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ORIGINAL PAPER

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

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Abstract In this work, we have characterized a diamine oxidase (DAO) from *Lathyrus sativus* and evaluated its use, for the first time, as biocatalytic component of an electrochemical biosensor for the determination of biogenic amines index in wine and beer samples. Firstly, DAO was electrokinetically characterized free in solution by means of a platinum electrode and then immobilized by using polyazetidine prepolimer on the surface of screen-printed electrodes constituted of two gold working electrodes. The amperometric measurements were carried out by using a flow system at a fixed potential of +600 mV vs the internal silver pseudo reference in phosphate buffer solution (0.1 mol l^{-1}, pH=7.4). The analysis of wine and beer samples were performed in flow injection system using the dual channel transducer providing simultaneous detection of sample and blank signal, and the resulting signal (after subtraction of the blank signal) was referred to that of putrescine. The results were compared with those obtained using a modified reference method based on gas chromatography-mass spectrometry analysis on the same samples. The results obtained in the analysis of Italian wines shows the better suitability of DAO-based biosensor in the determination of the biogenic amines (BAs) index expressed as putrescine equivalent in both red and white wines, being less efficient in beer samples where it underestimates by about 50% the BAs content.

Keywords Diamine oxidase · Biogenic amines index · Biosensors · Wine

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Introduction

Biogenic amines (BAs) are nitrogen-containing compounds produced during fermentation, from the decarboxylation of amino acids by yeast and bacteria [1, 2]. The main biogenic amines are tyramine, histamine, putrescine, cadaverine, tryptamine, and spermine (from which derives spermidine) synthesized by decarboxylation of the amino acids tyrosine, histidine, ornithine, lysine, tryptophan, phenylalanine, and arginine, respectively.

BAs have long been recognized as responsible for health problems in humans such as allergic or asthmatic events or food intolerances and are also potential precursors of the formation of carcinogenic *N*-nitroso compounds [3]. Formation of biogenic amines can occur during food processing and storage as a result of bacterial activities. Consequently, higher amounts of certain amines may be found in food as a consequence of the use of poor

quality raw materials, microbial contamination, and inappropriate food processing conditions or microbial contamination and inadequate storage conditions. There is evidence that a decrease in the product hygienic quality corresponds to an increase of biogenic amines content. For these reasons, monitoring the BAs content in food and beverages is becoming increasingly demanded by regulatory commissions (e.g., Commission Regulation (EC) 2073/2005). Under normal conditions, BAs are neutralized by the action of specific enzymes in the gut (e.g., diamine oxidase (DAO) metabolizes histamine [4], while the monoamine oxidase (MAO) metabolizes tyramine [5]). However, some pharmacological substances (MAO and DAO inhibitors) or foods in decomposition, rich of cadaverine and putrescine, may disrupt this mechanism [6–8].

BAs concentration is higher in fast-perishable foods, especially if fermented and rich in amino acids, such as fish, meat, fruit juices, wine, beer, cocoa, milk, and cheese [3]. In particular, BAs may be present in wines and beer at highly variable concentrations. An increase in BAs content often occurs in wines after malolactic fermentation, and it depends on the particular fermentation strain used [9–13]. Moreover, other conditions could increase the content of biogenic amines and therefore must be kept under control: poor hygiene during the fermentation period [14], long contact between wine and lees [15], high temperature [16], and high pH [17]. In beers, putrescine, spermine, and spermidine are naturally present because malt is a source of these substances, while tyramine, histamine, and cadaverine are formed during fermentation due to the contamination of lactic bacteria [14].

There are great numbers of analytical methods used for the determination of BAs for food and beverages samples [18, 19], and they are mainly based on gas chromatography [20, 21], high-performance liquid chromatography [22–29] with pre-column [30, 31] or post-column [32] derivatization, ion-pair chromatography [33, 34], and capillary electrophoresis [35–38]. For the detection, fluorescence [39–42], UV [43–45], mass-spectrometric [46, 47], and electrochemical detectors [34] are used. In all these analytical determinations, the analysis of BAs is not a simple task in that it entails a derivatization step or an extraction procedure or some other sample pretreatment.

