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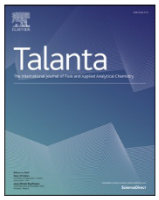

Ultrasensitive detection of soy traces by immunosensing of glycinin and β-conglycinin at disposable electrochemical platforms

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ABSTRACT

This work reports the first electrochemical bioplatform for the determination of soy traces in food. The bioplatform involves sandwich-type immunoassays using specific antibodies for β-conglycinin and glycinin, which are the main allergenic soy proteins, and carboxylic acid-modified magnetic microbeads. Amperometric detection at −0.20 V (*vs.* an Ag pseudo-reference electrode) was performed using single or dual screen-printed carbon electrodes and the H_2O_2/hydroquinone (HQ) system. The measured variation in the cathodic current was directly proportional to the concentration of target allergenic proteins. The developed bioplatforms exhibit a good selectivity and sensitivity providing limits of detection (LOD) values of 0.03 and 0.02 ng mL^{-1} for β-conglycinin and glycinin, respectively. The determination of both proteins can be carried out in only 1.5 h. The electrochemical bioplatforms allow their accurate determinations (with results statistically comparable to those provided by ELISA methodologies) in raw cookie dough and baked cookies enriched with soy flour. The results obtained confirm, in a pioneering way with electrochemical biosensors, the possibility of discriminating samples incurred with as little as 0.0005 ppm of a food allergen in model cookie extracts.

1. Introduction

The prevalence of food allergy has steadily increased during the last decades in developed countries and is considered a serious public health problem. Epidemiological studies in recent years indicate that 2–4% of the population suffers food allergy [1]. Soy is listed amongst the most frequent human food allergens which are known as the "big eight" because they account for 90% of all food allergies. Soy allergy has a prevalence of 0.5% among children of all ages in USA [2] and affect to one third of patients suffering from atopic dermatitis [3] and 10–14% of children with allergy to cow's milk proteins [4]. This high prevalence is probably because soy is widely used as an ingredient in the preparation of many foods such as meat, dairy and bakery products, edible spreads, cheese analogues, desserts, soups, etc. [5].

It is very common to find soy as a hidden allergen due to cross contamination or to poor cleaning of processing equipment which could endanger the lives of allergic consumers due to the intake of unintentional trace amounts [6]. In fact, 25% of bakery and 11% of snack products analyzed by Khuda et al. (2016) were positive for soy protein although it was not a declared ingredient [7]. EU and USA legislation on allergens labelling includes soy as one of the top allergens required to appear on the label of the food product as a preventive measure to avoid

Abbreviations: Blocking Buffer solution, BB; Capture antibody, cAb; Carboxylic acid-modified magnetic beads, HOOC−MBs; Detection antibody conjugated with peroxidase, HRP-dAb; Dual screen-printed carbon working electrodes, SPdCE; Homemade polymethylmethacrylate, PMMA; Horseradish peroxidase, HRP; Hydroquinone, HQ; Magnetic beads, MBs; N-(3-dimethyl-aminopropyl)-N′-ethylcarbodiimide, EDC; N-hydroxysulfosuccinimide, Sulfo-NHS; Phosphate buffer, PB; Phosphate buffer saline, PBS; Single screen-printed carbon working electrodes, SPCE.

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accidental ingestions, although allergic consumers find precautionary allergen labelling confusing and do not rely on it [8]. The threshold level of soy to cause allergic reactions has been reported to range between 0.0013 mg and 500 mg [9]. It is also important to note that according to the Scientific Panel of VITAL (2019) [10] the presence of 1 mg of protein from soybean flour would only affect 1% of soy allergy sufferers. However, there is no consensus among the scientific community on what level of allergens is tolerable in relation to the consequences of unintended allergen consumption and, therefore, highly sensitive detection methods are strongly demanded by regulatory agencies to protect allergic consumers as well as by the food industry for analytical control as part of the allergen management plans.

The soy proteins that are recognized by serum IgE of most soy-allergic individuals are glycinin and β-conglycinin [11]. These two proteins are the major storage globulins in soybean that account for about 70–80% of the total seed globulin fraction and they are referred to as 11 S and 7 S, respectively, according to their sedimentation coefficients [12].

