chemosensors

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Review

Thin Films Sensor Devices for Mycotoxins Detection in Foods: Applications and Challenges

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Abstract: Mycotoxins are a group of secondary metabolites produced by different species of filamentous fungi and pose serious threats to food safety due to their serious human and animal health impacts such as carcinogenic, teratogenic and hepatotoxic effects. Conventional methods for the detection of mycotoxins include gas chromatography and high-performance liquid chromatography coupled with mass spectrometry or other detectors (fluorescence or UV detection), thin layer chromatography and enzyme-linked immunosorbent assay. These techniques are generally straightforward and yield reliable results; however, they are time-consuming, require extensive preparation steps, use large-scale instruments, and consume large amounts of hazardous chemical reagents. Rapid detection of mycotoxins is becoming an increasingly important challenge for the food industry in order to effectively enforce regulations and ensure the safety of food and feed. In this sense, several studies have been done with the aim of developing strategies to detect mycotoxins using sensing devices that have high sensitivity and specificity, fast analysis, low cost and portability. The latter include the use of microarray chips, multiplex lateral flow, Surface Plasmon Resonance, Surface Enhanced Raman Scattering and biosensors using nanoparticles. In this perspective, thin film sensors have recently emerged as a good candidate technique to meet such requirements. This review summarizes the application and challenges of thin film sensor devices for detection of mycotoxins in food matrices.

Keywords: thin films; mycotoxins; food analysis; biosensors

1. Introduction

Mycotoxins are a group of secondary fungal metabolites produced by different species of filamentous fungi [1], among which the most important belong to the genera *Aspergillus*, *Fusarium*, and *Penicillium* [2].

Considering the agro-economic aspects and the impact on global agriculture, as well as the possible implications on the public health, the most relevant mycotoxins are aflatoxins (AFs), citrinin (CIT), deoxynivalenol (DON), fumonisin B1 (FB1), ochratoxin A (OTA), patulin (PAT), T-2 toxin (T-2), and zearalenone (ZEA) [3–5]. These toxins are found worldwide as natural contaminants in many food matrices of plant origin, like aromatic herbs, cereal grains, coffee beans, dried fruits, fruits, oilseeds,

spices, and vegetables, as well as in wine, and beer. Mycotoxins can also be found in animal-derived foods due to the intake of contaminated feeds [6–11].

The presence of mycotoxins in foods has severe implications on human and animal health even at very low levels, due to their mutagenic, teratogenic, carcinogenic, nephrotoxigenic, and immunosuppression effects [12]. Table 1 summarizes the main mycotoxins and their respective toxic effects, as well as the producing fungal species and the regulatory limit ranges established in the European Union (EU), when applicable [13–16].

Consequently, sensitive and accurate methods of analysis are needed to gather adequate information on the levels of exposure to mycotoxins and to assess the relevant toxicological risk for humans and animals. In addition, analytical methods should allow the measurement of such contaminants at levels lower than the legal limits fixed by the EU or other national or international regulations with good accuracy and precision, allowing us to establish monitoring programs and so to ensure international trade safety [17].

Table 1. Representative mycotoxins and their respective toxic effect and main producing fungal species.

Mycotoxin	Abbreviation	Food Matrix	Toxic effect	Limit EU *	Fungal Species	References
Aflatoxins	AFM1, AFM2, AFB1, AFB2, AFG1, AFG2	Peanuts, maize, milk and derived, cereals, oilseeds	Hepatotoxic, carcinogenic, probable immune suppression and childhood stunting reduced growth	AFB1: 2–12 µg/kg AFB1: 0.1 µg/kg for cereal-based foods for children and medicinal purposes AFM1: 0.05 µg/kg in milk and 0.025 µg/kg in foods for infants Total aflatoxin: 4–15 µg/kg	*Aspergillus flavus*, *A. parasiticus*	[15,18–21]
Ochratoxins	OTA, OTB, OTC	Cereals, coffee, cocoa, wine, beer, grapes, dried fruits	Nephrotoxic, hepatotoxic, neurotoxic, teratogenic, immunotoxic	0.5–10 µg/kg	*Aspergillus ochraceus*, *A. carbonarius*, *Penicillium verrucosum*	[12,13,22,23]
Fumonisins	FB1 e FB2	Maize and maize based food, rice, sorghum, soybeans	Neurotoxic, genotoxic, immunotoxic, carcinogenic, hepatotoxic, nephrotoxic	200–4000 µg/kg	*Fusarium verticillioides*, *F. proliferatum*	[14,24]
Trichothecenes	Type A: HT-2, T-2, Type B: DON	Wheat, barley, and maize and less often in oats, rice, rye, sorghum and triticale	Inhibition of protein synthesis, immunosuppressive and cytotoxic effect	DON: 200–1750 µg/kg	*Fusarium sporotrichioides*, *F. graminearum*, *F. culmorum*	[2,13,14,16,25]
Patulin	PAT	Apples and apple products, fruit juice	Genotoxic, embryotoxic, immunotoxic, teratogenic, carcinogenic	10–50 µg/kg	*Penicillium expansum*	[13,26]
Zearalenone	ZEA	Corn, oats	Hepatotoxic, genotoxic, immunotoxic, carcinogenic	20–400 µg/kg	*Fusarium graminearum*	[14,27]
Citrinin	CIT	Various commodities of plant origin, cereals, namely fermented rice	Nephrotoxic, neurotoxic, genotoxic, embryotoxic, immunotoxic	2000 µg/kg for rice	*P. citrinum*, *P. expansum*, *P. verrucosum*, *Aspergillus carneus*, *A. niveus*, *Monascus purpureus*	[12,16,28]

* The limits vary according to the food matrix [13–16,19].

