Detection of the peanut allergen Ara h 6 in foodstuffs using a voltammetric biosensing approach

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Abstract A voltammetric biosensor for Ara h 6 (a peanut allergen) detection in food samples was developed. Gold nanoparticle-modified screen-printed carbon electrodes were used to develop a sandwich-type immunoassay using two-monoclonal antibodies. The antibody-antigen interaction was detected through the electrochemical detection of enzymatically deposited silver. The immunosensor presented a linear range between 1 and 100 ng/ml, as well as high precision (inter-day RSD ≤9.8 %) and accuracy (recoveries ≥96.7 %). The detection and quantification limits were 0.27 and 0.88 ng/ml, respectively. It was possible to detect small levels of Ara h 6 in complex food matrices.

Keywords Electrochemical immunosensor · Ara h 6 · Voltammetry · Screen-printed carbon electrodes · Allergen · Peanut

Introduction

Food allergy is based on an immunological hypersensitivity to some food proteins or glycoproteins, generally mediated by immunoglobulin E. Symptoms usually involve the gastrointestinal tract (e.g., diarrhea, emesis), the skin (e.g., atopic dermatitis), and the respiratory system (e.g., asthma, rhinitis). Anaphylactic reactions are rare but particularly life threatening [1–3].

Peanut (*Arachis hypogaea*) is one of the most allergenic foods. Even very small amounts of peanut allergens can induce severe reactions in some allergic individuals [4, 5]. According to the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA 2004, Public Law 108–282, Title II) in the USA, and the Directive 2000/13/EC, as amended by Directives 2003/89/EC and 2007/68/EC, within the European Union, the presence of peanut in a food product has to be declared on the label. To date, the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Subcommittee have documented several peanut allergens [6]. Ara h 1 (cupin; vicillin-type 7S globulin), Ara h 2 (conglutin; 2S albumin), and Ara h 3 (cupin; legumin-type, 11S globulin), have been recognized as major allergens. More recently, Ara h 6 also emerged as an important allergen since it has a similar seroprevalence to Ara h 2 and has also been associated to clinical peanut allergy. Ara h 6 (14.5 kDa) is a conglutin (2S albumin) with heat-stable and immunogenic properties, being resistant to gut digestion [7, 8]. Due to its stability, Ara h 6 can be considered a suitable marker to identify the presence of peanut in food products and production lines.

Food allergies can be controlled not only by treating the manifested symptoms but also by avoiding allergen exposure. Therefore, accurate, highly sensitive, and selective methods to evaluate if a specific allergen is present in a foodstuff are of the utmost importance. Moreover, they can contribute to clarify situations such

as cross-contamination during food processing or the use of ingredients with "hidden" allergens that can put allergic consumers in danger.

Three main groups of methods for allergens detection are described in the literature, namely, immunoassays for protein detection [9–11], DNA-based methods [11, 12], and mass spectrometry [13]. Recently, biosensors appeared as great alternatives to classical methods, showing advantages such as a significant reduction of reagent consumption and the possibility of miniaturization and consequent portability [14]. However, their application in the field of food allergen analysis is still very limited. The development of fast, cheap, and environmentally friendly processes, able to detect vestigial amounts of allergens in complex food matrices, is still a major challenge.

Although there are some devices (immuno- and genosensors) described for the detection of some peanut allergens (e.g., Ara h 1, Ara h 3) [15–19], to the best of our knowledge, a biosensor for the specific detection of Ara h 6 was not described up to date.

Screen-printed carbon electrodes (SPCE) have been attracting increasing attention, due to its prominent characteristics, such as reduced dimensions, disposability, low-cost fabrication, possibility of mass production, and suitable practical application. Moreover, the steps of the immunoassay can be carried out by placing a single drop directly on the transducer's surface, which significantly lowers the consumption of expensive reagents, comparatively with other classical methods [20, 21]. The modification of the SPCE surface with gold nanoparticles electrochemically generated is a fast method to obtain gold nanostructures in a reproducible way. Interest in gold nanoparticles for biosensor construction has been increasing due to their high surface-to-volume ratio, long-term stability, high in-plane conductivity, and excellent biocompatibility. Indeed, a higher faradaic current is achieved and, therefore, higher peak current intensities (i_p) can be obtained, which makes them excellent platforms for different types of sensors. Gold nanoparticles also improve the electrochemical signal transduction of the binding reaction between antigens and antibodies and increase the amount of immobilized immunoreagents in a stable manner [22, 23].

