CROATICA CHEMICA ACTA
CCACAA, ISSN 0011-1643, e-ISSN 1334-417X
Croat. Chem. Acta **83** (2) (2010) 197–207.
CCA-3410

Original Scientific Article

Electrochemical and Antioxidant Properties of (+)-Catechin, Quercetin and Rutin

Martina Medvidović-Kosanović,* Marijan Šeruga, Lidija Jakobek, and Ivana Novak

Department of Applied Chemistry and Ecology, Faculty of Food Technology, J. J. Strossmayer University of Osijek, Kuhačeva 18, P.O. Box 709, HR-31000 Osijek, Croatia

RECEIVED JANUARY 28, 2009; REVISED JULY 22, 2009; ACCEPTED DECEMBER 14, 2009

Abstract. The electrochemical properties of three structurally different flavonoids ((+)-catechin, quercetin and rutin) on a glassy carbon electrode were studied at different pH by cyclic, differential pulse and square-wave voltammetry. It was determined that in all investigated compounds oxidation of catechol 3',4'-dihydroxy group on the B-ring (the first oxidation peak) occurs. This process is reversible, pH dependent and includes the transfer of $2e^-$ and $2H^+$. The products of electrochemical oxidation of all investigated flavonoids (especially of quercetin) strongly adsorb on the electrode surface. In terms of quantification, the absorbance value, proportional to the remaining ABTS+• concentration, is measured after a fixed reaction time. Results are expressed as Trolox equivalents (TEAC value), that is, as the concentration of Trolox solution (mmol dm^{-3}) with an antioxidant capacity equivalent to that found for 1.0 mmol dm^{-3} of the substance under investigation. It was found that the TEAC values depend on the concentration of the investigated compounds. The activity sequence of the investigated, structurally different, flavonoids follows the sequence: quercetin > (+)-catechin > rutin.

Keywords: flavonoids, electrochemistry, oxidation, antioxidant properties, TEAC assay

INTRODUCTION

Flavonoids are a class of secondary plant phenolics consisting of fifteen carbon atoms, arranged in a C_6-C_3-C_6 configuration. More than 5000 flavonoids have been identified up to date, they have been mostly found in leaves, seeds, bark and flowers of plants, where they have a protective role against ultraviolet radiation, pathogens and herbivors.[1,2] The anthocyanin copigments, which attract pollinating insects, are responsible for the characteristic red and blue colours of berries, wines and certain vegetables (major sources of flavonoids in human diet). Most of the beneficial health effects of flavonoids (anti-inflammatory, anti-cancer, cardio protective) are attributed to their antioxidant and chelating abilities.[3]

In the last two decades flavonoids were studied by different techniques. Electrochemical properties of flavonoids were studied by voltammetric techniques (cyclic voltammetry,[4–8,10–18,21–38,42] differential pulse voltammetry,[5,8,12–15,23–25,27,29,31,37,41] square wave voltammetry,[5,12,14,26,29,38] linear sweep voltammetry,[8,25,31,39,40] chronocoulometry,[30,42] chronoamperometry[17] AC impedance spectroscopy[20] and rotating disk electrode voltammetry[16,17]), UV-spectroelectrochemistry,[25,34] semi-empirical calculations[4] and biosensors.[9,12] Antioxidant capacity of flavonoids was studied by cyclic voltammetry,[19,43–45] differential pulse voltammetry,[46] Flow Injection Analysis (FIA),[47,48] chronoamperometry,[49] biosensors,[50] UV/Vis spectroscopy (with different reagents *e.g.* Folin-Ciocalteau,[19,50] FRAP,[43,51] DPPH,[19,49,52] ABTS,[19,50–52] *etc.*), photochemiluminescence[19], spectrofluorimetry (ORAC method)[50,52] and electron spin resonance (ESR) spectroscopy[39].

The structure of flavonoids is a key determinant of their radical scavenging and metal chelating activity, which is referred to as the structure-activity relationship (SAR). Some of the structural features and nature of substitutions on rings B and C which determine the antioxidant activity of flavonoids include the following: a) the degree of hydroxylation and the positions of the −OH groups in the B ring, in particular an ortho-dihydroxyl structure of ring B (catechol group) results in a higher activity; b) a double bond between C-2 and C-3, combined with a 3-OH, in ring C, enhances the active radical scavenging capacity of flavonoids; c) substitution of the 3-OH in ring C results in increase in torsion angle and loss of coplanarity, which subsequently reduces antioxidant activity and d) a double bond between C-2 and C-3, conjugated with the 4-oxo

* Author to whom correspondence should be addressed. (E-mail: mmkosano@kemija.unios.hr)

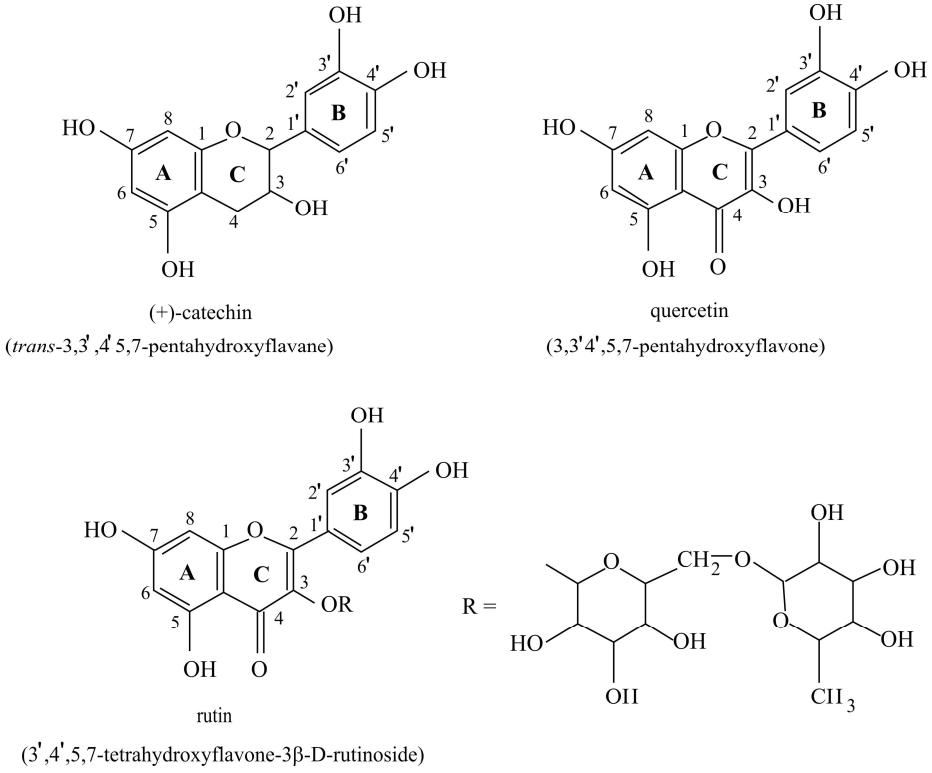

Figure 1. Molecular structures of (+)-catechin, quercetin and rutin.

group in ring C also enhances the radical scavenging capacity of flavonoids.[1]

Since the chemical activities of flavonoids in terms of their reducing properties as hydrogen or electron-donating agents could predict their potential to act as antioxidants (lower oxidation potential points to a higher antioxidant activity),[53] studying of electrochemical and antioxidant properties could help to better understand the mentioned group of compounds.

