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Review Article

Recent advances in electrochemical biosensing of aflatoxin M1 in milk – A mini review

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ABSTRACT

Aflatoxin, a member of mycotoxins produced by molds, is well known for its serious health implications for humans and animals alike. The global issue of aflatoxins entering the food chain poses a public health risk that should not be underestimated. It finds its way into the food chain through a variety of routes, including contaminated animal feed into milk. Given the hazardous nature of aflatoxin, the call for rapid and reliable detection methods of the toxin in milk is more important than ever. With the advent of nanotechnology, newly emerging electrochemical biosensors can present an answer to this call. In this review, immunosensors and aptasensors, the two most commonly developed electrochemical biosensors to detect aflatoxin in milk, are summarized. Expanding and complementing existing reviews, this article provides a compelling overview over the latest developments in the field of biosensors and discusses future directions as well as challenges that remain to be addressed.

1. Introduction

Aflatoxins have been discovered in the early 1960s in England when sudden death of more than 100,000 turkeys led to intensive research and investigation of the reason [1]. Eventually, the cause of the tragic mass mortality could be traced back to aflatoxins and numerous variants of the small molecule have been characterized since then [1]. Aflatoxins represent a subgroup of Mycotoxins, which are secondary metabolites of micro fungi. It is produced by several species of the fungus *Aspergillus* section *Flavi* and poses severe health risks to humans and animals alike [2–4]. Various reviews discussing interesting aspects of detection of aflatoxin using biosensors have been published. Liu et al. provides good coverage of electrochemical biosensing of aflatoxins for different sample types, including milk, wine, peanuts and maize [5]. Another excellent review by Perez-Fernandez et al. addresses electrochemical biosensors for aflatoxin detection, specifically emphasizing the use of nanomaterials to enhance sensor performance [6]. Focused reviews discussing biosensing in only milk as a substrate have also been published: Chen et al. expertly covered this topic in a recent review paper, providing a comprehensive picture of different types of novel sensors [7]. A compelling review by Vaz et al. provides an overview of various detection methods for AFM1 in dairy products. It also discusses biosensors and explores the underlying methodologies in detail in terms of sample preparation and extraction [8]. Another publication from Gurban et al. from 2017 gives detailed insight and coverage of several detection methods for aflatoxin M1 (AFM1) in milk, mainly focusing on electrochemical detection [9].

However, owing to fast sensor development in the recent years, several novel sensors have been designed since their work has been published. The ongoing and rapidly developing research in the field of electrochemical biosensors for AFM1 calls for an updated review. This mini review presents a focused view on solely electrochemical detection of AFM1 in milk and aims to summarize the most recent, state-of-the-art developments in this field. It provides a compelling summary of the latest electrochemical aptasensors and immunosensors, types of biosensors, bringing added value to the reviews already published. The sensors will be explored in more detail and compared to each other in terms of Limit of Detection under optimal conditions (LoD), biorecognition element, transducer platform and electrochemical methodology (Tables 1 and 2). To our best knowledge, enzymatic electrochemical sensors, another type of biosensors, for AFM1 detection in milk have not yet been reported and are therefore not included in the review.

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1.1. Aflatoxin contamination in food and feed

Aflatoxin contamination is a global threat compromising the safety of food and feed. Various environmental factors such as temperature, water availability and humidity impact growth of fungi and consequently production of mycotoxins [10]. Contamination is therefore prevalent in warm and humid regions [11]. Agricultural contamination can occur at almost every stage in the food-chain but is usually found in pre-harvested crops or during long-term storage of crops [12]. According to the Food and Agriculture Organization of the United Nations (FAO), approximately 25% of worldwide crop is contaminated with mycotoxins [13-15]. Aflatoxins can be found in a wide range of food commodities including cereals, oilseeds, various spices, nuts and milk products [12,16]. Studies report dangerous concentrations of the toxin in countries around the globe, for example, in pre-harvested maize in Tanzania [17], in dry fruits and edible nuts from Pakistan [18], in various nuts marketed in Italy [19] and in rice from Nigeria [20].