To date, enzyme-linked immunosorbent assays are preferred for soy allergen detection in food and several ELISA tests have been developed. They rely on the determination of single proteins such as glycinin [13–15], β-conglycinin [16–19], or the soybean trypsin inhibitor [20] or of the whole soy soluble proteins [21]. Most commercial ELISA tests have low detection limits ranging between 0.9 and 30 ng mL^{-1}. However, the cross-reactivity with other food matrix components can generate false positive results and the ELISA format used can also influence the final data readout [22]. In addition to ELISA methodology, other commercially available kits for the detection of soy allergens in food include the detection of characteristic fragments of genes that encode the food allergen expression by polymerase chain reaction (PCR) or the use of lateral flow devices, although they have some practical limitations [22,23]. For example, the degradation of proteins and DNA fragments during food processing can limit the applicability of PCR detection methods and the viscosity of food extracts can strongly influence the accuracy of LFD strips. In addition, PCR-based techniques are time-consuming, labor-intensive, and need expensive equipment.

In this context, electrochemical affinity biosensors have experienced an important growth in recent years due to their attributes of affordable cost, simplicity, reduced test time, ability for field operation and compatibility with multiplexed and multiomics analyses. Indeed, they are positioned as very competitive tools for the determination of food allergens at both protein and genetic levels [24]. So, electrochemical immunosensors exhibit high sensitivity and selectivity for the determination of the most relevant allergenic proteins in milk [25,26] peanuts [27], egg [28] and mustard seeds [29], among others. However, to our knowledge, no electrochemical biosensors have been reported so far for the determination of soy allergens.

This paper reports, for the first time, electrochemical immunosensing platforms for the simple and rapid single or dual determination of small concentrations of β-conglycinin and glycinin, the main soy allergens. Selective capture antibodies were used to build up sandwich immunosensing strategies using carboxylic acid-modified magnetic beads (HOOC−MBs). The same antibodies conjugated with horseradish peroxidase (HRP) were used as detector antibodies for each target protein. Amperometric measurements (at −0.20 V vs. the Ag pseudoreference electrode using the H_2O_2/hydroquinone (HQ) system) at single (SPCE) or dual (SPdCE) screen-printed carbon electrodes were used for the monitoring of the affinity reactions and the determination of the two allergenic proteins.

2. Experimental

2.1. Apparatus and electrodes

A multichannel potentiostat (model 1030B, CH Instruments, Austin, TX, USA) controlled by the CHI1030B software and a Magellan V 7.1 (TECAN) ELISA plate reader were used for the amperometric and spectrophotometric measurements, respectively. Single (SPCEs, DRP-110, ϕ 4 mm, active area working electrode 12.6 mm^2) and dual (SPdCEs, DRP-X1110, ϕ 2 mm, active area 6.3 mm^2 each working electrode) screen-printed carbon electrodes, and the respective DRP-CAC and DRP-BICAC specific connector cables were from Metrohm-DropSens S.L. (Oviedo, Asturias, Spain). A Wizard IR Vortex (VELP Scientifica), a Dynamag-2 Magnet magnetic separator (Invitrogen Dynal AS), a Basic 20+ (Crison) pH-meter, and a thermomixer MT100 incubator shaker (Universal Labortechnik) were employed. Homemade polymethylmethacrylate (PMMA) casings with one or two embedded neodymium magnets (AIMAN GZ) were also utilized.

2.2. Reagents and solutions

All the used reagents and solvents were of the highest available analytical grade. Carboxylic acid-modified MBs (HOOC-MBs, 2.7 μm Ø, Dynabeads M − 270 carboxylic acid, Cat.No. 14305D) were purchased from Invitrogen™. N-(3-dimethyl-aminopropyl)-N′-ethylcarbodiimide (EDC), N-hydroxysulfosuccinimide (Sulfo-NHS), ethanolamine, hydroquinone (HQ) and 30% w/v hydrogen peroxide (H_2O_2) were purchased from Sigma. NaH_2PO_4, Na_2HPO_4, NaCl, KCl, HCl and NaOH were all from Scharlab. 2-Morpholinoethanesulfonic acid (MES, from Gerbu) and Tris-HCl (from Panreac) were also employed.

Buffer solutions used, all prepared in Milli-Q water from a Millipore Milli-Q purification system 18.2 MΩ cm, include: 0.1 M phosphate buffer saline (PBS) solution pH 7.4 containing 0.137 M NaCl and 0.027 M KCl; 0.05 M phosphate buffer (PB) solution pH 6.0; 0.1 M PB solution pH 8.0; 0.025 M MES buffer solution pH 5.0 and 0.1 M Tris-HCl solution pH 7.2. Blocker™ Casein in PBS (a ready-to-use PBS solution containing 1.0% w/v purified casein, Blocking Buffer solution, BB solution) was purchased from Thermo Scientific.

