Recent advances in immunoassay-based mycotoxin analysis and toxicogenomic technologies

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Abstract

The co-occurrence and accumulation of mycotoxin in food and feed constitutes a major issue to food safety, food security, and public health. Accurate and sensitive mycotoxins analysis can avoid toxin contamination as well as reduce food wastage caused by false positive results. This mini review focuses on the recent advance in detection methods for multiple mycotoxins, which mainly depends on immunoassay technologies. Advance immunoassay technologies integrated in mycotoxin analysis enable simultaneous detection of multiple mycotoxins and enhance the outcomes' quality. It highlights toxicogenomic as novel approach for hazard assessment by utilizing computational methods to map molecular events and biological processes. Indeed, toxicogenomic is a powerful tool to understand health effects from mycotoxin exposure as it offers insight on the mechanisms by which mycotoxins exposures cause diseases.

Keywords: Analytical methods, Immunoassay, Mycotoxin detection, Multiple mycotoxins, Toxicogenomics

1. Introduction

Mycotoxins are naturally occurring toxins produced by certain fungi [1]. Pre-and post-harvest colonization of food commodities by fungi is largely influenced by various factors ranging from climatic and farm practice, handling and storage to processing and distribution of food [2]. Most mycotoxins are stable and survive food processing involves heat, physical, and chemical treatments [3]. Mycotoxin is one of the most dangerous contaminants of food and animal feed. Therefore, it is an important challenge and mission of many food safety specialists all over the world to protect humans or animals from dangerous contamination levels of various mycotoxins in the feeds or foods. Hazard analysis is a systematic approach to control food safety from biological, chemical, and physical hazards that can cause the end-product to be unsafe for consumption [4]. The analysis consists of identification, risk assessment, and the establishment of preventive measures toward the hazards and related causes [5]. Indeed, mycotoxin detection analysis plays an important role in reducing toxin exposure, wherein, improvement in technologies and techniques in the analytical process is crucial [6].

Mycotoxins exposure in humans may occur either directly by eating fungi-infected food or indirectly from animals that are fed with contaminated feed [7]. Of great significance, feed contamination poses an extra hazard for food safety due to the possible carry-over of mycotoxins to animal-derived products such as milk, meat, and egg, leading to mycotoxin intake by humans [8]. The negative effects on animal health and production have been documented in farmed animals such as poultry, swine, and cattle [9]. The phenomenon occurs due to the consumption of high levels of cereals and cereal by-products, that are prone to fungi infection in the daily diet of animals [10]. Animals showed varying susceptibilities to mycotoxins subjected to genetic, physiological, and environmental factors [11]. As animal species differ in terms of the absorption, metabolism, distribution, and excretion of mycotoxins, the sensitivity to the adverse effects of mycotoxins may vary [12]. Unfortunately, about 25% of the world's harvested crops are contaminated by mycotoxins each year, leading to huge agricultural

and industrial annual losses of around 1 billion metric tons of foods and food products [13].

The effects of some food-borne mycotoxins are acute with symptoms of severe illness appearing quickly after consumption of food products contaminated with mycotoxins [14]. Several mycotoxins are correlated with severe toxicological effects such as teratogenicity, hepatotoxicity, and carcinogenicity [15]. Other mycotoxins occurring in food have been linked to long-term effects on health, including immune deficiencies-related diseases [16]. Because of their severe effects on animal and human health, as well as their occurrences in food, a dozen have gained attention from the authorities. Hundreds of different mycotoxins have been identified, but the most observed mycotoxins that attract the concern to human health and livestock include aflatoxins, (AF), ochratoxin A (OTA), fumonisins (FUM), zearalenone (ZEN), and deoxynivalenol (DON) [17].

Mycotoxins have been detected in countless food commodities in every part of the world, thus poses critical challenge in regulation of mycotoxin contamination. Mycotoxins harm human and animal health, hamper economic development, as well as generate food wastage. Nonetheless, the co-occurrence of mycotoxins has further complicated the situation where the conventional mycotoxins analytical methods are no longer applicable. This review focuses on the recent advance in detection methods for multiple mycotoxins, which mainly depends on immunoassay technologies. Furthermore, it highlights toxicogenomic as a field which opens novel opportunities for hazard assessment by utilizing computational methods to map molecular events and biological processes.

2. Co-occurrence of mycotoxins

Risk assessments of mycotoxins in food done by the Joint FAO/WHO Expert Committee on Food Additives are used by governments and by the Codex Alimentarius Commission to establish maximum levels in food or provide other risk management advice to control or prevent contamination [18]. Codex standards are the international reference for national food supplies. For instance, total AFs in peanuts, almonds, hazelnuts, pistachios, Brazil nuts for further processing should not exceed 15 μg/kg. Meanwhile, OTA in raw wheat, barley, and rye should be lower than 5 μg/kg. The guidelines provided by the regulatory bodies only focus on the presence of single mycotoxin in food/feed [18].

However, the presence of multiple mycotoxins in raw ingredients and feeds is not a rare scenario.

