Electrochemical Biosensors for Food Security: Allergens and Adulterants Detection

Susana Campuzano, Víctor Ruiz-Valdepeñas Montiel, Rebeca Magnolia Torrente-Rodríguez, Ángel Julio Reviejo and José Manuel Pingarrón

Abstract Food safety plays an important role in public health and thus to society as a whole. Food may become unsafe due to the presence of allergens and adulterants amongst others and hence the determination of these analytes has gained great relevance in quality control and safety of food. Electrochemical biosensor devices are emerging as one of the foremost relevant techniques for monitoring food allergens and adulterants due to their quick, specific, sensitive and reliable performances, ease of mass fabrication, economics and field applicability. These electrochemical biosensors, based on the use of existing or recently developed bioreceptors in combination with nanomaterials and surface fabrication techniques are able to offer attractive and efficient platforms which may become a viable option for the development of simpler, faster, cheaper and more robust and reliable analytical methods for the detection of allergens and adulterants in food. This Chapter describes recent analytical strategies developed so far using electrochemical biosensors for the determination of potentially hazardous adulterants and allergenic food residues to ensure food safety. The main progresses achieved to date are presented, highlighting general considerations and potential prospects for the future. The variety of electrochemical biosensors that have appeared in recent years shows that it is a booming research area with still many challenges but also great opportunities to develop sensitive, reliable, robust, and cost effective food allergens and adulterants biosensing methodologies.

Keywords Electrochemical biosensors · Food security · Allergens · Adulterants

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1 Food Security

Ensuring food safety is nowadays a top priority of authorities and professional players in the food supply chain. Between the different targets of importance in food safety control: pathogens, allergens, adulterants, toxins and other forbidden contaminants, adulterants and allergens are growing food concerns.

Food allergy is nowadays regarded as a problem of public health importance due to its widespread among most countries and the concern raised by allergic consumers inadvertently exposed to the offending ingredient through allergen containing foods. Even little intake of offending allergen can trigger allergic reactions in allergic consumers; therefore the only effective way of prevention is the avoidance of the allergen containing food from the diet [1]. The risks associated with the presence of hidden allergens in the food chain have generated a high demand for fast, sensitive and reliable methods to trace food allergens in different commodities to be implemented by food industries [1, 2].

During the past decade, a considerable interest has been paid also to dairy products quality and methods of production due to the recent crises and scandals in food industry, which have seriously undermined consumer confidence. The need for rapid analytical techniques to determine the authenticity and to detect adulteration is greater than ever. The determination of authenticity of milk and dairy products requires appropriate analytical tools for analysis both during production and storage [3].

Then, one of the key challenges to determine the safety of food and guarantee a high level of consumer protection is the availability of fast, sensitive and reliable analytical methods to identify these specific risks associated to food before they become a health problem. The limitations of existing methods have encouraged the development of new technologies, such as biosensors, which represent an interesting alternative that may address a number of analytical measurement problems with a great potential for food safety. As compared to conventional analytical methods, biosensors are easy to handle, portable, quick and the user does not require special skills [4, 5].

Particularly, electrochemical biosensors have been emerging as powerful alternatives to standard methods for the detection and quantification of allergens and adulterants in foods because there are able to perform rapid and simple determinations with high sensitivity and selectivity and low-cost instrumentation. Moreover, they are easy miniaturized and automated, ideal to perform routine and decentralized analysis.

Because of these interesting advantages, the purpose of this chapter is to provide an overview of the variety of electrochemical biosensors-based analytical strategies developed so far for the detection of allergens and adulterants present in food, either accidentally or deliberately, which may be not acceptable to certain consumers. This chapter does not attempt to provide an exhaustive list of all the strategies developed given the numerous scientific papers already published in this area.

Instead, it will provide different trends in electrochemical biosensing strategies based on distinct approaches and what are the future prospects and potential barriers exist with respect to commercialization of such devices within the food sector.

2 Electrochemical Biosensors for Food Allergens

Nowadays, allergic diseases are a public health worldwide problem, getting diseases such as asthma, atopic eczema and respiratory or food allergies are no longer classified as rare diseases and to be considered common diseases in industrialized countries, affecting largely at children.

Food allergy is a type of adverse reaction to food, produced by an immunological mechanism resulting from ingestion, contact or inhalation of certain substances presented in food, known as allergens. Food allergy is nowadays regarded as a problem of public-health relevance, the main concern being the unintentional exposure of allergic consumers to the offending ingredient through allergen containing food. Food allergy affects about 1–10 % of the world population, being a growing public health problem in countries like US where more than 15 million affected people [6] and are suffered by about 4 % of adults and 8 % of children and responsible of about 30,000 cases of medical emergencies and between 150 and 200 deaths per year. Since today there is no cure for allergies, the strict elimination of these allergens from the diet and the availability of reliable methods for detection and quantification of food allergens are the only options to ensure compliance with food labeling and improve consumer protection [2]. A pivotal issue in food allergen quantification is the impossibility to define a useful threshold (a limit below which a stimulus causes no reaction) and valuable determination limits (LOQs). Since the sensitivity of a patient to a given allergen varies from one patient to another and over the time, it is difficult to define threshold doses for allergenic foods [7] and therefore, without well-established thresholds, these quantification methods must be as sensitive, accurate and reliable as possible.

In addition, currently there is a variety of allergenic foods in which different allergenic proteins of different properties were found; which further complicates the assessment of the allergenic potential of certain foods. While more than 160 foods can cause allergic reactions in people, there are eight major foods or food groups believed to account for 90 % of food allergies: cow's milk, eggs, peanuts, soybeans, wheat, tree nuts, fish and crustacean shellfish although a higher number of allergenic compounds have been identified so far [1]. On the other hand, food allergens can become part of the food through unintended exposure such as through contamination which can occur by raw materials or during the production process [8]. Therefore, analytical testing systems are needed by businesses in the food industry to enable them to test whether allergens are present in their raw materials and in the finished products and whether production lines have been correctly sanitized, by food inspection authorities for market surveillance, and by academia to enable and stimulate research into food allergy and allergen detection [9].