The purpose of this study was to show application of electrochemical methods (cyclic, differential pulse and square-wave voltammetry) in systematic investigation of electrochemical properties of three structurally different flavonoids (Figure 1), one flavanol ((+)-catechin) and two flavonols (rutin and quercetin) and to determine the antioxidant capacities of the investigated compounds by a Trolox equivalent antioxidant capacity (TEAC) assay.

EXPERIMENTAL

All chemicals were of the highest purity commercially available and were used without further purification. Rutin trihydrate ($C_{27}H_{30}O_{16}\cdot 3H_2O$), Quercetin dihydrate ($C_{15}H_{10}O_7\cdot 2H_2O$), (+)-Catechin hydrate ($C_{15}H_{14}O_6\cdot H_2O$) and 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Sigma (St. Louis, MO, SAD). (±)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Fluka (Buchs, Switzerland). All other chemicals were obtained from Kemika (Zagreb, Croatia). Stock solutions of investigated flavonoids (c = $1\cdot 10^{-2}$ mol dm^{-3}) were prepared in methanol and kept in the refrigerator (solutions were stable for at least 1 month). Prior to use, stock solutions were diluted to the desired concentration with a buffer supporting electrolyte (I_c = 0.1 mol dm^{-3}). Buffer supporting electrolyte solutions were prepared in high-purity water from a Millipore Milli-Q system (resistivity greater than or equal to 18 MΩ cm). A phosphate buffer solution of pH = 8.0 and 7.0, and an acetate buffer solution of pH = 5.5, 4.5 and 3.5 were used.

The electrochemical experiments were preformed with an EG & G Princeton Applied Research Model 273A potentiostat controlled by a computer in a three-electrode system, consisting of a glassy carbon working electrode (Bioanalytical System, d = 3 mm), a saturated calomel reference electrode (S.C.E.) and a platinum counter electrode. The glassy carbon working electrode was polished with α-Al_2O_3 powder (0.05 μm, Buehler, USA) before each measurement. The cyclic voltammetry scan rate was 50 mV/s. The differential pulse voltammetry conditions were: scan increment 5 mV, pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV/s. The applied square-wave voltammetry conditions used were: frequency 50 Hz, pulse amplitude 50 mV and scan increment 2 mV (effective scan rate of 100 mV/s).

Table 1a. Oxidation potential ($E_{p,a}$), half wave potential ($E_{p1/2}$), peak separation (ΔE_p), oxidation current ($I_{p,a}$) and the area under the anodic wave (S) values of the first oxidation peak for (+)-catechin as the function of pH values, obtained by cyclic voltammetry

pH	$E_{p,a}$ / V	$E_{1/2}$ / V	ΔE_p / V	$I_{p,a}$ / μA	S / μC
3.5	0.481	0.391	0.192	0.289	1.08
4.5	0.394	0.307	0.186	0.452	2.09
5.5	0.339	0.267	0.192	0.299	1.20
7.0	0.278	0.189	0.231	0.267	1.15
8.0	0.234	0.142	0.198	0.227	0.92

Table 1b. Oxidation potential ($E_{p,a}$), half wave potential ($E_{p1/2}$), peak separation (ΔE_p), oxidation current ($I_{p,a}$) and the area under the anodic wave (S) values of the first oxidation peak for quercetin as the function of pH values, obtained by cyclic voltammetry

pH	$E_{p,a}$ / V	$E_{1/2}$ / V	ΔE_p / V	$I_{p,a}$ / μA	S / μC
3.5	0.391	0.340	0.062	1.237	3.06
4.5	0.323	0.271	0.056	1.480	3.44
5.5	0.248	0.192	0.032	2.280	6.37
7.0	0.134	0.083	0.073	1.234	2.69
8.0	0.071	0.051	0.040	0.760	2.26

Table 1c. Oxidation potential ($E_{p,a}$), half wave potential ($E_{p1/2}$), peak separation (ΔE_p), oxidation current ($I_{p,a}$) and the area under the anodic wave (S) values of the first oxidation peak for rutin as the function of pH values, obtained by cyclic voltammetry

pH	$E_{p,a}$ / V	$E_{1/2}$ / V	ΔE_p / V	$I_{p,a}$ / μA	S / μC
3.5	0.459	0.419	0.064	0.374	0.65
4.5	0.401	0.361	0.037	0.327	0.71
5.5	0.318	0.279	0.037	0.350	0.69
7.0	0.226	0.188	0.047	0.292	0.53
8.0	0.182	0.141	0.075	0.234	0.76

Antioxidant properties of investigated flavonoids were studied by a Trolox equivalent antioxidant capacity (TEAC) decolourisation assay (the procedure was taken from Iveković *et al*.)[47] on J. P. Selecta UV/Vis spectrophotometer Model UV 2005. A stock solution of ABTS radical cation was prepared by mixing 0.2 ml ammonium peroxodisulfate solution (c = 65 mmol dm^{-3}) with 50 ml of ABTS solution (c =1 mmol dm^{-3}) in a phosphate buffer (I_c = 0.1 mol dm^{-3} NaH_2PO_4, pH = 7.40). The mixture was left to stand overnight. A 0.5 ml of ABTS radical cation stock solution was put into a 1 cm glass cuvette, diluted with 2 ml of phosphate buffer and the absorbance of the ABTS radical cation at 734 nm was read. Subsequently, a 0.1 ml of sample solution was added into the cuvette, the solution was quickly mixed and the absorbance was monitored at 734 nm for 60 s. The decrease of absorbance after 60 s (ΔA_{sample}) was compared with the decrease of absorbance caused by the addition of 0.1 ml of Trolox solution (c = 400 μmol dm^{-3}) (ΔA_{Trolox}) and the TEAC value was calculated according to the formula:[47]

$$\text{TEAC} = \frac{\Delta A_{\text{sample}} \cdot c_{\text{Trolox}}}{\Delta A_{\text{Trolox}} \cdot c_{\text{sample}}}$$

The pH measurements were carried out at the room temperature with the Metrel MA 5736 pH-meter.