Electrochemical biosensors for the determination of BAs have attracted much attention in the last years for their simplicity, reproducibility, low cost, short analysis time, and low detection limit; moreover, they could provide an upfront method for BAs determination, useful for field analysis in food and beverage industries. Biosensors are essentially based on the catalytic capabilities of amine oxidase [48, 49], diamine oxidase [50–57], or monoamine oxidase [58] and were used to determine BAs in food samples mainly on seafood or cheese.

Amine oxidases are a class of enzymes that catalyze the oxidation of primary amines in aldehydes, in the presence of molecular oxygen as electrons acceptor, with the formation of ammonia (NH_3) and hydrogen peroxide (H_2O_2). The enzymatic reaction can be followed with amperometric detection by means of electrochemical oxidation of H_2O_2 at 700 mV vs Ag/AgCl (Scheme 1).

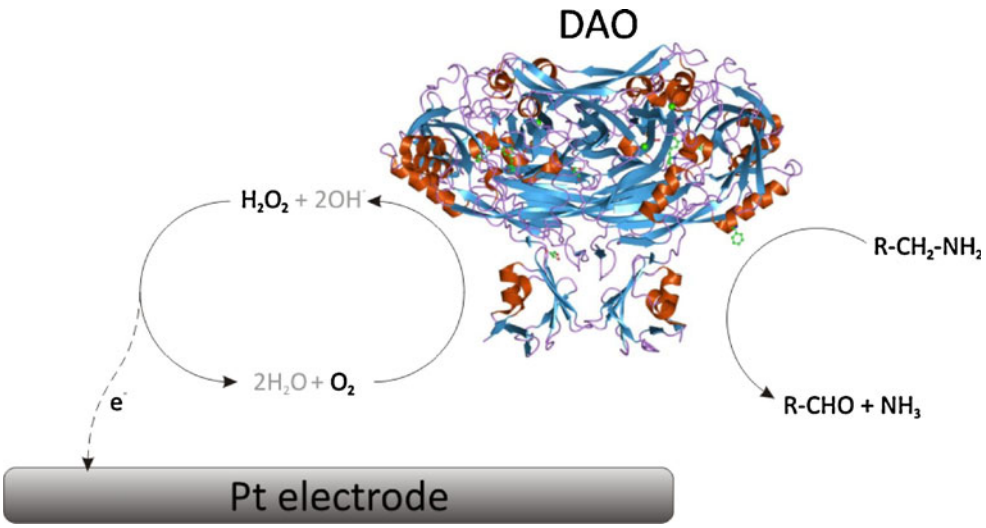
In this work, a diamine oxidase from *Lathyrus sativus* (EC 1.4.3.22) was electrokinetically characterized both free in solution and, for the first time, immobilized in a polyazetidine prepolimer (PAP) membrane, already used for analogous purpose in other works [59–62]. Hence, DAO was used to develop an electrochemical biosensor whose analytical performances were evaluated; finally, it was applied to the determination of the total amount of BAs in wine and beer samples. These samples are complex matrices with a lot of potentially interfering compounds. For this reason, we have developed an amperometric biosensor employing a double working electrode connected to a bipotentiostat able to measure two working channels simultaneously. This allows to subtract the sample signal (BAs+interference) to the blank one (interference) making possible to determine the total amount of BAs in the sample under analysis. Results are compared with a standardized gas chromatography-mass spectrometry (GC-MS) method on the same samples.

Experimental

Materials

Diamine oxidase from *L. sativus* (EC 1.4.3.22, activity, 131 U ml^{-1}; concentration, 2 mg ml^{-1}, Mr=148 kDa) was obtained from Molirom srl (Rome, Italy). Bovine serum albumin (BSA, EC 232-936-2), histamine, cadaverine, putrescine, spermine, and spermidine were purchased from Sigma and used as received. The polymeric film employed for protein entrapping was poly-1-(aminomethyl)-1-{2-[(6-oxyesane)amino]ethyl}-3-hydroxyazetidinium chloride (polyazetidine prepolymer, PAP®), donated by Hercules Inc. Wilmington DE (USA). Other chemicals were all of analytical grade. High purity deionized water (resistance, 18.2 MΩ×cm at 25 °C; TOC<10 μg/L) obtained from Millipore (France) has been used to prepare all the solutions.