At present, the main analytical techniques used to detect food allergens can be classified into protein-based or DNA-based assays. Protein-based assays detect either a specific target food allergen, using enzyme-linked immunosorbent assays (ELISAs), or total soluble allergenic proteins. DNA-based techniques detect the presence of allergens by amplifying a specific DNA fragment of the encoding gene for the target allergenic protein through polymerase chain reaction (PCR) [1]. However, false positive results (due to cross-reactivity with other co-extracted allergenic proteins) as well as large difference in quantitative results between available ELISA kits and the high numbers of replicates for samples or an external standard required by all the PCR methods described have hindered their application to processed foods or complex food matrices. Additional limitations of ELISA for food allergens detection include the possibility to evade detection in some ELISA formats of protein hydrolysates, heat-processed proteins or protein residues with lipophilic nature [10]. Furthermore, even though some level of automation has been achieved in the recent years, ELISAs remain laborious, time consuming and expensive, particularly when multiple targets need to be screened [2]. An additional limitation of DNA-based methods resides in the likelihood to find residual DNA encoding for the allergenic protein in the final processed food, PCR sensitivity strongly depending on the amount and the quality of the DNA isolated [1].

Therefore there is an urgent need to improve the robustness of the available analytical methods and to develop new standardized methods which must be fast, more sensitive, more accurate and more specific for better reliability to allow unambiguous identification of the allergens [7].

An emerging technology in food allergen analysis is the use of biosensors consisting of an integrated receptor-transducer device, which is capable of providing selective quantitative or semi-quantitative information using a biological recognition element [1]. Among these, the electrochemical biosensors have been well established as valid alternatives to classical analysis methods for detection of allergens, by offering the advantages of being easy to use, rapid, robust and often cheap multi-analyte testing [2, 11]. They hold great promise for reliable *point-of-care* sensor (POC) for household use, in particular, in applications where minimizing size and cost is crucial (e.g. *on-line* allergen-contamination monitoring). Electrochemical biosensors can be classified into potentiometric, amperometric, voltammetric and impedance types. Bioreceptors most commonly used in electrochemical biosensors for food allergen management include specific antibodies raised against an allergen [12–14], single-stranded DNA molecules capable of hybridizing with allergen-specific DNA fragments [15, 16], aptamer selected to recognize the target allergen directly [17, 18] and living cells which specifically recognize the target allergen and convert the recognition into a signal that can be electrochemically recorded and quantified [19]. Although the first applications of electrochemical biosensors to food-allergen management date back to 2008 [20, 21], in the following sub-sections, the most relevant electrochemical biosensors described for food allergens detection in the last 10 years, have been selected and classified according to the type of target and bioreceptor type employed.

2.1 Immunosensors

Already in 2008, Huang et al. [21] developed an impedimetric immunosensor for determination of peanut protein Ara h 1 by immobilizing a monoclonal antibody to Ara h 1 onto an Au electrode through amide bond formation to a carboxylate-terminated 11-mercaptoundecanoic acid (MUA) self-assembled monolayer (SAM). The detection limit (LOD) of this reagentless biosensor was estimated to be less than 0.3 nM.

Singh et al. reported the development of a nanopore immunosensor by covalent immobilization of peanut antibody within gold thin-film coated pores of commercial polycarbonate membranes [22]. In this approach protein Ara h 1 was detected by measuring the decrease in the pore conductivity as the peanut protein concentration increases due to the partial pore occlusion by antigen binding.

Direct casein detection was described by Cao et al. [23] by using an electrochemical immunosensor based on a glassy carbon electrode (GCE) functionalized with a gold nanoparticles (AuNPs) and poly(l-Arginine)/multi-walled carbon nanotubes (P-L-Arg/MWCNT) composite film through electropolymerization of L-arginine. Subsequently, AuNPs were adsorbed on the modified electrode in order to immobilize the capture antibody. Under optimized conditions and due to the formation of antibody–antigen complex on the modified electrode, peak currents of the redox couple (ferricyanide) obtained by differential pulse voltammetry (DPV) decreased linearly with increasing casein concentrations (in the range from 1×10^{-7} to 1×10^{-5} g mL^{-1}). This electrochemical immunosensor has a low LOD of 5×10^{-8} g mL^{-1} and was applied to the determination of casein in cheese samples with satisfactory results.

An electrochemical magnetoimmunosensor for the quantification of gliadin or small gliadin fragments in natural or pretreated food samples was described by Laube et al. [24]. The immunological reaction was performed on tosyl-activated magnetic beads (MBs) as solid support by the oriented covalent immobilization of the protein gliadin and using a specific HRP-labeled antibody for the detection. The modified MBs are then captured onto the surface of a magnetoelectrode based on graphite-epoxy composite (m-GEC) to perform the amperometric detection using H_2O_2 and HQ. Excellent LODs (in the order of μg L^{-1}) were achieved, according to the legislation for gluten-free products. The performance of this magnetoimmunosensor was successfully evaluated using spiked gluten-free foodstuffs (skimmed milk and beer), obtaining excellent recovery values.