2. Conventional Analytical Methods for Mycotoxins Detection

Chromatography is the most commonly used method used for mycotoxin analysis in food and feed [29]. The thin layer chromatography (TLC) was the first chromatographic method to be applied for mycotoxin determination and, nowadays, is still a routine technique used in several laboratories. TLC is presently used as a rapid visual screening method for certain mycotoxins (AFM1, AFG1, AFG2, FB1 and OTA) allowing a qualitative evaluation or, if coupled with instrumental densitometry, enabling a semi-quantitative assessment [30–32]. However, current trends in mycotoxin analysis in foods are focused on the application of robust, fast, easy to use, and cheap technologies that are able to detect and quantify simultaneously various mycotoxins with a high sensitivity and selectivity in a single run [33]. To meet those needs, many chromatographic methods such as high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV), diode array (DAD), fluorescence (FLD), or mass spectrometry (MS) detectors; and, ultra-HPLC (UHPLC) or UPLC with reduced column packing material (1–2 μm) have been developed [34]. Additionally, gas chromatography (GC) coupled with electron capture (ECD), flame ionization (FID), or MS detectors have been also used to identify and quantify volatile mycotoxins [34]. Due to the high polarity and low volatility of some mycotoxins, GC analysis often requires a derivatization step. Thus, this method is used rarely in mycotoxins analysis [35]. Mycotoxin analysis has been greatly enhanced by coupling liquid chromatography techniques with mass-spectrometry (e.g., LC-MS; LC-MS/MS) [29]. While HPLC-MS coupled with mass spectrometric or fluorescence detectors are usually applied for mycotoxins analysis in foods, other chromatographic techniques (e.g., TLC) are rarely used due to the limited sensitivity and specificity [34,36,37].

Chromatographic methods, namely LC and GC, typically require additional steps prior to detection, including extraction, clean-up and separation that are crucial, though time consuming, for a successful protocol, and directly affect the final choice for the detection procedure [29]. Although these methods have high sensitivity and selectivity, they are not suitable for rapid, on-site testing because they are laboratory-based and require skilled operators and expensive equipment. In addition, they are time-consuming, involve high costs, often require large amounts of hazardous reagents and solvents during the analysis process, and may exhibit a lack of accuracy for low analyte concentrations [38]. A comparison between the conventional analytical methods to detect mycotoxins in food and feed samples is summarized in Table 2. In this table, some examples of mycotoxin detection/quantification limits are given for the conventional analytical techniques commonly used, although it should be remarked that the referred techniques may allow achieving lower detection limits depending on the type of food matrix and/or on the target mycotoxin.

Additionally, enzyme-linked immunosorbent assays (ELISA) are an important analytical technique that has been widely used in the detection of mycotoxins [39,40]. The technique principles are based on the competitive interactions between mycotoxins (acting as an antigen) and specific antibodies labelled with toxin-enzyme conjugates [41]. ELISA can be performed in several ways such as direct assay, direct competitive assay, and indirect competitive assay, with competitive direct assay being the most commonly used method [42]. This technique provides a fast screening, and commercial kits are available. These have been validated for a wide variety of food matrices and are available for the detection and quantification of mycotoxins including AFs, DON, FB, OTA, T-2 toxin, and ZEA [33,41,43]. The ELISA-based method is user-friendly, less expensive, and less time-consuming than HPLC techniques, which, on the other hand, are much more reliable in terms of analyte quantification [44].

The availability of other fast, sensitive, simple, portable and cost-effective methods for rapid determination of mycotoxins and others contaminants is becoming an increasingly significant challenge for the food industry in order to ensure the safety of foods and feeds. Therefore, the use of analytical procedures based on sensors has recently gained increased interest, mainly due to their capability to overcome a large number of analytical challenges, including difficulties in detecting low-level mycotoxin contamination, and the co-occurrence of mycotoxins [45,46]. In this sense, many researchers

applied sensor-based devices like microarray chips [38], electrochemical sensors [47], biosensors [48], multiplex lateral flow [24,49], Surface Plasmon Resonance (SPR) [50], Total Internal Reflection Ellipsometry (TIRE) [51], and Surface Enhanced Raman Scattering (SERS) [32]. In this perspective, thin film sensors have recently emerged as good candidate techniques to fulfill such requirements, although not all of the above-mentioned types of sensor-based devices would fall within the thin-films sensors classification.

Table 2. Comparison between the conventional analytical methods for mycotoxins analysis.

Method	Advantages	Disadvantages	Mycotoxin/Matrix	LOD	LOQ	References
TLC	Simple and inexpensive Can be used as a rapid screening method	Poor sensitivity Poor precision Quantitative approach only if coupled with a densitometer	DON/Wheat flour	30 ng·mL^{-1}	100 ng·mL^{-1}	[30,52,53]
HPLC-FLD	Good selectivity Accurate identification Short analysis time Automatic analysis (autosampler) Official methods available	Expensive equipment Specialist expertise required Derivatization may be required	AFB1/Spices	0.04 ng·mL^{-1}	0.15 ng·mL^{-1}	[54,55]
LC-MS	Selective and sensitive detection Capability to generate structural information of the target analyte Low detection limits Simultaneous analysis of multiple mycotoxins Minimum sample pre-treatment steps	Expensive equipment Specialist expertise required Sensitivity depends on ionization technique	AFB1/Wheat grain	2 μg·kg^{-1}	3.5 μg·kg^{-1}	[55–57]
GC	Simultaneous analysis of multiple mycotoxins Selective and sensitive detection	Expensive equipment Specialist expertise required Derivatization required Non-linear calibration curve Carry over effects from previous sample	DON/Pasta	0.5 μg·kg^{-1}	1 μg·kg^{-1}	[4,35,58]
ELISA	Convenient and sensitive detection Ease of operation Rapid sample screening Simultaneous analysis of multiple mycotoxins Low use of organic solvents	Matrix interference problems Cross-reactivity with related mycotoxins Possible false positive/negative results Narrow operating range	OTA/Corn	4.0 ng·mL^{-1}	*Not specified*	[59]
Spectral analysis technology	Rapid screening of a large number of samples Qualitative and quantitative information about the structure of mycotoxins Can be used in situ	Complicated interpretation of spectral data Spectra overlapping Possible false positive/negative results	Fumonisin/Corn	100 μg·kg^{-1}	*Not specified*	[60,61]

ELISA—Enzyme-linked immunosorbent assay; GC—Gas chromatography; HPLC-FLD—High-performance liquid chromatography coupled with fluorescence detector; LC-MS—Liquid chromatography–mass spectrometry; TLC—Thin layer chromatography.

3. Thin Film Based Sensors

A thin film generally refers to a layer of material that ranges from a few nanometers to several micrometers in thickness. Thin film devices play an important role in many conventional and emerging technologies due to the constant advances in nanotechnology, among which the development of functional materials and the use of properties of thin films can be mentioned, such as high surface area, controlled nanostructure for effective charge transfer, and special physical and chemical properties [62].

