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Recent Innovations in Detection of Aflatoxins in Dairy and other Food Products

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REVIEW BASED BOOK CHAPTER

Recent Innovations in Detection of Aflatoxins in Dairy and other Food Products

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Abstract

Aflatoxins are naturally occurring secondary metabolites produced by Aspergillus flavus and Aspergillus parasitic, among other molds. The world's food safety and trading economy, particularly in developing nations, are seriously threatened by aflatoxins, the most dangerous natural mycotoxin yet identified. It contaminates a variety of products, including cotton, groundnuts, maize, and chilies, and has a devastating impact on both human and animal health. As a result, the global economy has suffered billion-dollar losses. Aflatoxin B1, B2, G1, and G2 are the most common and deadly of the over eighteen distinct forms of aflatoxins that have been identified so far. One of the most important aspects of controlling aflatoxin contamination is early fungal infection diagnosis. Aflatoxin contamination in crops and food items is therefore determined using a variety of techniques, such as chromatographic techniques, spectroscopic techniques, and immunochemical techniques. This chapter summarizes the recent methods for the detection and analysis of different aflatoxins in dairy and other food products.

Keywords
Aflatoxins, Food Safety, Detection Methods, Analytical Techniques, Biosensors

1. Introduction

A major family of secondary metabolites is known as aflatoxins, which are all derivatives of the difuran-coumarin and are mostly generated by Aspergillus flavus and Aspergillus parasiticus. While aflatoxins are nearly insoluble in water, they may be readily dissolved in a range of organic solvents including acetonitrile and methanol. The toxicity of food that has been tainted cannot be eliminated by cooking since they have a high thermal stability [1]. The most common naturally occurring toxins identified

in food and feed crops are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG2), and G2 (AFG2), which make up all of the known kinds of AFs. Furthermore, milk and milk products include the hydroxylated forms of AFB1 and AFB2, respectively, which are formed when grazing animals eat infected crops. These compounds are known as aflatoxin M1 (AFM1) and M2 (AFM2) [2].

The most powerful and common aflatoxin is aflatoxin B1 (AFB1). The International Agency for Research on Cancer (IARC) has categorized it as a Group 1 carcinogen, suggesting that it is a substance that is known to cause cancer in humans [3]. The most potent and well-studied natural carcinogen, AFB1, is known to cause hepatocellular carcinoma, which is closely related to immunosuppression, malnutrition, and impaired growth [4]. Aflatoxin B2 (AFB2) is structurally identical to AFB1 and less frequently discovered in tainted foods. Another important aflatoxin that endangers the health of both people and animals is aflatoxin G1 (AFG1). It is created as a result of bacteria converting AFB1 via metabolism. Of the four main aflatoxins, aflatoxin G2 (AFG2) is the least prevalent. Additionally, it is produced through the metabolic conversion of AFB1 [3]. Aflatoxin M1 (AFM1) is a toxin that develops from aflatoxin B1 (AFB1) in breastfeeding animals through the hydroxylation reaction, which is performed by liver cytochrome P450. Raw milk from cows fed AFB1-contaminated feed will be AFM1-contaminated [5].

Aflatoxins have been proven to have a variety of negative consequences on health, including hepatotoxicity, mutagenesis, carcinogenesis, immunosuppression, and neurotoxicity effects on epigenetics, reproductive problems, and stunted development [6]. Vomiting, stomach discomfort, pulmonary edema, coma, fatty liver, kidney, and heart diseases are symptoms of acute aflatoxicosis in humans. AFs also have a synergistic impact with hepatitis B and C in humans by boosting a person's vulnerability ten times or more to liver cancer. According to estimates, 25% of the world's food crops are harmed by aflatoxin (AF) contamination, which has been cited as one of the biggest threats to food safety [7]. Due to the significant financial losses and negative effects on human health, food contamination with AFs is a concern on a global scale [8]. Because of this the majority of regulatory organizations for food safety implement

tight guidelines to reduce dietary exposure in people. There are several different forms of AFs, but only four naturally exist, including aflatoxin B1 (AFB1, AFB2, AFG1, and AFG2), which is known to be harmful to human health. The primary cause of AF food contamination at both the pre and post-harvest stages is fungal diseases. Climate factors that encourage fungal growth and AF formation include humidity, precipitation, and high temperatures. The presence of AFs in food is also influenced by poor storage and transportation practices. As AFs are heat-resistant so standard household cooking techniques cannot destroy those [9].

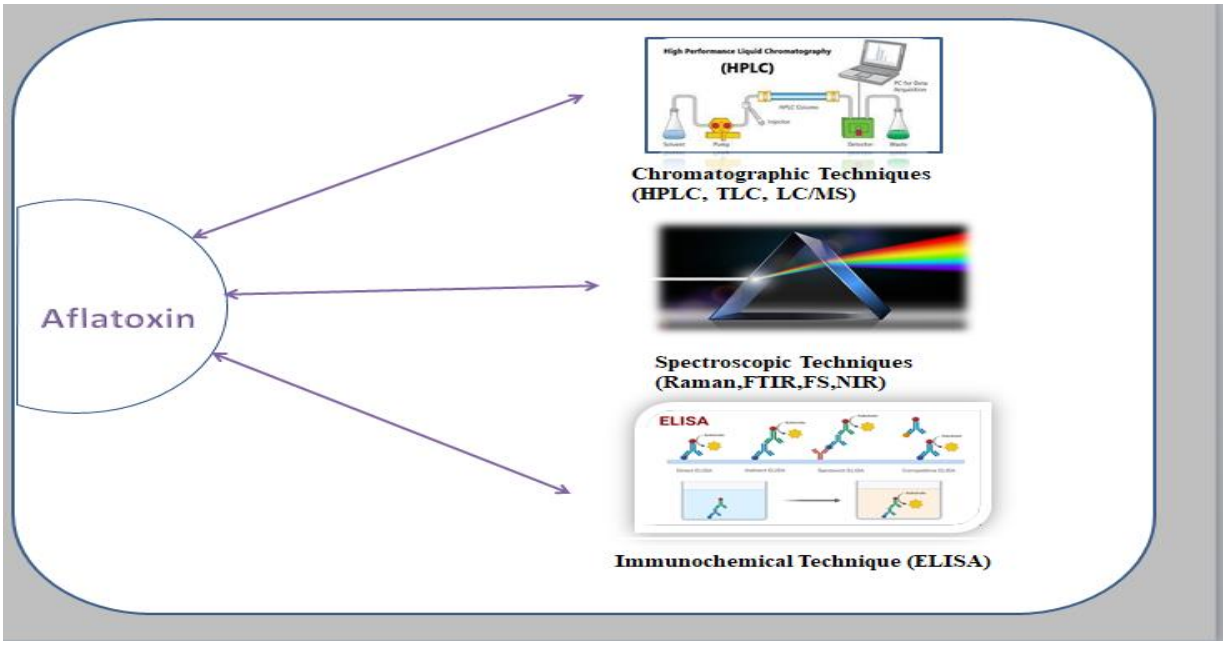

Figure 1 Detection of aflatoxin using chromatographic, spectroscopic and immunochemical techniques

