Journal of Biosensors & Bioelectronics
ISSN: 2155-6210

Ramírez, et al. J Biosens Bioelectron 2011, S3
DOI: 10.4172/2155-6210.S3-001

Research Article Open Access

Development of an Amperometric Biosensor Based on Peroxidases from *Brassica napus* for the Determination of Ochratoxin a Content in Peanut Samples

Eduardo A. Ramírez[1], Adrián M. Granero[2], María A. Zón[2]* and Héctor Fernández[2]*

[1]*Laboratorio de Nanoscopías y Fisicoquímica de Superficies, Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Diagonal 113 y calle 64 - La Plata, Argentina*

[2]*Departamento de Química, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Agencia Postal No. 3 (5800) - Río Cuarto, Argentina.*

Abstract

An amperometric biosensor based on *Brassica napus* hairy roots peroxidases to determine ochratoxin A is proposed by the first time. The enzymatic reaction rate was studied under different experimental conditions and the kinetics parameters were determined. The method employs a dialysis membrane covered, peroxidases entrapped and ferrocene-embedded carbon paste electrode (PBHR-Fc-CP) and is based on the fact that the decreased amount of H_2O_2 produced by the action of PBHR is proportional to the oxidised amount of ochratoxin A in the solution. The PBHR-Fc-CP biosensors showed a very good stability during at least five days. The reproducibility and the repeatability were 7.7% and 5.4%, respectively, showing a good biosensor performance. The calibration curve was linear in the ochratoxin A concentration range from 1 x 10^{-8} to 1,4 x 10^{-4} mol L^{-1}. The lowest concentration value measured experimentally for a signal to noise ratio of 3:1 was 5.7 x 10-9 mol L^{-1}.

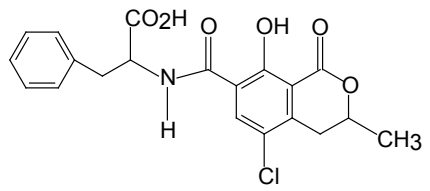
Keywords: Ochratoxin A; *Brassica napus* hairy roots peroxidases; Amperometric biosensor

Abbreviations: OTA: Ochratoxin A; PBHR: *Brassica napus* Hairy Roots Peroxidases; CP: Carbon Paste; HRP: Horseradish Peroxidase; Fc: Ferrocene; PBS: Phosphate Buffer Solution

Introduction

The ochratoxins are a group of highly toxic fungal secondary metabolites that are produced by several species of the *Aspergillus* and *Penicillium* genera [1,2]. Ochratoxin A (OTA) is the most toxic and prevalent among these toxins, and is receiving increasing attention because of the hazard imposed on both human and animal health [3].

OTA or 7-carboxyl-5-chloro-8-hydroxil-3,4-dihydro-3R-methyl-isocumarin-7-L-β-phenylalanine, is a colorless crystalline compound that belongs to a group of closely related derivatives of isocumarin linked to L-phenylalanine and classified as pentaketides [4] (Strcture 1).



Strcture 1

The toxin is potently nephrotoxic, teratogenic and an immunosuppressive agent [5-7]. It has been considered by the International Agency for Research on Cancer to be a potential carcinogen (group 2B) for human [8]. For this reason, many countries have restricted OTA leves in foods, with upper limits of 1 to 10 ppb depending on the type and quality of the foodstuff [9].

Peanuts are one of the most important agricultural products in Argentina. The central-south region of Córdoba Province produces 94% of the country's peanuts. The peanut industry exports 90% of its product, being Argentina the second largest exporter in the world [10]. Each year, a significant portion of the peanuts produced can not be marketed because of fungal disease at the postharvest stage and mycotoxin contamination [11]. With imports and exports of agricultural commodities increasing in recent years, monitoring of OTA is essential for food safety in the world-wide market.

Many research groups have been studying methods for monitoring naturally occurring OTA in a number of agricultural commodities [9,12]. Many different analytical methods are now available for the determination of OTA in a variety of matrices [9]. Chromatographic methods such as thin-layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography (HPLC) with immunoaffinity columns and fluorescence detection [13-17] provide sensitive and specific techniques, but generally require multiple steps prior to detection. Today, the electroanalytical methods are very valuable alternatives to other methods because generally no separation procedure is required prior to substrate determination. Furthermore, the required instrumentation is typically less expensive, smaller amounts of solvent are necessary and analysis time is shorter for electroanalytical methods over chromatographic measurements.

Biosensors have been proposed as an efficient analytical tool for the determination of polyphenolic compounds, exhibiting advantages

***Corresponding authors:** Héctor Fernández, Department of Chemistry, University of Río Cuarto, Tel: 54 358 467 6440; Fax: 54 358 467 6233; E-mail: hfernandez@exa.unrc.edu.ar

María A. Zón, Department of Chemistry, University of Río Cuarto, Tel: 54 358 467 6440; Fax: 54 358 467 6233; E-mail: azon@exa.unrc.edu.ar

Received July 12, 2011; **Accepted** August 12, 2011; **Published** August 15, 2011

Citation: Ramírez EA, Granero AM, Zón MA, Fernández H (2011) Development of an Amperometric Biosensor Based on Peroxidases from *Brassica napus* for the Determination of Ochratoxin a Content in Peanut Samples. J Biosens Bioelectron S3:001. doi: 10.4172/2155-6210.S3-001

such as the minimal preparation of the sample, selectivity, sensitivity, reproducibility, rapid time of response and simple use for continuous on-site analysis [18-25].

