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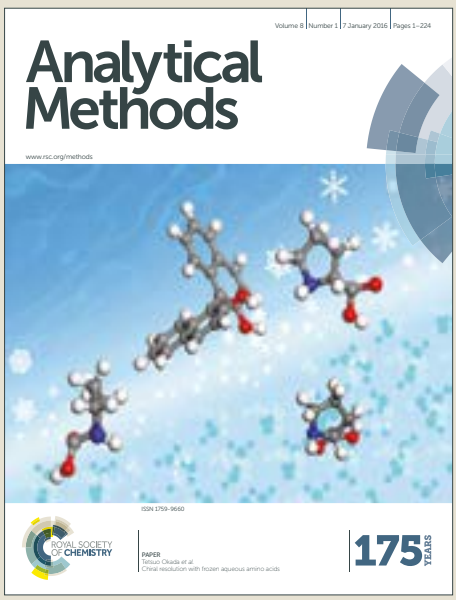

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Electrochemical immunosensor modified with carbon nanofibers coupled to a paper platform for the determination of gliadins in food samples

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Abstract

The gluten-free diet is a unique, effective treatment for different conditions related to gluten consumption. Therefore, it is crucial the availability of new methodologies for the sensitive and specific determination of gluten content in food samples.

Herein, a screen printed electrode modified with carbon nanofibers coupled to a paper immunoaffinity platform was reported for the determination of gliadin in foods samples. The paper microzone covalently functionalized with specific anti-gliadin antibodies was placed on the modified electrode surface for the electrochemical determination of gliadin. The surface of the electrode modified with carbon nanofibers was characterized by scanning electron microscopy (SEM) and cyclic voltammetry (CV), which showed the improved sensitivity of the modified surface. The developed device was evaluated using different flour samples obtaining a favorable response. The calculated limit of detection for the device in analyzed samples was 0.005 mg kg^{-1} and for the Enzyme-linked immunosorbent assay was 1.5 mg kg^{-1}. The coefficient of variation (CV) for the determination of 20 µg kg^{-1} of gliadin was 4.11 %.

The disposable electrochemical sensor developed, represents an easy-to-use and low-cost strategy for the determination of gliadin in food samples.

Keyword: gliadin, immunosensor, electrochemistry, carbon nanofiber, food

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Introduction

The restriction of the consumption of foods containing gluten is the appropriate treatment for two different disorders, one of them is celiac disease, and the other is non-celiac gluten sensitivity. Celiac disease is an autoimmune enteropathy caused by exposure to food containing gluten in genetically susceptible individuals [1]. The prevalence of this pathology is 1% of the world's population [2]. This enteropathy generates chronic inflammation in the small intestine with villous atrophy and therefore malabsorption syndrome. Although some patients could be asymptomatic [3,4], the frequent exposure to gluten cause symptoms as anemia, malnutrition, and alteration in growth. This disease can lead to significant complications as intestinal T-cell lymphoma and adenocarcinoma of the small intestine [5].

On the other hand, studies on non-celiac gluten sensitivity began in the early 1980s. Currently, the number of patients diagnosed with this condition is increasing. Its diagnosis is based on the exclusion of celiac disease and wheat allergy, due to the superposition of symptomatology among these. Compared with the above mentioned conditions, non-celiac gluten sensitivity is characterized by a negative result of the anti-transglutaminase antibody, and a standard IgE value, respectively. The absence of an accurate diagnostic form makes difficult the determination of the prevalence. Although, it is estimated that it is higher than for celiac disease [6].

Gluten proteins involved in the pathogenesis of this disease are contained in wheat, barley and rye grains. It is a complex protein, composed of two primary proteins: gliadins and glutenins contained in the endosperm of the seeds [7]. They are alcohol-soluble monomeric proteins, characterized by repetitive domains of proline and glutamine (prolamins). These amino acids cannot be degraded by pancreatic, gastric and intestinal enzymes, remaining in

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the intestinal lumen through the edge of the brush of the small intestine, which causes the inflammatory response [8–10].

As of today, the only way to manage celiac disease is the strict dietary abstinence from foods containing gluten[11]. Following this diet is difficult for celiac patients, due to variations in food labeling, misinformation, and cross-contamination [12].

According to the *Codex Alimentarius*, the products considered as free from gluten are those that do not exceed 20 mg kg^{-1} (20 ppm). Besides, it defines the concentrations that can contain foods labeled "reduced in gluten," between 20 mg kg^{-1} y 100 mg kg^{-1} [13]. Consequently, it is relevant to develop a sensitive and specific method to quantify gluten through detection of gliadin residues in food intended to celiac patients. The conversion factor prolamine/gluten widely used is 2, but the gluten composition can be affected by several parameters: botanical origin (*Triticum aestivum, Triticum aethiopicum, Triticum durum*) agricultural conditions and others [14].