Abbreviations	
AFB1	aflatoxin B1
OTA	ochratoxin A
FUM	fumonisins
ZEN	zearalenone
DON	deoxynivalenol
TCT	trichothecenes

Such an incident is due to the simultaneous production of several types of mycotoxins by a single fungus. For instance, *Fusarium* spp. produces a range of trichothecenes (TCT; DON, nivalenol, T-2, and HT-2 toxins), ZEN, and FUMs [19]. In addition, contamination may be caused by different fungal species concurrently. The manufacturing of compound feed involved mixing and milling feed materials. Such a process increases the susceptibility of various mycotoxins present in the feed. According to Smith et al. [20], the mixtures of AFs + FUM, DON + ZEA, AFs + OTA, and FUM + ZEA were abundantly detected in cereal and derived cereal product samples world widely. Besides, AFs + OTA was found mainly in dried fruits, herbs, and spices [19]. Moreover, a search in the literature from 1987 to 2016 showed that binary mixtures are the most common, while AFs are primarily found together with OTA or fusariotoxins (mainly FUM and ZEA) [20] in all food/feed. Meanwhile, the regional distribution pattern of mycotoxins' co-occurrence showed that AFs + OTA was the main mixture found in African samples. On the other hand, AFs + FUM mixture is the most prevalent in Africa, Asia, and South America [20]. AFs are a far greater problem in the tropics than in temperate zones of the world. However, because of the movement of agricultural commodities around the globe, no region of the world is AF-free. In more temperate and cold regions (Europe and North America), the mixture of TCTs or TCTs with ZEA are the most common, highlighting the importance of the climate conditions on fungal contamination, growth, metabolism, and thus mycotoxin production [21].

Such findings highlight the significance of mycotoxin co-occurrence-related study to provide the actual situations of health risks exposed to humans and animals. The combined toxicity effects are hardly predictable. Nevertheless, mycotoxin mixtures are found to have additive or synergistic effects, which are harmful to human and animal health. A previous study showed the mixture of DON and T-2 toxins significantly enhanced the mutagenic activity of AFB1 [22]. Although the screening of mycotoxins has been frequently performed, the legislation available over

the world only considers homogenous mycotoxin exposure data and does not address the combined effects of mycotoxins.

3. Development of mycotoxin analytical methods

Mycotoxins can be detected via two main techniques: conventional quantification in the laboratory and rapid screening. Conventional methods include thin-layer chromatography (TLC) [23], gas chromatography-tandem mass spectrometry (GC−MS) [24], high-performance liquid chromatography (HPLC) [25], liquid chromatography-mass spectrometry (LC-MS) [26], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [27], ultra-high liquid chromatography-tandem mass spectrometry (UHP-LC-MS/MS) [28]. Owing to their high accuracy, precision, and sensitivity, several conventional methods have been applied for the analysis of mycotoxins in many agricultural commodities and feedstuffs since the 1970s. LC and UHPLC were frequently used as these methods can identify regulated, unregulated, and emerging mycotoxins in one single run with small volume injection [29]. Yet, these conventional methods are deemed costly, tedious, complex, and impractical for fast screening in the field.

As a result, technologies in mycotoxin analysis have been continuously revolutionized with new concepts and approaches. The rapid screening methods are reliable tests that offer more sensitive and specific evaluation at a cheaper cost. Such methods offer effective monitoring of various mycotoxin contamination in food/feed on the spot. Recently, an increasing trend has been observed for the development of simultaneous detection of mycotoxins arising from the rapid immunoassay concept [30]. Enzyme-linked immunosorbent assay (ELISA) is a classic immunoassay that has been widely used [31]. Sensitive microtiter plate ELISA-based immunoassays are readily available in the market to measure a range of mycotoxins. These kits mainly function based on a competitive, heterogeneous ELISA format. The binding between the labelled toxin and antibody can be affected by the presence of interference compound via attachment towards the labelled toxin/antibody, antibody denaturation, and enzyme inhibition [32]. ELISA may overestimate levels of mycotoxin due to cross-reactivities and matrix dependency, therefore it is only recommended for comprehensively researched samples and the presence of sufficient standards [33]. Besides, ELISA also requires costly detectors, tedious procedures, and skilled technicians. Hence, the development of rapid methods has been established at an accelerated pace owing to

heavy demands. Advanced mycotoxin analyses developed in recent years are based on ELISA-inspired immunoassay theory.

4. Advance multi-mycotoxin immuno-based analysis

Emerging immunoassay platform technologies such as chemiluminescence (CL) immunoassay, fluorescence polarization (FP) immunoassay, lateral flow (LF) immunoassay, electrochemical (EC) immunosensor are useful for multiple mycotoxin detection [34]. The immunoassays used for multiplex mycotoxins analysis in recent years are summarized in Table 1. Immunoassay methods are developed based on antibody−antigen reactions. Mycotoxins are small non-immunogenic molecules [35]. The designing of specific antibodies against mycotoxins (analyte) and hapten is a crucial step for immunoassays. A hapten is a substance that can combine with a specific antibody but lacks antigenicity of its own [36]. An ideal hapten is closely similar to the target small molecule in terms of molecular structure and physicochemical properties with an attachment arm (4−6 carbon atoms) containing active group (−COOH and −NH2) to bind to the carrier protein [37].

