

Chapter 15

Aflatoxin: Occurrence, Regulation, and Detection in Food and Feed



Abdulahadi Yakubu and Ashish Vyas

Abstract The carcinogenic nature of aflatoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* is of great threat to humans and animals as well as an economic concern especially in tropical and subtropical countries where environmental conditions favor fungal growth. These conditions raises the possibility of contamination of aflatoxin in many agricultural foodstuffs like peanut, cereals, maize grains, and animal feed. Due to their low concentration in food and feeds, detection of aflatoxins in food and animal feeds must require highly sensitive, rapid, specific, portable, and inexpensive technique or device to meet the international maximum residue level (MRL). Several analytical techniques such as chromatography, mass spectrometry, infrared spectroscopy, capillary electrophoresis, immunoassays, and biosensors were used for the detection of aflatoxins. In this chapter, some aspects of aflatoxin occurrence in food and feeds, its current regulations by national and international regulatory bodies, and trends of some rapid and highly sensitive devices for easy detection of aflatoxins in food and feeds were discussed.

Keywords Aflatoxins · *Aspergillus* · Food · Feed · Occurrence · Biosensors · Regulations · Detection

A. Yakubu

Department of Microbiology, School of Bioscience and Bioengineering, Lovely Professional University, Phagwara, Punjab, India

Department of Science Laboratory Technology, College of Science and Technology, Jigawa State Polytechnic, Dutse, Nigeria

A. Vyas (✉)

Department of Microbiology, School of Bioscience and Bioengineering, Lovely Professional University, Phagwara, Punjab, India

e-mail: ashish.vyas@lpu.co.in

© Springer Nature Singapore Pte Ltd. 2020

J. Singh et al. (eds.), *Microbial Biotechnology: Basic Research and Applications*, Environmental and Microbial Biotechnology,

https://doi.org/10.1007/978-981-15-2817-0_15

337

15.1 Introduction

Mycotoxins are fungal secondary metabolites which may exhibit their effect as teratogens, carcinogens, mutagens, and estrogens. The presence of these toxins in foods may pose a serious health hazard to consumers and may lead to economic loss in food and feed industries. These include aflatoxins, patulin, ochratoxins, fumonisin, citrinin, trichothecenes, and zearalenone. In all these mycotoxins, aflatoxins are the most toxic and highly carcinogenic and therefore have a serious impact on human health (Nfossi et al. 2008; Paniel et al. 2010). Aflatoxins are produced by fungal species of *Aspergilli*, especially *Aspergillus flavus* and *Aspergillus parasiticus* (Nfossi et al. 2008; Paniel et al. 2010; Leong et al. 2010). Their contamination in food and feed has received public attention since few decades ago. Their presence in agricultural produce has great consequences on the economy of many affected areas mainly in the developing countries where there are poor pre- and post-harvest techniques (van Egmond 1983; Applebaum et al. 1982; Kumar et al. 2017). Up to now, 18 aflatoxins (AFs) have been identified, but only AFB₁, AFB₂, AFG₁, and AFG₂ are the most common of which among them, AFB₁ is the most toxic (Hansmann et al. 2009). When a cow ingested aflatoxin B₁ (AB₁) from contaminated feed, enzymatic hydroxylation will transform it to aflatoxin M₁ (AFM₁), now classified as group 1 carcinogenic agent by the International Agency for Research on Cancer (IARC 2002; Krishnamachari et al. 1975).

Aflatoxins were first discovered in the 1960s in England when an outbreak of Turkey “X” disease killed around 100,000 turkeys and other farm animals. Heavy ingested peanut containing *Aspergillus flavus* was found to be the feed component that caused the disease (van Egmond 1983; Hansmann et al. 2009). In India, Rajasthan and Gujarat states also recorded a case of hepatitis that resulted in the death of about 106 people due to the intake of food containing aflatoxin (Bhat and Vasanthi 2003). Preliminary analysis confirmed the presence of *Aspergillus flavus* in maize which is the major food staple of these states (Kumar et al. 2017; Krishnamachari et al. 1975). Fungal growth and production of aflatoxins are generally found in tropical regions where there are high environmental conditions temperature, moisture, relative humidity, unseasonal rain during harvest as well as flood. Fungal proliferation in food is mainly due to bad harvesting practices, lack of good storage facilities, and poor conditions in transportation and marketing (Mohamadi and Alizadeh 2010; Matabaro et al. 2017).

15.2 Global Aflatoxin Occurrence in Food and Feed

As defined by CODEX Alimentarius (2011), any substances that are accidentally found in human food or feed of food-producing animals due to production, manufacturing, processing, preparation, treatment, packaging, packing, transport, or holding of such food or feed or as a result of environmental conditions are called

contaminants. Contaminations in food and feeds with aflatoxins have a great negative impact economically and have received a lot of attentions since the previous decades. Global detection of these toxins in food commodities mainly in developing countries where pre- and post-harvest equipment are not enough to curtail the growth of these fungi is of great economic concern. Aflatoxins B1, B2, G1, and G2 occur naturally in foods and contaminate a large number of foods such as rice, wheat, corn, and peanuts (Schatzmayr and Streit 2013; Han et al. 2013) (Fig. 15.1). Table 15.1 shows recent investigations with different types of method performed globally for the detection of aflatoxins in food and feeds. The most toxic among them is AFB1 which is classified as group 1 liver carcinogen by the International Agency for Research on Cancer (IARC). Direct contact and indirect contact of human to AFs occur by consuming AF-contaminated foods and products from animals initially exposed to AF-contaminated feeds, respectively. Most developed countries set up a permissible level of AFs as low as possible because of their carcinogenic nature. A maximum permissible level of 2 $\mu\text{g}/\text{kg}$ for AFB1 as well as 4 $\mu\text{g}/\text{kg}$ for total AFB1, AFB2, AFG1, and AFG2 was approved by the European Union in a variety of products.