Through different crops, aflatoxins also find their way into animal feed. A study from 2015 [21] analyzed a total of 97 livestock feed (n = 48) and feed ingredients (n = 49) samples from different farms across the Indian states Andhra Pradesh and Telangana for aflatoxin B1 (AFMB1). With HPLC, 30% of livestock feed and 24.5% of feed ingredients have been found to be contaminated with high levels of the toxin [21]. Another study from Nakavuma et al. [22] found that all poultry feed samples (n = 27) from farmers of selected regions of Uganda showed aflatoxin contamination far above the legislation limit of 20 ppb (FAO 2004, [23]). Through conversations with the farmers, they report that every second of them had limited knowledge about the risk of aflatoxin contamination and its mitigation strategies [22].

1.2. Health risks associated with aflatoxins

Among various types of aflatoxins, B1/2 (AFB1/2) and its metabolized version M1/2 (AFM1/2) are the best-characterized group and present a major health risk for humans and animals alike.

Owing to its hepatocarcinogenic nature, it may play a causative role in 4.6 – 28.8 % of all global liver cancer incidents with most cases occurring in sub-Saharan Africa, Southeast Asia, and China where populations suffer from uncontrolled exposure to the toxin [24].

Next to its carcinogenicity, aflatoxins display well established immunosuppressive effects in animals which have been explained in a review article from 2004 [25]. There, they also summarize and discuss the suppressive effect of aflatoxin on antibodies against various diseases in response to vaccinations, causing decreased vaccine effectiveness in poultry and rabbits [25].

Additionally, aflatoxin exhibits severe effects on embryonic development in animals, such as bone malformations, visceral changes and low birth weight [26,27]. Da Silva et al. [26] reports that those effects have mainly been studied in animals like rats, mice and rabbits and more data on how aflatoxin affects human intrauterine development is needed. However, several studies report a correlation between chronic aflatoxin exposure and stunted child growth during early year child development [28–30]. A large body of research indicates that aflatoxin possesses broad spectrum cytotoxicity, impacting various cells of different types. One study from 2009 documents a reduction in bovine mammary epithelial cell viability upon impact of aflatoxin B1 [31]. Another study that assessed the adverse effects of aflatoxins on the intestine found that it causes DNA damage in Caco-2 cells before and after differentiation [32].

1.3. Aflatoxins in milk and other dairy products – Legal limits

In light of the numerous occurring instances of feed contamination with aflatoxins, it should come as no surprise that its metabolized form M1/2 can also be detected in animal byproducts such as bovine milk [33]. A review from 2021 that summarizes the global extent of aflatoxin occurrence in milk found that various countries belonging to the sub-Saharan region have aflatoxin levels significantly above the limits set by the EU and the United States [34]. To name an example, a study from Gizachew et al. in Ethiopia found that all feed (n = 156) had been contaminated with aflatoxin. Subsequent analysis of milk reported high contamination levels in milk indicating that the toxin had been passed on to the milk, posing a severe risk to end consumers [35]. This impacts not only the milk itself but all downstream dairy-based products such as yoghurt, cheese and infant formula [36].

Given the multifaceted adverse effects of aflatoxin on human health, the legal limits of the toxin in milk are particularly strictly regulated. In the United States, the Food and Drug Administration (FDA) limits the amount of aflatoxin in milk to 500ppt which translates to a concentration of 500 ng/L. In the EU, the European Community (EC) and Codex Alimentarius imposes a more stringent limit of 50ppt corresponding to 50 ng/L of aflatoxin in milk.

Table 1
Electrochemical immunosensors for aflatoxin detection in milk.

Target	Transducer-Platform	Biorecognition Element	Method of detection	LOD (optimized conditions)	Reference
AFM1	MoS_2 QD@UiO-66-NH_2 composite	Antibody	EIS	0.06 ng/mL	[52]
AFB1/2 & G/1/2 & AFM1	peptide amphiphile (C14R5) mediated rasAu-NP formation	Antibody	DPV	0.05 pg/mL	[53]
AFM1	SPE	Antibody	CA	25 pg/mL	[55]
AFM1	colloidal Au and Ag electrodisposition/SPE	Antibody	EIS	15 ng/L	[56]
AFM1	Au microelectrode array	antibody	CV	8 ng/L	[57]
AFM1	SPE/ELISA constructed on electrode (3,3,5′,5′-tetramethylbenzidine dihydrochloride)	antibody	CV	39 ng/L	[58]
AFM1	SPE/ GO-CS / CeO2-CS	antibody	DPV/CV	0.009 μg/L	[49]
AFM1	SWCNTs functionalized dispense-printed electrodes	antibody	CA	0.02 μg/L	[50]
AFM1	EC-ELISA / RCA DNAzyme coupled with COFs	antibody	DPV	0.15 ng/mL	[54]
AFM1	Silver wire	antibody	EIS	1 pg/mL	[59]
AFM1	Competitive immunoassay using HRP	antibody	Amperometry	0.01 μg/L	[60]
AFM1	SPE	antibody	Amperometry	0.01 μg/L	[61]
AFM1	Anti-idiotypic nanobodies /SPE	antibody	CA	0.09 ng/mL	[62]
AFM1	Ü-HPPA, polypyrrole-surface-working electrode	antibody	Potentiometry	40 pg/mL	[63]