By these reasons, in this work, a voltammetric gold nanoparticle-modified SPCE immunosensor was developed, validated, and used for Ara h 6 detection in real food matrices. First, the surface of the SPCE was modified with gold nanoparticles generated on the working electrode through electrochemical deposition of ionic gold. Then, a sandwich-type immunoassay, using two-monoclonal mouse IgG antibodies against Ara h 6 was developed. The detection antibody was labeled with alkaline phosphatase and the electrochemical detection relied on enzyme-catalyzed silver precipitation.

Materials and methods

Instrumentation

The electrochemical procedures were performed using a Metrohm Autolab PGSTAT12 potentiostat-galvanostat controlled by GPES4.9 software.

SPCEs (DropSens, Spain) were used as transducers of the biological interaction. These electrodes incorporate a conventional three-electrode electrochemical cell (50 μl) and are printed on ceramic substrates (3.4 × 1.0 cm) using carbon inks, for the working (d=4 mm) and counter electrodes, and a silver ink, for the pseudoreference electrode and the electrical contacts.

A scanning electron microscope (SEM) images were obtained at the "Centro de Materiais da Universidade do Porto (CEMUP)" using a FEI QUANTA 400 FEG/EDAX Pegasus X4M equipment.

Chemicals and reagents

Hydrochloric acid (37 %), 3-indoxyl phosphate (3-IP; ≥98 %), β-casein from bovine milk (≥98 %), magnesium nitrate hexahydrate (99 %), nitric acid (≥65 %), sodium chloride, streptavidin-alkaline phosphatase (S-AP) from *Streptomyces avidinii*, and tris(hydroxymethyl)aminomethane (Tris, ≥99.8 %) were all obtained from Sigma-Aldrich. Silver nitrate (≥99.9995 %) was from Alfa Aesar, and the gold ($H[AuCl_4]$) standard solution was acquired from Merck. Indoor Biotechnologies provided the immunoreagents: a mouse monoclonal anti-Ara h 6 IgG1 (clone 3B8 B5) antibody, a biotinylated monoclonal anti-Ara h 6 IgG1 (clone 3E12 C4 B3) antibody and naturally purified Ara h 6. Ultra-pure water (resistivity=18.2 MΩ cm) from a Millipore (Simplicity 185) water purification system was used for the preparation of solutions.

The reagents that were used in the immunoassay were prepared using a Tris-HNO_3 (pH 7.2) buffer (0.1 M), except the 3-IP/Ag^+ solution which was prepared in a Tris-HNO_3 (pH 9.8) buffer (0.1 M)+20 mM $MgNO_3$. These Tris-HNO_3 buffers were also used as the washing solutions in the immunoassay.

Sensor structure

The SPCEs were modified with gold nanoparticles by placing 40 μl of a 0.1 mM $[AuCl_4]^-$ solution (prepared in 0.1 M HCl) on the electrode and, subsequently, applying a constant current intensity of −100 μA for 240 s, followed by the application of a 0.1-V potential during 120 s to desorb the hydrogen formed during this procedure [22].

Afterwards, the resulting gold nanoparticle-modified SPCEs (SPCE-nAu) were rinsed with water and dried.

Nanoparticle analysis

The nanoparticle analysis was performed using ImageJ software. Firstly, threshold limit was determined by Huang method implemented in ImageJ, followed by particle analysis. A SEM image of a gold nanoparticle-deposited SPCE is shown in Fig. 1.

Samples

Cookies and chocolate were used to evaluate the immunosensor's performance. The cookies were composed of a complex matrix containing wheat flour, saccharose, vegetable oil, vitamin E, glucose, fructose, whey powder, salt, sunflower lecithin, sodium bicarbonate, caramel, sodium metabisulphite, and egg. Commercial chocolate samples with and without peanut as ingredient were also analyzed.

Sample preparation

Sample preparation was based on previously described procedures [19, 24], with minor adjustments. Briefly, after grinding in a mill (GM 200, Retsch, Germany) at 10,000 rpm during 20 s (3×), 1 g of sample was extracted with 10 ml of Tris-HNO_3 buffer (pH 8.2, 1 % NaCl) at 60 °C during 30 min. Subsequently, the samples were subjected to a first centrifugation step (5000 rpm) during 5 min (Labofuge Ae, Heraeus Sepatech, Germany). An aliquot (1 ml) of the supernatant was further centrifuged at 10,000 rpm for 3 min (Heraeus Fresco 17 Centrifuge, Thermo Fisher Scientific, Germany), and the resulting supernatant was used to perform the immunological detection. Dilutions of the extracts were performed when necessary.