Here, we report on the development of an amperometric biosensor based on peroxidases obtained from *Brassica napus* hairy roots (PBHR) to determine OTA content in solutions composed by comercial reagent and peanut samples.

The method employs carbon paste (CP) electrodes filled up with PBHR and ferrocene (Fc) at a given composition (PBHR–Fc–CP). The biosensor was covered externally with a dialysis membrane, which was fixed at the electrode body side part with a Teflon laboratory film and an O-ring. It is well known that phenolic and/or polyphenolic compounds can work as electron-donors for peroxidases in the catalytic reduction of H_2O_2 [26,27]. This approach allows detecting the decrease in H_2O_2 concentration in a solution after the oxidation of phenolic and/or polyphenolic compounds produced by the PBHR or horse radish peroxidase (HRP) in the presence of H_2O_2, given that HRP acts in cascade in the solution and PBHR at the electrode surface. The separation of the electrode surface from the solution by a semipermeable membrane allows minimizing the electrical noises as well as the fouling of the electrode surface.

Materials and Methods

Reagents and materials

Brassica napus hairy roots, obtained "*in vitro*" in our laboratory according to a procedure previously described by Agostini et al. [28] were used as the enzyme source.

Hydrogen peroxide (30%, v/v), pH 8.00 phosphate buffer solutions (PBS), sodium acetate, acetic acid, NaCl, HCl, NaOH and Na_2CO_3 were Merck p.a.; ochratoxin A (OTA), ferrocene (Fc) and o-dianisidine were purchased from Sigma and used as received. All solutions were prepared using water purified by a Labconco WaterPro Mobile System, Model 90901-01 (HPLC grade water). The concentration of H_2O_2 was determined spectrophotometrically at λ_{max} = 240nm (ε = 43.6 mol^{-1} cm^{-1}) [29]. Stock solutions of OTA were prepared in PBS and kept at a temperature of 40C. The electrode surface was covered with a dialysis membrane (Spectrum Co., Houston, TX, cut off molecular weight 100), which was fixed at the electrode body side part with a Teflon laboratory film and an O-ring.

Total extraction and purification of enzymes and determination of de peroxidase activity

Hairy roots were homogenized in a mortar with 10 mmol L^{-1} pH 4.00 sodium acetate/acetic acid buffer, containing 1 mol L^{-1} NaCl (1 g fresh roots per 3 mL of buffer) at 4 ºC. Homogenates were centrifuged at 5000 rpm for 5 min. The supernatants were considered as total peroxidase extracts (TPE). They were used in order to purify peroxidases and determine total peroxidase activity.

The purification of peroxidases was performed by molecular exclusion chromatography on Sephacryl S-200-HR (SIGMA) columns. Samples of 2.5 mL of TPE, previously dialyzed, were loaded on a Sephacryl S-200-HR column (2 cm × 32 cm) equilibrated with a 20 mmol L^{-1} pH 7.00 phosphate buffer. The column was washed with 100 mL of buffer at a flow rate of 1 mL min^{-1} controlled with a peristaltic pump (LKB 2232 Microperpex). Fractions of 5 mL were collected and monitored to determine their peroxidase activity using a qualitative method proposed by Forchetti and Tigier [30]. Fractions containing most peroxidases were mixed and lyophilized before they were used. These enzymes presented a peroxidase activity of 280 IU per solid mg and they were labeled as PBHR.

The total peroxidase activity was determined with o-dianisidine as substrate [31] and expressed in international unit (IU), defined as the amount of enzyme forming 1 mmol of product in 1 min under the experimental conditions employed.

Real samples

OTA production in peanut kernels: Fungal Strains: Four *Aspergillus* section *Nigr*i strains were evaluated. *A. carbonarius* strains RCPG and RCP203 and *A. niger* aggregates RCP42 and RCP191. All strains were isolated from peanut kernels in Argentina and were identified on morphological characters base [32]. OTA production was assayed on YES medium (2% yeast extract and 15% sucrose) [33]. Strains were maintained in glicerol (15%) at -80ºC and kept in the cultura collection at the Department of Microbiology and Immunology of the National University of Río Cuarto (Córdoba, Argentina).

Substrate: Peanut kernels (Runner variety) that had been irradiated (7 kGy) and retained their germinative capacity were used. Kernels were checked for sterility and the absence of OTA [34] and were kept at 4ºC until used. Irradiated peanut kernels were rehydrated to 0.93, 0.95, and 0.98 a_w by adding sterile distilled water. The a_w of representative samples of each treatment was checked at the beginning of the experiment with an AquaLabSeries 3 water activity meter (Decagon Devices, Inc., Pullman, WA).

