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Development of a QCM-D biosensor for Ochratoxin A detection in red wine

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ABSTRACT

Ochratoxin A (OTA), a highly toxic compound, is one of the most widely spread mycotoxins that contaminates a large variety of agricultural commodities. Due to its presence in the food chain, it imposes a hazard on both human and animal health. Therefore, there is a need for precise, fast and simple methods for toxin quantification. Herein, a novel sensor based on a quartz crystal microbalance with dissipation monitoring (QCM-D) and antibodies for specific analyte recognition was developed for rapid and sensitive detection of OTA in red wine.

The combination of indirect competitive assay with QCM-D gives a straightforward device, which can simultaneously measure frequency (Δf) and dissipation (ΔD) changes resulting in detailed information about the mass attached to the sensor surface as well as conformational changes, viscoelastic properties and the hydration state of the film.

Small molecules (such as OTA) suffer from poor LOD due to the high concentration of primary antibody needed to generate adequate signal. In the present study, amplification of the QCM-D signal was obtained by applying secondary antibodies conjugated with gold nanoparticles (AuNPs).

Thanks to this, a linear detection range of 0.2–40 ng mL^{-1} has been achieved with an excellent LOD of 0.16 ng $mL^{-1,}$ which is one order of magnitude lower than LOD specified by European Union legislation concerning the limit of OTA in food.

Moreover, a matrix effect (caused by the occurrence of polyphenols in wine) and associated non-specific interactions with the sensor surface was completely eliminated by a simple pre-treatment of the wine with the addition of 3% poly(vinylpyrrolidone) (PVP).

1. Introduction

Ochratoxins are a group of highly toxic fungal secondary metabolites produced mainly by *Aspergillus* (chiefly *A. ochraceus* and *A. niger*) and some *Penicillium* species (*P. verrucosum* and *P. carbonarius*) [1]. The most ubiquitous and toxic Ochratoxin occurring in agricultural products is Ochratoxin A (OTA). Other structurally related Ochratoxins (Ochratoxin B, C, α and β) are much less harmful or even do not exist in food [2]. The worldwide OTA occurrence in a broad range of raw and processed food commodities e.g. cereals, wine, coffee, dried fruits, beer, cocoa, nuts, beans, peas, bread and rice has already been amply described [3–6].

Studies show that this molecule can have several toxicological effects such as nephrotoxicity, hepatotoxicity, neurotoxicity, teratogenicity and immunotoxicity [2]. It is also suspected of being the main etiological factor responsible for Balkan endemic nephropathy and its association to urinary tract tumors has also been proven [7]. Due to the general concern about the OTA present in the food chain, its high stability and potentially negative effects on human and animal health, a number of countries have set up regulations including maximum permitted, or recommended levels for specific commodities. The European Union (E.U.) has established limits for OTA depending on the food product: 5 μg kg^{-1} for unprocessed cereals, 10 μg kg^{-1} for dried fruits, 15 μg kg^{-1} for spices, including chili powder, paprika, pepper or nutmeg, and 2 mg mL^{-1} for all types of wines [6].

Currently, routine analysis of OTA in foodstuff is mostly performed by chromatographical methods including thin layer chromatography (TLC) [8], liquid chromatography (LC) [9,10], gas chromatography (GC) [11] and high-performance liquid chromatography with fluorescence detection (HPLC) [12,13]. Those techniques are generally straightforward and yield reliable results, however, they require extensive preparation steps and are time-consuming. Thus, alternative approaches offering high sensitivity and simplicity such as enzyme-linked immunosorbent assay (ELISA) [14,15], electrochemistry [16],

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fluorescence [17] or chemiluminescence [18] have been developed. Moreover, label-free immunosensors for real-time toxins detection based on optical (e.g., surface plasmon resonance spectroscopy, SPR) [5] and piezoelectric or acoustic devices (e.g., quartz crystal microbalance, QCM) [19] have recently been investigated.