Common methods currently used for the detection of gluten content in foods are based on a sandwich or competitive enzyme-linked immune assay for gliadin [15,16] and wheat DNA recognition by polymerase chain reaction. These techniques present high sensitivity and specificity but require trained personnel, long incubation times and washing periods. For these reasons, it is crucial the availability of a fast, sensitive method to facilitate gluten detection, proper labeling and safe feeding for patients suffering from these conditions.

The paper matrix has been adopted as an attractive reaction and detection platform due to the following advantages: low cost, flexibility, biodegradability, porous permeability, and accessibility. The excellent chemical compatibility makes it a material extensively used in analytical and clinical chemistry [17,18]. The paper platforms are manufactured by modeling sheets of paper in a hydrophilic zone surrounded by hydrophobic barriers. Various techniques

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are used for this purpose, for example, wax printing, photolithography, polydimethylsiloxane printing (PDMS) and plasma treatment [19,20]. Wax printing is a quick and straightforward technique, carried out by printing wax patterns on the paper surface for the formation of hydrophobic barriers. Compared to other modeling techniques, this is inexpensive and suitable for producing a significant quantity of wax printed papers [21].

Recently, many efforts have been directed to combine different detection systems with paper platforms. Electrochemistry is widely used for this purpose due to its advantages like low cost, portability, high ability to detect low level concentrations and the possibility of miniaturization [22]. Electrochemistry offers the option to use different detection platforms as screen printed carbon electrodes (SPCE) modified with carbon-based nanomaterials [23,24]. CNFs present cylindrical shape characterized by different stacking arrangements of graphene sheets. Its mechanical resistance, chemical stability, and electrical proprieties are similar to the rest of carbon nanomaterials (single wall carbon nanotubes (SWCNT), multiwall carbon nanotubes (MWCNT), graphene (G), graphene oxide (GO)). However, CNFs have irregularities on their outer surfaces which significantly increase the efficiency of electron transfer on the electrode surface [25–29].

In this article, we developed an electrochemical disposable immunosensor to determine the gliadin content in foods intended for celiac patients. It combines the use of an SPCE modified with carbon CNFs with a paper immunorecognition support. The presence of the CNFs on SPCE provides an increase of the active area improving the sensitivity of the sensor. The paper platform represents a practical and efficient surface for the highly specific biorecognition of gliadin proteins present in food samples. In this sense, the sensor developed represents a promising resource to be applied for gluten detection in food production and for the control of marketed gluten-free food.

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Materials and methods

Reagents and solutions

All reagents used were of analytical reagent grade. Anti-gliadin (wheat) monoclonal antibody produced in mouse (Santa Cruz Biotechnology). Anti-gliadin peroxidase conjugate antibody, gliadin, bovine serum albumin (BSA), carbon tetrachloride, carbon nanofiber (graphitized (iron free) composed of conical platelets, D x L 100 nm x 20-200 µm), catechol (Q) and Whatman paper # 1 qualitative filter paper were purchased from Sigma-Aldrich. All buffer solutions were prepared with Milli-Q water.

The enzyme immunoassay for gliadin quantitative determination: R7001 RIDASCREEN® Gliadin, gliadin standard and the Set of 3 Gliadin Assay Controls RIDASCREEN® were purchased from R-Biopharm AG-Darmstadt Germany and was used according to the manufacturer's instructions.

Instrumentation

Electrochemical measurements were performed using BAS 100 B/W (Bioanalytical Analyzer Electrochemical System, West Lafayette, IN, USA). Cyclic voltammograms and amperograms were obtained using a screen printed carbon electrode (SPCE) ItalSens IS-C by PalmSens.

The ultrasonic bath (testlab, Argentina) model TB02 was used to achieve CNFs dispersion.

All pH measurements were made with an Orion Research Inc. (Orion Research Inc., Cambridge, MA, USA) Model EA 940 equipped with a glass combination electrode (Orion Research Inc.).

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The morphology of nanofiber films on the surface of the working electrode was studied by scanning electron microscopy LEO 1450VP (SEM). The paper microzones were printed with a Xerox ColorQube 8870 printer.

Preparation of carbon nanofiber dispersion and electrochemical reduction on SPCE surface

CNFs were chemically oxidized with 6 M HNO_3. This chemical agent generates groups rich in oxygen at the CNFs surface, increasing the capacity of dispersion and solubility of CNFs [25]. After that, were sonicated for 6 h, washed with bidistilled water until pH 7 and dried in an oven at 60°C. 30 µg mL^{-1} CNFs dispersion in carbon tetrachloride was prepared and sonicated (50-60 Hz) for about 2 h. After that, 5 µL of this dispersion were placed on SPCE surface (CNFs/SPCE) and dried at room temperature. The electrochemical reduction of CNFs was carried out by applying a constant potential of -1.2 V for 800 s, in 0.5 M $NaNO_3$ at pH 4.