Inoculation and Incubation conditions: Peanuts were conditioned with an appropriate amount of water far from the a_W levels. The substrate was kept at 4ºC for 48 h with manual periodic shaking to allow absorption and to reach equilibrium. 20 g of peanuts were placed as a monolayer into sterile Petri dishes. Peanuts were inoculated centrally with 5 µL of a spore suspension (1 x 10^6 spores/mL) from a 7-day-old culture growing on 2% malt extract agar (MEA) [35]. Inoculated peanuts plates with the same aW were sealed in plastic containers. Each container had beakers with a glycerol/water solution at the same aW as the peanuts, to maintain a constant relativity humidity. Temperatures tested were 18 and 25°C for a period of 30 days.

Ochratoxin A extraction: OTA production was analyzed after 30 days of incubation. Five grams of a finely ground peanut sample was added to a 250 mL Erlenmeyer flask along with 50 mL of an acetonitrile: water mixture (84:16). The mixture was shaken in an orbital shaker for 30 min and filtered through filter paper (Whatman No. 1). The extract (7 mL) was added to a clean up column (MycoSep ®229 Ochra column, MFC, Romer Labs®, Inc., MO., USA), and acidified with 70 µL of acetic acid. 4 mL of the purified extract was removed, evaporated to dryness, re-dissolved in the 200 µL of mobile phase (acetonitrile- water- acetic acid, 57: 41: 2), and injected into the HPLC.

OTA levels in each treatment were measured as previously described with minor modifications [34]. High performance liquid chromatography (HPLC) with fluorescence detection (λexc 330nm; λem 460nm) was applied. A C_{18} column (Supelcosil LC-ABZ, Supelco; 150 x 4.6 mm, 5 µm particle size) was used. The mobile phase was pumped at 1.0 mL/min. The injection volume was 100 µL and the retention time was 4 min. The detection limit was 1 ng/g.

Electrochemical instruments and experimental measurements

Cyclic voltammograms and amperometric measurements were performed with an Epsilon (BAS) potentiostat controlled by

electrochemical analysis software. Electrochemical measurements were carried out in a 2 mL Pyrex cell. The working electrode was a CP disk of 1.6 mm diameter obtained from Bioanalytical System (BAS), Inc. The counter electrode was a platinum foil of large area (~2 cm^2). An aqueous SCE was used as the reference electrode. Aliquots of 5 to 25 µL were added to the electrochemical cell for the determination of OTA content in solutions composed by comercial reagent and peanut samples contamined with the mycotoxin, respectively. Amperometric measurements were performed at a potential of −0.050 V *vs* SCE in solutions stirred at 1600 rpm. This operational applied potential was previously optimized by Granero et al. [36].

The Fc was used as a redox mediator. Biosensors were constructed by using the following procedure: PBHR, Fc and CP in different weight ratios were checked in order to obtain the best biosensor composition. Then, the optimal composition was used to fill up the CP electrodes. The biosensors were stored at 4ºC in a dry container when they were not in use. Experiments performed with and without bubbling pure nitrogen in solutions did not show any significant difference. Therefore, measurements were carried out in non-deoxygenated solutions. Experiments were performed at 25 ± 0,2ºC.

Results and Discussion

Response of PBHR-FC-CP biosensor versus H_2O_2 concentration

Paste electrodes were prepared by mixing different amounts of PBHR, Fc and CP. A cyclic voltammogram recorded in unstirred pH 8.00 PBS (blank solution) for the PBHR-Fc-CP biosensor showed an increase in the anodic current at potentials higher than 0.16 V *vs* SCE, which corresponds to the oxidation of Fc to Fc+. A cathodic peak with a peak potential at about 0.2 V was observed when the potential sweep direction was reversed at 0.6 V, which can be assigned to the reduction of Fc^+ to Fc at the electrode surface (Figure 1). On the other hand, a cyclic voltammogram recorded when H_2O_2 was added to the reaction médium showed a cathodic current at potentials more negative than -0.3 V *vs* SCE, which corresponds to the reduction of H_2O_2 at the biosensor surface. Therefore, the base current was practically zero between -0.3 and 0.2 V in pH 8.00 PBS. This behavior was similar to that previously found by our group when a similar biosensor was employed to determine t-Res using the comercial reagent [36].

On the other hand, four biosensors were constructed to optimize de amount of PBHR in the biosensor, for which the ratio of the other two components remained constant, i.e., 3 mg Fc and 45 mg CP. These biosensors were used to study their responses towars H_2O_2. Therefore, when only H_2O_2 was added to the stirred reaction medium, the enzimatic reaction took place between PBHR on the electrode surface and H_2O_2 penetrated the inner layer between the semi permeable membrane and the electrode surface. H_2O_2 was reduced to H_2O by PBHR and the enzyme was reduced to its native form by Fc, which was oxidized to Fc+. The Fc+ was then immediately reduced to Fc at the electrode surface held at a potential of -0.050 V *vs* SCE. Steady-

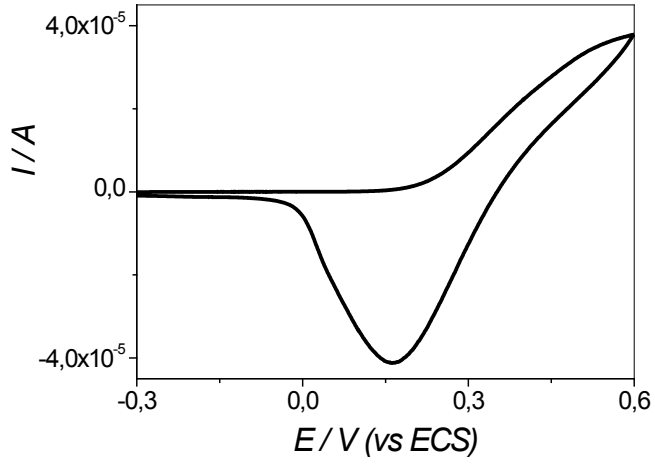


Figure 1: Cyclic voltammogram of PBHR-Fc-CP biosensor recorded in unstirred pH 8.00 PBS. v = 0.050 V s^{-1}.

