Chapter

Recent Biosensors Technologies for Detection of Mycotoxin in Food Products

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Abstract

Mycotoxins are chemically diverse and capable of inducing a wide diversity of acute and chronic symptoms, ranging from feed refusal to rapid death. Accurate detection and monitoring of mycotoxins is an essential component of the prevention, diagnosis, and remediation of mycotoxin-related issues in livestock and human food. Current trends in food analysis are focusing on the application of fast, simple procedure needed, and low-cost biosensor technologies that can detect with high sensitivity and selectivity different compounds associated with food safety. This chapter discussed the recent analytical methods-based biosensor technology for quantification of mycotoxins in food products. Mainly focus on the biosensor technology based on the immobilization of antibodies onto various nanomaterials such as nanoparticles, graphite, carbon nanotubes, and quantum dots. The nanomaterials are able to be functionalized with various biomolecules such as enzymes, antibodies, nucleic acids, DNA/RNA aptamers, bio- or artificial receptors that make them suitable for detection of various substances such as food toxins, bacteria, and other compounds important in food analysis. All the nanomaterials provide an effective platform for achieving high sensitivity that is similar and, in some cases, even better than conventional analytical methods. We believe that future trends will be emphasized on improving biosensor properties toward practical application in the food industry.

Keywords: mycotoxin, biosensor, nanomaterials, analytical methods, fungi

1. Introduction

Fungi are an organism that exists either in single-celled or complex multicellular organisms. This number of the organism may cause diseases by producing toxic substances which known as mycotoxins. Mycotoxins are toxic secondary metabolites of various fungi that significantly impact global food safety and security, from toxin exposure, economic loss of crops, or the salability of said crops. They are a widespread mixture of contaminants in various agricultural and food products, with both acute and chronic toxicological effects on human health [1]. Mycotoxin produced mainly by mycelial structure of filamentous fungi or specifically molds that may cause a harmful effect to animals as well as humans such as carcinogenic, nephrotoxicity, mutagenic, immunosuppressive, estrogenic neurotoxicity, reproductive and developmental toxicity, hepatotoxicity and indigestion [2].

Mycotoxins including aflatoxins (AFs), ochratoxins (OT), trichothecenes, zearalenone (ZEN), fumonisins (F), tremorgenic toxins, and ergot alkaloids mostly affect the public health and agro-economic significance. Factors affecting the magnitude of toxicity to the living organism are by consuming mycotoxin-contaminated foods or feeds, including species, mechanisms/modes of action, metabolism, and defense mechanisms [3]. Most of the countries agreed to set the limits of mycotoxins present in food because of the effects of the mycotoxins to human health. The permitted level is slightly different, which depends on the type of food products. The minimum limits for mycotoxins in single ppb (part per billion) and even below (0.05 ppb for infant foods) are established in EU, with similar standards in China and Japan [4].

Guan et al. [5] reported about 98% of the agricultural commodities, including corn, compound animal feeds, silage, cornmeal, puffed corn, wheat, bran, soybean meal, rapeseed meal, cottonseed meal and whole cottonseed content various group of mycotoxins. Besides, Smith et al. [6] stated that several mycotoxins contaminate approximately 48% of 7049 feedstuffs. Thus, it is essential to detect mycotoxins in the food industry to address the mycotoxin-related health issues to humans and animals effectively.

Conventional techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and mass spectrometry have been suggested by international organizations as standard approaches to study the occurrence of mycotoxins in food products [7]. Besides, enzyme-linked immunosorbent assay (ELISA) had been widely used to identify different types of mycotoxins. However, it has slight defects of cross-reactivity and possible false-positive or false-negative outcomes [8]. Also, those techniques usually costly and available in a specialized research laboratory needs highly personnel trained and laborious. Recently, advanced methods used to detect the presence of mycotoxins in food samples, which show high sensitivity, low cost, simple operation, and portable on-field use [9]. Besides, portable and easy-to-use biosensor devices suitable for express, in-field detection of mycotoxins. The development of biosensors for mycotoxins has risen sharply in the last decade with a large number of different bio-sensing technologies application. Zheng et al. (2006) reported biosensor as rapid methods which typically cost-effective, easy to be handled as well as a portable device to be used in an interchanging site compared to laboratory analysis.

