# PRECISION IN ANIMAL FEED SAFETY: EXPLORING MODERN TECHNIQUES FOR MYCOTOXIN ANALYSIS

## Abstract

Mycotoxins, noxious compounds synthesized by fungi, pose a significant threat to the safety and quality of food and feed products. This chapter delves into advanced analytical methods for the detection and quantification of diverse mycotoxins in feed samples. Mycotoxins, arising as by-products of fungal metabolism, can have severe health repercussions for animals and humans alike. The imperative to meet international regulatory standards has driven the evolution of rapid and sensitive mycotoxin analysis techniques to ensure consumer protection and product integrity. This chapter emphasizes the pivotal role of mycotoxin analysis across different phases of food production, emphasizing the necessity of tailored methodologies for varied sample types and objectives. Through a comprehensive examination of common mycotoxins such as aflatoxins, ochratoxin, zearalenone, deoxynivalenol, T-2 toxin, fumonisins, patulin, citrinin, and ergot alkaloids, their toxic effects on animals and potential impacts on human health are elucidated. The conclusion highlights the recent advancements in mycotoxin analysis, including the integration of omics tools to enhance our understanding of mycotoxin-producing species. The pressing need for rapid "point-on-demand" mycotoxin detection methods is underscored, while method validation's significance in ensuring reliable outcomes is emphasized. The dynamic field of mycotoxin analysis continues to evolve, prompting the exploration of innovative strategies to ensure the well-being of animals and consumers in an ever-changing landscape.

**Keywords:** Mycotoxins, Feed, Analytical methods, Quality assurance

## Authors

**Vidyarani H. B**
Division of Pathology
ICAR - Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh India.

**Bharat Shindhe**
Animal Nutrition Division
ICAR - Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh India.

**Prasad M. G**
Division of Livestock Products Technology
ICAR - Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh India.

## I. INTRODUCTION

Mycotoxins encompass a group of noxious compounds naturally synthesized by specific strains of fungi. These fungi flourish on diverse agricultural commodities like crops, nuts, and grains, both during cultivation and storage phases. Classified as secondary metabolites, mycotoxins deviate from directly contributing to the fungi's growth and advancement. Instead, they materialize as byproducts of fungal metabolic processes, often operating as defense mechanisms that empower the fungi to compete with rival microorganisms. Characteristic of mycotoxins is their relatively modest molecular mass, typically falling within the molecular weight range of 300 to 700 Daltons (Da). Despite their diminutive size, mycotoxins yield substantial detrimental consequences upon the well-being of animals and avian species that consume tainted fodder or edibles. Ingestion of substances contaminated with mycotoxins can precipitate an array of unfavorable outcomes, spanning from immediate poisoning to prolonged health complications. These include stunted growth rates, compromised reproductive capabilities, chronic health disorders, and, in extreme cases, fatality.

Owing to the conceivable hazards posed by mycotoxins to both human health and animal welfare, regulatory agencies and international entities have introduced standards and upper limits governing the presence of these toxins in victuals and animal feed. These thresholds are designed to assure the security of consumers and livestock, and they differ according to the specific type of mycotoxin and the nature of the food or feedstuff. The food and feed sectors are under duress to satisfy the benchmarks outlined by global organizations. Consequently, an imperative exists for analytical techniques that can reliably and expeditiously identify and quantify mycotoxins in assorted products. Established methodologies for mycotoxin assessment tend to be protracted and necessitate intricate sample preparation. To address these impediments, more sophisticated and efficient methodologies have emerged.

## II. CATALOG OF COMMON MYCOTOXINS AND THEIR IMPACT ON ANIMALS

1. **Aflatoxins: Detrimental Byproducts of *Aspergillus* Species:** Derived from *Aspergillus flavus* and *Aspergillus parasiticus*, aflatoxins emerge as potent carcinogens with high toxicity. Animals ingesting aflatoxins can suffer from liver impairment, suppressed immune response, hindered growth rates, reproductive disorders, and even mortality. Extended exposure can lead to the emergence of liver tumors.

2. **Ochratoxin: Nephrotoxic Effect from *Aspergillus and Penicillium* Strains:** Ascribed to *Aspergillus ochraceus* and *Penicillium verrucosum*, ochratoxin inflicts kidney damage and dysfunction on animals. It has been linked to kidney ailments and compromised immune function. Pigs, in particular, exhibit heightened sensitivity to ochratoxin.

