

Chapter

Detection and Detoxification Methods for Mycotoxins: From Classical to New Trends

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Abstract

Mycotoxins are toxic secondary metabolites produced by certain filamentous fungi. Hundreds of mycotoxins have been identified to date, and these mycotoxins have adversely affected human and animal health, as well as agriculture and the economy. Indeed, mycotoxin contamination in food and feed is pervasive and causes significant losses every year. As complete prevention of mycotoxin formation is close to impossible, researchers are actively developing new detection and detoxification techniques. In addition, mycotoxin detection methods often require some pretreatments for accurate measurement. On the other hand, in addition to physical, chemical and biological methods, mycotoxins can also be detoxified by other methods that have emerged in recent years. This study aims to highlight the detection and detoxification methods for mycotoxins and introduce innovative approaches in these areas.

Keywords: mycotoxins, detection, detoxification, classical, new trends

1. Introduction

Mycotoxin is derived from the terms “myco” meaning “fungus” and “toxin” meaning poison. Mycotoxins are potentially hazardous secondary metabolites produced by some types of filamentous fungi (molds) genera such as *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps*. Mycotoxins are the most toxic chemical agents found in food and feed, posing the greatest threat to human and animal health [1, 2]. More than 300 mycotoxins have been identified to date, the most common of which are aflatoxins, ochratoxins, fumonisins, trichothecenes (T2, DON, DAS, HT2), ergot alkaloids, alternaria, patulin, citrinin and zearalenone toxins are the most important mycotoxins related to agriculture, economics, and public health [2].

Mycotoxin contamination is undesirable in all foods. However, attaining this goal through natural and artificial methods is not feasible, and a relatively small amount of these mycotoxins will always be present. Each year mycotoxin contamination of food and feedstuff causes heavy losses worldwide at the level of humans, animals, agriculture, and industries. So widespread is the contamination, researchers are making a strong effort to develop new techniques for the detection and detoxification of mycotoxins [3].

Detection of mycotoxins may require some pretreatments such as liquid-liquid and solid-liquid extraction, supercritical fluid extraction, filtration, gel chromatography and immune affinity purification. The analytical methods most commonly used to determine mycotoxin presence are high-performance liquid chromatography (HPLC), liquid chromatography combined with mass spectrometry (LC/MS), gas chromatography (GC), gas chromatography combined with mass spectrometry (GC/MS), Enzyme-Linked Immunosorbent Assay (ELISA) and quick tests [4].

Mycotoxin detoxification can be achieved by using physical methods, chemical methods, biological methods and other emerging methods. In addition, there are approaches to examine the effects of some of these emerging technologies on mycotoxin detoxification by combining them with fermentation [5]. This study, aims to introduce the detection and detoxification methods of mycotoxins and new approaches toward these methods.

2. Pretreatments for mycotoxin detection

Pretreatments allow mycotoxins in food samples with complex biochemical structures to be isolated by improving their concentration and purity. This minimizes the risk of instrument contamination, signal interference and matrix effects in the analytical detection methods to be applied in the next steps (**Figure 1**).

2.1 Extraction

Extraction of mycotoxins from food samples is the first step in sample preparation. For the isolation of mycotoxins, liquid foods such as fruit juice, wine and milk are subjected to liquid-liquid extraction (LLE), while solid foods such as cereals and grains are subjected to solid-liquid extraction (SLE). Most mycotoxins are highly soluble in organic solvents such as methanol, acetonitrile (ACN), acetone, dichloromethane and ethyl acetate, while (with the exception of fumonisins and patulin) they are only slightly soluble in water. To improve extraction efficiency, certain amounts of acidic buffer or water are often added to organic solvents. Recently, many instrumental solvent extraction methods such as supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) have been used in mycotoxin analysis. Although these methods offer lower chemical solvent consumption and better extraction efficiency, these devices are costly and not easily available in common laboratories [6].