Different commercial wine and beer samples were acquired from local market. In the samples, the appropriate amount of phosphate salts was added to finally have a concentration of 0.1 mol l^{-1} phosphate, and then the pH value was adjusted to 7.4 using NaOH.



Scheme 1 The enzymatic reaction can be followed amperometrically by measuring the electrochemical oxidation current of H_2O_2

Apparatus

Electrokinetic experiments were performed by using a μ-Autolab type III potentiostat (Eco Chemie) controlled by means of the GPES Manager program (Eco Chemie) with a conventional three-electrode configuration. Platinum screen-printed electrodes (Pt-SPE, diameter of 1 mm; BVT Technologies (Brno, Czech Republic)) were used for the kinetic measurements with immobilized DAO, while a platinum disk (Pt, diameter of 3 mm, Metrohm, Switzerland) was used for the measurements with DAO free in solution. Graphite was used as counter electrode and Ag/AgCl (KCl_{sat}) (Metrohm, Switzerland, 198 mV vs NHE) as reference electrode.

The measurements with real samples were performed by using a μStat 400 bipotentiostat (DropSens (Oviedo, Spain)) controlled by means of the DropView 2.0 Software (DropSens). Screen Printed Electrodes (DualAu-SPE) (DropSens), constituted by two gold working electrode, gold as counter electrode and silver as pseudoreference one, were used.

GC-MS analyses were performed with an Agilent 6850A gas chromatograph coupled to a 5973 N quadrupole mass selective detector (Agilent Technologies, Palo Alto, CA, USA).

Chromatographic separations were carried out with an Agilent HP5ms fused-silica capillary column (30 m× 0.25 mm i.d.) coated with 5%-phenyl-95%-dimethylpolysiloxane (film thickness 0.25 μm) as stationary phase. Injection mode: splitless at a temperature of 260 °C. Column temperature program was at 70 °C (2 min) then to 300 °C at a rate of 15 °C/min and held for 5 min. The carrier gas was helium at a constant flow of 1.0 ml/min. The spectra were obtained in the electron impact mode at 70 eV ionization energy; ion source, 280 °C; ion source vacuum, 10^{-5} Torr.

MS analysis was performed simultaneously in TIC (mass range scan from *m/z* 50 to 800 at a rate of 0.42 scans s^{-1}) and SIM mode. For GC-SIM-MS analysis three characteristic ions were selected for each of the amines analyzed.

Biosensors preparation

DAO-based Pt and Pt-SPE biosensors were prepared by spreading 3 μl of a solution, containing 2 μl of enzyme 131 U ml^{-1} and 1 μl of PAP, onto the platinum working electrode surface to have a final amount of 0.26 U.

DAO-based dual Au-SPE biosensors were prepared by spreading 3 μl of a solution, containing 2 μl of enzyme 131 U ml^{-1} and 1 μl of PAP, onto one gold working electrode surface and a solution, containing 2 μl of BSA 2 mg ml^{-1} and 1 μl of PAP, onto the other gold working electrode surface.

All the electrodes were left to dry overnight at room temperature and, when not in use, were stored at 4 °C.

Electrochemical measurements

Chronoamperometric experiments were carried out at a fixed potential of +700 mV vs Ag/AgCl (KCl sat.) or +600 mV vs the internal silver pseudo reference. The potential of the internal pseudoreference electrode was checked in the chosen experimental conditions using ferrocyanide 1 mM as electrochemical probe, and it was determined to be +123 vs Ag/AgCl (KCl sat.). The kinetic measurements were performed under constant magnetic stirring in a 10-ml thermostated glass cell, at 25 °C in phosphate buffer solution 0.1 mol l^{-1}, pH=7.4. In the measurements with DAO free in solution, 2 μl of enzymatic solution were added to 2.5 ml of the buffer contained in measuring cell in order to have a final amount of 0.1 U ml^{-1}. Flow injection experiments were carried out using a

Table 1 Kinetic characteristics obtained with DAO free in solution, in phosphate buffer solution 0.1 M, pH 7.4

