Food Chemistry 190 (2016) 818–823

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Dual enzymatic biosensor for simultaneous amperometric determination of histamine and putrescine

W. Henao-Escobar, L. del Torno-de Román, O. Domínguez-Renedo *, M.A. Alonso-Lomillo, M.J. Arcos-Martínez

Analytical Chemistry Department, Faculty of Sciences, University of Burgos, Pza. Misael Bañuelos s/n, 09001 Burgos, Spain

ARTICLE INFO

Article history:
Received 24 February 2015
Received in revised form 11 June 2015
Accepted 13 June 2015
Available online 15 June 2015

Keywords:
Dual enzymatic biosensor
Histamine
Putrescine
Screen-printed electrodes
Simultaneous determination

ABSTRACT

A disposable electrodic system consisting of two working electrodes connected in array mode has been developed for the simultaneous determination of histamine (His) and putrescine (Put). Histamine deshydrogenase and putrescine oxidase enzymes were respectively immobilized by crosslinking on each working screen-printed electrode, both modified with tetrathiafulvalene. The dual system allowed the simultaneous amperometric determination of both species by measuring the oxidation current of the mediator in each working electrode. The effect of other potentially interfering biogenic amines was also evaluated. The capability of detection was of 8.1 ± 0.7 for His and 10 ± 0.6 μM for Put. The precision in terms of relative standard deviation was of 3.5% and 6.7% for His and Put, respectively. The developed biosensor was successfully applied to the determination of His and Put in different food samples.

1. Introduction

Biogenic amines (BAs) are organic bases with different structures that are mainly produced by microbial decarboxylation of their precursor amino acids (Calvo-Pérez, Domínguez-Renedo, Alonso-Lomillo, & Arcos-Martínez, 2013). These compounds are considered indicators of an earlier microbial decomposition in fish or shellfish, being also present in a great variety of food samples (Lange & Wittmann, 2002; Moret, Smela, Populin, & Conte, 2005) and beverages (Di Fusco, Federico, & Boffi, 2011; Kivirand & Rinken, 2011; López et al., 2012; Ordoñez, Callejon, Morales, & Garcia-Parrilla, 2013) such as meat, beer, chocolate, cheese or wine. The most important BAs related with spoilage in food are histamine (His), putrescine (Put) and cadaverine (Cad); the former being one of the most biologically active compounds (Pérez, Bartrolí, & Fábregas, 2013). The toxicological level of BAs is very difficult to establish. Nevertheless, a maximum total BAs (the sum of tyramine (Tyr, His, Put, and Cad) level of 750–900 mg/kg has been proposed as a safety limit (Gezginc, Akyol, Kuley, & Özogul, 2013). At low concentration levels, BAs may be considered essential for many physiological functions (Önal, Tekkeli, & Önal, 2013). However, if these compounds are consumed in high quantities, several toxicological problems arise. The symptoms of this foodborne chemical intoxication are allergy-like, characterized by cutaneous rash, gastrointestinal and neurological aspects such as nausea, vomiting, diarrhea, head-aches, palpitations, flushing, tingling, burning and itching, or by hypertension (Leuschner, Hristova, Robinson, & Hugas, 2013).

Analytical determination of BAs in food samples is not simple due to the great variety of chemical structures and the level of concentration at which these compounds are present in such complex matrices. High-performance liquid chromatography (HPLC) is one of the most often techniques used to determine these amines due to its high resolution and sensitivity (Hernández-Orte, Peña-Gallego, Ibarz, Cacho, & Ferreira, 2006; Önal et al., 2013). However, this technique usually requires sample preparation including a time-consuming pre-column derivatization. Electrochemical systems may be then presented as an interesting alternative for detecting BAs due to their simplicity, low cost, effectiveness and short time in the analysis of these kinds of compounds. In this way, electrochemical sensors based on the use of imprinted polymers have been developed for the selective determination of different BAs (Peeters et al., 2013; Ratautaite, Nesladek, Ramanaviciene, Baleviciute, & Ramanavicius, 2014). Electrochemical biosensors have been described as more simple devices in the determination of BAs in different samples. Thus, enzyme modified biosensors have attracted enormous attention in the electroanalysis of BAs during the recent years (Bóka, Adányi, Virag, Sebela, & Kiss, 2012; Calvo-Pérez et al., 2013; Dominguez-Renedo, Alonso-Lomillo, & Arcos-Martínez, 2007; Henao-Escobar, Domínguez-Renedo, Alonso-Lomillo, &

* Corresponding author.
E-mail address: olgado@ubu.es (O. Domínguez-Renedo).

http://dx.doi.org/10.1016/j.foodchem.2015.06.035
0308-8146/

Arcos-Martínez, 2013a; Henao-Escobar, Domínguez-Renedo, Alonso-Lomillo, & Arcos-Martínez, 2013b; Muresan, Ronda, & Frébort, 2008; Pérez et al., 2013; Rosini, Tonin, Vasylieva, Marinesco, & Pollegioni, 2013; Telsnig, Kalcher, Leitner, & Ortner, 2013; Telsnig et al., 2012; Young, Jiang, & Kirchhoff, 2013). As can be seen in Table 1, the main problem of the described biosensors comes from the low enzyme selectivity and the high working potentials needed for operation, leading to an increase in the effect caused by the presence of different interfering species.