Biosensors with QCM-based readout are gaining increasing popularity in the detection of chemicals and biomolecules. This technique is a powerful and well-established noninvasive tool for online monitoring and quantification of molecular interactions on a solid surfaces [20]. The transducer in a QCM sensor is an oscillating quartz crystal whose resonance frequency (Δf) changes with the change in the mass (Δm) according to the Sauerbrey equation [21]. In addition to adsorbed mass (ng cm^{-2} sensitivity), the damping behavior of the crystal related to the conformational or structural properties of the viscous layer can be defined by measuring the energy dissipation loss (ΔD) of the freely oscillating crystal [20,22,23]. The so-called QCM-D device enables simultaneous monitoring of the changes in frequency and dissipation, and thus provides additional information about the effective layer thickness, conformational changes, viscoelastic properties and the hydration state of the film [24].

Despite a wide variety of approaches, these sensors do not always fulfil the requirements of high sensitivity, especially regarding detection of small molecules. Mycotoxins are low molecular weight compounds and therefore, cannot generate sufficient QCM-D signal (frequency change). In order to obtain optimal assay sensitivity, that is, increase the signal, reduce the background, while keeping the detection time short, the competitive inhibition immunoassay format was applied in the present study. This format is based on antigen immobilization on the sensor surface followed by the injection of the mixture of primary antibody and sample containing free antigen [25]. To reach a lower limit of detection, the signal can be amplified by the use of additional high mass compounds such as a secondary antibody with or without conjugation to gold nanoparticles (AuNPs).

In this work, we present the development of a fast and sensitive QCM-D biosensor for the detection of Ochratoxin A in red wine. We have reached good QCM-D sensitivity with the specific detection format of indirect competitive assay. To increase sensitivity, the signal was further amplified by the implementation of a secondary antibody labeled with gold nanoparticles. With this system we were able to reach a detection limit at the ng mL^{-1} level. Additionally, by combining simultaneous monitoring of the frequency and dissipation changes, the mechanical and viscoelastic properties of the biofilm were characterized. Moreover, to minimize the matrix effect and non-specific adsorption of wine commodities such as polyphenols (resulting in antibodies inactivation), a very simple procedure (addition of 3% of binding agent poly(vinylpyrrolidone) (PVP)) was employed with no need for cleanup or preconcentration of the sample extract [5].

2. Material and methods

2.1. Reagents

All reagents were used as received without further purification. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were obtained from ThermoFisher Scientific (Germany). Thiols: (11-mercaptoundecyl)tetra(ethylene glycol) (MUTEG) and 16-mercaptohexadecanoic acid (MHDA), Ochratoxin A (OTA), the conjugate of OTA with bovine serum albumin (BSA-OTA), poly(vinylpyrrolidinone) (PVP), Tween 20, hydrogen peroxide (30%), ethanolamine and glycine were purchased from Sigma-Aldrich (Germany). The primary rabbit antibody against OTA (Ab_1) was from AntiProt. Gold nanoparticles (AuNPs, 20 nm)-labeled goat anti-rabbit secondary antibody (Ab_2-AuNPs) were from Abcam (UK). 20 mM acetate buffer (ACT, pH 4) was prepared from sodium acetate trihydrate and acetic acid (both from Sigma-Aldrich) and the pH was adjusted by HCl and NaOH. Phosphate-buffered saline pH 7.4 (PBS) (0.12 g KH_2PO_4, 0.72 g Na_2HPO_4, 4 g NaCl and 0.1 g KCl in 0.5 L distilled water) or PBS containing 0.1% Tween 20 (PBS-T) were used as running buffers. The ERM (European Reference Material) BD476 (OTA in red wine) was obtained from the Institute for Reference Materials and Measurements (Geel, Belgium). This material was prepared from commercial wine sources intended for human consumption and it was characterized by mass spectrometry and HPLC with optical detection. The concentration of OTA was determined as 0.52 ± 0.11 ng mL^{-1}.