There are two categories of multiple mycotoxin analysis, competitive and non-competitive formats. The competitive format has been widely applied to develop rapid immuno-based methods due to the characteristic of mycotoxin (which contain merely one binding epitope) [38]. The main immunoassay formats for mycotoxins detection can be referred in Fig. 1. In the direct competitive mode, the mycotoxin from the sample competes for a limited number of antibody-binding sites with the toxin-enzyme conjugate. A lower signal indicates a higher concentration of mycotoxin in the sample, as the mycotoxin in the sample reduces the availability of the immobilized antibody to bind the labelled mycotoxin [39]. The detected signal is inversely associated with the concentration of mycotoxin. For indirect competitive format, mycotoxins in a test sample compete with immobilized labelled mycotoxins or their conjugates to bind with a limited amount of specific antibodies in the system [40].

4.1. Chemiluminescence immunoassay

Mycotoxins detection using CL immunoassay employs simple optical equipment without an external light source [41]. Luminescence means that light is emitted by a substance when it returns from an excited state to a ground state [42]. A catalyst (enzyme, metal, and nanoparticles) is commonly

Table 1. List of the reviewed immunoassays for simultaneous detection of multiple mycotoxins in recent years.

Matrix	Analyte	Sample Preparation	Format	Signal	LOD	Linear range	Analysis Time (min)	Ref.
Chemiluminescence Immunoassay								
Corn	AFB1, OTA, ZEA, FUMB1	75% acetonitrile:-water (84:16); 5 min	Competitive; Quantitative	strep-HRP with enhanced CL	AFB1 (0.21 ng/mL), OTA (0.19 ng/mL), ZEA (0.09 ng/mL), FUMB1 (0.24 ng/mL)	AFB1 (0.47−55.69 ng/mL), OTA (0.48−127.11 ng/mL), ZEA (0.22−31.36 ng/mL), and FUMB1 (0.56−92.57 ng/mL)	60	[44]
Red yeast rice	CIT, AFB1, OTA	70% methanol solution (0.5 g sodium chloride); 30 min	Competitive; Quantitative	HRP with AuNP-IgG, tyramine, strep-HRP, CL substrate	CIT (0.00006 ng/mL), AFB1 (0.00008 ng/mL), OTA (0.00008 ng/mL)	0.0001−1.0 ng/mL	90	[45]
Maize	AFB1, AFG1, OTA, ZEA, T-2 toxin, FUMB1, FUMB2, DON	75% acetonitrile:methanol:-water (50:40:10); 10 min	Competitive; Quantitative	HRP with Investigador™ EV 4065, Evidence Investigator Myco 7	FB1+FB2 (250 µg/kg), AFB1 and AFG1 (1 µg/kg), OTA (1.5 µg/kg), ZEA (50 µg/kg), T-2 (25 µg/kg), DON (375 µg/kg)	FB1+FB2 (0−300 µg/kg), AFB1 (0−14 µg/kg), AFG1 (0−75 µg/kg), OTA (0−60 µg/kg), ZEA (0−150 µg/kg), T-2 (0−300 µg/kg), DON (0−7500 µg/kg)	90	[46]
Fluorescence Polarization Immunoassay								
Durum wheat	T-2 and HT-2 toxin	90% methanol or water	Competitive; Quantitative	HT-2 tracers	T-2 and HT-2 toxin (10 µg/kg)	50−200 µg/kg	15	[52]
Corn, soybean, sorghum, wheat, rice and oats	AFB1, ZEA, and OTA	75% methanol:-water (70:30); 10 min	Competitive; Quantitative	chicken IgY with Europium NP	AFB1 (0.04 µg/kg), ZEN (0.20 µg/kg), and OTA (0.10 µg/kg)	AFB1 (0.00387−0.06924 mg/L), ZEN (0.01435−0.28789 mg/L) and OTA (0.0099−0.20423 mg/L)	15	[53]
Maize	AFB1, ZEA, DON, T-2, FUMB1	80% methanol in 0.02 M PB; 3 min	Competitive; Quantitative	AuNPs	Visible: AFB1 (10 µg/kg), ZEA (2.5 µg/kg), DON (1.0 µg/kg), T-2 (10 µg/kg), FUMB1 (0.5 µg/kg); Quantitative: AFB1 (0.59 µg/kg), ZEA (0.24 µg/kg), DON (0.32 µg/kg), T-2 (0.9 µg/kg), FUMB1 (0.27 µg/kg)	NA	8	[54]

Lateral Flow Immunoassay

Wheat and wheat by-product	AFB1 and FUMB1	1% BSA and 2% PEG 10000 in PB; 2 min	Competitive; Qualitative	AuNPs	AFB1 (0.5 ng/mL) and FUMB1 (20 ng/mL)	NA	10	[60]
Buffer	AFB1, DON, FUMB1, T-2, and ZEA	NA	Competitive; Quantitative	Novel luminescent compound	AFB1 (1.3 ng/mL), DON (0.5 ng/mL), FUMB1 (0.4 ng/mL), T-2 (0.4 ng/mL), and ZEA (0.9 ng/mL)	AFB1 (5–40 ng/mL), DON (10–200 ng/mL), FUMB1 (0.5–10 ng/mL), T-2 (5–80 ng/mL), and ZEA (10–100 ng/mL)	NA	[61]
Maize	AFB1, DON, FUMB1, T-2, and ZEA	70% methanol; 5 min	Competitive; Quantitative	5,5-dithiobis-2-nitrobenzoic acid and 4-mercapto-benzoic acid	AFB1 (0.00096 ng/mL), DON (0.00011 ng/mL), FUMB1 (0.00026 ng/mL), T-2 (0.0086 ng/mL), and ZEA (0.0062 ng/mL)	AFB1 (0.0014–0.33 ng/mL), DON (0.14–33.3 ng/mL), FUMB1 (0.41–100 ng/mL), T-2 (0.014–33.3 ng/mL), and ZEA (0.015–3.7 ng/mL)	20	[62]