Nowadays, the main efforts in the development of biosensors for BAs are focused on the improvement of their sensitivity, but also of their selectivity, based upon the specificity of the enzyme used. In this work, two highly selective and specific enzymes were used for the analysis of Put and His, namely putrescine oxidase (PUO) from *Micrococcus rubens* and histamine deshydrogenase (HMD), respectively. These selected enzymes are pure active enzymes much more specific than the commonly used amino oxidases in the construction of biosensors for the determination of BAs (Kivirand & Rinken, 2011; Tsutsumi, Tsuse, Fujieda, & Kano, 2010).

Screen-printed carbon electrodes (SPCEs) have been used as electrochemical transducers, since they increase the above described advantages of the electrochemical biosensors in the determination of BAs such as His and Put (Alonso-Lomillo, Domínguez-Renedo, Matos, & Arcos-Martínez, 2010; Calvo-Pérez et al., 2013; Chemnitius & Bilitewski, 1996; Di Fusco et al., 2011; Henao-Escobar et al., 2013a; Henao-Escobar et al., 2013b; Keow et al., 2007; Keow et al., 2012; Lange & Wittmann, 2002; Piermarini, Volpe, Federico, Moscone, & Palleschi, 2010; Pérez et al., 2013; Telsnig et al., 2013). Tetrathiafluvalene (TTF) has been used as electrochemical mediator in order to decrease the working potential in the amperometric measurements. TTF was screen-printed by incorporating it into the carbon ink. PUO and HMD enzymes were immobilized on the TTF modified SPCEs (TTF/SPCEs) by cross-linking with glutaraldehyde (GA) and bovine serum albumin (BSA). The main novelty of this work resides in the employment of a dual electrode system consisting of two different working electrodes (PUO/TTF/SPCE and HMD/TTF/SPCE), which allowed the simultaneous determination of Put and His. This dual system represents, not only a simple method for the determination of Put and His but also, offers a rapid simultaneous determination of both BAs reducing the time needed for analysis. This last feature makes the developed method suitable for application in routine analysis in food control.

2. Experimental

2.1. Reagents

All reagents used were of analytical-reagent grade. Ultrapure water obtained from a Milli-Q water purifier (Millipore, Bedford, MA, USA) was used for the preparation of all solutions. The supporting electrolyte used for the measurements was a 100 mM KH_2PO_4 buffer solution (Sigma–Aldrich, Steinheim, Germany) containing 100 mM KCl (Merck, Darmstadt, Germany). The pH value was adjusted with a 1 M NaOH solution (J.T. Baker, Deventer, The Netherlands).

HMD (EC: HMD-IFO12069, activity 0.84 U/μL) was obtained from Biolan Microbiosensores (Scientific and Technological Park of Bizkaia, Zamudio, Spain), PUO from *M. rubens* (EC 1.4.3.10, activity 1.25 U/μL) was obtained from Inbiotec (León, Spain). Bovine serum albumin (BSA) and glutaraldehyde (GA), used in the immobilization procedure, were purchased from Sigma–Aldrich (Steinheim, Germany). Put and Cad were obtained from Sigma–Aldrich (Steinheim, Germany) as well. Tyr and His were acquired

Table 1
Electrochemical biosensors for the determination of BAs based on SPEs.

Enzyme	Electrode	Mediator	Immobilization	Potential (mV)	Capability of detection	Selectivity	Sample	References
PAO	SPCE	Ferrocene	Crosslinking with GA and BSA	+260	2.0 ± 0.18 μM (Tyr)	Total BAs	Brie Cheese	Calvo-Pérez et al. (2013)
PAO, TAO and DAO	Array of SPPtEs	–	Crosslinking with GA and transglutaminase	+700	10 mg/kg (His and Tyr) 5 mg/kg (Put)	Total BAs	Fish and meat	Lange and Wittmann (2002)
DAO	SPAuE	–	Immobilization in a polyazetine prepolymer	+600	0.2 mg/L (Put)	Total BAs	Wine and beer	Di Fusco et al. (2011)
DAO and HRP	SPCE	Ferrocene	Immobilization into a polysulfone/carbon nanotubes/ferrocene membrane	−50	0.17 μM (His)	–	Fish	Pérez et al. (2013)
MAO	SPCE	TTF	Crosslinking with GA and BSA	+250	17.2 ± 4.6 μM (Put)	Cad and Put interfere at a concentration level of 60 μM	Zucchini and anchovies	Henao-Escobar et al. (2013a)
PSAO	SPCE	MnO_2	Immobilized using a nafion solution	+400	0.3 μM (Cad and Put) 3 μM (Tyr and His)	Total BAs	Chicken	Telsnig et al. (2013)
MAO	Array of SPCEs modified with AuNPs	TTF	Crosslinking with GA and BSA	+250	9.9 μM (Put) 19.9 ± 0.9 μM (Cad)	Tyr interferes at a concentration level of 60 μM	Octopus	Henao-Escobar et al. (2013b)
DAO and HRP	SPCE	Ferrocene	Covalent immobilization using an aryldiazonium salt, hydroxysuccinimide and carbodiimide	+250	0.4 μM (His)	Total BAs	Anchovy samples	Alonso-Lomillo et al. (2010)
DAO	SPCE	Potassium ferricyanide	Entrapped in a photocured poly (2-hydroxyethyl) metacrylate film	+350	0.65 ppm (His)	–	Prawns	Keow et al. (2012), Keow et al. (2007)
DAO	SPCE	PB	Crosslinking with GA and BSA	−50	10 μM (Put)	Total BAs	Human saliva	Piermarini et al. (2010)