2.2. Apparatus

Piezoelectric measurements were performed with AT-cut gold-coated quartz crystals (Ø 14 mm, 100 nm thickness) with a resonance frequency of 5 MHz (LOT-Quantum Design GmbH, Germany) in flow-through mode (flow rate=50 μL min^{-1}) with a quartz crystal microbalance with dissipation monitoring QCM-D (E1 model, Q-Sense AB, Sweden) at 22 °C. In a QCM-D measurements, the fundamental resonance frequency of the crystal is excited and the variations in the resonance frequency, Δf, and energy dissipation ΔD, are recorded simultaneously for several overtones [26]. The oscillating frequency of the piezoelectric crystal decreases with the adsorption of a foreign substances on the surface. To calculate the mass uptakes (Δm) the simplified relation between the shift in frequency (Δf) and the mass of the adsorbed layer described by the Sauerbrey equation was used [21]:

$$\Delta m = \frac{-C \quad \Delta f_n}{n} \tag{1}$$

where C is the mass sensitivity constant (C=17.7 ng $cm^{-2}Hz^{-1}$ at f=5 MHz), n=1 for the fundamental frequency and $n > 1$ is the overtone number (n=3,5, ...). In the QCM-D measurements, Δf is not only related to the mass uptake but may also be caused by the hydration of proteins and water trapped in the pores of the layer. In very dissipative systems it is required to utilize a more complex model for surface mass density estimation, e.g. the Voigt model [27]. However, in the current paper the frequency shift of the 5th overtone was chosen (due to appropriate sensitivity and reproducibility as compared to other overtones) and the Sauerbrey equation was employed in the data analysis.

2.3. Sensor chip functionalization and detection format

As shown in Fig. 1, the gold surface of the sensor chip was modified with a mixed thiol self-assembled monolayer (SAM) to which the BSA-OTA conjugate was attached. Prior the functionalization, the QCM resonator wafers were cleaned with a 3:1 (v/v) piranha solution (concentrated H_2SO_4 and 30% H_2O_2) for 10 min, followed by rinsing with water. Afterwards, the sensor was dried in a nitrogen stream and treated with a UV/ozon cleaner for 30 min. The thiol SAM was formed on the gold surface upon overnight incubation in a mixture of MUTEG and MHDA (molar ratio 9:1) dissolved in ethanol at the total concentration of 1 mM. Afterwards, the sensor surface was rinsed with ethanol and dried in a stream of nitrogen. The carboxylic terminal

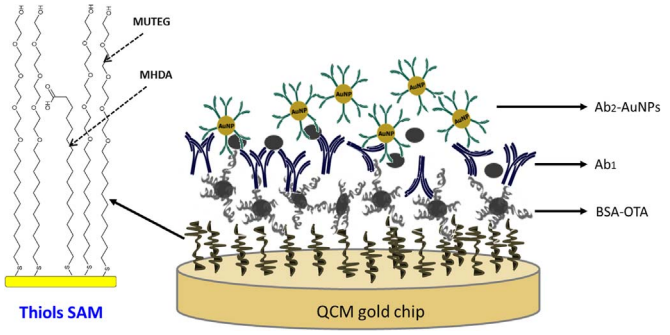

Fig. 1. Scheme of the interfacial molecular architecture for the detection of OTA by a competitive immunoassay utilizing QCM.

groups were activated by immersing the chip in EDC/NHS solution (concentrations in deionized water of 37.5 and 10.5 mg mL^{-1}, respectively) for 45 min. Then, the sensor was rinsed with water, dried in a nitrogen stream and inserted into the QCM-D cell. Subsequently, the immobilization of BSA-OTA (dissolved in ACT buffer at a concentration of 40 μg mL^{-1}) was done by 20 min circulation of the conjugate solution over the flow cell, followed by deactivation of unreacted active ester moieties using 10 min incubation in ethanolamine (1 M and pH 8.5).

For the detection of OTA, an inhibition immunoassay was applied. In this assay, the samples were pre-incubated with Ab_1 for 15 min (with an equal volume) followed by detection of the amount of unreacted Ab_1 antibody. PBS-T buffer or certified red wine standard were spiked with OTA at concentrations between 10^{-2} and 10^4 ng mL^{-1}. In order to decrease the influence of polyphenolic compound (matrix effect) that may interfere with the OTA analysis (inactivation of antibodies or unspecific sorption) in wine, samples were mixed with 3% of PVP and subsequently shaken for 5 min at room temperature, filtrated, and the pH of each aliquot was adjusted to 7.4 with NaOH prior to analysis. The mixture of Ab_1 with sample was pumped through the sensor for 10 min followed by washing with PBS-T buffer for 2 min to remove weakly bounded Ab_1 molecules. Afterward, the Ab_2–AuNPs antibody (concentration of 0.1 μg mL^{-1}) was flowed through the sensor for 10 min. Finally, the sensor surface was washed for 3 min with PBS-T buffer and the changes in the frequency owing to the binding of Ab_2-AuNPs to the captured Ab_1 were recorded. After each detection cycle, the sensor surface was regenerated by 10 min incubation in glycine buffer (pH 2.0, 20 mM).