RESULTS AND DISCUSSION

Cyclic Voltammetry

Cyclic voltammograms of (+)-catechin (Table 1a), quercetin (Table 1b) and rutin (Table 1c) (c = 1·10^{-5} mol dm^{-3}) were recorded in the buffer solutions (I_c = 0.1 mol dm^{-3}) in the pH range from pH = 3.5 to pH = 8.0. An example of their cyclic voltammograms recorded at pH = 5.5 is shown in Figure 2. Cyclic voltammetry of (+)-catechin showed two oxidation peaks associated with the oxidation centres present in the molecule, a reversible oxidation peak ($E_{p,a,1}$ = 0.339 V) which corresponds to the oxidation of the 3',4'-dihydroxy substitu-

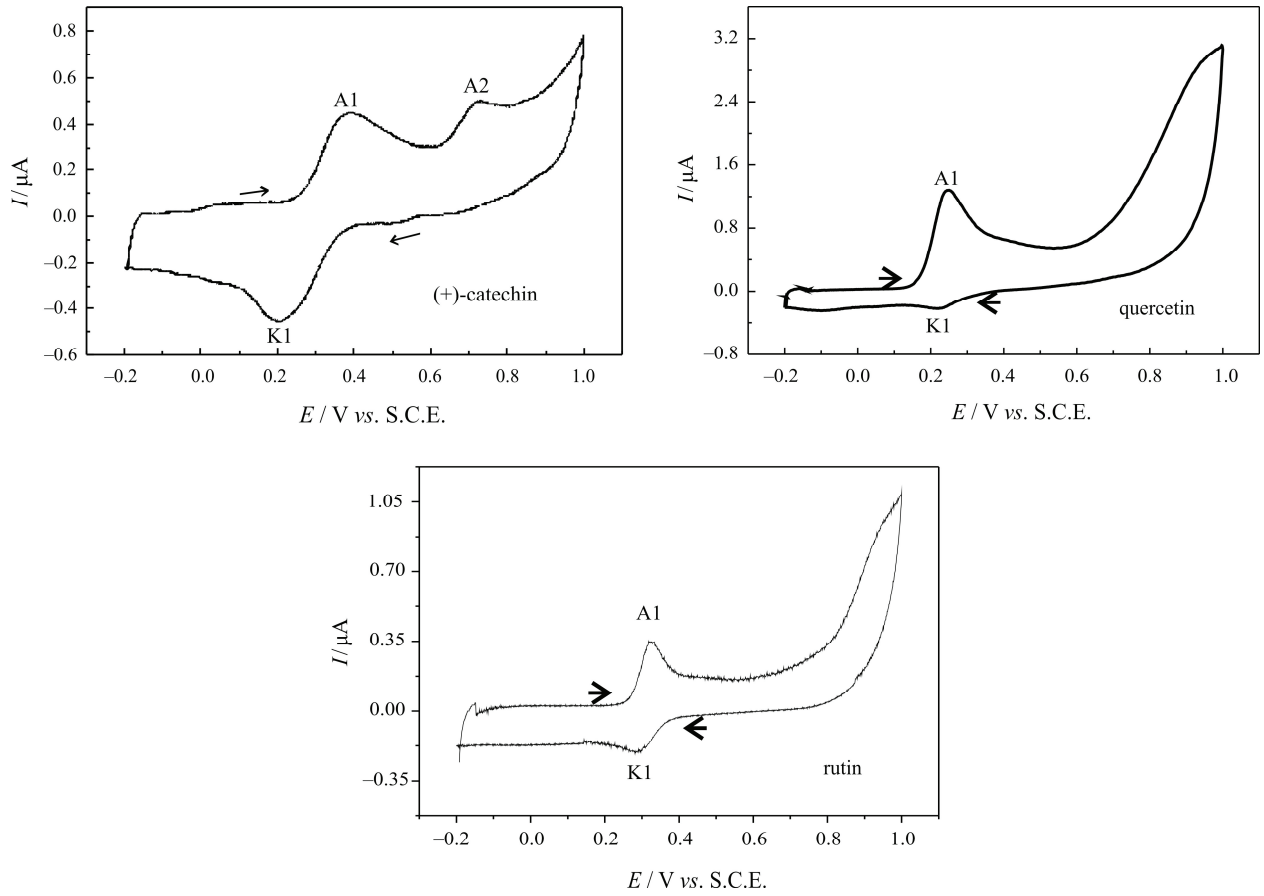

Figure 2. Cyclic voltammograms of (+)-catechin, quercetin and rutin (c = 1·10^{-5} mol dm^{-3}) in the acetate buffer (I_c = 0.1 mol dm^{-3}, pH = 5.5). The scan rate is 50 mV/s.

ent on the B-ring and an irreversible oxidation peak ($E_{p,a,2}$ = 0.715 V),[4,5,8] which some authors associate with the oxidation of OH group at the position 3 on the C ring[5] and other with the oxidation of 5,7-dihydroxy substituent on the A ring.[4,8] The reduction peak of the 3',4'-diquinone formed in the first oxidation peak appeared at $E_{p,c}$ = 0.147 V.[4,5,8] The peak separation is $\Delta E_p = E_{p,a} - E_{p,c}$ = 192 mV which agrees with some literature data.[8] Somewhat high peak separation value could point to the EC oxidation mechanism, which includes reversible electrochemical oxidation of (+)-catechin to *o*-quinon followed by a chemical reaction of catechin's oxidation products.

Cyclic voltammograms of rutin and quercetin have shown one reversible oxidation peak for rutin ($E_{p,a}$ = 0.318 V) and quercetin ($E_{p,a}$ = 0.248 V), which corresponds to the oxidation of the 3',4'-dihydroxy substituent on the B-ring. The reduction peak of the 3',4'-diquinone formed during the rutin or quercetin oxidation process, appeared at $E_{p,c}$ = 0.281 V for rutin and at $E_{p,c}$ = 0.218 V for quercetin. The peak separation is $\Delta E_p = E_{p,a} - E_{p,c}$ = 37 mV for rutin and 32 mV for quercetin, which points to a reversible electrode reaction involving two electrons on the glassy carbon electro-de.[14,16,25,29,31,33,41]

Results of this method show structural differences of the investigated flavonoids, since different values of oxidation ($E_{p,a}$) and reduction ($E_{p,c}$) peak potentials were observed, which can also be connected with their different antioxidant activities (lower oxidation potential points to the higher antioxidant activity).[53]

Differential Pulse Voltammetry

Differential-pulse voltammetry was used for the investigation of oxidation peak current (I_p) as a function of pH (from pH = 3.5 to pH = 8.0) and to study the adsorption of the investigated compounds on the glassy carbon electrode. As is shown in Table 2, the highest peak current of the first oxidation peak was around pH = 4.5 (for (+)-catechin) and around pH = 5.5 (for rutin and quercetin) and it decreased in more acidic and alkaline media. Oxidation peak potentials for all investigated compounds decreased with an increase of pH.

The plot of the peak potential (E_p) *vs*. pH for the first oxidation peak showed linearity with the slope 58.7 mV (for (+)-catechin), 73.1 mV (for quercetin) and 60.6 mV (for rutin), which corresponds to the mechanism involving the same number of protons and electrons and

Table 2. Oxidation current ($I_{p,a}$) and oxidation potential ($E_{p,a}$) values of the first oxidation peak for (+)-catechin, quercetin and rutin as the function of pH values, obtained by differential pulse voltammetry

	(+)-catechin		quercetin		rutin	
pH	$E_{p,a}$ / V	$I_{p,a}$ / μA	$E_{p,a}$ / V	$I_{p,a}$ / μA	$E_{p,a}$ / V	$I_{p,a}$ / μA
3.5	0.429	0.28	0.372	0.66	0.397	0.60
4.5	0.330	0.37	0.284	0.84	0.366	0.64
5.5	0.248	0.26	0.213	0.89	0.299	0.71
7.0	0.160	0.24	0.089	0.57	0.190	0.55
8.0	0.102	0.20	0.012	0.36	0.132	0.40

agrees with data found in literature.[4,5,14,16,25,29,31,33,37,41,42] The plot of the peak potential (E_p) *vs*. pH for the second oxidation peak of (+)-catechin showed linearity with the slope 63.3 mV which agrees with literature data[5] and also points to the oxidation mechanism which involves the exchange of the same number of protons and electrons.