3. **Zearalenone: Disruptive Impact of *Fusarium* Mycotoxins:** Originating from *Fusarium* species, zearalenone possesses estrogenic properties that disrupt animal reproductive systems. Infertility, hyperestrogenism, and other reproductive anomalies, especially in pigs and monogastric animals, result from exposure.

4. **Deoxynivalenol (DON): Vomitoxin's Effects by Fusarium StrainsL:** Produced by *Fusarium* species, DON, colloquially known as vomitoxin, induces feed refusal, vomiting, and compromised weight gain among animals. Gastrointestinal troubles and immune suppression can ensue.

5. **T-2 Toxin: Potent Peril from Fusarium Species:** Synthesized chiefly by *Fusarium sporotrichioides* and *Fusarium poae*, T-2 toxin poses formidable health threats to animals. Its consequences span feed rejection, hindered growth, immune suppression, and organ impairment.

6. **Fumonisins: Fusarium's Impact on Equine and Porcine Health:** Arising from Fusarium species, particularly *Fusarium verticillioides*, fumonisins induce equine leukoencephalomalacia (a neurological disorder in horses) and porcine pulmonary edema syndrome (lung swelling). Other animals also suffer reduced growth, immune suppression, and liver damage.

7. **Patulin: Gastric and Immune Disruption from *Penicillium and Aspergillus*:** Produced by *Penicillium* and *Aspergillus* species, patulin wreaks havoc on the gastrointestinal tract and immune system of animals. It also induces oxidative stress and DNA damage.

8. **Citrinin: Renal Impairment and Immune Consequences from Fungi:** Produced by *Penicillium*, *Aspergillus*, and *Monascus* species, citrinin leads to kidney impairment, compromises immune function, and triggers oxidative stress.

9. **Ergot Alkaloids: Vascular and Reproductive Disturbances from *Claviceps*:** Emanating from *Claviceps* species, ergot alkaloids provoke vasoconstriction and circulatory complications in animals. Symptoms encompass gangrene, convulsions, and fertility decline. It is imperative to recognize that the severity of these toxic impacts fluctuates based on factors such as mycotoxin type, concentration, animal species, age, exposure duration, and overall health status. Vigilant monitoring and management of mycotoxin contamination in feed and food items are critical to prevent adverse effects on animal health and productivity.

## III. EXTRACTION AND PRE CLEANING PROCEDURES

The typical procedures involved in extracting mycotoxins from feed (Figure 1) are as follows:
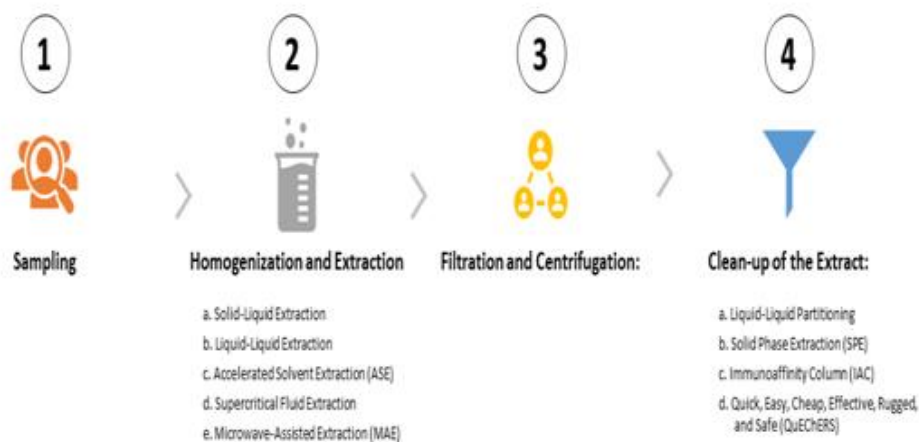
**Figure 1:** Schematic Representation of Standard Processes for Mycotoxin Extraction from Feed Samples

1. **Sampling:** This initial step holds pivotal importance in mycotoxin analysis. Due to the uneven distribution of fungal growth and mycotoxins in feed, obtaining a representative sample can be a challenge. Multiple sampling plans, as described by regulatory bodies like the FSSAI and EU (2020) under Commission Regulation (EC) No. 401/2006, have been formulated. Implementing a suitable sampling plan is crucial to obtain a representative sample and avoid false-negative outcomes (Alshannaq and Yu, 2017).