The urgent need for control measures against toxicogenic fungi especially aflatoxins was suggested in a research conducted in Eastern Ethiopia when a high concentration of total aflatoxin level was detected in 93 out of 120 samples of groundnut analyzed using ELISA test. As per variation of total aflatoxin level, between 15 mg/kg and 11,900 mg/kg is an indication of its high occurrence in Ethiopian groundnuts which is by far beyond the limits set by the European Union (EU) (4–15 mg/kg), Food and Agriculture Organization of the United Nations (FAO) (15 $\mu\text{g}/\text{kg}$), and World Health Organization (FAO/WHO) standard (15 $\mu\text{g}/\text{kg}$) (Chala et al. 2013). From a survey of 200 feeds and 200 milk samples in China, 40% of the feed samples have AFB1 in the range of 0.05–3.53 mg/kg, while 36% were positive for AFB2 in the range of 0.03–0.84 mg/kg. Although the amount of aflatoxin B1 was slightly

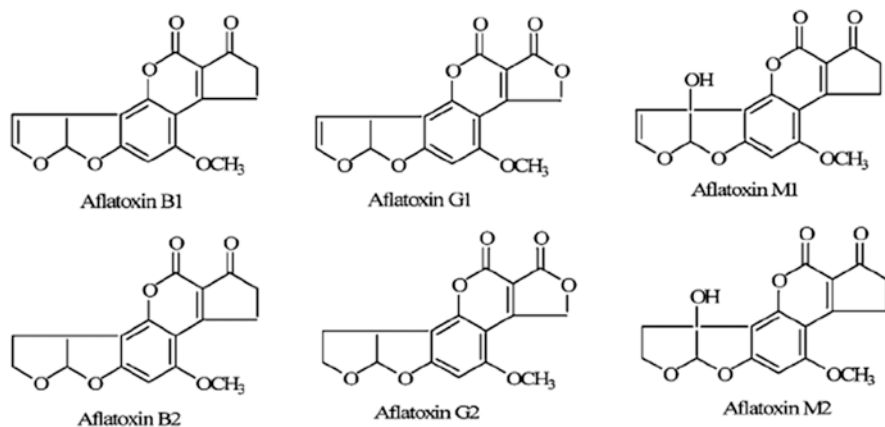


Fig. 15.1 Different types of aflatoxins (source: Zhang et al. 2014)

Table 15.1 Global occurrences of aflatoxins in food and feed in most countries

Country	Sample type	Type of aflatoxin	Number of samples (N)	Method of detection	Year	Range (ng/kg)	Mean	Reference
Portugal	Baby foods	M1	6	HPLC	2007	1.4	4.6	Alvito et al. (2010)
		B1	6				1.7	
Brazil	Skimmed yoghurt	M1	23	Fluorescence	2010	0.00–2.00		Iha et al. (2011)
Malaysia	Rice based	B1	13	ELISA	N/S	0.68–3.79	1.75	Reddy et al. (2011)
	Wheat based	B1	14				1.60	
	Peanut	B1	13				4.25	
India	UHT-treated milk	M1	45	ELISA	2010	5.00–15.00		Siddappa et al. (2016)
	Pasteurized milk	M1	7				3.00–4.00	
Serbia	Cow milk	M1	150	ELISA	2013	2.00–11.00	0.01–1.2	Kos et al. (2014)
Ethiopia	Stored groundnut	AFs	120	ELISA	N/S	15–1980	905	Chala et al. (2013)
						15–5560	1890	
China	Dairy cow feed	B1	200	ELISA	2010	3.53	0.31	Han et al. (2013)
		B2	200				0.14	
		M1	200				59.60	
		M1	21				0.00–12.00	
Zimbabwe	Peanut	B1		HPLC	2011			Mpunga et al. (2014)
		B2						
		G1						
		G2						
China	Rice	B1	370	HPLC	2009–2011	0.03–20.00	0.65	Lai et al. (2015)

higher than aflatoxin B2 in the feeds, it was still below the acceptable limits of set aside by European Union 5 µg/kg as well as 10 µg/kg for China, respectively. The total amount of aflatoxins was also below the US acceptable limit of 20 µg/kg (Han et al. 2013). In Malawi, Matumba et al. (2014) collected samples of locally processed and imported maize as well as groundnut-based food products. The extent of aflatoxin contamination was analyzed with the help of immunoaffinity-reversed-phase liquid chromatography. All imported baby cereal foods and locally processed de-hulled maize have low contents of AFs below acceptable limit, while that of locally processed maize-based foods was above the EU maximum acceptable limit of 0.1 mg/kg; monitoring of AFs in locally processed foods will likely reduce AF amount and also reduce the risk of eating AF-contaminated food and feeds.

In Zimbabwe, fungal contaminations and aflatoxin were detected using high-performance liquid chromatography-fluorescence and standard mycology culture methods, respectively. Four out of six peanuts examined for fungal contamination were infected with *Aspergillus flavus* or *Aspergillus parasiticus* ranging from 3 to 20% of the seeds studied, while 27% of the peanut butter samples were also infected with either *Aspergillus flavus* or *Aspergillus parasiticus*. The result also indicated that, 91% of peanut butter and 17% of peanut samples are contaminated with aflatoxins with mean values of 75.66 ng/kg, respectively. It was found to exceed the EU acceptable level and hence advice sensitization for manufacturers so as to reduce contamination level (Mupunga et al. 2014). A study of 45 samples of ultra-high treatment (UHT) milk from the Indian states of Karnataka and Tamil Nadu were analyzed for the detection of aflatoxin M1 by reversed-phase HPLC using fluorescent detector. All the UHT milk samples tested were positive for AFM1, and 38% of these contained AF levels higher than the acceptable limit of 0.5 µg/kg prescribed by the Codex and Indian regulatory commission (FSSAI 2011; Siddappa et al. 2016).

In Lebanon, Elkak et al. (2012) conducted a research to detect AFM1 in 111 randomly selected cheese samples from local small dairy farms and dairy industries as well as imported cheese. From the cheese samples analyzed, AFM1 was detected in 67.56% of which AFM1 levels in 17.33% of the samples exceeded the European Commission (EC) acceptable limit of 250 ng/kg. Frequent supervision of locally processed cheese in Lebanon may drastically reduce the health risk associated with AFM1. Vagef and Mahmoudi (2013) provide an update on the level of AFM1 in 144 fresh and pasteurized milk samples from western region of Iran using ELISA technique. They concluded that the amount of AFM1 in both fresh and pasteurized milk was higher than the tolerable level of 0.5 ppb where the contamination level was significantly higher in winter than in summer. In a similar research, cow milk samples were found to contain AFM1 in an amount ranging from 0.01 to 1.2 mg/kg, out of which, 86.0% of the milk samples contained high quantity of AFM1 higher than the tolerable limit of 0.05 mg/kg set by European Union (EU) regulations. Other types of milk samples indicated a percentage of AFM1 as 80.0%, 60.0%, and 60.0% in goat, donkey, and breast milk, respectively (Kos et al. 2014).