Thin layers applied in the thin film devices may comprise organic, inorganic, and composite thin layers, sharing analogous functionalities, properties, and fabrication routes. The combination between different thin films can produce a thin film device, like thin film solar cells, thermoelectric devices, actuators and also transistors, for the development of biosensors or other electrochemical sensors for mycotoxins detection [62]. The film layer(s) may be deposited using vapor, liquid or solid precursors or from a combination of several precursors phases, depending on their nature and on the desired functionality and specification of the thin film [62–64]. Conducting polymer thin films may be deposited using several solution-processed casting techniques, such as electrochemical deposition, Langmuir–Blodgett (LB) technique, layer-by-layer self-assembly, dip coating, spin coating, drop-casting, spray coating, inkjet printing, as well as using different thermal evaporation techniques at moderate temperatures [65]. In addition, some physical and chemical-vapor deposition methods (PVD and CVD, respectively), such as sputtering and molecular bean epitaxy modes, can also be applied to improve the quality of the deposition [62].

A biosensor is a bioanalytical device constituted by a biorecognition element (DNA, enzyme, antibody, etc.), which is responsible for recognizing an analyte, i.e., a bioreceptor; an immobilization matrix like conducting polymers [48,66], nanomaterials [67,68], sol–gel films [69], and self-assembled monolayers [70], which have been used for the immobilization of a biomolecule; and a transducer unit for converting the biochemical response into a recognizable electrochemical/electric signal.

Biosensors are commonly classified based on the type of transducer, as optical (colorimetry, fluorescence, luminescence, interferometry, spectroscopy, SPR, and TIRE); electrochemical (amperometry, conductimetry, potentiometry, and voltammetry); and piezoelectric (quartz crystal microbalance (QCM) [48,71].

3.1. Optical Sensors

Optical biosensors provide a powerful alternative to conventional methods such as ELISA and chromatographic techniques because they allow high sensitive, nondestructive, and real-time analysis of food toxins without needing extensive and complex sample preparation steps [72].

This kind of biosensor uses a transducer unit capable of converting the interaction between the biorecognition elements and the target analytes into a measurable optical signal. They are classified according to the optical method applied to detect the analyte of interest, which may be based on fluorescence, colorimetry, electrochemiluminescence, and SPR [73–75]. Among optical techniques, SPR and fluorescence are the most frequently used.

3.1.1. Fluorescence Sensors

Among the several optical detection methods, fluorescence sensing techniques attracted huge interest due to their easy operation, short analytical time and convenient signal reading [76]. Nanomaterials have been integrated into fluorescent biosensing devices, taking into account their inherent excellent optical and electronic properties [75]. Gold nanoparticles [77], dendrimers [78], quantum dots [79], and graphene oxide [80] have been employed in the mycotoxin sensing. In combination with nanoparticles, specific aptamers were also used to develop some fluorescence thin film sensors for mycotoxin detection [81,82]. The aptamer consists of a synthetic oligonucleotide ligand (either single stranded DNA (ssDNA) or RNA) that comprises less than 80 nucleotides and with less than 25 kDa and are known to exhibit high specificity and strong binding affinity [83].

Caputo et al. [84] developed a rapid, compact and innovative method for the detection of OTA based on hydrogenated amorphous silicon (a-Si:H) thin film sensors (Figure 1, reprinted with permission from [84] ©MDPI, 2018). The sensor was constituted by a stacked structure of p-type/intrinsic/n-type a-Si:H layers deposited by plasma enhanced chemical vapor technique (PECVD), which was deposited on a glass substrate allowing improvement of the detection limits. The metal contact surface comprised a three metal layer of chromium/aluminum/chromium, obtained by evaporation under vacuum [84]. As described by the researchers, a high-performance thin layer Chromatography (HPTLC) plate was placed on the silica side, being 2 µL of OTA solutions with different concentrations spotted. The incidence of an UV radiation, at 253.4 nm, allowed exciting the mycotoxin, which re-emitted light (mostly in the green range) passed through the glasses/TCO layers being absorbed by the a-Si:H photosensor, which is aligned with the OTA molecules dropped on the silica. According to Caputo et al. [84], the minimum detected OTA concentration was 0.25 $\mu g \cdot kg^{-1}$ (corresponding to 0.1 ng of OTA detected after an extraction process starting from 10 mL of red wine), showing that the presented system has the potential for a low-cost system suitable for the early detection of toxins in foods.

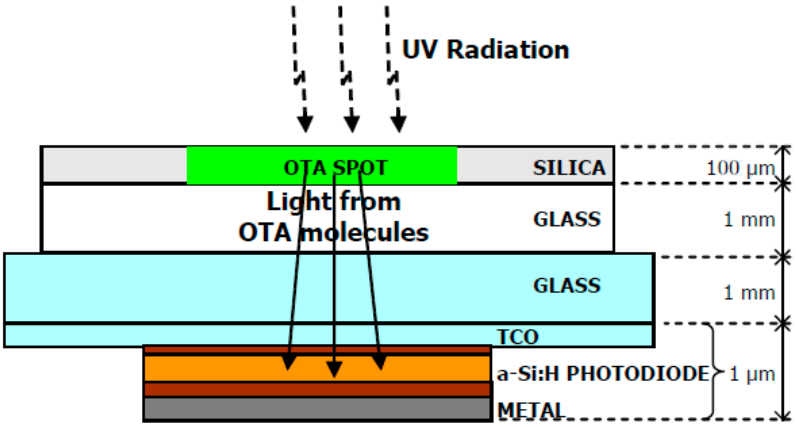

Figure 1. Qualitative scheme of the fluorescence detection system for OTA molecules. The analyte is confined in an HPTLC plate, optically coupled with a glass substrate, where the a-Si:H photosensor have been deposited. (reproduced with permission from Caputo et al. [84] ©MDPI, 2018).

Using the same system with a-Si:H photosensor, Caputo et al. [85] reported limits of detection of 0.1 ng and 1 ng in standard solutions or in contaminated red wine samples, respectively, allowing to infer if the OTA content in a red wine was above or below the legal limit (2 $\mu g \cdot kg^{-1}$) [86].