2. Mycotoxin

Fungal toxins are secondary metabolites, which can cause some diseases in living things known as mycoses; meanwhile, dietary exposure to such metabolites produces the disease named mycotoxicoses. Mycotoxins are known as secondary metabolites, produced from microfungi and able to cause–effect human health as well as animals. Mycotoxins are commonly used as antibiotics and growth promotants because of their unique characteristics in pharmacological activity. Most of the mycotoxin are found as natural contaminant food, mainly in vegetable and feed. Nut, cereals, oilseeds, dried fruits, spices, and food from animal origins for example milk, egg, and meat are also may contain mycotoxin either outside or inside the product [10, 11]. A mycotoxin is believed no function in the life of a producer cell, unlike primary metabolites [12]. There are few types of mycotoxin such as aflatoxins (AFs), zearalenone (ZEA), deoxnivalenol (DON), ochratoxin (OTA) and T-2 toxin (trichothecene mycotoxin) which are a significant threat to the life and health of human and live stocks [13]. Mycotoxins are low molecular weight and thermal-stable secondary metabolite of toxic molds that belong to genera *Aspergillus*,

Penicillium, Alternaria, and *Fusarium*. These toxins are present in the mycelium and spore of the mold. Mycotoxin may become a biological weapon in bioterrorism because of its acute and chronic toxicities [14].

3. Types of mycotoxin

The established mycotoxins for agriculture and public health concerns including aflatoxins, ochratoxins, zearalenone, T-2 and HT-2 toxin, deoxynivalenol, fumonisins, citrinin, patulin, and ergot alkaloids shown in **Figure 1**. Aflatoxins B1 and M1 (AFT B1 & M1) [15] produced by *Aspergillus flavus* and *A. parasiticus* species grown on grains and cereals, spices, tree nuts. Aflatoxin B1(AFB1) is one of the most carcinogenic substances produced by fungi and results in inevitable contamination of food and feed at deficient concentrations. Four main types of aflatoxin naturally contaminate foods which are aflatoxin B1 (AFB1), G1 (AFG1) and their dihydroderivatives B2 (AFB2) and G2 (AFG2). Others without additional metabolites known as Aflatoxin M1 and Aflatoxin M2 [16]. AFT M1 being a 4-hydroxylated metabolite of AFT B1, is found in cow and sheep milk and milk products. Some studied had been identified there is 20 aflatoxins that belongs to a group called highly substituted difuranocoumarins. The International Agency for Research on Cancer (IARC) had been classified aflatoxin as very toxic compounds in group 1 due to evidence that shows the carcinogenicity in human [17].

Ochratoxin A (OTA) produced by *Aspergillus ochraceus, A. carbonarius,* and *Penicillium verrucosum* is one of the most abundant contaminants in grain and pork products, coffee, dried grapes, as well in wine and beer at humidity around 15–19%