2. Detection Techniques

2.1 Chromatographic Techniques

2.1.1 High-Performance Liquid Chromatography (HPLC)

A frequently used analytical technique for the separation, identification, and quantification of different chemicals, including aflatoxins, is high-performance liquid

chromatography (HPLC). The HPLC method makes use of liquid chromatography principles to separate the mixture's components [3].

Mycotoxins have been extensively analyzed over the past ten years using HPLC and other adsorbents. With either normal or reverse-phase HPLC, toxins have been separated based on their polarity [10]. Due to the composition of the milk or milk products, the procedures employed for HPLC detection of AFM1 may differ somewhat or need a different polarity of the mobile phase [11]. The majority of mycotoxins already contain natural fluorescence and may be identified using HPLC_FD, which is dependent on the existence of a chromophore in the molecules for fluorescence detection. The ability to integrate different detection methods allowing for the detection of many chemicals from a single component is the major benefit of employing HPLC, in addition to the high quality of separation and low LOD [12]. AFM1 was detected qualitatively and quantitatively in 130 samples of milk and some dairy products using the techniques of thin layer chromatography (TLC), high-performance]. liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). Additionally, the most significant amount of AFM1 in the local cheese was discovered with ELISA at 939.67ng/L and with HPLC at 300.7ng/L. Yogurt and cheese exhibited higher levels of AFM1 contamination than other items. The percentages of positive findings (contaminated with AFM1) were 38.5%, 50.5%, and 53.8%, in that order [13].

Because of its accuracy, sensitivity, and more precisely, the ability to identify aflatoxins as long as the concentration surpasses 0.015 ppb in ultra/high-performance liquid chromatography (UPLC/HPLC) has become an incredibly popular detection technology in recent years [1]. The variations in their physicochemical characteristics, such as polarity and hydrophobicity allow aflatoxins to be separated by HPLC. Usually, a reverse-phase HPLC column is employed, with the stationary phase being non-polar (hydrophobic) and the mobile phase being a polar solvent or their combination. Based on their respective polarity, this configuration enables the retention and extraction of aflatoxins [3]. A total of 40 samples were examined using the ELISA screening test and the confirmatory technique of high-performance liquid chromatography (HPLC) with fluorescence detection (FLD) following immunoaffinity column (IAC) clean-up. The

findings showed that there was no evidence of contamination in the butter samples from the Black Sea region with better qualitative characteristics in terms of AFM1, which proved as suitable for the detection of AFM1 in butter is the precise and sensitive IAC/HPLC-FLD technique [14]. 170 raw cow's milk samples from the Qazvin province's dairy farms, factories, milk collection facilities, and milk supply centers were gathered for this study during the winter months of 2013, and all samples were tested for AFM1. High-performance liquid chromatography and a commercially available competitive ELISA kit were used to evaluate the samples. All milk samples had AFM1 contamination. AFM1 contamination was found in 57 milk samples (33.52%), exceeding the threshold set by Iran's Institute of Standards and Industrial Research (0.5 ng/ml), whereas AFM1 content was within the allowable limits in 113 milk samples (66.481%) [15]. To identify AFM1 in milk and cheese, an HPLC technique with fluorescence detection was used after post-column derivatization with pyridinium hydrobromide perbromide. The average recoveries were 90% and 76%, respectively, for milk and cheese, which had detection limits of 0.001 g/kg and 0.005 g/kg, respectively. Additionally, the accuracy (RSDr) for cheese varied from 3.5% to 6.5% and for milk, it was from 1.7% to 2.6%. The experiment showed that the procedure was straightforward and amenable to automation, making it suitable for the precise and accurate study of AFM1 in milk and cheese [16]. The detection of AFM1 in milk and milk-based yogurt and cheese products was accomplished in this study using highly accurate and sensitive HPLC-FLD techniques along with IAC clean-up. In terms of parameters like specificity, sensitivity, accuracy, and precision of the analytical techniques for determining the presence of AFM1 in milk, yoghurt, and cheese, the fluorescence detection approach employing IAC clean-up produced good results. The techniques were tested for three distinct matrices at concentrations of AFM1 in milk that were permitted by European law. AFM1, which was identified in the dairy samples by HPLC-FLD at levels above LOD, was verified by LC-MS-MS [17].

2.1.2 Thin Layer Chromatography (TLC)

Aflatoxins fluoresce under UV light (365nm wavelength), which separates them on the thin plate due to their distinct adsorption capacities [1]. Thin-layer chromatography

(TLC), in line with AOAC standards, has been a recognized and frequently used technique for AF analysis since the 1990s. It is frequently utilized in laboratories throughout the globe to provide quality control of different food items and to conduct qualitative analysis [18]. The process uses a stationary phase usually made of glass or plastic or cellulose, silica, or alumina immobilized on it. While moving through the solid stationary phase, the analyte is transported by the mobile phase, which consists of water and an organic solvent combination.

Different kinds of AFs have varying affinities for the stationary phase, which causes them to either firmly attach to it or stay in the mobile phase. These affinities are caused by variances in the structure of their molecules and connections to the stationary and mobile phases. Thin-layer chromatography can effectively and efficiently separate mycotoxins due to this distinct behavior [19]. Thin-layer chromatography has been widely used to determine the amount of AF in a variety of food and feed samples, with reported detection limits as low as 1–20 ppb [20]. TLC techniques were mostly used to determine the presence of AFB1 in maize samples in 1978. TLC technique utilization has been observed to fall with time, from 53% in 1989 to 7% in 2002. TLC techniques are still advised at the moment for the identification of AF in any plant material. TLC techniques are occasionally used for mycotoxin screening in natural herbal medicines when fungal analysis has a cheap cost of detection and requires less equipment, it is researched together with concurrent mycotoxin contamination [21].