Glycinin and β-conglycinin used as standards were isolated using acid precipitation and salting-out followed by gel filtration on a Sepharose CL-6B column) as previously described [13,19]. The degree of purity of isolated proteins was higher than 96% as determined by SDS-PAGE and densitometry using an EPSON EU-88 Image Scanner III (Long Beach, California).

Antibodies against glycinin and β-conglycinin were obtained in rabbits by immunization with the corresponding purified proteins as previously described by Wehbi et al. [30]. All procedures were conducted under Project Licence 30/19 approved by the Ethic Committee for Animal Experiments from the University of Zaragoza. The care and use of animals were performed accordingly with the Spanish Policy for Animal Protection RD53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Specific antibodies to glycinin and β-conglycinin were purified by affinity chromatography using a HiTrap NHS activated HP column previously coupled with each protein, as previously described [18]. Purified antibodies were conjugated with horseradish peroxidase (HRP) using the Lighting-link HRP conjugation Kit (Innova Biosciences, Cambridge, UK).

Washing buffer was composed of 1.5 mM potassium phosphate, 8 mM sodium phosphate, 0.14 mM potassium chloride and 0.14 mM sodium chloride, pH 7.4 (PBS); elution buffer 0.1 M HCl-glycine buffer, pH 2.8 containing 0.5 M NaCl, was employed. To neutralized elution buffer 0.5 M Tris buffer, pH 8.0 was used.

Soybean flour with 37.6% protein was purchased from Bionsan.

2.3. Preparation of the magnetic bioconjugates

The determination of both allergenic proteins required the formation of sandwich immunocomplexes on HOOC-MBs using similar protocols. The corresponding specific antibody to the target protein was used unmodified as capture antibody (cAb) or conjugated with HRP as detection

antibody (HRP-dAb).

The formation of the sandwich immunocomplexes onto MBs was performed in microcentrifuge tubes in successive incubation steps carried out at room temperature and under constant stirring (950 rpm, 25 °C) followed by washings where the microcentrifuge tubes were placed on a magnetic rack to remove the supernatant without losing MBs. All incubation steps were carried out with 25 μL of the corresponding solution and the washing steps with 50 μL.

Briefly, each determination was carried out by placing 3 μL of the HOOC-MBs suspension in a microcentrifuge tube. Two washings with MES solution were performed for 10 min, and the HOOC groups of MBs were activated by incubation for 35 min in EDC/Sulfo-NHS solution (prepared in MES). After two washings with MES solution, the activated MBs were incubated with 2.5 μg mL^{-1} solutions of the corresponding cAb (prepared in MES) for 15 or 30 min (β-conglycinin and glycinin, respectively). Upon two further washings with MES solution, the residual active groups of the cAb-MBs were blocked by incubation for 1 h with 1 M ethanolamine solution (in 0.1 M PB, pH 8.0). After one washing with Tris-HCl buffer (pH 7.2) and two further washings with BB solution, the as prepared cAb-MB were filtered and stored at 4 °C until use.

The cAb-MBs were incubated for 15 min with the standard solution of the target allergenic protein or the analyzed food extract (prepared in BB solution). Upon two washings with BB solution, the modified MBs were incubated with a 1/1000 diluted solution of the corresponding HRP-dAb for 15 min. After two washings with BB solution, the MBs carrying the sandwich immunoconjugates of each allergenic protein were re-suspended in the appropriate volume (50 μL or 5 μL) of 0.05 M PB (pH 6.0) to perform the single or dual amperometric detection, respectively.

2.4. Amperometric measurements

50 or 5 μL of the magnetic immunoconjugate suspension were deposited onto the surface of the SPCE or SPdCE (previously placed in the corresponding PMMA casing) working electrodes, respectively. The SP(*d*)CE/casing assembly was connected to the potentiostat and immersed in an electrochemical cell containing 10 mL of 0.05 M PB (pH 6.0) and 1 mM HQ. Amperometric measurements were performed at room temperature in stirred solutions at −0.20 V vs. the Ag pseudoreference electrode by monitoring the cathodic current variation occurring upon the addition of 50 μL of a 0.1 M H_2O_2 solution prepared in 0.05 M PB pH 6.0. To ensure their stability, HQ and H_2O_2 solutions were prepared just before making the amperometric detection. The presented results correspond to the mean value of the difference between the steady state and the background current measured for three replicates, and the error bars were estimated as three times the standard deviation of each set of replicates ($\alpha = 0.05$).