2. **Homogenization and Extraction:** Sample preparation comprises two primary steps, including, extraction and clean-up. Extraction typically employs organic solvents such as methanol, acetonitrile, or acetone, depending on the target analyte. Many mycotoxins require non-polar organic solvents, while polar ones like fumonisins and deoxynivalenol (DON) can be extracted with water. Mixtures of organic solvents with water or acidic buffers are often used to enhance mycotoxin extraction. For samples rich in lipids, non-polar solvents like hexane and cyclohexane are preferred (Alshannaq and Yu, 2017). Various extraction methods exist, including solid-liquid extraction and liquid-liquid extraction. Advanced automated methods like Supercritical Fluid Extraction (SFE), Accelerated Solvent Extraction (ASE), and Microwave-Assisted Extraction (MAE) are also employed (Alshannaq and Yu, 2017).

   - **Solid-Liquid Extraction:** Primarily used for solid materials such as grains and cereal foodstuffs. Involves disposable cartridges filled with materials like silica gel or bonded phases. High binding capacity for small molecules.

   - **Liquid-Liquid Extraction:** Employed for liquid samples. Capitalizes on the differential solubility of toxins in two immiscible solvent phases.

   - **Accelerated Solvent Extraction (ASE):** Utilizes conventional solvents at elevated temperatures (100-180°C) and pressures (1500-2000 psi) to enhance analyte extraction from the matrix.

- **Supercritical Fluid Extraction:** Utilizes unique properties of supercritical fluids, often carbon dioxide under critical conditions, to assist analyte extraction.

- **Microwave-Assisted Extraction (MAE):** Uses electromagnetic energy to convert into thermal energy for extraction within a vessel where samples and extractant interact.

3. **Filtration and Centrifugation:** Following mycotoxin extraction, filtration and centrifugation are essential to remove interfering particles before proceeding with further clean-up steps (Alshannaq and Yu, 2017).

4. **Cleanup of the Extract:** This step aims to eliminate substances that could interfere with the subsequent mycotoxin detection. Multiple cleanup techniques are available.

- **Liquid-Liquid Partitioning:** Separates compounds based on their solubility in two immiscible liquids (organic solvent and water).

- **Solid Phase Extraction (SPE):** Analytes bind to the column's surface due to interactions with sorbent functional groups.

- **Immunoaffinity Column (IAC):** Utilizes antigen-antibody interactions. Offers advantages like minimal mycotoxin loss, complete removal of interfering substances, and enhanced accuracy compared to SPE extraction (Turner *et al.*, 2015).

- **Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS):** This technique combines extraction and clean-up for mycotoxins from diverse food matrices. A buffered MeCN extraction is conducted, with anhydrous magnesium sulfate (MgSO4) used to induce liquid-liquid partitioning by removing water. The cleanup phase employs dispersive solid-phase extraction (dispersive-SPE) with primary secondary amine (PSA) and C18 sorbents to eliminate fatty acids and other components (Alshannaq and Yu, 2017).

## IV. ANALYTICAL METHODS

1. **Chromatographic Methods:** Chromatographic separation combined with a compatible detection system stands as the extensively employed approach for mycotoxin analysis. It not only validates positive results but also serves as a benchmark method for confirming other tests. Among the available techniques, liquid chromatography (LC) stands out as the prevalent choice, although gas chromatography (GC) and thin-layer chromatography (TLC) continue to be recognized (Singh and Mehta, 2020).

- **Thin Layer Chromatography:** This technique remains in prevalent use within developing and underdeveloped nations. Thin-layer chromatography involves employing a sheet composed of an unreactive substrate, such as glass, plastic, or aluminum foil. This sheet is coated with a slim layer of adsorbent material, which can include substances like silica gel, alumina (aluminum oxide), or cellulose. This adsorbent layer is referred to as the stationary phase. Once the sample has been placed

onto the plate, a solvent or combination of solvents (known as the mobile phase) moves up the plate through capillary action. Some of the advantages of this method includes rapid analysis of numerous samples within a short span of time is achievable. The cost per sample analyzed is kept low. The process allows for straightforward and uncomplicated estimation. Drawbacks of the method include relatively low sensitivity and reproducibility of the method. A considerable amount of solvent is required for the procedure. The process encounters challenges when it comes to automation.