3. Results and discussion

3.1. Sensor chip and assay characterization

The QCM-D is an acoustic wave device measuring the resonance of a piezoelectric quartz crystal upon electrical excitation (bulk excitation). The resonance frequency decreases linearly with addition of mass on the sensor surface. BSA-OTA immobilization showed a slight increase in dissipation energy (ΔD=10.77×10^{-6}) therefore, the frequency measurement was directly converted to mass by using the Sauerbrey equation (Eq.1) and the recorded frequency shift (Δf =57.31 Hz). The surface coverage of the covalently bound antigen conjugate was calculated to be 202.84 ng cm^{-1}. A typical QCM-D plot, with real-time changes in the frequency and dissipation of adsorption sequence is shown in the Fig. 2. Due to the very low concentration of the primary antibody, the recorded signal for both the frequency and dissipation is weak (Δf =17.97 Hz and ΔD=2.02×10^{-6}). Hence, an additional high mass provided by Ab_2-AuNPs injection was applied resulting in a signal enhancement (Δf=52.53 Hz) and consequently an improvement of immunoassay sensitivity.

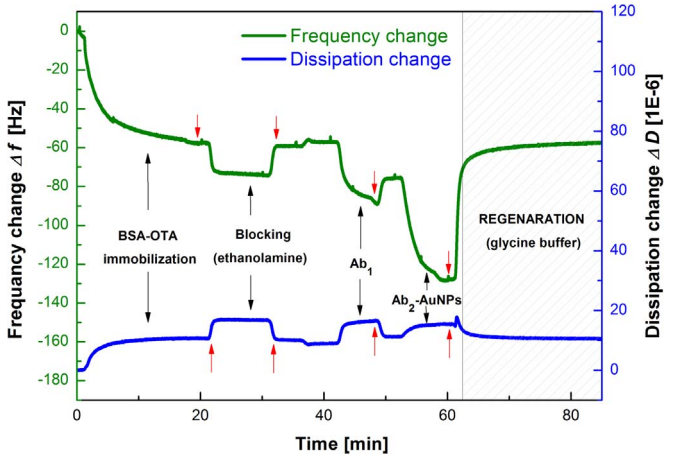

Fig. 2. QCM-D curves showing the frequency (Δf, green curve) and dissipation (ΔD, blue curve) changes during the adsorption sequence of the indirect immunoassay. Red arrows represent the washing steps. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The combined Δf and ΔD data analysis provide also information about the viscoelastic and mechanical properties of the protein layer. Moreover, according to the general understanding of the dissipative behavior of the biofilm, water is known to be trapped and sensed as additional mass [28]. A larger dissipation or $\Delta D/\Delta f$ ratio are usually correlated with high water content, loose bindings between interacting molecules or creation of a soft and dissipative film. On the other hand, the low dissipation per mass unit could be attributed to the formation of a well-structured complex with a high affinity binding and low degree of hydration [20]. Estimated $\Delta D/\Delta f$=1.7×10^{-7} for BSA-OTA layer indicates formation of a homogeneous and fairly rigid film.

Surface regeneration for cyclic use of the same chip is an important part of QCM-D immunoassays. In order to reuse the sensor chip surface, a simple regeneration protocol based on breaking the strong non-covalent interaction between toxin and antibody was applied [29]. This was accomplished by injection of glycine buffer at pH 2.0 over the sensor surface. As shown in Fig. 2, after the regeneration step the frequency and dissipation signals return almost to the original baseline level (negligible increase of Δf and ΔD≈0. 1%) indicating complete breakdown of the immuno-complex interaction and revealing fully reversible assay cycle. In this case, the immobilized antigen was still present at the sensor surface and hence, the assay procedure for the next cycle started with the injection of the primary antibody.