AuNPs: gold nanoparticles; DAO: diamine oxidase; HRP: horseradish peroxidase; MAO: monoamine oxidase; PAO: plasma amino oxidase; PB: prussian blue; PSAO: diamine oxidase extracted from pea seedlings; SPPtEs: screen-printed platinum electrodes; SPAuE: screen-printed gold electrode; TAO: tyramine oxidase.

from Fluka (Steinheim, Germany). Other BAs, including spermidine (Spd), spermine (Spm) and tryptamine (Tryp) were purchased from Acros Organics (Geel, Belgium).

Different inks were used to construct the TTF/SPCEs. C200802P2 carbon ink and D2071120D1 dielectric ink were obtained from Gwent Electronic Materials (Torfaen, UK). Silver 5029 conductor paste was purchased from Dupont Limited (Bristol, UK). Electrodag 6037 SS (Ag/AgCl ink) from Acheson Colloiden (Scheemda, The Netherlands) was also used. TTF was provided by Sigma–Aldrich (Steinheim, Germany).

$HClO_4$ from Panreac (Barcelona, Spain), ethyl acetate and NaCl from Fluka (Steinheim, Germany), ammonia solution, acetone, HCl and Na_2SO_4 from Merck (Darmstadt, Germany) were used for BAs extraction in food samples.

$NaHCO_3$ (Panreac, Barcelona, Spain), dansyl chloride (Sigma–Aldrich, Madrid, Spain) reagent (5 mg mL^{-1} in acetone), proline (Sigma–Aldrich, Madrid, Spain) solution (100 mg mL^{-1}), diethyl ether (Fisher Chemical, Loughborough, UK) and acetonitrile (Sigma–Aldrich, Madrid, Spain) were used in the derivatization procedure of the samples for HPLC analysis.

Fresh octopus and red wine samples were obtained from a local market. Certified canned fish aqueous extracts samples from FAPAS® (8.7 mg/kg) were also analyzed.

2.2. Apparatus

Electrochemical measurements were performed with a PalmSens potentiostat driven by the PS Trace program (PalmSens® Instruments BV, Houten, The Netherlands). pH of buffer solutions were measured with a HI 221 pHmeter (HANNA Instruments, USA). The TTF/SPCEs were produced on a DEK 248 printing machine (DEK, Weymouth, UK) using screen polyester mesh and polyurethane squeegees.

Quantitative analysis of BAs was carried out using an HPLC system (1260) from Agilent Technologies (Waldbronn, Germany) equipped with an auto-sampler, a quaternary pump and a diode array detector. Separation was achieved using a reversed phase column (Chromolith (R) high Resolution RP-18 endcapped 100–4.6 mm (Merck KGaA, Darmstadt, Germany)). The detection was performed with a UV–Vis system set at 240 nm.

2.3. Biosensor preparation

Two different electrodic systems have been used in this work: the first one (System I) was used in the His determination and the second one (System II) in the His and Put simultaneous determination.

2.3.1. System I

A three screen-printed electrodes (SPEs) configuration system was used for the determination of His consisting of a Ag/AgCl reference electrode (Ag/AgCl SPE), a carbon ink (counter electrode), and a carbon ink modified with 5% TTF (working electrode). This electrodic system was constructed following previously described procedures (Asturias-Arribas, Alonso-Lomillo, Domínguez-Renedo, & Arcos-Martínez, 2013; del Torno-de Román, Alonso-Lomillo, Domínguez-Renedo, & Arcos-Martínez, 2013; Domínguez-Renedo & Arcos-Martínez, 2007; Henao-Escobar et al., 2013b; Molinero-Abad, Alonso-Lomillo, Domínguez-Renedo, & Arcos-Martínez, 2014). HMD was then immobilized on TTF/SPCEs, obtaining HMD/TTF/SPCEs for the determination of His. The immobilization of the enzyme was performed using a crosslinking process with GA and BSA. A volume of 5 μL of a solution containing HMD (0.84 U/μL); BSA (3% w/v prepared in 100 mM pH 6 phosphate buffer) and GA (2.5% v/v prepared in water), in the volume ratio 1:1:2 was deposited on the working electrode surface. Once deposited, the mixture was allowed to dry for 90 min at 4 °C.

