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Polarographic study of hydrogen peroxide anodic current and its application to antioxidant activity determination

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ABSTRACT

Behavior of hydrogen peroxide in alkaline medium has been studied by direct current (DC) polarography with dropping mercury electrode (DME) aiming to apply it in antioxidant (AO) activity determination. Development of a peroxide anodic current having form of a peak, instead of common polarographic wave, has been investigated. As a base for this investigation the interaction of H_2O_2 with anodically dissolved mercury was followed. Formation of mercury complex [$Hg(O_2H)(OH)$] has been confirmed. The relevant experimental conditions, such as temperature, concentration and pH dependence, as well as time stability of hydrogen peroxide anodic current, have been assessed. Development of an AO assay based on decrease of anodic current of hydrogen peroxide in the presence of antioxidants (AOs) has been described. Under optimized working conditions, a series of benzoic acids along with corresponding cinnamate analogues have been tested for hydrogen peroxide scavenging activity. In addition, the assay versatility has been confirmed on various complex samples.

1. Introduction

A substantial body of data obtained applying numerous antioxidant (AO) assays on biological, food and pharmacological samples, natural products and individual AOs has appeared in literature during the last few decades. Variable behavior of different AOs in various assays performed under different conditions make comparison between results challenging. Determined AO activity, of either individual AOs or complex samples, depends on method applied for its measurement. AOs can exert multifunctionality, i.e. various mechanisms of AO activity. Insight into multifunctional behavior of AOs requires multilateral approach. More over, wide variety of AOs is present in complex food or biological matrices and credible evaluation of AO activity still demands application of more than one method. A widely accepted standardized method has not yet been established despite the persistent efforts of the scientific community to come to an agreement regarding standardization [1].

The most widely applied AO assays until know are spectrophotometric ones, based on stable organic radicals scavenging. As opposed to scavenging of artificial radical species, scavenging of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radical and singlet oxygen has been less often used. Determination of ability of AOs to prevent oxidation thorough non-radical reaction were neglected as well, but has been applied more widely during the last decade. Assays based on biologically relevant, stable reactive oxygen species, hydrogen peroxide becomes more popular due to advantages over assays based on non-physiological organic radicals or unstable ROS.

Several methodologies to determine hydrogen peroxide scavenging (HPS) have been reported until now, some of them only recently. Generally, HPS assays can be divided on peroxidase-based and enzyme-free. Peroxidase-based HPS employs horseradish peroxidase to achieve oxidation of scopoletin or homovanillic acid [2]. Reaction of oxidation inhibited by AOs present has been followed fluorimetrically. Using peroxidase-based assay Sroka and Cisowski investigated HPS of some phenolic acids [3]. Limitations of peroxidase-based system, for example presence of interfering compounds, have been surpassed using enzyme free methodologies. Direct reaction of peroxide with Ti (IV) surveyed spectrophotometrically has been applied to access AO activity of some complex food samples [4]. Highly sensitive chemiluminescent (CL) assays destined to either polar or nonpolar environment represent a significant advancement over previously used enzyme based HPS assays, since the effects caused by radical reaction were eliminated. A transition metal enhanced luminol CL-based assay [5] has been applied on various samples successfully. More recently developed assay relied on the peroxyoxalate chemiluminescence using 9,10-diphenylantracene and imidazole as the flourophore and catalyst, has been used to asses peroxide quenching activity of lipid-soluble AOs [6]. Structure–activity relationship for cinnamic

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and benzoic acids has been studied by former one [7]. Various hydrophilic and hydrophobic compounds have been studied using the chemiluminogenic reaction of lucigenin with hydrogen peroxide and relationship structure–activity established [8,9].

Due to the high sensitivity, quickness, and possibility of direct determination, various electrochemical techniques can be applied for evaluating the AO status. Reports on development and application of AO assays based on electrochemical techniques are numerous [10] but successful electrochemical measurement of HPS has been reported for the first time in 2009 [11]. Kinetics of hydrogen peroxide elimination after its injections into various samples has been followed by the application of electrochemical sensors using chronoamperometry. More recently, hydrogen peroxide scavenging activity has been determined using direct current (DC) polarography. DC polarographic assay, based on decrease of anodic current of hydrogen peroxide in the presence AOs, has been applied on complex samples such as beer, wine, strong alcoholics and raspberry extracts [12–15], while research related to fundamental aspects of assay development will be the subject of this report.

