Chapter

Recent Biosensors Technologies for Detection of Mycotoxin in Food Products

Kobun Rovina, Sulaiman Nurul Shaera, Joseph Merrylin Vonnie and Su Xin Yi

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 promoters 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, fumonisons, 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 $\geq 15^\circ\text{C}$ [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 immunosorbents and molecular imprint 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.
Recent Biosensors Technologies for Detection of Mycotoxin in Food Products
DOI: http://dx.doi.org/10.5772/intechopen.89022

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
Mycotoxins and Food Safety

6

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 $6.9 \times 10^{-9}$ to $5.0 \times 10^{-7}$ mol L$^{-1}$. The limit of detection was $2.3 \times 10^{-9}$ 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. Aptsensor 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 aptsensor 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 FB₁) 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 monoclonal 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.
Acknowledgements

The authors would like to thank the Universiti Malaysia Sabah for the support of this study.

Conflict of interest

The authors declare no conflict of interest.

Author details

Kobun Rovina*, Sulaiman Nurul Shaera, Joseph Merrylin Vonnie and Su Xin Yi
Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

*Address all correspondence to: rovinaruby@ums.edu.my

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
References

[1] IARC (International Agency for Research on Cancer). Mycotoxin control in low- and middle-income countries. IARC Working Group Report No. 9. 2015

[2] Pleadin J, Frece J, Markov K. Mycotoxins in food and feed. Advances in Food and Nutrition Research. Available online 6 March 2019. In Press, Corrected Proof. 2019

[3] Hussein HS, Brasel JM. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology. 2001;167(2):101-134. DOI: 10.1016/S0300-483X(01)00471-1

[4] Nabok A, Al-Rubaye AG, Al-Jawdah AM, Tsargorodska A, Marty JL, Catanante G, et al. Novel optical biosensing technologies for detection of mycotoxins. Optics and Laser Technology. 2019;109:212-221. DOI: 10.1016/j.optlastec.2018.07.076

[5] Guan S, Gong M, Yin Y, Huang R, Ruan Z, Zhou T, et al. Occurrence of mycotoxins in feeds and feed ingredients in China. Journal of Food, Agriculture and Environment. 2011;9(2):163-167. DOI: 10.1186/2049-1891-5-37

[6] Smith MC, Madec S, Coton E, Hymery N. Natural co-occurrence of mycotoxins in foods and feeds and their in vitro combined toxicological effects. Toxins. 2016;8(4):94

[7] Ferreira I, Fernandes JO, Cunha SC. Optimization and validation of a method based in a QuEChERS procedure and gas chromatography–mass spectrometry for the determination of multi-mycotoxins in popcorn. Food Control. 2012;27(1):188-193. DOI: 10.1016/j.foodcont.2012.03.014

[8] Ran R, Wang C, Han Z, Wu A, Zhang D, Shi J. Determination of deoxynivalenol (DON) and its derivatives: Current status of analytical methods. Food Control. 2013;34(1):138-148. DOI: 10.1016/j.foodcont.2013.04.026

[9] Guan J, Wang YC, Gunasekaran S. Using L-arginine-functionalized gold nanorods for visible detection of mercury (II) ions. Journal of Food Science. 2015;80(4):N828-N833. DOI: 10.1111/1750-3841.12811

[10] Adeyeye SA. Fungal mycotoxins in foods: A review. Cogent Food and Agriculture. 2016;2(1):1213127. DOI: 10.1080/23311932.2016.1213127

[11] Freire FDCO, da Rocha ME. Impact of mycotoxins on human health. Fungal Metabolites. 2017;1:239-261. DOI: 10.1007/978-3-319-25001-4_21

[12] Goyal S, Ramawat KG, Mérillon JM. Different shades of fungal metabolites: An overview. Fungal Metabolites. 2016;1:1-29. DOI: 10.1007/978-3-319-25001-4_34

[13] Anfossi L, Baggiani C, Giovannoli C, D’Arco G, Giraudi G. Lateral-flow immunoassays for mycotoxins and phycotoxins: A review. Analytical and Bioanalytical Chemistry. 2013;405(2-3):467-480. DOI: 10.1007/s00216-012-6033-4