2.3.2. System II

A four SPEs configuration system was used for the simultaneous determination of His and Put consisting of two different working electrodes (dual-TTF/SPCEs) following the procedure described in previous works (Calvo-Pérez, Domínguez-Renedo, Alonso-Lomillo, & Arcos-Martínez, 2014; del Torno-de Román, Alonso-Lomillo, Domínguez-Renedo, & Arcos-Martínez, 2015; Henao-Escobar et al., 2013b). This fabrication procedure is similar to the one used in the conventional three-electrode configuration development, including a second screen-printed carbon working electrode. One working electrode was modified with HMD (HMD/TTF/SPCEs) and the other one with PUO (PUO/TTF/SPCEs) by crosslinking using GA and BSA (Fig. 1). HMD immobilization was performed by depositing a volume of 5 μL of a solution containing HMD (0.84 U/μL); BSA (3% w/v prepared in 100 mM pH 6 phosphate buffer) and GA (2.5% v/v prepared in water) in the volume ratio 1:2:2, on one working electrode surface. In the same way, PUO immobilization was carried out by depositing a volume of 5 μL of a solution containing PUO (1.25 U/μL): BSA (3% w/v prepared in 100 mM pH 6 phosphate buffer): GA (2.5% v/v prepared in water) in the volume ratio 1:2:2, on the other working electrode surface. Once deposited, the mixture was allowed to dry for 90 min at 4 °C.

2.4. Electrochemical measurements

Amperometric measurements using system I, for the determination of His, were made at room temperature (approx. 20 °C) in a cell containing 5 mL of the supporting electrolyte solution (100 mM phosphate buffer and 100 mM KCl) of the desired pH, under constant stirring. The amperometric detection was performed by measuring the anodic current due to the oxidation of the mediator TTF (Scheme 1), operated at a potential of +130 mV *vs.* Ag/AgCl SPE, except for the experimental variables optimization process.

His and Put were simultaneously determined using a dual-TTF/SPE (system II) based on the amperometric measurement of the anodic current due to the oxidation of the mediator TTF in both working electrodes. The above-described experimental conditions for HMD/TTF/SPCEs in system I, were also used for the amperometric determination of His using the dual system. In the case of amperometric measurements of Put, the working potential was fixed at +300 mV, as it has been described in a previous work for the single analysis of this compound (Henao-Escobar et al., 2015).

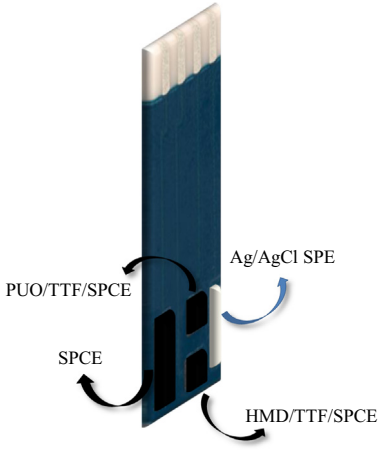
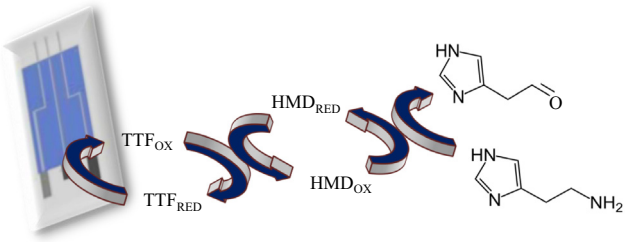


Fig. 1. Scheme of a homemade dual-TTF/SPCE (1.3 cm × 2.8 cm).



Scheme 1. Enzymatic mechanism of HMD/TTF/SPCEs biosensors.

2.5. Sample preparation

Extraction of His and Put in fresh octopus samples was carried out using an ethyl acetate-acetone mixture, according to procedure described for Yigit and Ersoy (2003). In this way, 10 g of the previously homogenized sample and 10 mL of a 5 % $HClO_4$ solution were mixed in a vortex mixer for 1 min. The mixture was placed in an ultrasonic bath for 10 min and then, centrifuged for 10 min at 7000 rpm. The supernatant was recovered and neutralized with a 3% ammonia solution. Then, it was saturated with NaCl and extracted with 10 mL of an ethyl acetate–acetone (2:1) solvent system in a vortex mixer for 1 min. Then the mixture was briefly centrifuged, and the organic phase was separated. Finally, the solvent was evaporated.

In the case of the wine samples a previous pre-treatment step with activated carbon was necessary. In this way, 50 mg of this compound were added to 1 mL of the wine sample. The mixture was then centrifuged at 13,000 rpm for 2 min. The supernatant was finally analyzed using the developed biosensors.

3. Result and discussion

3.1. Determination of histamine using system I

3.1.1. Optimization and characterization process

The developed HMD/TTF/SPCEs biosensors produced an amperometric signal related to the mediator oxidation (Fig. 2), which can be associated with the concentration of His.

The amperometric response obtained by means of these biosensors depends on several experimental parameters, which can define the sensitivity but also the selectivity of these systems. The most influence experimental factors are pH value of the buffer solution and the working potential. Therefore, an optimization procedure of these experimental variables was carried out. These factors were optimized by means of a 2^2 central composite design, taking as response variable the intensity of oxidation obtained for a 100 μM His solution. The values which correspond to the high (+) and low (−) levels and to the central point (0) for each factor were:

$$\text{Working potential}\,(+) = +300\ \text{mV} \qquad \text{pH}\,(+) = 9$$

$$\text{Working potential}\,(-) = -100\ \text{mV} \qquad \text{pH}\,(-) = 5$$

$$\text{Working potential}\,(0) = 100\ \text{mV} \qquad \text{pH}\,(0) = 7$$

Then 11 experiments derived from all the possible combinations of each factor were carried out and evaluated in terms of analysis of variance. The combination of factors levels that maximizes the response over the experimental region were a pH value of 6.8 with a working potential of +130 mV *vs.* a Ag/AgCl SPE for the determination of His using HMD/TTF/SPCEs.