In Brazil, a research was conducted to check the incidence and occurrence of aflatoxin M1 in cheese, yoghurt, and dairy drinks. A total of 123 samples were collected and analyzed using different methods in which all the samples tested have

AFM1 higher than the acceptable level. Although there is lack of regulatory limit of aflatoxins in Brazil, this survey offered some useful information on the occurrence of AFM1 in Brazilian dairy products with potential risk to consumers as well as an insight into the need for establishment of Brazilian Maximum Residue Level (MRL) of AFs in food and feeds (Iha et al. 2011). Also in Croatia, Bilandzir et al. (2014) found a high concentration of AFM1 above tolerable limit when different animals were studied for the presence of AFM1 in their milk from July to September 2013. The result indicated that high level of this toxin in cow milk shows the use of contaminated feedstuff in some farms within the studied period.

15.3 Regulations

International and national regulatory bodies set maximum tolerable limits of aflatoxins as their carcinogenic and hazardous nature was detected in food and feed of humans and other animals, respectively. The joint FAO/WHO expert committee stated that the presence of aflatoxins in food should be limited to tolerable limit defined as the amount of a substance that cannot be removed from food or feed without discarding that particular food or compromising the exact availability of main food supplies. The first legal act on aflatoxin was established by United States Food and Drug Administration (USFDA) in 1965 when a maximum residual level (MRL) of 30 µg/kg for total aflatoxin (B1 + B2 + G1 + G2) was proposed. Since then, the maximum residual level has been regularly revised. The current regulations for aflatoxin approved by the Joint FAO/WHO Committee (CODEX), FDA, and some other countries are given in Table 15.2.

Many countries have set their maximum acceptable limit of aflatoxins in food and feeds. Industrialized nations set lower limit and regularly monitor and update their acceptable limit for import and export of food commodities likely to contain

Table 15.2 Standard ranges of aflatoxins set by international organizations

Regulatory body	Type of AF	Food/feed items	Standard range (ppb)	Year	Source
CODEX (WHO/FAO)	Aflatoxin total and AFM1	Milk, almonds, Brazil nuts, hazelnuts, peanuts, etc.	0.5–15	2015	Stan (2015)
EU	B1 (sum of B1, B2, G1, and G2) and M1	Peanuts and other oilseeds, almonds, dried fruits, corn, infant formula milk, etc.	0.5–15	2006	European (2006)
FDA	B1, total, and M1	Milk, peanut, peanut products, foods, Brazil and pistachio nuts	0.5–300	2011	USFDA (2011)
FSSAI	Total and M1	Milk, cereals and cereal products, pulses, nuts, etc.	From 0.5 in milk to 30 in spices	2011	FSSAI (2011)

Table 15.3 Maximum regulation limits for aflatoxins in food and feed in some countries

Country	Aflatoxin	MRL (ppb)	Types of food	Reference
India	B1	30	All	Anukul et al. (2013)
Switzerland	B1 B1	n/a	All Maize and cereals	Creppy (2002)
Malaysia	Total	35	All	Anukul et al. (2013)
Taiwan	Total	15	Peanut, corn	Anukul et al. (2013)
South Korea	B1	0.1	Baby food	Anukul et al. (2013)
Sweden	M1	n/a	Liquid milk products	Creppy (2002)
Czech Republic	M1 M1	n/a	Children milk Adult milk	Creppy (2002)
China	B1 M1	20 0.5	Maize, peanuts Infant food	Anukul et al. (2013)
Philippines	Total	20	Human foods	Anukul et al. (2013)
Austria	B1	n/a	All Cereals and nuts	Creppy (2002)
Japan	Total B1 B1	10 10 5	All Rice Other grains	Anukul et al. (2013)

aflatoxin than developing and underdeveloped countries. In Table 15.3, for example, countries like China and the Philippines as reported by Anukul et al. (2013) set a tolerance level of 20 ppb for total aflatoxins in all human foods, while 30 and 35 ppb are the tolerance levels of aflatoxin B for all human foods in India and Malaysia, respectively. However, this difference of acceptable limit among countries brings about difficulties in the trades of commodities from one country to another. Even though in 2011, the Serbian Government has changed and harmonized their tolerable limit for AFM1 in milk with the European Union (EU) regulation, the 2013 occurrence of AFM1 in Serbian milk led to change in regulation where MRL was changed from 0.05 to 0.5 mg/kg. Kos et al. (2014) suggest permanent and harmonious regulations of AFM1 in milk and that of other aflatoxins in animal feeds with that of the EU taking into account that milk is one of the major food staples for Serbians.

15.4 Aflatoxin Detection in Food and Feed

15.4.1 Sampling and Sample Preparation

The main problem with aflatoxin analysis is obtaining a representative sample from the said commodity. A large amount of commodity may be required to increase the chances of toxin detection since a very small quantity may differ widely within any batch of food and feed. Detailed procedures for sampling and preparation of aflatoxin analysis can be found in FAO/WHO standard regulation 20/2015. Based

on USFDA (2011) criterion, three steps which include sample size reduction, sample particle reduction, and sample homogenization for uniformity are vital in preparing any sample for aflatoxin detection.

15.4.2 Extraction of Aflatoxin from Food and Feed

An efficient extraction step is required in order to detect and quantify aflatoxins in any food samples. In polar protic solvents like methanol, acetone, chloroform, and acetonitrile, aflatoxins are normally and easily soluble. Aflatoxin extraction involves the use of organic solvents like methanol, acetonitrile, or acetone mixed in different proportion with a small quantity of water (Bertuzzi et al. 2011).