Here, the behavior of hydrogen peroxide polarographic current in alkaline conditions and its relevance to HPS assay development have been reported. Electrode reaction of hydrogen peroxide at dropping mercury electrode (DME) has been followed using DC polarography. Interaction between H_2O_2 and anodically dissolved mercury have been studied with the aim to confirm formation of mercury complex [$Hg(O_2H)(OH]$, assumed by Kikuchi and Murayama [16]. Phenomenon of a peak current development, instead of common polarographic wave, i.e. the nature of hydrogen peroxide anodic current has been enlightened. Temperature, concentration and pH dependence, as well as time stability of peroxide anodic current, which sensitivity to the presence of AOs enabled the AO assay development, has been assessed. Under working conditions optimized within the scope of this study, HPS activity of series of benzoic and cinnamic acids has been determined. In order to demonstrate the assay versatility various complex samples have been included into the study as well.

2. Experimental

2.1. Reagents and materials

Hydrogen peroxide was the medical grade 35% (v/v) solution, purchased from BELINKA (Slovenia). Universal Britton Robinson (BR) buffer was prepared by titration of acidic solution (0.4 mol l^{-1} acetic, phosphoric and boric acid) with 0.2 mol l^{-1} NaOH solution to desired pH. Clark and Lubs (CL) buffer was made from the solution of 0.2 mol l^{-1} boric acid and potassium chloride by addition of 0.2 mol l^{-1} NaOH to desired pH value. For preparation of CL buffer pH 9.8, in 50.00 ml of the 0.2 mol l^{-1} solution of H_3BO_3 and KCl 40.80 ml of 0.2 mol l^{-1} NaOH was added. The substances for buffer preparations were of analytical grade quality.

Phenolic compounds were from Sigma (St. Loius, MO, USA). Working standard solutions of phenolics were prepared before each experiment in water, ethanol or working buffer solution. Solutions of myrcetin, ellagic acid and cinnamic acids (*o*-coumaric, *m*-coumaric, *p*-coumaric, caffeic and ferulic) were 2.0 mmol l^{-1}, while solutions of benzoic acids (2-hydroxybenzoic, 3-hydroxybenzoic, 4-hydroxybenzoic, protocatechuic and syringic) were 4.0 mmol l^{-1}. For preparation of all solutions deionized water was used.

Extraction of maté (Roasted maté) was carried out by pouring 200 ml of boiled distilled water over the plant samples (2 g) at room temperature. After extraction (10 min), the infusions was filtered through a tea strainer. Instant cofee and coffee (7 g) were extracted in 50 ml of distilled boiling water for 10 min and decanted. White and red wine was purchased from Serbian markets and used without pretreatment.

The inert atmosphere in the electrolytic cell was maintained with pure gaseous nitrogen (>99.995%, Messer, Serbia).

2.2. Polarography

Polarographic current–potential (*i*–*E*) curves were recorded by Princeton Applied Research (PAR) 174A Polarographic Analyzer equipped with X–Y recorder, Houston Instrument, Omnigraph 2000. Electrolytic cell of the volume 30 ml adapted for thermostating was used. The working electrode was the dropping mercury electrode (DME), with capillary characteristics of $m = 2.5$ mg s^{-1} at mercury reservoir height (h_r) 75 cm. Mechanically programmed drop time (τ) in all cases was 1 s, except when influences of τ and h_r on *i*–*E* curve characteristics were followed. Mercury reservoir height h_r was 45 cm in all experiments, except when influence of its square root $(h_r)^{1/2}$ on height of examined H_2O_2 anodic current, was followed. The saturated calomel electrode (SCE) as reference, and a Pt-foil as auxiliary electrode, completed the three electrode system.

The *i*–*E* curves recording starting potential was 0.25, 0.20 or 0.10 V given for each particular case. Potential scanning rate was 10 mV s^{-1}. Current oscillations of DME were filtered with filter (lpf) of instrument used by keeping it at 3 s. At the beginning of every experiment buffer solution in the cell was deaerated with nitrogen by bubbling it during 5 min. After addition of H_2O_2 solution, as well as after every addition of analyzed sample, the stream of nitrogen was passed through the solution for 30 s. Samples were gradually added in equal aliquots. The inert atmosphere above analyzed solution was kept during *i*–*E* curves recording. Percentage of decrease of the limiting anodic current of H_2O_2 (i_l) obtained upon each addition was plotted against volume or amount of tested samples. The slope of the linear part of every graph obtained was used as a measure of HPS activity. All experiments, except these in which the influence of temperature was followed, were performed at the room temperature (22 ± 0.5) °C.