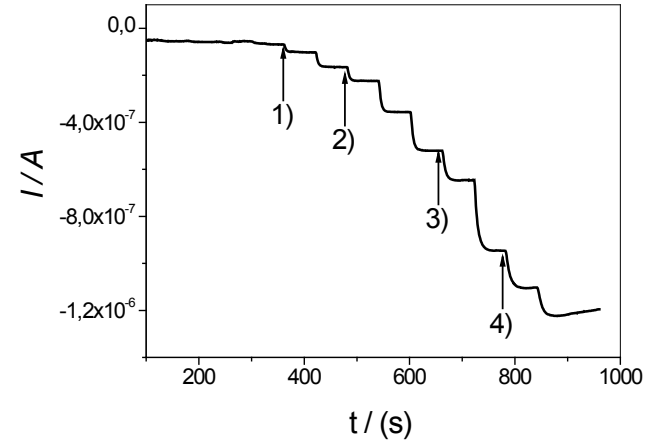


Figure 2: Steady-state current responses on the addition of different H2O2 concentrations at the stirred pH 8.00 PBS reaction medium measured with a dyalisis membrane covered PBHR-Fc-CP (8-6-45) biosensor. $c^*_{H_2O_2}$: (1) 61.8 µmol L^{-1}; (2) 163.5 µmol L^{-1}; (3) 743 µmol L^{-1} and (4) 1443 µmol L^{-1}.

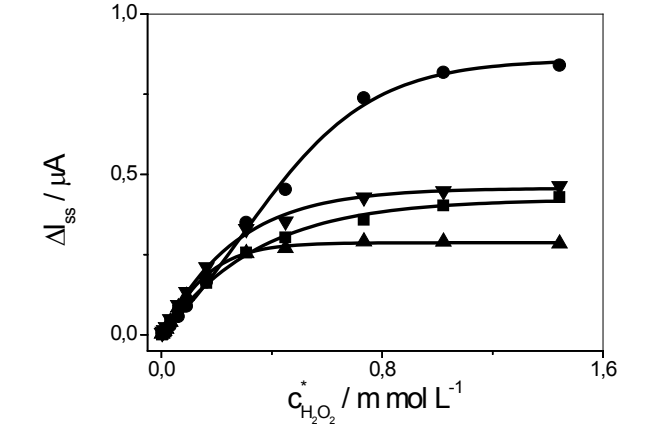


Figure 3: Differences between the base and the steady-state currents, ΔI_{ss}, as a function of under the same experimental conditions as in Figure 2. The PBHR-Fc-CP compositions were: (▲) 3-3-45 mg (▼) 6-3-45 mg (●) 8-3-45 mg (■) 10-3-45 mg.

PBHR-Fc-CP biosensor composition (mg)	I_{max,H_2O_2} (µA)	K_{A,H_2O_2} (mmol L^{-1})
3-3-45	0.33	0.11
6-3-45	0.53	0.19
8-3-45	0.98	0.25
10-3-45	0.51	0.35

Table 1: I_{max,H_2O_2} and K_{A,H_2O_2} kinetics parameters obtained for different biosensor compositions shown in Figure 3 after adding different aliquots of H_2O_2 to pH 8.00 PBS.

state reduction currents (I_{ss}) obtained after the addition of different aliquots of H_2O_2 are shown in Figure 2. The differences between the initial base current and the corresponding I_{ss} (ΔI_{ss}) were proportional to the H_2O_2 bulk concentration (c^*_{OTA}), showing a Michaelis-Menten type saturation (Figure 3). Plots of I_{ss} *vs* I_{ss} / (Eadie-Hofstee plots) were linear and from them we calculate the kinetic parameters of the PBHR-Fc-CP biosensor for H_2O_2 which are summarized in Table 1. Average values of H_2O_2 maximum current (I_{max,H_2O_2}) and Michaelis-Menten apparent constant (K_{A,H_2O_2}) were calculated from the intercept and the slope of Eadie-Hofstee plots for five replicate measurements. Therefore, from results shown in Table 1, biosensors constructed using 8-3-45 mg of PBHR, Fc and CP, respectively, were employed in the next experiments, considering that this electrode composition gave the maximum current value. Then, similar experiments were performed to optimize the Fc amount. Thus, four biosensors were constructed for which the ratio of the other two components remained constant, i.e., 8 mg PBHR and 45 mg CP. The differences between the initial base current and the corresponding I_{ss} (ΔI_{ss}) were proportional to the H_2O_2 bulk concentration ($c^*_{H_2O_2}$), showing a Michaelis-Menten type saturation (Figure 4). Plots of I_{ss} *vs* I_{ss}/ (Eadie-Hofstee plots) were linear. From those plots kinetic parameters of the PBHR-Fc-CP biosensor for H_2O_2 were calculated. They are summarized in Table 2. Average values of H_2O_2 maximum current (I_{max,H_2O_2}) and Michaelis-Menten apparent constant (K_{A,H_2O_2}) were calculated from the intercept and the slope of Eadie-Hofstee plots for five replicate measurements. Therefore, from results shown in Table 2, biosensors constructed using 8-6-45 mg of PBHR, Fc and CP, respectively, were employed in the next experiments, considering that this electrode composition gave the maximum current value. It should be noted that when the biosensor was built with HRP instead of PBHR as the recognition element, it showed less affinity by H_2O_2, as has been previously reported [36]. For this reason PBHR was selected as the recognition element for the assembly of the biosensor.