In the specific case of chocolate, samples were frozen at −20 °C before being ground, and 1 g of skimmed milk powder (Nestlé) was added before the extraction step to block the high amount of phenolic compounds (e.g., tannins) present in this matrix, which have ability to bind to the allergens and/or antibodies [24].

Immunosensor assay

Each SPCE-nAu was coated with a monoclonal anti-Ara h 6 IgG (3B8 B5) solution (25 μg/ml) and left to incubate overnight at 4 °C. After rinsing the sensor (Tris-HNO_3 (pH 7.2) buffer), surface blocking was carried out using a 2 % β-casein solution during 30 min. Then, the sensor was washed and incubated (60 min) with 40 μl of a food sample extract, Ara h 6 standard (calibration assay), or Tris-HNO_3 (pH 7.2) buffer (blank assay). After a new washing, a 40-μl drop of biotinylated monoclonal anti-Ara h 6 IgG (3E12 C4 B3; 1:25000) was placed on the sensor and left to incubate for 60 min. After

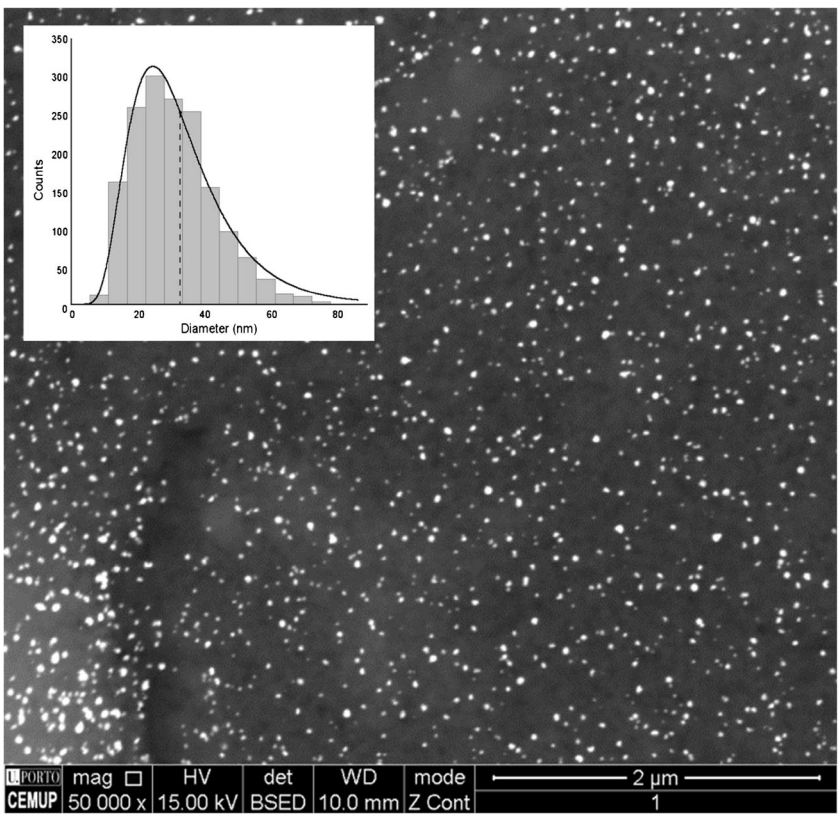

Fig. 1 SEM image of a gold nanoparticle-modified screen-printed carbon electrode. The histogram was obtained for ca. 1600 particles by analyzing three images from three distinct electrodes where particles were deposited under same conditions

rinsing, 40 μl of S-AP (1×10^{-10} M) were added and left to react for 60 min. The sensor was then rinsed with Tris-HNO_3 (pH 9.8) buffer, and the enzymatic reaction was carried out by placing 40 μl of a 3-IP (1.0×10^{-3} M) and silver nitrate (4.0×10^{-4} M) solution on the immunosensor's surface. After 20 min, a linear sweep voltammogram was recorded from −0.02 to +0.4 V, at a scan rate of 50 mV/s, to obtain the electrochemical current of the enzymatically deposited silver [25]. Figure 2 depicts a schematic representation of this immunoassay. Analyses were always performed in triplicate, and measurements were carried out at controlled temperature (21± 1 °C).