A novel label-free voltammetric immunosensor for sensitive detection of β-lactoglobulin (β-LG) based on electrografting of aryl diazonium salt organic film on graphene modified screen printed electrodes has also been developed [25]. The derivatization of the graphene electrode surface was achieved by electrochemical reduction of in situ generated 4-nitrophenyl diazonium cations in aqueous acidic solution, followed by electrochemical reduction of the terminal nitro groups to amines, which were activated using glutaraldehyde and used for the covalent immobilization of the capture antibody. Results demonstrated that the DPV reduction peak current of $[Fe(CN)_6]^{3-/4-}$ decreased linearly with increasing

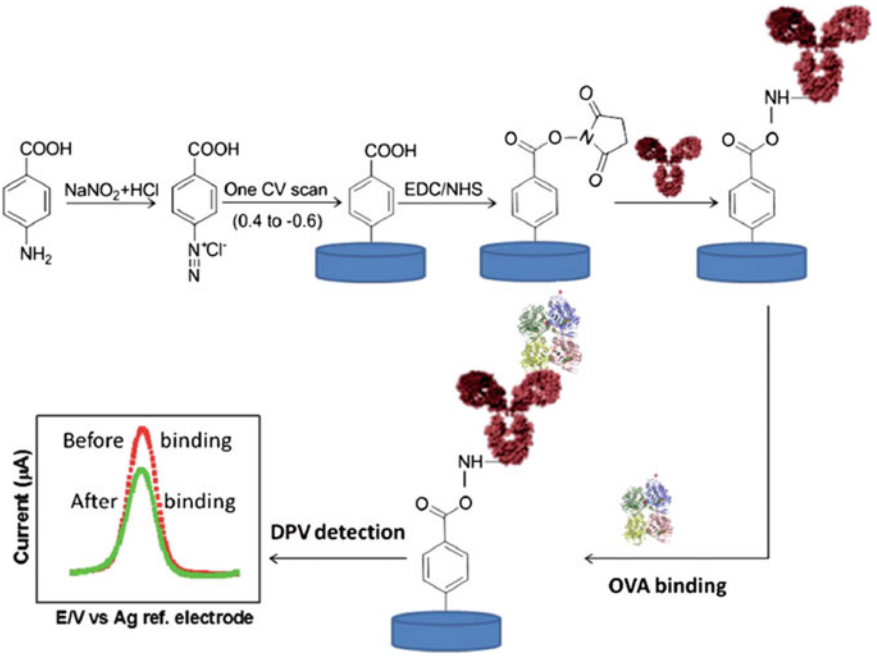

Fig. 1 Scheme of the ovalbumin immunosensor based on the use of carboxyphenyl modified graphene (Reprinted from [26] with permission. Copyright 2013 RSC)

concentrations of the target protein due to the formation of antibody–antigen complex on the modified electrode surface. This label-free voltammetric immunosensor enabled a LOD of 0.85 pg mL^{-1} and a dynamic range from 1 pg mL^{-1} to 100 ng mL^{-1}. Noteworthily, the immunosensor was also evaluated for the determination of β-LG in several milk-containing food products (cake, cheese, snacks and biscuits) and results obtained showed excellent correlation with those provided by a commercial ELISA kit. These authors reported also a label-free voltammetric immunosensor for ovalbumin by using carboxyphenyl modified graphene. In this approach, graphene-modified screen printed carbon electrodes were covalently functionalized using electrochemical reduction of in situ generated aryl diazonium salt forming a carboxyphenyl film on the graphene surface (Fig. 1) [26]. The terminal carboxylic groups on the graphene surface were activated using EDC/NHS and used to immobilize the capture antibody using the decrease in the $[Fe(CN)_6]^{3-/4-}$ DPV reduction peak current after the immunochemical reaction for the ovalbumin detection. The developed immunosensor has been used for ovalbumin detection in the concentration range of 1 pg mL^{-1} to 0.5 mg mL^{-1} with a LOD of 0.83 pg mL^{-1} and applied to the determination of spiked cake extracts.

Ruiz-Valdepeñas Montiel et al. proposed a disposable amperometric magnetoimmunosensor for the determination of β-LG using a sandwich configuration, SPCEs and HOOC-modified MBs (HOOC-MBs) [12]. This methodology involved

the immobilization of the capture antibody onto the activated carboxylic-modified MBs (HOOC-MBs) and successive incubation steps of the modified MBs with the analyte and a specific HRP-labeled detector antibody. The resulting modified MBs are captured by a magnet placed under the surface of a disposable carbon screen-printed electrode (SPCE) and the amperometric responses are measured at −0.20 V (vs. Ag pseudo-reference electrode), upon addition of hydroquinone (HQ) as electron transfer mediator and H_2O_2 as the enzyme substrate. The β-LG magnetoimmunosensor exhibited a wide range of linearity (2.8–100 ng mL^{-1}) and a low LOD of 0.8 ng mL^{-1}. The magnetoimmunosensing platform was successfully applied for the detection of β-LG in different milk samples without any matrix effect after just a sample dilution. The results correlated properly with those provided by a commercial ELISA spectrophotometric kit offering a truthful analytical screening tool.

Same authors reported also a similar configuration for the sensitive determination of Ara h 1 in only 2 h [27]. This Ara h 1 magnetoimmunosensor exhibits a wide range of linearity (20.8–1000.0 ng mL^{-1}) Ara h 1, a LOD of 6.3 ng mL^{-1}, a great selectivity and a useful lifetime of 25 days. The usefulness of the immunosensor was demonstrated by determining the endogenous Ara h 1 in different matrices (diluted food extracts and undiluted saliva samples). The results correlated properly with those provided by a commercial ELISA method offering a reliable and promising analytical screening tool in the development of user-friendly devices for on-site determination of Ara h 1.

Other MBs-based electrochemical immunosensor has been developed for ovalbumin determination [13]. A sandwich-type immunocomplex formed by specific anti-ovalbumin immunoglobulin G, ovalbumin and secondary anti-ovalbumin antibodies conjugated with HRP was performed onto the surface of activated carboxylate-modified MBs. The electrochemical signal proportional to the enzymatic reaction of HRP during the reduction of H_2O_2 with thionine as electron mediator was measured by linear sweep voltammetry at screen-printed platinum electrodes. This method was suitable for quantification of ovalbumin in the range of 11 to 222 nM and a LOD of 5 nM.

Alves et al. have developed recently integrated voltammetric immunosensors for the determination of Ara h 6 [14] and Ara h 1 [28]. AuNPs-modified screen-printed carbon electrodes were used to develop sandwich-type immunoassays using specific capture antibodies and ALP-labeled detector antibodies. The electrochemical detection of the antibody-antigen interaction was performed by applying an anodic (stripping) voltammetric potential scan of the enzymatically deposited silver in the presence of 3-indoxyl-phosphate (3-IP) and silver ions. Both methodologies were reproducible, presented a good repeatability, provided accurate results, very low LOD (0.27 and 3.8 ng mL^{-1} of Ara h 6 and Ara h 1, respectively) and were successfully applied to determination in complex food matrices, such as cookies and chocolate.