A portable thin film device for detecting OTA was developed by De Cesare et al. [44]. The device allowed assessing the mycotoxin level of different food commodities, even if it was below the maximum limit imposed by the EU Commission [86] for the specific food evaluated. The prototype device included three main parts: a UV light source that supplied the radiation to induce the OTA fluorescence; a commercial HPTLC plate made of a silica gel-covered glass; an array of amorphous silicon photodetectors (a-Si:H photosensors), positioned behind the back side of the TLC plate. The photosensors were deposited by PECVD on a glass that was optically coupled to the TLC plate. The system performance was evaluated using OTA standard solutions, as well as with OTA extracts from fortified wine or beer samples. The results revealed a quantification limit of 0.2 ng in 2 µL of extracted sample solution (red wine or beer fortified with OTA) which would correspond to 10 ng of OTA in 5 mL of real matrix, being, according to the researchers, a value of 5.5 mL (admitting a 90% extraction efficiency) of sample enough to determine if the OTA concentration was lower than the legal threshold (2 $\mu g \cdot kg^{-1}$) [86].

Some fluorescence sensing assays are based on Fluorescence Resonance Energy Transfer (FRET) [80]. In this manner, one chemical group provides energy and the other one accepts the transferred energy. A large overlap between receiver excitation and donor emission must exist in order to achieve FRET. When the distance between donor and acceptor is close enough, the energy will be transferred from

the donor to the acceptor. Furthermore, the acceptor and donor in the FRET system can be designed in a biunique or one-to-multiple manners, enabling the simultaneous application of multiple mycotoxin sensing methods [76]. The FRET technique was applied by several researchers, allowing detection of different mycotoxins (e.g., AFM1, AFB1, OTA and FB1) in foods (e.g., milk, peanuts, rice, and/or maize). Antibodies or aptamers were immobilized onto fluorescent nanoparticles-graphene oxide, quantum dots-AuNPs or nanogold-strips, forming thin films and enabling achieving low detection limits (e.g., 0.02 to 0.1 ng·mL^{-1}, depending on the method/mycotoxin) [80,87,88].

3.1.2. Surface Plasmon Resonance (SPR) Sensors

Biosensors based on SPR use a thin metal (usually gold or silver) film between two transparent media with different refractive indices, like, a glass prism and the sample solution. When a polarized light beam passes through the higher refractive index medium (e.g., glass prism) it can undergo a total internal reflection if the incidence angle is above the critical angle, generating an evanescence wave that penetrates the metal layer [89,90]. The interaction of this wave with free oscillating electrons at the metal film surface at a specific angle of incidence will cause the excitation of the plasmon surface, resulting in a decrease in the reflected light intensity. A SPR system thus detects changes in the refractive index of the surface layer of a solution in contact with the sensor chip [81]. Figure 2 (reproduced with permission from reference [91] ©Nature, 2018) shows the SPR biosensor principle, including a typical set-up as well as a common binding cycle.

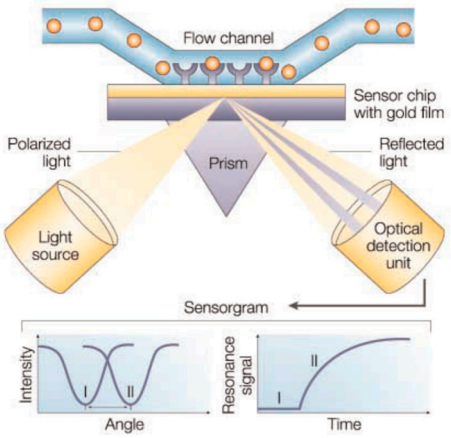

Figure 2. SPR biosensor principle. Typical set-up for an SPR biosensor. (reproduced with permission from Cooper [91] ©Nature, 2018).

Small compounds, such as mycotoxins, usually do not generate a sufficient change in the refractive index and thus their detection using SPR is more challenging, due to the low signal intensity and poor sensitivity [92]. However, a clear enhancement of the method sensitivity can be achieved by using SPR combined with competitive or inhibitive detection tools together with the use of additional high mass labels.

Indeed, SPR sensing has been used with competitive-inhibition assays and sandwich assays to determine mycotoxins. In competitive methodologies, the sensor surface is coated with an antibody that interacts with the mycotoxin. When a conjugated antigen is added to the sample, it competes with the mycotoxin for the limited number of biding sites on the surface. Therefore, the recorded signal is inversely proportional to the mycotoxin concentration. The inhibition assay relies on mixing a known concentration of antibody with a sample containing an unknown concentration of antigen that is subsequently injected into the flow cell and passed through a sensor surface, to which antigen is immobilized. Then, the amount of bounded antigen to the modified surface antibody is measured and the obtained signal is proportional to the concentration of the mycotoxin [76,93]. In the sandwich format, the antibody is added to the sample to recognize and react with the target, forming a primary

complex [50,94,95]. Then, the primary complex is placed in contact with the sensor chip to generate the sandwich complex, which results in a measurable signal [76].

Other approaches to amplify the SPR signal response involve the use of fluorescence or modifications of the sensor chip with metallic nanoparticles. The latter ones are widely used in the construction of biosensors due to their unique physical and chemical properties, good biocompatibility and high catalytic activity for many chemical reactions. Among the large variety of nanomaterials currently available, the implementation of gold nanoparticles (AuNPs) has gained huge relevance [75]. The application of silver film over nanospheres has also been a very effective practice due to the high enhancement factor achieved ($\geq 10^4$), the high stability within a wide temperature range and wide analyte concentration ranges, and the good shelf-life (higher than 40 days) [96].

The nanomaterial-based enhanced SPR biosensing systems may be achieved using two common strategies. One promotes the substrate SPR biosensing by applying nanomaterials and, the other uses nanomaterials as amplification labels for SPR biosensing enhancement. As SPR substrate, nanomaterials with large surface area allow the immobilization of several biorecognition elements.

Fu et al. [97] reported a SPR biosensor for OTA detection based on gold hollow balls (AuHBs) with dendritic surface. An electropolymerized thionine (PTh) film was deposited onto a gold electrode, forming a PTh-modified electrode surface with several amino groups. The deposition was achieved using voltammetric cycles between 0 and 1.5 V at a scan rate of 50 mV/s in 0.1 M thionine aqueous solution. The AuHBs were immobilized using a thionine thin film electropolymerized onto the SPR probe surface. Then, anti-OTA monoclonal antibody (anti-OTA, linked to AuHBs, was also immobilized onto the SPR-probe surface. The developed SPR biosensor exhibited a linear detection range from 0.05 to 7.5 ng mL^{-1} with a low limit of detection (LOD) (0.01 ng mL^{-1}), under the optimum operating conditions. Furthermore, this SPR biosensor also enabled a fast detection (<30 min) of OTA in milk samples.