3. Results and discussion

Influence of hydrogen peroxide concentration, pH of supporting electrolyte and temperature on hydrogen peroxide anodic current has been studied with the aim to optimize conditions for further use in determination of AO activity based on decrease of the peroxide current in the presence of AOs.

3.1. pH influence on H_2O_2 anodic current

Influence of pH on anodic current of 5.0 mmol l^{-1} hydrogen peroxide has been studied in BR buffer. pH from 5.0 to 11.0 has been considered (Fig. 1). No difference in *i*–*E* curve before and after addition of H_2O_2 has been noticed at pH 5.0 (Fig. 1, curves 1). A small anodic current, having the shape like a peak, appears at pH 6.0 (Fig. 1, thick curve 2) and increases in height till pH reach 10 (thick curve 6). At pH 11.0 instead of current peak one regular DC polarographic wave was observed (Fig. 1, thick curve 7). Oxygen reduction current noticed at higher pH resulted from peroxide instability (Fig. 2b).

3.2. Influence of experimental time duration on H_2O_2 anodic current

The influence of experimental time duration on the observed anodic current height at pH 10.0 and 11.0 shows that peroxide solution is more stable at pH 10.0 (Fig. 2a) than at 11.0 (Fig. 2b). Even superior stability of the anodic current peak height of H_2O_2 has

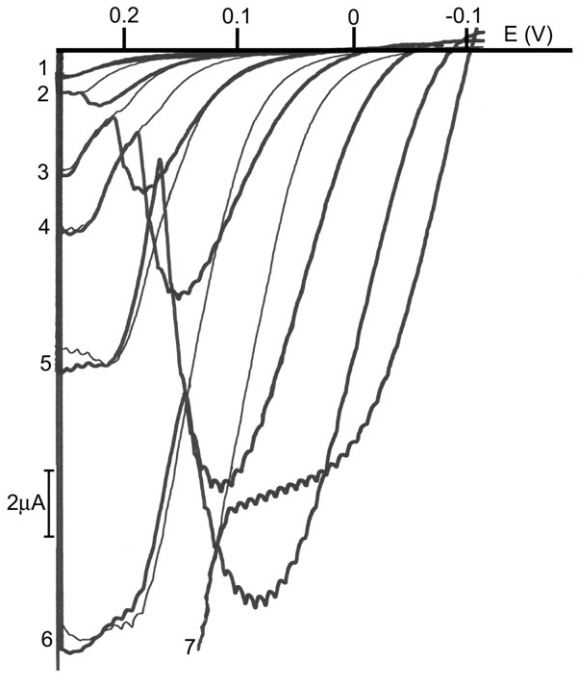

Fig. 1. Dependence of anodic current of 5.0 mmol l^{-1} H_2O_2 in Univ. BR buffer as supporting electrolyte on pH: (1) 5.0, (2) 6.0, (3) 7.0, (4) 8.0, (5) 9.0, (6) 10.0, (7) 11.0. Polarograms were recorded without (thin lines) and in the presence of hydrogen peroxide (thick lines).

been noticed in the CL buffer pH 9.8. Relative standard deviation of 33 *i–E* curves of 5.0 mmol l^{-1} H_2O_2 in CL buffer recorded successively during 30 min was 1.16%. Thereafter, CL buffer (pH 9.8) has been used in further experiments. Since the obtained curves of hydrogen peroxide, in the applied pH interval and peroxide concentration, do not have a characteristic shape (wave) some of relevant polarographic current characteristics have not been evaluated exactly.

3.3. Concentration dependence of H_2O_2 anodic current

The dependence of H_2O_2 anodic current on its concentration has been studied in the range 0.25–5.0 mmol l^{-1} in CL buffer (pH 9.8) as supporting electrolyte. At low H_2O_2 concentrations ($\leq$1.0 mmol l^{-1}) one well defined anodic wave appears at 0.08 V *vs* SCE (Fig. 3, curve 2). At higher H_2O_2 concentrations the current peak