Wax patterning and antibody immobilization

The wax patterns were printed on Whatman paper # 1 qualitative filter paper using a Xerox ColorQube 8870 printer. Previously, a 6 mm diameter microzone was designed with Corel Draw 9.

The paper surface offers hydroxyl groups for bioconjugation process, but in pure cellulose, they are unreactive [30]. For this reason, it is necessary an activation procedure by plasma oxidation which allows the covalent bonding of antibodies on the paper cellulose surface. The plasma treatment induces the rupture of the union between C3 and C4 of the pyranose ring, forming a carbon radical and oxygen radical. Both radicals combine for the formation of aldehyde groups. These aldehyde groups which form Schiff bases with the amino groups of the antibodies. [31]. Firstly, the paper was treated for 2 min by oxygen plasma

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whose excitation frequency and power were 100 W and 13.56 MHz respectively. Secondly, 5 µL of anti-gliadin antibodies solution of 10 µg mL^{-1} were added to the paper surface and incubated in a humid chamber for 30 min. Finally, it was washed with PBS buffer pH 7.2.

The modified paper surface obtained represents a practical and versatile platform to perform the immunorecognition process before the electrochemical determination.

Gliadin content determination

The processed samples were common wheat flour, gluten-free flour, manioc flour, rice flour and control flour samples of known concentrations of gliadin. The extraction process was carried out with 60% ethanol because the used samples (flours) are raw materials. In the case of foods samples exposed to enzymatic degradation, heat treatments, mechanical and chemical processes, the use of a specific extraction solution is required (Mendez Cocktail extraction solution – R-Biopharm, Germany).

In the first case, the gluten extraction was as follows: 0.3 g of each sample was weighed and mixed with 3 mL of 60% ethanol. After that, the mixture was incubated at room temperature for 30 min under continuous stirring. Finally, it was centrifuged at 8000 rpm for 10 min. The gliadin concentrations were determined in the obtained supernatants diluted 1:50. Following the procedure described above, the resulting dilution factor was 500.

The gliadin content determination using the functionalized paper platform and the modified electrode was achieved with the following stages (Figure 1).

The functionalized paper support was subjected to a blocking procedure with BSA 1%, incubated in a humid chamber for 5 min and washed with buffer PBS. In the next step, 5 µL of the samples were added in the microzone and incubated in the same conditions for 10 min. The functionalized paper platform was washed with PBS buffer pH 7.2. After that,

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5 µL of anti-gliadin antibody conjugate with HRP were added, incubated in a humid chamber for 5 min and washed with PBS buffer pH 7.2. (Table 1, Electronic supplementary Data A)

Finally, the paper platform was placed on the CNFs/SPCE surface for the gliadin electrochemical determination. To perform amperometric measurements the paper platform was exposed to the addition of 5 µL of a 1 mM citrate-phosphate buffer solution pH 5 applying a detection potential of - 0.15 V. Once the background current stabilized (20 s approximately), 5 µL of a revealing solution containing 1 mM Q and 1 mM H_2O_2 were incorporated. The beginning of the enzymatic product reduction could be observed at 25 s. Finally, the reduction current of o- benzoquinone (BQ) was measured at 60 s.

A dilution factor of 500 must further multiply the gliadin concentration values (µg kg^{-1} (ppb)) obtained from the calibration curve. Considering that gliadin usually represents 50 % of the proteins present in gluten, this result should be multiplied by 2 to get the gluten concentration.

Results and discussion

Modified electrode characterization

In this work, we use CNFs as a nanomaterial for the modification of the SPCE surface. The CNFs/SPCE was also electrochemically characterized by CV of 1mM ferri/ferrocyanide redox couple ($[Fe(CN)_6]^{4-/3-}$) in PBS, pH 7.2. The potential scan was ranged from -0.15 to 0.8 V at a scan rate of 0.075 V s^{-1}. Figure 2 (a) shows the voltammograms corresponding to a blank signal for CNFs/SPCE (green line), unmodified (black line) and modified SPCE (red line). In this figure, it can be observed an improved peak current for CNFs/SPCE compared with unmodified SPCE, indicating that the incorporation of CNFs in the SPCE surface, improved the conductivity and increased the active surface area of the electrode.