Substrate	v_{max} (mM s^{-1})	K_M (mM)	v_{max}/K_M (nA mM^{-1})
Histamine	$(2.53\pm0.02)\times10^{-4}$	$(1.12\pm0.06)\times10^{-1}$	2.26×10^{-3}
Cadaverine	$(3.42\pm0.24)\times10^{-3}$	$(1.72\pm0.20)\times10^{-1}$	1.99×10^{-2}
Putrescine	$(2.46\pm0.19)\times10^{-3}$	$(2.19\pm0.17)\times10^{-1}$	1.12×10^{-2}
Spermine	$(1.38\pm0.12)\times10^{-4}$	$(5.87\pm0.29)\times10^{-2}$	2.35×10^{-3}
Spermidine	$(7.60\pm0.32)\times10^{-4}$	$(1.00\pm0.06)\times10^{-1}$	7.60×10^{-3}

suitable microliter cell (DropSens) connected to an Omnifit sample injection valve (DropSens) and a Gilson Minipuls-3 peristaltic pump with a flow rate of 4 μl s^{-1}. The carrier buffer was 0.1 mol l^{-1} phosphate solution, pH 7.4 and aliquots of 250 μl of putrescine standard solutions at different concentrations in the same buffer were used to obtain the calibration plot.

Analysis of real samples by electrochemical measurements

The analysis of wine and beer samples were performed in flow injection system using the dual channel transducer providing simultaneous detection of sample and blank signal; appropriately diluted samples were injected in the microliter cell by means of the injection valve, and the resulting signal (after subtraction of the blank signal) was referred to that of putrescine.

Analysis of real samples by GC-MS

Amines were analyzed according to the method of Paik et al. [63] with slight modification. Briefly, 0.5 ml aliquots of wine, spiked with internal standard (IS) 1,6-diaminohexane (300 ng), were added with 0.5 ml 5 M NaOH and immediately subjected to sequential *N*-ethoxycarbonylation and *N*-pentafluoropropionylation. *N*-ethoxycarbonylation was performed in one step by adding to the aqueous phase ethyl chloroformate (20 μl), dissolved in dichloromethane (1 ml). After vortex mixing (2 min), the mixture was saturated with NaCl and extracted with diethyl ether (3 ml) and ethyl acetate (2 ml). The organic extracts were combined and dried under reduced pressure. Then, the sample was subjected to the second derivatization step by adding 20 μl pentafluoropropionyl anhydride (65 °C for 60 min).

A stock solution of the seven amines was prepared at concentration of 10 mg ml^{-1}, each in 0.1 M HCl. This solution was used to prepare working solutions ranging from 10 to 100 ng ml^{-1} in HCl 0.1 M. The internal standard working solution was prepared by dissolving stock solution of 1,6-diaminohexane at 15 ng μl in 0.1 M HCl. The calibration samples were prepared at eight different concentrations in the range 25–20,000 ng ml^{-1}.

Results and discussion

DAO free in solution

The free enzyme in solution was bioelectrochemically characterized in the presence of several typical substrates.

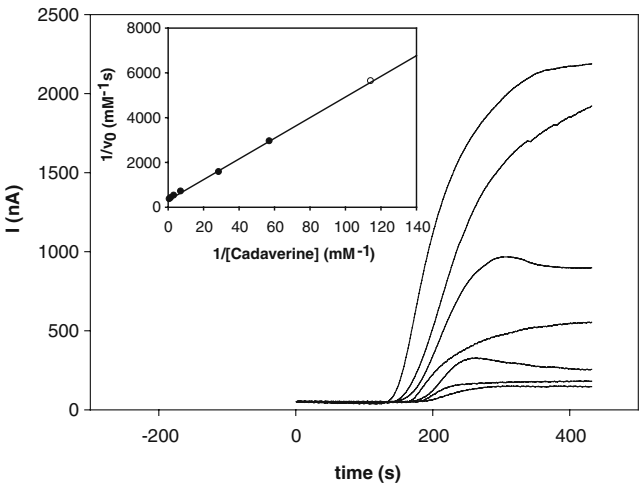


Fig. 1 Amperometric response using 0.26 U of DAO free in solution for different additions of cadaverine. *Inset*: linearization of Lineweaver-Burk