Determination of aflatoxins based on immunoassay technique requires extraction using mixture of ethanol and water. This is due to the fact that methanol has lesser negative effect on antibodies compared to acetone (Stroka et al. 1999; Lee et al. 2004). The clean-up technique which uses immune-affinity column (IAC) chromatography is usually followed after extraction (Ma et al. 2013). This immune-affinity column chromatography can bind with antigen and antibody with high reversibility and specificity which can separate as well as purify target analytes from matrices (Shelver et al. 1998). The crude sample is mainly applied to the column having a specific antibody to aflatoxins immobilized on a solid support like silica during clean-up. Aflatoxin binds onto the column and is retained with the sample moving beneath the column. The other washing step is usually required to remove impurities and unbound proteins when conducted with appropriate buffers and ionic strengths.

15.4.3 Trends in the Methods Used for Aflatoxin Detection

Over some decades, a lot of analytical methods and devices have been developed for the detection and separation of aflatoxins in food and feeds. Monitoring its presence in various commodities is important for the protection of consumer as well as producing raw materials prior to cost intensive processing or transport. Most of the devices or methods described below have advantages and disadvantages over one another in terms of their mode of operation, utilization, purchase, reliability, duration, and acceptability explained in many literatures.

15.4.3.1 Chromatographic Methods

A separation technique that involves the physical interaction between a mobile phase and a stationary phase is known as chromatographic technique. The separated components are to be distributed between mobile phase which is usually fluid

passing along stationary phase (Braithwaite and Smith 2000). In practical point of view, the analyzed sample usually dissolved in mobile phase and applied as a spot on the stationary phase. Sorbents are the partitions between solid and liquid stationary phase when samples are analyzed in mobile phase. The most common chromatographic methods for evaluations of aflatoxins are described below.

Thin-Layer Chromatography (TLC)

De Iongh first used thin-layer chromatography (TLC) in 1964, and TLC was regarded as the best method for aflatoxin detection in 1990 by the Association of Official Analytical Chemists (AOAC). This separation technique usually depends on silica, aluminum, or cellulose as stationary matrix, while the mobile phase consists of a mixture of methanol, acetonitrile, and water immobilized on plastic or glass. Aflatoxin movement within these phases is based mainly on changes of aflatoxin solubility in the two phases. This technique has an application for the measurement of aflatoxins in agricultural produce and can also detect as small as 1–20 ppb of aflatoxin. It can however need a well-trained personnel and tedious sample pretreatment, and sometimes, it is not accurate since there is probability that errors may occur at multiple points along the process. This technique has application in measurement of already known aflatoxins at high concentrations, especially if new equipment is not readily available (Wacoo et al. 2014).

High-Performance Thin-Layer Chromatography (HPTLC)

This technique is like thin-layer chromatography, but unlike TLC, all the separation processes such as plate development, application of sample, as well as interpretation of data were carried out automatically in a precise and efficient way. This method is time-consuming and laborious and requires well-trained personnel and expensive instrumentations (Badea et al. 2004). Other limitations of this method are requirement of complex gradient mobile phase, large amount of organic solvent, and regular maintenance of equipment (Wacoo et al. 2014). However, some of these limitations were overcome by the use of gas chromatography.

Gas Chromatography (GC)

Gas chromatography mainly separates aflatoxins by the movement of carrier gas acting as mobile phase through the column acting as stationary phase that has a liquid coated onto inert solid particles (Cunha and Fernandes 2010). Electron capture detector (ECD) or flame ionization detectors (FID) are usually used to detect aflatoxins while gases are separated from other samples as they move along the column. Separation of aflatoxins in gas chromatography requires molecule derivatization to a detectable volatile form since most of the toxins are not volatile.

High-Performance Liquid Chromatography (HPLC)

Just like similar chromatographic methods, this method also separates aflatoxins as mobile phase moves along stationary one. This movement mainly consists of adsorbents depending on the chemical and physical structure of aflatoxins (Gamliel et al. 2017). The sample normally in liquid form moves along the column by carrier solvents where the aflatoxins separate from the main components during extraction. The procedure applied in this separation technique is usually differentiated from other techniques based on their column types and carrier liquid since only few detectors like ultraviolet and fluorescent light are coupled to HPLC.

15.4.3.2 Spectroscopic Methods

Fluorescence Spectrophotometry

This method can quantitatively measure from 5 to 5000 ppb of aflatoxin in 5 minutes and remains to be the most pivotal technique in the analysis and characterization of molecules that emit energy at a certain wavelength in peanuts and grains for aflatoxin analysis. As per Gamliel et al. (2017), derivatization may be required for best analysis of aflatoxin using fluorometry for improved aflatoxin fluorescence. As per approval by European High commission, aflatoxin detection limit using this method is moderately high than the limit of 4 µg/kg.

Frontier Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) is the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample, and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint, no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis (Mirghani et al. 2001) reported the use of frontier infrared spectroscopy for the analysis of aflatoxins in peanuts and peanut cake which use total internal reflectance. Detection of aflatoxins was also reported by Pearson et al. (2001) using transmittance and reflectance in single corn kernels.

Infrared Spectroscopy

Infrared (IR)-based methods require little sample preparation with extensive calibration. Near-infrared (NIR) and mid-infrared (MIR) are rapid and nondestructive analytical techniques and, hence, usually used for food quality (McMullin et al.

2015). Due to their limited sensitivity, commonly used IR-based method cannot detect mycotoxin directly in a food and plant tissues. However, it can be used as a detection tool following appropriate separation procedures like HPLC. The major limitation of this method is the limited sensitivity of detection.

15.4.3.3 Immunochemical Methods

According to Wacoo et al. (2014), this method of detection depends mainly on specific binding between antigens and antibodies. Various immunochemical methods have been developed because of the high affinity and specificity of antibodies on antigens. However, this binding specificity is not limited to antibodies and antigens but also on ligands and receptors which also have such affinity and high specificity (Sargent and Sadik 1999).