[14] Overy DP, Seifert KA, Savard ME, Frisvad JC. Spoilage fungi and their mycotoxins in commercially marketed chestnuts. International Journal of Food Microbiology. 2003;88(1):69-77. DOI: 10.1016/S0168-1605(03)00086-2

[15] Yin YN, Yan LY, Jiang JH, Ma ZH. Biological control of aflatoxin contamination of crops. Journal of Zhejiang University. Science. B. 2008;9(10):787-792. DOI: 10.1631/jzus.B0860003
[16] Yagati A, Chavan S, Baek C, Lee MH, Min J. Label-free impedance sensing of aflatoxin B1 with polyaniline nanofibers/Au nanoparticle electrode array. Sensors. 2018;18(5):1320. DOI: 10.3390/s18051320

[17] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Chemical Agents and Related Occupations. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 100 (PT F), 9. 2012

[18] Bayman P, Baker JL. Ochratoxins: A global perspective. Mycopathologia. 2006;162(3):215-223. DOI: 10.1007/s11046-006-0055-4

[19] Mateo R, Medina Á, Mateo EM, Mateo F, Jiménez M. An overview of ochratoxin A in beer and wine. International Journal of Food Microbiology. 2007;119(1-2):79-83. DOI: 10.1016/j.ijfoodmicro.2007.07.029

[20] Alhamoud Y, Yang D, Kenston SS, Liu G, Liu L, Zhou H, et al. Advances in biosensors for the detection of ochratoxin A: Bio-receptors, nanomaterials, and their applications. Biosensors and Bioelectronics. 2019:111418. DOI: 10.1016/j.bios.2019.111418

[21] Desjardins AE, Proctor RH. Molecular biology of Fusarium mycotoxins. International Journal of Food Microbiology. 2007;119(1-2):47-50. DOI: 10.1016/j.ijfoodmicro.2007.07.024

[22] Katererere DR, Stockenström S, Shephard GS. HPLC-DAD method for the determination of patulin in dried apple rings. Food Control. 2008;19(4):389-392. DOI: 10.1016/j.foodcont.2007.04.015

[23] Hernández MJ, García-Moreno MV, Durán E, Guillén D, Barroso CG. Validation of two analytical methods for the determination of ochratoxin A by reversed-phased high-performance liquid chromatography coupled to fluorescence detection in musts and sweet wines from Andalusia. Analytica Chimica Acta. 2006;566(1):117-121. DOI: 10.1016/j.aca.2006.02.002

[24] Leitner A, Zöllner P, Paolillo A, Stroka J, Papadopoulou-Bouraoui A, Jaborek S, et al. Comparison of methods for the determination of ochratoxin A in wine. Analytica Chimica Acta. 2002;453(1):33-41. DOI: 10.1016/S0003-2670(01)01483-0

[25] Turner NW, Subrahmanyam S, Piletsky SA. Analytical methods for determination of mycotoxins: A review. Analytica Chimica Acta. 2009;632(2):168-180. DOI: 10.1016/j.aca.2008.11.010

[26] Tang S, Zhang H, Lee HK. Advances in sample extraction. Analytical chemistry. 2015;88(1):228-249

[27] Ezekiel CN, Sulyok M, Ogora IM, Abia WA, Warth B, Šarkanj B, et al. Mycotoxins in uncooked and plate-ready household food from rural northern Nigeria. Food and Chemical Toxicology. 2019;128:171-179. DOI: 10.1016/j.fct.2019.04.002

[28] Anklam E, Berg H, Mathiasson L, Sharman M, Ulberth F. Supercritical fluid extraction (SFE) in food analysis: A review. Food Additives and Contaminants. 1998;15(6):729-750. DOI: 10.1080/02652039809374703

[29] Taylor SL, King JW, Richard JL, Greer JI. Analytical-scale supercritical fluid extraction of aflatoxin B1 from field-inoculated corn. Journal of Agricultural and Food Chemistry. 1993;41(6):910-913. DOI: 10.1021/jf00030a014

[30] Rotariu L, Lagarde F, Jaffrezic-Renault N, Bala C.
Electrochemical biosensors for fast detection of food contaminants—trends and perspective. TrAC, Trends in Analytical Chemistry. 2016;79:80-87. DOI: 10.1016/j.trac.2015.12.017