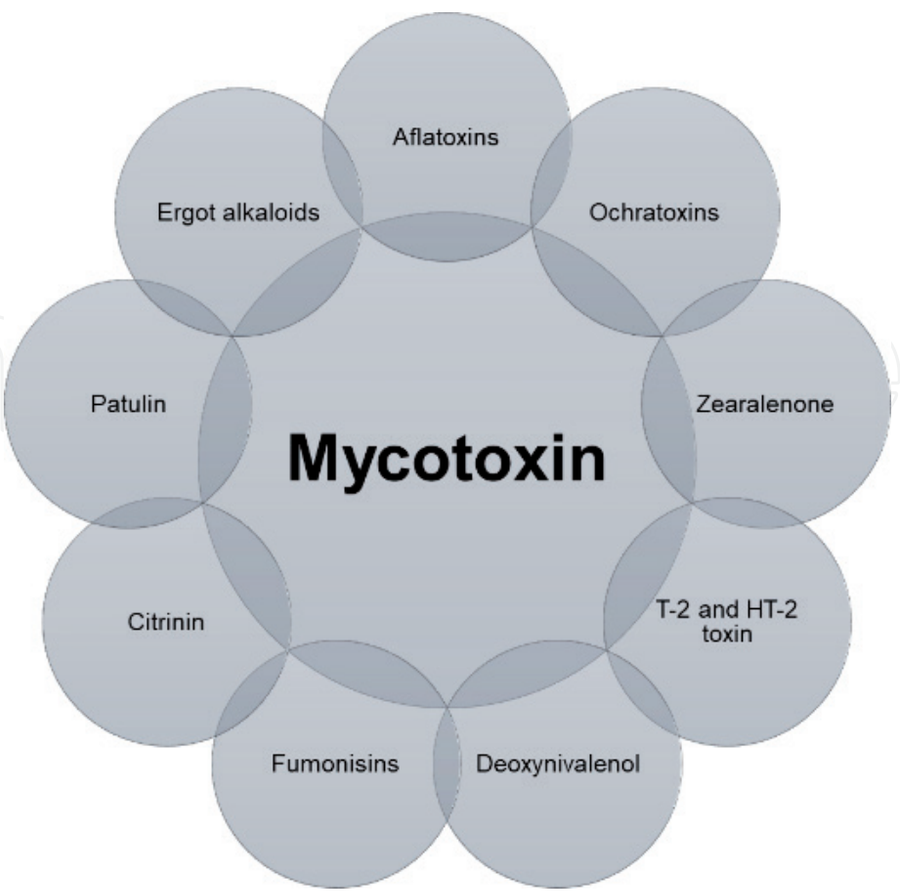

Figure 1.
Primary groups of mycotoxins in various food products.

and temperature ≥15°C [18]. OTA is carcinogenic and neurotoxic for humans, and immunotoxic for animals [19]. OTA can cause various forms of kidney, liver, and brain diseases in both humans and animals, although the trace amount of OTA usually is present in food [20].

Zearalenone (ZEN) produced by *Fusarium* or *Giberella* species grown on crops (maize, barley, oats, wheat, rice, also bread) is a potent estrogen metabolite causing infertility in swine and poultry [21].

4. Isolation of a mycotoxin from real samples

4.1 Solid-phase extraction (SPE)

A variation of chromatographic techniques based on small disposable cartridges packed with silica gel or bonded phase, which in the stationary phase is the basic principle of solid-phase extraction. The sample loaded in one solvent under low pressure and rinsed to remove the most of contaminant are moved and eluted in another solvent. These cartridges have a high capacity for small binding molecules. Different bonding phase such as silica gel, aminopropyl, florisil, phenyl, ion exchange materials, anionic and cationic to affinity materials including immunoadsorbents and molecular imprinted polymers (MIPs) are available in SPE cartridges [22]. OTA formation occurs in some Spanish sweet, which going drying process. C-18 column had been shown successful recovery above 90% of OTA, which enables to be isolated from the matrix [23]. Silica gel frequently used in SPE because the surface of silica particles is heterogeneous with a variety of silanol group which can bind target compound through multiple electrostatic interactions. Generally, silica gel was used directly or after modification, and it is a hydrophobic phase which used in environmental and food analysis of toxin, which performed both polar and non-polar solvents. Previous research conducted by Leitner et al. [24] showed that the use of C-18 reverse-phase in the extraction of OTA from wine and offer good result with combination with mass spectroscopy.

4.2 Liquid: liquid extraction (LLE)

Liquid–liquid extraction (LLE) or also known as solvent extraction agitating different solubility of toxin in the aqueous phase and an immiscible organic phase to extract the compound into one solvent and leaving the rest of matrix in others phase. A solvent such as hexane and cyclohexane are used to remove non-polar contaminant or molecule, for example, lipids, and cholesterol [25]. The common goal of LLE is sample clean-up and analyte component pre-concentration. Sample clean-up requires high selectivity of partitioning analyte component over potential interferents while analyte component pre-concentration require high distribution ratio to analyte can be extracted from a large volume of sample too small volume of extractant. Two bulk-liquid phases at least which are an aqueous phase that contains dissolved sample an organic extractant phase. The variety of condition will decide either the agitated mixture become the dispersed phase and another continuous phase. The thermodynamic driving force is resulting from the movement of chemical species from one bulk phase to another in two ways either by the difference in chemical potential for neutral species or electrochemical for ionic species [26]. Lately, Ezekiel et al. [27] used acetonitrile/water/acetic acid 79:20:1, (v/v/v) in a 50 mL polypropylene for the metabolites extraction and determination of apparent recoveries.