Electrochemical Immunosensor

|								
Corn	FUMB1 and DON	NA	Non-Competitive; Quantitative	AuNP and poly-pyrrole-electro-chemically reduced graphene oxide	FUMB1 (4.2 ng/mL) and DON (8.8 ng/mL)	FUMB1 (200–4500 ng/mL) and DON (50–1000 ng/mL)	NA	[68]
Corn and wheat	AFB1, OTA, ZEN and DON	20% methanol, 5 min	Non-Competitive; Quantitative	surface plasmon resonance (Hydra-zone connection)	AFB1 (0.59 ng/mL), OTA (1.27 ng/mL), ZEA (7.07 ng/mL) and DON (3.26 ng/mL)	AFB1 (0.99–21.92 ng/mL), OTA (1.98–28.22 ng/mL), ZEA (10.37–103.31 ng/mL) and DON (5.31–99.37 ng/mL)	NA	[69]
Corn	FUMB1 and DON	80% methanol, 15 min	Non-Competitive; Quantitative	Indium tin oxide and poly-dimethylsiloxane electrodes	FUMB1 (0.097 ng/mL) and DON (0.035 ng/mL)	FUMB1 (0.3–140 ng/mL; 0.2–60 ng/mL)	NA	[70]
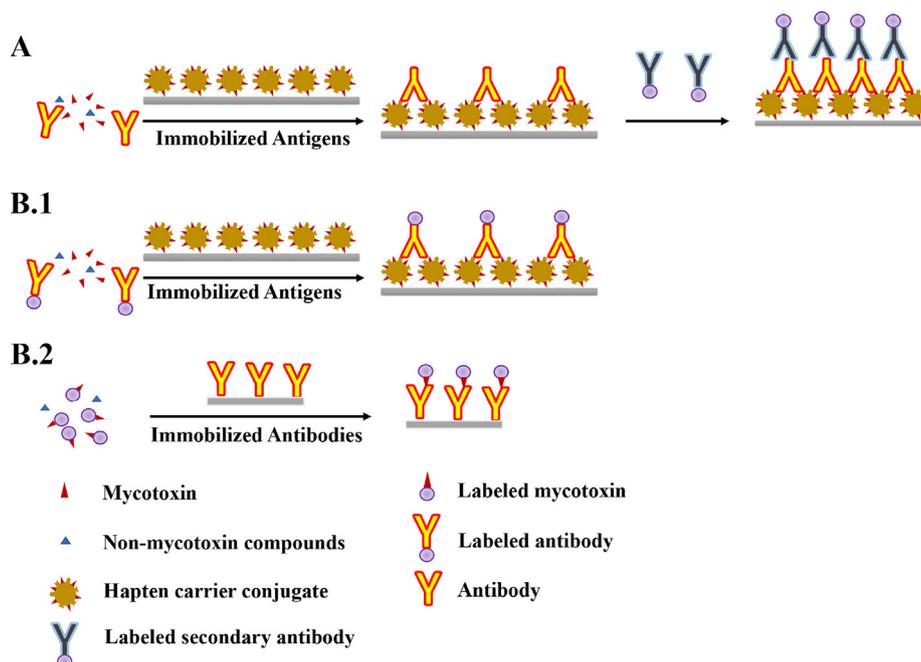
Fig. 1. Basic immunoassay concepts for mycotoxins detection: competitive indirect (A) and competitive direct (B.1 and B.2). Immunoassays are based on immunological principles that use antibodies to recognize and capture target antigens or haptens.

used in a CL immunoassay to enhance the photon intensity. The catalyst added also acts as an enzyme protector and allows the reaction to maintain for a longer period [43]. Chemical reaction-induced CL intensity in the immunoassay is directly measured using a luminescent signal instrument. The luminous intensity is directly linked to the concentration of the measured substance.

Zhang and colleagues have successfully developed a simultaneous mycotoxin (AFB1, OTA, ZEN, and FUMB1) quantitative detection in corn samples [44]. The sensitivity of the CL immunoassay kit was enhanced by both the biotin-streptavidin system and enhanced CL. Results showed the detection limits for AFB1, OTA, ZEN, and FUMB1 were 0.21, 0.19, 0.09, and 0.24 ng/mL, with detection ranges of 0.47−55.69, 0.48−127.11, 0.22−31.36, and 0.56−92.57 ng/mL, respectively. The limit of detection (LOD) of this antibody microarray for AFB1, OTA, ZEN, and FUMB1 in corn was 5.25, 4.75, 2.25, and 6 μg/kg, respectively, with 79.2−113.4% recovery rates.