Abbreviations: MoS_2, molybdenum disulfide; QDs, quantum dots; UiO-66-NH_2, zirconium-based metal–organic framework; rasAu-NP, raspberry-like gold nanoparticles; SPE, screen-printed-electrode; ELISA, enzyme-linked immunosorbent assay; GO-CS, graphene oxide-chitosan; CeO_2-CS, cerium oxide-chitosan; SWCNTs, single-walled carbon nanotubes; RCA, rolling-circle amplification; COFs, covalent organic framework; HRP, horse-radish peroxidase p-HPPA, 3-(4-hydroxyphenyl)propionic acid; AFM1, Aflatoxin M1; AFB1/2, Aflatoxin B1/2; AFG1/2, Aflatoxin G1/2; CV, cyclic voltammetry; DPV, differential pulse voltammetry; EIS, electrochemical impedance spectroscopy; SWV, square wave voltammetry; CA, chronoamperometry.

1.4. Detection of aflatoxin in milk – From conventional methods to biosensors

Milk constitutes a significant part of the diet of various countries around the world in both adults and young children. Due to consensus regarding the detrimental impact of aflatoxin on human health it is clear that reliable quantitative detection of aflatoxin in milk becomes increasingly important. Various methods are being used for this purpose, including Thin-Layer Chromatography (TLC) [37] and High-Pressure Liquid Chromatography (HPLC) coupled with either Mass Spectrometry (MS) or fluorescent detectors [38–40]. These methods are high in cost, non-portable and time consuming to operate, but offer great sensitivity and reproducibility. Beyond that, the enzyme-linked immunosorbent assays (ELISA) has become a widely used technique to detect aflatoxins in milk all around the world [41,42]. ELISA is easy to perform and offers high specificity and sensitivity but comes with limitations such as high possibility of false positives/negatives, antibody instability and refrigerated transport and storage [43].

During the last decade various types of biosensors to reliably detect analytes have become increasingly popular. Leland C. Clark Jr., who is considered one of the founding fathers of the field of biosensors, developed a biosensor for oxygen detection as early as 1956 [44]. Today, it is impossible to imagine life without them as they play an indispensable role in health care and disease diagnosis, environmental monitoring and, ultimately food safety [45].

Many ways to classify biosensors exist: One way is based on their receptor, an element that imparts selectivity to the sensor. Possible receptors can be target specific antibodies, enzymes, aptamers or molecular imprinted polymers (MIP), with which the sensor can be functionalized. Another way to classify biosensors is based on the type of transducer used for the sensor. A transducer converts input into signals that provide quantitative insight about the concentration of the analyte of interest and can broadly be categorized into electrochemical, optical, gravimetric, thermal or electronic based on its working principle [45]. If further, more in-depth reading is required, the reader is hereby referred to Naresh et al. who provides a holistic overview of biosensor classification, design and working principle [45].

Biosensors could present an answer to the call for aflatoxin detection, as they offer a number of advantages over conventional detection methods. Combined with the advent of nanotechnology, biosensors are rapidly improving in terms of measurement speed and sensitivity. In addition, biosensors offer the possibility of point-of care detection and do not require professionals to operate. As biosensors usually require little equipment, they also present an attractive solution that could be used in technically challenged areas. It is therefore safe to say that biosensors mark a step in the right direction to become established as a cheap and reliable method for aflatoxin detection in milk.

