Full Paper

On the Electrochemical Oxidation of Resveratrol

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Abstract
Resveratrol (3,5,4′-trihydroxystilbene) is an organic metabolite produced by plants in response to fungal infection. It is found in various plant fruits and is abundant in the skins of unripe grapes and related products. This photosensitive molecule exists in two isomeric forms, *trans* and *cis*-resveratrol. The antioxidant activity of resveratrol, both *trans* and *cis* forms, was evaluated by means of cyclic, differential pulse and square-wave voltammetry over a wide pH range, using a glassy carbon electrode. Voltammograms of resveratrol presented two oxidation peaks; the first oxidation peak corresponds to the oxidation of the phenol group and was shown to undergo an irreversible oxidation reaction. The second oxidation peak corresponds to the oxidation of the resorcinol moiety and is also irreversible. The influence of pH on the electrochemical oxidation process of resveratrol was investigated.

Keywords: *trans*-Resveratrol, *cis*-Resveratrol, Electrochemistry, pH dependent antioxidant activity

1. Introduction

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a naturally occurring phytoalexin produced by a limited number of plants as a response to stress or injury, fungal attack or UV exposure [1, 2]. It can be found in vines, eucalyptus, some pines and berries, in the roots of the Asian medicinal plant *Polygonum cuspidatum* [3], grapes, peanuts and their derived products.

Various studies reported resveratrol to have anticancer activity [4, 5], to prevent heart and blood diseases, to be a neuroprotective agent [6, 7], hormone [8], gene and enzyme modulator [9], and possibly to be a longevity activator [10]. Being a phenolic compound, it may act as an antioxidant that can protect cell membranes and cellular DNA from the damaging effects of free radical induced oxidation [11].

Resveratrol exists as *trans*- and *cis*-isomers (Scheme 1) and both isomeric forms have been detected in wines [12], with their concentration in a direct relationship to the weather conditions [1 – 3]. Physiologically, the *cis*- form seems to act in the same way as *trans*-resveratrol, although it is less effective [13].

Electrochemical measurements leading to the quantification and determination of physicochemical parameters of antioxidants are relevant for the evaluation of the antioxidative properties of polyphenols [14]. In order to act as an antioxidant, resveratrol itself has to undergo oxidation. In a scavenging reaction a hydrogen atom is donated to the radical that becomes a nonradical. The hydrogen atoms are supplied by dissociation of the −OH groups, a process that takes place both in scavenging reactions and electrochemical oxidation. Because the hydroxyl groups are electron donating, the phenoxyl radicals are preferred targets for the oxidants, but they may also form polymeric products that adsorb on the surface of the electrode [15, 16].

Solid state voltammetry consists in mechanically immobilizing microparticles of resveratrol onto the glassy carbon surface. Abrasive stripping voltammetry can be employed
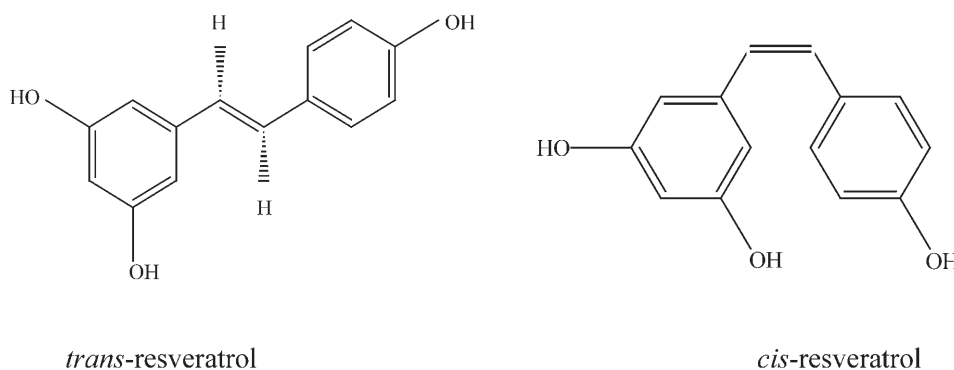
Scheme 1. Molecular structure of resveratrol *trans*- and *cis*-isomers.

for a direct study of solid samples that contain electrochemically active elements [17–20]. This method was used for an initial characterization of resveratrol electrochemical oxidation properties. The main disadvantage of this technique is the impossibility to know the amount of immobilized compound and therefore its concentration.