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Another electrochemical study of CNFs/SPCE was the effect of the scan rate on CVs (Figure 2b). As can be appreciated, the oxidation and reduction peak currents show a linear correlation with the square of scan rate (Figure 2b inset) in the evaluated range (0.02-0.3 V s^{-1}). The obtained results expose the existence of a fast electrochemical and diffusion-controlled process. Figure 2(c) displays the evaluation of the ratio of the anodic and cathodic peak currents (Ipa/Ipc) as a function of the CNFs concentration. For this, we used 1mM $[Fe(CN)_6]^{4-/3-}$ in PBS, pH 7.2. The electrochemical reversibility decay when the CNFs concentration is higher than 30 µg mL^{-1}. Thus, 30 µg mL^{-1} of a CNFs solution were used for electrode modification.

The proposed method employs an enzymatic mediator with electrochemical activity, which is incorporated into the developer solution. Therefore, it is necessary to evaluate the behavior of the same in the CNFs/SPCE surface by cyclic voltammetry. This study was performed with a solution of 1 mM Q in 1 mM citrate-phosphate buffer pH 5 in the same experimental condition described for the obtaining of figure 2(a). The Figure 2 (d) shows the voltammograms corresponding to a blank signal for CNFs/SPCE (green line), unmodified (black line) and modified SPCE (red line). This cyclic voltammetry show one anodic and a corresponding cathodic peak which corresponds to the transformation of Q to BQ and vice-versa within a quasi-reversible two-electron process[32]. Likewise, to the figure 2 a, an improved peak current for CNFs/SPCE compared with unmodified SPCE was obtained. Additionally, the effect of the paper platform placed on the surface of the CNF / SPCE was evaluated. This comparative study revealed an insignificant variation in the obtained signals. (Electronic supplementary Data B).

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To characterize the surface morphology of SPCE before and after of CNFs incorporation, was carried out by scanning electron microscopy (SEM). Figure 3 (a) shows the image of unmodified SPCE. Figure 3 (b) shows the image of CNFs/SPCE, which reveals the adequate distribution of CNFs on the SPCE surface by generating a homogeneous CNFs film with a compressed three-dimensional structure. The CNFs have an average diameter of 100 nm. These nanomaterials could be an excellent platform for electrochemical transduction, as they significantly increase the active surface of the electrode. Besides, as mentioned above, CNFs improve conductivity by providing a possible pathway for electron transfer.

Optimization

Electrode modification conditions

Several variables were optimized to maximize the sensitivity of the proposed method. One of these was the optimum dispersion media of CNFs. This parameter was evaluated using 60 µg of CNFs and 2 mL of methanol, dimethylformamide and carbon tetrachloride. A better dispersion media found was carbon tetrachloride. This dispersion was dropped on the SCPE and dried for 4 min at room temperature.

The CNFs electroreduction on the electrode surface is strongly affected by several parameters, such as the reduction time and reduction potential. Both factors have been optimized to obtain the best analytical performance. For the optimization of the reduction time the potential was set at -1.2 V and the reduction time was evaluated in a range of 100-900 s using 1mM $[Fe(CN)_6]^{4-/3-}$ in PBS, pH 7.2. As figure 3 (c) shows, the current grows with the increase of the reduction time until a value of 800 s, and then it remained constant. Therefore, a reduction time of 800 s was selected as optimum time. Regarding the reduction

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potential, the time used was 800 s, and the working electrode potential was varied from -0.7 to -1.5 V in the experimental conditions described for electroreduction time optimization. As shows the Figure 3(d), the current increased slowly by increasing potential up to a value of -0.9 V, then grew rapidly from -0.9 to -1.2 V and remained constant from -1.2 to -1.4 V. Therefore, a reduction potential of -1.2 V was selected as optimum potential.

Immunoassay optimization

The antibody concentration to be immobilized on the paper surface represents a relevant parameter to be optimized. Higher concentrations of antibodies cause adsorption in multiple layers, which would generate interference [33]. In our case, the optimum concentration of anti-gliadin antibody to be immobilized was evaluated by HRP saturation method. For this purpose, increasing antibody concentrations (1 - 14 μg mL^{-1}) were added in the paper microzone. Later, a constant and saturating amount of Horseradish peroxidase (HRP) (5 mg in 0.1 mL of PBS) was incorporated. HRP adsorbed in the available sites that were not previously occupied by antibodies. After that, paper microzones were placed on the electrode surface. The substrate solution containing 1 mM H_2O_2 and 1 mM Q in 1 mM citrate-phosphate buffer pH 5 was added. HRP in the presence of H_2O_2 catalyzes the oxidation of Q to BQ. The electrochemical reduction back to Q was detected on CNFs/SPCE at -0.15 V. As can be seen in fig.2(a) in electronic supplementary materials (C) the generated current reduces when antibody concentration increase, due to the lower availability of sites for HRP adsorption. Therefore, the generated current was inversely proportional to the amount of immobilized antibody. The optimum value of immobilized anti-gliadin antibodies was 10 μg mL^{-1}.