Differential pulse voltammetry revealed a second oxidation peak of rutin and quercetin around 0.9 V which corresponds to oxidation of 5,7-dihydroxyl group on the A-ring and agrees with literature data.[14,16,25,29] Both peaks of rutin, quercetin and (+)-catechin decreased with successive scans (Figure 3) for all pH values studied, which confirmed adsorption of their oxidation products on the glassy carbon electrode surface (the decrease of the oxidation peaks in the second scan was the most visible for quercetin, which confirms that the products of quercetin oxidation block the electrode surface and agrees with literature data).[14] Even though, two other oxidation peaks of quercetin, peak 2 around 0.3 V (*vs*. Ag/AgCl), which corresponds to oxidation of 3-OH group at ring C and peak 3 around 0.6 V (*vs*. Ag/AgCl), which corresponds to oxidation of 5-OH group at ring A, were found by Brett et all,[14] those peaks were not observed in our study at any pH value. The oxidation products of investigated flavonoids can also

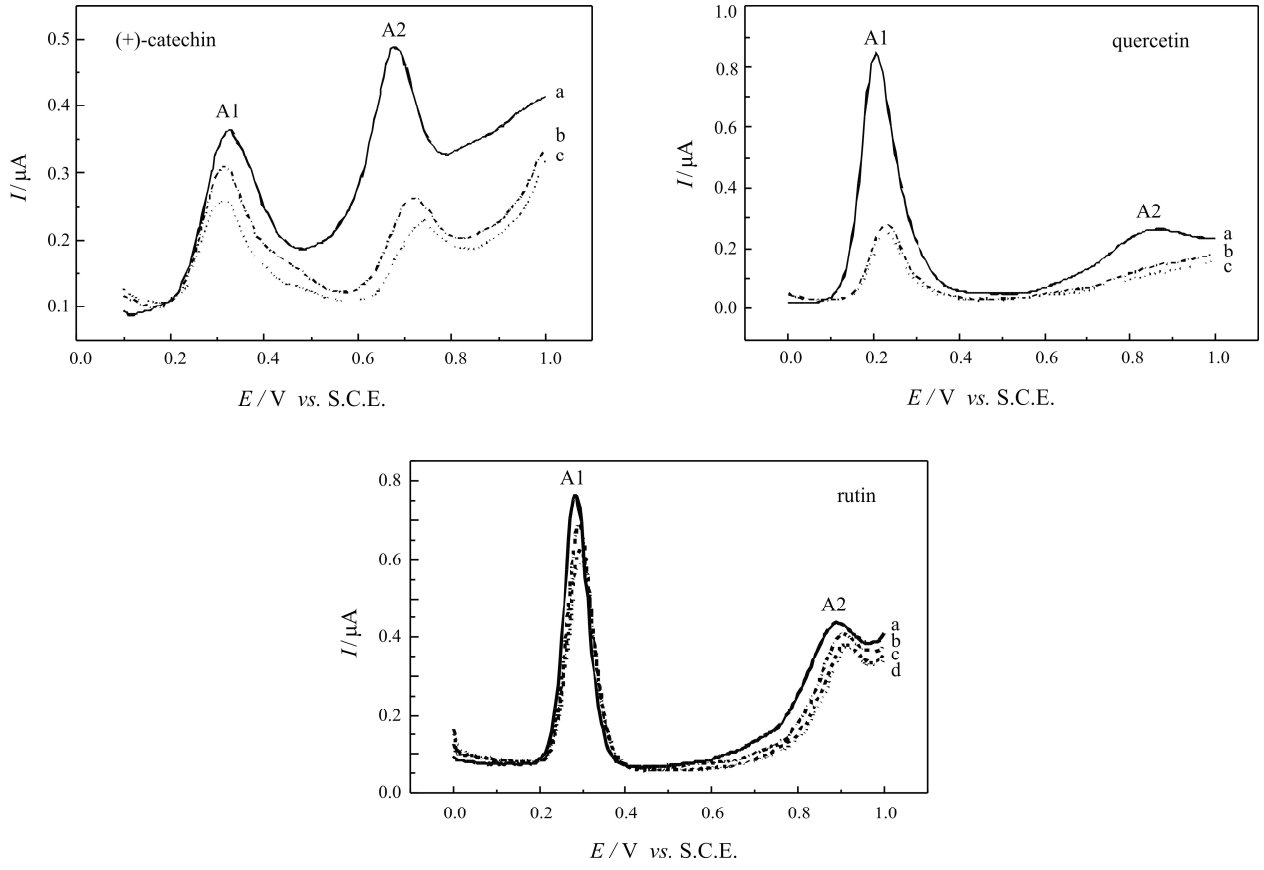

Figure 3. Diferential pulse voltamograms of (+)-catechin, quercetin and rutin ($c = 1 \cdot 10^{-5}$ mol dm^{-3}) in the acetate buffer ($I_c = 0.1$ mol dm^{-3}; pH = 4.5 for (+)-catechin; pH = 5.5 for rutin and quercetin) at the scan rate of 5 mV/s.

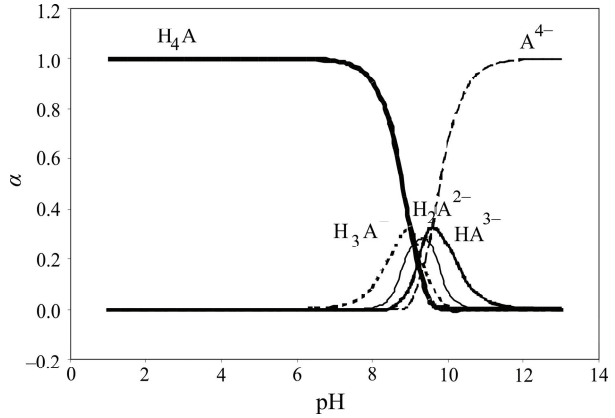

Figure 4. Dissociation diagram of (+)-catechin: (H_4A) undissociated form of (+)-catechin, (H_3A^-, H_2A^{2-}, HA^{3-} and A^{4-}) deprotonated forms of (+)-catechin. Dissociation constants ($pK_{a3'}$ = 9.02; $pK_{a4'}$ = 9.12; pK_{a5} = 9.43 and pK_{a7} = 9.58) were taken from the literature.[54]