The mechanism of electrochemical oxidation of resveratrol, in the *trans-* and *cis-* configurations has been studied at a glassy carbon electrode using cyclic, differential pulse and square-wave voltammetry over a wide range of pH.

2. Experimental

2.1. Reagents

Resveratrol $C_{14}H_{12}O_2$ (3,5,4′-trihydroxystilbene) was purchased from Extrasynthèse, Genay, France and all the other reagents were Merck analytical grade. All the solutions were prepared with high-purity deionized water from a Millipore Milli-Q purification system (resistivity ≥ 18 MΩ/cm).

A stock solution of resveratrol, 4.4×10^{-3} M, was prepared in ethanol and kept at $-4\,°$C. This solution was diluted to the desired concentration by mixing buffer supporting electrolyte.

Solutions of buffer supporting electrolyte of ionic strength 0.2 M were used in all experiments (see Table 1), and were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system.

2.2. Instrumentation

The electrochemical experiments were done using a μAutolab type II PGSTAT running with GPES (General Purpose Electrochemical System) version 4.9 software PG (Eco-Chemie, Utrecht, The Netherlands).

Voltammograms were recorded using a three-electrode configuration (Cypress System, Inc., USA). The working electrode was a glassy carbon electrode (GCE) of 1.5 mm diameter; Ag/AgCl (saturated KCl) was used as a reference

Table 1. Supporting electrolyte solutions 0.2 M ionic strength.

Composition	pH
0.2 M HCl + 0.2 M KCl	1.2
0.2 M HCl + 0.2 M KCl	2.1
0.2 M NaAcO + 0.2 M HAcO	3.2
0.2 M NaAcO + 0.2 M HAcO	3.9
0.2 M NaAcO + 0.2 M HAcO	5.1
0.2 M Na$_2$HPO$_4$ + 0.2 M NaH$_2$PO$_4$	5.9
0.2 M Na$_2$HPO$_4$ + 0.2 M NaH$_2$PO$_4$	7.0
0.2 M Na$_2$HPO$_4$ + 0.2 M NaH$_2$PO$_4$	8.2
2 M NH$_4$Cl + 2 M NH$_3$	9.2
2 M NH$_4$Cl + 2 M NH$_3$	10.3
0.2 M KCl + 0.2 M NaOH	11.0
0.2 M KCl + 0.2 M NaOH	11.9

electrode and a platinum wire as a counter electrode. The electrodes were used in an electrochemical cell of 2 mL capacity.

UV measurements were performed with an UV-VIS Spectrophotometer SPECORD S100 running with Aspect Plus Version 1.5 (Analytik Jena GmbH, Jena, Germany).

The pH measurements were carried out with a CRISON GLP 21 pH-meter at room temperature.

2.3. Procedure

All experiments were performed at room temperature (ca. $23 \pm 2\,°$C) and in the presence of oxygen.

The solid-state electrochemistry of resveratrol was performed by attaching the aqueous insoluble solid to the surface of the working electrode by mechanical pressing.

Measurements in solution were carried out after diluting the resveratrol ethanol stock solution to the desired concentration by mixing buffer supporting electrolyte.

Cyclic voltammograms were recorded at scan rates of 25, 50 and 100 mV s^{-1}. The differential pulse voltammetry conditions were: pulse amplitude 50 mV, pulse width 70 ms and scan rate of 5 mV s^{-1}. Square-wave voltammetry conditions were: frequencies of 13, 25 and 50 Hz, amplitude 50 mV and a potential increment of 2 mV (effective scan rates of 25, 50 and 100 mV s^{-1}, respectively). A current response was recorded in the range -1.0 to $+1.35$ V. All potentials were reported vs. Ag/AgCl (sat KCl) electrode.

The glassy carbon working electrode was polished with diamond spray (3 and 1 μm) prior to each measurement and abundantly washed with deionized water.

3. Results and Discussion

3.1. Solid State Electrochemistry

The resveratrol molecule contains both resorcinol and phenol moieties. Because of the conjugated double bond between these, resveratrol has a planar structure (Scheme 1). All three functional −OH groups attached to the ring structures are electroactive.

Abrasive stripping voltammetry was done by mechanical immobilization of microparticles of *trans-*resveratrol onto the surface of the working electrode followed by immersion in electrolyte solution. Since the microcrystals of resveratrol are insoluble in water after mechanical pressing they remain attached to the electrode surface [17]. The measurements were carried out in the dark in order to ensure only the presence of the *trans-* isomer.