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Finally, the incubation time was evaluated due to it is an essential factor when the reduction of the assay time is required. This parameter was evaluated using three different standard concentrations. For low concentration standards, the signal growth with the increase of gliadin concentration, while, for high concentration standard, the intensity of the current increased until 10 min of incubation time due to saturation of the specific antibody binding sites. Thus, the optimal reaction time was 10 min (Electronic supplementary Data C (b)).

Analytical performance

Gliadin quantitative detection in food samples was performed with the designed electrochemical immunosensor. A linear relation, i (nA) = 2.7718 + 11.45 × C gliadin (figure 4a), was observed between the current signal and the gliadin concentration in the range of 0 and 80 μg kg^{-1}. The correlation coefficient (r) for this plot was 0.998. The coefficient of variation (CV) for the determination of 20 μg kg^{-1} of gliadin was 4.11 % (six replicates). Furthermore, the limit of detection (LOD) for the electrochemical device in analyzed samples was 0.005 mg kg^{-1}, considering LOD as the concentration that gives a signal 3.29 times the standard deviation of the blank above its signal.

The accuracy of the electrochemical immunosensor was tested with a dilution test, which was performed with 20 μg kg^{-1}gliadin standard concentration serially diluted in 0.01 M PBS pH 7.2. (Electronic supplementary Data D).

ELISA procedure was also carried out. Absorbance changes were plotted against the corresponding gliadin concentration, and a calibration curve was constructed. The linear regression equation was $A = 0.165 + 0.026 \times C_{gliadina}$ with the linear relation coefficient r^2 = 0.991, the CV for the determination of 20 μg kg^{-1} of gliadin was 5.3 % (six replicates). For ELISA procedure, the LOD was 1.5 mg kg^{-1}.

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The obtained gliadin concentration values for samples using the immunosensor were compared with those obtained by the official Type I method for the determination of gliadin, endorsed by the *Codex Alimentarius Commission* (ELISA R5 Méndez) as the official analysis method for quality assessment of the gluten-free food. The slope obtained was reasonably close to 1, indicating good correspondence between the two methods (Electronic supplementary Data E). Compared with spectrophotometric ELISA method, the immunosensor showed improved sensitivity, which allows the determination of very low levels of gliadin and consequently the gluten content (Electronic supplementary Data F).

Selectivity, reproducibility, and stability

The selectivity, reproducibility, and stability are also critical analytical factors for gliadin determination. The selectivity of the system was investigated against the following reagents: albumin, casein, glutenin from wheat, gliadin, β lactoglobulin, and folic acid. The experiment was tested by the solutions with gliadin (40 μg kg^{-1}) and different interference substances (40 μg kg^{-1}). As figure 4(b) shows, only casein exhibited an increase of 39.9% in the analytical signal. This result represents a relevant data due to skin milk which contains casein is one of the agents widely used for blocking procedure. For this reason, BSA was selected as a blocking agent. This result is consistent with previously reported data [34]. The other agents displayed negligible signals. The results indicated the high selectivity of the sensor.

The precision of the disposable immunosensor was evaluated with intra and inter-assay approaches. These were performed by replicating the experiment six times using the immunofuntionalized paper microzone incorporated on CNFs-SPCE for each analyte concentration. The intra and inter-assay CV% obtained from six replicates using three

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gliadin standard concentrations (5 μg kg^{-1}, 20 μg kg^{-1} and 80 μg kg^{-1}) were in a range of 3.87% and 6.56%, respectively (Electronic supplementary Data G). These results expose the satisfactory repeatability and reproducibility of the sensor.

The stability of the device was also evaluated. For this purpose, six lyophilized paper microzones in PBS buffer pH 7 and six CNFs-SPCE were stored for three months at 4°C. The devices showed the same currents as those used immediately after its design. The signals were obtained in the same conditions described before for the flour samples.

Real samples analysis

To demonstrate the applicability of the designed electrochemical device the gliadin concentration was measured in 11 flour samples (manioc flour, rice flour, gluten-free flour, and common wheat flour) and 3 control samples (Figure 4c). It is relevant to note that the analyzed samples did not contain casein. The samples were spiked with a gliadin concentration of 10 mg kg^{-1} after extraction procedure to obtain relative recovery. The gliadin concentration values founded should be multiplied by a factor of 2 [35]. The relative recoveries of the spiked samples ranged from 98.50% to 102.10% with a CV fewer than 4.93%, which showed an adequate accuracy for gliadin determination in food samples (Table 1).

Discussion

During the last 10 years, numerous studies describing different methodologies for the determination of gliadins in food matrices have been published. Among them, it is essential to mention commercial immunoassay kits which include multiple steps, long incubation times and a large amount of reactive and samples[33]. Moreover, complex methods as LC-MS/MS have been reported. This methodology represents a powerful tool, making possible to detect individually wheat, oats, barley, and rye in a single chromatographic run. Although,

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it requires trained personnel, expensive equipment and includes cumbersome sample pretreatment and time-consuming extraction and digestion steps[36].