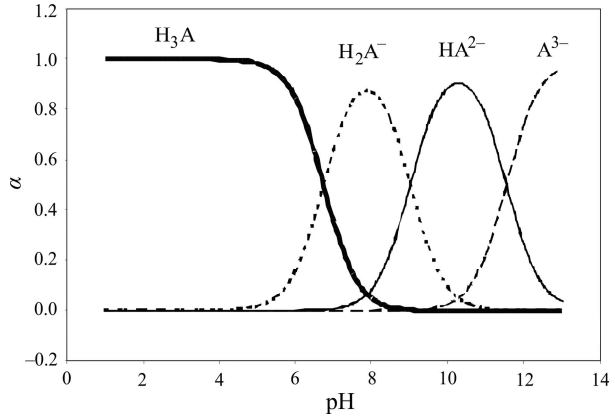

Figure 5. Dissociation diagram of quercetin: (H_3A) undissociated form of quercetin, (H_2A^-, HA^{2-} and A^{3-}) deprotonated forms of quercetin. Dissociation constants ($pK_{a3'}$ = 6.74; $pK_{a4'}$ = 9.02 and $pK_{a5,7}$ = 11.55) were taken from the literature.[55]

undergo homogenous chemical reactions with water following their oxidation at a glassy carbon electrode.[5,14,29,31]

Dissociation diagrams of (+)-catechin, quercetin and rutin (Figures 4−6), calculated from their microscopic dissociation constants deduced from NMR measurements for (+)-catechin[54] ($pK_{a3'}$ = 9.02; $pK_{a4'}$ = 9.12; pK_{a5} = 9.43 and pK_{a7} = 9.58) and from potentiometry and spectrophotometry for quercetin[55] ($pK_{a3'}$ = 6.74; $pK_{a4'}$ = 9.02 and $pK_{a5,7}$ = 11.55) and rutin[56] (pK_{a7} = 7.35; $pK_{a4'}$ = 8.80; $pK_{a3'}$ = 11.04 and pK_{a5} = 11.90) show that their spontaneous deprotonation doesn't occur below pH = 6 for (+)-catechin (the similar result was obtained by Martinez *et al.*)[4] and below pH = 5 for quercetin and rutin. It means that the neutral molecules of the investigated flavonoids participate in an electrochemical oxidation reaction and adsorption processes up to pH = 6

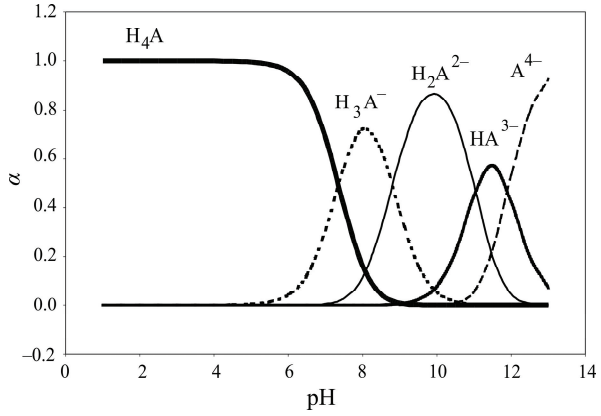

Figure 6. Dissociation diagram of rutin: (H_4A) undissociated form of rutin, (H_3A^-, H_2A^{2-}, HA^{3-} and A^{4-}) deprotonated forms of rutin. Dissociation constants (pK_{a7} = 7.35; $pK_{a4'}$ = 8.80; $pK_{a3'}$ = 11.04 and pK_{a5} = 11.90) were taken from the literature.[56]

for (+)-catechin and up to pH = 5 for quercetin and rutin. At higher pH values, spontaneous deprotonation of flavonoids occur, different dissociated species of (+)-catechin, quercetin and rutin are formed and the possibility of occurrence of much more complex electrochemical and adsorption processes exists, which agrees with the results of Martinez *et al.*[4] for (+)-catechin (they assumed the possibility of occurrence of electron or hydrogen abstraction reactions of catechin in the range of physiological pH).

Square-Wave Voltammetry

Square-wave voltammograms were recorded over the same pH interval (Table 3) investigated by cyclic voltammetry and differential-pulse voltammetry. The results have confirmed adsorption of oxidation products of investigated flavonoids on the electrode surface since the oxidation peak currents decreased with increasing scan number. The advantage of this method compared to differential pulse and cyclic voltammetry lies in greater speed of analysis, lower consumption of electroactive species and reduced problems with blocking of the electrode surface (the current is sampled in positive and negative-going pulses, so the oxidation and reduction peaks of electroactive species can be obtained at the same time). The reversibility of the first oxidation peak of (+)-catechin, quercetin and rutin was confirmed since both the oxidation (forward current) and reduction (backward current) peaks appeared at the same potential, E_p = 0.34 V for (+)-catechin (at pH = 4.5), E_p = 0.25 V for quercetin (at pH =5.5) and E_p = 0.33 V for rutin (at pH =5.5) (Figure 7). The square-wave voltammograms have confirmed the irreversibility of the second oxidation peak of (+)-catechin, since there was no corresponding reduction peak in the investigated pH interval.

Table 3. Oxidation current ($I_{p,a}$) and oxidation potential ($E_{p,a}$) values of the first oxidation peak for (+)-catechin, quercetin and rutin as the function of pH values, obtained by square wave voltammetry

	(+)-catechin		quercetin		rutin	
pH	$E_{p,a}$ / V	$I_{p,a}$ / μA	$E_{p,a}$ / V	$I_{p,a}$ / μA	$E_{p,a}$ / V	$I_{p,a}$ / μA
3.5	0.393	2.223	0.369	3.441	0.469	2.999
4.5	0.338	3.377	0.319	5.916	0.400	3.316
5.5	0.268	2.604	0.245	9.271	0.330	3.693
7.0	0.186	1.987	0.134	5.489	0.230	2.765
8.0	0.130	1.983	0.074	3.130	0.189	2.162

A possible oxidation mechanism of (+)-catechin, which corresponds to the oxidation of 3',4'-dihydroxy substituent on the B-ring and includes transfer of two electrons and two protons is given in Figure 8. The mechanism involves ionization of (+)-catechin, losing a proton to give the monoanionic species followed by a one electron, one proton oxidation of the monoanionic species to form a radical anion. This then undergoes a second reversible one-electron oxidation to give dehydro-form of (+)-catechin. The latter species is rapidly protonated and then dehydrated to yield the final product *o*-quinone.[4,5,8]

The mentioned oxidation mechanism is pH dependent (the highest oxidation peak current was obtained around pH= 4.5). According to some authors,[4] at lower pH values the first oxidation step of (+)-catechin