Results and discussion

Nanoparticle characterization

In scanning electron microscopy, backscattered electron (BSE) images are quite useful to identify and determine the shape of metallic nanoparticles electrodeposited on soft materials (carbon), mainly due to high contrast provided by BSE. Such images may be used to determine size distribution of nanoparticles [26].

In nanoparticle synthesis, several normally distributed variables may influence the particles growth rate. Therefore, the size distribution of a nanoparticle synthesis follows a lognormal distribution, which probability density function (PDF) can be written as:

$$y = \frac{A}{\sqrt{2\pi}\sigma x} e^{-\frac{(\ln(x)-\mu)^2}{2\sigma^2}}$$

where, μ is the location parameter and σ is the scale parameter (they should not be confused with the mean and standard deviation of a normal distribution). In the lognormal distribution, the logarithm of a variable x (size) is normally distributed, and because it is an asymmetric distribution, the mean and standard deviation differ from a normal distribution. In Fig. 1, a SEM image of a gold nanoparticle-deposited SPCE is shown, as well as a histogram obtained by analysis of *ca.* 1600 particles. The analysis was carried out in three different images from three distinct electrodes where particles were deposited under the same conditions. The histogram shows the particles counting (gray bars), the fitted distribution (black line), and the mean particle size (dashed line). The particle size was found at 33.4±15.4 nm, where 33.4 nm is the arithmetic mean and 15.4 the arithmetic standard deviation (the square root of variance).

Optimization of the immunosensing strategy

The immunosensing strategy was based on a sandwich-type format. Monoclonal antibodies were selected due to their specificity for a single epitope of a certain protein, which, in principle, allows the reduction of cross-reactions and non-specific binding, compared with polyclonal antibodies [14]. The antibodies were immobilized on the gold-nanostructured sensor surface through chemisorption: the high affinity between gold and thiol groups of the antibody provides a stable immobilization strategy.

In the first phase of the immunosensor development, the assay conditions were tested as follows: after the immobilization of the capture antibody (25 μg/ml) and surface blocking with casein (2 %), a standard Ara h 6 solution (50 ng/ml) was placed on the sensor's surface for 60 min; after rinsing, the immunosensor was incubated with the biotinylated detection antibody (different dilutions tested separately: 1:1000, 1:2500, 1:5000, 1:10,000, 1:25,000, and 1:50,000), for 60 min; after a

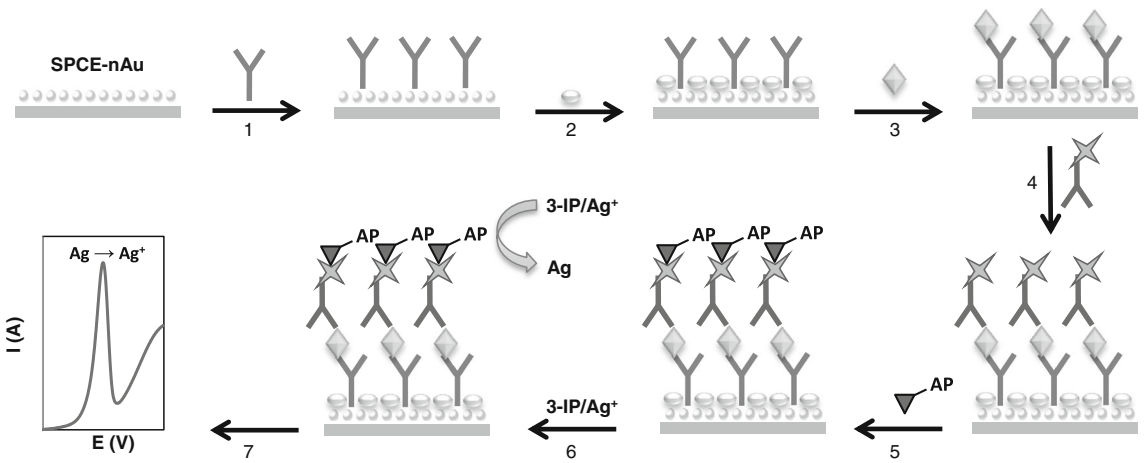

Fig. 2 Schematic representation of the developed immunoassay. Legend: *1*, immobilization of capture antibody; *2*, surface blocking with casein; *3*, antigen incubation (Ara h 6); *4*, biotinylated detection antibody incubation; *5*, addition of streptavidin-alkaline phosphatase; *6*; addition of the enzymatic substrate and silver ion; and *7*, voltammetric detection of deposited silver

new washing step, S-AP (2×10^{-10} M) was added (60 min) and, after rinsing, the enzymatic reaction was carried out, during 20 min, by using 3-IP (1.0×10^{-3} M) and silver nitrate (4.0×10^{-4} M). Voltammetric measurements were subsequently performed. The results (for this and all the subsequent experiments) are expressed as mean value±standard deviation, calculated from triplicate analyses.

