-103.9	Cho et al., 2008

Aflatoxins	Matrix	Sample preparation (sample mass, type and volume of extractor solvent)	Clean-up	Matrix Effect	Detection	LOQ	R%	Reference
AFB1, AFB2, AFG1, AFG2	maize, wheat, rye, rice, oat, barley, soya, and infant cereals	QuEChERS - 5 g 10 mL ACN 0.5% CH ₃ COOH ASE - 5 g ACN:H ₂ O:CH ₃ COOH (80:19:0.5, v/v/v)	defatting step with n-hexane	Standard addition	LC-ESI-MS/MS	1.0 - 2.0 µg kg ⁻¹	QuEChERS 89-116 ASE 67-107	Desmarchelier et al., 2010
AFB1, AFB2, AFG1, AFG2, AFM1	Maize Walnuts Biscuits Breakfasts cereals	5 g 10 mL ACN:H ₂ O (80:20, v/v) biscuit samples - 20 mL ACN:H ₂ O (80:20, v/v) -	-	UHPLC-MS/MS	Matrix-matched calibration	0.03-3.5 µg kg ⁻¹	71.3-104.7	Frenich et al., 2009
AFB1, AFB2, AFG1, AFG2	Corn peanuts	25 g 80 mL ACN:H ₂ O (84:16, v/v)	Immunoaffinity column	-	UPLC-UV	0.63-1.07 µg kg ⁻¹	83.4-94.7	Fu et al., 2008
AFB1, AFB2, AFG1, AFG2	Sorghum pistachios	10 g 40 mL MeOH: H ₂ O (80:20, v/v) 1 g NaCl 20 mL n-hexane	immunoaffinity column	-	HPLC-FLD	0.08-0.16 µg kg ⁻¹	68.3-87.7	Ghali et al., 2009
AFB1, AFB2, AFG1, AFG2	Cassava flour	10 g 1 g NaCl 25 mL MeOH:H ₂ O (80:20, v/v)	immunoaffinity column	-	HPLC-FLD Post columns PHRED	5.0 µg kg ⁻¹	52-89	Gnonlonfin et al., 2010
AFB1, AFB2, AFG1, AFG2	Peanuts	25 g 5 g NaCl 125 mL MeOH:H ₂ O (7:3 v/v)	immunoaffinity columns	-	HPLC-UV-FLD	0.1-3.5 ng mL ⁻¹	65-90	Gonzalez et al., 2008
AFB1, AFB2	Groundnut	5 g 10 mL MeOH:H ₂ O (80:20, v/v) 5 mL hexane	-	Diluted 10-fold to avoid interferences	Adsorptive stripping voltametry	0.1-0.115 ng mL ⁻¹ (LOD)	-	Hajian & Ensafi, 2009
AFB1, AFB2, AFG1, AFG2, AFM1, AFM2	Traditional Chinese medicines	2 g 10 mL ACN:H ₂ O (84:16, v/v)	SPE	Internal standard [¹³ C ₁₇]-AFB1	UHPLC-ESI-MS/MS	0.1-0.39 µg kg ⁻¹	85.6-117.6	Han et al., 2010
AFB1, AFB2, AFG1, AFG2, AFM, AFM2	Peanuts and their derivative products	2.5 g 10 mL ACN:H ₂ O (84:16, v/v)	SPE	Matrix-matched calibration	UHPLC-ESI-MS/MS	0.012-0.273 µg kg ⁻¹	74.7-86.8	Huang et al., 2010
AFB1, AFB2, AFG1, AFG2	barley	10 g 50 mL ACN:H ₂ O (60:40, v/v)	immunoaffinity column	-	UHPLC-FLD	0.038 - 0.15 µg kg ⁻¹	71.7-99.6	Ibáñez-Vea et al., 2011
AFB1, AFB2, AFG1, AFG2	Cereals Wheat Barley Oats	Automatic ASE 10 g sample Extraction with acetonitrile	-	Matrix-matched calibration	LC-ESI-MS/MS	20 - 65 µg kg ⁻¹	61-94	Kokkonen & Jestoi, 2009
AFB1, AFB2, AFG1, AFG2	nuts, cereals, dried fruits, and spices	0.5 g 1 mL MeOH:H ₂ O (80:20, v/v) SPME	-	AFM1 (I.S.)	LC-ESI-MS	2.1 - 2.8 pg mL ⁻¹ (LOD)	80.8-109.1	Nonaka et al., 2009
AFB1	Corn	25 g 100 mL ACN:H ₂ O (84:16, v/v)	Mycosep columns	Matrix-matched calibration	ELIME-array	1.5 ng mL ⁻¹	95-114%	Piermarini et al., 2009
AFB1, AFB2, AFG1, AFG2	Cereal flours	2 g 10 mL of MeOH:PB1 (80:20, v/v) Ultrasonic bath (20 min) SPME	Immunoaffinity column	-	-	0.1-0.63 µg kg ⁻¹	49-59	Quinto et al., 2009
AFB1, AFB2, AFG1, AFG2	rice	50 g 100 mL MeOH:H ₂ O (80:20, v/v)	immunoaffinity columns	-	HPLC-FLD	0.44-0.6 µg kg ⁻¹	83-102	Reiter et al., 2010