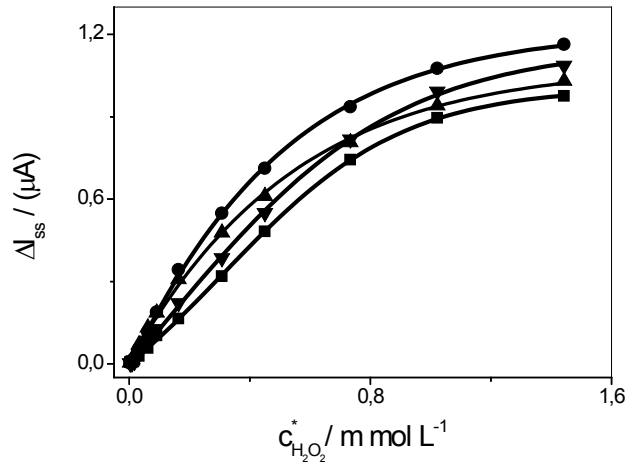


Figure 4: Differences between the base and the steady-state currents, ΔI_{ss}, as a function of $c^*_{H_2O_2}$ under the same experimental conditions as Figure 2. The PBHR-Fc-CP compositions were: (▲) 8-1.5-45 mg (■) 8-3-45 mg (●) 8-6-45 mg (▼)8-9-45 mg.

PBHR-Fc-CP biosensor composition (mg)	I_{max,H_2O_2} (μA)	K_{A,H_2O_2} (mmol L^{-1})
8-1.5-45	1.64	0.7
8-3-45	0.98	0.25
8-6-45	2.17	0.8
8-9-45	1.72	0.79

Table 2: I_{max,H_2O_2} and K_{A,H_2O_2} kinetics parameters obtained for different biosensor compositions shown in Figure 4 after adding different aliquots of H_2O_2 to pH 8.00 PBS.

Responses of PBHR-Fc-CP biosensors versus OTA concentration

When both HRP and OTA were added to the stirred reaction médium composed by pH 8.00 PBS and a given H_2O_2 bulk concentration, the enzimatic catalytic cycle also took place in the solution bulk. The oxidized HRP, produced as a result of its reaction with H_2O_2, is reduced back to its native state by OTA. The decrease of H_2O_2 in the solution bulk was detected as a decrease in the Fc+ reduction current at the biosensor surface [36]. The optimum HRP concentration in solution was 10 nmol L^{-1}. Thus, the addition of H_2O_2 to the pH 8.00 PBS + 10 nmol L^{-1} HRP reaction medium produced a steady limiting current ($I_{s,lim}$) at -0,050 V *vs* SCE after 180 s, which corresponds to the reduction of Fc+ generated by HRP catalyzed reduction of H_2O_2 to H_2O (Figure 5). The difference between Is,lim with and without OTA ($\Delta I_{s,lim}$) corresponds to the H_2O_2 concentration decrease given that the enzimatic reaction was taking place in the bulk solution. The steady state

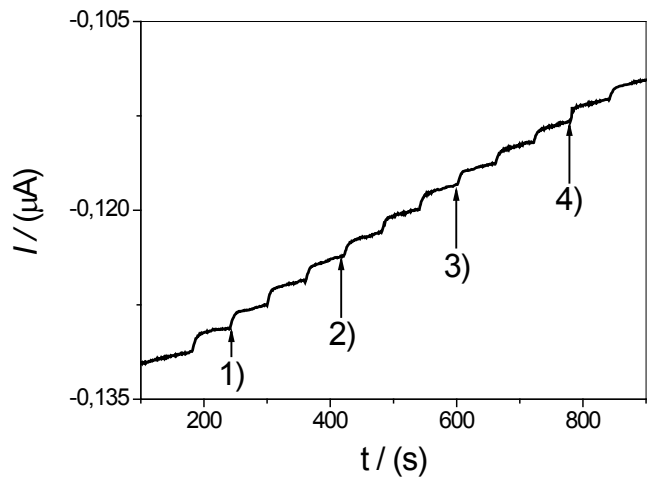


Figure 5: Steady-state current responses on the addition of different OTA concentrations in a reaction medium under stirring containing 10 nmol L^{-1} HRP + 100 μmol L^{-1} H_2O_2 in pH 8.00 PBS with a dyalisis membrane covered PBHR-Fc-CP (8-6-45) biosensor. c^*_{OTA}: (1) 30 μmol L^{-1}; (2) 73 μmol L^{-1}; (3) 113 μmol L^{-1} and (4) 150 μmol L^{-1}