Although the first four dilutions of biotinylated antibody (1:1000, 1:2500, 1:5000, and 1:10,000) resulted in higher peak current intensities (i_p; 49.9±1.1, 52.2±2.5, 52.7±1.4, and 46.5±2.5 μA, respectively), than the 1:25,000 dilution (39.6±1.2 μA), the respective i_p values of the blank assays were also significantly ($p<0.05$) higher (25.5±3.2, 19.5±5.1, 20.4±4.0, and 14.4±2.3 μA, correspondingly) than those obtained with the 1:25,000 dilution (11.5±1.5 μA). In the case of the 1:50,000 dilution, a decrease of both signals was obtained (8.3±1.2 μA for blank and 16.7±2.3 μA), in the presence of the allergen. Lower blank signals and, simultaneously, high values in the presence of the allergen represent a better performance of the immunoassay (better surface blocking, less unspecific reactions), since lower detection limits and higher reproducibility can be achieved. Therefore, the 1:25,000 dilution of the detection antibody was selected to perform the following studies, which aimed to lower even more the blank signal, still obtaining a good response in the presence of Ara h 6.

In a second test, using the optimized dilution of biotinylated antibody, different concentrations of capture antibody were assayed, namely, 1, 5, 10, 25, and 50 μg/ml. The results showed no significant differences ($p>0.05$) between the i_p values of the blank assays when the different concentrations of the capture antibody were used. However, in the presence of the allergen (50 ng/ml), an increasing response was noticed up to 25 μg/ml of capture antibody (40.9±2.5 μA), followed by a significant decrease for 50 μg/ml of antibody (31.8±0.80 μA), which means that a response saturation occurred. Indeed, an excessive amount of adsorbed antibody may block the binding sites for Ara h 6. In this test, it was also found that a concentration of capture antibody higher than 1 μg/ml is needed to obtain a response, since, in this particular case, no significant differences ($p>0.05$) were observed between the i_p values measured in the absence (blank) and presence of Ara h 6 (50 ng/ml).

Because the analytical signal was relatively near for 10 and 25 μg/ml of capture antibody (37.9±1.1 and 40.9±2.5 μA, respectively), both concentrations were selected to proceed the studies.

With the selected capture (10 and 25 μg/ml) and detection antibody (1:25,000) concentrations, two different levels of S-AP (2×10^{-10} and 1×10^{-10} M) were tested to evaluate the possibility to lower the blank signals. The results are depicted in Fig. 3. Indeed, a decrease in the amount of S-AP used, resulted in a reduction of the blank signals of ~60 and 80 % (for 10 and 25 μg/ml of capture antibody, respectively), whereas the i_p obtained in assays 2 and 4 (with 50 ng/ml of Ara h 6) remained about 60–70 % of the original ones (assays 1 and 3, respectively). Assay 4, in which 25 μg/ml of capture antibody and 1×10^{-10} M of S-PA were both employed, showed the best results regarding the blank signal, which probably resulted from a higher surface blocking due to a higher concentration of the capture antibody together with casein. Therefore, these conditions were chosen to proceed with the following tests.

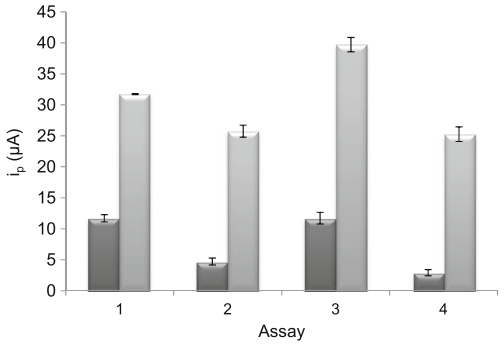

Fig. 3 Peak current intensities (i_p) obtained for different capture antibody (10 and 25 μg/ml) and streptavidin-alkaline phosphatase concentrations (1×10^{-10} and 2×10^{-10} M). Legend: *Dark gray bars*, blank assays. *Light gray bars*, Ara h 6 (50 ng/ml). Results are presented as average±standard deviation ($n=3$). Experimental conditions: capture antibody (assays 1 and 2, 10 μg/ml; assays 3 and 4, 25 μg/ml); β-casein (2 %); Ara h 6 (0 (blank) and 50 ng/ml); biotinylated detection antibody (1:25000); S-AP (assays 1 and 3, 2×10^{-10} M; assays 2 and 4, 1×10^{-10} M, respectively); 3-IP (1.0×10^{-3} M), and silver nitrate (4.0×10^{-4} M)