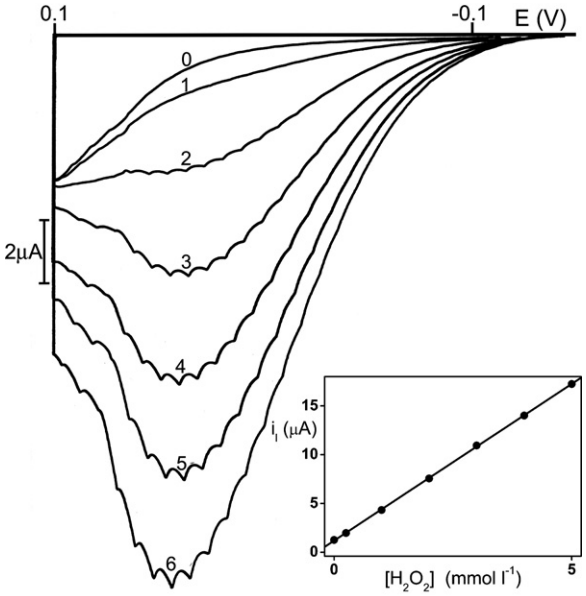

Fig. 3. Dependence of hydrogen peroxide anodic current on its concentration from *CL* buffer (pH 9.8): (1) 0.25, (2) 1.0, (3) 2.0, (4) 3.0, (5) 4.0 and (6) 5.0 mmol l^{-1} (insert: dependence of limiting anodic current i_l on H_2O_2 concentration).

instead of wave appears (Fig. 2, curves 3–6). Conclusion that the peak current obtained does not represent polarographic maximum has been based on observation that 0.005% of Triton-X100 does not have any influence on its shape. CL buffer alone gives anodic current close to H_2O_2 whose influence on peroxide current shape is rather obvious. Alongside the obtained shape of DC anodic current (peak) it is well reproducible, showing very good linear dependence on H_2O_2 concentration (R = 0.9999), as shown in Fig. 3 (insert).

3.4. Interaction of H_2O_2 with anodically dissolved mercury

Since in alkaline medium H_2O_2 predominantly exist as perhydroxyl ion, HO_2^-, the assumption was made that peroxide anodic current more probably originated from the mixed complex [Hg(O_2H)(OH)] formation [16]. Aiming to prove the existence of mixed perhydroxyl-hydroxyl complex of mercury, H_2O_2 solution (1.0 mmol l^{-1}) has been titrated with $HgCl_2$ standard solution (10 mmol l^{-1}) (Fig. 4a, curves 0–6). The reverse titration of buffered $HgCl_2$ solution (1.0 mmol l^{-1}) with standard H_2O_2 solution (10^2 mmol l^{-1}) has been shown in Fig. 4b (curves 0–9). In the first

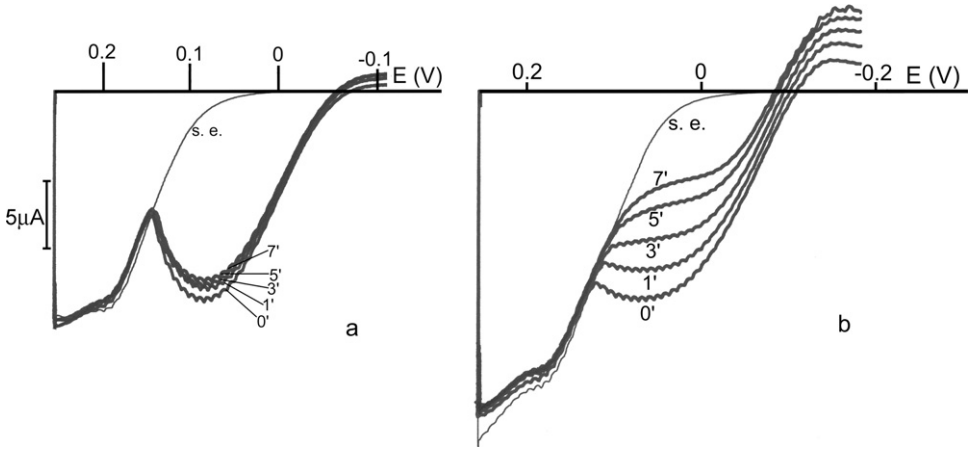

Fig. 2. Influence of experimental time on anodic current of 5.0 mmol l^{-1} H_2O_2 in BR buffer pH 10.0 (a) and pH 11.0 (b).