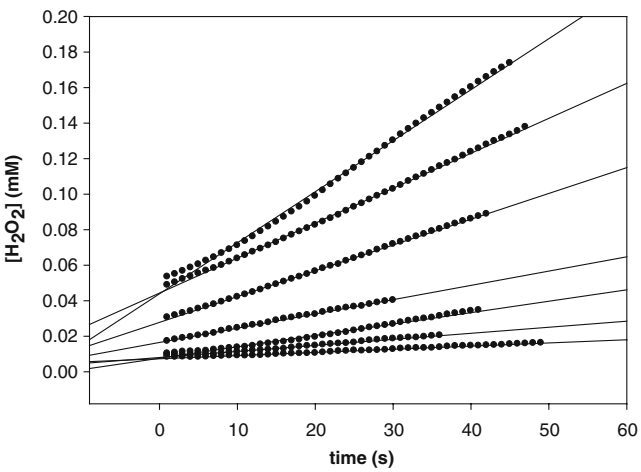


Fig. 2 Initial rates extrapolated from the amperometric responses of DAO free in solution with different additions of cadaverine

Table 2 Kinetic and analytical characteristics obtained with DAO-modified electrodes in phosphate buffer solution 0.1 mol l^{-1}, pH 7.4

Substrate	I_{max} (nA)	K_M^{app} (mM)	I_{max}/K_M^{app} (nA mM^{-1})	*h* Value	Sensitivity (nA mM^{-1})
Histamine	386.7±17.8	1.05±0.05	368.3	1.02	305.5±22.3
Cadaverine	748.4±24.9	2.84±0.12	263.5	0.99	294.7±1.2
Putrescine	633.4±16.7	1.58±0.03	400.9	1.01	354.7±10.2
Spermine	295.8±7.7	1.14±0.08	259.5	1.04	228.3±1.7
Spermidine	590.4±23.6	1.77±0.19	333.6	1.02	231.4±16.3
Tyramine	117.7±0.6	0.53±0.03	222.1	1.03	185.3±0.7
Phenylethylamine	126.1±2.2	0.84±0.02	150.1	1.01	114.9±9.1

The kinetic parameters were obtained by Lineweaver–Burk linearization of the classical Michaelis–Menten kinetics. In these experiments, the variation of H_2O_2 produced by the enzymatic reaction was monitored by detecting the corresponding oxidation current at a fixed potential. Before every measurement, it was necessary to construct a calibration plot for hydrogen peroxide under the same conditions: this allowed to modify the I vs. time responses obtained from the experiments, in $[H_2O_2]$ vs. time curves and consequently, to determine the initial rate of the enzymatic reaction (v_0).

In Table 1, the main kinetic parameters obtained with a series of substrates and DAO free in solution are summarized. In Figs. 1 and 2, the amperometric responses obtained for different concentration of cadaverine and the slopes determined from them for v_0 calculation are reported, respectively.

Comparing the data reported in Table 1, obtained with the use of DAO from *L. sativus* (LSAO), with those reported in literature for amine oxidase from *Lathyrus cicera* (LCAO), [64] which belongs to the same enzymatic family, it can be observed that their behaviors are quite similar.

Immobilized DAO

The determination of the kinetic parameters of immobilized DAO was carried out using histamine, cadaverine, putrescine, spermine, spermidine, tyramine, and phenylethylamine as substrates. In order to evaluate the applicability of the Michaelis–Menten approach to describe the kinetic behaviour of immobilized DAO, we have employed the Hill's equation [65]. This equation is often used in enzyme kinetics to describe the dependence of the steady-state rate of an enzymatic reaction on the substrate concentration (Eq. 1):

$$v = v_{max}\left([S]/[S]_{0.5}\right)^h / \left\{\left(1 + [S]/[S]_{0.5}\right)^h\right\} \quad (1)$$