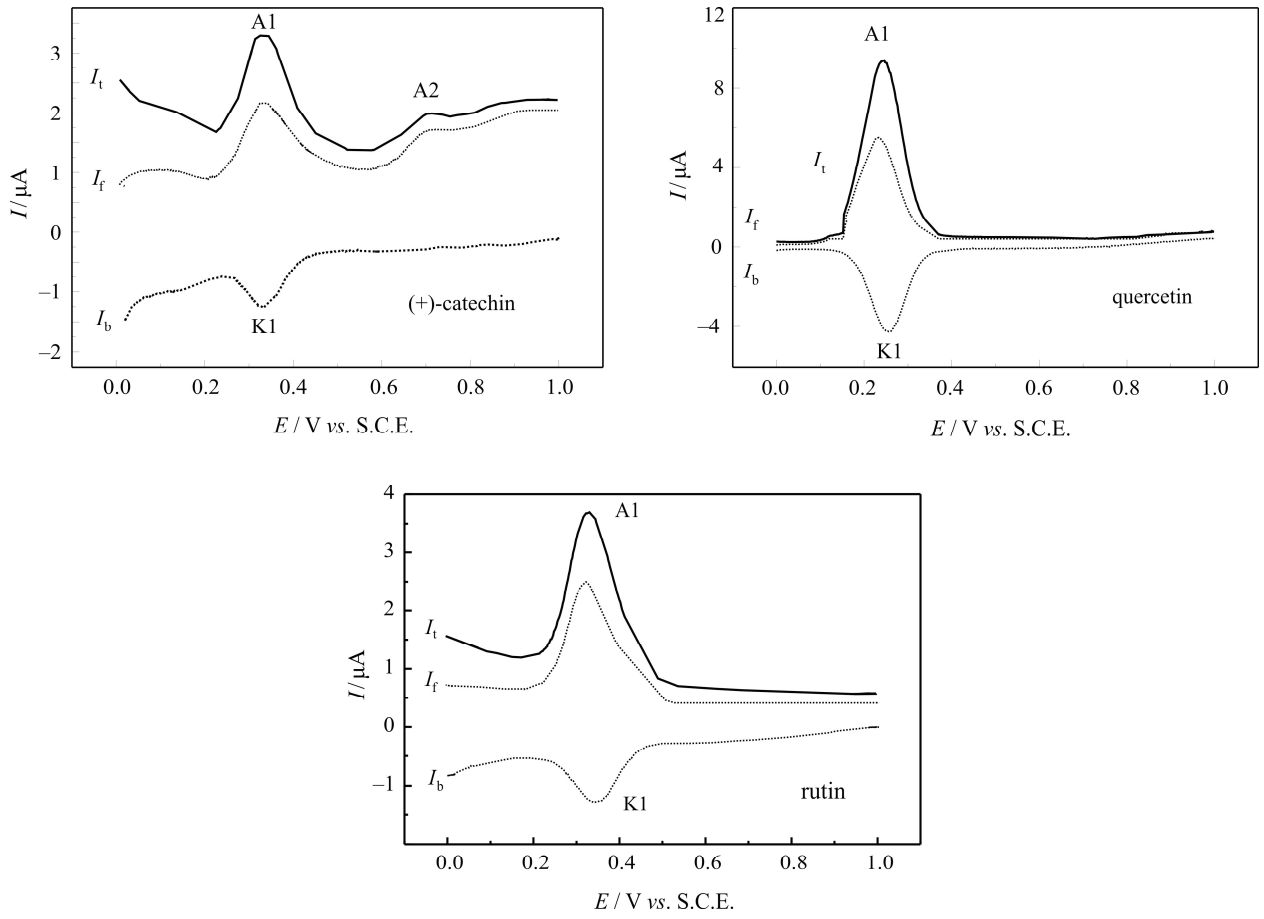

Figure 7. Square wave voltamograms of (+)-catechin, quercetin and rutin ($c = 1 \cdot 10^{-5}$ mol dm^{-3}) in the acetate buffer ($I_c = 0.1$, pH = 4.5 for (+)-catechin and pH = 5.5 for quercetin and rutin) at the frequency of 50 Hz, pulse amplitude of 50 mV and scan increment of 2 mV (the effective scan rate is 100 mV/s). (I_t) total current, (I_f) forward current and (I_b) backward current.

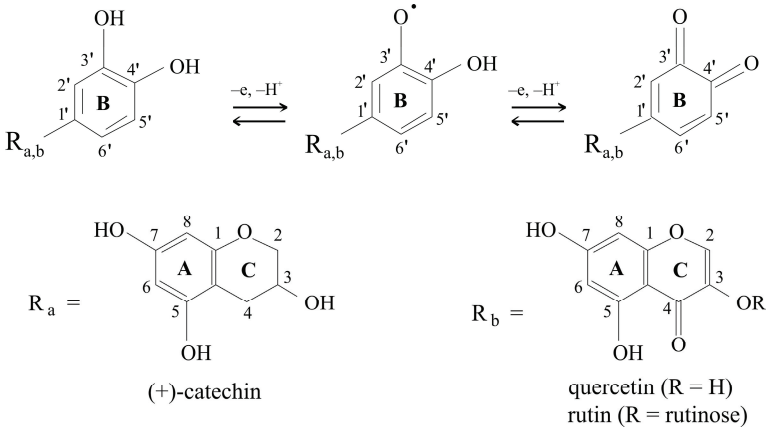

Figure 8. A possible mechanism of electrochemical oxidation of (+)-catechin (R_a), quercetin (R_b) and rutin (R_b). R = H in quercetin and rutinose ($C_{12}O_9H_{21}$) in rutin.

follows the eH mechanism (neutral molecule of (+)-catechin losses one electron first and after that one proton). At higher pH values (pH = 7.4) the first oxidation step follows two parallel reaction paths, - the eH mechanism (phenol/radical cation/phenoxyl radical) and the He mechanism (phenol/phenolat anion/phenoxyl radical). The second oxidation step of (+)-catechin follows the He mechanism (neutral molecule of (+)-catechin losses one proton and after that one electron). The coproportionation reaction between *o*-quinone and (+)-catechine is also possible, which leads to a semi-quinonic radical, which is able to give an electroinactive dimer.[8]

The second irreversible oxidation peak of (+)-catechin which some authors associate with the oxidation of OH group at the position 3 on the C ring[5] and other with the oxidation of 5,7-dihydroxy substituent on the A ring,[4,8] also includes transfer of two protons and two electrons. The final oxidation product is a catechin polymer which is the result of head to tail polymerization mechanism (repeated condensation reactions between the A-ring of one unit and the B-ring of another unit).[8]

A possible oxidation mechanism of quercetin and rutin, which corresponds to the oxidation of 3',4'-dihydroxy substituent on the B-ring and includes transfer of two electrons and protons is given in Figure 8. The mechanism involves ionization of quercetin /rutin, losing a proton to give the monoanionic species followed by a one electron, one proton oxidation of the monoanionic species to form a radical anion. This then undergoes a second reversible one-electron oxidation to give dehydro-form of quercetin/rutin. The latter species is rapidly protonated and then dehydrated to yield the final product *o*-quinone[14,27] and in the quercetin oxidation mechanism semi-quinone and *p*-quinone methide could also be formed[27] (it was found that quercetin/rutin and their oxidation products are both adsorbed on the electrode surface).[14,29,31,33,23] Quercetin can react as a nucleofil with its oxidation product quercetin-*o*-quinone to form a dimer[17] and can be degraded by air oxygen to produce relatively smaller phenolic acids or 3,4-dihydroxy and 2,4,6-trihydroxyphenylglyoxylic acid.[27] The oxidation mechanism is pH dependent and the optimal pH value for the oxidation process of both flavonoids was found to be around pH=5.5 which agrees with data found in literature.[15,41] The second oxidation peak of quercetin and rutin found in differential pulse voltammograms, which corresponds to the oxidation of 5,7-dihydroxy group on the A-ring is an irreversible oxidation process.[16,29,37]