A novel CL immunoassay combined with a dual-signal amplification strategy was developed for rapid and ultrasensitive detection of multiple mycotoxins in herbal medicine [45]. The immunosensor array uses multi-HRP wrapped AuNPs as the primary signal tag to amplify the CL signals. The primary amplification is subjected to secondary amplification using the tyramine signal amplification (TSA) technique. Such an array tested on herbs containing citrinin, AFB1, and OTA provides

excellent sensitivity (50−57-fold signal amplification and detection limits down to sub-pM level), small amounts of reagents (3.5 μL for each test), simple sample pretreatment [45].

In other study, Freitas et al. [46] have employed a biochip chemiluminescent immunoassay for multi-mycotoxins screening in maize. This immunoassay (Investigador™ EV 4065, Evidence Investigator Myco 7) is based on the Evidence Investigator Biochip Array technology and uses a Randox Biochip containing immobilized antibodies specific to mycotoxins. Spiked samples were fortified at 250 μg/kg for FB1 + FB2, 1 μg/kg for AFB1 and AFG1, 1.5 μg/kg for OTA, 50 μg/kg for ZEA, 25 μg/kg for T2 and 375 μg/kg for DON. Besides, low false results rate (<5%) was achieved and the obtained precision data is in agreement with EU legislation performance criteria.

CL immunosensors have obtained extensive focus, as well as a large number of successful examples for the sensitive detection of mycotoxins. However, CL immunosensors are limited by high background signal and poor reagent stability [47].

4.2. Fluorescence polarization (FP) immunoassay

The FP immunoassay is based on the principle that when a fluorescent molecule in solution is exposed to polarized light at excitation wavelength the resulting emission is depolarized [48]. The orientation of the fluorescence emission (horizontal and vertical directions) determines the polarization

[49]. Small-sized fluorescent molecules such as my-cotoxins showed higher rotation and low polarization than larger molecules [50]. Meanwhile, the polar-isation of mycotoxins can be increased via the inter-action with antibodies. FP immunoassay involves the competition for binding sites on a mycotoxin-specific antibody between mycotoxin in the sample with a mycotoxin-fluorophore tracer [51]. The polarization value is inversely proportional to mycotoxin con-centration. FP immunoassay is a non-enzymatic ho-mologous immunoassay where the free and bound tracer needless to be separated. Removal of washing steps reduces assay time significantly.

For example, Lippolis and colleagues have devel-oped a portable FP analyzer for the simultaneous determination of T-2 toxin, HT-2 toxin, and relevant glucosides in wheat [52]. Antibody used in the study showed high sensitivity (IC50 = 2.0 ng/mL) and cross-reactivity (100% for T-2 toxin and 80% for T-2 and HT-2 glucosides). The result revealed that the immunoassay developed displayed high sensitivity (LOD 10 μg/kg) with a recovery rate of 92–97%, which meet the criteria set by the European Union.

A multiplex quadruple-label time-resolved FP immunoassay has been established for simultaneous quantitative detection of AFB1, ZEN, and OTA in grains [53]. This assay was developed based on quadruple-label probes coupled with time-resolved fluorescent nanobeads. The probes were specific to chicken IgY-specific antibody (C line signal), AFB1, ZEN, and OTA monoclonal antibodies (T line signal). The ratios of T/C value determine the concentrations of mycotoxins. The LODs for AFB1, ZEN, and OTA were 0.04 μg/kg, 0.20 μg/kg, and 0.10 μg/kg in 6 grains (corn, soybean, sorghum, wheat, rice, and oats), respectively. The recovery rates ranged from 71.60-to 119.98%. The results obtained from this study were similar to that of LC-MS/MS [53].

A smartphone-based quantitative dual detection mode device has been developed by Liu et al. [54]. The device is integrated with gold nanoparticles and time-resolved fluorescence microspheres. The sig-nals were detected using visible light and fluores-cence, then presented in the smartphone's app-lication. The device can be used to detect AFB1, ZEN, DON, T-2, and FB1. The visible LODs were 10/2.5/1.0/10/0.5 and 2.5/0.5/0.5/2.5/0.5 μg/kg for the two methods, respectively. The quantitative limits of detection (qLODs) were 0.59/0.24/0.32/0.9/0.27, 0.42/0.10/0.05/0.75/0.04 μg/kg, respectively. The re-coveries of both immunoassays (visible light and fluorescence) ranged from 84.0% to 110.0%. It is worth noting that validation of FP immunoassay is required for every food commodity as the polar-isation of tracers can be affected by the matrix.

FL offers great advantages and broad application. Nevertheless, some challenges or limitations are yet to be solved. The background fluorescence and complex matrix components of the tested samples may interfere the fluorescence signals [55]. More-over, the corresponding antibody of multiple my-cotoxins need to be prepared for simultaneous detection. Hence, the development of novel fluo-rescent reporters will be the focused in future FL immunoassay.