2.5. Analysis of samples

The amperometric immunosensing platforms were applied to the determination of β-conglycinin and glycinin in cookies samples with increasing amounts (0.0005–5000 ppm) of soybean flour before and after baking. For their preparation, the flour was added to the ingredients before processing to obtain the desired final concentrations. 200 g of wheat flour, 100 g of sugar, 10 g of yeast, 70 g of sunflower oil, and 1 egg, were mixed, left for 1 h at 4 °C and baked at 180 °C for 12 min. To obtain the sample extracts, 3 g of accurately weighted sample cookie were introduced in plastic tubes, incubated with 30 mL of PBS (pH 7.4), shaken in a vortex for 1 min and heated in a water bath at 60 °C for 15 min. The supernatant obtained after centrifuging at 3000 g for 15 min was directly analyzed according to the protocol described in section 2.3.

3. Results and discussion

This paper reports the preparation of first electrochemical bioplatforms developed to date for the individual or simultaneous determination of the two major soy allergenic proteins. The bioplatforms were optimized and characterized for the individual determination of both proteins and their coupling on dual platforms was evaluated to allow their simultaneous determination. The bioplatforms relied on the preparation of independent magnetic bioconjugates (sandwich immunocomplexes immobilized on HOOC-MBs) for the determination of each target protein and their coupling to perform individual or dual amperometric transduction at disposable electrodes (Scheme 1).

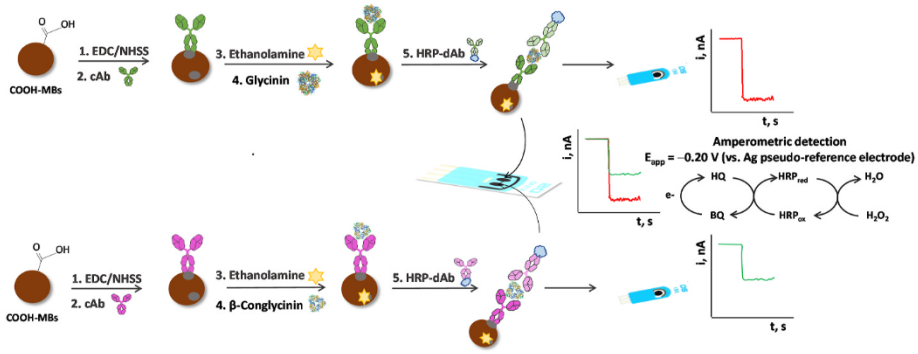
3.1. Evaluation of key experimental variables

The variables involved in the amperometric transduction using the H_2O_2/HQ system at the same electrode substrates (applied potential, pH, and composition of the supporting electrolyte) were optimized in previous works [31–33]. Thus, only key conditions involved in the formation of the sandwich immunocomplexes on the surface of HOOC-MBs were optimized in this work. These conditions included cAb concentration and incubation time with activated HOOC-MBs for their immobilization, number of steps for the formation of the sandwich immunocomplexes, incubation time of cAb-MBs with the protein standard solution for their immunorecognition, and dilution and incubation time with HRP-dAb for the formation of the HRP-labelled sandwich immunocomplexes.

The results obtained are shown in Figures S1 and S2 (in the Supporting Information), where the single amperometric responses provided by the resulting bioplatforms in the presence of 0 (white signal, B) and 50 (signal S) ng mL^{-1} of each allergenic protein standard, as well as the corresponding S/B ratio, are displayed. Larger S/B ratios were selected as the criterion to choose the value of each variable for further work.