Nowadays, it relevant the availability of fast, sensitive and specific methodologies for routine control of gluten in food samples, providing the correct labeling for safe feeding. This requirement has generated an exponential grow of sensor technologies development. The bioanalytical sensor for gluten detection could be classified according to biorecognition agent employed. For gliadin detection immunosensors and aptasensors have been developed. Recently aptasensors coupled to electrochemical detection based on competitive format have been described [37–39]. These sensors allowed the determination of hydrolyzed and whole gluten with adequate LODs. However, the same articles have reported that the immobilization of aptamer generates a deleterious effect over its affinity, being more advantageous the attachment of the complementary peptide in the competitive assay.

Immunosensors with competitive and sandwich format couplet to different detection system have also been reported. The selection of the immunological model depends on the sample type. When samples contain whole gluten, the sandwich format and ELISA R5 Méndez confirmation are adequate, while for samples with hydrolyzed gluten the competitive form represents the best choice. In this sense, several articles related to immunosensors designed have been reported. Electrochemical immunosensor using magnetic particles modified with the anti-gliadin antibody [40] was described. The use of these particles requires long incubation periods for the gliadin capture and detection. Microfluidic immunosensor with impedance spectroscopy was also described [41]. This device represents a portable tool which requires the use of different techniques and materials for the microfluidic platform

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construction and pump system for the flow generation. Immunosensors can also be coupled with a fluorescence detection system [42], which offers is characteristic sensitivity.

The electrochemical device developed in this work is the only one based on the paper support for the immunorecognition process. This platform of high availability, accessibility, and low cost was covalently functionalized with anti-gliadin antibodies. The analyzed samples contain whole gluten, being the sandwich type format the suitable for this application. The electrochemical detection was performed by SPCE modified with CNFs which allowed us to reach the less LOD compared to the described articles (Table 2). Finally, the proposed system requires an analysis time of 28 min, less than the compared methods.

Conclusion

In this work, a novel electrochemical disposable device was developed based on an innovative paper immunoaffinity reaction platform combined with the use of SPCE modified with CNFs as the detection system. The wax printing technique and plasma oxidation treatment allowed to obtain a delimited reaction area in the paper surface, where antibodies were covalently immobilized. This stable, specific and practical recognition platform was implemented to perform the gliadin determination by using a non competitive assay format in flour samples. The incorporation of CNFs allowed obtaining the increase of electron transfer efficiency and the active area enabling the determination of low levels of gliadin in food samples. Besides, the electrochemical detection can be done within 1 min and the complete assay in 28 min, much less than the reported methodologies. These features revealed the valuable contribution of this technology for gluten-free food control applications requiring a disposable device to perform fast, sensitive and selective determinations.

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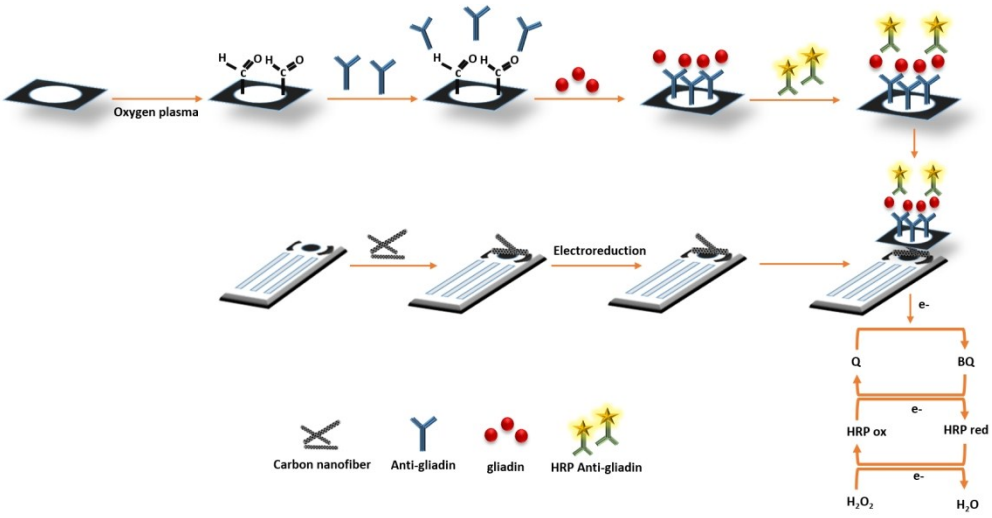

Schematic representation of the electrode modification and gliadin determination procedures.