4.3. Lateral flow immunoassay

LF immunoassay is a simple one-step immuno-chromatographic paper assay that does not require complex instruments [56]. Its outstanding advantages of convenience and rapidity are especially suitable for on-site monitoring of mycotoxin contamination in food. These test strips are mainly labelled with gold or enzyme [57]. The basic LF immunoassay equipment consists of sample coating pads, conjugate-release pads, absorbent pads, and nitrocellulose membranes [58]. Typically, LF immunoassay is a competitive-based mycotoxin detection assay where mycotoxin in the sample and the mycotoxin-conjugate immobi-lized on the test line compete for the specific anti-body. The absence of colour on the test line indicates the presence of an analyte, while the colour devel-oped on the test line and the control line indicates a negative result [59]. Some existing LF immunoassays have been considerably improved by combining different kinds of nanosensors or strategies for increasing sensitivity or efficiency.

LFIA-based multiplex detection of AFB1 and FUM in a single test line with multi-coloured gold nano-particles signals has been reported [60]. The nano-particles (red and blue) were attached to antibodies against the analytes. The detection of AFB1 and FUM contamination in raw and processed food was achieved with visual cut-off levels at 1 ng/mL and 50 ng/mL, respectively. Another study has com-bined microarray with LF immunoassay for simul-taneous mycotoxins detection (AFB1, DON, FUMB1, T-2, and ZEA) [61]. A strong fluorescence organic compound that can be read under UV light was utilized. The LOD of AFB1, DON, FUMB1, T-2, and ZEA were 1.3, 0.5, 0.4, 0.4, and 0.9 ppb, respectively. The recoveries of these five mycotoxins were 70.7–119.5% and 80.4–124.8% for intra-assay and inter-assay, respectively. The device offers high specificity and high sensitivity.

A multiplex LFIA-based on surface-enhanced Raman scattering was established to detect six main fungal toxins in maize [62]. The Au@Ag nuclear shell nanoparticles used in the LFIA are composed of two

types of Raman reporter molecules, 5,5-dithiobis-2-nitrobenzoic acid and 4-mercaptobenzoic acid, which distributed on three test lines of nitrocellulose membrane. Upon optimisation, the limits of detection were as low as 0.96 pg/mL for AFB1, 6.2 pg/mL for ZEN, 0.26 ng/mL for FUMB1, 0.11 ng/mL for DON, 15.7 pg/mL for OTA, and 8.6 pg/mL for T-2 toxin, respectively. The spiking experiment showed high accuracy with recovery of 78.9−106.2% and satisfactory assay precision with the coefficient of variations below 16%.

A recent review has summarized the recent development of LFIA for both single and mutiplex mycotoxin analysis [63]. The development of LFIA provides a promising technique for multiplex, highly sensitive, and on-site detection of mycotoxins.

4.4. Electrochemical immunosensor

The EC immunosensor, a type of biosensor, employs the antibody as a capture agent and quantitatively measures the electrical signal resulting from the binding event between the antibody and analyte [64]. EC sensors are characterized by simple operation, outstanding sensitivity, low cost, and facile miniaturization and have become a promising strategy for addressing specificity and sensitivity in detection. EC sensors are solid-state devices containing specific recognition elements, signal transducer, and electrochemical display [65]. The EC compounds are produced upon interactions between mycotoxin and antibody/aptamer on the transducer surface [66]. Aptamers are short, single-stranded DNA or RNA (ssDNA or ssRNA) molecules that can selectively bind to the analyte [67]. Electrochemical signal transducers, on the other hand, are normally made of Au, indium tin oxide, and carbon.

The first mycotoxin multiplex EC immunosensor was reported for the detection of FUMB1 and DON [68]. A disposable screen-printed carbon electrode modified by nanoparticles (Au and polypyrrole-electrochemically reduced graphene oxide) was used as sensing platform. Such modification enhanced anti-toxin antibody immobilization, electrical conductivity, and biocompatibility. The LODs were 4.2 ppb for FUMB1 and 8.6 ppb for DON.

In other study, Wei et al. [69] established a simultaneous detection method of multi-residue using surface plasmon resonance technique to measure AFB1, OTA, ZEN and DON in corn and wheat with high sensitivity, accuracy and specificity. Surface plasmon resonance sensor chip was fabricated based on self-assembled monolayer. The LODs for AFB1 (0.59 ng/mL), OTA (1.27 ng/mL), ZEA (7.07 ng/mL) and DON (3.26 ng/mL) were

identified. In addition, low cross-reactivity for all four mycotoxins were demonstrated in the study.

A dual-channel three-electrode electrochemical sensor pattern was etched on a transparent indium tin oxide-coated glass via photolithography and was integrated with capillary-driven polydimethylsiloxane microfluidic channel [70]. The two working electrodes were functionalized with gold nanoparticles and anti-FB1 and anti-DON antibodies. Tests were performed by incubating the working electrodes in a sample solution introduced in the PDMS channel. The formation of toxin-antibody immunocomplexes on the working electrode surface produced electrochemical signal responses, which were recorded and compared with the control signal to quantify individual mycotoxin concentrations. Such dual-channel ITO-microfluidic electrochemical immunosensor able to achieve LODs of 97 pg/mL and 35 pg/mL, respectively for FB1 and DON, and their corresponding linear ranges of detection were 0.3−140 ppb and 0.2−60 ppb.