Cyclic voltammograms of *trans-*resveratrol at pH 3.2 showed the presence of two oxidation peaks associated with the two moieties of the molecule. Both peaks, P_1 at $+0.65$ V and P_2 at $+0.92$ V, are irreversible. The second scan shows the absence of both oxidation peaks probably due to the formation of a non-electroactive film on the surface of the electrode, Figure 1.

Both oxidation processes of resveratrol, observed using solid state cyclic voltammetry, are affected by the pH of the buffer aqueous solution, Figure 2, the peak potentials being displaced to more negative potentials with increasing pH values.

Square-wave voltammetry is a fast, low-consumption technique that can be employed with success for the examination of the solid electroactive species [21, 22]. The irreversibility of both peaks was confirmed over the whole pH range by solid state square-wave voltammetry measurements of mechanically attached resveratrol. The total current response shows two peaks with potentials close to those obtained by cyclic voltammetry, Figure 3. The starting potential was at $+0.2$ V for pH 3.2 acetate buffer and the
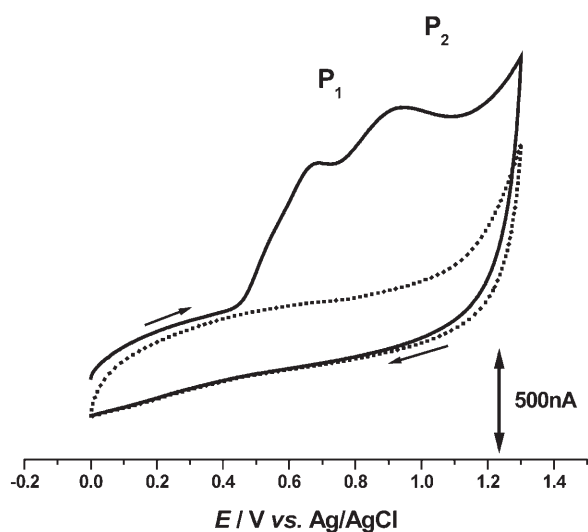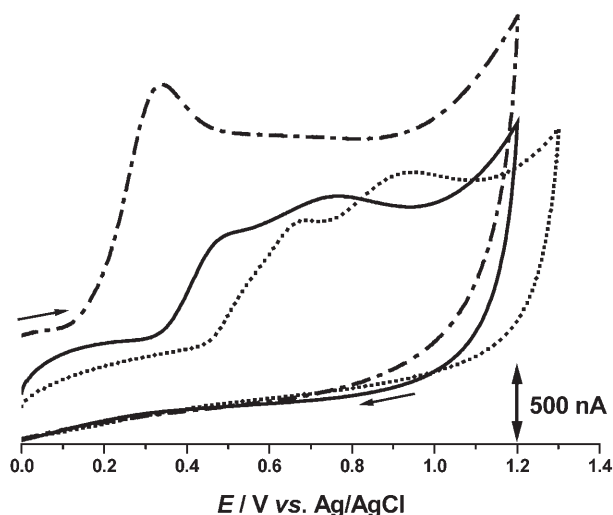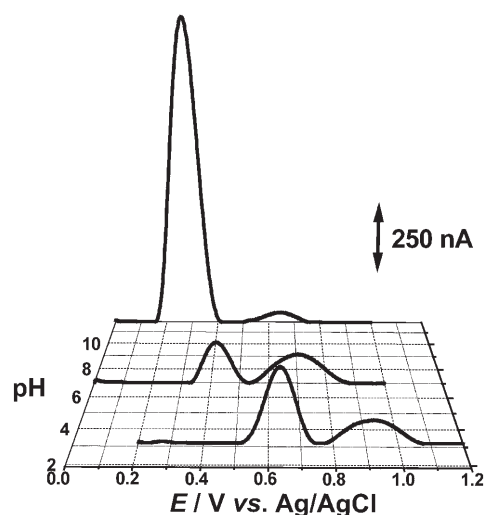

Fig. 3. 3D plot of solid state square-wave voltammograms of *trans*-resveratrol in different buffer solutions. Effective scan rate: 25mVs^{-1}.

frequency was 13 Hz, which corresponds to an effective scan rate of 25 mV s^{-1}. The forward scan clearly shows the presence of both oxidation peaks and the lack of backward current the behavior of an irreversible reaction.

3.2. Measurements of Resveratrol in Solution

Spectrophotometric and voltammetric measurements of resveratrol in a mixed ethanol/buffer solution were performed in order to complement the data obtained by solid state electrochemistry.