Figures S1a and S2a show that, for both proteins, larger S/B ratios were found for 2.5 μg mL^{-1} of cAb solutions in the preparation of cAb-MBs. The responses in the absence of allergen did not vary significantly but the ratios slightly decreased above this concentration. Figures S1b and S2b show that better S/B ratios were obtained when the activated HOOC-MBs were incubated with 2.5 μg mL^{-1} cAb solution during 15 and 30 min for β-conglycinin and glycinin, respectively. The decrease in the S/B ratio value observed for both proteins with longer incubation times may be attributed to the worse recognition of the target protein due to steric hindrance when large concentrations of cAb are immobilized on the MBs [34]. Bars 0 in Figures S1a, S1b, S2a and S2b reveal that it was not possible to detect the target proteins in the absence of immobilized cAb. The results obtained in the evaluation of the number of steps employed for the formation of the sandwich immunocomplexes are shown in Figures S1c and S2c. For both proteins, a larger S/B ratio was found when the capture of the target protein and the enzymatic labelling with HRP-dAb were made in independent steps. This can be explained because a worse recognition of the target protein by cAb-MBs when it is tagged with HRP-dAb due to steric hindrance. The results in Figures S1d and S2d show that an efficient capture of both allergenic proteins by cAb-MBs occurred in 15 min. The slight decrease in the responses measured in the presence of allergen for longer incubation times is most likely due to aggregation phenomena. Moreover, the results displayed in Figures S1e, S1f, S2e and S2f show that incubation of allergenic proteins-cAb-MBs in a 1/1000 dilution of HRP-dAb for 15 min for the formation of sandwich immunocomplexes provided larger S/B ratios. The smaller ratios observed for larger HRP-dAb concentrations (Figs. S1e and S2e) or longer incubation times (Figs. S1f and S2f) can be explained by a higher contribution of nonspecific adsorptions (larger B signals) under such conditions.

Table 1 summarizes the optimized variables. Interestingly, all the selected values, except for the incubation time with the cAb, were the



Scheme 1. Schematic display of the fundamentals involved in the immunosensing platforms developed for the amperometric determination of β-conglycinin and glycinin at SP(*d*)CE.

Table 1
Optimized key variables, evaluated ranges, and selected values for the single amperometric determination of β-conglycinin and glycinin standards with the developed bioplatforms.

Variable	Tested range	Selected value
[cAb], μg mL^{-1}	1–10	2.5
Incubation time with cAb, min	15–60	15[a]/30[b]
Number of steps	1–2	2
Incubation time with allergenic protein, min	15–60	15
HRP-dAb dilution	1/5000–1/500	1/1000
Incubation time with HRP-dAb, min	15–60	15

[a] β-Conglycinin.
[b] Glycinin.

same for both proteins which greatly facilitated their simultaneous determination in the same platform.

3.2. Analytical and operational characteristics

The amperometric bioplatforms developed for the single determination of β-conglycinin and glycinin allowed the construction of the calibration plots displayed in Fig. 1. The corresponding analytical characteristics are summarized in Table 2. The LOD values were estimated according to the 3 × s_b/m criterion, where s_b was the standard

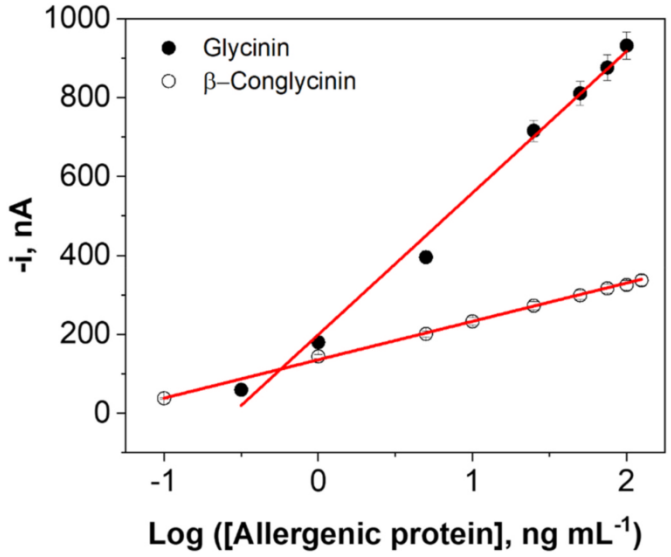


Fig. 1. Calibration graphs constructed with the developed bioplatforms for the amperometric determination of β-conglycinin (empty circles) and glycinin (full circles) standards.

Table 2
Analytical characteristics achieved with the developed bioplatforms for the single amperometric determination of β-conglycinin and glycinin standards.

Parameter	β-Conglycinin	Glycinin
Linear range, ng mL^{-1}	0.1–125	0.1–100
Slope, nA mL ng^{-1}	95 ± 1	359 ± 13
Intercept, nA	137 ± 2	199 ± 19
R^2	0.999	0.996
LOD, ng mL^{-1}[a]	0.03	0.02

[a] 1 ng mL^{-1} <> 0.001 ppm.

deviation of 10 measurements obtained in the absence of allergenic protein, and m the slope of the respective calibration plot.