Where v_{max} is the maximum rate, [S] the substrate concentration, and $[S]_{0.5}$ the substrate concentration producing half occupation of the binding sites and *h* is the Hill coefficient, describing the cooperativity of the system enzyme-substrate. At $h=1$, the enzymatic reaction is non-cooperative reaction, meaning that once one ligand molecule is bound to the enzyme, its affinity for other ligand molecules neither increases nor decreases; in this case, the

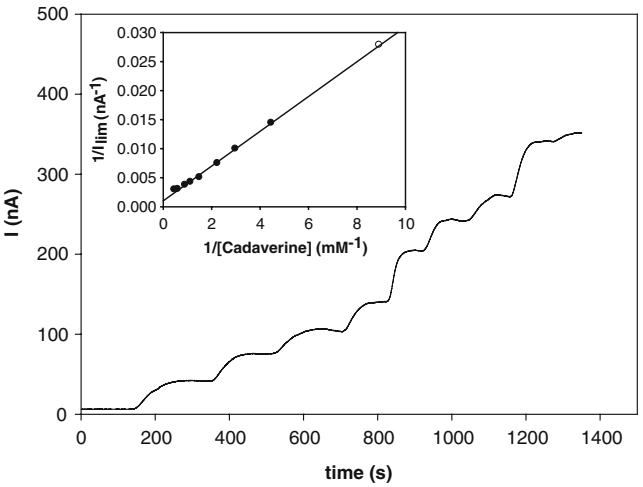


Fig. 3 Amperometric response using DAO-modified Pt-SPE electrode for different additions of cadaverine. *Inset*: linearization according to Lineweaver-Burk

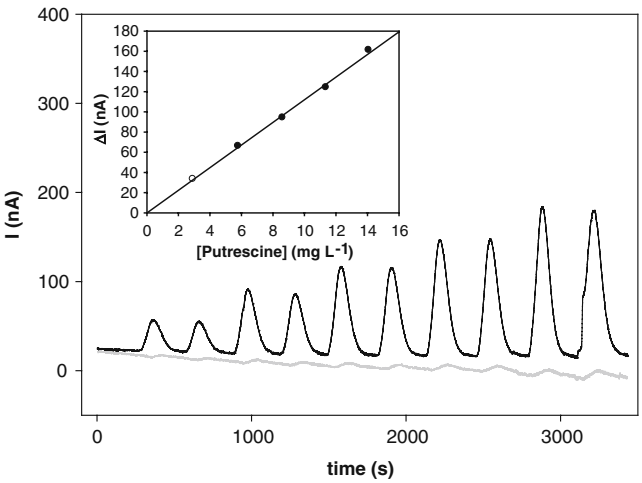


Fig. 4 Amperometric responses using DualAu-SPE for five double additions of putrescine obtained with DAO-PAP (*black line*) and BSA-PAP (*gray line*). *Inset*: calibration plot

Table 3 Total amount of BAs in wine and beer obtained in batch and flow injection systems

Sample	[Total BAs as putrescine] mg l^{-1}	
	FI	GC-MS
White wine 1	15.0±0.8	12.6±0.5
White wine 2	6.6±0.7	6.0±0.4
Red wine 1	26.5±6.5	21.8±1.6
Red wine 2	43.0±5.4	44.9±3.1
Blonde beer 1	7.8±2.2	15.1±0.9
Blonde beer 2	3.8±0.1	8.1±0.8

Hill's equation corresponds to the classic Michaelis–Menten equation ($[S]_{0.5}$ corresponding to the Michaelis constant K_M). The Hill coefficient (h) was experimentally obtained for several different substrates by fitting the $\log(I/I_{max}-I)$ vs. $\log [S_{ox}]$ and h values ranging from 0.99 to 1.04 were obtained (Table 2). From these results, we can assess that DAO immobilized onto the electrode surface follows the Michaelis–Menten kinetic model.

The DAO-modified Pt-SPE electrodes were bioelectrochemically characterized in the presence of a series of different substrates. The kinetic parameters were calculated according to a simplified approach [66] which entails the application of Eq. 2 and its the Lineweaver-Burk-type linearization of as expressed in Eq. 3:

$$I_{lim} = \frac{I_{max}\,[S_{ox}]}{[S_{ox}] + K_M^{app}} \tag{2}$$

$$\frac{1}{I_{lim}} = \frac{1}{I_{max}} + \frac{K_M^{app}}{I_{max}\,[S_{ox}]} \tag{3}$$

where $[S_{ox}]$ is the concentration of the oxidized substrate, I_{lim} is the limiting current measured after every addition of substrate, K_M^{app} is the apparent Michaelis–Menten constant for the enzymatic reaction, and I_{max} is the steady-state current.