Thin layer chromatography was the first chromatographic technique, and it is still used today to quickly test for specific mycotoxins using either instrumented densitometry or eye inspection. The implementation of reliable, quick, simple, and low-cost methods that in a single run can identify and measure many mycotoxins with high selectivity and sensitivity is the focus of current trends in mycotoxin analysis in food [22]. When aflatoxins were first recognized as food contamination, thin-layer chromatography was the most widely used chromatographic method. Since then, advances in chromatographic technology have kept pace with advancements in mycotoxin analysis [23]. Aflatoxin may be found via thin-layer chromatography, which is a quick and easy procedure. In a nutshell, each test sample's aflatoxin is extracted using

organic solvents, spread, and identified at a particular wavelength via chromatography (e.g., 365 nm) following a series of steps. Aflatoxin will produce fluorescence that could be utilized to estimate the amount of aflatoxin [24].

Thin-layer chromatography is regarded as the first chromatographic method and is useful for quick mycotoxin screening. Despite being a cheap procedure, the measurements cannot be regarded as sensitive and precise. Each mycotoxin's physical and chemical makeup determines the appropriate cleanup phase and the sample preparation step is also important [25]. There are several screening techniques based on TLC that are applicable to AFM1 in milk, but only a few laboratories employ them since they don't offer a sufficient quantification limit (LC). A study involving 14 laboratories from 11 different countries was initiated in 2004 by the Food and Agriculture Organization (FAO), the International Atomic Energy Agency (IAEA), the International Union of Pure and Applied Chemistry (IUPAC), and the Committee on Food Chemistry. The goal of the study was to create a technique that might integrate the purification of immune affinity to ascertain AFM1 in milk using these three organizations' respective food chemistry committees [26]. The separation, evaluation of purity, and identification of organic compounds may all be done using thin-layer chromatography, a fairly ancient technique. In reality, it represented one of the techniques used for separation in previous AF analyses that was most often utilized. The Association of Analytical Communities (AOAC) also recognized thin-layer chromatography as an approved technique and the preferred approach for identifying and quantifying AFs at concentrations as low as 1 mg/g in 1990 [12]. A combination of methanol, acetonitrile, and water makes up the mobile phase of thin-layer chromatography that transports the sample while moving along the stationary phase. Thin-layer chromatography is a highly sensitive test that needs skilled specialists to perform, yet it is beneficial to identify many forms of mycotoxins in one test [27].

2.1.3 Liquid Chromatography/Mass Spectroscopy (LC/MS OR LC/MS/MS)

The material is extracted using the LC system; after ionization, the parent ions and fragments are separated using the MS system's mass analyzer based on the mass charge ratio [1]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has

become a popular method for determining aflatoxins in a variety of food matrices, including which includes fruits, vegetables, grains, and products made from them, as well as Chinese herbal remedies [28]. Aflatoxin detection, both quantitative and qualitative, has been the subject of a few researches on tea samples. Furthermore, one of the biggest obstacles to overcome before performing an LC-MS/MS quantitative analysis on a sample is the reduction of matrix effect. For this reason, an effective, precise, easy-to-use, and affordable pretreatment method is crucial to the success of LC-MS in determining the presence of aflatoxin in tea. Our objective was the development of a simple and efficient LC-MS/MS approach for the identification of aflatoxins in fermented tea by utilizing a special pretreatment methodology to achieve low threshold detection, high sensitivity, and the reduction of matrix effects [29].

The detection of aflatoxins (AFB1, AFB2, AFG1, and AFG2) in maize has been improved and verified using an LC-MS/MS technique. The method most frequently employed to identify mycotoxins nowadays is liquid chromatography linked to tandem mass spectrometry or LC MS/MS. Due to its great selectivity and sensitivity, it may be administered without a clean-up step or derivatization [30]. The experimental work revealed the effectiveness of the LC-MS/MS approach for the precise measurement of mycotoxin binders in the presence of aflatoxins: efficient sample preparation and administration. When developing a technique for quantifying mycotoxins in raw materials with mycotoxin binders, it is advised to employ LC-MS/MS as it enables the simultaneous determination of numerous mycotoxins [31]. The fastest-growing approach for mycotoxin analysis has been LC-MS since the 1980s. The advantages of mycotoxin analysis using LC-MS have long been acknowledged and utilized. It is possible to determine numerous mycotoxins at once using the approach by using LC isolation and the targeted analyte mass to charge (m/z). Compared to the extrinsic qualities employed by LC-UV/FLD, that include visible and ultraviolet absorption of light or fluorescence emission, this intrinsic attribute enables a more precise determination by considering the molecular weight of the target analyte [18]. The interfaces between LC and MS detectors, the restricted ionization efficiency of MS detectors, and the weak and unstable response of many mycotoxins due to inadequate desolvation were the constraints that hampered the adoption of LC-MS in the 1980s [32].

At the moment, LC-MS is the industry standard for mycotoxin analysis due to these extremely desirable properties. To determine the presence of aflatoxin B1 and M1 in milk, fresh milk, and milk powder samples, a new, completely automated approach based on dual-column switching employing online turbulent flow chromatography and LC-MS/MS was implemented [33]. AFM1 analysis in dairy products has been made possible by the growth of many LC-MS or LC-MS/MS techniques following the advent of mass spectrometry (MS) and its connection with LC [16]. To determine any possible correlations between each mycotoxin, this study examined the levels of contamination and co-occurrence of many mycotoxins using AFB1-positive samples from three different Traditional Chinese medicinal material (TCM) matrices [34]. Three distinct TCM matrices Polygalae Radix (PR), Coicis Semen (CS), and Eupolyphaga Steleophaga (ES) represent different matrices of wood fiber, starches, and proteins, respectively, and their respective mycotoxin levels were examined using the aforementioned method. The results of this study were utilized to forecast the likelihood of mycotoxin contamination and serve as a benchmark for the safe monitoring of TCMs and the establishment of guidelines and limitations [35].