Aflatoxins	Matrix	Sample preparation (sample mass, type and volume of extractor solvent)	Clean-up	Matrix Effect	Detection	LOQ	R%	Reference
AFB1, AFB2, AFG1, AFG2	cereals	MSPD (1 g C18) 1 g 10 mL ACN		Matrix-matched calibration	LC-ESI-MS/MS	1 µg kg ⁻¹	64-91	Rubert et al., 2010
AFB1	Chili	2 g 5 mL MeOH:H ₂ O (80:20, v/v)			Membrane-based immunoassay	2 µg kg ⁻¹	88-101	Saha et al., 2007
AFB1, AFB2, AFG1, AFG2	Tigernuts and Their Beverages	MSPD (2 g C18) 1 g or 1 mL 10 mL hexane 10 mL ACN			LC-FLD	0.21-1.49 µg kg ⁻¹ tigernuts 0.13-0.57 µg L ⁻¹ beverages	72.3-82.1 (tigernuts) 74.0-86.3 (beverages)	Sebastià et al., 2010
AFB1, AFB2	pistachio	PFE 7 g 5 mL n-hexane MeOH:H ₂ O (80:20, v/v)	purified with chloroform		HPLC-FLD		100	Sheibani & Ghiasiaskar, 2009
AFB1, AFB2, AFG1, AFG2	paprika	25 g 100 mL of MeOH:H ₂ O (60:40, v/v)	immunoaffinity column	-	HPLC-FLD	0.23-0.45 µg kg ⁻¹	75.6-108	Shundo et al., 2009
AFB1, AFB2, AFG1, AFG2, AFM1	bread, fruits, vegetables, jam, cheese, chestnuts red wine	0.5 g 2 mL ACN:H ₂ O:CH ₃ COOH (79:20:1, v/v/v)	-	Matrix matched calibration	HPLC/ESI-MS/MS	0.7-1.5 µg kg ⁻¹ (LOD)	97-100	Sulyok et al., 2007
AFB1	Rice	1 g 5 mL MeOH:H ₂ O (80:20, v/v)			Electrochemical sensor	0.1 µg L ⁻¹ (LOD)	88.5-112	Tan et al., 2009
AFB1, AFB2, AFG1, AFG2	beer	4 mL beer 16 mL ACN		Matrix matched calibration	U-HPLC-orbitrapMS	0.5 - 3.0 µg L ⁻¹	90-117	Zachariasova et al., 2010
AFB1, AFB2, AFG1, AFG2	wheat flour, corn flour, poultry feeds	50 g 250 mL MeOH:H ₂ O (80:20, v/v)	immunoaffinity column	-	LC-FLD	0.01 - 0.01 µg kg ⁻¹	>65%	Zinedine et al., 2007

Table 1. Main parameters about extraction and determination of aflatoxins from 2007 to the present.

3.5 Conclusions and analysis tools of tomorrow

Determination of aflatoxins has been carried out using TLC, HPLC, LC-MS, LC-MS-MS, and immunological methods. Each one of the techniques has advantages and disadvantages. TLC provides an economical screening method. HPLC methods coupled with fluorescence detection are sensitive and the most widely used methods, but most require a derivatization step. Immunoassays provide rapid screening for total aflatoxin, but they may not be sufficiently reliable as quantitative methods for individual aflatoxins. LC-MS methods are specific and sensitive, and their use is becoming increasingly widespread. However, due to the low levels and the number of interferences from the matrices, usually, a sample preparation step is required to allow the extraction, preconcentration, and clean-up, enhancing the sensitivity and selectivity.

The advance in the extraction and determination of aflatoxins will continue increasing together with the improvement of analytical science. The search for sample preparation methods that allow fast extraction, good accuracy and precision, low extraction of interferences, low consumption of solvents will continue together with the increase in

detection techniques with higher accuracy and sensibility. So, the determination of aflatoxins in foods will continue to be developed and improved.