In Table 2, the data obtained with DAO-modified electrode towards several substrates are summarized; as an example, in Fig. 3, the Lineweaver-Burk linearization plot obtained for cadaverine is also reported.

As observed, the affinity of the enzyme (correlated to K_M^{app}) towards the selected substrates is essentially the same as with free enzyme in solution, with the exception of cadaverine. On the other hand, the efficiency (roughly evaluated by I_{max}/K_M^{app}) is maximum for putrescine, suggesting that the latter should be considered as a standard to be used for expressing the total content of BAs in real samples analysis.

Biosensor characterization

The DAO-biosensor was analytically characterized, using putrescine as standard, under flow injection conditions. In order to reduce the influence of matrix effect, which could affect the detection of biogenic amines in real samples, a special SPE employing two working electrodes was used as transducer. This dual-working electrode SPE coupled to the proper multimeter (μStat 400 bipotentiostat (Dropsens, Oviedo (Spain))) and driven by the DropView™ Software allows the simultaneous registration of both sample and blank signals and eventually to subtract the latter on the fly. Unfortunately, only a few limited types of dual-SPEs are available so far, and among them, a dual platinum SPE is still missing. Hence, a dual gold electrode, in place of platinum, was chosen although in the detection of hydrogen peroxide; gold entails a slight loss in the sensitivity [67], but it is still useful for our purposes.

The following analytical parameters were obtained using the dual-Au-SPE and putrescine as substrate: sensitivity 11.2±0.4 nA mg^{-1} l, linear range 0.7–20.0 mg l^{-1}, and LOD 0.2 mg l^{-1}. The corresponding chronoamperogram obtained and the calibration plot thereof are reported in Fig. 4.

The biosensor has been used for ten consecutive calibration plots without a significant loss of performances for the first five confirming a good reproducibility taking into account that it has been obtained using a screen-printed disposable transducer.

Table 4 Determination of biogenic amines in wine and beer obtained by GC/MS analysis, expressed as milligrams per liter of putrescine (n=3)

	Histamine	Cadaverine	Putrescine	Spermine	Spermidine	Tyramine	Phenyletylamine
White wine 1	0.39±0.04	0.16±0.01	8.0±0.2	Nd[a]	0.14±0.01	2.1±0.2	1.78±0.09
White wine 2	1.22±0.04	0.08±0.01	3.1±0.2	0.08±0.01	0.12±0.01	1.2±0.1	0.16±0.01
Red wine 1	3.9±0.2	0.43±0.03	12.7±0.5	0.09±0.01	0.24±0.03	4.1±0.4	0.27±0.01
Red wine 2	3.7±0.1	0.92±0.05	24.0±0.5	0.08±0.01	0.31±0.03	14.9±0.9	0.90±0.02
Blonde beer 1	0.8±0.1	0.88±0.03	7.5±0.2	0.80±0.03	1.8±0.1	3.2±0.6	0.12±0.03
Blonde beer 2	Nd[a]	0.36±0.02	5.6±0.3	0.39±0.01	0.57±0.01	1.13±0.09	0.07±0.01

[a] Below the limit of detection

Real samples analysis

In order to reduce any kind of interference, the analysis of real samples was carried out by a differential method employing the dual SPE-based biosensor above described. The measurements were carried out on two white wines, two red wines, and two blonde beers. The registered amperometric signals were referred to that one of putrescine thus obtaining the total amount of BAs, considering that the developed biosensor displays the highest catalytic efficiency towards putrescine (Table 1).