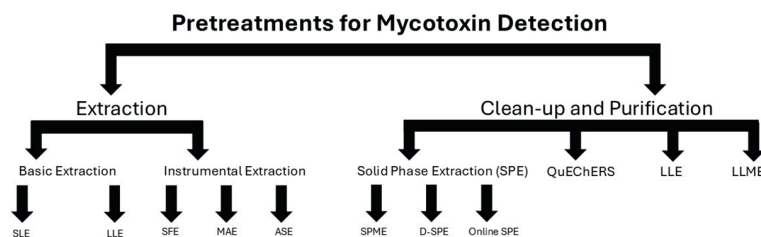


Figure 1.
Various pretreatments for the detection of mycotoxins.

2.2 Clean-up and purification

After extraction, filtration and centrifugation can be applied to remove coarse impurities before performing further clean-up steps. In the further clean-up steps, different techniques such as solid phase extraction (SPE), solid phase micro extraction (SPME), dispersive solid phase extraction (D-SPE), QuEChERS (quick, easy, cheap, effective, rugged and safe), liquid-liquid extraction (LLE), dispersive liquid-liquid micro extraction (DLLME) can be applied.

2.2.1 Solid-phase extraction (SPE)

SPE has been recognized as a beneficial alternative to classic liquid-liquid extraction due to the avoidance of large solvent consumption, tedious process and long operation time. With the development of SPE, new perspectives and progress are provided as methodological solutions in mycotoxin analysis. Significant progress has been observed in the simplification, automation and miniaturization of the original formats [7].

The adsorbent is the most important factor of the SPE method. According to the polarity of materials, commercial SPE adsorbents can be classified into four categories: (1) reversed-phase adsorbents for non-polar or weakly polar compounds, such as C18 and C8, (2) positive-phase adsorbents for polar compounds, such as CN, NH₂ and Florisil, (3) ion-exchange adsorbents, such as SAX, PRS, and PSA, and (4) additional adsorbents, such as Oasis Hydrophilic Lipophilic Balance (HLB) and immunoaffinity columns (IACs). Apart from these, there are different adsorbents such as molecular recognition-based polymers (such as molecularly imprinted polymers and aptamers), nanoparticles-based adsorbents (such as graphene and its composites, activated carbon and its composites), bonded silica-based adsorbents (such as humic acid bound silica), hyper crosslinked polymers (such as synthesized from heterocyclic phenyl-imidazole monomers) which are being studied and promising [8].

2.2.1.1 Solid phase micro extraction (SPME) techniques

Since SPME combines sampling, extraction, sample cleaning and enrichment in a single step, it is one of the most simple and effective techniques. The efficiency of SPME has been supported further by the development of matrix-compatible coatings in which sorbent particles of a few or tens of μm in diameter are embedded in a polymeric binder like polyacrylonitrile (PAN). In these coatings, the binder forms a thin film that allows extraction and enrichment of only small molecules while avoiding interaction with large molecules (such as protein and cell matter) from the sample matrix [9].

2.2.1.2 Dispersive solid-phase extraction (D-SPE) techniques

Dispersive SPE (d-SPE) improves sensitivity and reduces sorption time by allowing sorbents to interact directly and quickly with targets. It avoids issues like high backpressure or clogging found in conventional SPE. d-SPE can be used alone or with other methods and is commonly applied in mycotoxin analysis through two approaches: dispersive micro-SPE (D- μ -SPE) and magnetic SPE (m-SPE). D- μ -SPE uses small amounts of micro- or nanomaterials, making the properties of sorbents critical for the accurate and efficient detection of mycotoxins. Nanomaterials in

D- μ -SPE offer rapid extraction and high efficiency, overcoming issues like back-pressure, and are more cost-effective and time-saving compared to traditional SPE cartridges. To improve the mass transfer kinetics, ultrasonication or vortex can be applied as an assistive technique for D- μ -SPE. M-SPE, which eliminates the need for centrifugation or filtration, is widely used in mycotoxin analysis. It incorporates magnetic properties with functional materials, and its application in this field is more extensively studied than D- μ -SPE, using magnetized carbon nanoparticles, functionalized magnetic nanoparticles, and magnetized nanoporous materials [7].