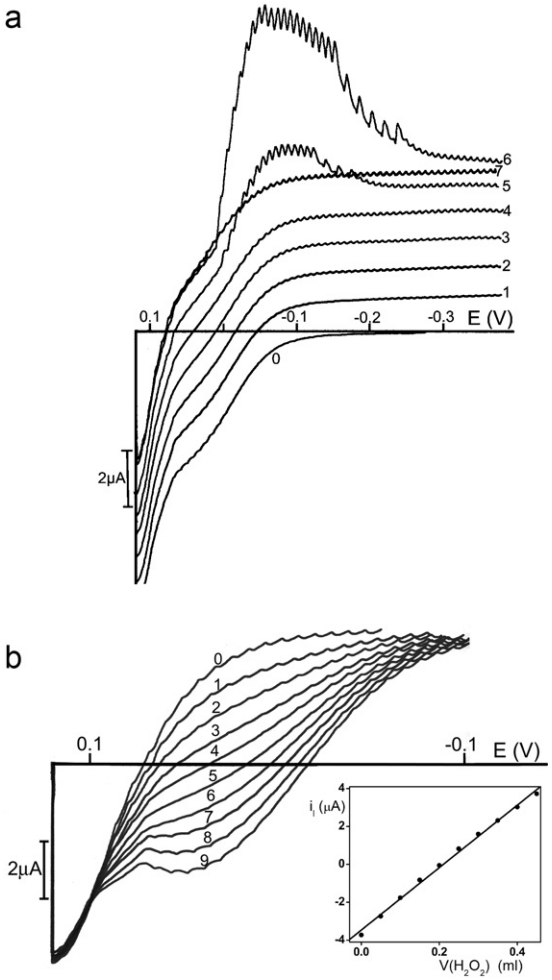

Fig. 4. Titration curves of (a) 1.0 mmol l^{-1} H_2O_2 in 20.0 ml of CL buffer (pH 9.8) with 10 mmol l^{-1} $HgCl_2$: (1) 0.0, (2) 0.5, (3) 1.5, (4) 2.0, (5) 2.5, (6) 3.0 ml of $HgCl_2$, (7) 0.005% of Triton X-100 added in solution (6) and (b) 1.0 mmol l^{-1} $HgCl_2$ with 10^2 mmol l^{-1} H_2O_2: (1) 0.0, (2) 0.05, (3) 0.10, (4) 0.15, (5) 0.20, (6) 0.25, (7) 0.30, (8) 0.35, (9) 0.4 ml H_2O_2 (insert: i_l in function of H_2O_2 volume).

case (Fig. 4a) when concentration of Hg(II) increases the anodic current decreases with simultaneous rise of cathodic current, while the sum of these two currents remains constant. After the anodic current disappearing at the molar ratio $H_2O_2/HgCl_2$ equal one (Fig. 4a, curve 4), a very intense current at the beginning of cathodic current plateau appears upon further addition of Hg (II) (Fig. 4a, curves 5 and 6). This current disappears completely upon addition of 0.005% Triton-X100 (Fig. 4a, curve 7) indicating that it belongs to polarographic maximum appearing at higher Hg(II)-concentrations. In the opposite titration (Fig. 4b), starting concentration of Hg(II) was 1 mmol l^{-1} and the characteristic maximum was not observed. Titration curves presented in Fig. 4b (curves 0–9) shows that the anodic current of peroxide appearing after reaching reactant molar ratio 1:1 (Fig. 4b, curve 4) rises linearly upon further addition of peroxide standard solution (Fig. 4b, curves 5–9). The curves presented in Fig. 4 (a and b), clearly demonstrate the formation of the above mentioned complex, having reactants molar ratio 1:1.

3.5. Influence of temperature on H_2O_2 anodic current

Under the experimental conditions described above (Fig. 4a and b), the formation of mercury perhydroxyl-hydroxyl complex has been found responsible for H_2O_2 anodic current development. The influence of temperature of experimental solution on current characteristics is important for further consideration of the electrode reaction and thermal stability of complex formed.

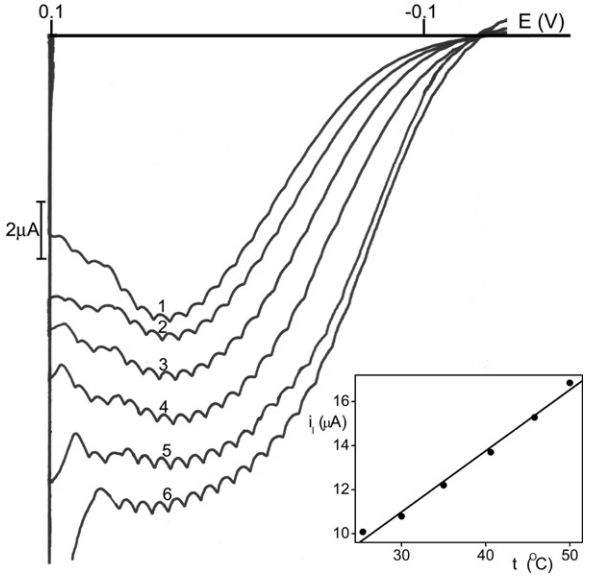

Fig. 5. Dependence of the anodic limiting current, i_l of 3.0 mmol l^{-1} H_2O_2 in CL buffer (pH 9.8) on temperature: (1) 25.4, (2) 30, (3) 35, (4) 40.6, (5) 45.8, (6) 50 °C (insert: dependence of i_l on t).