4.3 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) had been used for years for industrial-scale separation and isolation of variety compound. SFE also has been utilizing in the field of food science to isolate not only natural food component but also unnatural compound like organic contaminants. SFE was developing and used as an alternative to extraction using liquid solvents. SFE considered an up-and-coming technique for the future because supercritical fluids have useful physical properties such as high viscosity and high diffusion constant for sample extraction which result in faster mass transport than regular and shorter the time for extraction. Using compressible gas like carbon dioxide (CO_2), the solvation power can be changed by altering the density or decrease the pressure to atmospheric pressure [28].

Most common supercritical fluid (SF) used is SC-CO_2, which is a suitable substituent for halogenated solvents. This is because the carbon dioxide is non-toxic, non-flammable, not significantly contribute to global warming and might be the cheapest solvent except for water. The usage of SFE to extract mycotoxin are very limited until recently because of the relative polar nature of mycotoxin and relative non-polar nature of food commodities such as nut and nut product. Taylor et al. [29] investigated the use of analytical SFE to remove aflatoxin Bi from field inoculated corn samples. Modification using a combination of various pressures "(2000-15,000 psi), temperatures (40–80°C), the quantity of SC-CO_2 (50–500 ml), and organic modifiers were used to optimize the extraction method. Optimal conditions were 5000 psi at 80°C with 15% modifier (acetonitrile/methanol 2:1) and a liquid carbon dioxide volume of 100 ml. The result gained from the extraction was 94.6% (RSD 6.2%, n = 5) of aflatoxin Bi could be recovered from ground corn contaminated at a level of approximately 500 μg/kg when using these settings.

5. Advanced techniques for detection of mycotoxin based biosensor

The integration of bioreceptors, nanomaterials, and different read-out techniques is capable of accomplishing the rapid, sensitive, and multiplexed detection of mycotoxins. In this section, the advanced applications of different read-out biosensors, including optical, EC, mass-sensitivity, and surface-enhanced Raman spectroscopy biosensors, integrated with the bio-receptors above and nanomaterials, are discussed (**Figure 2**).

5.1 Electrochemical biosensors

A biosensor is an analytical device that incorporating a bio-component or bio-receptor such as isolated enzymes, whole cell, tissues, aptamers with a suitable transducing system to detect chemical compound [30]. Measurement of the signal is generally electrochemical for biological, and this bio-electrochemical serves as transduction component in electrochemical biosensors. The biological reaction generates change in signal for conductance or impedance, measurable current or change accumulation, which can be measured by conductometric, potentiometric, or amperometric techniques [31]. The interaction between the target molecule and the electrical signal of bio-component produced can be measured.

Electrochemistry has been widely used in various fields, due to their high selectivity and sensitivity, high signal-to-noise ratio, simplicity, miniaturization, low cost, robust to liquid samples and more feasible for on-site application [20]. The electrochemical technique requires a reference, auxiliary, and a working electrode. Two exciting compounds are analyzed using compound biosensors that have interest