Under these optimized conditions, calibration parameters were calculated by the robust regression least median square regression

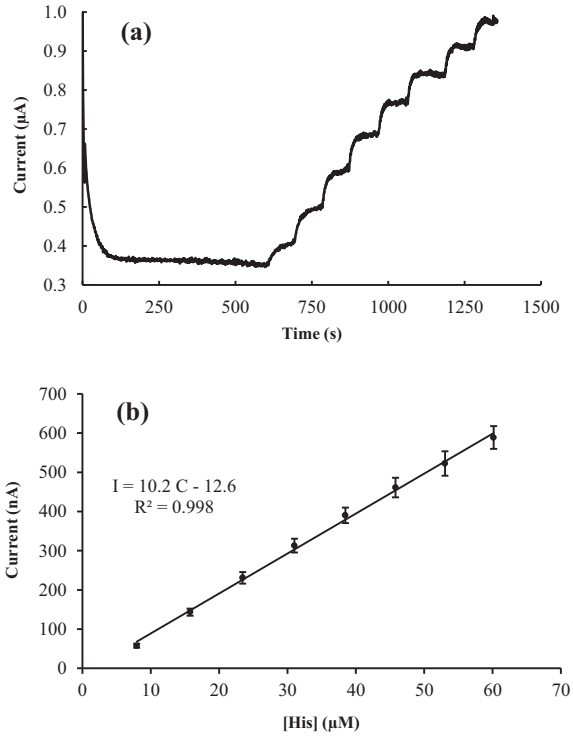


Fig. 2. (a) Chronoamperogram recorded on HMD/TTF/SPCEs for successive additions of 40 μL of a 1 mM His solution. (b) Relation between amperometric current intensity and concentration of His in HMD/TTF/SPCEs biosensors (pH, 6.8; working potential, +130 mV *vs.* Ag/AgCl SPE).

(LMS) using the program Progress (Rousseeuw & Leroy, 1987). LMS presents the advantage of being able to detect anomalous points. Once those anomalous points are removed from the calibration set, a regression based on the least squared criterion was performed in order to obtain a correct evaluation of the slope (sensitivity) and the independent term of calibration. Both terms are important for judging the quality of calibration and, indeed, the analytical performance (Asturias, Alonso, Domínguez, & Arcos, 2011). In this way, a linear response in the range from 8 to 60 μM was obtained for His. The precision of the method was also calculated in terms of reproducibility and repeatability using the slopes of three calibration sets constructed using different biosensors and the same biosensor, respectively. The sensors showed an acceptable relative standard deviation (RSD) value of 3.5% ($n = 3$) and 2.5% ($n = 3$) for reproducibility and repeatability, respectively. The capability of detection was calculated for a probability of false positive (α) and negative (β) equal to 0.05, according to the literature (ISO11843, Part I 1997 and Part II 2000). This biosensor showed a capability of detection of 8.1 ± 0.7 μM for His.

3.2. Analytical application

The developed HMD/TTF/SPCEs biosensors were applied in the analysis of His content in fish and red wine samples. In this way, the viability of the HMD/TTF/SPCE biosensor was tested in the determination of the content of His in samples of red wine (9.3 mg/L (83.7 μM) of His, tested by HPLC), using the method of standard additions.

The presence of possible interferents in the wine samples was eliminated by using activated carbon as it has been described in Section 2.5. Different quantities of this compound were tested in order to find the best possible analytical conditions. In this way, 4, 8, 16, 50 and 100 mg of activated carbon were added to 1 mL of red wine and, a 50 mg treatment was found to be adequate.

Chronoamperograms were then recorded under the optimum conditions by means of an addition of a volume of 100 μL of pretreated wine into the electrochemical cell, containing 5 mL of buffer solution at pH 6.8. Next, successive additions of 40 μL of a 1 mM His solution were performed. The concentration of His found was 9.0 ± 0.7 mg/L ($\alpha = 0.05$, $n = 3$). This value is in good agreement with the one obtained by HPLC.

A similar procedure was applied for the analysis of a certified Canned Fish sample (FAPAS®, 8.7 mg/kg). The standard addition method was performed by means of the construction of calibration sets based on a first addition of 100 μL of FAPAS® into the electrochemical cell, containing 5 mL of buffer solution pH 6.8. Then, successive additions of 40 μL of a 1 mM His solution, were carried out. The concentration reported for this sample was 8.9 ± 0.4 mg/kg ($\alpha = 0.05$, $n = 4$), which agrees with the value certified by the supplier. Therefore, the developed HMD/TTF/SPCE is suitable for use in the determination of His in these types of complex samples, considering the associated uncertainties.

3.3. Simultaneous determination of histamine and putrescine using a dual-TTF/SPCEs biosensor (system II)

The main objective of this work has been the development of an electrochemical method for the simultaneous determination of His and Put. In this way, the dual-TTF/SPCEs system constructed as it has been described in Section 2.3 allowed the selective analysis of both BAs in a same sample.