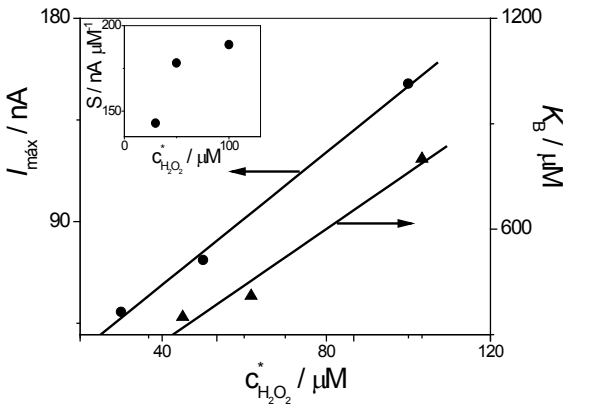


Figure 6: Dependece of $I_{max,OTA}$ obtained for OTA through Eadie-Hofstee plots with H_2O_2 concentration. Insert: plot of sensitivity as a function of $c^*_{H_2O_2}$.

currents obtained after the addition of OTA were reached at about 60 s.

Plots of $\Delta I_{s,lim}$ *vs* OTA concentration (c^*_{OTA}) also showed a Michaelis-Menten saturation. Figure 6 displays the máximum current ($I_{\max,OTA}$) and the apparent Michaelis Menten constant ($K_{B,OTA}$) values obtained from the addition of different aliquots of OTA to the solution composed by pH 8.00 PBS + 10 nmol L^{-1} HRP in the presence on different H_2O_2 bulk concentration. For the sensitivity ($S = I_{\max,OTA} / K_{B,OTA}$ *vs* $c^*_{H_2O_2}$, see insert of Figure 6) [37], the plateau was reached at 100-150 µmol L^{-1} of H_2O_2. This means that at these peroxide concentrations, the bioelectrode responses are limited by the enzymatic kinetics rate. Therefore, a concentration of 100 µmol L^{-1} of H_2O_2 was chosen as the best peroxide level in the reaction medium. It should be noted that when PBHR is used as biological material in solution, after the addition of OTA there were no apreciables changes in the amperometric response, which would indicate that OTA is not a good substrate for this enzyme. On the other hand, it has been found that OTA behave as a good substrate for HRP. Thus, we decided to use HRP as biological material in solution.

Biosensor statistical parameters

The PBHR-Fc-CP biosensor reproducibility was tested by measuring the calibration curve slope for OTA of five different bioelectrodes in a solution of pH 8.00 PBS + 10 nmol L^{-1} HRP + 100 µmol L^{-1} H_2O_2 as the reaction media. Percent relative standard deviations (%RSD) of 7.7% were obtained for OTA. The repeatability assays were performed carrying out six consecutive amperometric measurements on the same biosensor. In this case, %RSD of calibration curve slopes was 5.4%, when a PBHR-Fc-CP biosensor was used in PBS pH 8.00 + 10 nmol L^{-1} HRP + 100 µmol L^{-1} H_2O_2. The stability of PBHR-Fc-CP biosensor was tested by using the same biosensor to determined the slopes from several calibration curves (n = 5) for OTA. The slopes obtained were practically constant in the order of the experimental error until about five days, showing a good stability of PBHR biosensor. A noticeable decrese in the slope started from the fifth day and about 40% decrease was obtained on the seventh day. A linear variation was found for $\Delta I_{s,lim}$ *vs* OTA concentration (c^*_{OTA}) in the range from $1x10^{-8}$ to $1.4x10^{-4}$ mol L^{-1}. The lowest concentration value measured with PBHR-Fc-CP biosensor for a signal to noise ratio of 3:1 was 5.7 x 10^{-9} mol L^{-1} (2.3 ppb).

In order to achieve another criterion to test the accuracy of the biosensor method for OTA quantification in peanut samples the results were also investigated by the HPLC method [34]. Five samples were used to perform this analysis. The concentrations determined by HPLC-immunoaffinity column cleanup were plotted *vs* those obtained from the biosensor method. We found a linear variation with slope = 0.931 and correlation coefficient close to 1 (r = 0.9973) indicating a good correspondence between methods (Figure 7).

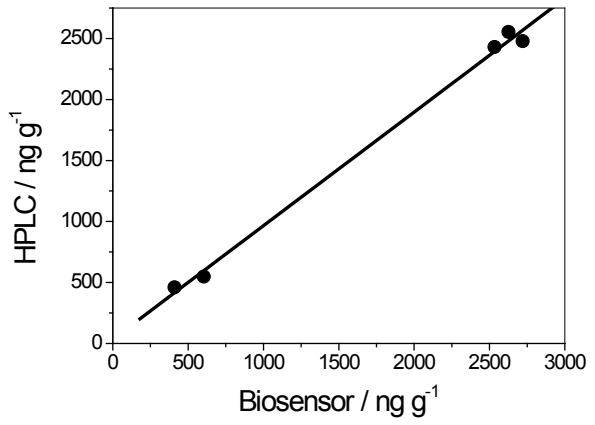


Figure 7: Amount of OTA in peanut samples measured by HPLC and PBHR-Fc-CP (8-6-45) biosensor.