[31] Bard AJ, Faulkner LR, Leddy J, Zoski CG. Electrochemical Methods: Fundamentals and Applications. New York: wiley; 1980

[32] Mishra G, Barfidokht A, Tehrani F, Mishra R. Food safety analysis using electrochemical biosensors. Food. 2018;7(9):141. DOI: 10.3390/foods7090141

[33] Lu L, Gunasekaran S. Dual-channel ITO-microfluidic electrochemical immunosensor for simultaneous detection of two mycotoxins. Talanta. 2019;194:709-716. DOI: 10.1016/j.talanta.2018.10.091

[34] Nieto CD, Granero AM, Garcia D, Nesci A, Barros G, Zon MA, et al. Development of a third-generation biosensor to determine sterigmatocystin mycotoxin: An early warning system to detect aflatoxin B1. Talanta. 2019;194:253-258. DOI: 10.1016/j.talanta.2018.10.032

[35] Peltomaa R, Benito-Peña E, Moreno-Bondi MC. Bioinspired recognition elements for mycotoxin sensors. Analytical and Bioanalytical Chemistry. 2018;410(3):747-771. DOI: 10.1007/s00216-017-0701-3

[36] Pfeiffer F, Mayer G. Selection and biosensor application of aptamers for small molecules. Frontiers in Chemistry. 2016;4:25. DOI: 10.3389/fchem.2016.00025

[37] McKeague M, Bradley CR, Girolamo AD, Visconti A, Miller JD, DeRosa MC. Screening and initial binding assessment of fumonisin B1 aptamers. International Journal of Molecular Sciences. 2010;11(12):4864-4881. DOI: 10.3390/ijms11124864

[38] Sabet FS, Hosseini M, Khabbaz H, Dadmehr M, Ganjali MR. FRET-based aptamer biosensor for selective and sensitive detection of aflatoxin B1 in peanut and rice. Food Chemistry. 2017;220:527-532. DOI: 10.1016/j.foodchem.2016.10.004

[39] Chen X, Bai X, Li H, Zhang B. Aptamer-based microcantilever array biosensor for detection of fumonisin B1. RSC Advances. 2015;5(45):35448-36452. DOI: 10.1039/C5RA04278J

[40] Guo X, Wen F, Zheng N, Li S, Fauconnier ML, Wang J. A qPCR aptasensor for sensitive detection of aflatoxin M1. Analytical and Bioanalytical Chemistry. 2016;408(20):5577-5584. DOI: 10.1007/s00216-016-9656-z

[41] Samokhvalov AV, Safenkova IV, Eremin SA, Zherdev AV, Dzantiev BB. Use of anchor protein modules in fluorescence polarisation aptamer assay for ochratoxin A determination. Analytica Chimica Acta. 2017;962:80-87. DOI: 10.1016/j.aca.2017.01.024

[42] Wang S, Zhang Y, Pang G, Zhang Y, Guo S. Tuning the aggregation/disaggregation behavior of graphene quantum dots by structure-switching aptamer for high-sensitivity fluorescent ochratoxin A sensor. Analytical Chemistry. 2017;89(3):1704-1709. DOI: 10.1021/acs.analchem.6b03913

[43] Duan H, Li Y, Shao Y, Huang X, Xiong Y. Multicolor quantum dot nanobeads for simultaneous multiplex immunochromatographic detection of mycotoxins in maize. Sensors and Actuators B: Chemical. 2019;291:411-417. DOI: 10.1016/j.snb.2019.04.101

[44] GoryachevaIY, SaegerSD, EreminSA, PeteghemCV. Immunochemical methods for rapid mycotoxin detection: Evolution from single to multiple analyte screening: A review. Food Additives and Contaminants. 2007;24(10):1169-1183. DOI: 10.1080/02652030701557179
Recent Biosensors Technologies for Detection of Mycotoxin in Food Products
DOI: http://dx.doi.org/10.5772/intechopen.89022

[45] Garden SR, Strachan NJ. Novel colorimetric immunoassay for the detection of aflatoxin B1. Analytica Chimica Acta. 2001;444(2):187-191. DOI: 10.1016/S0003-2670(01)01231-4

[46] Homola J, Yee SS, Gauglitz G. Surface plasmon resonance sensors. Sensors and Actuators B: Chemical. 1999;54(1-2):3-15. DOI: 10.1016/S0925-4005(98)00321-9