- **Gas Chromatography:** Primarily employed for the separation of volatile substances, this method necessitates a derivatization process (specifically silylation and acylation) to transform mycotoxins into derivatives that are more volatile, less polar, and thermally stable. When coupled with detectors such as a Mass Spectrometry (MS) detector, known as GC-MS (Gas Chromatography-Mass Spectrometry), this technique enables both the identification and quantification of compounds to take place simultaneously. This approach offers notable advantages, including, a remarkable degree of specificity and sensitivity for all types of mycotoxins. The capability to conduct a simultaneous analysis of multiple mycotoxins. There are several considerations to be aware of in this method, where skilled technicians are essential to perform the analysis effectively. The potential for properties from previous samples to linger can pose challenges. Additional techniques such as derivatization may be necessary. The method can be expensive to implement. There's an increased risk of column blockage and contamination in comparison to HPLC and LC methods (Singh and Mehta, 2020).

- **Liquid Chromatography:** This technique serves as the primary method of separation in mycotoxin analysis. High-performance liquid chromatography (HPLC) stands as the widely adopted technique for mycotoxin identification and quantification. It frequently employs reverse-phase columns, most commonly C18 columns, coupled with mobile phases containing well-balanced mixtures of water and either MeOH or MeCN. Spectrometric detectors such as UV (HPLC-UV) or fluorescence are employed based on the specific analyte. Advantages of this method are mainly sensitivity and versatility in its application. However, it is not portable and involves a significant cost. Additionally, skilled technicians are required to perform the analysis, and the procedure is time-consuming.

- **Ultrafast Liquid Chromatography:** This method employs narrow-bore columns that contain sub-2-μm particles, along with mobile-phase delivery systems that function under significantly elevated backpressures, surpassing those encountered in HPLC. Benefits of this technique encompass amplified peak resolving capabilities, the generation of narrower peaks, expedited analyses, and heightened sensitivity. However, there are downsides associated with this approach. The utilization of smaller particle sizes leads to the development of elevated pressure. This increase in pressure can result in a reduced lifespan for the columns (Izzo *et al.*, 2020).

2. **Immunological Techniques:** These approaches rely on the interaction between specific antibodies and mycotoxins functioning as antigens, aided by markers (such as radioactive, chromogenic, fluorescent, or enzymatic).These techniques exhibit simplicity, cost-

effectiveness, and sensitivity. Moreover, they do not demand advanced equipment or highly skilled personnel. However, they are susceptible to interference from co-extractives and can be affected by non-specific interactions or matrix effects (Singh and Mehta, 2020).

- **ELISA (Enzyme Linked Immuno-Sorbent Assay):** This method is widely employed in immunoassays. The ELISA assay allows for direct analysis of the sample, negating the need for a cleanup procedure. The competitive ELISA format is predominantly favored. The benefits encompass specificity, reduced requirement for cleanup steps, cost-effective measurements, and straightforward application. Nevertheless, this method tends to lack precision at lower concentrations. It can be influenced by structurally similar mycotoxins or interference from matrices. The kits are designed for single use, and the potential for false positive and false negative outcomes necessitates supplementary confirmatory analyses.

- **Radioimmunoassay (RIA):** Among immunological approaches, radioimmunoassay was the initial technique to be developed. It revolves around competitive binding interactions between a radioactive-labeled antigen and an unlabeled nonradioactive antigen. In this process, the radioactive-labeled antigen competes with the nonradioactive one for a predetermined quantity of antibodies. This method offers the capability to conduct multiple analyses simultaneously. It also provides elevated levels of sensitivity and specificity. Some disadvantages of this method are label composed of a radioactive isotope is employed, introducing potential health hazards. Furthermore, issues arise concerning the storage and disposal of the generated low-level radioactive waste (Singh and Mehta, 2020).

- **Lateral Flow Immunoassay (Immunodipsticks):** Commercially accessible kits offer membrane-based test strips. These kits provide results in a visual and qualitative manner, indicating whether a specific mycotoxin is present or absent below a predefined threshold. The sample travels along the strip through capillary migration, giving rise to two lines. One is the test line, which correlates with the mycotoxin concentration, while the other is the control line, serving to validate the assay. Advantages of this approach include its ability to facilitate swift and on-site mycotoxin analysis. Moreover, it streamlines the process by enabling a single-step analysis. However, this method comes with certain drawbacks. Notably, there is a lack of reproducibility in the results, and it can be plagued by sensitivity issues (Koczula and Gallotta et al., 2016; Liao and Li, 2010).