Table 1 Chromatographic techniques for detecting aflatoxins in food and food products

Detection Techniques	Food Products	Aflatoxin	Reference
1. HPLC	1. Milk 2. Yogurt 3. Cheese 4. Butter	AFM1 AFM1 AFM1 AFM1	[12, 13, 14]
2. TLC	1. Maize 2. Milk	AFB1 AFM1	[21, 26]
3. LC/MS	1. Maize 2. Milk 3. Fresh milk 4. Milk powders	AFB1, AFB2, AFG1, AFG2 AFM1	[30, 33]

The detection of aflatoxins is a common usage of tandem mass spectrometry in conjunction with liquid chromatography, or LC/MS/MS, a potent analytical method. Aflatoxin analysis that is extremely selective and sensitive is achieved by combining the mass spectrometry's sensitivity and specificity with liquid chromatography's separation capabilities using the LC/MS/MS approach [3].

2.2 Spectroscopic Techniques

2.2.1 Raman Spectroscopy

Usually producing a faint signal, inelastic light scattering causes molecular vibrations that are studied using Raman spectroscopy. However, the strong electromagnetic field produced by the stimulation of the localized surface plasmon resonance (LSPR) can greatly increase the Raman signals when a sample is placed close to or on rough and noble metals nano-substrates [36].

2.2.1.1 Raman Spectroscopy in different food products

Surface-enhanced Raman scattering (SERS) has become a potent analytical method for the sensitive and quick identification of a wide range of food pollutants, including pathogenic microorganisms, allergies, pesticides, and microplastics [37]. Research work involved the quick detection of three prevalent mycotoxin types observed on maize at the same time: ochratoxin A (OTA), zearalenone (ZEN), and aflatoxin B1 (AFB1). It was achieved by developing a technique utilizing label-free surface-enhanced Raman spectroscopy. Each mycotoxin's inherent chemical fingerprint was identified by its distinct Raman spectra, which made it possible to distinguish between them with ease. For corn, the limit of detection (LOD) for AFB1, ZEN, and OTA were 10 ppb (32 nM), 20 ppb (64 nM), and 100 ppb (248 nM), in that order. The SERS spectra of known concentrations were utilized to predict AFB1, ZEN, and OTA concentrations up to 1.5 ppm (4.8 μM) using multivariate statistical analysis; the resulting correlation coefficients were 0.74, 0.89, and 0.72, respectively. Each sample was sampled in less than 30 minutes on average. A potential technique for the quick and simultaneous identification of mycotoxins in maize is the use of label-free SERS and multivariate analysis. This technique may be expanded to include additional mycotoxin kinds and

crops [36]. SERS has been used to check aflatoxins qualitatively with increased sensitivity and specificity by conjugating probe molecules with biomolecular targeting ligands (such as antibodies) and using core-shell nanoparticles. In the standard AFB1 detection procedure, AFB1 is incubated with the SERS aptasensor for a predetermined amount of time, and the resulting complex is then rinsed with buffer solution and re-dissolved in it. The next step is to use a Raman spectrometer to measure SERS by placing many drops on an aluminum plate [24]. Surface-impaired a relatively recent analytical method called Raman scattering has drawn interest as a possible mycotoxin detection substitute. Because surface plasmons are produced when incoming light interacts with a metallic surface, surface plasmon resonance spectroscopy amplifies the Raman signal of molecules adsorbed onto that surface. A broad variety of mycotoxins may be detected by SERS, which has extremely sensitive detection limits in the femtomolar to picomolar range. In addition, it takes less sample preparation time and is non-destructive. SERS has many benefits over conventional analytical techniques for the identification of mycotoxins, such as high sensitivity, low sample preparation requirements, and the ability to detect mycotoxins in situ and in real time [38].

2.2.2 Fourier Transform Infrared Spectroscopy (FTIR)

2.2.2.1 FTIR in food products

The analysis of food components and adulterants has been greatly enhanced by the development of Fourier transform infrared spectroscopy (FTIR) spectroscopy as a potent analytical instrument. Food research has made extensive use of it [39]. The detection of aflatoxin in maize and peanuts has been accomplished with success using Fourier transform infrared spectroscopy [40]. A fast way for determining and measuring aflatoxin contamination in peanuts was devised; utilizing multivariate data processing and Fourier transform infrared spectroscopy. Because this approach is thought to be nondestructive, it was suggested as an alternative to the chromatographic method because it is quicker, more feasible, and requires lesser reagent. With an attenuated total reflectance in the mid-infrared range (4,000–475 cm−1), an FTIR spectrophotometer was used to scan the sample spectra [41]. AFB1 contamination in pure milk was identified and measured using a Fourier transform infrared spectroscopy

in conjunction with multivariate analysis. The AFB1 concentrations of 10, 20, 30, 40, and 50 parts per billion were added to milk, and the FTIR spectra of the contaminated and pure milk samples showed variations in absorption, particularly in the 1800–1331 cm−1 wave number band. The milk samples infected with AFB1 were found to be separated and grouped at the 5% significance level according to principal component analysis. It was determined if it would be possible to detect AFB1 in pure milk using soft independent modeling by class analogies, and the models that were created were able to classify infected samples of milk with certainty [42].

2.2.3 Fluorescence Spectroscopy

One of the most often utilized detection methods in food physical and chemical inspection standards is fluorescence detection. Fluorescence spectroscopy (FS) is another method that may be used to identify aflatoxins like AFM1 and AFM2. The material is generally extracted, centrifuged, filtered, and then run over an immune-affinity column. There, aflatoxin binds with a particular antibody to produce an antibody-antigen complex, which is then passed into a liquid chromatography-fluorescence detection system [43]. The sensitivity can be increased by post-column derivatization with pyridine bromide or iodine; the LOD is around 0.001 µg/kg. Aflatoxin may be found in milk, almonds, animal feed, grain, and oil using this technique [24]. Various fluorescent substances generate energy at distinct wavelengths, making fluorescence spectrophotometry a suitable method for identifying AF. Fluorescence spectrophotometry can quantify AFs in the range of 5 ppb to 5000 ppb in less than 5 minutes [44]. This work examined the use of for the first time in the concurrent detection of aflatoxin B1 (AFB1) contamination in rice and Aspergillus molds using laser-induced fluorescence (LIF) technology. A variety of Aspergillus strains, both toxic and non-toxic, were intentionally injected into rice samples and allowed to develop into different mold and AFB1 infection levels [45]. LIF spectroscopy might be used to track mold activity, according to spectral analysis and principal component analysis (PCA). The kind of infected mold, the degree of mold infection, and the concentration of AFB1 were then used to categorize rice samples using three different classification techniques based on linear and non-linear algorithms. At less than 2%, the greatest accurate classification

rate of 97% was attained. Furthermore, samples of rice flour fared better under discrimination than samples of kernels did. The results of this study confirm that LIF spectroscopy may be used to quickly and non-destructively identify mold and aflatoxin contamination in grains without the need for laborious pre-processing [46].