2. Electrochemical biosensors

IUPAC has defined an electrochemical biosensor as a device that is "capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element [46]". In addition to the most common biological recognition elements, antibodies and aptamers, Thévenot et al. also lists biocatalytic elements such as enzymes, whole cells and tissue in a detailed technical report on electrochemical biosensors [46]. Upon binding of the target substance to the biorecognition element, a change in electric parameters like current or impedance can be measured, giving quantitative insight about the analyte concentration (Fig. 2). Analytical detection methods for electrochemical biosensors mainly include various types of voltammetry namely cyclic voltammetry (CV), square wave voltammetry (SWV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS). Since there already exist extensive literature on the working principles behind these methods [47] it will not be discussed in depth in this mini review.

2.1. Electrochemical immunosensors for aflatoxin detection in milk

In an electrochemical immunosensor, the working electrode is functionalized with antibodies serving as a target specific capture agent. In Table 1 recent electrochemical sensors employing antibodies as a biorecognition element are listed. In this chapter several studies developing electrochemical immunosensors have been selected to show how versatile and creative aflatoxin detection can get.

Screen printed electrodes (SPEs) have revolutionized the biosensor industry due to their low cost, their rapid production, their small size and their ability to be easily modified, the latter achieving a variety of improvements in overall performance [48]. For an immunosensor developed by An et al,the electrodes have been modified with a nanocomposite of graphene oxide-chitosan (GO-CS) and cerium oxide-chitosan (CeO2-CS), which lead to an improved electrochemical response and an increase in antibodies linked to the electrode surface [49]. They employed differential pulse voltammetry (DPV) and cyclic voltammetry (CV) to investigate aflatoxin concentration and were able

Table 2
Electrochemical aptasensors for aflatoxin detection in milk.

Target	Transducer-platform	Biorecognition element	Method of detection	LOD	Reference
AFM1	Au-NPs	aptamer	DPV	0.9 nM	[66]
AFB1	Au-NPs	aptamer	–	2 pg/mL	[66]
AFM1	label free rGO/Au-NPs	aptamer	EIS	0.3 ng/L	[74]
AFB1	Cu_2O NCs/GCE	MIPs/aptamer	EIS	12.0 pg/L	[75]
AFM1	Au-NPs/ ECNF	aptamer	CV	0.6 pg/ml	[69]
AFM1	GCE / Neutral Red dye / polycarboxylated pillar[5]arene	aptamer	EIS	0.5 ng/L	[72]
AFM1	hexaethyleneglycol-modified 21-mer oligonucleotide immobilized on CSPE	aptamer	EIS	1.15 ng/L	[76]
AFM1	Au-NPs	aptamer	DPV	0.9 ng/L	[77]
AFM1	ferrocene/silicon NPs	aptamer	EIS	4.53 fM	[73]
AFM1	Pt-NPs on GCE modified with MIL-101(Fe)	aptamer	EIS	2 pg/mL	[71]
AFM1	Polyaniline electrodeposited on GCE	aptamer	Voltammetry/EIS	1–5 ng/L	[78]
AFM1	Polyamidoamine dendrimers-based signal amplification	aptamer	EIS/DPV	8.47 & 8.67 ng/L	[79]
AFM1	ss-HSDNA/self-assembled monolayer of cysteamine and Au-NPs	aptamer	EIS	–	[80]
AFM1	Quantum-Dot Au-NPs composite	aptamer	DPV/CV	0.3 nM	[81]
AFM1	DNA-Au@Ag conjugates	aptamer	DPV	0.02 ng/mL	[82]
AFM1	*in situ* construction of COFs TpBD on GCE	aptamer	DPV	0.15 ng/mL	[83]

Abbreviations: Au-NPs, gold-nanoparticles; rGO, reduced graphene oxide; Cu_2O, copper oxide; NCs, nanocubes; GCE, glassy carbon electrodes; ECNF, electrospun carbon nanofibers; CSPE, carbon screen printed electrode; NPs, nanoparticles; Pt-NPs, platinum nanoparticles; MIL-101(Fe), Fe-based metal–organic framework; ss-HSDNA, thiol-modified single stranded DNA; COFs, covalent organic frameworks; AFM1, Aflatoxin M1; AFB1, Aflatoxin B1; CV, cyclic voltammetry; DPV, differential pulse voltammetry; EIS, electrochemical impedance spectroscopy; SWV, square wave voltammetry.

to achieve a detection limit of 0.009 μg/L under optimal conditions. Testing their sensor in pure milk samples revealed high accuracy and recovery rates for concentrations ranging from 0.02 μg/L to 0.5 μg/L, further underlining its promising potential for the detection of AFM1 in milk [49].