The LOD values achieved with the developed bioplatforms are lower than those claimed for ELISA methods (estimated using different criteria). Thus, LODs of 0.5–2.0 ng mL^{-1} for β-conglycinin [17,18] and 0.3 ng mL^{-1} for glycinin [15] were reported employing direct [17], competitive [15,16] or sandwich [18] immunoassay formats. It is important to note that the ELISA tests that use the same immunoreagents and assay format that those employed in the preparation of the developed bioplatforms report LOD values of 0.9 and 6.0 ng mL^{-1} for β-conglycinin [19] and glycinin [13], respectively, i.e. 30 and 300 times higher than those achieved with the electrochemical bioplatforms. This high sensitivity, together with the affordable cost, the shorter assay time, the point-of-care (POC) suitability and the compatibility with multiplexed determinations make the developed bioplatforms particularly attractive tools compared with the widely employed ELISA methodologies for the detection of trace levels of both soy proteins. In addition, the commercial ELISA kit available for the detection of soy, based on the determination of β-conglycinin using the same immunoreagents as the developed immunoplatform (Proteon Soy from Zeulab S.L.), exhibits a 100-fold higher LOD (3 ng mL^{-1}).

It should be emphasized that the detection levels achieved with the developed bioplatforms (0.00003 and 0.00002 ppm for β-conglycinin and glycinin, respectively) are significantly lower than those established by standardization bodies for analytical methods that can detect food allergens in the low ppm range (1–10 mg allergenic ingredient per Kg food product) [35,36].

The protocols employed for the preparation of the magnetic bioconjugates and to perform the amperometric transduction allow reproducible measurements to be obtained, with RSD values of 3.8 and 3.7% calculated from the amperometric responses for 25 ng mL^{-1} standards of β-conglycinin and glycinin, respectively, provided by 5 bioplatforms prepared in the same way. Moreover, the storage stability of the cAb-MBs bioconjugates was checked by monitoring the amperometric responses obtained with the bioplatforms prepared using the stored bioconjugates in the absence and in the presence of 25 ng mL^{-1} of β-conglycinin and glycinin. Figure S3 (in the Supporting Information)

shows as the responses were statistically comparable for at least 42 days after the bioconjugates preparation (no longer times were tested), thus allowing the determination of both allergenic proteins to be completed in only 30 min.

3.3. Application to the analysis of soy protein in incurred cookies

The bioplatforms developed for the single determination of the two allergenic proteins were applied to the analysis of uncooked cookie dough and baked cookies both unincurred and incurred with different percentages of soy flour (Table 3).

Table S1 (in the Supporting Information) shows as an important matrix effect was observed in the extracts prepared from raw and cooked matrices for both allergenic proteins (see slope values from the corresponding calibration graphs). Accordingly, the determinations were performed by interpolating the responses obtained for the extracts of each sample into the corresponding calibration graph constructed in the respective matrix (raw cookie dough or cooked cookie extracts).

Importantly, as shown by the results summarized in the Table S1 (in the Supporting Information), the sensitivity decreases by about 65% in baked samples compared to raw samples due to the denaturation and/or aggregation of these allergenic proteins (denaturation temperature of glycinin: 80–90 °C, and of β-conglycinin: 60–75 °C [37]. Similar results have been described for baked bread, where recovery decreased to 54.5 ± 0.5% and glycinin could not be detected in sterilized pate. In contrast the concentration of glycinin in pasteurized sausage was similar to that of unprocessed sample (106.0 ± 4.1%) [37].

The amperometric responses obtained with the developed bioplatforms for the single determination of each allergenic protein in all the analyzed sample extracts are shown in Fig. 2. In addition, Table 4 summarizes the concentration values of the soy proteins determined with the developed bioplatforms and the commercial ELISA methodology in the raw and cooked samples incurred with 0.05 and 0.5 ppm of soy flour (samples 4, 5, 13 and 14). These samples were selected because they are most probable in unintentional contamination events. Good recovery values were found for the raw samples thus confirming the good accuracy of the results provided by the bioplatforms. In the case of baked samples and despite the applied treatment, both proteins could be detected to estimate the recoveries. The good agreement of these results with those obtained with the ELISA methodology using the same immunoreagents ($t_{exp} < t_{tab}$ values in Table 4 and correlations shown in Figure S4, in the Supporting Information) further confirmed the accuracy of the results provided by the developed immunoplatforms.