EC immunosensor provides reliable quantitative results at low cost of assay and equipment. However, EC immunosensor is sensitive towards pH, ions and temperature of the samples [71]. Therefore, such factors should be taken into account during mycotoxin quantification.

5. Pitfalls of mycotoxin analysis

Various problems have been found in the analytical chemistry of mycotoxin. The accuracy of the mycotoxin analysis is greatly dependent on the accuracy of sampling. The distribution of analytes in the food/feed commodities is highly heterogeneous [72]. A previous study showed that the coefficient of variation in the determination of AF concentration in peanuts is between 60% and 120% for sampling, dropped to 20% for subsampling, and remained 10% for the analysis [73]. Inappropriate sampling techniques may lead to inaccurate determination of mycotoxin contents in the food/feed. Such items available in the market pose a huge health risk to consumers. The common issues in sample preparation include inadequate purification, presence of artifacts, loss of analyte, and false recovery calculation [74]. The introduction of internal standards in the sample before the extraction and clean-up may solve part of the problem.

All analytical methods of mycotoxins should be fully validated by establishing compliance to various criteria: selectivity, linearity, the limit of detection, the limit of quantification, decision limit, detection capability, intra-/interday precision, recovery, accuracy, and robustness [75]. It is worth noting that

the golden standard of validations and certified reference materials are necessary for the process of method validation. Besides, interlaboratory studies and comparisons could assist in identifying possible analytical issues with mycotoxin analysis [76].

Noteworthy, mycotoxins may be converted into modified forms by plant detoxification systems [77]. Such modifications lead to modified chromatographic profiles, epitope conformation, or polarity, these mycotoxin derivatives usually escape conventional analytical methods and are not regulated by legislation and thus are called "masked" mycotoxins.

6. Toxicogenomics

Toxicogenomics is a new paradigm of toxicology inspired by the rapid development of microarray technologies in toxicology study. Today the field has expanded to include proteomics, genomics, transcriptomics, metabolomics, as well as epigenomics [78]. It is a sub-discipline of toxicology that deals with hazard identification, mechanistic toxicology, and risk assessment. Various toxicogenomics technologies measure hundreds to thousands of molecules (eg, DNA, RNA, proteins, lipids, and metabolites) from non-clinical and environmental toxicity studies. Since its introduction, toxicogenomics is frequently applied as a biomarker discovery tool that diagnoses or predicts disease. Furthermore, it has been extremely useful to shed light on the cellular and molecular mechanisms that are responsible for the toxicity [79]. With the presence of advanced developments in bioinformatics and analytical technologies, omics studies analyses can now be employed to understand the roles of biomolecules. Besides, the technologies help to recognize the relationships, systemic effects, and mechanisms of actions of compounds/agents [80].

These molecular techniques can be categorized into targeted and non-targeted [81]. Untargeted experiments intend to quantify the broadest range of changes in proteome, genome, transcriptome, or metabolome found in an extracted sample without prior knowledge of the possible outcomes. Undeniably, the results can be affected by the methods of extraction and analysis. The complex data gained from the results need computational software and knowledge to recognize and link the biomolecules between samples. At the same time, their interconnectivity in pathways related to the phenotype or aberrant process can be examined [82]. Meanwhile, targeted omics experiments provide higher selectivity and sensitivity compared to untargeted experiments [83]. The biomolecules analyzed are based on information gathered from the literature review. Besides, optimal methods are established for the analysis of targeted biomolecules and pathways. This review focus on untargeted omic toxicogenomic studies performed in recent years.

A recent study revealed that ZEA treatment for up to 40 µg/mL resulted in significant increase in generation time and decrease in egg production of *Caenorhabditis elegans* [84]. There were 3149 gene expressions altered by ZEA. The most affected genes were involved in development and reproduction. The genes responsible for collagen synthetic pathway was 20 folds lower compared to untreated *C. elegans.* The results demonstrate that disruption of the collagen biosynthetic pathway might be a key mechanism in ZEA-induced reproductive and developmental toxic effects in *C. elegans*. Another study by Liew et al. [85] also revealed that AFB1 altered gut proteomes of the rats via several pathways related to the occurrence of inflammation, cancer, and ROS generation as described in Fig. 2. Proteomic profiling in the study was performed using LC/MS/MS. Results showed that AFB1 downregulated five pathways and upregulated 19 pathways found in Reactome database.