After six cycles, the used quartz crystals were cleaned with piranha solution to remove all adsorbed on the gold surface materials including antigen (BSA-OTA) as well as SAM. SAMs are mechanically fragile surfaces, and thus, techniques for polishing or roughening surfaces of metals can remove the SAM and expose a clean surface on bulk metal substrates [30]. There are a number of different techniques for removing SAMs from gold substrates such as: thermal desorption [31], ion sputtering [32] or UV/ozonolysis [33]. Chemical oxidants or reductants such as concentrated acids or bases or piranha solutions also are effective for cleaning substrates [34]. In our work piranha solution was used both for cleaning the surface before SAM functionalization as well was for removing thiols from the sensor surface. Moreover, a gold surface exposed to UV light (also used in this study) can be purged of sulfur impurities via oxidation of chemisorbed sulfur to sulfonates which can then be removed by washing with water [35]. The UV/ozone method allows one to remove old thiol monolayers and to provide a fresh surface for additional experiments. After such a treatment, the gold surface could be reused – a new SAM could be created followed by a fresh antigen solution immobilization.

The detection scheme is based on an indirect competitive format where Ab_2-AuNPs are used as a signal enhancement tag. The antigen attached to the surface (BSA-OTA conjugate) competes with toxins from the sample to bind with the added primary antibodies. Subsequently, captured Ab_1 bind with secondary antibodies labeled with AuNPs resulting in signal amplification and improvement of the assay sensitivity. In order to check the specificity of antibodies a simple experiment was performed. Briefly, BSA was immobilized on a chip, followed by Ab_1 injection and incubation for 10 min. After washing with PBS-T, only a negligible increase of the signal was recorded, indicating no adsorption of Ab_1. Naturally, in the absence of Ab_1, no non-specific binding of the secondary antibodies was observed (data not shown).

Due to the fact that wine is a very complex beverage containing a large variety of macromolecules which can interfere with the affinity binding on the surface, it is required to implement a pre-treatment of the wine samples prior the analysis. It might also seem that the percentage of alcohol (≈12%) in wine can influence the proper functionality of the assay. However, Ngundi et al. [36] demonstrated

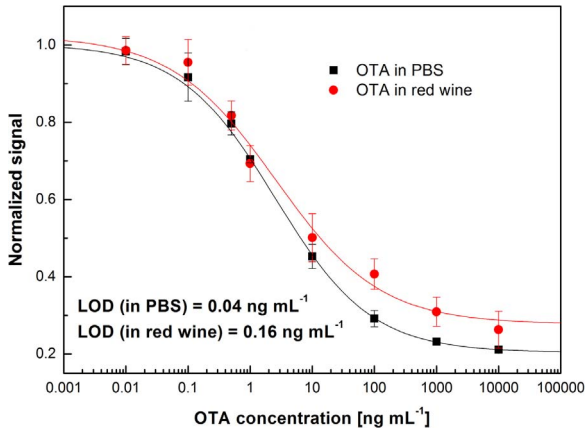

Fig. 3. Normalized calibration curves for the detection of OTA using inhibition immunoassay performed in PBS-T buffer (black squares) and red wine (red circles). Each point is an average of three replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that the amount of ethanol is not a major factor which hampers the analysis. On the other hand, Ogunjimi and Choudary [37] have shown antibody inactivation due to the presence of polyphenols in fruit juice, wines and vegetables. Thus, a simple pre-treatment of wine samples involving the use of 3% PVP, a binding agent commonly used to remove polyphenols from plant extracts, was applied. We have previously proved an excellent ability of PVP to eliminate unwanted compounds from the solution and therefore preserve antibody activity [5].

3.2. Ochratoxin A detection

The sensor response was measured for series of buffer and wine samples spiked with OTA at concentrations ranging from 10^{-2} to 10^{4} ng mL^{-1}. Fig. 3 shows the calibration curves normalized with the sensor response for the blank sample (not spiked with OTA). The calibration curves were fitted with a sigmoidal function. The limit of the detection (LOD) and the limit of quantification (LOQ) were defined as the concentration of OTA equivalent to three times (for LOD) and ten times (for LOQ) the value of the standard deviation (SD), measured in the absence of OTA.