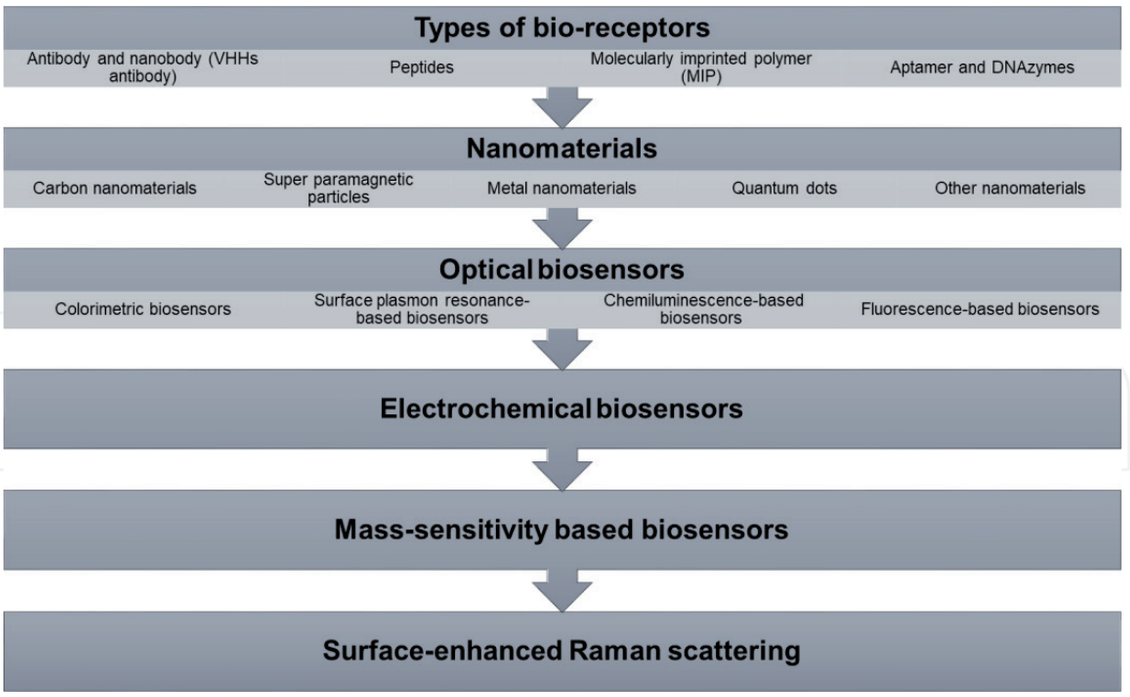

Figure 2.
The applications of different read-out biosensors integrated with bioreceptors and nanomaterials.

for nutritional food quality and contaminant such as toxin or pathogen that supposed not to be found in food products [30]. Selection of suitable working electrode is a crucial part of successful electrochemical measurement either by modification in working electrode materials or traditional metals such as mercury or gold [32].

Due to the widely occurring co-contamination of mycotoxins in raw food materials, Lu and Gunasekaran [33] designed and fabricated of an electrochemical immunosensor for simultaneous detection of two mycotoxins, fumonisin B1 (FB1) and deoxynivalenol (DON), in a single test. A dual-channel three-electrode electrochemical sensor pattern was etched on a transparent indium tin oxide (ITO)-coated glass via photolithography and was integrated with capillary-driven polydimethylsiloxane (PDMS) microfluidic channel. The achieved detection limits found 97 and 35 pg./mL, respectively. Besides, Nieto et al. [34] A third-generation enzymatic biosensor were developed to quantify sterigmatocystin (STEH). It was based on a glassy carbon electrode modified with a composite of the soybean peroxidase enzyme (SPE) and chemically reduced graphene oxide. A third-generation enzymatic biosensor to quantify STEH in corn samples spiked with the mycotoxin. The biosensor was based on glassy carbon (GC) electrode modified with a composite of SPE and chemically reduced graphene oxide (CRGO). The biosensor was also used to determine STEH in corn samples inoculated with Aspergillus flavus, which is an aflatoxins fungus producer. The biosensor showed a linear response in the concentration range from 6.9×10^{-9} to 5.0×10^{-7} mol L^{-1}. The limit of detection was 2.3×10^{-9} mol L^{-1} for a signal: noise ratio of 3:1.

5.2 Aptasensor

The aptamer is referred to the Latin word, aptus means "to fit," which relationship between aptamers and their target look like "lock-and-key" theory [35]. Aptamers usually single-stranded RNA or DNA, which consist of 2–60 nucleotides, which specifically bind to the target, including organic molecules and cells. Aptasensors referred to biosensors using aptamers as biorecognition element and aptasensor were described in 1996 [36] which had been used in multiple sensing applications.