As it has been described above, the optimum pH value for the analysis of His using HMD/TTF/SPCEs is 6.8. However, the determination of Put using PUO/TTF/SPCEs implies working at pH 10 values. Thus, a study of the optimum conditions for the simultaneous determination of both BAs was necessary. In this way, a pH 8 value was found to be adequate to obtain chronoamperometric responses with the necessary analytical quality.

The dual-TTF/SPCEs system resulted very selective towards His and Put determination. In this way, the response of both working electrodes (HMD/TTF/SPCE and PUO/TTF/SPCE) towards different BAs was analyzed. In the case of HMD/TTF/SPCE, this analysis was performed in the concentration range from 10 up to 200 μM for each BA. The BAs selected in this work were those most commonly found in food samples, namely Tyr, Cad, Put, Spd, Tryp and Spm. Under optimal operational conditions, the HMD/TTF/SPCE biosensor presented a slight signal for Put at concentration levels higher than 190 μM, this signal corresponds to 3% of that recorded for His in the same concentration (Fig. 3). In the same way the PUO/TTF/SPCE resulted very selective for the analysis of Put being only Tyr considered as an interferent at the concentration level of 150 μM.

The performance of the dual system has been also demonstrated by means of its application in the simultaneous analysis of His and Put in a complex matrix such as an octopus sample.

Fig. 4 shows the amperometric response recorded using the dual-TTF/SPCE system II developed biosensor for the simultaneous determination of His and Put by standard addition in octopus samples. The octopus samples analyzed were previously treated according the procedure described in Section 2.5. The extract obtained was dissolved in 2 mL of water. A volume of 200 μL of this extract was placed into the electrochemical cell, containing 5 mL of buffer solution pH 8, and following by successive additions of 20 μL of a 10 mM Put and 15 mM His solution.

The levels of Put and His found in octopus samples are shown in Table 2. These values are in good agreement with those obtained by HPLC. In this context, the concentration values obtained using dual-TTF/SPCEs and those obtained by HPLC are shown to be equal since the null hypothesis of the *t*-test cannot be rejected at the 95% confidence level. Thus, the developed dual biosensor is both accurate and suitable for the simultaneous determination of Put and His in complex samples.

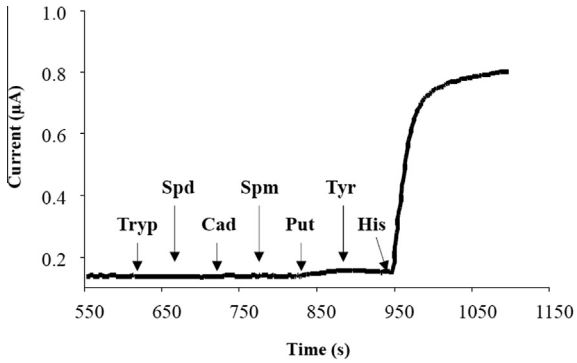


Fig. 3. Chronoampeometric response obtained on HMD/TTF/SPCEs for different BAs at a 190 μM concentration level (pH, 6.8; working potential, +130 mV *vs.* Ag/AgCl SPE).

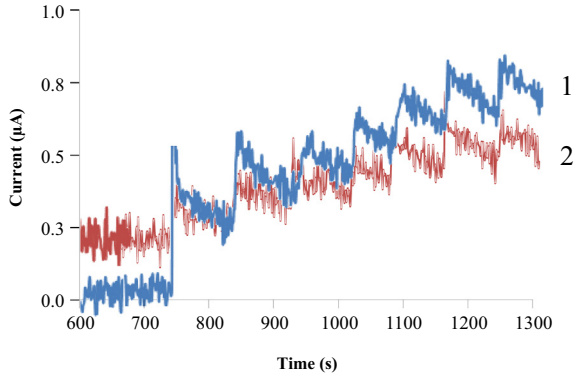


Fig. 4. Chronoamperograms recorded for the simultaneous determination of His and Put in octopus samples using a dual-TTF/SPCEs biosensor. (1) Amperometric response of Put on PUO//TTF/SPCE (pH, 8.0; working potential, +300 mV *vs.* Ag/AgCl SPE) and (2) Amperometric response of His on HMD/TTF/SPCE (pH 8.0; working potential, +130 mV *vs.* Ag/AgCl SPE). The first addition (720 s) corresponds to 200 μL of the sample. Successive additions correspond to a volume of 20 μL of a 10 mM Put and 15 mM His solution.

Table 2
Content of His and Put in octopus samples.

Biogenic amine	Analytical method	
	Dual-TTF/SPCEs (mg/kg; $\alpha = 0.05$, $n = 3$)	HPLC (mg/kg; $\alpha = 0.05$, $n = 3$)
Put	16 ± 1	16 ± 1
His	15 ± 1	13 ± 2

4. Conclusions

This work reports the development of a novel and very sensitive and selective biosensor for the determination of His based on the modification of SPCEs with HMD. The developed biosensor shows two important advantages: on one hand its disposable character, which makes it very useful in routine analysis of His. On the other hand the selectivity is sufficiently high to meet all analytical requirements for food samples. This selectivity is based on the employment of TTF as mediator but also in the very selective enzyme (HMD) used. The suitability of the disposable biosensor has been also demonstrated in the successful determination of His in samples of wine and fish requiring a very simple preparation of the sample.