Results obtained show the high sensitivity of the developed bioplatforms in food matrices allowing the discrimination of cookie extracts (either baked or raw dough) incurred or not with as little as 0.0005 ppm of soy flour. Importantly, the soy detectability provided by the bioplatforms in food extracts is considerably higher (260-2000 times) than that estimated for other bioplatforms for food allergenic proteins. It is the case of peanut traces through the determination of Ara h 1 and Ara h 2 (1 ppm of peanut flour in wheat flour) [27], or egg traces through ovomucoid determination (0.17 and 0.13 ppm of lyophilized egg in wheat flour and baked bread, respectively) [28]. It is also relevant to note that ELISA methodologies using the same immunoreagents detected 50 and 500 ppm of soy protein isolate in incurred pasteurized sausages and baked bread by targeting β-conglycinin [19] or 50 ppm in cookies spiked with cookies containing soy seeds according to the label, by targeting glycinin [13]. These concentration values are about 100, 000 and 1,000,000 times larger than those detected with the developed bioplatforms (cookies incurred with 0.0005 ppm of soybean flour).

Interestingly, it was possible to improve the discrimination between unincurred and incurred with 0.0005 ppm soybean samples if the specific magnetic immunoconjugates ($cAb_{\beta\text{-conglicinin}}$-MBs + $cAb_{glicinin}$-MBs) were mixed and incubated with the sample and with the two HRP-AbD solutions, and further capturing on the SPCE surface for the amperometric detection (Figure S5, in the Supporting Information).

Table 3
Samples prepared for the analysis of β-conglycinin and glycinin using the developed bioplatforms.

Code	Sample	$[Soy\ flour]_{added}$, ppm
1	Baked cookie	–
2		0.0005
3		0.005
4		0.05
5		0.5
6		5
7		50
8		500
9		5000
10	Raw cookie dough	–
11		0.0005
12		0.005
13		0.05
14		0.5
15		5
16		50
17		500
18		5000

3.4. Dual bioplatform for the simultaneous determination of β-conglycinin and glycinin

The implementation of the simultaneous determination of β-conglycinin and glycinin in dual detection platforms was carried out. Figure S6 (in the Supporting Information) allow us to deduce that no apparent cross-reactivity occurred between the two adjacent SPdCE electrodes. Both the analytical and operational characteristics (Table S2 in the Supporting Information) and the ability to discriminate between unincurred and incurred samples with 0.0005 ppm soybean (Figure S7 in the Supporting Information) were comparable to those obtained with the single bioplatforms. Since the employed magnetic bioconjugates were the same, the slight decrease in sensitivity found for the simultaneous determination of the two soy proteins at the dual platform should be only attributed to the smaller surface area of the SPdCEs working electrodes compared to the SPCEs (6.3 vs. 12.6 mm^2).

4. Conclusions

This work reports the first electrochemical bioplatforms described to date for the detection of soy traces based on the determination of its two main allergenic proteins: β-conglycinin and glycinin. The rationale is based on the capture and selective labelling of each target protein using a specific unconjugated or HRP-labelled antibody, respectively, on the MBs surface. The resulting magnetic bioconjugates bearing the sandwich immunocomplexes were captured on the working electrode surface of screen-printed electrodes for single or dual amperometric detection. Evaluation of the analytical and operational characteristics of the developed bioplatforms demonstrates reproducible fabrication protocols, improved sensitivities respect to commercial ELISA kits for the determination of these allergens, and at least a 42 days storage stability of the magnetic immunoconjugates (cAb-MBs). The usefulness of the bioplatforms was evaluated through the analysis of raw cookie dough or baked cookies samples incurred or not with different percentages of soy flour. The obtained results showed the ability for detecting this important allergenic food in both matrices at levels as low as 0.0005 ppm. This level of detection is significantly lower than that achieved with ELISA methodologies using the same immunoreagents (50–500 ppm). It is important to note that, counting since the prepared cAb-MBs, the determination can be performed in a short time as 30 min by direct assaying the food extract and using low-cost and simple instrumentation which is compatible with multiplexed determinations and POC operation. In addition, due to the versatility of the developed methodology and the type of electrodes and transduction used, these bioplatforms