3.2.1. Spectrophotometric Measurements

The solution of *trans*-resveratrol was prepared and protected from light to avoid photon-induced isomerization and the voltammetric measurements were performed initially in the dark. UV spectra recorded after each set of electrochemical measurements confirmed the presence of only the *trans*- isomer. The UV spectrum exhibits two maxima at 306 nm and 320 nm, which are characteristic of the *trans*-isomer. The solution was then exposed to diffuse daylight for 8–10 h so the photo-isomerization occurred and a new UV spectrum was recorded, showing a maximum around 290 nm, characteristic of the *cis*- isomer, as reported in the literature [23].

3.2.2. Voltammetry of trans-Resveratrol and cis-Resveratrol

Differential pulse voltammograms were recorded for different pHs (Table 1) to confirm the dependence of the oxidation mechanism of resveratrol with pH. In all buffer solutions the voltammograms showed the presence of two oxidation peaks, as obtained by solid-state voltammetry. The oxidation of the phenol moiety occurs first, at a potential below $+0.7$ V depending on pH and corresponds

Fig. 1. Solid state cyclic voltammograms of *trans*-resveratrol in pH 3.2, 0.2 M acetate buffer solution: (—) first and (·····) second scan. Scan rate: 25 mV s^{-1}.

Fig. 2. Solid state cyclic voltammograms of *trans*-resveratrol in different buffer solutions: (·····) pH 3.2, (—) pH 7.0, and (·–·) pH 11.9. Scan rate: 25 mV s^{-1}.

to peak P_1. The resorcinol group is oxidized at higher positive potentials, but below $+0.9$ V, depending on pH and corresponds to peak P_2.

It can be observed in Figure 4a that a peak P_3 appeared at $+0.94$ V, in pH 3.2 acetate buffer. The oxidation peak current for both peaks decreased in the second scan, due to the adsorption of the oxidation products at the electrode surface, which are oxidized at $+0.40$ V, peak P_4.

A differential pulse voltammogram of resveratrol at pH 3.2 was recorded, where the scan was stopped at $+0.65$ V, immediately after peak P_1. The subsequent scans, recorded in the same conditions, showed the absence of any peaks related to the oxidation products of resveratrol.

Control measurements with resorcinol at pH 3.2 show only one peak in the first scan at $+0.82$ V, at almost the same potential as the peak P_2 of resveratrol, Figure 4b. In the second scan of resorcinol a peak appears, at $+0.45$ V, due to the oxidation of resorcinol oxidation products formed in the first scan.

These control experiments led to the conclusion that peak P_2 is due to the oxidation of the resorcinol moiety of resveratrol and that peak P_4 formed in the second scan is related to further oxidation of this oxidation product.

The peak P_3 of resveratrol only occurs in acid media and was not observed in the control measurements with resorcinol for the same pH. This behavior was exhibited by both trans- and cis-isomers, Figure 4c. For increasing pH values, peak P_3 diminishes and remains only as a small shoulder of P_2 over the rest of the pH range studied. The occurrence of peak P_3 can be explained by the rearrangement of the molecule of resveratrol, parallel and perpendicular on the electrode surface, due to the presence of the double bond between the phenol and resorcinol moieties, which leads to different oxidation potentials of the two hydroxyl groups presented by the resorcinol moiety.

Baseline-corrected differential pulse voltammograms of trans-resveratrol as a function of pH, Figure 5, show a decrease of the oxidation potentials with increasing pH. It was also observed that peak P_1 gives higher currents in basic media, whereas for peak P_2 the currents were higher in acid and neutral media. The number of electrons involved in the oxidation process, using the relation of peak width at half height [24], was determined to be $1e^-$ for peak P_1 and $2e^-$ for P_2, over the whole pH range studied.