Experimental results obtained demonstrate that our enzymatic bioelectrode method is a very useful technique to detect and quantify OTA in peanut samples. The correlation between biosensor and HPLC method [34] indicates the effectiveness of the enzimatic electrode for OTA determination. While the detection limit determined is higher than values in some previous reports [38,39] still this electrode shows important advantages by using it for screening purposes due to its very simple construction and rapid measurements.

Conclusions

It is concluded that the PBHR biosensor exhibited a good performance, stability, reproducibility, repeatability, detection limit and linear range for the quantification of OTA. This good analytical performance allowed us to estimate the OTA in peanut samples using a very simple experimental procedure. The contents of OTA in samples of peanuts determined with the biosensor show a good correlation with values obtained with HPLC method. In addition, this method offers some advantages over the HPLC method, such as: short detection time (~1 min), small sample volumes, reduced use of solvents and low cost. These advantages indicate that a PBHR biosensor can be used as a useful tool for a rapid screening in the determination of OTA in peanuts samples.

Acknowledgements

Financial support from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) and Secretaría de Ciencia y Técnica (SECyT) from the Universidad Nacional de Río Cuarto is gratefully acknowledged. E. A. R. thanks CONICET for research fellowships.

References

1. Van der Merwe K, Steyn P, Fourie L, Scott D (1965) Ochratoxin A: a toxic metabolite produced by *Aspergillus ochraceus*. Willh Nature J Theron, Nature 205: 1112-1113.
2. Stormer FC (1992) Ochratoxin A-a mycotoxin of concern, in: D. Bhatnagar, Lillehoj EB, Arora DK (Eds.), Handbook of Applied Mycology, vol. 5: Mycotoxins in Ecological Systems, Marcel Dekker, Inc., New York, p. 403.
3. Steyn PS, Stander MA (1999) Mycotoxins as causal factors of diseases in humans. J Toxicol Toxin Rev, 18: 229-243.
4. Steyn P S (1984) Ochratoxins and related dihydro-isocoumarins, in: Bettina V (Ed.), Mycotoxins: Production, Isolation, Separation and Purification, Elsevier, New York, p. 183.
5. Harvey RB, Elissalde MH, Kubena LF, Weaver EA, Cirrier DE, et al. (1992) Immunotoxicity of ochratoxin A to growing gilts. Am J Vet Res 53: 1966-1970.
6. Størmer FC, Lea T (1995) Effects of ochratoxin A upon early and late events in human T-cell proliferation. Toxicol 95: 45-50.
7. Müller G, Kielstein P, Rosner H, Berndt A, Heller M, et al.(1999) Studies of the influence of ochratoxin A on immune and defence reactions in weaners. Mycoses, 495-505.
8. International Agency for Research on Cancer, Some Naturally Occurring Substances: Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, Monographs on the Evaluation of Carcinogenic Risk to Humans, vol. 56, IARC, Lyon, France, 1993, p. 489.
9. Turner N W, Subrahmanyam S, Piletsky S A (2009) Analytical methods for determination of mycotoxins: A review. Anal Chim Acta 632: 168-180.

10. Bongiovani RM, Gillet M, Argentina Association of Agricultural Economics. Economic analysis of the peanut crop under different rotations and tillage systems. Available at: ~http://agro.uncor.edu/aaea2007/Comunicaciones20A/Bongiovani.pdf. Accessed 15 November 2009.

11. Secretaria de Agricultura, Ganadería, Pezca y Alimentación (2009). Avaible at: http//www.sagpya.mecon.gov.ar. Accessed 26 November 2009.

12. Shephard GS, Berthiller F, Corner J, Krska R, Lombaert GA, et al. (2009) Developments in mycotoxin analysis: An update for 2008-2009. World Mycotoxin J 2: 3-23.

13. Shephard GS, Fabiani A, Stockenstrom S, Mshicilele N, Sewram V (2003) Quantitation of ochratoxin A in South African wines. J Agric Food Chem 51: 1102-1106.

14. Braicu C, Puia C, Bodoki E, Socaciu C (2008) Screening and quantification of aflatoxins and ochratoxin A in different cereals cultivated in Romania using thin-layer chromatography-densitometry. J Food Qual 31: 108-120.

15. Olsson J, Börjesson T, Lundstedt T, Schnürer J (2002) Detection and quantification of ochratoxin A and deoxynivalenol in barley grains by GC-MS and electronic nose. Int J Food Microbiol 72: 203-214.

16. González-Osnaya L, Soriano JM, Moltó JC, Mañes J (2008) Simple liquid chromatography assay for analyzing ochratoxin A in bovine milk. Analytical Methods, Food Chem 108: 272-276.

17. Solfrizzo M, Panzarini G, Visconti A (2008) Determination of ochratoxin A in grapes, dried vine fruits, and winery byproducts by high-performance liquid chromatography with fluorometric detection (HPLC-FLD) and immunoaffinity cleanup. J Agric Food Chem 56: 11081-11086.