2.2.4 Near-Infrared Spectroscopy (NIR)

A chemometric framework based on a near-infrared (NIR) spectroscopy approach is designed in this study to quantitatively evaluate aflatoxin B1 (AFB1) in peanuts. With the use of a portable NIR spectrometer, the NIR spectra of peanut samples showing various degrees of fungal infection were recorded [47]. Then, for data refining, the proper pre-processing methods were used. To simplify the study, a preliminary screening of the pre-processed NIR spectra was carried out using the iterative variable subset optimization (IVSO) approach, which removed a large number of unnecessary variables. To enhance this initial screening procedure, the chosen feature variables were further optimized using the beluga whale optimization (BWO) method. Based on the improved near-infrared spectral characteristics, support vector machine (SVM) models were then created to assess AFB1 in peanuts quantitatively. According to the findings, the SVM model considerably enhances generalization ability and detection efficiency, especially following secondary optimization using BWO-IVSO. With a correlation value of 0.9761, a relative percent deviation of 4.6999, and a root mean square error of prediction of 24.6322 μg·kg−1, the SVM model generated following BWO-IVSO optimization demonstrated the most remarkable amount of generalization among the models examined [48]. One major factor contributing to liver cancer in humans is aflatoxin B1 (AFB1). Based on Fourier transform near-infrared (FT-NIR) spectroscopy technology, this work suggests a quantitative detection strategy for the AFB1 in maize. With an FT-NIR spectrometer, we first collected spectrum data on samples of maize with different levels of mildew. Next, we optimized the characteristic wavelengths of the spectra following SNV treatment using the ant colony optimization (ACO) and NSGA-Ⅱ algorithms, respectively. To achieve precise AFB1 detection in corn, back propagation neural network (BPNN) models were ultimately developed utilizing the optimal wavelengths. The resulting data demonstrated that the BPNN model optimized by the

NSGA-Ⅱ algorithm using the four distinct wavelength variables had the best prediction performance. The correlation coefficient of prediction (RP) of the optimal NSGA-Ⅱ-BPNN model is 0.9951, and its root mean square error of prediction (RMSEP) is 1.5606 μg kg−1. Overall, the results show that it is possible to detect AFB1 quantitatively in corn using the FT-NIR technique. Moreover, the NSGA-Ⅱ algorithm offers special benefits for optimizing spectral characteristics, as it can yield characteristic wavelength variables that are both small and highly pertinence [49].

Table 2 Spectroscopic techniques for the detection of aflatoxin in food products

Spectroscopic Detection Techniques	Food Products	Aflatoxin	Chemometric Techniques	Reference
1. Raman	1. Maize	Ochratoxin A, Zearalenone (ZEN), AFB1	Principal Component Analysis (PCA)	[36]
2. Fourier Transform Infrared Spectroscopy (FTIR)	1. Maize 2. Peanut butter 3. Milk	AFB1 AFB1, AFB2 AFM1 AFB1	Principal Component Analysis (PCA)	[40, 42]
3. Florescence Spectroscopy (FS)	1. Rice	AFB1	Principal Component Analysis (PCA)	[46]
4. Near- Infrared Spectroscopy (NIR)	1. Peanuts 2. Maize 3. Corn	AFB1 AFB1 AFB1	-	[48, 49]

2.2.5 Immunochemical Method

2.2.5.1 Enzyme-linked Immunosorbent Assay (ELISA)

The foundation of ELISA is the competitive interactions that occur between mycotoxins, which function as antigens and designated antibodies that are labeled with toxin-enzyme conjugate for several binding sites [22]. The fundamental idea is that an antibody binds to an antigen. The very effective bio-catalytic characteristics of the enzyme and its incredibly precise antigen-antibody recognition serve as the foundation

for this method. After the enzyme and antibody are attached, the color-emitting substrate is transformed into a visible colorimetric output, which is then used to enhance the signal [24].

2.2.5.2 ELISA in food products

Aflatoxin M1 (AFM1), a toxic chemical found in milk, may make it unsafe for consumption. This investigation aimed to evaluate the concentrations of AFM1 in milk, specifically those that surpass the benchmarks established by the European Union (50 ng/L), the Food and Drug Administration (500 ng/L), and the Iranian National Standards Organization (100 ng/L). A total of 180 raw cow's milk samples, 45 from each season, were gathered from several retail dairy markets in Gorgan for the study. The enzyme-linked immunosorbent assay (ELISA) method was used to determine the amount of aflatoxin M1 present in the samples. 139 (72.2%) raw cow milk samples had AFM1 identified in them, ranging from 3.5 to 357 ng/L. The FDA's 500 ng/L maximum limit for aflatoxin M1 concentration was not exceeded in any of the samples that were collected. But when it came to aflatoxin M1 in raw cow's milk, 41 samples (22.7%) went above the EU's 50 ng/L standard and 26 samples (14.4%) over the INSO's 100 ng/L limit. The summer season, which comprised 32 (71.1%) and 38 (84.4%) samples, had the lowest and highest AFM1 levels of contamination, respectively [50]. One of the easiest, fastest, and most popular techniques for identifying aflatoxins is the enzyme-linked immunosorbent assay. The fundamental idea is that an antibody binds to an antigen. The effective bio-catalytic characteristics of the enzyme and its incredibly precise antigen-antibody recognition serve as the foundation for this method [51]. After the enzyme and antibody are attached, the color-emitting substrate is transformed into a visible colorimetric output, which is then used to enhance the signal. The sample was pretreated before being added to the enzyme-linked immunosorbent assay plate containing the immobilized antigen. In the dark, the sample reacted with the enzyme-substrate and antibodies working solution. By evaluating the color-developed solution and stop solution, that were added after the unreacted enzyme and antibodies were rinsed away in the eluent, the color depth of the sample may be used to estimate the quantity of aflatoxin present. Two categories of ELISA exist: direct and indirect. The