4. Legislation, desintoxication and control

Concern about the potential hazards posed by dietary aflatoxins started in the 1960s after some 100000 turkey poults in Great Britain died as a result of aflatoxin exposure from their feed. When it became evident that aflatoxin exposure caused cancer in many species, most countries, established various regulations for aflatoxin levels (either total aflatoxins or for AFB1) in food and/or feed in order to limit exposure to this group of mycotoxins (Van-Egmond et al., 2007). These initial regulations on aflatoxins were not based on the derivation of a TDI (estimated tolerable daily intake), but rather on a desire to keep levels as low as technologically feasible (basis for regulations in some countries), or 'free' of aflatoxins by not allowing residues above the analytical detection limit (basis for regulations in some other countries). The early prudent actions regarding aflatoxins by governments have been justified, since AFB1 has been found to be a potent genotoxic agent and carcinogen in many test systems and animal species (Kuiper-Goodman, 1995; Wogan, 1974).

Worldwide, aflatoxins because of their prevalence and toxicity are important in public health. Public health concerns center on both primary poisoning from aflatoxins in commodities, food and feed stuffs, and relay poisoning from aflatoxins in milk. The allowable levels of aflatoxins in animal feedstuff and human foods vary with governmental jurisdictions (Coppock & Christian, 2007).

Aflatoxins are of great concern because of their detrimental effects on the health of humans and animals, including carcinogenic, mutagenic, teratogenic and immunosuppressive effects. AFB1 is the most potent hepatocarcinogen known in mammals and is classified by the International Agency of Research on Cancer as Group 1 carcinogen (Eaton & Gallagher, 1994 as cited in Zinedine, 2009).

The hazardous nature of aflatoxin to humans and animals has necessitated the need for establishment of control measures and tolerance levels by national and international authorities. Different countries have different regulations for aflatoxin. The general trend is that industrialized countries usually set lower tolerance levels than the developing countries, where most of the susceptible commodities are produced. However, such lack of harmony may give rise to difficulties in the trade of some commodities (Aibara & Maeda, 1989; Ismail, 1997).

The first legislative act was undertaken in 1965 by the Food and Drug Administration (FDA) of the USA, which proposed a tolerance level of 30 $\mu\text{g kg}^{-1}$ of total aflatoxins (B1 + G1 + B2 + G2). With increasing awareness of aflatoxins as potent toxic substances, the proposed level was lowered to 20 $\mu\text{g kg}^{-1}$ in 1969. The FDA has action levels for aflatoxins regulating the levels and species to which contaminated feeds may be fed (Table 2). In 1973, the European Economic Community (EEC) established legislation on maximum permitted levels of AFB1 in different types of feedstuffs. The legislation has been frequently amended since then (EEC, 1974; FDA, 1977; Ismail 1997).

The European Community levels are more restrictive (Tables 3 and 4), 4 $\mu\text{g kg}^{-1}$ total aflatoxin in food for human consumption are the maximum acceptable limits in the EU, the strictest in standard worldwide. Human foods are allowed 4–30 ppb aflatoxin, depending on the country involved (John, 2007).

Commodity	Concentration ($\mu\text{g kg}^{-1}$)
Cottonseed meal as a feed ingredient	300
Corn and peanut products for finishing beef cattle	300
Corn and peanut products for finishing swine	200
Corn and peanut products for breeding beef cattle, swine and mature poultry	100
Corn for immature animals and dairy cattle	20
All products, except milk, designated for humans	20
All other feedstuffs	20
Milk	0.5

Table 2. U.S. Food and Drug Administration action levels for total aflatoxins in food and feed ($\mu\text{g kg}^{-1}$).

Human food	AFB1 ($\mu\text{g kg}^{-1}$)	AFB1, B2, G1, G2 ($\mu\text{g kg}^{-1}$)	M1 ($\mu\text{g kg}^{-1}$)
Groundnuts, dried fruit and processed products thereof	2	4	-
Groundnuts subjected to sorting or phytic treating	8	15	-
As above but for nuts and dried fruits	5	10	-
Cereals (including maize) and processed products thereof	2	4	-
Milk	-	-	0,05

Table 3. European Union for aflatoxins in human food ($\mu\text{g kg}^{-1}$).

The Brazilian National Agency for Sanitary Vigilance established the Resolution (RDC) n° 7 of February 2011 which provides for the maximum permissible (LMT) for aflatoxins (Table 5) and other mycotoxins in food.