Todescato et al. [98] developed a new sensor prototype capable of detecting OTA, at levels lower than the legal threshold of 0.5 μg/kg, in different food matrices (dried milk, juices, and wheat milk): The sensor device comprised a silver film over nanospheres plasmonic substrates functionalized with a specific anti-OTA antibody (Ag-FON), able to bind with the complex OTA-Alexa Fluor (AF) 647. Briefly, as described by the researchers, polystyrene nanospheres, in an aqueous solution, were spin-coated on top of the clean glass slides to form a self-assembled stack. Silver thin films were then deposited onto the substrates using an Edwards E306A coating system with a bare pressure of 6×10^{-5} mbar. The metal deposition rate was approximately 1 Å/s and the final Ag thicknesses, evaluated by means of a quartz crystal microbalance, were approximately equal to 50, 100 and 150 nm. In the work, OTA concentrations ranging from 0 to 5 $\mu g \cdot kg^{-1}$ were incubated on commercial micro arrays or on Ag-FON slides. The Ag-FON array slides allowed detecting OTA concentration as low as 0.05 $\mu g \cdot kg^{-1}$, 10 folds lower than the detectable concentrations using commercial microarray slides (0.5 $\mu g \cdot kg^{-1}$). Indeed, the limit of quantification (LOQ) of OTA using Ag-FON substrates was of 3.6 $ng \cdot kg^{-1}$ that is 20 times lower than the LOQ observed for commercial microarray slides (70.7 $ng \cdot kg^{-1}$). To evaluate the feasibility of the detection strategy for real samples, the authors spiked milk, juices and wheat mix samples with unlabeled OTA and the results showed that the proposed methodology was able to detect OTA concentrations as low as 0.5 $\mu g \cdot kg^{-1}$ (E.U. legislation lower tolerable limit) in the spiked samples, whose levels were statistically higher than those observed in the control samples (not spiked with OTA). These findings showed that OTA concentrations higher than 0.5 $\mu g \cdot kg^{-1}$ could be detectable in these food matrices when compared to control samples [98].

Karczmarczyk et al. [99] developed an indirect OTA detection method for red wines analysis, based on an AuNPs-enhanced SPR device. The sensor chip comprised a BK7 glass substrate that was coated by applying a sputtering deposition method, with a thick gold film (37 nm). The setup allowed secondary antibodies, conjugated with AuNPs, to interact with antibody–OTA complexes immobilized on a gold sensor chip surface, via self-assembled thiol monolayer (SAM). In order to optimize the enhanced SPR biosensor response for the OTA immunoassay, Ab2-AuNPs conjugates with different diameters (10–40 nm) were used. The LODs were obtained in PBS as well as in red wine samples and

were equal to 0.068 and 0.19 ng·mL^{-1}, respectively. The highest signal amplification was obtained for diameters of 40 nm and for distances greater than 50 nm between the nanoparticles and the gold surface leading to enhancement factors greater than 100.

A similar AuNPs enhanced SPR thin film immunosensor was constructed for a fast and sensitive detection of aflatoxin M1 (AFM1) in milk and dairy products by Karczmarczyk et al. [100]. In this work, and similarly to reference [97], the sensor chip was prepared on the top of a BK7 glass substrate that was coated with a 41 nm thick gold layer. Two surface architectures were used for the immobilization of AFM1 and of the primary antibody on the gold surface. The first, (A), was based on a bicomponent SAM with polyethylene glycol (PEG) moieties and, the second, (B), used poly(2-hydroxyethyl methacrylate) marked as p(HEMA) brush (Figure 3; reproduced with permission from [100] ©Elsevier, 2018). Both sensors were characterized in terms of surface mass density of the immobilized AFM1 conjugate as well as affinity bound of primary and secondary antibodies. The sensor with thiol mixed SAM with PEG moieties showed a LOD of 26 pg·mL^{-1} and 38 pg·mL^{-1} in buffer standard solutions and in milk samples, respectively. On the other hand, the sensor with p(HEMA) exhibited a LOD of 18 pg·mL^{-1}, which is more than two-times lower compared to that on thiol SAM with PEG groups. The biosensor [100] was highly sensitive towards AFM1 in milk, allowing its detection in a low time-period (55 min), showing a sensitivity of at least one order of magnitude higher compared to the those reported by other methods, including electrochemistry [101] or indirect and direct ELISA [102].

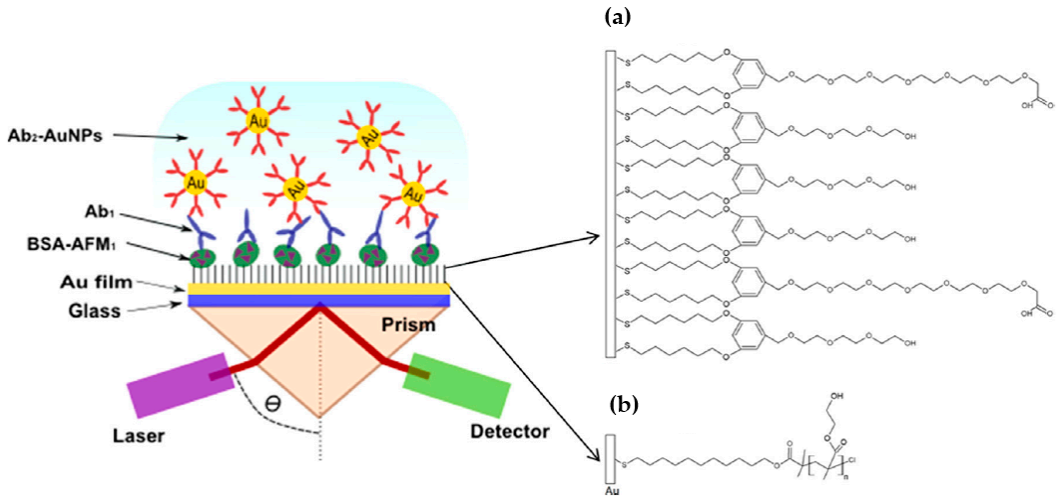

Figure 3. Scheme of the optical setup and sensor chip with different surface architectures: (**a**) mixed SAM and (**b**) p (HEMA) brushes (reproduced with permission from Karczmarczyk et al. [100] ©Elsevier, 2018).

Another interesting optical approach is the use of Localized Surface Plasmon Resonance (LSPR), which has been attracting the attention of researchers because of its potentially high sensitivity [103,104].