Advantages using aptamers are aptamers can provide high stability and affinity. Aptamers also provide simplicity, low cost, and excellent batch-to-batch reproducibility. Aptasensor can attract massive attention because of excellent binding constant toward most mycotoxins. The critical step in the design of biosensors is immobilization of aptamers because this factor can affect the affinity of the aptamer for target and long-term stability for real sample. There are several immobilization strategies affect the used for aptasensor development. Firstly, the adsorption or π-π interaction between DNA bases aptamer and graphene oxide (GO)-modified interfaces [37]. The covalent linkage of the aptamer to the carboxylic acid group that presents on surface or nanomaterial [38] and thiolated binding aptamers to CdTe quantum dots (QDs) or Au-based materials [39]. Besides, affinity binding based on biotin-streptavidin or other affinity interaction [40, 41] and hybridization of partially complementary single-stranded DNA which immobilized on surface or nanoparticle [42]. Duan et al. [43] developed multicolor quantum dot nanobeads for simultaneous qualitative immunochromatographic detection of mycotoxins (ZEN, OTA, and FB_1) in corn samples with detection limits reached up to 5, 20, and 10 ng/mL within 10 min, respectively.

5.3 Immunosensor

Immunosensors are devices based on the detection of analyte-antibody interaction. Three main groups have been developing, which are luminescent or colorimetric sensors, surface plasmon resonance, and electrochemical sensors. The sensor usually combined with simple methanol–water for the extraction of a mycotoxin from food samples. Colorimetric and luminescent are based on the visible or UV light transformation into an analytical signal [44]. A colorimetric sensor developed for AFB1 detection using direct competitive ELISA principle. The color was detected and measured with spectrometer by reading absorbance at 620 nm. According to Garden and Strachen [45], this method could detect AFB1 as low as 0.2 ng/mL in artificially contaminated food material as compared to the sensitivity of a microtitre plate ELISA.

Surface plasmon resonance (SPR) is an optical phenomenon which used for measure changes on the surface of thin metal films (Au or Ag) under condition total internal reflection [46]. The sensitivity of SPR sensors and microtiter plate ELISAs were compared for detection of AFB1 using same immunoreagents, which are a polyclonal antibody and AFB1-BSA conjugate. As a result, the SPR sensor (3.0–49 ng/mL) is a more sensitive but narrow and linear range of detection compared to ELISA (12–25,000 ng/mL) [47]. Electrochemical immunosensor for mycotoxin are based on competitive ELISA principle, which electrochemical transducer allows detection redox directly [44]. Pemberton et al. [48] in their study, a calibration plot AFB1 obtained over the concentration range from 0.15 to 2.5 ng/mL, which give detection limit around 0.15 ng/mL in buffer solution.

OTA is small molecules that possess one epitope and no more than one antibody can bind due to their small molecular size. This molecule was detected using a competitive assay rather than a sandwich assay format. The competitive assay is based on the competition of immobilized antigen and a free antigen for the antibody in solution. One of the critical parameters to determine the sensitivity and limit of detection (LOD) is antibody concentration. The excessive antibody in solution may cause more antigen needed to create a measurable difference in signal. Therefore, to increase the binding capacity, protein conjugate such as SPR sensor development was used which the OTA either directly conjugated to BSA or PEG. The sensitivity increased with decreasing antibody concentration because the PEG-linked surface needs less initial antibody concentration for efficient analysis. Pirincci et al. [49]

described that the OTA-sensitive QCM sensor was developed by direct immobilization of OTA to the sensor surface.

5.4 Molecularly imprinted polymer (MIP)

Molecular imprinted polymer (MIP) is a method which is described as a method that highly efficient in producing functional material that able to mimic natural recognition entities, such as antibodies and biological receptors [50] which equipped with particular identification characteristics. In 2009, an electrochemical sensor was built by Pardieu et al. [51] for the method of detection. Thus, this method is used to recognize a specific element for template molecule detection.

MIP is used in various field of application to recognize biological and chemical molecules including amino acids and proteins [52], nucleotide derivatives, pollutants, drugs and foods [53]. Molecularly imprinted polymer method had been applied in chromatography for HPLC and GC, Solid phase extraction, Chemical sensor systems, catalysis, drug delivery, antibodies, and receptors system [54]. The formation of a complex between an analyte and the functional monomer determines the Molecularly imprinted polymer. A three-dimensional polymer network is formed due to the presence of a significant excess of a cross-linking agent [55]. A specific recognition site is formed which complementary in shape, size, and chemical functionality to the template molecule as the template being removed from the polymer after the polymerization process occurs as shown in the figure. The recognition phenomena occur when the intermolecular interactions such as hydrogen bonds, dipole–dipole, and ionic interactions between the template molecule and the functional groups present in the polymer matrix. This method is used due to their high selectivity and affinity for the target molecules. Therefore, the recognized polymer will bind to the template molecule only selectively.