Moreover the combination of two different biosensors in a dual electrodic system has permitted the simultaneous determination of two different BAs, Put and His, even in complex samples such as wine or fish. The described electrochemical dual biosensor allows a very novel, sensitive and selective determination of both compounds using a very simple methodology, which would permit a rapid screening and monitoring of both analytes in food industry.

Acknowledgements

Authors would like to acknowledge funding via Junta de Castilla y León (BU212A12-2), Ministerio de Ciencia e Innovación (MICINN) and Fondo Europeo de Desarrollo Regional (FEDER) INNPACTO (SERIBIO), TEC2009-12029 and TEC2013-40561-P. Wilder Henao Escobar would like also to acknowledge funding through his MICINN scholarship FPI.

References

Alonso-Lomillo, M. A., Domínguez-Renedo, O., Matos, P., & Arcos-Martínez, M. J. (2010). Disposable biosensors for determination of biogenic amines. *Analytica Chimica Acta, 665*, 26–31.

Asturias, L., Alonso, M. A., Domínguez, O., & Arcos, M. J. (2011). CYP450 biosensors based on screen-printed carbon electrodes for the determination of cocaine. *Analytica Chimica Acta, 685*, 15–20.

Asturias-Arribas, L., Alonso-Lomillo, M. A., Domínguez-Renedo, O., & Arcos-Martínez, M. J. (2013). Screen-printed biosensor based on the inhibition of the acetylcholinesterase activity for the determination of codeine. *Talanta, 111*, 8–12.

Bóka, B., Adányi, N., Virag, N., Sebela, M., & Kiss, A. (2012). Spoilage detection with biogenic amine biosensors, comparison of different enzyme electrodes. *Electroanalysis, 24*, 181–186.

Calvo-Pérez, A., Domínguez-Renedo, O., Alonso-Lomillo, M. A., & Arcos-Martínez, M. J. (2013). Disposable amperometric biosensor for the determination of tyramine using plasma amino oxidase. *Microchimica Acta, 180*, 253–259.

Calvo-Pérez, A., Domínguez-Renedo, O., Alonso-Lomillo, M. A., & Arcos-Martínez, M. J. (2014). Speciation of chromium using chronoamperometric biosensors based on screen-printed electrodes. *Analytica Chimica Acta, 833*, 15–21.

Chemnitius, G. C., & Bilitewski, U. (1996). Development of screen-printed enzyme electrodes for the estimation of fish quality. *Sensors and Actuators B: Chemical, 32*, 107–113.

del Torno-de Román, L., Alonso-Lomillo, M. A., Domínguez-Renedo, O., & Arcos-Martínez, M. J. (2013). Gluconic acid determination in wine by electrochemical biosensing. *Sensors and Actuators B: Chemical, 176*, 858–862.

del Torno-de Román, L., Alonso-Lomillo, M. A., Domínguez-Renedo, O., & Arcos-Martínez, M. J. (2015). Dual biosensing device for the speciation of arsenic. *Electroanalysis, 27*, 302–308.

Di Fusco, M., Federico, R., & Boffi, A. (2011). Characterization and application of a diamine oxidase from *Lathyrus sativus* as component of an electrochemical biosensor for the determination of biogenic amines in wine and beer. *Analytical Bioanalytical Chemistry, 401*, 707–716.

Dominguez-Renedo, O., Alonso-Lomillo, M. A., & Arcos-Martínez, M. J. (2007). Recent developments in the field of screen-printed electrodes and their related applications. *Talanta, 73*, 202–219.

Domínguez-Renedo, O., & Arcos-Martínez, M. J. (2007). Anodic stripping voltammetry of antimony using gold nanoparticle-modified carbon screen-printed electrodes. *Analytica Chimica Acta, 589*, 255–260.

Gezginc, Y., Akyol, I., Kuley, E., & Özogul, F. (2013). Biogenic amines formation in Streptococcus thermophilus isolated from home-made natural yogurt. *Food Chemistry, 138*, 655–662.

Henao-Escobar, W., Domínguez-Renedo, O., Alonso-Lomillo, M. A., & Arcos-Martínez, M. J. (2013a). A screen-printed disposable biosensor for selective determination of putrescine. *Microchimica Acta, 180*, 687–693.

Henao-Escobar, W., Domínguez-Renedo, O., Alonso-Lomillo, M. A., & Arcos-Martínez, M. J. (2013b). Simultaneous determination of cadaverine and putrescine using a disposable monoamine oxidase based biosensor. *Talanta, 117*, 405–411.

Henao-Escobar, W., Domínguez-Renedo, O., Alonso-Lomillo, M. A., Cascalheira, J. F., Dias-Cabral, A. C., & Arcos-Martínez, M. J. (2015). Characterization of a disposable electrochemical biosensor based on putrescine oxidase from *Micrococcus rubens* for the determination of putrescine. *Electroanalysis, 27*, 368–377.

Hernández-Orte, P., Peña-Gallego, A., Ibarz, M. J., Cacho, J., & Ferreira, V. (2006). Determination of the biogenic amines in musts and wines before and after malolactic fermentation using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate as the derivatizing agent. *Journal of Chromatography A, 1129*, 160–164.

ISO11843, (Part I 1997 and Part II 2000). Capability of detection, Genève, Switzerland.