Different assay formats were afterwards studied in order to evaluate the possibility of reducing the steps and/or the total time of the immunoassay. Three different strategies were tested and compared with the previously described assay (format A): format B—mixture of antigen and detection antibody before application on the sensor surface; format C—previous mixture of detection antibody and S-AP; and format D—previous mixture of antigen, detection antibody and S-AP. The obtained results are shown in Fig. 4.

As can be observed, in general, no significant differences ($p<0.05$) were found between the i_p values of the blanks for all the assay formats. The first format tested (A), in which the immunoreagents were added separately, provided the highest i_p values in the presence of Ara h 6. A significant loss of signal is notorious in both formats C and D, which was probably due to a steric hindrance when the different reagents were mixed before being placed on the sensor's surface. Although the total assay time was reduced by 1 h with format B, the signal obtained in the presence of the allergen decreased ~20 %. Indeed, reducing the analysis time will only compensate if the signal was higher, or at least similar, and the reagent consumption was equivalent. In turn, a loss of signal of about 20 % was observed from formats A to B, for the same

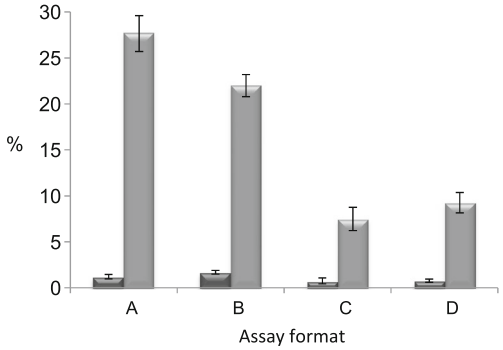

Fig. 4 Comparison of peak current intensities (i_p; data presented in relative percentages) of four different assay formats. Legend: *Dark gray bars*, blank assays. *Light gray bars*, Ara h 6 (50 ng/ml). Experimental conditions for all assays: capture antibody (25 μg/ml); β-casein (2 %); Ara h 6 (0 (blank) and 50 ng/ml); biotinylated detection antibody (1:25000); S-AP (1×10^{-10} M); 3-IP (1.0×10^{-3} M) and silver nitrate (4.0×10^{-4} M). Differences between assays: **A** the addition of immunoreagents was performed in separate steps; **B** antigen and detection antibody were previously mixed before application on the sensor surface; **C** detection antibody and S-AP were previously mixed; **D** antigen, detection antibody, and S-AP were previously mixed

concentrations of capture and detection antibodies. Moreover, the used volume of detection antibody will have to be higher since it will have to be mixed with the real samples, individually and in a reproducible way, before the mixture was applied on the sensor surface.

For all these reasons, and based on the linear range obtained for format A (described below), this assay was selected to be validated and applied to real samples. Additionally, shorter reaction times were tested in order to evaluate the possibility to decrease the total time of the assay. The time of reaction of capture antibody/allergen and allergen/detection antibody were reduced, separately or simultaneously, from 60 to 30 min. In situations in which only one of the steps was shorter, an approximately 40 % reduction of the signal was obtained. When decreasing the time of both steps at the same time, the signal was reduced in 72 %. This showed that at least 60 min were necessary for a complete reaction between the antibodies and the allergen.

Method validation

The final optimized method used to analyze the samples is detailed in the "Materials and methods." The precision of the methodology was evaluated in terms of repeatability and reproducibility using a 50-ng/ml Ara h 6 solution. The repeatability was evaluated by performing six successive inter-electrodic measurements using separate immunosensors and the reproducibility was assessed through six inter-day evaluations. Relative standard deviations were 1.9 and 9.8 %, respectively, showing that the immunosensor provides precise results.