The influence of temperature on anodic current of 3.0 mmol l^{-1} H_2O_2 in CL buffer pH 9.8 was followed from 20.5 to 50.0 °C. The obtained anodic polarographic curves in the temperature interval considered are presented (Fig. 5) together with dependence of their peak current on t (Fig. 5, insert).

When temperature increases the observed anodic current raise in height. At temperatures lower that 50.0 °C, temperature coefficient (ω = 1.5%/°C) is characteristic for diffusion limited electrode processes. At higher t (≥50 °C) anodic current decreases with simultaneously rise of oxygen reduction current (not shown) pointed out either the instability of free hydrogen peroxide or its mixed complex formed.

Also, the nature of peroxide anodic current has been determined based on its dependence from mercury reservoir height. Linear dependence (R = 0.9982) of the anodic limiting current i_l on square root of mercury reservoir height further confirm its diffusion control.

3.6. Influence of phenolic compounds on H_2O_2 anodic current

In the last years, H_2O_2 scavenging activity becomes a key aspect of the total AO activity determination. Various AO assays, based on H_2O_2 scavenge, have been developed recently [17]. A polarographic approach to determination of AO activity has been demonstrated [18,19], while here the main polarographic methodology has been successfully applied to determine H_2O_2 scavenging activity of phenolic compounds under founded optimal working conditions. As supporting electrolyte CL buffer pH 9.8 has been chosen since it enables development of the anodic current as a peak whose height linearly depends on peroxide concentration from 0.25 to 5.0 mmol l^{-1} having good stability during the experimental time. As AOs cause decreases of peak height the highest concentration of peroxide (5.0 mmol l^{-1}) has been used as starting.

Influence of a series of benzoic acid (2-, 3- and 4-hydroxybenzoic, protocatechuic, vanillic and syringic) along with cinnamate analogous (*o*-, *m*- and *p*-coumaric, caffeic and ferulic acid) on peroxide current has been examined. Ellagic acid, lactone of gallic acid, as well as flavonone myricetine has been included

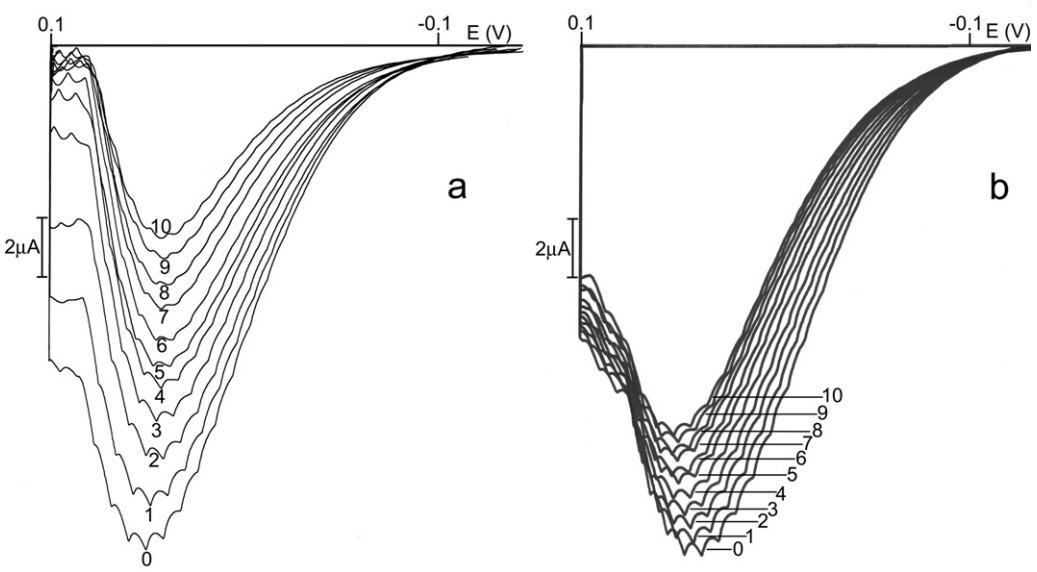

Fig. 6. Anodic current of 5.0 mM H_2O_2 in CL buffer (pH 9.8) before (0) and after addition of ten equal aliquots of 100 μl of 2.0 mmol l^{-1}: (a) myricetin (1–10) and (b) *p*-coumaric acid.

into the study in order to demonstrate more clearly influence of molecular structure on HPS activity. The effect of myricetine and *p*-coumaric acid has been shown in Fig. 6. After recording polarogram of anodic current of starting H_2O_2 concentration in CL buffer (Fig. 6 a and b, curve 0), phenolic compounds have been introduced into the cell solution in equal aliquots.