18. Gomes SASS, Nogueira JMF, Rebelo MFJ (2004) An amperometric biosensor for polyphenolic compounds in red wine. Biosens Bioelectron20: 1211-1216.

19. Freire RS, Ferreira MM, Durán N, Kubota LT (2003) Dual amperometric biosensor device for analysis of binary mixtures of phenols by multivariate calibration using partial least squares. Anal Chim Acta 485: 263-269.

20. Abhijith KS, Kumar PV, Kumar MA, Thakur MS (2007) Immobilised tyrosinase-based biosensor for the detection of tea polyphenols. Anal Bioanal Chem 389: 2227-2234.

21. Berend S, Grabaric Z (2008) Determination of total polyphenol content in food with the flow-injection method. Arh Hig Rada Toksikol 59: 205-212.

22. Korkut S, Keskinler B, Erhan E (2008) An amperometric biosensor based on multiwalled carbon nanotube-poly (pyrrole) - horseradish peroxidase nanobiocomposite film for determination of phenol derivatives. Talanta 76: 1147-1152.

23. Di Fusco M, Tortolini C, Deriu D, Mazzei F (2010) Laccase-based biosensor for the determination of polyphenol index in wine. Talanta 81: 235-240.

24. Nalewajko-Sieliwoniuk E, Tarasewicz I, Kojilo A (2010) Flow injection chemiluminiscence determination of the total phenolic levels in plant-derived beverages using soluble manganese (IV). Anal Chim Acta 668: 19-25.

25. Ibarra-Escutia P, Juarez Gómez J, Calas-Blanchard C, Mary JL, Ramírez-Silva M T (2010) Amperometric biosensor based on a high resolution photopolymer deposited onto a screen-printed electrode for phenolic compounds monitoring in tea infusions. Talanta 81: 1636-1642.

26. Kulys J, Bilitewski U, Schmid RD (1991) The kinetics of simultaneous conversion of hydrogen peroxide and aromatic compounds at peroxidase electrodes. Bioelectrochem Bioenerg 321: 277-286.

27. Marko-Varga G, Emneus J, Gorton L, Ruzgas T (1995) Development of enzyme-based amperometric sensors for the determination of phenolic compounds. Trends Anal Chem 14: 319-328.

28. Agostini E, Milrad de Forchetti S, Tigier H (1997) Production of peroxidases by hairy roots of *Brassica napus*. Plant Cell Tiss Organ Cult 47: 177-182.

29. Agostini E, Hernández Ruíz J, Arnao MB, Milrad SR, Tigier HA, Acosta M (2002) A peroxidase isoenzyme secreted by turnip (*Brassica napus*) hairy-root cultures: inactivation by hydrogen peroxide and application in diagnostic kits. Biotechnol Appl Biochem 35: 1-7.

30. Milrad de Forchetti S, Tigier HA (1990) Indole-3 acetic acid oxidase and syringaldazine oxidase of peroxidase isozymes in soybean root nodules. Physiol Plant 79: 327-339.

31. González PS, Capozucca CE, Tigier HA, Milrad SR, Agostini E (2006) Phytoremediation of phenol from wastewater by peroxidases of tomato hairy root cultures. Enzyme Microb Technol 39: 647-653.

32. Klich MA (2002) Identification of common *Aspergillus* species. Centraalbureeau voor Schimmelculture, Utrecht, The Netherlands.

33. Magnoli C, Astoreca A, Ponsone L, Fernández-Juri MG, Barberis CL, et al. (2007) Ochratoxin A and *Aspergillus* section Nigri in peanut seeds at different months of storage in Córdoba, Argentina. Int J Food Microbiol 119: 213-218.

34. Scudamore K A, MacDonal SJ (1998) A collaborative study of an HPLC method for determination of ochratoxin A in wheat using immunoaffinity column clean-up. Food Addit Contam 15: 401-410.

35. Pitt JI, Hocking AD (1997) Fungi and food spoilage. Blackie Academic and Professional, London.

36. Granero AM, Fernández H, Agostini E, Zón MA (2008) An Amperometric Biosensor for trans-Resveratrol Determination in Aqueous Solutions by Means of Carbon Paste Electrodes Modified with Peroxidase Basic Isoenzymes from *Brassica napus*. Electroanalysis 20: 858-864.

37. Ruzgas T, Emnéus J, Gorton L, Marko-Varga G (1995) The development of a peroxidase biosensor for monitoring phenol and related aromatic compounds. Anal Chim Acta 311: 245-253.

38. Ansari A, Kaushik A, Solanki P, Malhotra B (2010) Nanostructured zinc oxide platform for mycotoxin detection. Bioelectrochem 77: 75-81.

39. Perrotta P, Vettorazzi N, Arévalo F, Granero A, Chulze S, et al. (2011) Electrochemical Studies of Ochratoxin a Mycotoxin at Gold Electrodes Modified with Cysteamine Self-Assembled Monolayers: Its Ultrasensitive Quantification in Red Wine Samples. Electroanal 23:1585-1592.

This article was originally published in a special issue, **Biosensors: Applications in Imaging Techniques** handled by Editor(s). Dr. Yuan Zhen, University of Florida, USA