3.1.3. Total Internal Reflection Ellipsometry (TIRE)

The method of Total Internal Reflection Ellipsometry (TIRE) combines the advantages of SPR and spectroscopic ellipsometry and allows achieving sensitivities 10 times higher than SPR and, therefore, it became particularly suitable for detection of low molecular weight molecules such as mycotoxins [105].

Al Rubaye et al. [82] reported for the first time a label-free optical detection of OTA using a direct assay with highly specific aptamers using the TIRE method (Figure 4; (reproduced with permission from reference [82] ©Elsevier, 2018). Gold layers, of about 25 nm of thickness, were formed by evaporation on glass slides using an Edwards E306A equipment. A thin layer of chromium (2–3 nm) was obtained by evaporation, allowing improvement of the adhesion of the gold layer on the glass surface. Immobilization

was carried out by casting the aptamer solution onto the gold coated slides. The reported results showed that the detection of OTA using this aptamer-TIRE method was successful and OTA concentrations as low as 0.01 ng·mL^{-1} could be detected. This same research group [51] also developed a highly sensitive analytical TIRE method, which combined with LSPR and using nanostructured gold films allowed detecting aflatoxin B1 and M1 in a direct assay based on the use of specific aptamers immobilized on a gold sensor surface. The concentration range for aflatoxin B1 detection ranged from 0.01 ng·mL^{-1} to 100 ng·mL^{-1}, being obtained LODs of 0.01 ng·mL^{-1}, which is remarkably low for LSPR-based biosensors.

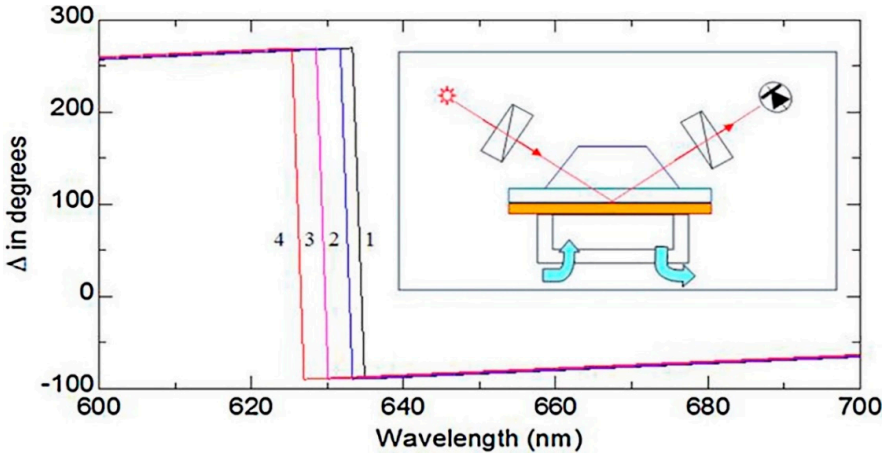

Figure 4. Total Internal Reflection Ellipsometry (TIRE) spectra recorded on aptamer layer (1) and after binding Ochratoxin A (OTA) of 0.01 ng/mL (2), 1 ng/mL (3) and 10 ng/mL (4). (reproduced with permission from Al Rubaye et al. [82] ©Elsevier, 2018).

3.2. Electrochemical Biosensors

The use of electrochemical biosensors is based on the electroactive characteristics of several analytes, which may be oxidized or reduced on a working electrode surface, generating a measurable electrochemical signal [106]. Most of electrochemical biosensors developed for mycotoxins detection are based on the use of specific antibodies, aptamers or artificial receptors like molecularly imprinted polymers (MIPs) as affinity ligands that allow binding the analyte to the sensor, with negligible interference from other components that may be present in the sample [107,108].

To transform the mycotoxin interaction into a measurable analytical signal, different electrochemical techniques have been used like (1) *amperometric*, which measures the changes in the current at a given applied voltage resulting from the oxidation or reduction of an electroactive biological element providing specific quantitative analytical information; (2) *potentiometric*, which measures the changes in the voltage between the working and the reference electrodes due to the establishment of an electrostatic interaction; (3) *conductometric*, which measures the changes in the capability of the sensing material to transport charge (electron); and (4) *impedimetric*, which measures the resistance of the generated electric current at certain applied voltage and combines the analysis of both the resistive and capacitive properties of the materials [109–113]. Many review articles have focused on mycotoxin detection using different electrochemical biosensors [47,107,114,115] and on the application of nanomaterial-mediated bio and immunosensors [21,75,76].

Table 3 summarizes research regarding electrochemical sensors devices recently used for mycotoxin analysis in food samples. It should be remarked that, concerning the electrochemical devices, the majority of the works do not give information regarding the thickness of the detection thin-films formed, usually only reporting the thickness sensor surface where the film layers are formed. Nevertheless, from the overall information gathered for most devices, it could be assumed that the thickness of the film layers (nanocomposites are usually reported) is at nanometer-micrometer level, although for few devices it could be at a millimeter level.

Table 3. Electrochemical thin film sensors devices recently used in the mycotoxins analysis in food samples.