2.2.1.3 Online SPE

SPE techniques can be combined with the online system, which provides some advantages. Analysis time, sample loss and the amount of solvent required can be reduced, as well as sensitivity, accuracy, and precision can be increased by eliminating personal errors [10, 11]. Furthermore, the online system allows partial or total automation of analytical steps with some special facilities such as automatic sample loading, washing of interference, analyte elution, separation and detection by the analytical system [12, 13].

2.2.2 QuEChERS

The QuEChERS (quick, easy, cheap, effective, rugged and safe) method is one of the most widely used methods for the extraction of both solid and liquid food samples. This method is fast and requires less use of solvents, so it is both economical and environmentally friendly (since it produces less waste). This method involves two steps. In the first step, a separation based on inorganic and organic phases can be obtained by using salt solution and solvent. Moreover, solvents together with salts allow the separation of analytes with different polarity. The second step includes the clean-up stage, using various absorbents such as C18 (Octadecyl silica), PSA (N-Propylethane-1,2-diamine) and SPE cartridge. C18 is used to remove non-polar compounds, and PSA is used to remove sugars, lipids and organic acid. However, in some cases, it may be preferable not to have a clean-up step in this method [14].

2.2.3 Liquid-liquid extraction (LLE)

Liquid-liquid extraction (LLE) utilizes the differing solubility of a toxin in an aqueous phase versus an immiscible organic phase to isolate the compound into one solvent, while leaving the remaining matrix in the other [15]. For example, solvents like hexane and cyclohexane are employed to eliminate non-polar impurities, such as lipids and cholesterol. This method is effective for various toxins and is suitable for small-scale preparations. However, it can be time-consuming and depends on the specific matrix and compounds being analyzed. Another drawback is the potential loss of samples due to adsorption onto glassware [16].

2.2.4 Liquid phase microextraction (LPME)

Recently, liquid phase microextraction (LPME) methods have gained popularity and are increasingly being utilized. In these LPME techniques, analytes are extracted from an aqueous solution (donor phase) into a μ L-level of a water-immiscible organic

solvent (acceptor phase). Single-drop microextraction, hollow fiber-LPME, and dispersive liquid-liquid microextraction (DLLME) are three primary types of LPME techniques. Of these, DLLME has gained significant attention since it was developed in 2006. Its main advantages include simplicity, high extraction recovery (ER), enrichment factor (EF) and speed. However, DLLME has drawbacks, including the use of toxic solvents and a time-consuming centrifugation process. To address these issues, new methods like air-assisted liquid-liquid microextraction, ultrasound-assisted DLLME and effervescence-assisted DLLME (EA-DLLME) have been developed [17].

3. Mycotoxin detection methods

3.1 Analytical methods

Different kinds of analytical approaches such as thin layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC) and liquid chromatography coupled with mass spectrometry (LC-MS) have been used for mycotoxins detection in various samples.

TLC is a basic method, but it is generally considered to be less practical or to have insufficient sensitivity compared to LC methods. Nevertheless, a small number of researchers are investigating this method as it still has uses in certain situations [18].

Gas chromatographic methods based on Flame Ionization Detector (FID), Electron Capture Detector (ECD) and Mass Spectrometry (MS) detection are widely used methods for the quantitative simultaneous determination of mycotoxins. These methods require a preliminary clean-up of extracts and pre-column derivatization of the purified extract with specific reagents. Mass spectrometry (MS), or tandem mass spectrometry (MS/MS), offers an advantage in confirming the identity of chromatographic peaks [19].

HPLC is the most popular method for analyzing mycotoxins in food and feed. It is a quantitative technique ideal for the online clean-up of sample extracts and can be paired with various detectors, such as DAD, UV and FLD [20]. Fluorescence detectors are undoubtedly the most sensitive among the modern HPLC sensors [21]. Although HPLC-FLD offers sufficient sensitivity, the presence of various interferences in sample matrixes limits their direct application in food samples [22].