A portable indirect competitive amperometric immunosensor using rabbit polyclonal anti-bovine β-casein, goat anti-rabbit IgG-HRP conjugate antibodies and a set of 8 carbon working electrodes screen printed on alumina able to perform β-casein detection in eight different samples has been recently published by

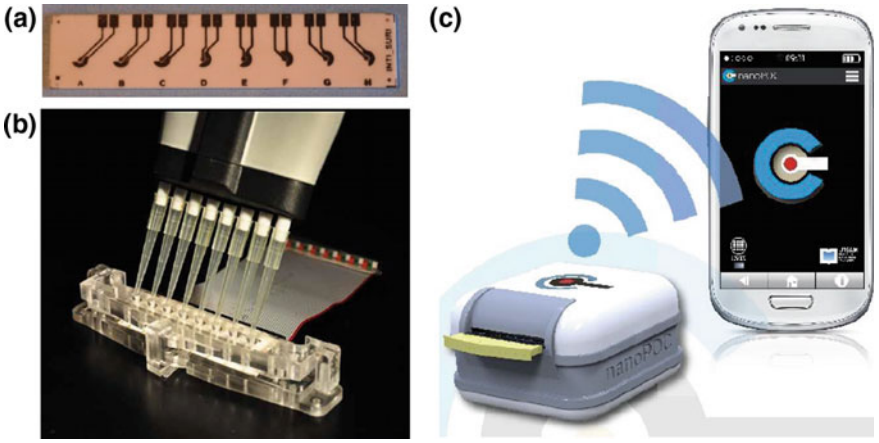

Fig. 2 Set of working and auxiliary electrodes screen printed on alumina, electrochemical cells and portable potentiostat connected to a smartphone and controlled by bluetooth. (Reprinted from [29] with permission. Copyright 2015 Creative Commons Attribution Licence)

Molinari et al. [29]. Commercial β-casein is covalently immobilized onto the electrode surface using the EDC/NHS method after the functionalization of the sensor surfaces with carboxylic groups by plasma treatment and an indirect competitive reaction between soluble and electrode surface immobilized antigen to the binding sites of the anti-bovine β-casein antibodies which are then recognized by HRP-conjugated secondary antibodies, took place. The amperometric signal detected, due to the enzymatic reduction of the redox mediator after adding H_2O_2, is inversely proportional to the β-casein concentration present in the sample solutions. Although this approach was not particularly sensitive (0–10 ppm range), no complex electronic instrumentation such as computer was required in this methodology since the eight electrochemical cells are integrated into a small-size portable potentiostat controlled by a smartphone via Bluetooth communication (Fig. 2).

2.2 DNA Sensors

Electrochemical DNA-based biosensors have been widely used for detecting specific genes associated with target allergens. Betazzi et al. [20] developed an electrochemical genosensor platform for the simultaneous, sensitive and specific detection of PCR amplicons obtained from cDNA of the major hazelnut protein allergens (Cor a 1.04 and Cor a 1.03) in foodstuffs. The used platform was a low density array of eight individually addressable gold working electrodes, enabling the simultaneous analysis of different samples. Unmodified PCR products were captured at the sensor interface via sandwich hybridization with thiol-tethered DNA capture probes assembled on the gold electrodes and biotinylated signaling probes.

The resulting biotinylated hybrids were coupled with a streptavidin–alkaline phosphatase conjugate and DPV was finally used to detect the α-naphthol signal produced by enzymatic reaction from α-naphthyl phosphate. LODs of 0.3 and 0.1 nmol L^{-1} were obtained for Cor a 1.03 and Cor a 1.04, respectively. Storage stability studies demonstrated that the capture probe-modified chip provided a stable response for up to 4 months. The genosensor was applied to detect the presence of these allergens in several commercial foodstuffs, obtaining a good agreement with the results obtained with a standard ELISA kit.

Sun et al. [30] developed a very sensitive DNA sensor, based on the use of a dually-labeled stem-loop probe, for detecting peanut-allergen Ara h 1 using a probe. The probe (modified with a thiol group at its 5′ end and a biotin tag at its 3′ end) assembled on a gold electrode was "closed" when the target oligonucleotide was absent, and "open" after hybridization with the target, thus moving away from the electrode surface the biotin group at its 3′ end. The electron transfer efficiency changes resulting from the detachment of biotin tags from the electrode surface were measured by faradaic EIS. This method with a linear response in the range of 10^{-15}–10^{-10} M, a LOD of 0.35 fM and the ability to discriminate a single-base mismatch, was successfully applied to detect the target allergen in a peanut-milk beverage.

A sandwich selective electrochemical genosensor has been developed also for the detection of an 86-mer DNA peanut sequence encoding part of the allergen Ara h 2 (conglutin-homolog protein) [31]. The method is based on the use of thiolated capture and biotinylated detector probes and screen-printed gold electrodes. The electrochemical signal monitored corresponded to the oxidation of 1-naphthol generated through the hydrolysis of 1-naphthyl phosphate in the presence of Strep-ALP (use to label the biotinylated detector probe attached to the electrode surface). The proposed sensor showed a sensitivity as high as 3 mA nM^{-1}, a linear range from 5×10^{-11} to 5×10^{-8} M and a LOD of 10 pM.

Sun et al. developed a stem-loop DNA probe biosensor for peanut allergen Ara h 1 using a multilayer graphene–gold nanocomposite as a signal amplification material [15]. In this case a thiolated hairpin DNA-biotin probe was immobilized onto a multilayer graphene–gold nanocomposite prepared by a cycle-alternate electrodeposition method to deposit monolayers of graphene and AuNPs on a GCE. The prepared biosensor, based on an "off" state in the presence of the target DNA demonstrated a linear response ranging from 10^{-16} to 10^{-13} M, an ultrasensitive LOD of 0.041 fM, a one-base mismatch selectivity and successful recoveries (86.8–110.4 %) of the Ara h 1 gene from a peanut milk beverage.