Method	Mycotoxin	Bioreceptor	Interface Material	Sample Type	Limit of Detection (LOD)	Linearity Range	Reference
Amperometric	CIT	Antibody	Au surface electrodeposited on a GCE	Rice	0.1 $ng \cdot mL^{-1}$	0.5–50 $ng \cdot mL^{-1}$	[116]
	AFM1	Antibody	SPCE	Milk	0.039·$ng \cdot mL^{-1}$	1–10.000 $ng \cdot L^{-1}$	[117]
	ZEA	Antibody	Au@AgPt	Milk	0.0017 $ng \cdot mL^{-1}$	0.005–15 $ng \cdot mL^{-1}$	[118]
	AFB1	Antibody	Chitosan-AuNPs modified gold microelectrode	Wheat	0.15 $ng \cdot mL^{-1}$	1.6–32 $ng \cdot mL^{-1}$	[119]
Conductometric	OTA	Enzyme	TLN/AuNPs/(PVA/PEI)	Olive oil	1 nM	2–100 nM	[120]
	AFB1	Enzyme	Au + Pyroceramic + Cr	Standard solution	50 $ng \cdot mL^{-1}$	0.25–1 mM	[121]
Impedimetric	OTA	Antibody	SAM (AUT/Au)	Coffee	0.0008 $ng \cdot mL^{-1}$	0.5–6.0 $ng \cdot dL^{-1}$	[122]
	OTA	Aptamers	SPCE	Cocoa beans	0.15 $ng \cdot mL^{-1}$	0.15–2.5 $ng \cdot mL^{-1}$	[123]
	AFM1	Aptamers	SPCE	Milk	*Not specified*	20–1000 $ng \cdot kg^{-1}$	[124]
	AFM1	Aptamers	SPCE	Buffer	1.15 $ng \cdot L^{-1}$	2–150 $ng \cdot L^{-1}$	[124]
	AFM1	Antibody	Ag wire	Milk	0.001 $ng \cdot mL^{-1}$	6.25–100 $pg \cdot mL^{-1}$	[125]
	OTA	Aptamers	AuNPs–cPC	Soybean	10^{-8} $ng \cdot mL^{-1}$	10^{-8}–0.1 $ng \cdot mL^{-1}$	[126]
	OTA	Aptamers	Au electrode + AuNPs/Boltorn H30®	Beer	0.02 nM	0.1–100 nM	[127]
	OTA	Aptamers	SPCE/PTH + IrO_2 NPs	White wine	0.014 nM; 5.65 $ng \cdot kg^{-1}$	0.01–100 nM	[128]
	DON	Antibody	GCE + AuNPs/G/$PhNO_2$	Cereals	0.3 $ng \cdot mL^{-1}$	6–30 $ng \cdot mL^{-1}$	[129]
	AFB1	Antibody	CD-trodes modified with lipoic acid SAM	Peanut	0.11 $ng \cdot mL^{-1}$	1.56–31.2 $ng \cdot mL^{-1}$	[130]
	OTA	Antibody	AuSPCE/BSA	Plant extracts	*Not specified*	2.5–100 $ng \cdot mL^{-1}$	[131]
Potentiometric	AFM1	Antibody	Ag/AgCl	Standard solution	0.04 $ng \cdot mL^{-1}$	0.25–2 $ng \cdot mL^{-1}$	[132]
	AFM1	Antibody	Ag/AgCl	Milk	0.5 $ng \cdot mL^{-1}$	*Not specified*	[132]
	AFM1	-	GCE + GQDs-α-CD + AgNPs	Milk	2000 nM	0.015–25 mM	[133]
	OTA	Aptamers	GCE + AuNPs/$MoSe_2$	Red wine	0.00008 nM	0.0001–1 nM	[134]

Table 3. *Cont.*

Method	Mycotoxin	Bioreceptor	Interface Material	Sample Type	Limit of Detection (LOD)	Linearity Range	Reference
Voltammetry	DON	Antibody	GCE + SWNTs/CS	Sorghum	0.005 $ng \cdot mL^{-1}$	0.01–1000 $ng \cdot mL^{-1}$	[135]
	AFB1	Antibody	SPCE modified with AuNPs and PPy/ErGO film	Corn	4.2 $ng \cdot mL^{-1}$	200–4500 $ng \cdot mL^{-1}$	[136]
	DON	Antibody	SPCE modified with AuNPs and PPy/ErGO film	Corn	8.6 $ng \cdot mL^{-1}$	50–1000 $ng \cdot mL^{-1}$	[136]
	OTA	Antibody	GCE + PTH/AuNPs	Corn	0.2 $ng \cdot mL^{-1}$	1–1000 $ng \cdot mL^{-1}$	[137]
	OTA	-	GCE + MIP/MWCNT	Beer and wine	4.1 nM (1.7 $ng \cdot mL^{-1}$)	50–1000 nM	[138]
	AFB1	Antibody	Au electrode + CNTs/PDDA/PdeAu	Standard solution	0.03 $ng \cdot mL^{-1}$	0.05–25 $ng \cdot mL^{-1}$	[139]
	AFB1	Antibody	Au electrode + CNTs/PDDA/PdeAu	Rice	1250 $ng \cdot kg^{-1}$	*Not specified*	[139]
	OTA	Antibody + enzyme	AuSPE	Red wine	15 $ng \cdot mL^{-1}$	10^{-2}–10^{3} $ng \cdot mL^{-1}$	[140]
	AFM1	Antibody + enzyme	AuSPE	Milk	0.037 $ng \cdot mL^{-1}$	10^{-2}–10^{3} $ng \cdot mL^{-1}$	[140]
	AFB1	Aptamers	PAMAM G4 + Au electrode + cystamine	Peanuts	0.40 nM	0.1–10 nM	[141]

Au—Gold; Au@AgPt—Gold core and imperfect Silver/Platinum shell structure; AuNPs/G/$PhNO_2$—Gold nanoparticles-dotted 4-nitrophenylazo functionalized graphene; AUT—11-amino-1-undecanethiol; BSA—bovine serum albumin; CD—recordable compact disks; CDs—Cyclodextrins; CNTs/PDDA/PdeAu—Carbon nanotubes; Cr—Chromium; ErGO—Electrochemically reduced graphene oxide; GCE—Glassy carbon electrode; GQDs—graphene quantum dots; IrO_2 NPs—Iridium oxide nanoparticles; MIP—molecularly imprinted polymer; $MoSe_2$—Molybdenum diselenide; MWCNTs—Multiwalled carbon nanotubes; NPs—Nanoparticles; PAMAM G4—Poly(amidoamine) dendrimers of fourth generation; Pd—Palladium; PDDA—poly(diallyldimethylammoniumchloride); PPy—Polypyrrole; PTH—Polythionine; PTH—Polythionine; PVA/PEI—polyvinyl alcohol/polyethylenimine; SAM—Self assembled monolayer; SPCE—Screen printed carbon electrodes; SPE—Screen printed electrodes; SWNTs/CS—Single-walled carbon nanotubes/chitosan; TLN—thermolysin.

3.3. Mass-Based Piezoelectric Biosensors (Quartz Crystal Microbalance)

A Quartz Crystal Microbalance (QCM) consists of a thin quartz disk where electrodes are placed. The application of an external electrical potential to a piezoelectric material produces an internal mechanical stress. As the QCM is piezoelectric, an oscillating electric field applied across the device induces an acoustic wave that propagates through the crystal and reaches a minimum impedance when the thickness of the device is a multiple of a half wavelength of the acoustic wave. Another advantage of QCM is its ability to carry out real-time measurements [142].