In another immunosensor developed by Abera et al, the electrodes have been functionalized with single-walled carbon nanotubes (SWCNTs) to significantly improve sensitivity for AFM1 [50]. They employed a special electrochemical technique termed chronoamperometry, in which a square-wave potential is applied to the working electrode and the change in current is then measured as a function of time [51]. The chronoamperometric technique revealed the same working range of 0.01–1 μg/L for both buffer and spiked milk. Given that the sensitivity was in line with legal AFM1 concentration limits in milk, the sensor represents a promising candidate in the field of electrochemical biosensing [50].

It becomes clear that the advent of nanotechnology pushes sensor development to newly found limits. An example of this is a recent study of Kaur et al, in which they explore molybdenum disulfide (MoS_2) and metal–organic frameworks (MOFs) nanocomposites for development of an AFM1 immunosensor [52]. Using EIS as the method of choice, they achieved a detection range of 0.2–10 ng/mL with a limit of detection of 0.06 ng/mL under optimized conditions. Verified by HPLC, the sensor performed well in spiked milk samples, displaying similar R2 values when compared to optimized buffer conditions [52].

A prime example of how nanomaterials are used to enhance aflatoxin immunosensor performance to unparalleled limits can be found in a sensor developed by Mao et al. [53]. They used self-assembling amphiphilic peptides (PAs) to form nano structures, in this case PA-gold nano particle (PA-AuNPs). This mediated formation of monodispersed hollow raspberry-like AuNPs (rasAuNPs), resulting in two benefits: The blank signal current was lowered while simultaneously causing more antibodies to bind [53]. These advantages allowed for ultra-sensitive detection limits of different aflatoxins (AFB1/2, AFG2/2 and AFM1/2) between 0.082 and 0.14 pg/mL and validated by HPLC, they achieved similar results in spiked peanut milk [53].

The following study by Pang et al. shows how diverse and multifaceted the field of electrochemical immunosensors can get [54]. They developed a complex and innovative sensor platform based on the principle of rolling circle amplification (RCA). Upon binding of aflatoxin to the target antibody fixated to a microplate, primer-AuNPs-aptamers trigger the production of ssDNA which, under optimal conditions, folds into a peroxidase-mimicking DNAzyme. The DNAzyme can then catalyze the oxidation of 2-aminophenol to 3-aminophenylhydrazine, which ultimately accumulates at the electrode, causing a measurable change in current [54]. Using this technique under optimal conditions, Pang et al. achieved AFM1 detection as low as 0.15 ng/mL. As reflected in the high recovery rates, they report accurate determination of AFM1 in spiked milk samples at concentrations ranging from 20 to 60 ng/mL [54].

2.2. Electrochemical aptasensors for aflatoxin detection in milk

In addition to the immunosensors discussed in the previous section, the working electrode of a sensor can also be functionalized with aptamers, generally referred to as an aptasensor. Aptamers are small oligonucleotides with high target specificity due to their formation of tertiary structures. In an iterative process termed Systematic Evolution of Ligands by Exponential Enrichment (SELEX), invented by Tuerk & Gold [64], a pool of random oligonucleotides is subjected to repeated selection against a target of interest. As a result of their high target affinity, ease of modification and robust, animal-free production, aptamers have gained increasing acceptance to become a promising option alongside to antibodies for sensor development and various other applications [65]. Table 2 represents a comprehensive list of novel electrochemical sensors using aptamers as a biorecognition element.

The following chapter aims to discuss some outstanding electrochemical aptasensors using a variety of creative approaches to achieve AFM1 detection in milk.

Ramezani and colleagues developed an electrochemical aptasensor for AFM1 where they employed an innovative method using a combination of the AFM1 aptamer a complementary strand to the aptamer (CS) modified with gold nanoparticles (AuNPs) [66]. The hairpin structure of the aptamer inhibits binding of the AuNP-CS complex. Upon binding AFM1 to the aptamer, its hairpin structure collapses, which facilitates convergence of the AuNP-CS to the electrode surface [66]. Aided by addition of the redox agent methylene blue, changes in current can be measured. Differential pulse voltammetry (DPV) and cyclic voltammetry (CV) revealed a limit of detection of 0.9 nM under optimal conditions and 1.8 nM for milk samples, which is well below the legal limits [66].