- **Fluorescence Polarization Immunoassay (FPI):** This method indirectly gauges the rate of rotation of a fluorescent marker in a solution. It operates by pitting the free mycotoxin from the sample against the mycotoxin marked with the tracer, all in competition for a particular antibody. When the sample contains a higher quantity of mycotoxin, more tracer-marked mycotoxins remain unbound, leading to a reduction in the value of the fluorescence signal. The fluorescence polarization signal value demonstrates an inverse relationship with the quantity of mycotoxin present in the sample. This method presents several advantages. It is known for its reliability, speed, and ease of execution. Furthermore, it is reasonably compatible with automation

processes. However, there are certain drawbacks associated with this method. Notably, it can be costly to implement (Maragos, 2009; Singh and Mehta, 2020).

3. **Biosensors and Biosensor-based Methods:** Biosensors are analytical tools that consist of an antibody, acting as a recognition element for a specific target mycotoxin. These devices also incorporate a transducing component that converts the alteration in a physical property, resulting from the reaction, into a quantifiable signal. Biosensors are categorized according to the method of signal transduction they employ. They are grouped into three main types: piezoelectric, optical, and electrochemical sensors.

- **Piezoelectric Quartz Crystal Microbalances (QCMs):** This method entails the direct detection of antigens. The piezoelectric quartz crystal operates based on variations in mass occurring on the electrode surface upon interaction between an antigen and an antibody immobilized on the quartz crystal's surface. The alteration in mass is directly proportionate to the concentration of the formed antigen-antibody complex.

- **Optical Immunosensors:** The Surface Plasmon Resonance (SPR) platform operates by detecting alterations in the refractive index that arise from the interaction between an analyte and its bio-specific counterpart, which is immobilized on the sensor surface.

- **Electrochemical Immunosensors:** An electrochemical immunosensor is a device that uses antibodies incorporated into a biorecognition layer to produce electroactive signals detectable by transducers (amplifiers), which generate measurable signals. Biosensors offer several benefits like they deliver results swiftly, are cost-effective, exhibit heightened sensitivity, and possess portability. However, biosensors also come with certain drawbacks. They rely on specialized analytical equipment and can exhibit limitations in terms of selectivity and reproducibility (Li et al., 2021).

4. **Spectroscopic Methods**

- **Infrared Spectroscopy/ Fourier Transform Near Infrared spectrometry:** Near-infrared (NIR) spectroscopy is employed either independently or in conjunction with Fourier-transform (FT-NIR). The analysis of mycotoxins relies on assessing the absorption or reflectance of incident NIR radiation within the sample (Agriopoulou *et al.*, 2019). Advantages of this approach include its high level of accuracy and the fact that it requires minimal expert training. However, there are disadvantages associated with it including, spectra overlapping and potential interference from other functional chemical groups can hinder the interpretation process. Additionally, this method is most effective when dealing with high contamination levels of the analyte.

- **Fluorescence Spectrophotometry:** In certain molecules, the absorption process is succeeded by the emission of light at a distinct wavelength, rendering these molecules fluorescent. Fluorescence plays a crucial role in the characterization and analysis of molecules emitting energy at specific wavelengths. This phenomenon is utilized for

the analysis of aflatoxins in grains and raw peanuts, as they exhibit fluorescence properties (Smeesters *et al.*, 2015).

- **Raman Spectroscopy:** The underlying principle in this process entails exposing a substance to monochromatic light, leading to the identification of energy loss in the form of scattered light. Benefits of this approach encompass furnishing valuable qualitative and quantitative insights into the chemical functional groups of mycotoxin compounds during analysis. The method itself is characterized by speed, sensitivity, and simplicity. Nonetheless, there is a limitation in terms of weak Raman scattering, which poses challenges in measurement. To address this issue, Surface-Enhanced Raman Scattering (SERS) has been developed as a solution (Wu *et al.*, 2021).

- **Ion-Mobility Spectrometry (IMS):** This technique involves the electrophoretic separation of ionized compounds within a neutral gas phase, typically carried out at atmospheric or nearly atmospheric pressure. Benefits of this method include its straightforward nature, rapid response, sensitivity, and cost-effectiveness. However, there are drawbacks associated with this technique. It offers limited resolving power and selectivity (Singh and Mehta, 2019).