Very recently, the first electrochemical genosensor able to quantify gluten in food products was developed [16]. This approach used a sandwich-based format, involving the use of a capture probe immobilized onto the screen-printed gold electrode, and a signaling probe functionalized with fluorescein isothiocyanate (FITC), both able to hybridize with the target analyte, a fragment encoding the immunodominant peptide of α2-gliadin amplified by a tailored PCR. The sensor was able to reliable detect as low as 0.001 % (w/w) of wheat flour in an inert matrix

and to analyze processed food samples, without complicated pretreatment protocols, giving results in agreement with the Codex recommended method.

Among the DNA-based biosensors, aptameric ones have attained also great attention in the determination of food allergens and will be discussed in the following subsection [5].

2.2.1 Aptasensors

Aptamers are small single-stranded DNA or RNA sequences which inherently adopt stable three dimensional sequence-dependent structures and bind a target ligand with high affinity [32].

Amaya-Gonzalez et al. [33] developed a competitive electrochemical aptaassay based on MBs for determination of gliadin in real samples. Two different aptamer sequences (Gli1 and Gli4) against the immunodominant peptide from wheat gliadin, the 33-mer, that also recognize celiac disease related proteins from barley, rye and oat were selected [34]. The competition is established between the biotinylated peptide immobilized on the surface of streptavidin-modified MBs (Strep-MBs) and gliadin in solution in the presence of a fixed amount of the specific biotinylated aptamer. The electrochemical signal was achieved by chronoamperometry using the system H_2O_2/TMB after enzymatic labeling with streptavidin-HRP of the biotinylated aptamer fraction bound to the 33-mer modified MBs. In the presence of increasing concentrations of gliadin, the amount of free aptamer available to bind the 33-mer immobilized peptide on the Strep-MBs diminishes resulting in a decreasing analytical signal with analyte concentration. This competitive electrochemical magnetoassay did not show any cross-reactivity with non-triggering celiac disease proteins from soy, rice or maize. Moreover, the results achieved demonstrated that the assays based on the two aptamer sequences, Gli1 and Gli4 are complementary: while the assay using the Gli1 aptamer is suitable for quantifying gluten in hydrolysed samples (LOD of 4.9 ppm), the method based on the Gli4 aptamer is more sensitive (LOD of 0.5 ppm) and allows the quantification of gluten in heated foods thus providing answers to one of the main challenges associated with analytical determination of gluten, namely detection of gliadin in heated and hydrolysed foods. Aptamer-based sensors for the label-free determination of Ara h 1 have been developed by immobilizing on gold surfaces a thiolated aptamer [17] or an amino (NH_2)-terminated 77-base DNA aptamer covalently to a carboxylated SAM employing carbodiimide chemistry [35]. Both platforms are based on the use of an 80-base DNA aptamer and impedimetric detection. The fast and low-cost aptameric platform based on the amino-modified aptamer can detect Ara h 1 with a LOD of $\sim$1 nM and up to concentrations of $\sim$15 nM.

A novel aptasensor for lysozyme detection was reported based on the immobilization of the aptamer onto the surface of a screen-printed carbon electrode by covalent binding via diazonium salt chemistry [18]. The developed aptasensor exhibited a very good linearity (0.025–0.8 μM) and a LOD of 25 nM far below the maximum amount allowed by the International Organization of Vine and Wine

(OIV). This disposable platform demonstrated promising results in the analysis of spiked wine samples. Same authors developed also other impedimetric aptasensor for lysozyme detection based on a sandwich format using a capture aptamer immobilized covalently via diazonium salt chemistry on a screen-printed carbon electrode and a biotinylated detector antibody [36]. Detection was performed by monitoring by DPV the electrochemical oxidation signals of 1-naphthol after labeling the biotinylated antibody with avidin marked with alkaline phosphatase and using 1-naphthyl phosphate as the enzymatic substrate. This aptasensor, characterized by a wide detection range, from 5 fM to 5 nM and a LOD of 4.3 fM was also applied to the determination in spiked wine samples.

2.3 Whole Cell-Based Sensors

Jiang et al. developed a novel cell-based impedimetric biosensor to quantify major shrimp allergen Pen a 1 (tropomyosin) [37]. This aptasensor was based on the immobilization of anti-shrimp tropomyosin IgE pre-sensitized rat basophilic leukemia (RBL-2H3) living mast cells, encapsulated in type I collagen, on a self-assembled L-cysteine/AuNPs (AuNPsCys)-modified gold electrode (see Fig. 3). In the presence of antigen, the sensitized immobilized cells lead to initiation of signaling cascade resulting in the degranulation of secretory vesicles and releasing of inflammatory molecules, which can be monitorized by the increase in the impedance value (R_{et}). Results presented demonstrated that R_{et} values increased with the concentration of purified shrimp allergen Pen a 1 in the range of 0.5–0.25 μg mL^{-1} with an LOD of 0.15 μg mL^{-1}.

Same authors developed also a very attractive fluorescent magnetic nanobeads-based mast cell biosensor for electrochemical detection of activated cells that were employed to quantify both shrimp allergen tropomyosin (Pen a 1) and fish allergen parvalbumin (PV) [19]. In this approach cationic magnetic fluorescent nanoparticles (CMFNPs) were used for transfecting RBL-2H3 cells and the resulting CMFNP-transfected RBL-2H3 cells (pre-sensitized by anti-Pen a 1 IgE or Anti-PV IgE) were magnetically captured on a magnetic glassy carbon electrode (MGCE). The response induced by the activated cells in the presence of the target allergen was monitorized by EIS. Results show high detection accuracy for these targets, with linear ranges from 0.5 to 10 ng mL^{-1} and 0.1 to 6 μg mL^{-1} and LODs of 0.16 μg mL^{-1} and 0.03 ng mL^{-1} for the PV and Pen a 1, respectively.