Feed	AFB1 ($\mu\text{g kg}^{-1}$)	Feed	AFB1 ($\mu\text{g kg}^{-1}$)
Feed (exceptions below)	50	Complete feedstuff for pigs and poultry	20
Groundnuts, copra, palm kernel, cottonseed, babasu, maize and products derived from processing thereof	20	Other complete feedstuffs	10
Complete dairy feed	5	Complementary feedstuffs for cattle, sheep, goats (except dairy, calves and lambs)	50
Complete feed for lambs and calves	10	Complementary feedstuffs for pigs and poultry (except for young animals)	30

Table 4. European Union regulations for aflatoxins in feeds ($\mu\text{g kg}^{-1}$).

It is estimated that about 35% of human cancers are directly related to diet, and the presence of aflatoxins in foods is considered an important factor in the formation of liver cancer, mainly in tropical countries. The reduction of population exposure to aflatoxin, and the consequent reduction of health risks will only be possible with a job with the food producers and efficient actions of sanitary vigilance (Doll & Peto, 1981).

Mycotoxin	Commodity	Maximum limit tolerated ($\mu\text{g kg}^{-1}$)
AFB1, B2, G1, G2	Cereals and cereal products, except corn and derivatives, including malted barley	5
	Beans	5
	Chestnuts except Brazil-nut, including walnuts, pistachios, hazelnuts and almonds	10
	Dried and dehydrated fruits	10
	Brazil-nut shell for direct consumption	20
	Brazil-nut shelled for direct consumption	10
	Brazil-nut shelled for further processing	15
	Cereal-based foods for infant feeding (infants and toddlers)	1
	Infant formulas and follow-up formula for infants and toddlers	1
	Cocoa beans	10
	Cocoa and chocolate	5
	Spices: <i>Capsicum</i> spp. (dried fruits, whole or ground, including peppers, chili powder, cayenne and paprika), <i>Piper</i> spp. (the fruit, including white pepper and black pepper) <i>Myristica fragrans</i> (nutmeg) <i>Zingiber officinale</i> (ginger) <i>Curcuma longa</i> (turmeric). Spice mixtures that containing one or more of the spices listed above.	20
	Groundnut (in shell), (peeled, raw or roasted), peanut butter or peanut butter.	20
Corn, grain (whole, broken, crushed, ground), flour or corn meal	20	
Aflatoxin M1	Fluid milk	0,5
	Milk powder	5
	Cheese	2,5

Table 5. Maximum permitted (LMT) for aflatoxin in Brazil.

Aflatoxins can be detoxified or removed from contaminated food and nutrients by physical, chemical or biological methods. The inactivation of these compounds by physical and chemical methods have not proved to be effective and economically viable (Mishra & Das, 2003). However, biological degradation offers an attractive alternative to eliminate these

toxins retaining food nutritional value. In the last decade it became clear that fungi are among the microorganisms that play a major role in mycotoxin degradation in particular AFB1 (Zucchi et al., 2008).

Aflatoxins are thermostable, so the physical treatment by heat results in only small changes in their levels (Tripathi & Mishra, 2010). Chemical treatments using solvents are able to extract these compounds causing minimal effect on nutritional quality, however, this technology is still impractical and expensive, besides inducing odors and flavors. Ammoniation is also used as an effective and practical application for decontamination of agricultural products containing aflatoxins (Allameh et al., 2005). Ozonation is the chemical method that has been most studied for the decontamination of aflatoxins in foods, once ozone has been recognized as safe by the Food and Drug Administration in 2001 (Zorlugenc et al., 2008).

Currently, several studies have shown that aflatoxins are susceptible to some microorganisms such as fungi, bacteria and yeasts, being for this reason studied as a form of biological degradation. Taylor et al. (2010) studied some enzymes belonging to the group of actinomycetales specifically *Mycobacterium smegmatis* which is capable of catalyzing the ester group of aflatoxins by activating the molecules for the spontaneous hydrolysis and subsequent decontamination. Niu et al. (2008) studied several microorganisms from microbial sources that have coumarin as a carbon source. The results indicated that degradation was performed enzymatically by protease. Cacciamani et al. (2007) evaluated AFB1 and ochratoxin A degradation by solid fermentation using *A. oryzae* and *Rhizopus sp.* The first showed higher AFB1 decontamination (80%). There are several alternatives for detoxification of aflatoxins in foods, such as the use of acids and bases in the industry, being replaced by processes that involve components such as ozone GRAS and the use of fungi, bacteria or yeasts.

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