TEAC assay

In this study, the antioxidant capacities of (+)-catechin, quercetin and rutin solutions of different concentrations, expressed as Trolox equivalents, were determined by the classic TEAC decolourisation assay. It was found that TEAC values of all investigated compounds are concentration dependent (Figure 9), which agrees with data found in literature.[57] Due to the concentration dependency of the TEAC values of investigated compounds, the TEAC values measured at concentration, c = 100 μmol dm^{-3} were used for comparison and they amounted to: 3.42 for quercetin, 2.95 for (+)-catechin and 2.87 for rutin. Since a higher TEAC value points to a higher antioxidant capacity, it can be concluded that quercetin has the highest antioxidant capacity followed by (+)-catechin and rutin. The TEAC assay gave the same relative order of antioxidant capacities of investigated flavonoids as obtained by cyclic voltammetry, since a lower half-wave potential value ($E_{1/2}$) of the first oxidation wave, also points to the higher antioxidant capacity[53] (quercetin has the lowest $E_{1/2}$ values (Table 1b), followed by (+)-catechin (Table 1a) and rutin (Table 1c). The obtained results also agree with data found in literature.[21,43,44,47,51]

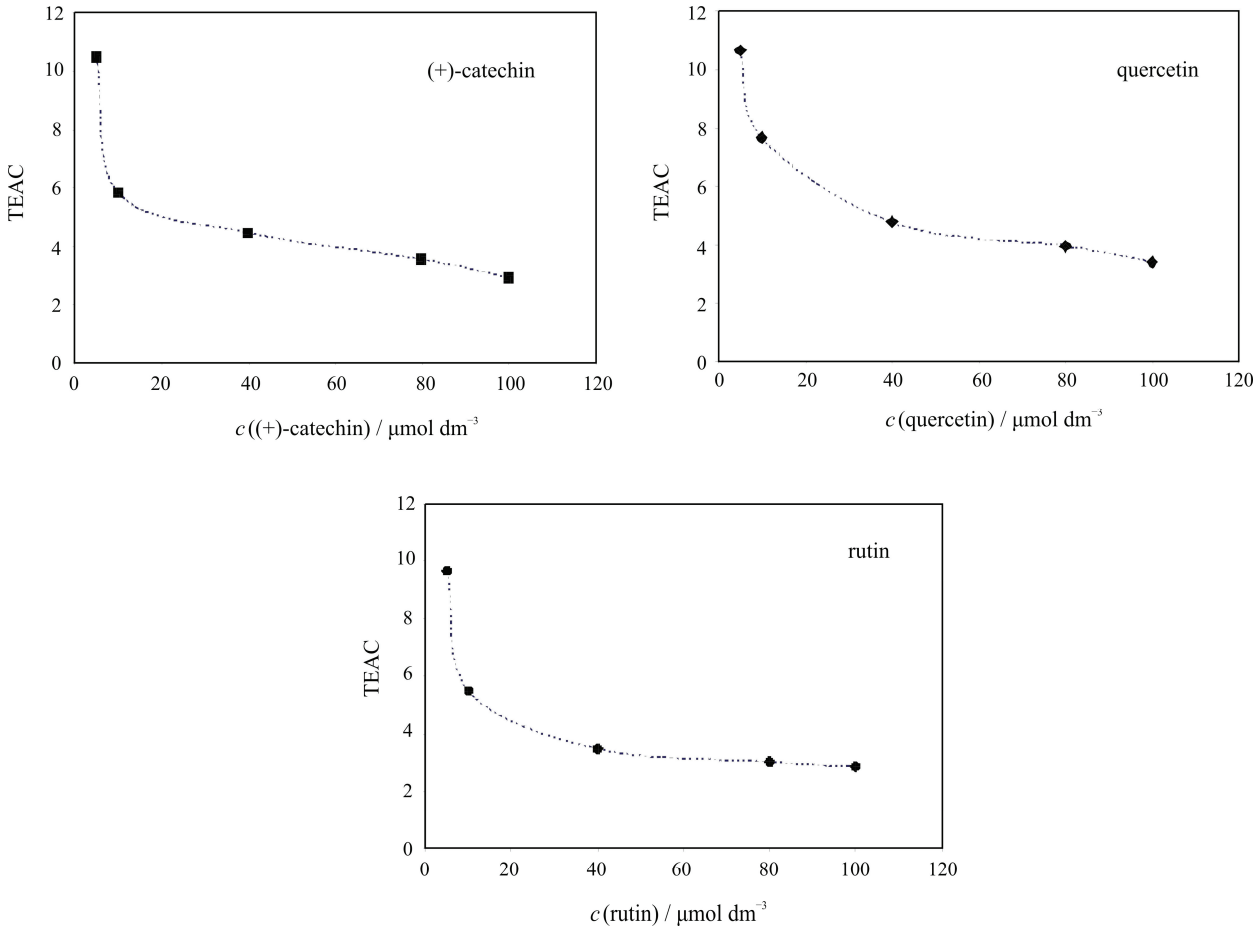

Figure 9. The TEAC values of (+)-catechin, quercetin and rutin (n = 3, SD = ± 0.05 for (+)-catechin and rutin; SD = ± 0.30 for quercetin) measured after 1 min in different concentrations by TEAC assay.

Different antioxidant capacities of investigated flavonoids, obtained in this study, can be explained by their structural differences. Antioxidant activity of (+)-catechin, quercetin and rutin can be ascribed to common 3',4'-catechol structure of the B-ring, which indicates a higher antioxidant activity (during the oxidation process on the B-ring of a flavonoid molecule, *o*-semiquinone radical is formed, which is a good antioxidant). Different antioxidant capacities can be explained by the presence of a double bond between C-2 and C-3, conjugated with the 4-oxo group in the ring C (present in quercetin and rutin molecules), as well as with the presence of 3-OH group in ring C (present in quercetin molecule) which enhance the radical scavenging capacity of flavonoids. Lower antioxidant capacity of rutin compared to quercetin and (+)-catechin can be explained by a lower number of –OH groups in a rutin molecule (quercetin and (+)-catechin have five –OH groups and rutin has four) and by supstitution of 3-OH in ring C with rutinose, which increases the steric tension and reduces the antioxidant activity. Higher antioxidant capacity of quercetin compared to (+)-catechin, can be explained by the presence of a double bond between C-2 and C-3, conjugated with the 4-oxo group in ring C in a quercetin molecule which enhances its antioxidant activity.[58,59]

CONCLUSIONS

The results of this study have shown that the electrochemical oxidation of (+)-catechin, quercetin and rutin on a glassy carbon electrode, which corresponds to oxidation of 3',4'-dihydroxy substituent on the B-ring (the first oxidation peak), is pH dependent, includes the transfer of two electrons and two protons and that the oxidation products of all flavonoids studied are strongly adsorbed on the electrode surface. Peak current dependence on pH revealed the maximum at around pH = 4.5 for (+)-catechin and pH = 5.5 for quercetin and rutin (it decreased in more acidic and alkaline media). Structural differences of the investigated flavonoids were shown through obtained different values of their oxidation and reduction potentials. TEAC assay results have shown that the TEAC values of all three investigated compounds are concentration dependent. The activity se-

quence of the investigated, structurally different, flavonoids is: quercetin > (+)-catechin > rutin.