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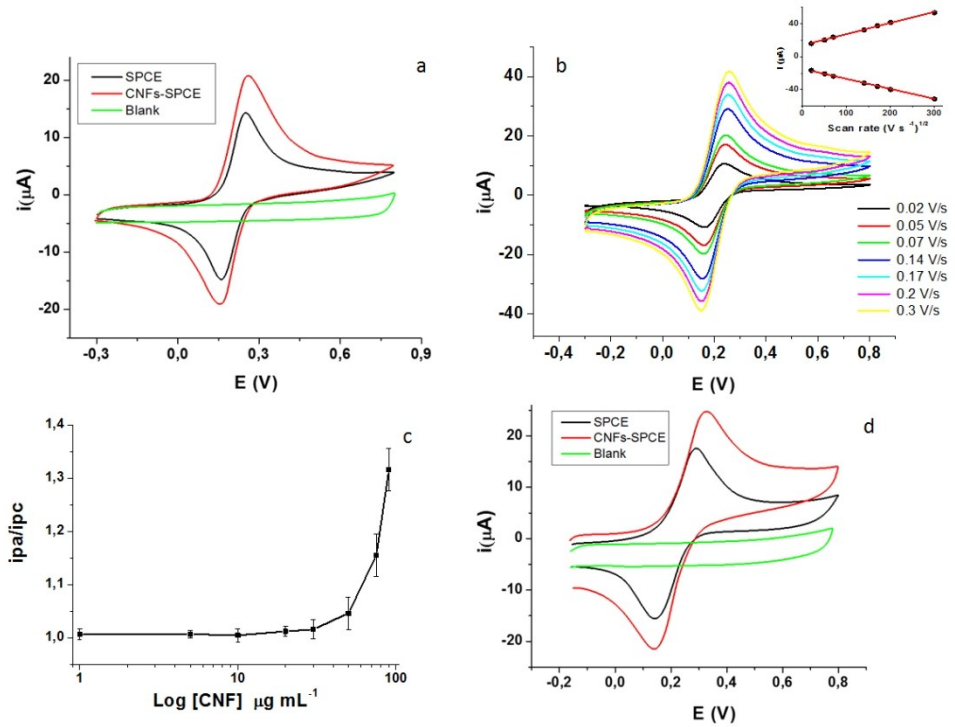

(a) Cyclic voltammograms (CVs) obtained with 1mM [Fe(CN)6]4-/3- in PBS, pH 7.2 at 0.075 V s−1 for unmodified SPCE (black line), CNFs / SPCE (red line) and blank signal for CNFs/SPCE (green line), (b) CVs of CNFs/SPCE with 1 mM [Fe(CN)6]4-/3- in PBS pH 7.2 at different scan rates from 0.02 to 0.3 V s-1. Insert: plot of peak current vs. scan rate. (c) Optimization of the CNFs concentration employing the current anodic and cathodic peak ratio using 1mM [Fe(CN)6]4-/3- in PBS, pH 7.2. (d) CVs obtained in a solution of 1 mM Q in 1 mM citrate-phosphate buffer pH 5 at 0.075 V s−1 for unmodified SPCE (black line), CNFs / SPCE (red line) and blank signal blank signal for CNFs/SPCE (green line).

318x232mm (96 x 96 DPI)

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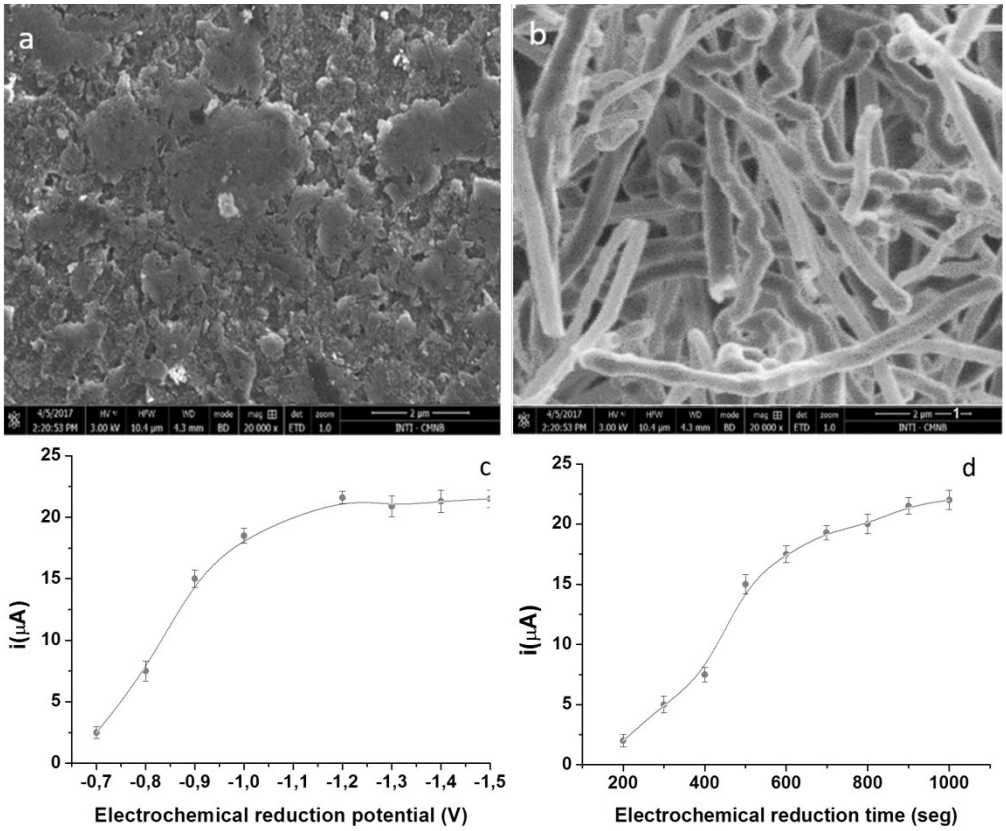