Fig. 3 clearly shows that the recorded signal is proportional to the amount of bound antibody (gradual decrease when increasing the concentration of OTA in the sample assigned to the blocking of Ab_1 binding sites). The LOD and LOQ for the assay established in a PBS-T buffer were 0.04 and 0.13 ng mL^{-1}, respectively. The same parameters estimated for experiments performed in red wine samples were 0.16 and 0.55 ng mL^{-1} with the working linear range (IC20-IC80) between 0.2–40 ng mL^{-1}. The coefficient of variation (CV) of the standard points of the calibration curves were 8.77% and 3.79% for the assay carried out in red wine and PBS-T, respectively, showing a good repeatability of the detection method. A midpoint value (IC50) of 2.53 and 2.58 ng mL^{-1} were obtained during competition assays performed in PBS and red wine, respectively. The higher LOD value obtained from wine measurements indicates adsorption of wine components on the sensor surface causing a somewhat higher background. However, this value is still one order of magnitude lower than the maximum residue level of OTA required by the European Commission.

The presented QCM-D biosensor offers higher sensitivity, shorter analysis time and avoids complicated and time consuming pre-cleaning methods with respect to other reports on the detection of this toxin [38–43]. High-performance liquid chromatography (HPLC) [44], gas chromatography-mass spectrometry, thin-layer chromatography (TLC) [45], enzyme linked immunosorbent assay (ELISA) [46] and immunochromatographic assays as lateral flow strips [47] show good performance for OTA detection however, they all require multiple steps prior to the detection (including extraction and clean-up procedures), what extend the analysis time and causing extra costs. Compared to other alternative methods such as capillary electrophoresis with diode array detection [48], electrochemical immunosensors [49] or optical techniques like surface plasmon resonance (SPR) [5,6], our QCM sensor offers a similar detection limit but shorter detection time.

4. Conclusion

An indirect competitive bioassay was successfully established for the detection of Ochratoxin A in red wine utilizing gold nanoparticles for QCM-D signal enhancement. Small molecules (such as OTA) suffer from poor LOD due to the high concentration of Ab_1 being needed to generate adequate signal. To overcome this problem, in the present study we have reported implementation of a secondary antibody with gold nanoparticle label resulting in the enhancement of the sensitivity of the low molecular weight compound assay. The decrease of the frequency and dissipation changes (Δf and ΔD) are proportional to the mass of molecules adsorbed on the chip surface and therefore, an additional mass provided by AuNPs results in a great signal amplification in comparison to the assay only with Ab_1. A linear range 0.2–40 ng mL^{-1} has been achieved with excellent LOD of 0.16 ng mL^{-1}, which is one order of magnitude lower then LOD specified by E.U. legislation concerning limit of exposure in food. Additionally, the biosensor can be easily regenerated with 10 min incubation in glycine buffer. Moreover, a real-world application of the system was tested with the determination of OTA in spiked red wine samples. Finally, a matrix effect (caused by the occurrence of polyphenol in wine) and associated non-specific interactions with the sensor surface was minimized by a simple pre-treatment of the wine with the addition of 3% of the binding agent PVP.

The method described here is a rapid (less than one hour detection time), sensitive and cost effective (inexpensive reagents, reduction of the concentration of the valuable primary antibody) alternative for the detection of various mycotoxins in food and beverages and therefore, an important tool in the field of food security and thus, human health.

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References

[1] M. Weidenbörner, Encyclopedia of Food Mycotoxins, Springer Science & Business Media, Berlin, Heidelberg, 2001.
[2] A. El Khoury, A. Atoui, Ochratoxin A: general overview and actual molecular status, Toxins 2 (4) (2010) 461–493.
[3] A. Pittet, Natural Occurrence of Mycotoxins in Foods and Feeds: an Update Review, Revue de Medecine Veterinaire, France, 1998.
[4] M. Lobeau, et al., Development of a new clean-up tandem assay column for the detection of Ochratoxin A in roasted coffee, Anal. Chim. Acta 538 (1) (2005) 57–61.
[5] A. Karczmarczyk, et al., Fast and sensitive detection of Ochratoxin A in red wine by nanoparticle-enhanced SPR, Anal. Chim. Acta 937 (2016) 143–150.
[6] J. Yuan, et al., Surface plasmon resonance biosensor for the detection of Ochratoxin A in cereals and beverages, Anal. Chim. Acta 656 (1) (2009) 63–71.
[7] H. Bacha, et al. Human ochratoxicosis and its pathologies. in Colloquium INSERM, 1993.
[8] A. Pittet, D. Royer, Rapid, low cost thin-layer chromatographic screening method for the detection of Ochratoxin A in green coffee at a control level of 10 µg/kg, J. Agric. Food Chem. 50 (2) (2002) 243–247.
[9] J. Blesa, et al., Rapid determination of Ochratoxin A in cereals and cereal products by liquid chromatography, J. Chromatogr. A 1046 (1) (2004) 127–131.
[10] A. Visconti, M. Pascale, G. Centonze, Determination of Ochratoxin A in domestic and imported beers in Italy by immunoaffinity clean-up and liquid chromatography, J. Chromatogr. A 888 (1) (2000) 321–326.
[11] Y. Jiao, et al., Identification of Ochratoxin A in food samples by chemical