[47] Daly SJ, Keating GJ, Dillon PP, Manning BM, O’Kennedy R, Lee HA, et al. Development of surface plasmon resonance-based immunoassay for aflatoxin B1. Journal of Agricultural and Food Chemistry. 2000;48(11):5097-5104. DOI: 10.1021/jf9911693

[48] Pemberton RM, Pittson R, Biddle N, Drago GA, Hart JP. Studies towards the development of a screen-printed carbon electrochemical immunosensor array for mycotoxins: A sensor for aflatoxin B1. Analytical Letters. 2006;39(8):1573-1586. DOI: 10.1080/00032710601713289

[49] Pirincci SS, Ertekin O, Laguna DE, Ozen FS, Guloglu FB, Ozturk ZZ, et al. Label-free QCM Immunosensor for the detection of ochratoxin A. Multidisciplinary Digital Publishing Institute Proceedings. 2017;1(8):706. DOI: 10.3390/s18041161

[50] Poma A, Turner AP, Piletsky SA. Advances in the manufacture of MIP nanoparticles. Trends in Biotechnology. 2010;28(12):629-637. DOI: 10.1016/j.tibtech.2010.08.006

[51] Pardieu E, Cheap H, Vedrine C, Lazerges M, Latchat Y, Garnier F, et al. Molecularly imprinted conducting polymer based electrochemical sensor for detection of atrazine. Analytica Chimica Acta. 2009;649(2):236-245. DOI: 10.1016/j.aca.2009.07.029

[52] Bossi A, Bonini F, Turner AP, Piletsky SA. Molecularly imprinted polymers for the recognition of proteins: The state of the art. Biosensors and Bioelectronics. 2007;22(6):1131-1137. DOI: 10.1016/j.bios.2006.06.023

[53] Baggiani C, Anfossi L, Giovannoli C. Solid phase extraction of food contaminants using molecular imprinted polymers. Analytica Chimica Acta. 2007;591(1):29-39. DOI: 10.1016/j.aca.2007.01.056

[54] Ge Y, Turner AP. Molecularly imprinted sorbent assays: Recent developments and applications. Chemistry—A European Journal. 2009;15(33):8100-8107. DOI: 10.1002/chem.200802401

[55] Ramström O, Mosbach K. Synthesis and catalysis by molecularly imprinted materials. Current Opinion in Chemical Biology. 1999;3(6):759-764. DOI: 10.1016/S1367-5931(99)00037-X

[56] Kriz D, Ramstroem O, Svensson A, Mosbach K. A biomimetic sensor based on a molecularly imprinted polymer as a recognition element combined with fiber-optic detection. Analytical Chemistry. 1995;67(13):2142-2144. DOI: 10.1021/ac00109a037

[57] Dey D, Goswami T. Optical biosensors: A revolution towards quantum nanoscale electronics device fabrication. BioMed Research International. 2011;2011:7. Article ID 348218. DOI: 10.1155/2011/348218

[58] Luo XL, Xu JJ, Zhao W, Chen HY. Glucose biosensor based on ENFET doped with SiO2 nanoparticles. Sensors and Actuators B: Chemical. 2004;97(2-3):249-255. DOI: 10.1016/j.snb.2003.08.024

[59] Sant W, Pourciel ML, Launay J, Do Conto T, Martinez A, Temple-Boyer P. Development of chemical field effect transistors for the detection of urea. Sensors and Actuators B: Chemical. 2003;95(1-3):309-314. DOI: 10.1016/S0925-4005(03)00430-1
[60] Nath N, Chilkoti A. A colorimetric gold nanoparticle sensor to interrogate biomolecular interactions in real time on a surface. Analytical Chemistry. 2002;74(3):504-509. DOI: 10.1021/ac015657x

[61] Li M, Chen L, Zhang W, Chou SY. Pattern transfer fidelity of nanoimprint lithography on six-inch wafers. Nanotechnology. 2002;14(1):33. DOI: 10.1088/0957-4484/14/1/308

[62] Turner DC, Chang C, Fang K, Brandow SL, Murphy DB. Selective adhesion of functional microtubules to patterned silane surfaces. Biophysical Journal. 1995;69(6):2782-2789. DOI: 10.1016/S0006-3495(95)80151-7