LC-MS technique coupling of LC and MS provides a great opportunity for the analysis of mycotoxins. HPLC with MS detection eliminates the need for sample derivatization for fluorescence activity enhancement. Furthermore, the use of LC and tandem mass spectrometry enables a very selective and sensitive detection. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is a useful technique for the identification and quantification of chemicals such as mycotoxins. Before the mass spectrometer, HPLC will separate the sample into chemical compounds. Then mass spectrometer will ionize molecules and sort, and identify them according to their mass-to-charge ratio (m/z) (**Figure 2**) [23].

3.2 Mycotoxin sensors

Biosensors are analytical devices that detect and measure target molecules using biological recognition elements and a signal transducer. Recently, there have been

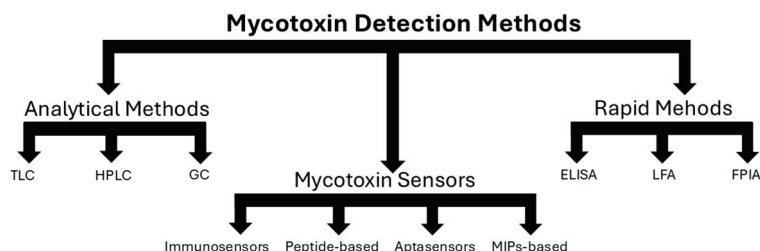


Figure 2.
Various methods for detection of mycotoxins.

efforts to develop different types of biosensors, including immunosensors, peptide-based sensors, aptasensors and molecularly imprinted polymer-based (MIPs-based) sensors for mycotoxin detection. They provide real-time monitoring of reactions by creating digital output formats. Mycotoxin screening methods can be advanced by improving sensitivity, robustness, simplicity and reusability. Various transducers for the development of mycotoxin sensors continue to be investigated, including electrochemical, calorimetric, optical and magnetic [24].

3.3 Rapid methods

3.3.1 Enzyme-linked immunosorbent assay (ELISA)

ELISA, developed by Engvall and Perlmann in 1971, is an immunological technique used to analyze protein samples in microplate wells with specific antibodies. The use of ELISA has become indispensable in food safety as well as in different fields over time. Typically conducted in 96-well plates, ELISA can involve direct labeling of targets or competitive binding with labeled secondary antibodies. Its simplicity, speed, high throughput, and low cost make it popular for rapid mycotoxin detection [25].

3.3.2 Lateral flow assay (LFA)

LFA-based instruments are ideal for rapid mycotoxin detection in food, providing results in 5–30 minutes without the need for complex preparation. These tools are stable, require minimal sample input and are user-friendly, making them accessible even in low-resource settings. Their portability and cost-effectiveness contribute to their growing popularity for on-site testing outside of laboratories. LFAs are categorized into sandwich and competitive types, with sandwich LFAs suited for larger molecules and competitive LFAs for smaller ones like mycotoxins [26].

3.3.3 Fluorescent polarization immunoassay (FPIA)

FPIA operates on the principle that exposing a fluorescent molecule in solution to polarized light at its excitation wavelength results in depolarized emission. Small fluorescent molecules rotate quickly and exhibit lower polarization, while their interaction with larger molecules like antibodies slows their rotation, increasing polarization. This increase can be measured, making it useful for developing competitive immunoassays for mycotoxin detection. In this competitive format, mycotoxins in the sample compete with a fluorophore-labeled tracer for binding sites on specific