Among numerous nanomaterials, carbon fibers have excellent reputation due to their superior material properties and their ultra-light weight [67]. They are fabricated in a versatile process called electrospinning, which has been covered by Xue and colleagues in great detail [68]. Rahmani et al. used an electrospun carbon nanofiber (ECNF) mat as a novel electrode base material and decorated it with gold nanoparticles and immobilized thiol-modified single strand DNA (ss-HSDNA) [69]. With cyclic voltammetry as the analytical method, a linear range of 1–100 pg/mL and a limit of detection of 0.6 pg/mL has been achieved. The feasibility of the sensor for practical applications was investigated by measuring spiked milk samples supplemented with 10–40 pg/mL AFM1 and yielded good recovery rates [69].

Another nano composite that found its way into sensor development is the metal–organic framework (MOFs), an ordered structure formed by single metal ions. It has several useful applications ranging from drug delivery to gas storage and ultimately sensing [70,71]. By using a platinum nanoparticle decorated glassy carbon electrode modified with MOFs, Jahangiri et al. developed an electrochemical aptasensor for AFM1. Employing electrochemical impedance spectroscopy (EIS), a linear calibration range of 0.1–80 nM/mL and a detection limit of 2 pg/mL have been obtained. Furthermore, their data reports good recovery percentages and standard deviations when using the sensor to detect AFM1 in spiked, pasteurized milk samples [71].

Using a novel approach to electrochemical aptasensing, Smolko and colleagues achieved reliable detection of AFM1 under optimal conditions and in milk with detection limits of 0.5 ng/L and 40 ng/L, respectively [72]. In the presence of the carrier complex polycarboxylated pillar[5]aren (P[5]A-COOH), they covered a glassy carbon electrode (GCE) with polymeric Neutral red (NR). Two different aptamers, one against AFM1 and on against NR were covalently linked to the carrier and their interaction with AFM1 could be measured by the change in redox activity of the layer [72].

A prime example of ultra-low detection limits for AFM1 is a study from Aissa et al., in which they developed a sensor achieving a linear detection range of 10–500 fM with a detection limit of 4.35 fM and a quantification limit of 14.95 fM. To amplify the signal originating from aptameric interactions, they used ferrocene-modified silicon nanoparticles attached to a polymer-functionalized screen-printed carbon electrode. This novel transduction system is based on electrochemical impedance spectroscopy (ECS) and the authors believe it as one of the main reasons for the unmatched sensor performance [73]. Another advantage next to the femtomolar detection range, is that the sensor only needs to be incubated for 30 min before recording their capacitive response and only amounts as low as 50 μL of analyte solution are needed for successful measurement. They also show that their sensor platform can be effectively used for commercial, pasteurized milk samples [73], making it the most promising sensor up to date.

3. Future perspectives and concluding remarks

This mini review aims to provide a multifaceted overview of the

global issue of aflatoxin contamination in food and feed and summarizes recent advances in development of novel electrochemical biosensors successfully used to detect aflatoxin in milk.

The two most prominent sensor types for electrochemical aflatoxin detection in milk developed in recent years are relying on either antibodies or aptamers as a biorecognition element to confer selectivity and sensitivity to the sensor (Figs. 1 and 2). Although both sensor types have achieved excellent results in terms of detection limit and linear range, aptamers excel in terms of low detection limits. In addition, aptamers are cheaper and offer more flexibility without the ethical issues associated with the production of antibodies in animals. Their flexibility stems from them being easily modifiable and also interchangeable with variants binding to different targets [65].

Despite promising advancements in sensor development, there are still challenges remaining that need to be addressed.

While various sensors discussed in this review are reporting excellent results of aflatoxin detection in milk in recovery studies, the next step towards field testing has yet to be made. This would include more research revolving around on-site testing, digitalization, portability and ease of use for non-professionals in technically challenged areas around the world. A recent publication from Ramalingam et al. [81] moves into the right direction by enhancing portability of the detection system by introducing microfluidic aptasensing.

Another issue that needs to be addressed when exploring on-site testing is to prolong shelf life of functionalized sensors. Some studies presented in this review report stability of sensors between 2 days and 9 weeks [49,53,59,62,69,71,74,75,77,78,83]. Successful implementation at point-of-care facilities like farms or factories would benefit from significantly longer storage times, underlining the importance of additional data on how long-time storage affects sensor performance and reliability.