Karczmarczyk et al. [143] developed a novel sensor device based on a quartz crystal microbalance with dissipation monitoring (QCM-D). The sensor consisted of a gold surface modified with a mixed thiol self-assembled monolayer (SAM) to which the BSA-OTA conjugate was attached as shown in Figure 5 (reproduced with permission from Karczmarczyk et al. [143] ©Elsevier, 2018). Antibodies for specific analyte recognition were used, allowing fast and sensitive detection of OTA in red wine. The amplification of the QCM-D signal was achieved by applying secondary antibodies conjugated with AuNPs. The developed QCM-D biosensor exhibited a linear detection range of 0.2–40 ng·mL^{-1} and a LOD of 0.16 ng·mL^{-1}.

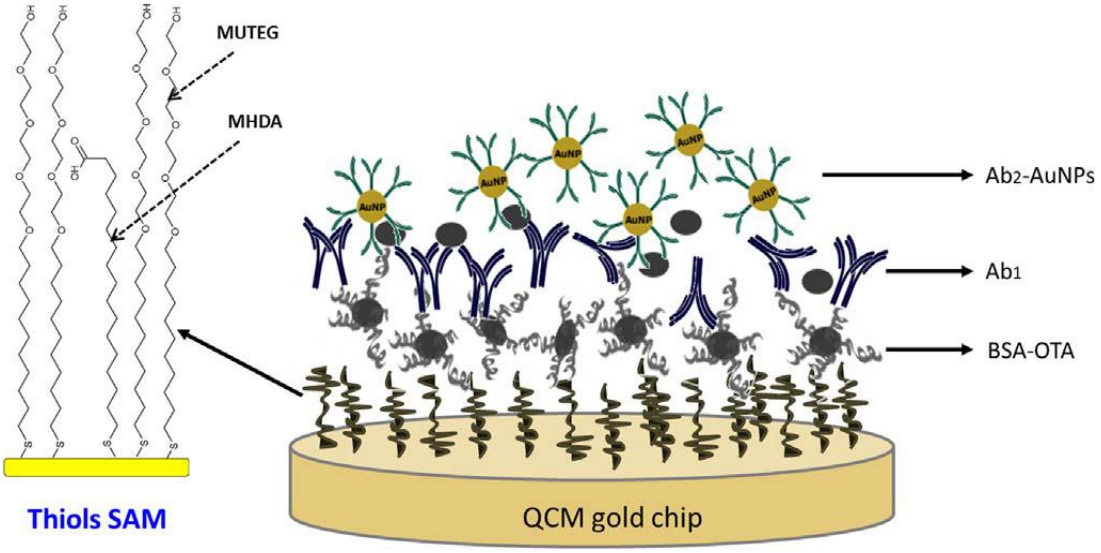

Figure 5. Scheme of the interfacial molecular architecture for the detection of OTA using a competitive immunoassay-QCM approach (reproduced with permission from Karczmarczyk et al. [143] ©Elsevier, 2018).

A simple and sensitive QCM immunosensing platform was designed by Tang et al. [144] for detecting AFB1 in foodstuffs. Initially, the phenoxy-derived dextran molecule was immobilized on the surface of QCM gold substrate. Then, AFB1-bovine serum albumin (AFB1-BSA) conjugated concanavalin A (Con A) was assembled onto the QCM probe through the dextran-Con A interaction. Glucose-loaded nanoliposome, labeled with monoclonal anti-AFB1 antibody, was used for the amplification of QCM signal. The observed dynamic ranged from 1.0 ng·kg^{-1} to 10 μg·kg^{-1} and the LOD was 0.83 ng·kg^{-1}. The accuracy of the immunoassay was evaluated with peanut samples, including naturally contaminated peanut samples and spiked peanut samples. The immunosensing platform showed similar results compared to commercial AFB1 ELISA kits.

4. Conclusions and Future Perspectives

The need of controlling food contamination is a current and growing worldwide challenge. Therefore, the availability of fast and accurate analytical devices that could be used as a warning preliminary screening tool, allowing the detection of food contamination is of utmost relevance.

Thin film biosensor devices are currently one of the most active research areas within the mycotoxins analysis. Comparing with the traditional analytical methods used for mycotoxins analysis, the main advantages of biosensors include the fast analysis time and rapid detection, high sensitivity, easy sample preparation, reusability and low cost. Nanomaterials such as gold, silver, metal oxides and quantum dots have been extensively employed for enhancing the detection capability of biosensors due to their remarkable optical, electronic, thermal, and mechanical properties, allowing the increase of their sensitivity, stability, and selectivity towards mycotoxins. In addition, several studies have been carried out focusing in the use of aptamers, antibodies, and MIPs together with nanoparticles in order to amplify the signal responses and thus guaranteeing a greater selectivity of thin film biosensors. Finally, it should also be mentioned that sensors merging electrochemical and optic detection systems (e.g., electrochemiluminescence and SPR assays) seem to be very promising approaches, demonstrating reliable and sensitive mycotoxins detection, possessing self-checking and self-calibration capabilities. These devices can also be designed for single or multi-analyte testing, single-use or reusable procedures, even allowing continuous monitoring assays. However, some drawbacks must be overcome before these devices may be used as routine techniques for mycotoxins detection. An effort must be made by researchers to convince the industrial partners of the advantages of thin film sensor devices, allowing their commercial exploitation. The use of several non-standard deposition techniques for obtaining the thin layers, the need of incorporating antibodies or aptamers as recognition elements coupled with the nano/microscale of the films poses some practical limitations. Moreover, the availability of user-friendly commercial devices, which are currently not a reality, requires merging several fields of knowledge (e.g., artificial intelligence, digital electronic sensors design, material sciences, microcircuit design, software innovations, and electronic systems integration) that at the actual research and development level is not a straightforward task. Furthermore, although the thin film sensor devices may enhance the mycotoxins detection performance, it should be remarked that the conventional analytical techniques (e.g., ELISA, HPLC, GC, etc.) allow for achieving detection limits of the same order of magnitude ($ng \cdot mL^{-1}$ or $\mu g \cdot kg^{-1}$), fulfilling the legal detection thresholds and so, limiting the need of establishing novel detection strategies. Similarly to other emerging techniques, the key challenge in the near future would be reaching the market, which would require unequivocally demonstrating the practical detection feasibility of such devices as well as the possibility of producing them at an industrial level, or the huge advantages of delivering personalized solutions upon a specific request from a client. Nevertheless, the reported overall satisfactory performances achieved with thin-film sensor based devices for mycotoxins detection in food matrices, even at levels lower than the legally regulated thresholds, allow foreseeing their future practical application, as a routine analytical procedure.

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