Percentage of decrease of anodic current has been calculated upon each aliquot addition according to the following equation:

$$\%\ \text{scavenged}\,[H_2O_2] = \left(1 - \frac{i_I}{i_{I0}}\right) \times 100$$

Dose–response curves (percentage of i_I decrease *vs* amount (μmol)) for myrcetin and ellagic, protocatechuic and ferulic acids have been shown in Fig. 7a. HPS activity of benzoic acid derivatives, expressed as the slope of dose–response curves, has been compared with the activity of cinnamic acid derivatives (Table 1). Observation that cinnamate derivatives are stronger in relation with their benzoic analogues corroborates with study by Sroka and Cisowski [3], while Mansouri et al. [7] reported opposite finding. Among tested benzoates syringic acid has been found to be the most effective whereas *m*-hydroxybenzoic exhibited the weakest activity. Following rank of order has been obtained for benzoic acids: syringic > vanillic > protocatechuic > 2-hydroxybenzoic > 4-hydroxybenzoic > 3-hydroxybenzoic. Ferulic acid is the strongest AOs among tested cinnamates as opposed to *m*-coumaric acid. The order of efficacy of cinnamic acids is: ferulic > caffeic > *o*-coumaric > *p*-coumaric > *m*-coumaric (Table 1). The activity correlates positively with number of substituents bonded to the aromatic ring. Finding that compounds with one hydroxy group exhibit the lowest HPS activity (coumaric acids and hydroxybenzoic acids) corroborate with previous studies [3]. Since flavonoids have almost all of the main structural characteristics of the strong AOs (*ortho* OH groups-B ring), OH groups in position 3, 5, 7 and almost planar molecular geometry [20], remarkably higher HPS activity relative to phenolic acids has been expected. Activity of myrcetine, a representative of flavonoids, has been found an order of magnitude higher than for example hydroxybenzoic acids and protocatechuic acid. Also, dilactone ellagic acid superior activity in comparison to either benzoates or cinnamates has been expected.

The study of wide range of non phenolic and phenolic substances including complex carbohydrates, amino acids, peptides and proteins, as well as various classes of phenolics such as non flavonoids (phenolic alcohols and aldehydes, benzoic and cinnamic

Table 1
Hydrogen peroxide scavenging activity of flavonon myrcetine, benzoic and cinnamic acids, expressed as the slope of the dose-response curves (percentage of i_I decrease *vs* amount of tested compound (μmol)).

	Intercept (%)	Slope (% μmol^{-1})	*R*
Benzoic acids			
3-Hydroxybenzoic	0.3 ± 0.5	2.88 ± 0.36	0.9844
4-Hydroxybenzoic	1.4 ± 1.3	3.28 ± 1.38	0.8594
2-Hydroxybenzoic (salicylic)	−0.2 ± 0.3	3.51 ± 0.28	0.9876
3,4-Dyhydroxybenzoic (protocatechuic)	0.0 ± 0.4	5.92 ± 0.25	0.9982
4-Hydroxy-3-methoxybenzoic (vanillic)	−0.6 ± 0.5	10.29 ± 0.89	0.9852
4-Hydroxy-3,5-dimethoxybenzoic (syringic)	0.4 ± 0.9	13.48 ± 1.80	0.9912
Ellagic acid	−0.3 ± 0.3	21.51 ± 0.38	0.9992
Cinnamic acids			
3-Hydroxycinnamic (*m*-coumaric)	0.4 ± 0.4	8.00 ± 0.45	0.9953
4-Hydroxycinnamic (*p*-coumaric)	0.9 ± 0.7	9.02 ± 0.68	0.9915
2-Hydroxycinnamic (*o*-coumaric)	0.0 ± 0.7	9.80 ± 0.62	0.9921
3,4-Dyhydroxycinnamic (caffeic)	−5.1 ± 0.8	12.65 ± 0.75	0.9965
4-Hydroxy-3-methoxycinnamic (Ferulic)	−0.9 ± 0.3	13.86 ± 0.52	0.9958
Flavonoid			
Myricetin	0.4 ± 0.8	46.84 ± 3.35	0.9924