Radioimmunoassay (RIA)

Rauch et al. (1987) invented RIA with its application in the qualitative determination of insulin in human blood with further extension for aflatoxin in contaminated food items. Langone and Vunakis (1976) confirmed through their studies the use of solid phase radioimmunoassay technique in determining aflatoxin B1 in peanut, and a limit of detection of 1 µg/kg was achieved. In addition, Rauch et al. (1987) reported the use of radioimmunoassays for the qualitative and quantitative determination of aflatoxin B1 and aflatoxin M1 levels. This immunochemical method depends on competitive binding among radioactive-labelled and nonradioactive antigens. Berson and Yalow (1968) reported that for a set number of antibody or antigen binding sites on the same antibody, radioactive-labelled antigen takes part with unlabelled nonradioactive antigen. A measured amount of labelled antigen and an unknown quantity of unlabelled antigen with that of standards react competitively with a known and small amount of the antibody. These labelled amounts of antigen are in inverse proportion to the amount of unlabelled antigen in the test. The advantage of this method is its capacity to run many examinations simultaneously with high reactivity and precision. One negative aspect of RIA is it requires antigen in pure form and used label isotopes associated with possible endangerment as well as problem associated with storage and disposing radioactive waste (Wacoo et al. 2014).

Enzyme-Linked Immunosorbent Assay (ELISA)

Avrameas (1969) invented the ELISA using enzyme-antigen conjugates and enzyme-antibody conjugates. ELISA method relies on the preciseness of antibodies for antigens, and the reactivity of the assay is enhanced by tagging either the antibodies or the antigens with an enzyme that can be simply evaluated by use of specific substrates. Hence, an antibody which is immobilized onto a stable support may take an unlabelled

antigen in the analyte, which is subsequently distinguished by a labelled antibody. As per Devi et al. (1999), mean immobilized antibody onto a solid support may capture an unlabelled antigen in the analyte, which later identified by a labelled antibody. ELISA method is presently used in the identification of aflatoxin in agricultural products (Anjaiah et al. 1989; Thirumala-Devi et al. 2002; Ondieki et al. 2014). Some commercially available ELISA kits that use enzymes alkaline phosphatase and horseradish peroxidase as labels in analysis of aflatoxins based on a competitive immunoassay format are extensively used (Ostadrahimi et al. 2014) and (Huybrechts 2011). The ELISA technique has advantages; that is, it is feasible to achieve the test on a 96-well assay platform resulting in the analysis of a large number of concurrent samples; the ELISA kits are cheap and easy to use, and most importantly, there is no possibility of health hazards associated with enzyme label.

15.4.3.4 Immunosensors

An international scientific endeavor, i.e., International Union of Pure and Applied Chemistry (IUPAC), defined a biosensor as any device that can provide précised quantitative and semi-quantitative interpretive data using biological understanding element in explicit connection with transducers (Shruthi et al. 2014). This device is based on interaction between biological components and transducers. Biological components such as enzymes, antibodies, and tissue slices are used to recognize and interact with a specific analyte, while transducers convert this interaction into a signal that can be amplified with respect to the concentration of the analyte (Shruthi et al. 2014). Amperometric, optical, potentiometric, magnetic, and colorimetric devices are normally used as transducers. Magliulo et al. (2005) reported a rapid and highly sensitive chemiluminescent enzyme immunoassay for the determination of AFM1 in a milk sample. Similar work using these transducers was described by Parker et al. (2009), Zangheri et al. (2014), Vdovenko et al. (2014), Mavrikou et al. (2017), and Stepurska et al. (2015). It is found to be highly sensitive, accurate, cost-effective, sensitive, and throughput in the screening of AFM1 as compared with other immunoassay. Cuccioloni et al. (2008) designed an assay for analytical test of aflatoxins B1 and G1 which is an alternate screening technique for mycotoxins. This determination approach to monitor toxins was based on surface plasmon resonance using neutrophil porcine elastase as bait. Its applications include moderate speed, recycling of the capturing surface and cost effective. Stepurska et al. (2015) has designed a potentiometric biosensor based on a pH-sensitive field-effect transistor and an enzyme acetylcholinesterase for the detection of aflatoxin B1 in real samples. It was proved to be very stable and highly sensitive when tested in the determination of AFB1 in walnut, sesame, and peanut. The application of protein for creating a highly sensitive site against AFB1 produced through bioimprinting as a means of detecting AFB1 by capacitive biosensors was reported by Gutierrez et al. (2016). This biosensor has the ability to generate specific interactions with aflatoxin B1 demonstrated in a linear relation between log concentration and signal registered of the target aflatoxin in a concentration ranges between 3.2×10^6 to 3.2×10^9 M

when using ovalbumin as framework for bioimprinting. Other biosensors developed for aflatoxin detections include an aptamer for detection of AFB1 (Castillo et al. 2015) and an electrical immunosensor for detection of ultra-trace quantity of AFM1 in food products (Paniel et al. 2010). This immunosensor has a low detection limit of 0.01 ppb which is under the recommended level of 0.05 ppb and has good reproducibility.

15.5 Conclusion and Future Challenges

The occurrence of aflatoxins in food and feeds is of global concern both in terms of health implication and economic consequences especially in developing countries where pre- and post-harvesting practices are poor. These lead to the establishment of various regulatory bodies in different countries for the sole purpose of regulating and controlling risk associated with consumption of aflatoxins in food or feeds. Different analytical methods that are usually applied for identification of toxins in food and feeds include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC), which are largely time-consuming and expensive and require trained personnel and also a series of sample preparation. Based on these mentioned problems, it is important to develop better methods based on sensitivity and cost. This leads to the development of more improved analytical techniques that are rapid, more sensitive, specific, and on-site immunoassay. RIA and ELISA suffer a setback as they require pure state of antigen, used label isotopes associated with possible health hazard, and multiple washing steps, which sometimes prove laborious and time-consuming. The development of immunosensors like biosensors has brought about an opportunity for more rapid, highly sensitive, inexpensive and rapid on-site technique for easy detection of aflatoxins in food and animal feeds. This technique is based on interaction between biological components such as enzymes, antibodies, microorganisms, and tissue slices which recognize and react with a transducer like amperometric, optical, potentiometric, and magnetic devices. The biological component reacts and recognizes a specific analyte, while transducers convert this interaction into signal. However, these biosensors have some setbacks as some transducers are expensive and lose activity after some time except when stored under a better condition, require purification and isolation cost, and have slow response to time and longer recovery time.