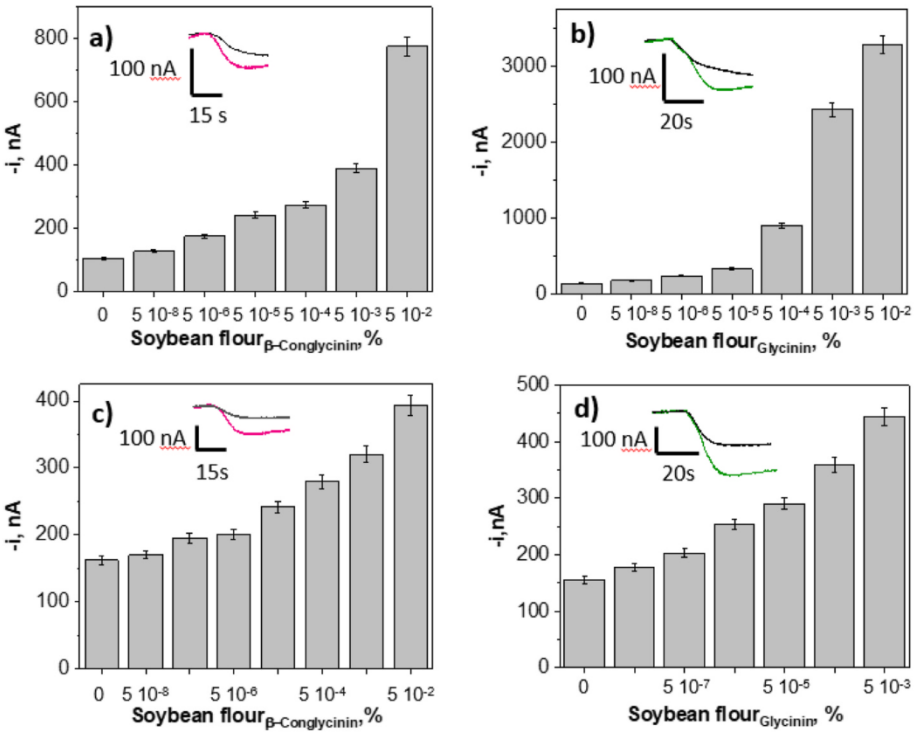


Fig. 2. Amperometric responses measured with the bioplatforms developed for the determination of β-conglycinin (a,c) and glycinin (b,d) in extracts of (unbaked) incurred cookie dough (a,b) and baked incurred cookies (c,d). Inset: actual amperometric traces recorded for raw cookie dough (top) and baked cookie (down) samples unincurred (in black) and incurred (in colour) with 0.0005 ppm soybean flour. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 4
β-Conglycinin and glycinin concentrations (in ng mL^{-1}) provided by the developed bioplatforms and conventional ELISA methodology in the incurred samples.

Sample	Biosensor			ELISA		t_{ext} [b]	Biosensor			ELISA		t_{exp} [b]
	[β-Conglycinin]	RSD, %	Recovery, % [a]	[β-Conglycinin]	RSD, %		[Glycinin]	RSD, %	Recovery, % [a]	[Glycinin]	RSD, %	
13	1.23 ± 0.08	2.9	93 ± 7	1.2 ± 0.3	9.4	0.419	1.33 ± 0.06	1.8	101 ± 4	1.3 ± 0.1	7.7	1.110
14	13.1 ± 0.6	1.9	99 ± 5	13 ± 1	4.6	0.046	13.3 ± 0.7	2.1	101 ± 5	13.1 ± 0.7	5.3	0.483
4	0.51 ± 0.04	3.2	–	0.49 ± 0.05	4.3	1.161	0.46 ± 0.02	2.2	–	0.50 ± 0.03	6.0	2.310
5	3.6 ± 0.4	5.0	–	3.7 ± 0.7	7.5	0.608	4.9 ± 0.2	1.4	–	4.8 ± 0.3	5.8	0.249

(mean value ± $t \times s/\sqrt{n}$, $n = 3$, $\alpha = 0.05$).
[a] Recoveries were estimated considering that the protein content of the flour is 37.6% and that 70% of this is β-conglycinin and glycinin.
[b] $t_{exp} < t_{tab}$ of 4.303 ($n = 3$, $\alpha = 0.05$).

may be extended to a higher multiplexing degree by testing other soy allergenic proteins or other relevant allergens in the matrix to be analyzed. These unique features position the developed bioplatforms as highly attractive analytical tools to ensure food safety for manufacturers, distributors, and consumers.

Credit author statement

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2022.123226.

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