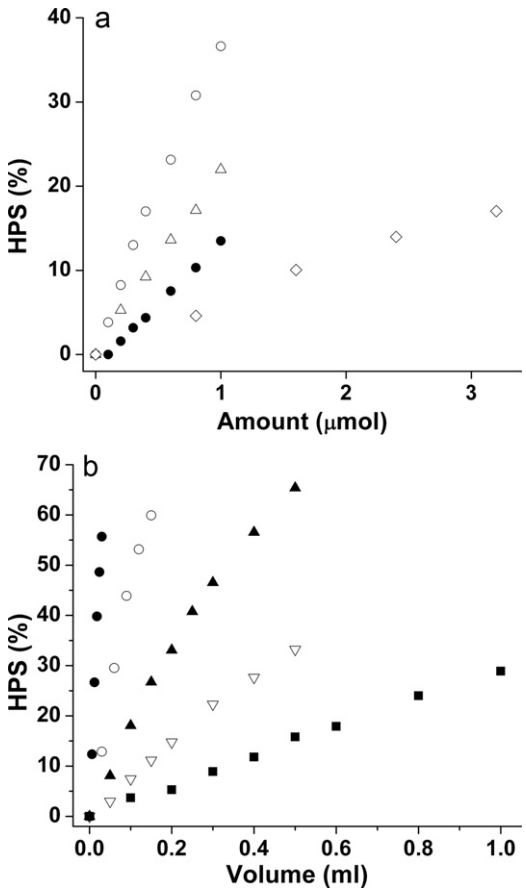

Fig. 7. Percentage of H_2O_2 anodic limiting current decrease against: (a) amount of phenolic compounds (μmol) added (myrcetin ○, ellagic acid △, ferulic acid ●, protochatechuic acid ◊); (b) volume of complex samples added (ml) (instant coffee ●, coffee ○, red wine ▲, white wine ▽, maté ■).

acid derivatives) and flavonoids (flavonols, flavanons, flavonones), aiming to establish structure–activity relationship, is currently in progress.

Aiming to introduce the slope as appropriate way to express HPS activity, decrease of anodic current of peroxide obtained upon addition of various complex samples (red and white wine, instant coffee, coffee and maté) has been demonstrated in Fig. 7b. Prominent difference between dose response curves indicate that even in the case of complex samples the slope can be used as measure of HPS activity. Besides as the slope, there are various ways to express activity of either pure compounds or complex samples such as (i) volume or amount of sample required for 50% decrease of i_{l0}, assigned as AO_{50} (μl or μM), or its reciprocal value $1/AO_{50}$ (μl^{-1} or μM^{-1}); (ii) the anodic current decrease of 20% that corresponds to 1.0 mmol l^{-1} of peroxide concentration; (iii) decrease of anodic current obtained upon addition of aliquot whose volume or concentration has been estimated previously as appropriate. Among various ways to express results, the slope has been chosen as the most reliable. Until now, HPS activity of complex samples (beer, wine, strong alcoholics, raspberry extracts) [12–15] was expressed as volume required for 50% of decrease (and its reciprocal value), or decrease of anodic current obtained after addition of 500 μl of samples (HPS500). Those results were validated through correlation with total phenolic content (FC assay) and standard AO assays (DPPH, ABTS, FRAP). For the sake of comparison between different studies, we suggest introduction of the slope as practical, simple and adequate measure of HPS activity of both individual substances and complex samples even in the case when their activity varies highly.

The assay developed and optimized within the scope of this study can be recommended as simple, rapid, reliable and inexpensive. The proposed assay does not require enzymes, artificial radical species, chemiluminescent reagents or biological tissue. Also, relative to optical methods the assay is superior in evaluation of HPS activity of colored and turbid samples.

4. Conclusion

DC polarographic behavior of hydrogen peroxide on dropping mercury electrode, in alkaline buffered solutions, has been systematically studied. The origin of the anodic current and influence of different experimental conditions on it (pH of medium, H_2O_2 concentration, electrolyte solution temperature) have been followed. Confirmation that anodic current appears from mixed complex [Hg(O_2H)(OH)], formed with anodically produced Hg (II) in alkaline medium has been provided. Development of enzyme-free AO assay based on peroxide anodic current decrease in the presence of AOs has been described. Under founded optimal working conditions benzoic and cinnamic acids have been tested for their ability to react with hydrogen peroxide. Antioxidant activity of complex samples has been considered and the assay versatility demonstrated. According to results obtained, the assay can be recommended as simple, rapid, reliable and inexpensive alternative to conventional spectrophotometric and chemiluminescent H_2O_2 scavenging assays.

Acknowledgments

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