The effects of multiple mycotoxins on human and animals have attracted the attention of researchers. A study through integrated metabolic pathways has suggested that compared with individual mycotoxins, the combination of DON and ZEN group weakens the toxic effect in mouse liver, indicating the antagonistic effect of DON + ZEN treatment [86]. The mice were subjected to combined 2 mg/kg DON and 20 mg/kg ZEN for 21 days. The metabolic pathway analysis was performed with MetaMapp and drawn by CytoScape. The metabolic pathway analysis demonstrated that the combined DON and ZEN treatment could down-regulate the valine, leucine and isoleucine biosynthesis, glycine, serine and threonine metabolism, and O-glycosyl compounds related glucose metabolism in liver tissue. The metabolic profiling in serum confirmed the finding that the combined DON and ZEN treatment has an "antagonistic effect" on liver metabolism of mice. On the other hand, Gao et al. performed transcriptomic (RNA) and proteomic profiling (iTRAQ) on intestinal epithelial cells (Caco-2 cells), stimulated with aflatoxin M1 (AFM1) (4 µg/mL) and OTA (4 µg/mL) [87]. Such cross-omics analysis identified several mechanisms induced by AFM1+OTA. Up to 10,906 genes were altered by AFM1+OTA, where 10,004 genes were downregulated. The highest number of genes were related to inflammation. Meanwhile, transcriptomic analysis demonstrated that AFM1 and OTA primarily activated the signaling pathways involved in immunity/inflammation, including
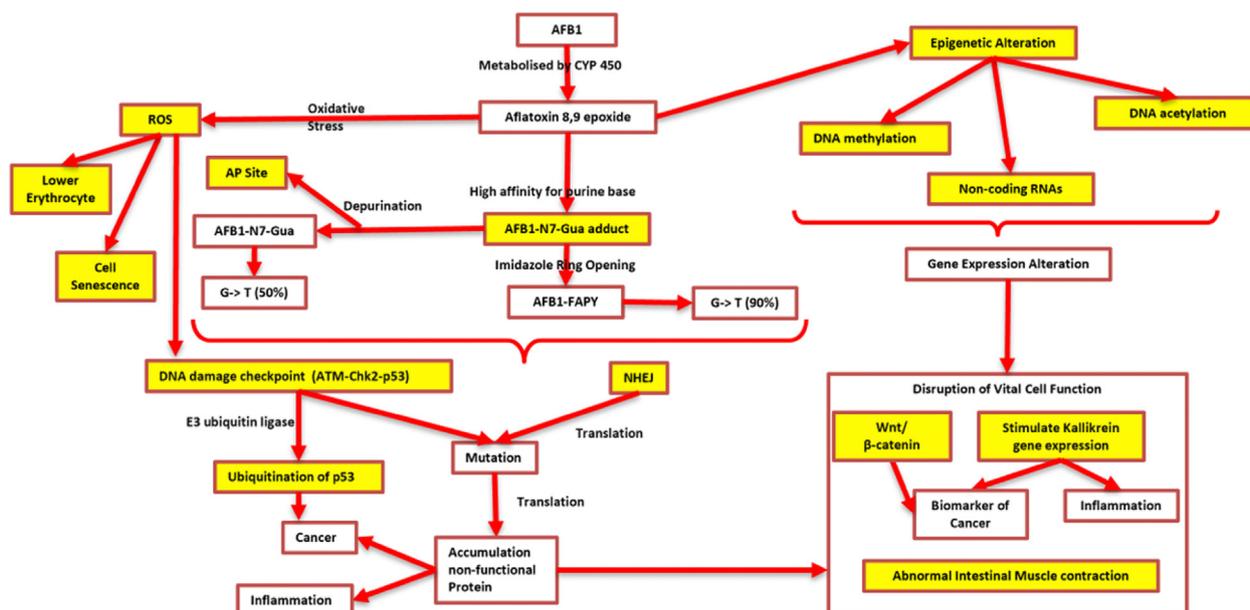
Fig. 2. Reactome pathways related to AFB1-induced toxicity (highlighted in yellow). Adopted from Liew et al. [85] https://doi.org/10.1016/J.FCT.2022.112808, with permission from Elsevier.

complement and coagulation cascades and the TNF, transforming growth factor (TGF)-β, T cell receptor, B cell receptor, and Notch signaling pathways. The transcriptomic and proteomic profiling results in this study also showed that the genes and proteins altered by single mycotoxins is different from combination of mycotoxins [87]. This highlights the need for toxicogenomic applications in co-occurrence of mycotoxins.

Toxicogenomic studies provide a new aspect in the assessment of environmental exposure. These studies offer valuable information at molecular level which can be used to identify the mode of toxicity mechanism of mycotoxin. Due of their sensitivity, toxicogenomic technologies are expected to reveal more than has been possible to date about the potential effects of exposure to toxic substances even in the early stage. Such data also improves the understanding of the variability in reactions of humans and animals towards mycotoxins.

7. Conclusions and perspectives

Mycotoxins in food currently present a global threat and require sensitive and selective detection. Importantly, the isolation and extraction method of mycotoxin should be optimized and reproducible for further detection. The market today for mycotoxin test kits is already a competitive one with multiple companies selling variations of similar products. Conventional mycotoxin detection methods used in regulatory agencies has been deemed unsuitable for the current situation where multiple mycotoxins are

present in the food/feed. Immunoassay techniques able to improve the situation by providing higher sensitivity, precision, and simplicity as well as cost-effective. Growing progress in immunoassay technologies is keeping its pace over the past 5 years for reliable mycotoxin detection. Although pioneering studies have recently been performed, immunoassays with specificity and high throughput for multiple mycotoxin detection need to be further investigated.

Based on the literature, on-site monitoring of multiple mycotoxins using hand-held digital biosensors is the key development to control risk assessment. Nonetheless, immunoassays are still the current detection method of choice. Besides, toxicogenomic is likely to play a role in mycotoxin regulation and litigation. In fact, the development of tools and approaches for elucidating the mechanisms involved in toxic responses helps to enhance risk assessment by regulatory authority.

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Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Disclaimer

Conflict of interest

Acknowledgments

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