derivatization and gas chromatography-mass spectrometry, J. Chromatogr. A 595 (1–2) (1992) 364–367.
[12] A. Visconti, M. Pascale, G. Centonze, Determination of Ochratoxin A in wine by means of immunoaffinity column clean-up and high-performance liquid chromatography, J. Chromatogr. A 864 (1) (1999) 89–101.
[13] P. Markaki, et al., Determination of Ochratoxin A in red wine and vinegar by immunoaffinity high-pressure liquid chromatography, J. Food Protection® 64 (4) (2001) 533–537.
[14] D. Flajs, et al., ELISA and HPLC analysis of Ochratoxin A in red wines of Croatia, Food Control 20 (6) (2009) 590–592.
[15] I. Barna-Vetró, et al., Sensitive ELISA test for determination of Ochratoxin A, J. Agric. Food Chem. 44 (12) (1996) 4071–4074.
[16] M. Heurich, M.K.A. Kadir, I.E. Tothill, An electrochemical sensor based on carboxymethylated dextran modified gold surface for Ochratoxin A analysis, Sens. Actuators B: Chem. 156 (1) (2011) 162–168.
[17] C. Tessini, et al., Alternatives for sample pre-treatment and HPLC determination of Ochratoxin A in red wine using fluorescence detection, Anal. Chim. Acta 660 (1) (2010) 119–126.
[18] J.C. Sauceda-Friebe, et al., Regenerable immuno-biochip for screening Ochratoxin A in green coffee extract using an automated microarray chip reader with chemiluminescence detection, Anal. Chim. Acta 689 (2) (2011) 234–242.
[19] W.C. Tsai, C.K. Hsieh, QCM-based immunosensor for the determination of ochratoxin A, Anal. Lett. 40 (10) (2007) 1979–1991.
[20] X. Su, et al., Enzyme immobilization on poly (ethylene-co-acrylic acid) films studied by quartz crystal microbalance with dissipation monitoring, J. Colloid Interface Sci. 287 (1) (2005) 35–42.
[21] G. Sauerbrey, Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung, Z. für Phys. 155 (2) (1959) 206–222.
[22] Ö. Ertekin, S. Öztürk, Z.Z. Öztürk, Label Free QCM Immunobiosensor for AFB1 Detection Using Monoclonal IgA Antibody as Recognition Element, Sensors 16 (8) (2016) 1274.
[23] M.C. Dixon, Quartz crystal microbalance with dissipation monitoring: enabling real-time characterization of biological materials and their interactions, J. Biomol. Tech. 19 (3) (2008) 151.
[24] A. Sweity, et al., Relation between EPS adherence, viscoelastic properties, and MBR operation: Biofouling study with QCM-D, Water Res. 45 (19) (2011) 6430–6440.
[25] J. Mitchell, Small molecule immunosensing using surface plasmon resonance, Sensors 10 (8) (2010) 7323–7346.
[26] C. Montis, et al., Nucleolipid bilayers: a quartz crystal microbalance and neutron reflectometry study, Colloids Surf. B: Biointerfaces 137 (2016) 203–213.
[27] M.V. Voinova, et al., Viscoelastic acoustic response of layered polymer films at fluid-solid interfaces: continuum mechanics approach, Physica Scr. 59 (5) (1999) 391.
[28] A.A. Feiler, et al., Adsorption and viscoelastic properties of fractionated mucin (BSM) and bovine serum albumin (BSA) studied with quartz crystal microbalance (QCM-D), J. Colloid Interface Sci. 315 (2) (2007) 475–481.
[29] K. Andersson, D. Areskoug, E. Hardenborg, Exploring buffer space for molecular interactions, J. Mol. Recognit. 12 (5) (1999) 310–315.