The same study was done for cis-resveratrol and only a small difference was observed in the peak potentials which is not sufficient to allow a distinction between trans- and cis-isomers by means of electrochemistry, Figure 6. The relation between the oxidation potential E_p and pH is linear with a slope of -45 mV per pH unit for peak P_1, for both trans- and
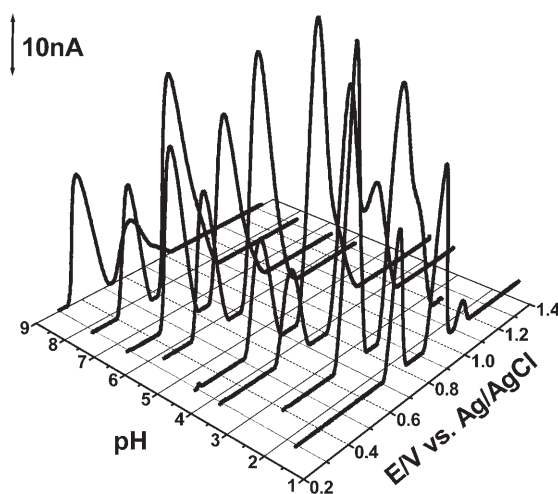

Fig. 5.   3D plot of differential pulse voltammograms of 10 μM trans-resveratrol in ethanol/buffer supporting electrolyte as a function of pH. Scan rate: 5 mV s^{-1}.
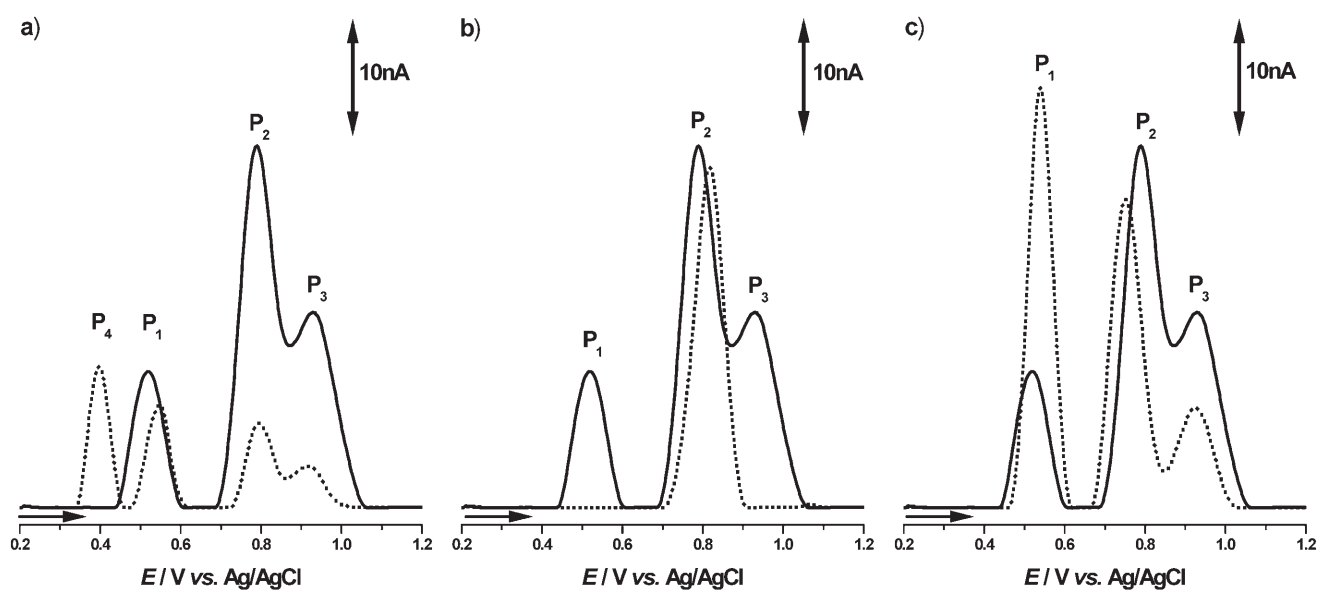