The molecularly imprinted materials have excellent physical and chemical characteristics. The materials can resist high physical and chemical reaction against external degrading factor. Thus, the molecularly imprinted polymer is stable against mechanical stress, high temperature, and pressure, resistant against treatment with acid, base, or metal ions, and also stable in a wide range of solvents [56]. Sellergren firstly reported the application of MIP in solid phase extraction in 1994. Generally, the MIP as a sorbent was recognized as an accurate, selective, and sensitive pre-treatment method in detecting trace amounts of chemicals in the matrix. The application of MIP in solid phase extraction is used for veterinary residues, pesticides residue, illegal drugs, mycotoxins, and persistent organic pollutants had been published.

5.5 Optical biosensors

Biosensors can be divided into different groups, which are electrochemical, optical, thermometric, piezoelectric, or magnetic [57]. Somehow, the optical biosensor is the most preferred among the other methods. This is because it has powerful analytical techniques which have a high specification, sensitivity, small size, and cost-effectiveness [58, 59]. An optical biosensor is a device which is selective and sensitive that can detect deficient levels of chemicals and biological substances and for the measurement of molecular interactions in situ and in real time [60].

Optical methods, such as colorimetric, fluorescent, chemiluminescent, and surface plasmon resonant strategies, are proper techniques for mycotoxins detection due to their simplicity, rapidity, reliability, and high sensitivity. An optical biosensor is a system which combined various entities in a single system such as sampling, a biosensor, a system for replenishing information, and a data analysis system which to implement a biological model that provides information for human

or machine [57]. The biosensor systems are developed by crucial attributes, which are the integration of fluidics, electronics, separation technology, and biological sub-systems. An optical biosensor is a compact analytical device, having a biological sensing element, integrated or connected to an optical transducer system. In this method, the analyte of interest that binds to the complementary optical bio-recognition element is recognized as immobilized on a suitable optical substrate [61]. An electronic signal is produced which the magnitude of the frequency is proportional that correspond to the concentration of an analyte or a group of analytes, to which the element will bind is the objective of optical biosensors [62]. Meanwhile, enzyme, substrate, antibody, and nucleic acids are used as the primary biological materials in optical biosensor technology [57]. The detection usually relies on an enzyme system which converts the analytes to products catalytically and can be oxidized or reduced at a working electrode.

Optical biosensing has two general modes, which are label-free and label-based. For label-free mode, the interaction of the analyzed material with the transducer will generate a detectable signal. On the contrary, the use of the label and the optical signal then generated by a colorimetric, fluorescent, or luminescent method are involved in label-based sensing [63]. The usage of optical biosensor depends on the different fields of use. This is because it has own requirements in term of measuring analysis, required precision of output, the sample concentration required, the time taken to complete the probe, the time necessary to prepare and reuse the biosensor, and the cleaning requirements of the system [57].

In the food industry, this method is used for the direct detection of bacteria in products. Optical biosensor used to detect the changes of refractive indices as the cell bind to the receptor, which is immobilized on the transducer [49]. The advantages of using optical biosensors are their speed, immunity of signal to electrical or magnetic interference. Besides, it is highly sensitive, reproducible, and simple-to-operate analytical tools. Somehow, some instrumentation involved in this method high in cost. Nabok et al. [4] reviewed the recent progress in the development of novel optical biosensing technologies for the detection of mycotoxins indirect assay with either specific antibodies or aptamers.