[30] J.C. Love, et al., Self-assembled monolayers of thiolates on metals as a form of nanotechnology, Chem. Rev. 105 (4) (2005) 1103–1170.
[31] M.R. Shadnam, A. Amirfazli, Kinetics of alkanethiol monolayer desorption from gold in air, Chem. Commun. 38 (2005) 4869–4871.
[32] S. Chenakin, B. Heinz, H. Morgner, Sputtering of hexadecanethiol monolayers self-assembled onto Ag (111), Surf. Sci. 397 (1) (1998) 84–100.
[33] D.E. King, Oxidation of gold by ultraviolet light and ozone at 25 C, J. Vac. Sci. Technol. A 13 (3) (1995) 1247–1253.
[34] T.W. Schneider, D.A. Buttry, Electrochemical quartz crystal microbalance studies of adsorption and desorption of self-assembled monolayers of alkyl thiols on gold, J. Am. Chem. Soc. 115 (26) (1993) 12391–12397.
[35] C.G. Worley, R.W. Linton, Removing sulfur from gold using ultraviolet/ozone cleaning, J. Vac. Sci. Technol.-Sect. A-Vac. Surf. Films 13 (4) (1995) 2281–2284.
[36] M.M. Ngundi, et al., Array biosensor for detection of Ochratoxin A in cereals and beverages, Anal. Chem. 77 (1) (2005) 148–154.
[37] A.A. Ogunjimi, P.V. Choudary, Adsorption of endogenous polyphenols relieves the inhibition by fruit juices and fresh produce of immuno-PCR detection of Escherichia coli 0157: H7, FEMS Immunol. Med. Microbiol. 23 (3) (1999) 213–220.
[38] N. Belli, et al., Review: Ochratoxin A (OTA) in wines, musts and grape juices: occurrence, regulations and methods of analysis, Food Sci. Technol. Int. 8 (6) (2002) 325–335.
[39] N.W. Turner, S. Subrahmanyam, S.A. Piletsky, Analytical methods for determination of mycotoxins: a review, Anal. Chim. Acta 632 (2) (2009) 168–180.
[40] E.P. Meulenberg, Immunochemical methods for Ochratoxin A detection: a review, Toxins 4 (4) (2012) 244–266.
[41] S. Duarte, A. Pena, C. Lino, A review on Ochratoxin A occurrence and effects of processing of cereal and cereal derived food products, Food Microbiol. 27 (2) (2010) 187–198.
[42] R. Köppen, et al., Determination of mycotoxins in foods: current state of analytical methods and limitations, Appl. Microbiol. Biotechnol. 86 (6) (2010) 1595–1612.
[43] M.Z. Zheng, J.L. Richard, J. Binder, A review of rapid methods for the analysis of mycotoxins, Mycopathologia 161 (5) (2006) 261–273.
[44] M. Sieber, et al., Metabonomic study of Ochratoxin A toxicity in rats after repeated administration: phenotypic anchoring enhances the ability for biomarker discovery, Chem. Res. Toxicol. 22 (7) (2009) 1221–1231.
[45] L. Keller, et al., Fungal and mycotoxins contamination in corn silage: monitoring risk before and after fermentation, J. Stored Prod. Res. 52 (2013) 42–47.
[46] M.R. Morgan, Mycotoxin immunoassays (with special reference to elisas), Tetrahedron 45 (8) (1989) 2237–2249.
[47] A. Laura, et al., A lateral flow immunoassay for measuring Ochratoxin A: development of a single system for maize, wheat and durum wheat, Food Control 22 (12) (2011) 1965–1970.
[48] E. González-Peñas, et al., Comparison between capillary electrophoresis and HPLC-FL for Ochratoxin A quantification in wine, Food Chem. 97 (2) (2006) 349–354.
[49] S.H. Alarcón, et al., Development of an electrochemical immunosensor for Ochratoxin A, Anal. Lett. 37 (8) (2004) 1545–1558.