In Table 3, the results obtained with the different methods in the detection of BAs in real samples of wine and beer both using flow injection (FI) system are reported and compared with the results obtained by GC-MS as reference method. Also, in the case of GC-MS data, the total amount of BAs was expressed as putrescine even if this technique is able to provide the concentration of each different amine eventually present in the sample; in particular, for each real sample, the obtained amounts of phenylethylamine, tyramine, cadaverine, histamine, spermidine, and spermine were expressed in molar concentration, summed and multiplied by putrescine molecular weight thus obtaining the total amount of BAs as putrescine in milligrams per liter; data obtained for different amines are reported in Table 4.

By comparing the data obtained with the proposed biosensor either in flow conditions to those obtained with the reference method, it can be observed that in the case of both white and red wines the data are in a satisfactory agreement; conversely, in the case of blonde beers, data obtained using GC-MS are twice as much those obtained with FI. It must be remembered that the biosensor data are obtained as pool of all the biogenic amines present in the real sample even if they are expressed as total amount of putrescine; hence, they could be affected by a systematic error due to the different sensitivities of the enzyme chosen to assemble the biosensor towards the different amines (see Table 2); this could explain the underestimation obtained using biosensor method in the case of blonde beer. Taking into account the different combination of biogenic amines in the considered real samples (as detected by GC-MS, see Table 4), it can be assumed that, in beer, some interfering compounds could be present affecting the enzymatic efficiency. Being putrescine the most efficiently detected amine (by the biosensor) among those present in the sample, in presence of enzymatic inhibition putrescine is practically the only amine revealed thus explaining the underestimation of total BAs content occurring in the case of beers.

In this context, it should be borne in mind that biosensors are often accounted as upfront methods whose results could be used to identify a safe concentration level useful to locate those real samples that should be analyzed by a reference method.

In addition, if our biosensor performances are compared with those reported in literature using different diamine oxidase biosensors in food analysis (see Table 5), it emerges clearly that also in these cases a possible source of systematic error is represented by the relative lack of selectivity of this class of enzymes, leading to different sensitivities of biosensors towards the several biogenic amines usually present in real food samples. Nevertheless, those sensors have been proposed and applied to evaluate the freshness and quality of foods, although, in some cases, they display recovery values much below 50%.

Conclusions

Diamine oxidase from *L. sativus* (EC 1.4.3.22) was amperometrically characterized; the obtained results allowed to compare the enzyme to other amine oxidases and to evaluate the selectivity towards different typical

Table 5 Amperometric biosensors based on different diamine oxidases for determination of biogenic amines in real samples

Electrode	Sensitivity	Sample	E (V) vs Ag/AgCl	Recovery (%)	Ref.
Pt	0.304 AU $mM^{-1a,b}$ 0.259 AU $mM^{-1a,c}$ 0.236 AU $mM^{-1a,d}$	Fish fillet	+0.4	–	51
CP SPE[e]	5.56 nA ppm^{-1b}	Tiger prawn	+0.35	99	52
Pt	265.1 nA mM^{-1b} 114.2 nA mM^{-1c} 57.5 nA mM^{-1d}	Caciocavallo cheese and anchovy	+0.7	34–100	53
Pt	–	Anchovy	+0.65	84–126	55
Pt	1.95 nA mM^{-1b}	Fish	+0.7	–	56
GC[f]	1.45 nA mM^{-1b} 8.14 nA mM^{-1c} 8.05 nA mM^{-1d}	Cheese	+0.65	–	57

[a] (AU = arbitrary unit); [b] vs histamine; [c] vs putrescine; [d] vs cadaverine; [e] carbon paste screen printed electrode; [f] glassy carbon

biogenic amines. Secondly, the DAO was immobilized and employed to develop a novel DAO-based amperometric biosensor; the latter was characterized both from a kinetic and an analytical point of view, and it was tested for the analysis of BAs in real samples of beverages. Biosensor's performances were validated using a modified reference method based on GC-MS analysis concluding that the biosensor accurately measure the overall BAs content expressed as putrescine equivalent in both red and white wines, being less efficient in beer samples where it underestimates of about 50% the BAs content. The fast response, minimal sample treatment, and high sensitivity of the apparatus indicate that it may conveniently be employed within production plants or wineries in order to provide a reliable estimate of the overall BAs content, a parameter that is being increasingly demanded by food quality commissions worldwide.

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