[63] Damborský P, Švitel J, Katrlík J. Optical biosensors. Essays in biochemistry. 2016;60(1):91-100

[64] Chauhan R, Singh J, Sachdev T, Basu T, Malhotra BD. Recent advances in mycotoxins detection. Biosensors and Bioelectronics. 2016;81:532-545. DOI: 10.1016/j.bios.2016.03.004

[65] Puiu M, Istrate O, Rotariu L, Bala C. Kinetic approach of aflatoxin B1–acetylcholinesterase interaction: A tool for developing surface plasmon resonance biosensors. Analytical Biochemistry. 2012;421(2):587-594. DOI: 10.1016/j.ab.2011.10.035

[66] Moscone D, Arduini F, Amine A. A rapid enzymatic method for aflatoxin B detection. In: Microbial Toxins. Totowa, NJ: Humana Press; 2011. pp. 217-235. DOI: 10.1007/978-1-61779-102-4_20

[67] Stepurska KV, Soldatkin OO, Kucherenko IS, Arkhypova VM, Dzyadevych SV, Soldatkin AP. Feasibility of application of conductometric biosensor based on acetylcholinesterase for the inhibitory analysis of toxic compounds of different nature. Analytica Chimica Acta. 2015;854:161-168. DOI: 10.1016/j.aca.2014.11.027

[68] Soldatkin OO, Burdak OS, Sergeyeva TA, Arkhypova VM, Dzyadevych SV, Soldatkin AP. Acetylcholinesterase-based conductometric biosensor for determination of aflatoxin B1. Sensors and Actuators B: Chemical. 2013;188:999-1003. DOI: 10.1016/j.snb.2013.06.107

[69] Egbunike GN, Ikekuguonu FI. Effect of aflatoxicosis on acetylcholinesterase activity in the brain and adenohypophysis of the male rat. Neuroscience Letters. 1984;52(1-2):171-174. DOI: 10.1016/0304-3940(84)90369-0

[70] Szegletes T, Mallender WD, Rosenberry TL. Nonequilibrium analysis alters the mechanistic interpretation of inhibition of acetylcholinesterase by peripheral site ligands. Biochemistry. 1998;37(12):4206-4616. DOI: 10.1021/bi972158a

[71] Hansmann T, Sanson B, Stojan J, Weik M, Marty JL, Fournier D. Kinetic insight into the mechanism of cholinesterasterase inhibition by aflatoxin B1 to develop biosensors. Biosensors and Bioelectronics. 2009;24(7):2119-2124. DOI: 10.1016/j.bios.2008.11.006

[72] Bourne Y, Taylor P, Radić Z, Marchot P. Structural insights into ligand interactions at the acetylcholinesterase peripheral anionic site. The EMBO Journal. 2003;22(1):1-2. DOI: 10.1093/emboj/cdg005

[73] Kramer A, Schneider-Mergener J. Synthesis and screening of peptide libraries on continuous cellulose membrane supports. In: Combinatorial Peptide Library Protocols. Vol. 87. Humana Press; 1998. pp. 25-39. DOI: 10.1385/0-89603-392-9:25

[74] Sellrie F, Schenk JA, Behrsing O, Böttger V, Micheil B. A competitive immunoassay to detect a hapten using
an enzyme-labelled peptide mimotope as tracer. Journal of Immunological Methods. 2002;261(1-2):141-144. DOI: 10.1016/S0022-1759(01)00561-0

[75] Kieber-Emmons T, Monzavi-Karbassi B, Wang B, Luo P, Weiner DB. Cutting edge: DNA immunization with minigenes of carbohydrate mimotopes induce functional anti-carbohydrate antibody response. The Journal of Immunology. 2000;165(2):623-627. DOI: 10.4049/jimmunol.165.2.623

[76] Yuan Q, Pestka JJ, Hespenheide BM, Kuhn LA, Linz JE, Hart LP. Identification of mimotope peptides which bind to the mycotoxin deoxynivalenol-specific monoclonal antibody. Applied and Environmental Microbiology. 1999;65(8):3279-3286. Available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC91492/

[77] Wild D. The Immunoassay Handbook. New York: Stockton Press; 1994