For calibration purposes, Ara h 6 standard solutions with increasing concentrations (1, 5, 10, 50, and 100 ng/ml) were prepared and analyzed with the immunosensor. A linear semi-logarithmic relationship between Ara h 6 concentration ([C]) and the i_p was obtained between 1 and 100 ng/ml:

$$\begin{aligned} i_p(\mu A) &= (8.0117 \pm 0.5652) \times \log[C](ng/ml) \\ &\quad + (7.6206 \pm 0.4326), r \\ &= 0.998 \end{aligned}$$

The limits of detection (LOD) and the quantification (LOQ) were calculated from the calibration plot using the following equations: LOD=3 s/m and LOQ=10 s/m, where s is the standard deviation of the intercept and m is the slope of the calibration plot. The LOD and LOQ values obtained were 0.27 and 0.88 ng/ml, respectively.

Due to the lack of other biosensors for Ara h 6 detection, the LOD and LOQ values cannot be compared with those of other devices. Nevertheless, they are similar to those described for the commercial ELISA kit that detects this protein (from Indoor Biotechnologies). However, comparison is possible with immunosensors that have been developed to detect other peanut allergens. For example, Singh et al. [17] and Huang et al. [18] described two impedimetric immunosensors for Ara h 1 detection with LODs of 40 ng/ml and <0.3 nM (<19 ng/ml), respectively. Pollet et al. [15] developed an optical biosensor for Ara h 1 with an LOD of 90 ng/ml, and Mohammed et al. [26] developed an optical immunosensor able to detect 0.7 μg of peanut allergens/ml of extract. Although not directly comparable, because different proteins are being analyzed, the LOD of the sensor developed in this work was significantly lower than those cited above.

Independently of the efficiency of β-casein as blocking agent, the presence of other proteins in food can lead to non-specific adsorptions on the sensor's surface, prejudicing the detection of Ara h 6. Due to the absence of a reference material, recovery experiments to evaluate accuracy were performed. For this purpose cookies (containing, among other ingredients, listed in the "Materials and methods," whey powder and egg, potential allergenic ingredients) were used.

Cookies were analyzed before and after being spiked with three increasing amounts of the Ara h 6 standard (5, 10, and 50 ng/ml). The non-spiked sample gave negative results (i.e., no significant differences ($p>0.05$) when compared with the blank assays).

The recoveries obtained for the three levels of addition were 96.7, 98.8, and 97.6 %, respectively, showing the high accuracy of the sensor.

In an additional test, cookie samples were analyzed before and after being mixed with 0.1 % of peanut (instead of the Ara h 6 standard, as performed above). Once

more, before peanut addition, a negative result was obtained. Nevertheless, the immunosensor easily detected the presence of Ara h 6 from peanut (not standard), even when the sample extract was diluted 100-fold before being applied on the electrode surface.

The developed immunosensor was also used to detect Ara h 6 in chocolate samples with and without peanut as ingredient, as described on the label. The chocolate sample without peanut gave a negative result (no significant differences ($p>0.05$) when compared with the blank assays). On the other hand, in the chocolate sample containing peanut as ingredient, 3.7 μg of Ara h 6/g of chocolate was detected.

As referred before, there is not a reference material that could be used to estimate accuracy of the methods developed for Ara h 6 quantification. Nevertheless, we believe that these data show an accurate, precise, and linear response of the sensor to presence of this allergen in complex food matrices.

Conclusions

In this work, gold nanoparticle-modified screen-printed carbon electrodes were used to develop a two-monoclonal antibody sandwich-type electrochemical immunosensor for Ara h 6 (a major peanut allergen) detection. The validation studies indicated that the proposed methodology provides precise (good repeatability and reproducibility) and accurate results. The developed immunosensor achieved a very low LOD (0.27 ng/ml) and was successfully applied to the Ara h 6 detection in complex food matrices, such as cookies and chocolate.