Detection method	Advantages	Disadvantages
TLC	Quick, simple, and qualitative method	Low sensitivity and no quantification
HPLC	Good sensitivity, selectivity and repeatability, can be automated (autosampler), short analysis times, official methods available, provides confirmation (by different detectors).	Requirement expensive equipment, specialist expertise, pretreatment and derivatization.
GC	Simultaneous analysis, good sensitivity, can be automated (autosampler), provides confirmation (by different detectors).	Requirement expensive equipment, specialist expertise, pretreatment and derivatization. Matrix interference problems, calibration difficulties, variation in repeatability.
Sensors	Real time monitoring, suitable for development.	Cross-reactivity with related mycotoxins, (sometimes) clean-up needed, variation in repeatability.
ELISA	User-friendly, inexpensive equipment, high sensitivity, simultaneous analysis, suitable for screening, limited use of organic solvents.	Cross-reactivity with related mycotoxins, matrix interference problems, possible false results, confirmatory LC analysis required.
LFA	Rapid detection, no need complex preparation, require minimal sample input, user-friendly, no expensive equipment required.	Cross-reactivity with related mycotoxins, validation required for additional matrices.
FPIA	Rapid, no clean-up required.	Poor sensitivity in some cases, cross-reactivity with related mycotoxins, matrix interference problems.

Table 1.
Advantages and disadvantages of mycotoxin detection methods.

antibodies, affecting polarization levels. An increase in polarization indicates a negative sample, while a decrease signals the presence of mycotoxins, making polarization inversely proportional to their concentration (**Table 1**) [27].

4. Mycotoxin detoxification methods

4.1 Physical methods

Contaminated grain does not have the same color or density as safe grain. Thus, grain can be sorted according to appearance or density. In addition to basic sorting, the sieving cleaning technique can be applied by utilizing the size difference of the grains, and the flotation technique can be applied by utilizing the density difference. When mycotoxin contamination is heterogeneous, the removal of the contaminated portion may reduce the level of mycotoxin in the final product. Washing food or grain can also reduce mycotoxin levels. Indeed, water-soluble mycotoxins can be partially washed off the surface of grains. There are studies on the use of distilled water for this process, as well as the use of solutions with different contents [28].

The adsorption is another physical method applied for mycotoxin removal. The application of adsorption agents has become popular since the European Union (EU)

allowed substrates that suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action to be used as feed additives [29].

Mycotoxins are generally very stable and are rarely eliminated by thermic treatment. Little or no reduction in mycotoxin levels occurs as a result of normal cooking conditions, such as boiling, frying. For efficiency of thermal processes, the initial level of contamination, type of mycotoxin and its concentration, heating temperature and time, the degree of heat penetration, moisture content, pH and ionic strength of the food all play a significant role in toxin degradation. Similarly, most mycotoxins are not often affected by irradiation. Radiolysis of water produces free radicals that could react with mycotoxins. In addition to UV, X and gamma-rays, microwave applications are also known to be effective in the detoxification of some mycotoxins [30].

Cold plasma is basically created by atmospheric dielectric discharge, with synthetic air as a working gas [31]. There are studies proving that cold plasma has antimicrobial and antimycotoxic effects on food surfaces. However, its potential cytotoxicity effect is still unclear because of the limited number of studies published [32].

4.2 Chemical methods

Various acidic, alkaline and oxidizing agent treatments have been used so far for the detoxification of mycotoxins. Some of these applications have also been supported by heating. Acids such as lactic acid, citric acid, hydrochloric acid have been used effectively in acidic treatments. For alkaline treatments, chemicals such as sodium hydroxide and ammonia have been used, with the most effective results obtained with ammonia. The use of ozone has also been widely involved in the detoxification of various mycotoxins. Although the results obtained in mycotoxin detoxification by chemical methods are highly successful, they have disadvantages such as the potential to form degradation products (conversion to another mycotoxin) and the high risk of producing chemical waste [33].

4.3 Biological methods

4.3.1 Microbial detoxifications

Microbial detoxification involves utilizing the metabolites or enzymes released by microorganisms during their growth, or the inherent traits of the microorganisms, to break down or inhibit the formation of mycotoxins, thus minimizing mycotoxin contamination. Additionally, microorganisms can directly adsorb mycotoxins from samples, aiding in their removal or reduction. Various wild-type bacterial species (especially lactic acid bacteria), yeasts (especially *Saccharomyces cerevisiae*), molds and actinomycetes can be employed for mycotoxin detoxification. With the rapid advancement of genetic engineering techniques, there are ongoing studies aimed at enhancing the detoxification abilities of these microorganisms [34–36].