antibody is conjugated directly to the enzyme in a direct ELISA. The direct ELISA, in contrast, uses a labeled secondary antibody that is coupled with the detecting enzyme during the main antibody binding process [24]. This research set out to quantify aflatoxin M1 (AFM1) in pasteurized milk from Mashad, northeastern Iran. In order to do this, 42 milk samples were taken from retail establishments in the fall of 2011 and tested using an enzyme-linked immunosorbent assay method for AFM1. Each analysis was conducted twice. Based on an average formulation of 23 ± 16 ppt and a contaminant level from 6 to 71 ppt, the results indicated the detection of AFM1 in 97.6% of the milk samples analyzed. All samples had AFM1 concentrations below the FDA's and the Iranian national standard (500 ppt), with only 3 (1.6%) samples having AFM1 concentrations above the EU's and the Codex Alimentarius Commission's maximum tolerance limit (50 ppt). Based on prior research and our results, AFM1-contaminated milk is not a problem in this area and the regional threshold for AFM1-contaminated milk may be lowered to less than 100 ppt [52]. The most widely used approach for determining mycotoxin immunological techniques is undoubtedly the enzyme-linked immunosorbent test. ELISA offers quick screening, and a variety of commercial kits are available for the detection and measurement of important mycotoxins, such as fumonisins, AFs, AFM1, OTA, ZEA, DON, and T-2 toxins. A large range of food matrices have been used to verify ELISA procedures. The most popular method for doing ELISA is a competitive direct assay, while other methods include direct assay, competitive direct assay, and competitive indirect assay. This method for analyzing mycotoxins in food is quick, precise, and very simple to apply [22]. The reason for this investigation was the identification of aflatoxin B1 (AFB1) in 40 Iranian Tarom rice samples. ELISA, or enzyme-linked immunosorbent assay, was used to examine AFB1 in the specimens. Two analyses of each were performed. In every rice sample, aflatoxin B1 was detected; its concentration varied between 0.29 and 2.92 mg/kg. The 2013 rice samples had a mean AFB1 content that was greater than the 2012 rice samples. But out of the 40 samples, 25 went above the maximum allowed level, which is 2 mg/kg according to EU regulations, and the maximum allowable level was not attained by any of the samples, which is 5 mg/kg according to the Institute of Standards and Industrial Research of Iran (ISIRI) for aflatoxin B1 [53].

Table 3 Immunochemical technique for detecting aflatoxins in foods

Detection techniques	Food Products	Aflatoxin	Reference
1. Enzyme-Linked Immunosorbent Assay (ELISA)	1. Milk 2. Rice	AFM1 AFB1	[24, 53]

Conclusion

Although aflatoxins will always be a problem for public health across the world, every country and area has different difficulties. The problem has grown increasingly complicated as a result of international commerce, climate change, and various regulatory frameworks. To tackle the problem of mycotoxin contamination, several analytical tools are therefore required. Numerous spectroscopic, immunochemical, and chromatographic techniques have been developed to identify aflatoxins. In chromatographic techniques, the HPLC method offers great automation, high sensitivity, and high accuracy in estimating aflatoxins. Rapid, precise, and trustworthy aflatoxin findings are delivered by HPLC in a brief amount of time. However, HPLC-MS/MS is an expensive technology that should only be used by professionals who possess the necessary training and qualifications. Moreover, this limits its application to well-equipped laboratory settings only, excluding outdoor settings. The most rapidly expanding method for analyzing mycotoxins is now the LC-MS approach. Mycotoxin analysis has long recognized and benefited from the potential advantages of the LC-MS approach. A lot of work has gone into quantifying aflatoxins using this method, despite the high initial expenditures of LC-MS equipment. The ability of TLC to detect various mycotoxin kinds with superb resolution and excellent sensitivity is its main benefit. In addition, costly equipment, a qualified technician, and sample pretreatment are needed. Additionally, TLC has several disadvantages that might arise during the production, spotting, and interpretation of TLC plates. In spectroscopic techniques,

detecting molecular vibrations that lead to changes in polarizability which is water-insensitive and produces fewer overlapping bands is clearly advantageous when using Raman spectroscopy. Fast and non-destructive, Raman spectroscopy does not require sample pre-processing for detection. It has been widely employed in a variety of scientific domains and in the quantitative identification of mycotoxins. The benefits of FTIR include high resolution, high radiant flux, minimal stray radiation, and quick scanning. In immunochemical methods, ELISA has the advantage of being an inexpensive, quick approach with low sample quantities and comparatively fewer preparation steps than other methods. It also has good repeatability and repeatability, as well as high specificity and sensitivity. Though its accuracy and repeatability can be increased by having a prior separation step, the nature of the mycotoxin, the sample preparation method, and the material itself can occasionally affect the ELISA's accuracy. These techniques work well for both regulatory and research applications.

Author Contributions

All authors equally contributed for manuscript preparation and editing.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Peng, Z., Zhang, Y., Ai, Z., Pandiselvam, R., Guo, J., Kothakota, A., & Liu, Y. (2023). Current physical techniques for the degradation of aflatoxins in food and feed: Safety evaluation methods, degradation mechanisms and products. Comprehensive Reviews in Food Science and Food Safety.
2. Bhardwaj, K., Meneely, J. P., Haughey, S. A., Dean, M., Wall, P., Zhang, G., ... & Elliott, C. T. (2023). Risk assessments for the dietary intake aflatoxins in food: A systematic review (2016–2022). Food Control, 109687.
3. Koli, R., & Mannur, V. S. (2023). Determination of aflatoxins using chromatographic methods in several foods, feed and herbal medicine products: an analytical review (from 2010 to 2022). Journal of Liquid Chromatography & Related Technologies, 46(11-15), 238-254.
4. Wu, X., Meng, W., Duan, C., Cao, J., Wei, Y., Cui, X., ... & Zhang, X. (2023). AFG1- induced TNF-a-mediated inflammation enhances gastric epithelial cell injury via CYP2E1. Food and Chemical Toxicology, 176, 113756.
5. Chen, Q., Meng, M., Li, W., Xiong, Y., Fang, Y., & Lin, Q. (2023). Emerging biosensors to detect aflatoxin M1 in milk and dairy products. Food Chemistry, 398, 133848.
6. Nazhand, A., Durazzo, A., Lucarini, M., Souto, E. B., & Santini, A. (2020). Characteristics, occurrence, detection and detoxification of aflatoxins in foods and feeds. Foods, 9(5), 644.
7. Tiwari, S., Singh, B. K., & Dubey, N. K. (2023). Aflatoxins in food systems: recent advances in toxicology, biosynthesis, regulation and mitigation through green nanoformulations. Journal of the Science of Food and Agriculture, 103(4), 1621-1630.