(a) Characterization SEM image of unmodified SPCE, (b) SEM image of CNFs / SPCE. (c) Effect of electroreduction potential with the presence of 1 mM [Fe(CN)6]4-/3- in PBS pH 7.2 and an electroreduction time of 800 s from -0.7 V to -1.5 V (d) Effect of electrochemical reduction time with the presence of 1 mM [Fe(CN)6]4-/3- in PBS pH 7.2 and an electrochemical reduction potential -1.2 V from 200 s to 1000 s.

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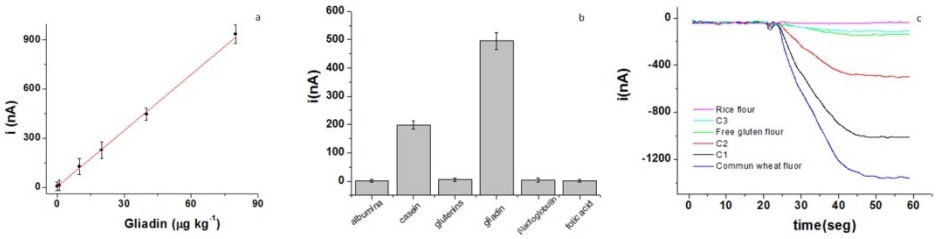

(a) Calibration curve of the immunosensor using different gliadin standard concentrations Error bar = SD (n = 5). (b)The selectivity of the system was evaluated against 40 µg kg-1 albumin, casein, glutenin from wheat, gliadin, β lactoglobulin and folic acid, following the procedure described for the electrochemical determination of gliadin content at a potential value of -0.15 V and (c) Amperometric response of the immunosensor to gliadin: Rice flour (pink line), Gluten free flour (green line), Control sample 5.5 ppm (light blue line), Control sample 20 ppm (red line) Control Sample 50 ppm (black line), Common wheat flour (Blue line). Error bar = SD (n = 5).

447x120mm (96 x 96 DPI)

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Samples no.	Gliadin content mg kg^{-1}	Found with spiked gliadin[a] mg kg^{-1}	Recovery (%)	CV (%) (n=6)
Manioc flour (2)	Nd	10.21	102.10	4.71
Rice flour (2)	Nd	9.85	98.50	3.80
Gluten free flour (3)	3.01	12.87	98.92	4.93
Common wheat flour (3)	59.06	69.56	100.72	3.98

[a] The data was obtained from six independent experiments (n = 6). The samples were spiked with 10 mg kg^{-1} of gliadin.

Nd: Not detected

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System	Detection	Sample	LOD **	Ref.
ELISA - Competitive	Spectrometry	Different food samples	2.9 ppm	(33)
(LC-ESI-MS/MS)*	Mass spectrometry	Flours and seeds, pasta, biscuits, cookies	5 ppm	(36)
Aptasensor	Amperometric	Fixamyl, rolled oats, fit Snack	0.113 ppm	(37)
Aptasensor	Impedance	Beer, toasted bread, rice and corn flour	0.05 ppm	(38)
Competitive magneto inmunosensor	Optical detection	Beer and skimmed milk	0.0057 ppm	(40)
Immunosensor	Impedance	Beer	0.2 ppm	(41)
Immunosensor	Fluorescence	Beer, flour, and noodles.	0.025 ppm	(42)
Immunosensor	Amperometry	Flour	0.005 ppm	-

*Liquid chromatography-electrospray ionization-tandem mass spectrometry.

** LOD calculated for food samples taking all dilutions into consideration.