- **Mass Spectrometry/Tandem Mass Spectrometry:** In the realm of mass spectrometry, the procedure entails transforming the molecules of the analyte into a charged (ionized) state during the ionization process. Following this, the analysis revolves around the ions and any fragment ions, based on their mass-to-charge ratio (m/z). Tandem mass spectrometry, often referred to as MS/MS or MS2, is an approach that involves coupling two or more mass analyzers together. This coupling is achieved by introducing an additional reaction step, thereby enhancing the capacity of these analyzers to scrutinize chemical samples (Arroyo-Manzanares *et al.*, 2021).

5. **Nanoparticles based Detection Methods:** Nanomaterials are materials characterized by their small size, typically below 100 nanometers in length. They are synthesized using both inorganic and organic substances. These materials have found successful application in advancing sample pre-treatment techniques and in devising methods for precise mycotoxin detection. This integration notably enhances detection capabilities in terms of sensitivity, detection speed, and accuracy.

- **Nanomaterials-based Immunoassays:** Incorporating nanomaterials into immunoassays offers a range of distinctive attributes. These include heightened surface reactivity, diverse sizes, robust electrical conductivity, intense fluorescence, capability for immobilizing biomolecules, modulation of electron transfer, signal generation, and amplification. Notable examples encompass metal nanomaterials, carbon nanomaterials, covalent organic frameworks (COFs), up conversion nanoparticles (UCNPs), magnetic particles, silica nanoparticles, and quantum dots (Zhang et al., 2020).

- **Nanomaterials-based Aptasensors:** Aptamers, which consist of peptide or nucleic acid molecules, represent a category of molecular recognition components. Their distinct recognition specificity for various analytes has led to their extensive use as

recognition elements in the creation of aptasensors. In order to bolster the capabilities of aptasensors, such as selectivity and sensitivity, an array of nanomaterials has been incorporated. These materials serve purposes such as immobilization support, signal generation, signal amplification, fluorescence quenching, and as alternatives to enzyme labels. However, it's important to note that this method does have some drawbacks. It can be time-consuming and may result in nonspecific interactions.

- **Nanomaterials in MIPs-based Mycotoxin Sensors:** Molecularly imprinted polymers (MIPs) represent a synthetic technique intended to replicate natural recognition agents, such as antibodies and biological receptors, by attaining specificities akin to those observed in antibody-antigen interactions. These polymers are widely embraced as recognition components in biosensors. The advantages of MIPs encompass their economical nature, pronounced specificity, straightforward preparation, robust stability, swift binding kinetics, and high affinity. They are emerging as viable alternatives to antibodies in sensor development, with significant strides achieved in their application for mycotoxin detection. To further enhance the capabilities of MIP-based mycotoxin sensors, the signal generation and amplification properties of nanoparticles (NPs) have been effectively integrated (Zhang *et al*., 2020).

6. **Microfluidic "Lab-on-A-Chip":** The utilization of microfluidic "lab-on-a-chip" platforms for mycotoxin detection in food has gained momentum. The conception of a microfluidic analytical platform originates from the concept of a Total Analysis System (TAS). The overarching goal is to condense and amalgamate all essential stages of chemical analysis onto a singular device, with channel dimensions ranging from 100 nanometers to 500 micrometers. Within these microfluidic devices, immunological-based separation elements primarily revolve around the use of antibodies. A critical aspect is the requirement for a miniaturized and highly sensitive detector. This approach offers various advantages, including diminished consumption of samples and reagents, cost-effective fabrication, versatile design with multifunctionality, rapid analysis and response times, capacity for high-throughput screening, and portability, allowing for convenient in-field detection. However, a significant challenge of this technology arises from the complexity of food matrices. Microfluidic systems can be susceptible to blockage from minuscule particles or vapor bubbles, which is a notable concern (Guo *et al*., 2015).

7. **Genomic Methods:** Genomic techniques encompass the initial extraction of mold DNA or RNA from food samples, succeeded by detection utilizing methods such as polymerase chain reaction (PCR), quantitative PCR (qPCR), loop-mediated isothermal amplification (LAMP), and reverse transcription PCR (RT-PCR) (Agriopoulou et al., 2020).

8. **Transcriptomics Approach:** This area of study encompasses the comprehensive analysis of the entire set of RNA transcripts produced by genes in a specific tissue or cell type under particular conditions. In the realm of mycotoxin research, methods like RT-qPCR and high-throughput microarray analysis have found widespread and extensive use (Lancova et al., 2011).