Future research on recent recognition elements such as bacteriophages and aptamers should be focused where more robust, rapid, highly sensitive, cost-effective, and miniaturized biosensors for on-site detection of aflatoxins can be developed. The development of biosensors based on interactions between nanomaterials and biomolecules on the surface of nanofilms may also attract attention in future researches for aflatoxin detection in food and animal feeds.

References

- Alvito PC, Sizoo EA, Almeida CMM, Van Egmond HP (2010) Occurrence of aflatoxins and ochratoxin A in baby foods in Portugal. *Food Anal Methods* 3:22–30. <https://doi.org/10.1007/s12161-008-9064-x>
- Anjaiah V, Mehan VK, Jayanthi S, Reddy DVR, McDonald D (1989) Enzyme-linked immunosorbent assay (ELISA) for aflatoxin B1 estimation in groundnuts. In ICRISAT Conference Paper no: CP 432, Patancheru, India, pp 183–189. <https://doi.org/10.1057/9781137313256>
- Anukul N, Vangnai K, Mahakarnchandkul W (2013) Significance of regulation limits in mycotoxin contamination in Asia and risk management programs at the national level. *J Food Drug Anal* 21(3):227–241. <https://doi.org/10.1016/j.jfda.2013.07.009>
- Applebaum RS, Brackett RE, Wiseman DW, Marth EH (1982) Aflatoxin: toxicity to dairy cattle and occurrence in milk and milk products - a review. *J Food Prot* 45(8):752–777. <https://doi.org/10.4315/0362-028X-45.8.752>
- Avrameas S (1969) Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry* 6(1):43–52. [https://doi.org/10.1016/0019-2791\(69\)90177-3](https://doi.org/10.1016/0019-2791(69)90177-3)
- Badea M, Micheli L, Cristina M, Candigliota T, Marconi E, Mottram T, Palleschi G (2004) Aflatoxin M1 determination in raw milk using a flow-injection immunoassay system. *Analysis* 520:141–148. <https://doi.org/10.1016/j.aca.2004.05.068>
- Berson SA, Yalow RS (1968) General principles of radioimmunoassay. *Clin Chim Acta* 369(2):125–143. <https://doi.org/10.1016/j.cca.2006.05.002>
- Bertuzzi T, Rastelli S, Mulazzi A, Donadini G, Pietri A (2011) Mycotoxin occurrence in beer produced in several European countries. *Food Control* 22(12):2059–2064. <https://doi.org/10.1016/j.foodcont.2011.06.002>
- Bhat RV, Vasanthi S (2003) Mycotoxin food safety risk in developing countries. *Food Saf Food Secur Food Trade*. <https://doi.org/10.1016/j.fm/2004.01.012>
- Bilandzir N, Bozic D, Dokic M, Sedak M, Kolanovic BS, Varenina I, Cvetnic Z (2014) Assessment of aflatoxin M1 contamination in the milk of four dairy species in Croatia. *Food Control* 43:18–21. <https://doi.org/10.1016/j.foodcont.2014.02.044>
- Braithwaite A, Smith FJ (2000) *Chromatographic methods*, vol 11, 5th edn. Springer, Cham. <https://doi.org/10.1007/978-94-011-0599-6>
- Castillo G, Spinella K, Poturnayová A, Šnejdárková M, Mosiello L, Hianik T (2015) Detection of aflatoxin B1 by aptamer-based biosensor using PAMAM dendrimers as immobilization platform. *Food Control* 52:9–18. <https://doi.org/10.1016/j.foodcont.2014.12.008>
- Chala A, Mohammed A, Ayalew A, Skinnis H (2013) Natural occurrence of aflatoxins in groundnut (*Arachis hypogaea* L.) from eastern Ethiopia. *Food Control* 30(2):602–605. <https://doi.org/10.1016/j.foodcont.2012.08.023>
- Creppy EE (2002) Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol Lett* 127:19–28
- Cuccioloni M, Mozzicafreddo M, Barocci S, Ciuti F, Pecorelli I, Eleuteri AM, Angeletti M (2008) Biosensor-based screening method for the detection of aflatoxins B1–G1. *Anal Chem* 80(23):9250–9256
- Cunha SC, Fernandes JO (2010) Development and validation of a method based on a QuEChERS procedure and heart-cutting GC-MS for determination of five mycotoxins in cereal products. *J Sep Sci* 33(4–5):600–609. <https://doi.org/10.1002/jssc.200900695>
- Devi KT, Mayo MA, Reddy KLN, Delfosse P, Reddy G, Reddy SV, Reddy DVR (1999) Production and characterization of monoclonal antibodies for aflatoxin B1. *Lett Appl Microbiol* 29(5):284–288. <https://doi.org/10.1046/j.1472-765X.1999.00685.x>
- Elkak A, El O, Habib J, Abbas M (2012) Occurrence of aflatoxin M1 in cheese processed and marketed in Lebanon. *Food Control* 25(1):140–143. <https://doi.org/10.1016/j.foodcont.2011.10.033>
- European CR (2006) European Commission Regulation (EC): setting maximum levels for certain contaminants in foodstuffs. *Eur Union Law* 364:5–24. <https://doi.org/10.2203/dose-response.06-012.Hanekamp>