8. Eskola, M., Kos, G., Elliott, C. T., Hajšlová, J., Mayar, S., & Krska, R. (2020). Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate'of 25%. Critical reviews in food science and nutrition, 60(16), 2773-2789.
9. Alamir, J., Almaiman, L., Alrujib, Y., Alhamidi, R., Alowais, B., Alhussain, S. & Bineid, M. (2023). Aflatoxins in food products consumed in the Kingdom of Saudi Arabia: A preliminary dietary risk assessment. Food Science & Nutrition.
10. Thangadurai, D., Divya, D., Dabire, S. S., Sangeetha, J., Dodamani, M., Islam, S., . . . Chowdhury, Z. Z. (2022). HPLC for Detection and Quantification of Food Toxins of Plant Origin Analysis of Naturally Occurring Food Toxins of Plant Origin (pp. 143-160): CRC Press.
11. Pickova, D., Ostry, V., Toman, J., & Malir, F. (2020). Presence of mycotoxins in milk thistle (Silybum marianum) food supplements: A review. Toxins, 12(12), 782.
12. Iqbal, S. Z., Jinap, S., Pirouz, A. A., & Faizal, A. A. (2015). Aflatoxin M1 in milk and dairy products, occurrence and recent challenges: A review. Trends in Food Science & Technology, 46(1), 110-119.
13. Al-Mossawei, M. T., Al-Zubaidi, L. A., Hamza, I. S., & Abduljaleel, S. Y. (2016). Detection of AFM 1 in Milk and Some Dairy Products in Iraq using different techniques. Advances in Life Science and Technology, 41, 74-81.
14. Aksoy, A., Atmaca, E., Yazici, F., GÜVENÇ, D., GÜL, O., & DERVİŞOĞLU, M. (2016). Comparative analysis of aflatoxin M1 in marketed butter by ELISA and HPLC. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 22(4).
15. Norian, R., Mahmoudi, R., Porfarzaneh, A., Mashatian, F., Kaboudari, A., Rahimi Pir Mahalleh, S. F., & Katiraee, F. (2015). Determination of aflatoxin M1 levels in raw milk samples using ELISA and high-performance liquid chromatography in Qazvin, Iran. Journal of Mycology Research, 2(1), 41-48.
16. Vaz, A., Cabral Silva, A. C., Rodrigues, P., & Venâncio, A. (2020). Detection methods for aflatoxin M1 in dairy products. Microorganisms, 8(2), 246.
17. Kim–Soo, K. R. L. M., & chung, H. (2016). Aflatoxin M1 levels in dairy products from South Korea determined by high performance liquid chromatography with fluorescence detection. Journal of Food and Nutrition Research, 55(2), 171-180.
18. Zhang, K., & Banerjee, K. (2020). A review: Sample preparation and chromatographic technologies for detection of aflatoxins in foods. Toxins, 12(9), 539.
19. Ochoa, G. S., & Synovec, R. E. (2023). Investigating analyte breakthrough under non-linear isotherm conditions during solid phase extraction facilitated by non-targeted analysis with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. Talanta, 259, 124525.
20. Okechukwu, V. O., Adelusi, O. A., Kappo, A. P., Njobeh, P. B., & Mamo, M. A. (2023). Aflatoxins: Occurrence, Biosynthesis, Mechanism of Action and Effects, Conventional/Emerging Detection Techniques. Food Chemistry, 137775.
21. Zhang, L., Dou, X. W., Zhang, C., Logrieco, A. F., & Yang, M. H. (2018). A review of current methods for analysis of mycotoxins in herbal medicines. Toxins, 10(2), 65.
22. Alshannaq, A., & Yu, J. H. (2017). Occurrence, toxicity, and analysis of major mycotoxins in food. International journal of environmental research and public health, 14(6), 632.
23. Shephard, G. S. (2016). Current status of mycotoxin analysis: a critical review. Journal of AOAC International, 99(4), 842-848.
24. Liu, S., Jiang, S., Yao, Z., & Liu, M. (2023). Aflatoxin detection technologies: recent advances and future prospects. Environmental Science and Pollution Research, 1-27.
25. Agriopoulou, S., Stamatelopoulou, E., & Varzakas, T. (2020). Advances in analysis and detection of major mycotoxins in foods. Foods, 9(4), 518.
26. Ketney, O., Santini, A., & Oancea, S. (2017). Recent aflatoxin survey data in milk and milk products: A review. International Journal of Dairy Technology, 70(3), 320-331.