Consensus exists on how to treat milk samples prior to measurement, namely by dilution in methanol, followed by centrifugation to get rid of lipids present in the milk. However, owing to the complexity of milk, elucidating effects of matrix interference on electrochemical measurements must yet be explored in greater detail. Parker et al. [58] investigated such effects for their sensor and proposed a general approach to suppress interference, paving the way for numerous research possibilities in the near future. Notably, the sensor developed by Aissa et al. [73] circumvents matrix interferences by its ability to quantify in extremely diluted samples.

Adding to the unwanted effects of matrix interference on electrochemical measurements, milk composition can considerably be affected by numerous factors. These include genetic factors such as different breeds of dairy cattle, their stage of lactation, age, feeding regimes and diseases [84]. Various studies included in this review have reported outstanding sensor performance for local milk samples of different types such as milk powder, raw milk and pasteurized milk. However, more efforts should be expended to broaden milk sampling not only by type of milk but also by region across the world to allow for a more complete picture of sensor performance.

Ultra-sensitive detection methods often go hand in hand with complex, non-trivial functionalization protocols that come at a higher price point and consume more time. Since it is not always important to detect below certain concentration levels, a middle ground must be found between complexity and performance. A simpler functionalization protocol would also facilitate automation, making upscaling and commercialization easier.

It is not only the complexity of functionalization protocols that needs to be addressed in future research. Mao et al. [53] discusses in a recent publication that due to strong binding forces between bioreceptor and target, it is often difficult to regenerate the sensing interface, resulting in