5.6 Enzymatic inhibition

There are a variety of enzymes such as cholinesterase, urease, glucose oxidase and more that have been applied in an enzymatic inhibition analysis and this method is pretty standard [64]. According to Puiu et al. [65], Acetylcholinesterase (AChE) is the most commonly used enzyme, and the reason is it is susceptible toward mycotoxin which is becoming the preferred method for mycotoxin detection. This statement is also supported by [66], which stated that biosensors for Aflatoxin B1 (a type of mycotoxin) or AFB1, in short, is developed by using AChE due to the inhibitory effect of AFB1 to AChE enzymatic activity. Also, the inhibitory effect of mycotoxin is a reversible process due to the non-covalently binding nature to the enzyme [67]. Soldatkin et al. [68] stated that aflatoxin showed the highest sensitivity toward enzymatic inhibition method among the other groups of toxins. A past study conducted by Egbunike and Ikegwuonu [69] also suggests that usage of cholinesterase in biosensor method as the biological component is usable as AFB1 detector as aflatoxicosis has been reported to be correlated with a significant reduction of acetylcholine turnover in rat brain.

Based on the previous research, it is proven that AChE is inhibited by the AFB1 from binding at the external site, which is located at the active site gorge entrance located at the tryptophan residue. The inhibitory effect of the AFB1 can be seen by its action where the toxin blocks the entrance to the active site so that the substrate

cannot enter to participate to the catalytic site result in the choline unable to exit as proposed by the steric blockade model [70]. Based on the observation in the study conducted by Hansmann et al. [71], their results lead them to two findings. The first observation is the addition of AFB1 in the binding site of the active site did not fulfill the description for inhibitory activity, and this suggests that the AFB1 does not slide to the catalytic site. As for the second observation, mutation of Trp321 to alanine in Dm-AChE put a stop on the inhibitory activity at 10 μM concentration, and AFB1 at a concentration of 100 μM does not inhibit Hu-BuChE enzymatic activity. Also, the researchers assumed that AFB1 could not enter into the active site due to its relatively big size, especially when considering the hydrophilic shell might be further increased in size. Due to this condition, aflatoxin is grouped as a ligand which binds on the external site of the cholinesterase [72].

5.7 Mimotope

Mimotope or also known as peptide-displaying phage or synthetic peptides [73] is now one of the most reliable methods that are used to identify epitopes which are detected by monoclonal antibodies which are antibodies that made by the same immune cell is given that they are clones of one single parent cell. Next, the usage of mimotope in mycotoxin detection involves the usage of peptides which are identified to be structurally not identical to the original epitope of mycotoxin but at least have the properties to mimic the epitope by binding to the antibodies [74]. Generally, this method shared instead of the same concept with enzymatic inhibition, which in this case, the mimotope will be the one that elicits antibody. Also, this method is beneficial when the original epitopes (example from a mycotoxin) are hard to be isolated and at the same time only available in minimal amount [75]. The first assay that using mimotope for detection is being done by Yuan et al. [76], where a mimotope is used to identify the mycotoxin deoxynivalenol.

A study has been conducted by Sellrie et al. [74] which aims to describe a competitive immunoassay for identification of hapten fluorescein by utilizing a monoclonal anti-fluorescein antibody B13-DE1 and a mimotope peptide which act by binding to the antibody. Based on their findings, the peptide mimotope was conjugated to horseradish peroxidase (HRP) which is then competing for binding to monoclonial antibody B13-DE1 with fluorescein. Based on the result, they have proven that mimotopes can be used to utilization in simple yet sensitive immune assays in order to quantitatively identify and determine substance with low molecular weights. As for the reliability and reproducibility, the assay was proved by validation data and found to be in the range which is described in the literature for conventional competitive immunoassays by Wild [77].

6. Advanced techniques for detection of mycotoxin based biosensor

During the last few decades, consumers have become more aware of health and food quality, consequently, research on food safety augmented. The variety of contaminants in many food products requires the development of high-throughput, real-time, and portable detection methods. The evaluation of the different mycotoxins residues in foodstuffs became an essential factor in guaranteeing the products' quality. Hence, it is essential to improve the analytical standards to detect and quantify the presence of a mycotoxin. The operation procedure should be simplified continuously for the convenience of users. The biosensor based nanotechnology can be extensively used in food contaminants monitoring and eventually become effectively routine analysis tools that could meet numerous challenges.

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Conflict of interest

The authors declare no conflict of interest.

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