Keow, C. M., Abu Bakar, F., Salleh, A. B., Heng, L. Y., Wagiran, R., & Bean, L. S. (2007). An amperometric biosensor for the rapid assessment of histamine level in tiger prawn (*Penaeus monodon*) spoilage. *Food Chemistry, 105*, 1636–1641.

Keow, C. M., Abu Bakar, F., Salleh, A., Heng, L. Y., Wagiran, R., & Siddiquee, S. (2012). Screen-printed histamine biosensors fabricated from the entrapment of diamine oxidase in a photocured poly(HEMA) film. *International Journal of Electrochemical Science, 7*, 4702–4715.

Kivirand, K., & Rinken, T. (2011). Biosensor for biogenic amines: The present state of art Mini-Review. *Analytical Letters, 44*, 2821–2833.

Lange, J., & Wittmann, C. (2002). Enzyme sensor array for the determination of biogenic amines in food samples. *Analytical Bioanalytical Chemistry, 372*, 276–283.

Leuschner, R. G. K., Hristova, A., Robinson, T., & Hugas, M. (2013). The Rapid Alert System for Food and Feed (RASFF) database in support of risk analysis of biogenic amines in food. *Journal of Food Composition and Analysis, 29*, 37–42.

López, R., Tenorio, C., Gutiérrez, A. R., Garde-Cerdán, T., Garijo, P., González-Arenzana, L., et al. (2012). Elaboration of Tempranillo wines at two different pHs. Influence on biogenic amine contents. *Food Control, 25*, 583–590.

Molinero-Abad, B., Alonso-Lomillo, M. A., Domínguez-Renedo, O., & Arcos-Martínez, M. J. (2014). Malate quinone oxidoreductase biosensors based on tetrathiafulvalene and gold nanoparticles modified screen-printed carbon electrodes for malic acid determination in wine. *Sensors and Actuators B: Chemical, 202*, 971–975.

Moret, S., Smela, D., Populin, T., & Conte, L. S. (2005). A survey on free biogenic amine content of fresh and preserved vegetables. *Food Chemistry, 89*, 355–361.

Muresan, L., Ronda, R., & Frébort, I. (2008). Amine oxidase amperometric biosensor coupled to liquid chromatography for biogenic amines determination. *Microchimica Acta, 163*, 219–225.

Önal, A., Tekkeli, S. E. K., & Önal, C. (2013). A review of the liquid chromatographic methods for the determination of biogenic amines in foods. *Food Chemistry, 138*, 509–515.

Ordoñez, J. L., Callejon, R. M., Morales, M. L., & Garcia-Parrilla, M. C. (2013). A survey of biogenic amines in vinegars. *Food Chemistry, 141*, 2713–2719.

Peeters, M., Troost, F. J., Mingels, R. H., Welsch, T., van Grinsven, B., Vranken, T., et al. (2013). Impedimetric detection of histamine in bowel fluids using synthetic receptors with pH-optimized binding characteristics. *Analytical Chemistry, 85*, 1475–1483.

Pérez, S., Bartrolí, J., & Fábregas, E. (2013). Amperometric biosensor for the determination of histamine in fish samples. *Food Chemistry, 141*, 4066–4072.

Piermarini, S., Volpe, G., Federico, R., Moscone, D., & Palleschi, G. (2010). Detection of biogenic amines in human saliva using a screen-printed biosensor. *Analytical Letters, 43*, 1310–1316.

Ratautaite, V., Nesladek, M., Ramanaviciene, A., Baleviciute, I., & Ramanavicius, A. (2014). Evaluation of histamine imprinted polypyrrole deposited on boron doped nanocrystalline diamond. *Electroanalysis, 26*, 2458–2464.

Rosini, E., Tonin, F., Vasylieva, N., Marinesco, S., & Pollegioni, L. (2013). Evolution of histamine oxidase activity for biotechnological applications. *Applied Microbiology and Biotechnology, 98*, 739–748.

Rousseeuw, P. J., & Leroy, A. M. (1987). *Robust regression and outlier detection*. New York; Chichester: Wiley.

Telsnig, D., Kalcher, K., Leitner, A., & Ortner, A. (2013). Design of an amperometric biosensor for the determination of biogenic amines using screen printed carbon working electrodes. *Electroanalysis, 25*, 47–50.

Telsnig, D., Terzic, A., Krenn, T., Kassarnig, V., Kalcher, K., & Ortner, A. (2012). Development of a voltammetric amine oxidase-modified biosensor for the determination of biogenic amines in food. *International Journal of Electrochemical Science, 7*, 6893–6903.

Tsutsumi, M., Tsuse, N., Fujieda, N., & Kano, K. (2010). Site-directed mutation at residues near the catalytic site of histamine dehydrogenase from Nocardioides simplex and its effects on substrate inhibition. *Journal of Biochemistry, 147*, 257–264.

Yigit, M., & Ersoy, L. (2003). Determination of tyramine in cheese by LC–UV. *Journal of Pharmaceutical and Biomedical Analysis, 31*, 1223–1228.

Young, J. A., Jiang, X., & Kirchhoff, J. R. (2013). Amperometric detection of histamine with a pyrroloquinoline-quinone modified electrode. *Electroanalysis, 25*, 1589–1593.