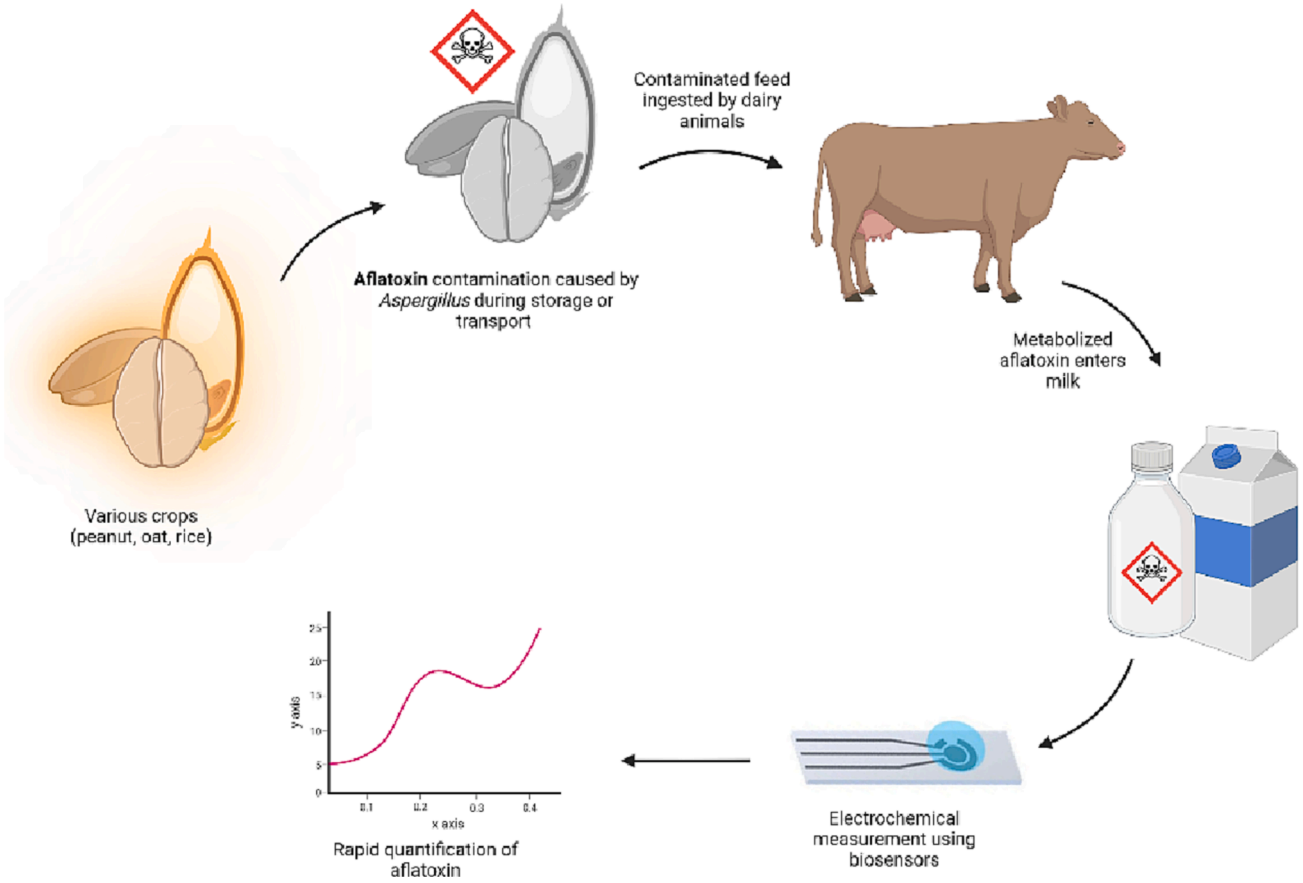

Fig. 1. Aflatoxin entering the food chain through milk can be rapidly quantified using electrochemical biosensors. Created with BioRender.com.

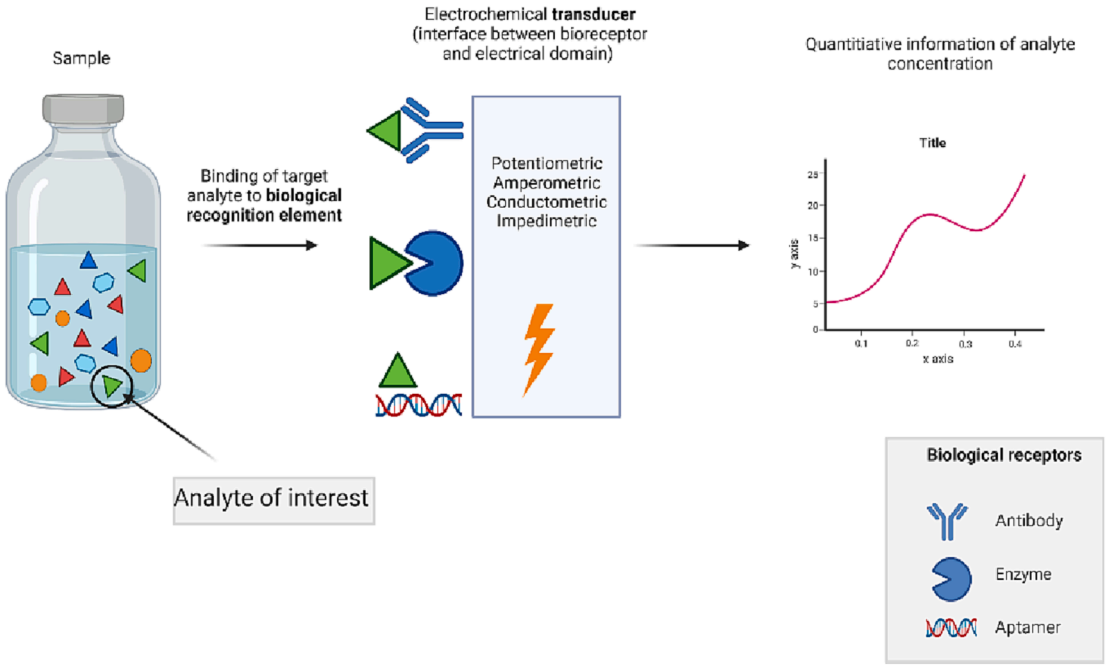

Fig. 2. Working principle of electrochemical biosensors. Created with Biorender.com.

disposable, single-use sensors. Future research should be dedicated to avoiding those issues by altering the preparation protocol of the sensor interface to allow for multiple sensor usages. This would not only reduce fabrication and material costs, but also minimize the negative impact on the environment.

Aflatoxin contamination constitutes a severe global health risk to humans and animals alike and the demand for reliable and rapid detection methods is more important than ever. Hand in hand with the advent of nanotechnology, the boundaries of electrochemical sensing of aflatoxin in milk are being pushed to newfound limits. The publications discussed in this review have laid solid groundwork for aflatoxin detection. Future research should be dedicated to build upon this foundation to develop cheap, portable, easy to use and eco-friendly systems.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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