2.4 Other Biosensors

Apart from the most commonly bioreceptors mentioned in Sect. 2, lectins have been also applied to develop electrochemical biosensors for food allergens determination. Xu and co-workers [38] developed an electrochemical biosensor for the direct

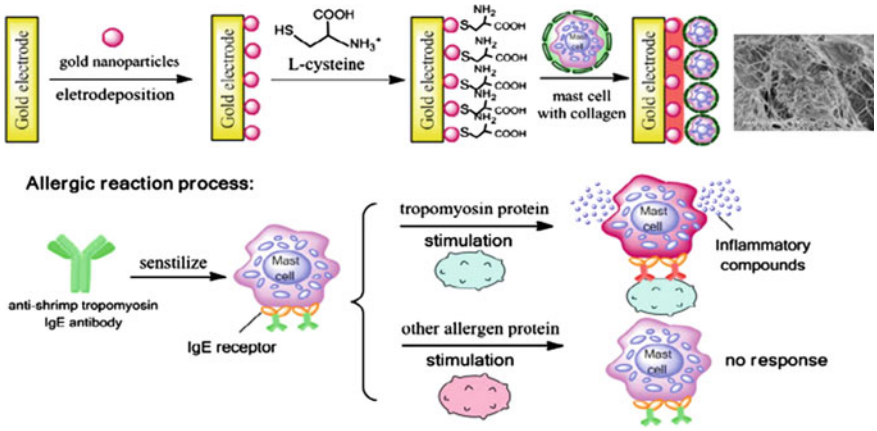

Fig. 3 Schematic illustration of the preparation of modified electrode (above) and principle of allergen reaction process (below). (Reprinted from [37] with permission. Copyright 2013 Elsevier)

Fig. 4 Scheme of assembly and electrochemical strategy for detection of CHOM. (Reprinted from [38] with permission. Copyright 2011 Elsevier)

detection of chicken ovomucoid (CHOM) using ZnO quantum dots (QDs) bioconjugates and Concanavalin A as a recognition element. The novel bioconjugates were synthetized by self-assembling of CHOM onto ZnO QDs through electrostatic interaction between positively charged QDs surface and negatively charged protein. This ZnO-QD/CHOM bio-conjugate was captured on a Concanavalin A-modified electrode and the extent of the biorecognition was accomplished by square wave voltammetric (SWV) of captured QDs after acidic dissolution (see Fig. 4). The zinc SWV peaks monitored were dependent on the CHOM concentration in the 1–140 ng mL^{-1} range. The proposed method, with a LOD of 0.1 ng mL^{-1}, offers a simpler platform for the detection of other glycoprotein allergens.

Recently Sugawara et al. [39] developed an electrochemical biosensor to detect ovalbumin (OVA) by using a specific peptide probe (peptide-1) conjugated with

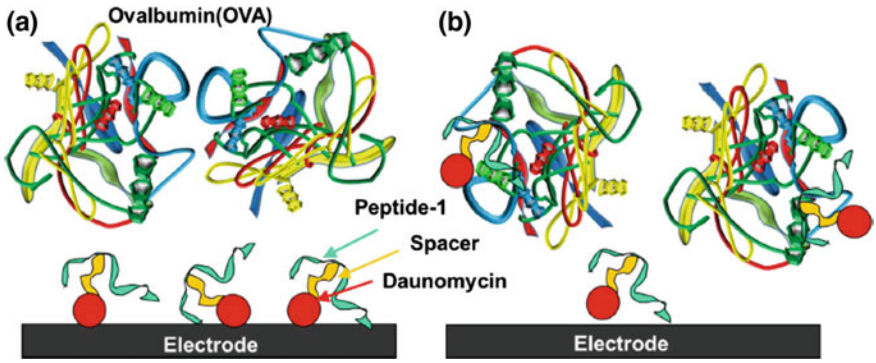

Fig. 5 Principle of the OVA detection by using a peptide-based biosensor. (Reprinted from [39] with permission. Copyright 2015 Elsevier)

daunomycin adsorbed on a GCE. The DPV peak current of the daunomycin moiety decreased as the concentration of OVA increased due to the binding between the OVA and the peptide probe which was released from the electrode surface (Fig. 5) The calibration curve of the OVA using the peptide probe was linear and ranged from 1.5×10^{-11} to 3.0×10^{-10} M. Furthermore, this method could be applied to the electrochemical sensing of the OVA in egg whites and in fetal bovine serum.

3 Electrochemical Biosensors for Food Adulterants

Food adulteration, which induced lost large of money as well as the confidence of consumers, has been practiced since a long time ago and becomes increasingly in the last years more sophisticated. Nowadays European consumers are increasingly demanding information and reassurance not only on the origin but also on the content of their food. Protecting consumer rights and preventing fraudulent or deceptive practices such as food adulteration are important and challenging issues facing the European food industry, as manufacturers are required to provide and confirm the authenticity and point of origin of food products and their components [40].

Non-authentic food products arise from the adulteration and fraud. The replacement of original substance partially or completely with more easily available and cheap substance is the most common procedure performed by defrauders such as the addition of: (i) flavors/aromas to improve the value of cheap products; and/or (ii) cheap substances to the food products. Foods and ingredients presenting high-value are the most vulnerable for adulteration. Therefore, nowadays, the determination of the authenticity and the detection of adulteration of dairy products is a major concern in order to: (i) assure the traceability system from milk to fork; and (ii) ensure that dairy products are correctly labeled in terms of which animals are actually processed for consumption [3]. Indeed, milk and meat are between the

25 top foods commonly adulterated [41]. To authenticate food commodities several standard analytical techniques such as physicochemical, sensory, chromatography, stable isotope analysis, immunological and enzymatic techniques, DNA and protein based assays and triacylglycerol analysis have been applied [3, 40]. Although these methods are considered as the reference ones and able to detect low levels of adulteration, they are expensive, time consuming, destructive, sophisticated, laborious, and technically demanding and are therefore not suited for on-line or large-scale operations [3, 40]. Furthermore, adulterants can be revealed with great difficulty in the context of methods commonly applied in laboratories [40].