4.3.2 Enzymatic detoxifications

Research into enzymes capable of converting mycotoxins into less harmful or non-toxic products has become increasingly significant. Various enzymes, such as laccase, manganese peroxidase (MnP) and oxidase, have been identified for this purpose. While identifying and purifying these mycotoxin-degrading enzymes (MDE) can be time-consuming and costly, using pure enzymes presents advantages over

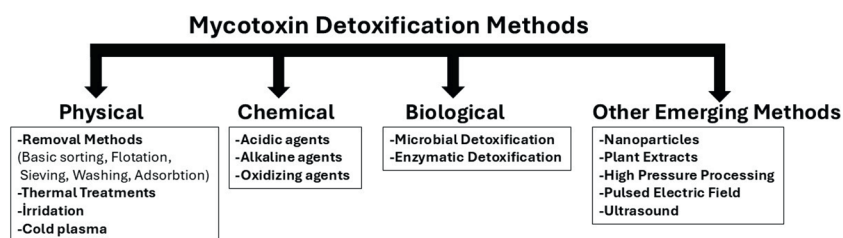


Figure 3.
Various methods for detoxification of mycotoxins.

whole bacterial cells, particularly in environments unsuitable for microbial survival. Enzymatic degradation offers benefits like ease of handling, consistent performance, high efficiency and specificity. However, many MDEs are stored intracellularly in fungi, making extraction challenging. Although some natural MDEs have been purified, their use in complex food and feed matrixes remains limited. Recombinant enzymes are particularly appealing because genetic engineering allows for efficient, cost-effective production. Recent studies have focused on enhancing yield and reducing purification costs, but commercial applications of recombinant MDEs are still not fully realized [37].

4.4 Other emerging methods

New approaches such as the use of nanoparticles and plant extracts are also being tested in mycotoxin detoxification. There are studies examining the effects of nanoparticles coated with some special substances (chitosan, etc.) to adsorb mycotoxins, inhibit mycotoxin-producing microorganisms and degrade mycotoxins. On the other hand, there are studies on the inactivation of mycotoxin-producing microorganisms and mycotoxins by components such as plant extracts, essential oils and secondary metabolites. Moreover, the use of plant extracts is attractive due to their extremely low toxicity and environmental friendliness compared to chemical methods [38].

High-pressure-processing (HPP), pulsed electric field (PEF), as well as ultrasound methods, have been identified as emerging and green technologies useful in controlling microorganisms and mycotoxins in foodstuffs. Using HPP, the structure of mycotoxins can be modified, which reduces both their toxicity and their ability to thrive in the environment. Moreover, this technique can be combined with moderate heating to deactivate heat-resistant microorganisms. PEF leads to a transmembrane voltage between the inner and outer parts of the cell membrane, which, after a certain point, leads to permanent structural changes and cell death. Although this method is more prominent in ensuring the death of mycotoxin-producing microorganisms in a short time, it has also been shown to be effective in mycotoxin degradation under some special conditions. The use of ultrasound has been shown to decontaminate cereal products and thereby prevent mycotoxin formation (**Figure 3**) [39].

5. Conclusion

Mycotoxins not only cause economic losses but also adversely affect human health. Moreover, the inevitability of mycotoxin formation in food products has necessitated

the development of effective practices for the pre-production, during-production and post-production stages of food products. Therefore, rapid and accurate detection of mycotoxins and detoxification of mycotoxin-contaminated food products are of utmost importance. Although scientists have been working on both of these important factors for many years, due to the structural differences of each type of food product and mycotoxin, it has so far not been possible to determine the optimal type of detection and detoxification method, since it is not possible to obtain the same response after each treatment. Therefore, further studies on these issues and the development of new techniques remain important to shorten processing time, increase sensitivity and efficiency, and reduce processing costs. While it is encouraging that recent studies have shown promising results in these areas, the permanence of new techniques depends on meeting important criteria such as the absence of side effects and sustainable cost-effectiveness.

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