- FSSAI (2011) Food safety and standards (contaminants, toxins and residues) regulations. New Delhi. Retrieved from https://fssai.gov.in/hi/dam/jcr...5b17.../Compendium_Contaminants_Regulations.pdf. Accessed 12 Mar 2018
- Gamliel A, Dehne HW, Karlovsky P, Fletcher J (2017) Detection of mycotoxins in food: applications of rapid and reliable tools in a biosecurity context. Springer, Cham. <https://doi.org/10.1007/978-3-319-46897-6>
- Gutierrez AVR, Hedstrum M, Mattiasson B (2016) Bioimprinting as a tool for the detection of aflatoxin B1 using a capacitive biosensor. *Biotechnol Rep* 11:12–17. <https://doi.org/10.1016/j.btre.2016.05.006>
- Han RW, Zheng N, Wang JQ, Zhen YP (2013) Survey of aflatoxin in dairy cow feed and raw milk in China. *Food Control* 34:35–39. <https://doi.org/10.1016/j.foodcont.2013.04.008>
- Hansmann T, Sanson B, Stojan J, Weik M, Marty JL, Fournier D (2009) Kinetic insight into the mechanism of cholinesterase inhibition by aflatoxin B1 to develop biosensors. *Biosens Bioelectron* 24(7):2119–2124. <https://doi.org/10.1016/j.bios.2008.11.006>
- Huybrechts B (2011) Evaluation of immunoassay kits for aflatoxin determination in corn & rice. Tervuren, Belgium
- IARC (2002) International Agency for Research on Cancer: monographs on the evaluation of carcinogenic risks to humans. *Int Agency Res Cancer* 96:1–390. <https://doi.org/10.1002/food.19940380335>
- Iha MH, Barbosa CB, Okada IA, Trucksess MW (2011) Occurrence of aflatoxin M1 in dairy products in Brazil. *Food Control* 22:1971–1974. <https://doi.org/10.1016/j.foodcont.2011.05.013>
- Kos J, Levi J, Đuragi O, Koki B, Miladinovi I (2014) Occurrence and estimation of aflatoxin M1 exposure in milk in Serbia. *Food Control* 38:41–46. <https://doi.org/10.1016/j.foodcont.2013.09.060>
- Krishnamachari KA, Bhat RV, Nagarajan V, Tilak TBG (1975) Hepatitis due to aflatoxicosis: an outbreak in Western India. *Lancet* 5:1061–1063. [https://doi.org/10.1016/S0140-6736\(75\)91829-2](https://doi.org/10.1016/S0140-6736(75)91829-2)
- Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG (2017) Aflatoxins: a global concern for food safety, human health and their management. *Front Microbiol* 7(1):1–10. <https://doi.org/10.3389/fmicb.2016.02170>
- Lai X, Zhang H, Liu R, Liu C (2015) Potential for aflatoxin B1 and B2 production by *Aspergillus flavus* strains isolated from rice samples. *Saudi J Biol Sci* 22(2):176–180. <https://doi.org/10.1016/j.sjbs.2014.09.013>
- Langone JJ, Vunakis H (1976) Aflatoxin B1: specific antibodies and their use in radioimmunoassay. *J Natl Cancer Inst* 56(3):591–595
- Lee NA, Wang S, Allan RD, Kennedy IR (2004) A rapid aflatoxin B1 ELISA: development and validation with reduced matrix effects for peanuts, corn, pistachio and soybeans. *J Agric Food Chem* 52:2746–2755
- Leong Y, Ismail N, Latif AA, Ahmad R (2010) Aflatoxin occurrence in nuts and commercial nutty products in Malaysia. *Food Control* 21(3):334–338. <https://doi.org/10.1016/j.foodcont.2009.06.002>
- Ma F, Chen R, Li P, Zhang Q, Zhang W, Hu X (2013) Preparation of an immunoaffinity column with amino-silica gel microparticles and its application in sample cleanup for aflatoxin detection in agri-products. *Molecules* 18(2):2222–2235. <https://doi.org/10.3390/molecules18022222>
- Magliulo M, Mirasoli M, Simoni P, Lelli R, Portanti O, Roda A (2005) Development and validation of an ultrasensitive chemiluminescent enzyme immunoassay for Aflatoxin M1 in milk. *J Agric Food Chem* 53(CI):3300–3305
- Matabaro E, Ishimwe N, Uwimbabazi E, Lee BH (2017) Current immunoassay methods for the rapid detection of aflatoxin in milk and dairy products. *Compr Rev Food Sci Food Saf* 11:1–13. <https://doi.org/10.1111/1541-4337.12287>
- Matumba L, Monjerezi M, Biswick T, Mwatseteza J, Makumba W, Kamangira D, Mtukuso A (2014) A survey of the incidence and level of aflatoxin contamination in a range of locally and imported processed foods on Malawian retail market. *Food Control* 39:87–91. <https://doi.org/10.1016/j.foodcont.2013.09.068>
- Mavrikou S, Flampouri E, Iconomou D, Kintzios S (2017) Development of a cellular biosensor for the detection of aflatoxin B1, based on the interaction of membrane engineered Vero cells