27. Shabeer, S., Asad, S., Jamal, A., & Ali, A. (2022). Aflatoxin contamination, its impact and management strategies: an updated review. Toxins, 14(5), 307.
28. Jimenez Medina, M. L., Lafarga, T., Garrido Frenich, A., & Romero-González, R. (2021). Natural occurrence, legislation, and determination of aflatoxins using chromatographic methods in food: a review (from 2010 to 2019). Food Reviews International, 37(3), 244-275.
29. Zhou, H., Liu, N., Yan, Z., Yu, D., Wang, L., Wang, K., ... & Wu, A. (2021). Development and validation of the one-step purification method coupled to LC-MS/MS for simultaneous determination of four aflatoxins in fermented tea. Food Chemistry, 354, 129497.
30. Ouakhssase, A., Chahid, A., Choubbane, H., Aitmazirt, A., & Addi, E. A. (2019). Optimization and validation of a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination of aflatoxins in maize. Heliyon, 5(5).
31. Peng, H., Chang, Y., Baker, R. C., & Zhang, G. (2020). Interference of mycotoxin binders with ELISA, HPLC and LC-MS/MS analysis of aflatoxins in maize and maize gluten. Food Additives & Contaminants: Part A, 37(3), 496-506.
32. Beccaria, M., & Cabooter, D. (2020). Current developments in LC-MS for pharmaceutical analysis. Analyst, 145(4), 1129-1157.
33. Fan, S., Li, Q., Sun, L., Du, Y., Xia, J., & Zhang, Y. (2015). Simultaneous determination of aflatoxin B1 and M1 in milk, fresh milk and milk powder by LC-MS/MS utilising online turbulent flow chromatography. Food Additives & Contaminants: Part A, 32(7), 1175-1184.
34. Miklós, G., Angeli, C., Ambrus, Á., Nagy, A., Kardos, V., Zentai, A., . . . Bartók, T. (2020). Detection of aflatoxins in different matrices and food-chain positions. Frontiers in Microbiology, 11, 1916.
35. Zhao, D. T., Gao, Y. J., Zhang, W. J., Bi, T. C., Wang, X., Ma, C. X., & Rong, R. (2021). Development a multi-immunoaffinity column LC-MS-MS method for comprehensive investigation of mycotoxins contamination and co-occurrence in traditional Chinese medicinal materials. Journal of Chromatography B, 1178, 122730.
36. Gabbitas, A., Ahlborn, G., Allen, K., & Pang, S. (2023). Advancing Mycotoxin Detection: Multivariate Rapid Analysis on Corn Using Surface Enhanced Raman Spectroscopy (SERS). Toxins, 15(10), 610.
37. Huang, Y. (2022). Application of Surface-Enhanced Raman Spectroscopy in Food Safety. Highlights in Science, Engineering and Technology, 26, 407-413.
38. Adade, S. Y. S. S., Lin, H., Haruna, S. A., Johnson, N. A. N., Chen, Z., Fuyun, W., ... & Chen, Q. Simultaneous Detection of Aflatoxin B1 and Ochratoxin a in Edible Crude Palm Oil Using Modified Dispersive Solid-Phase Extraction and Sers Sensor Coupled with Pattern Recognition Algorithms. Available at SSRN 4484203.
39. Valand, R., Tanna, S., Lawson, G., & Bengtström, L. (2020). A review of Fourier Transform Infrared (FTIR) spectroscopy used in food adulteration and authenticity investigations. Food Additives & Contaminants: Part A, 37(1), 19-38.
40. Jaiswal, P., Jha, S. N., Kaur, J., Borah, A., & Ramya, H. G. (2018). Detection of aflatoxin M1 in milk using spectroscopy and multivariate analyses. Food Chemistry, 238, 209-214.
41. Sulistiawan, A., Setyaningsih, W., & Rohman, A. (2022). A new FTIR method combined with multivariate data analysis for determining aflatoxins in peanuts (Arachis hypogaea). Journal of Applied Pharmaceutical Science, 12(7), 199-206.
42. Jha, S. N., Jaiswal, P., Kaur, J., & Ramya, H. G. (2021). Rapid detection and quantification of aflatoxin B1 in milk using fourier transform infrared spectroscopy. Journal of The Institution of Engineers (India): Series A, 102, 259-265.
43. Janik, E., Niemcewicz, M., Podogrocki, M., Ceremuga, M., Gorniak, L., Stela, M., & Bijak,.
M. (2021). The existing methods and novel approaches in mycotoxins' detection. Molecules, 26(13), 3981.

44. Alameri, M. M., Kong, A. S. Y., Aljaafari, M. N., Ali, H. A., Eid, K., Sallagi, M. A., ... & Lai, K. S. (2023). Aflatoxin Contamination: An Overview on Health Issues, Detection and Management Strategies. Toxins, 15(4), 246.
45. Benkerroum, N. (2020). Aflatoxins: Producing-molds, structure, health issues and incidence in Southeast Asian and Sub-Saharan African countries. International journal of environmental research and public health, 17(4), 1215.
46. Wang, B., Shen, F., He, X., Fang, Y., Hu, Q., & Liu, X. (2023). Simultaneous detection of Aspergillus moulds and aflatoxin B1 contamination in rice by laser induced fluorescence spectroscopy. Food Control, 145, 109485.
47. Liu, J., Xie, J., Han, J., Wang, H., Sun, J., Li, R., & Li, S. (2020). Visible and near-infrared spectroscopy with chemometrics are able to predict soil physical and chemical properties. Journal of Soils and Sediments, 20, 2749-2760.
48. Li, J., Deng, J., Bai, X., Monteiro, D. D. G. N., & Jiang, H. (2023). Quantitative analysis of aflatoxin B1 of peanut by optimized support vector machine models based on near-infrared spectral features. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 303, 123208.
49. Liu, T., He, J., Yao, W., Jiang, H., & Chen, Q. (2022). Determination of aflatoxin B1 value in corn based on Fourier transform near-infrared spectroscopy: Comparison of optimization effect of characteristic wavelengths. Lwt, 164, 113657.
50. Rahimzadeh Barzoki, H., Faraji, H., Beirami, S., Keramati, F. Z., Nayik, G. A., Izadi Yazdanaabadi, Z., & Mozaffari Nejad, A. S. (2023). Seasonal study of aflatoxin M1 contamination in cow milk on the retail dairy market in Gorgan, Iran. Dairy, 4(4), 571-580.
51. Qazi, R. A., Aman, N., Ullah, N., Jamila, N., & Bibi, N. (2023). Recent advancement for enhanced e. Coli detection in electrochemical biosensors. Microchemical Journal, 109673.
52. Mohamadi Sani, A., Khezri, M., & Moradnia, H. (2012). Determination of aflatoxin in milk by ELISA technique in Mashad (Northeast of Iran). International Scholarly Research Notices, 2012.
53. Eslami, M., Mashak, Z., Heshmati, A., Shokrzadeh, M., & Mozaffari Nejad, A. S. (2015). Determination of aflatoxin B1 levels in Iranian rice by ELISA method. Toxin Reviews, 34(3), 125-128.