For all these reasons, there is a need to develop cheap, fast and efficient analytical methods for the detection of frauds and authentication of food products. In comparison with the conventional methods, electrochemical biosensors constitute very promising tools to determine and/or to detect the authenticity and adulteration of milk and dairy products; they are rapid, non-destructive, effective and reliable, require only basic training in a user-friendly software, and low cost per assay.

This book chapter will provide also a comprehensive overview of the applications of different electrochemical biosensors to determine the authenticity and to detect the adulteration of dairy products during the last 10 years. Actual examples illustrating the utilization of these techniques mainly in laboratory environments will be discussed as well as their advantages and disadvantages.

Electronic aptamer based (E-AB) sensors were developed for the determination of cocaine [42, 43]. E-AB sensors comprise an aptamer that is attached at one end to an electrode surface. The distal end of the aptamer probe is modified with an electroactive redox marker for signal transduction [43]. These sensors are based on the use of a thiolated DNA strand assembled on gold electrodes and modified at the other end with methylene blue (MB). In the absence of target, the aptamer is thought to remain partially unfolded, in the presence of target the rearrangement of the aptamer structure brings the redox tag in close proximity of the electrode thus resulting in an increase of the MB reduction peak. While Baker et al. fabricated the sensor on a ~ 1 mm^2 gold electrode, White et al. compared the performance of three different E-AB architectures (aptamer, concaptamer and pseudosandwich) on array-based chips containing 36 working gold electrodes. Baker et al. demonstrated that the "signal-on" sensor responded rapidly (seconds) and specifically to micromolar cocaine in adulterated samples and was regenerated via a brief, room temperature wash.

Cao et al. developed a novel and simple electrochemical method for determination of melamine based on oligonucleotides ($d(T)_{20}$) film modified gold electrodes [44]. The proved interactions between oligonucleotides and melamine, via electrostatic and hydrogen-bonding interactions, lead to the increase in the peak currents of ferricyanide, measured by differential pulse stripping voltammetry (DPSV), which could be used for electrochemical sensing of melamine. This approach provided a linear range from 3.9×10^{-8} to 3.3×10^{-6} M and a LOD of 9.6×10^{-9} M. The proposed electrochemical biosensor is rapid, convenient and low-cost for effective sensing of melamine and was applied successfully to the determination of melamine in milk products, with a recovery of 95 %.

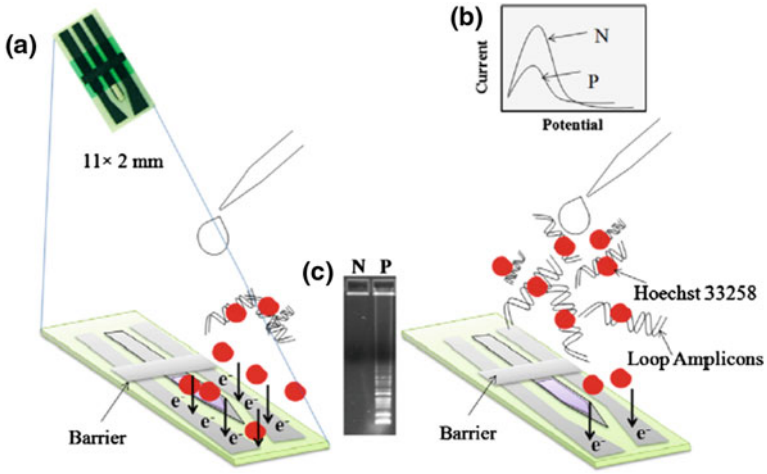

Fig. 6 Species-specific identification using a LAMP amplification-based electrochemical genosensor. (Reprinted from [46] with permission. Copyright 2010 Elsevier). **a** Without amplification product (N). **b** With amplification product (P). **c** Gel electrophoresis analysis of loop amplicons

Singh et al. described the use of electrochemically prepared PANi/ClO_4 doped films onto ITO electrodes to immobilize DNA as biosensing platform for the detection of sanguinarine in adulterated multard oil [45]. The ds-DNA was covalently immobilized on the PANi film using EDC and NHS and the basis of this methodology was the decrease of sanguinarine electrochemical signal after its binding to ds-DNA by intercalation with insertion between adjacent base pairs of DNA duplex strand. This genosensor showed acceptable linearity from 1 to 40 μg and the recovery experiment results found recoveries between 89 and 121 % in spiked edible mustard oil sources.

An easy, rapid and sensitive method of detection of the presence of meat species in raw or processed foods was developed by Ahmed et al. [46]. This strategy, based on the use of loop-mediated isothermal amplification (LAMP) and disposable electrochemical genosensors, detected by Linear Sweep Voltammetry (LSV) the change in the anodic peak current of the DNA binder H33258 after its interaction in solution with the generated loop amplicons (Fig. 6). The comparison with the multiplex-PCR (M-PCR) detection method demonstrated that this approach was more specific, reduced the cross-reactivity and avoided the formation of non-specific amplicons in the detection of meat species in meat containing raw and processed foods. Moreover, this method, which gave LODs of ~20.33 ng μL^{-1}, 78.68 pg μL^{-1} and 23.63 pg μL^{-1} for pork, chicken and bovine species, respectively, took only an hour and being isothermal may be a good candidate to become a portable biosensor for on-site monitoring of meat species identification in raw and processed foods.