9. **Proteomic Methods:** This process entails the initial extraction of mold peptides/proteins from food samples, followed by subsequent analysis using matrix-assisted laser desorption or ionization time-of-flight mass spectrometry (MALDI-TOF MS). The technique of MALDI-TOF MS rapidly identifies fungal isolates and mycotoxins with remarkable accuracy, serving as an alternative to chromatographic methods. However, it is worth noting that this approach can be costly, and there exists a requirement to establish a comprehensive public database for optimal utilization (Eshelli *et al*., 2020).

10. **Electronic Nose:** The physicochemical attributes of secondary fungal metabolites can be evaluated using an electronic nose. This device operates in a manner akin to a gas chromatography (GC) system, detecting volatile compounds emitted by contaminated food through a solid-state sensor. By detecting the distinct aroma, preliminary insights into the category of produced metabolites can be gained (Agriopoulou *et al*., 2020).

11. **Aggregation-Induced Emission Dye:** Aggregation-induced emission (AIE) exploits the phenomenon where the fluorescence of a group of fluorescent dyes is significantly enhanced when they are in an aggregated state. This intensified fluorescence is often a result of restricted intramolecular rotations within the aggregates. Examples of fluorescent dyes utilized in their aggregated form include 9,10-distyrylanthracene (DSA), tetraphenylethene (TPE), and silacyclopentadiene (silole). An apta-sensor for the detection of ochratoxin has been developed based on AIE dyes (Agriopoulou *et al*., 2020).

12. **Quantitative NMR:** This serves as the fundamental approach for organic compound identification, utilizing nuclear magnetic resonance (NMR) for quantitative analysis. An advantage of this technique is the ability for quantification without the need for standards, along with dependable identification of isolated metabolites. However, it's important to note that drawbacks include low sensitivity, a scarcity of available commercial software, and a restricted range of quantification methods (Agriopoulou *et al*., 2020).

13. **Hyperspectral Imaging:** The spectral reflectance of every individual pixel is captured across a spectrum of wavelengths in the electromagnetic range. A wider array of wavelengths is scanned for each pixel. The outcome is an image composed of pixel values, reflecting the intensity of reflectance at each wavelength of the spectrum. Benefits of this approach encompass the amalgamation of both spectral and spatial attributes (imaging component) of a specific sample. Moreover, it's a swift and non-destructive process. Nevertheless, challenges arise in the need for a cost-effective design and the development of efficient multivariate algorithms that can enhance the performance of the algorithm (Xing *et al*., 2017).

## V. QUALITY ASSURANCE

Method validation is an essential prerequisite to ensure the production of reliable results that are characterized by comparability and traceability. This validation process involves assessing various performance characteristics, including but not limited to the limit of detection and quantification (LOD/LOQ), linearity, precision (both repeatability and reproducibility), selectivity (considering interference from other compounds and/or matrix constituents), robustness or ruggedness, working range, and trueness or bias.

Numerous protocols and guidelines for conducting method validation have been documented. For example, the ISO standard 5725 provides valuable guidance in this regard (ISO, 2019). Diverse methods for mycotoxin analysis have been validated and are officially recognized by authoritative bodies such as CEN, AOAC, and ISO (Gilbert, 2002).

## VI. CONCLUSION AND FUTURE PERSPECTIVES

Mycotoxins, potent toxins originating from fungi, pose a pervasive threat to food and feed supplies. The imperative to align with international regulatory benchmarks has fueled the innovation of quicker and more sensitive mycotoxin analysis methods, ensuring both consumer safety and the integrity of food and feed commodities. Given their involvement in the contamination of consumables, the detection of both targeted and untargeted mycotoxins has acquired paramount significance pre-harvest, during harvesting, and post-harvest. However, advocating for a universally applicable method across all sample types proves impractical. Instead, the choice of methodology must be tailored to factors such as the distinct sample type, the intended purpose, and the resources at hand within the laboratory.

Recent years have witnessed substantial strides in mycotoxin analysis, with omics tools significantly augmenting our understanding of mycotoxin-associated challenges, especially in the identification of mycotoxin-producing species. The demand for expedited mycotoxin identification in a "point-on-demand" manner has become unmistakable. Furthermore, it's crucial to underscore the indispensability of method validation, a prerequisite to yield reliable outcomes, ensuring consistency and traceability in results. As we navigate the dynamic landscape of mycotoxin analysis, the continual pursuit of novel methodologies and strategies remains pivotal to safeguarding both human and animal well-being.

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