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References

1. Chafen JJ, Newberry SJ, Riedl MA, Bravata DM, Maglione M, Suttorp MJ, Sundaram V, Paige NM, Towfigh A, Hulley BJ, Shekelle PG (2010) Diagnosing and managing common food allergies: a systematic review. JAMA 303:1848–1856
2. Berin MC, Sicherer S (2011) Food allergy: mechanisms and therapeutics. Curr Opin Immunol 23:794–800
3. Leung A, Kamat D (2008) In: Chesterton CM (ed) Food allergies: new research. New York, Nova Science Publishers Inc
4. de Leon MP, Rolland JM, O'Hehir RE (2007) The peanut allergy epidemic: allergen molecular characterization and prospects for specific therapy. Expert Rev Mol Med 9:1–18
5. Khodoun M, Strait R, Orekov T, Hogan S, Karasuyama H, Herbert DR, Jörg Köhl MD, Finkelman FD (2009) Peanuts can contribute to anaphylactic shock by activating complement. J Allergy Clin Immunol 123:342–351
6. Allergen nomenclature. IUIS allergen nomenclature sub-committee. http://www.allergen.org/. Accessed 20 Feb 2015
7. Koid AE, Chapman MD, Hamilton RG, van Ree R, Versteeg SA, Dreskin SC, Koppelman SJ, Wünschmann S (2014) Ara h 6 complements Ara h 2 as an important marker for IgE reactivity to peanut. J Agric Food Chem 62:206–213
8. Hed J (2009) Cross-reactivity in plant food allergy—clinical impact of component resolved diagnostics (CRD). Phadia AB, Sweden
9. Besler M, Kasel U, Wichmann G (2002) Determination of hidden allergens in foods by immunoassays. Int Symp Food Allergens 4:1–18
10. Taylor SL, Nordlee JA, Niemann LM, Lambrecht DM (2009) Allergen immunoassays—considerations for use of naturally incurred standards. Anal Bioanal Chem 395:83–92
11. Monaci L, Visconti A (2010) Immunochemical and DNA-based methods in food allergen analysis and quality assurance perspectives. Trends Food Sci Technol 21:272–283
12. Słowianek M, Majak I (2011) Methods of allergen detection based on DNA analysis. Food Sci 75:39–44
13. Monaci L, Visconti A (2009) Mass spectrometry-based proteomics methods for analysis of food allergens. TrAC 28:581–591
14. Alves RC, Barroso F, González-García MB, Oliveira MBPP, Delerue-Matos C (2015) New treds in food allergens detection: towards biosensing strategies. Crit Rev Food Sci Nutr. doi:10.1080/10408398.2013.831026
15. Pollet J, Delport F, Janssen KP, Tran DT, Wouters J, Verbiest T, Lammertyn J (2011) Fast and accurate peanut allergen detection with nanobead enhanced optical fiber SPR biosensor. Talanta 83:1436–1441
16. Wang W, Han J, Wu Y, Yuan F, Chen Y, Ge Y (2011) Simultaneous detection of eight food allergens using optical thin-film biosensor chips. J Agric Food Chem 59:6889–6894
17. Singh R, Sharma PP, Baltus RE, Suni II (2010) Nanopore immunosensor for peanut protein Ara h 1. Sensors Actuators B 145:98–103
18. Huang Y, Bell MC, Suni II (2008) Impedance biosensor for peanut protein Ara h 1. Anal Chem 80:9157–9161
19. Alves RC, Pimentel FB, Nouws HPA, Marques RCB, González-García MB, Oliveira MBPP (2015) Detection of Ara h 1 (a major peanut allergen) in food using an electrochemical gold nanoparticle-coated screen-printed. Biosens Bioelectron 64:19–24
20. Díaz-González M, González-García MB, Costa-García A (2005) Recent advances in electrochemical enzyme immunosensors. Electroanalysis 17:1901–1918
21. Ricci F, Adornetto G, Palleschi G (2012) A review of experimental aspects of electrochemical immunosensors. Electrochim Acta 84:74–83
22. Martínez-Paredes G, González-García MB, Costa-García A (2009) In situ electrochemical generation of gold nanostructured screen-printed carbon electrodes. Application to the detection of lead underpotential deposition. Electrochim Acta 54:4801–4808
23. Pingarrón JM, Yáñez-Sedeño P, González-Cortés A (2008) Gold nanoparticle-based electrochemical biosensors. Electrochim Acta 53:5848–5866
24. Pele M, Brohee M, Anklam E, van Hengel AJ (2007) Peanut and hazelnut traces in cookies and chocolates: relationship between analytical results and declaration of food allergens on product labels. J Food Addit Contam 24:1334–1344
25. Fanjul-Bolado P, Hernández-Santos D, González-García MB, Costa-García A (2007) Alkaline phosphatase-catalyzed silver deposition for electrochemical detection. Anal Chem 79:5272–5277
26. Govatsi K, Chrissanthopoulos A, Dracopoulos V, Yannopoulos SN (2014) The influence of Au film thickness and annealing conditions on the VLS-assisted growth of ZnO nanostructures. Nanotechnology 25:215601