4 General Considerations

Although a great variety of bioreceptors have been explored to the development of electrochemical biosensors: antibodies, single-stranded DNA and aptamer sequences, whole viable cells and lectins, since the increase in allergen awareness and regulations, immunosensors are the electrochemical biosensors most commonly used. Recently, the selection of aptamers for this group of ingredients is emerging. Electrochemical aptasensors may become a viable option for the development of more powerful, cleaner and cheaper analytical methods for the detection of allergens and adulterants in food. Particular relevant is that this electrochemical aptasensors have demonstrated to be good candidates to rival the established methods of analysis for more reliable quantitation of gluten content in food and ensure the safety of a wider sector of the celiac disease sensitized population [33]. However, only preliminary assays based on a reduced number of suited aptamers and at a purely academic stage have been described. There is a clear need for selection of new aptamers against the great variety of allergenic and adulterant residues that can be found in food, which is continuously increasing.

It is important to mention also that although quantification of food allergens using genosensors is a challenging task that only rarely devices have addressed and results in terms of DNA concentration are meaningless for consumers and often used as yes/no tools, a genosensor that allows for the first time to quantify the gluten content in food products has been described recently [16].

In addition, given the sensitivity of persons with allergen sensitivity it can be predicted that biosensor devices with improved sensitivity based on the use of nanomaterials, such as QDs [39], AuNPs [14, 19, 23, 28, 37] carbon nanotubes [23] and graphene [15, 25, 26] will find widespread utility. Moreover, although very scarcely explored until now, the use of lectins as recognition elements is very promising to develop simple and cheap biosensing platforms for glycoprotein allergens determination.

Regarding the electrochemical biosensors for determining adulterants it is important to mention that the type of bioreceptors applied for these target analytes is limited to DNA sequences and aptamers. In order to cope with the crucial step of DNA-probe immobilization, surface-confined stem-loop DNA structures have been designed and proposed as capture probes with performance superior to linear probes in terms of ability to discriminate mismatches [15, 30]. Although the applicability of food adulterants electrochemical biosensors have been less explored, they have demonstrated great potential to detect melamine in milk and to identify meat species of particular interest in the food industry.

Worth to mention also the increasing tendency to fabricate these electrochemical biosensors onto screen-printed electrodes providing the advantages of being disposable and mass produced, with a low manufacturing cost and require low volume of reagents and samples. Magnetobiosensors have also been described for food allergens determination [12, 19, 24, 27, 33] making use of the advantages of MBs in the final performance of electrochemical biosensors in terms of sensitivity,

reduced assay time and minimization of matrix effects which is essential for the analysis in complex matrices such as food extracts. Regarding the formats of assay while direct, sandwich and competitive formats have been employed in electrochemical immunosensors and aptasensors developed for allergens determination; all the approaches reported for adulterants are based on direct assays.

5 Conclusions and Future Prospects

Food quality and safety is of paramount importance from health as well as economic point of view. Particularly, the detection of food allergens and adulterants is an upcoming field, since standard techniques are not able to fulfill current standards in their determination. The present chapter has reviewed the most recent literature (over the past 10 years) on electrochemical biosensors development for food-allergens and adulterants management using a bioreceptor-based classification and highlighting the most important achievements and the new research trends.

From the discussed literature it is clear that electrochemical biosensors can play a very helpful role in the detection of food allergens and adulterants and a vital role in assuring food safety and help to take quicker preventive actions when required. Featuring high speed of execution, good sensitivity and selectivity, robustness, ease of use and high degree of automation and portability for in-field applications, they have demonstrated all the potential for direct, real-time, on-line, monitoring of food allergens and adulterants along the production chain. However, despite the great efforts performed in the last years, there are still some important challenges that should be faced in this innovative application field for electrochemical biosensors. Off-line measurements, long incubation times and sensitivity to non-specific binding from matrix components are common open issues, which often constrain the use of electrochemical biosensors to preliminary proof-of-concept investigations on standard solutions. Most of the described assays have proved the quantification of targets in aqueous solutions and only a few faces the analysis of spiked samples. Moreover, in some cases, the contaminant was added in an intermediate step or even at the end of sample preparation. There are two relevant issues associated with real sample analysis: possible electrochemical interferences (1) and efficient extraction of target analyte from the complex food matrix (2). To avoid electrochemical interferences, surface chemistry needs to be carefully optimized, in conjunction with sample pre-treatment and cleanup. Sample preparation and efficient extraction of the target analytes remain being the limiting factors for the total analysis time and final performance of the electrochemical biosensor. While for liquid samples often times a simple filtration and dilution is sufficient, for solid samples several steps of extraction and cleanup are necessary. The development of online sample preparation systems or easy to use kits for rapid sample extraction and cleanup will also be required in order to facilitate the development of commercial devices and alleviate current problems related to complex and time demanding sample pre-treatment protocols and the presence of false negative results

(attributed to non-effective extractions from the food matrix). Moreover, in order to validate the claim that electrochemical biosensors are reliable alternatives to existing methods, more efforts should be directed towards participation in proficiency testings, comparative studies using current approaches, analysis of certified reference materials and more closely consideration of the reproducibility issue.

A great deal of effort should be devoted in these directions in order to exploit all the attractive features and great potential of electrochemical biosensors in screening for food allergens and adulterants. Analogously, nanomaterial-based biosensors were shown to be promising tools for improving sensing performance but there are still in their infancy in their application to food matrices. Detailed investigations on the interferences in real sample analysis and evaluation of technological issues related to the final application to food must be addressed before biosensors can fully benefit from integrating nanotechnology.

Apart from facing all these challenges, recent trends in the development of electrochemical biosensors for food field quality and safety include the development of portable biosensors, with multiplexing capabilities and amplified analytical signals.

Finally, giving the on-going demand for devices that can be used outside the laboratory environment to assess the safety and quality of foods on-site, next years will witness a steep increase in the number of research studies devoted to electrochemical biosensors for this purpose, where the start of the race to bring to market aptamer-based assays for different targets of importance in food safety control will occur. However, it is worth to mention that the transfer of this attractive technology to market will require also overcoming the resistance of the agri-food sector, a conservative sector mainly relying on well-known processes and not focused on emerging technologies. But as food analysts are facing increasingly complex challenges, they will need the best available technology and this is where electrochemical biosensors will find their niche.

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