Fig. 4.   Baseline-corrected differential pulse voltammograms of 10 μM trans-resveratrol (—) first scan and (·····): a) second scan, b) 10 μM resorcinol, c) 10 μM cis-resveratrol. Scan rate: 5 mV s^{-1}, in ethanol/pH 3.2 0.2 M acetate buffer solution.
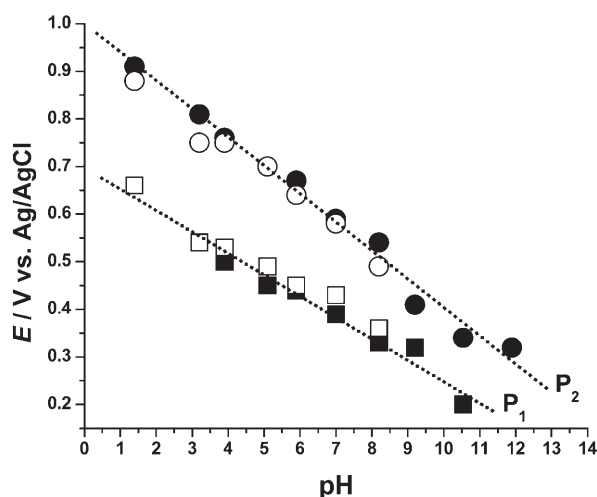
Fig. 6. Dependence of differential pulse potentials on pH of *trans*- and *cis*-resveratrol in ethanol/buffer supporting electrolyte: *trans*-resveratrol (■, P_1) peak 1 and (●, P_2) peak 2, and *cis*-resveratrol (□, P_1) peak 1 and (○, P_2) peak 2.
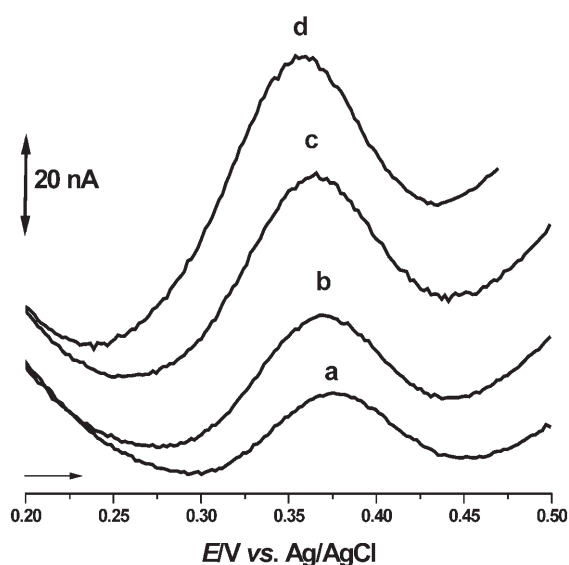

Fig. 7. Differential pulse voltammograms in ethanol/pH 7.0 0.2 M phosphate buffer solution: a) 1.5, b) 2, c) 3 and d) 5 μM *trans*-resveratrol. Scan rate 5 mV s^{-1}.

cis-resveratrol, probably due to a possible dimer formation. For peak P_2 a slope of -59 mV per pH unit was found, leading to the conclusion that during the oxidation process protons are also released from the molecule, corresponding to a mechanism with the same number of protons and electrons.

3.3. Analytical Determination of Resveratrol

For the analytical determination of resveratrol the oxidation peak P_1 was chosen, since it occurs at low oxidation potentials.

Differential pulse voltammograms were recorded for standard additions of resveratrol corresponding to bulk concentrations between 0.3 and 41 μM resveratrol in ethanol/pH 7.0 0.2 M phosphate buffer. After each measurement the electrode surface was polished and thoroughly rinsed with deionized water in order to ensure a clean GCE surface. Differential pulse voltammograms obtained for different concentrations of resveratrol are shown in Figure 7 curves a – d.

For each concentration three measurements were performed. Good linearity was found between peak current and concentration described by the equation: $I_{pc}(A) = 3.93 \times 10^{-9} + 9.03 \times 10^{-3}$ [*trans*-resveratrol](M), where $R = 0.99$, $N = 9$, $P < 0.0001$ and $SD = 4.86 \times 10^{-10}$. The parameters to define the sensitivity were calculated and the value obtained for the limit of detection, $LOD = 1.78 \times 10^{-7}$ M, was based on three times the noise level, and the limit of quantification was $LOQ = 5.86 \times 10^{-6}$, based on ten times the noise level.

4. Conclusions

The present voltammetric study aimed to characterize the electrochemical properties of resveratrol, a compound of

great interest mainly because of its presence in red wines and for its potential medical benefits.

The oxidation process of resveratrol is complex, pH dependent, all steps being irreversible. Resveratrol presents two oxidation peaks corresponding to its oxidizable moieties, a phenol and a resorcinol. From the point of view of free radical chemistry, resveratrol is a moderate antioxidant with relatively high oxidation potentials, which depend on the pH of the buffer supporting electrolyte. Resveratrol adsorbs strongly on the electrode surface and the final oxidation products block the electrode surface, as shown by the decrease of all oxidation peaks in successive scans.

Also, it has been shown that there is no appreciable difference in oxidation potentials between the *trans*- and the *cis*- forms of resveratrol which would allow an electrochemical identification of the isomers.

5. Acknowledgements

6. References

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