- with anti-AFB1 antibodies on the surface of gold nanoparticle screen printed electrodes. *Food Control* 73:64–70. <https://doi.org/10.1016/j.foodcont.2016.06.002>
- McMullin D, Mizaikoff B, Krska R (2015) Advancements in IR spectroscopic approaches for the determination of fungal derived contaminations in food crops. *Anal Bioanal Chem* 407(3):653–660. <https://doi.org/10.1007/s00216-014-8145-5>
- Mirghani MES, Che Ma YB, Jinap S, Baharin BS, Bakar J (2001) A new method for determining aflatoxins in groundnut and groundnut cake using Fourier transform infrared spectroscopy with attenuated total reflectance. *JAOCS* 78(10):985–992. <https://doi.org/10.1007/s11746-001-0376-y>
- Mohamadi H, Alizadeh M (2010) A study of the occurrence of aflatoxin M1 in dairy products marketed in Urmia. *Iran J Agr Sci Tech* 12:579–593
- Mupunga I, Lebelo SL, Mngqawa P, Rheeder JP, Katerere DR (2014) Natural occurrence of aflatoxins in peanuts and peanut butter from Bulawayo, Zimbabwe. *J Food Prot* 77(10):1814–1818. <https://doi.org/10.4315/0362-028X.JFP-14-129>
- Nfossi LA, Alderara MAC, Aggiani CLB, Iovannoli CRG, Rletti ENA, Iraudi GIG (2008) Development and application of solvent-free extraction for the detection of aflatoxin M1 in dairy products by enzyme immunoassay. *J Agric Food Chem* 56:1852–1857
- Ondieki D, Lutta ST, Okoth MO, Kering P (2014) Rapid assessment of aflatoxin contamination of groundnut by thin layer chromatography and competitive enzyme linked immunosorbent assay from selected divisions of Busia County in Kenya. *Afr J Food Sci Technol* 5(1):12–20. <https://doi.org/10.14303/ajfst.2014.008>
- Ostadrhiri A, Ashrafnejad F, Kazemi A, Sargheini N, Mahdavi R, Farshchian M, Mahluji S (2014) Aflatoxin in raw and salt-roasted nuts (pistachios, peanuts and walnuts) sold in markets of Tabriz, Iran. *Jundishapur J Microbiol* 7(1):1–6. <https://doi.org/10.5812/jjm.8674>
- Paniel N, Radoi A, Marty J (2010) Development of an electrochemical biosensor for the detection of aflatoxin M1 in milk. *Sensors* 10:9439–9448. <https://doi.org/10.3390/s101009439>
- Parker CO, Lanyon YH, Manning M, Arrigan DWM, Tothill IE (2009) Electrochemical immunochip sensor for aflatoxin M1 detection. *Anal Chem* 81(13):5291–5298
- Pearson TC, Wicklow DT, Maghirang EB, Xie F, Dowell FE (2001) Detecting aflatoxin in single corn kernels by transmittance and reflectance spectroscopy. *Trans ASAE* 44(5):1247–1254. <https://doi.org/10.13031/2013.6418>
- Rauch P, Fukal L, Prosek J, Brezina P, Kas J (1987) Radioimmunoassay of aflatoxin M1. *J Radioanal Nucl Chem* 117(3):163–169. <https://doi.org/10.1007/BF02165370>
- Reddy KRN, Farhana NI, Salleh B (2011) Occurrence of *Aspergillus* spp. and aflatoxin B1 in Malaysian foods used for human consumption. *J Food Sci* 76(4):T99–T104. <https://doi.org/10.1111/j.1750-3841.2011.02133.x>
- Sargent A, Sadik OA (1999) Monitoring antibody-antigen reactions at conducting polymer-based immunosensors using impedance spectroscopy. *Electrochim Acta* 44(26):4667–4675. [https://doi.org/10.1016/S0013-4686\(99\)00265-0](https://doi.org/10.1016/S0013-4686(99)00265-0)
- Schatzmayr G, Streit E (2013) Global occurrence of mycotoxins in the food and feed chain: facts and figures. *World Mycotoxin J* 6:213–222. <https://doi.org/10.3920/WMJ2013.1572>
- Shelver WL, Larsen GL, Huwe JK (1998) Use of an immunoaffinity column for tetrachlorodibenzo-p-dioxin serum sample cleanup. *J Chromatogr B Biomed Appl* 705(2):261–268. [https://doi.org/10.1016/S0378-4347\(97\)00540-9](https://doi.org/10.1016/S0378-4347(97)00540-9)
- Shruthi GS, Amitha CV, Blessy BM (2014) Biosensors a modern day achievement. *J Instrum Technol* 2(1):26–39. <https://doi.org/10.12691/jit-2-1-5>
- Siddappa V, Nanjegowda DK, Viswanath P (2016) Occurrence of aflatoxin M1 in some samples of UHT, raw & pasteurized milk from Indian states of Karnataka and Tamil Nadu. *Food Chem Toxicol* 50(11):4158–4162. <https://doi.org/10.1016/j.fct.2012.08.034>
- Stan C (2015) General standard for contaminants and toxins in food and feed (Codex Stan 193-1995). New York City. Retrieved http://www.fao.org/input/download/standards/17/CXS_193e_2015.pdf. Accessed 12 Mar 2018
- Stepurska KV, Soldatkin OO, Arkhypova VM, Soldatkin AP, Lagarde F, Jaffrezic-Renault N, Dzyadevych SV (2015) Development of novel enzyme potentiometric biosensor based

- on pH-sensitive field-effect transistors for aflatoxin B1 analysis in real samples. *Talanta* 144:1079–1084. <https://doi.org/10.1016/j.talanta.2015.07.068>
- Stroka J, Petz M, Joerissen U, Anklam E (1999) Investigation of various extractants for the analysis of aflatoxin B1 in different food and feed matrices. *Food Addit Contam* 16(8):331–338. <https://doi.org/10.1080/026520399283902>
- Thirumala-Devi K, Mayo MA, Hall AJ, Craufurd PQ, Wheeler TR, Waliyar F, Reddy D (2002) Development and application of an indirect competitive enzyme-linked immunoassay for aflatoxin M1 in milk and milk-based confectionery. *J Agric Food Chem* 50(4):933–937. <https://doi.org/10.1021/jf011139b>
- USFDA (2011) Mycotoxin regulatory guidance: a guide for grain elevators, feed manufacturers, grain processors and exporters. Washington DC. Retrieved <https://www.ngfa.org/wp-content/uploads/NGFAComplianceGuide-FDARegulatoryGuidanceforMycotoxins8-2011.pdf>. Accessed 12 Mar 2018
- Vagef R, Mahmoudi R (2013) Occurrence of aflatoxin M1 in raw and pasteurized milk produced in west region of Iran (during summer and winter). *Int Food Res J* 20(3):1421–1425
- van Egmond HP (1983) Mycotoxins in dairy products. *Food Chem* 11(4):289–307. [https://doi.org/10.1016/0308-8146\(83\)90076-6](https://doi.org/10.1016/0308-8146(83)90076-6)
- Vdovenko MM, Lu C, Yu F, Yu I (2014) Development of ultrasensitive direct chemiluminescent enzyme immunoassay for determination of aflatoxin M1 in milk. *Food Chem* 158:310–314. <https://doi.org/10.1016/j.foodchem.2014.02.128>
- Wacoo AP, Wendi D, Vuzi PC, Hawumba JF (2014) Methods for detection of aflatoxins in agricultural food crops. *J Appl Chem* 2014:1–15. <https://doi.org/10.1155/2014/706291>
- Zangheri M, Di Nardo F, Anfossi L, Giovannoli C, Baggiani C, Roda A, Mirasoli M (2014) Multiplex chemiluminescent biosensor for type B-fumonisins and aflatoxin B1 quantitative detection in maize flour. *R Soc Chem* 140:1–9. <https://doi.org/10.1039/b000000x>
- Zhang X, Kuča K, Dohnal V, Dohnalová L, Wu Q, Wu C (2014) Military potential of biological toxins. *J Appl Biomed* 12(2):63–77. <https://doi.org/10.1016/j.jab.2014.02.005>