REFERENCES

1. N. Balasundram, K. Sundram, and S. Samman, *Food Chem*. **99** (2006) 191−203.
2. J. B. Harborne and C. A. Williams, *Phytochemistry* **55** (2000) 481−504.
3. C. A. Rice-Evans, N. J. Miller, and G. Paganga, *Trends Plant Sci*. **2** (4) (1997) 152−159.
4. S. Martinez, L. Valek, M. Metikoš-Huković, and J. Piljac, *J. Electroanal. Chem*. **584** (2005) 92−99.
5. P. Janeiro and A. M. Oliveira Brett, *Anal. Chim. Acta* **518** (2004) 109−115.
6. P. A. Kilmartin, H. Zou, and A. L. Waterhouse, A. *J. Enol. Vitic*. **53** (4) (2002) 294−302.
7. P. A. Kilmartin and C. F. Hsu, *Food Chem*. **82** (2003) 501−512.
8. V. Castaignède, H. Durliat, and M. Comtat, *Anal. Lett.* **36** (9) (2003) 1707−1720.
9. A. Jarosz-Wilkołazka, T. Ruzgas, and L. Gorton, *Enzym Microb Tech*. **35** (2004) 238−241.
10. J. Piljac, S. Martinez, T. Stipčević, Ž. Petrović, and M. Metikoš-Huković, *Am. J. Enol. Vitic*. **55** (4) (2004) 417−422.
11. J. Wu, H. Wang, L. Fu, Z. Chen, J.H. Jiang, G. Shen, and R. Yu, *Talanta* **65** (2005) 511−517.
12. D. A. El-Hady, *Anal. Chim. Acta* **593** (2007) 178−187.
13. L.-J. Yang, C. Tang, H.-Y. Xiong, X.-H. Zhang, and S.-F. Wang, *Bioelectrochemistry* **75** (2009) 158−162.
14. A. M. Oliveira Brett and M.-E. Ghica, *Electroanalysis* **15** (22) (2003) 1745−1750.
15. N. E. Zoulis and C. E. Efstathiou, *Anal. Chim. Acta* **320** (1996) 255−261.
16. H. P. Hendrickson, A. D. Kaufman, and C. E. Lunte, *J. Pharm. Biomed. Anal*. **12** (3) (1994) 325−334.
17. H. R. Zare, M. Namazian, and N. Nasirizadeh, *J. Electroanal. Chem*. **584** (2005) 77−83.
18. D. Zielińska, L. Nagels, and M. K. Piskuła, *Anal. Chim. Acta* **617** (2008) 22−31.
19. D. Zielińska, W. Wiczkowski, and M. K. Piskuła, *J. Agric. Food Chem*. **56** (2008) 3524−3531.
20. D. Zielińska and B. Pierozynski, *J. Electroanal. Chem* . **625** (2009) 149−155.
21. A. Simić, D. Manojlović, D. Šegan, and M. Todorović, *Molecules* **12** (2007) 2327−2340.
22. H. P. Hendrickson, M. Sahafayen, M. A. Bel, A. D. Kaufman, M. E. Hadwiger, and C. E. Lunte, *J Pharmaceut Biomed Anal.* **12** (3) (1994) 335−341.
23. J.-B. He, X.-Q. Lin, and J. Pan, *Electroanalysis* **17** (18) (2005) 1681−1686.
24. X.-Q. Lin, J.-B. He, and Z.-G. Zha, *Sens. Actuators, B* **119** (2006) 608−614.
25. G.-P. Jin, J.-B. He, Z.-B. Rui, and F.-S. Meng, *Electrochim. Acta* **51** (2006) 4341−4346.
26. V. Adam, R. Mikelova, J. Hubalek, P. Hanustiak, M. Beklova, P. Hodek, A. Horna, L. Trnkova, M. Stiborova, L. Zeman, and R. Kizek, *Sensors* **7** (2007) 2402−2418.
27. A. Zhou, S. Kikandi, and O. A. Sadik, *Electrochem. Comm.* **9** (2007) 2246−2255.
28. Y. Ni, X. Zhang, and S. Kokot, *Spectrochim. Acta A* **71** (2009) 1865−1872.
29. M.-E. Ghica and A. M. Oliveira Brett, *Electroanalysis* **17** (4) (2005) 313−318.
30. B. Zeng, S. Wei, F. Xiao, and F.Zhao, *Sens. Actuators, B* **115** (2006) 240−246.
31. J. Kang, X. Lu, H. Zeng, H. Liu, and B. Lu, *Anal. Lett*. **35** (4) (2002) 677−686.
32. J. Tang, Z. Wu, J. Wang, and E. Wang, *Electroanalysis* **13** (16) (2001) 1315−1318.
33. X. Bao, Z. Zhu, N.-Q. Li, and J. Chen, *Talanta* **54** (2001) 591−596.
34. J.-B. He, Y. Wang, N. Deng, and X.-Q. Lin, Bioelectrochemistry **71** (2007) 157−163.
35. A. C. Franzoi, A. Spinelli, and I.C. Vieira, *J Pharmaceut Biomed Anal.* **47** (2008) 973−977.
36. M. Namazian, H. R. Zare, and M. L. Coote, *Biophys. Chem.* **132** (2008) 64−68.
37. X. Tian, F. Li, L. Zhu, and B. Ye, *J. Electroanal. Chem.* **621** (2008) 1−6.
38. Y. Zhang and J. Zheng, *Talanta* **77** (2008) 325−330.
39. D. B. McPhail, R. C. Hartley, P. T. Gardner, and G. G. Duthie, *J. Agric. Food Chem*. **51** (2003) 1684−1690.
40. A. Gomes, E. Fernandes, M. B. Q. Garcia, A. M. S. Silva, D. C. G. A. Pinto, C. M. M. Santos, J. A. S. Cavaleiro, and J. L. F. C. Lima, *Bioorg Med Chem.* **16** (2008) 7939−7943.
41. G. J. Volikakis and C. E. Efstathiou, *Talanta* **51** (2000) 775−785.
42. W. Sun, M. Yang, Y. Li, Q. Jiang, S. Liu, and K. Jiao, *J Pharmaceut Biomed Anal.* **48** (2008) 1326−1331.
43. O. Firuzi, A. Lacanna, R. Petrucci, G. Marrosu, and L. Saso, *Biochim. Biophys. Acta* **1721** (2005) 174−184.
44. H. Hotta, S. Nagano, M. Ueda, Y. Tsujino, J. Koyama, and T. Osakai, *Biochim. Biophys. Acta* **1572** (2002) 123−132.
45. C. Le Bourvellec, D. Hauchard, A. Darchen, J.-L. Burgot, and M.-L. Abasq, *Talanta* **75** (2008) 1098−1103.
46. A. J. Blasco, M.C. González, and A. Escarpa, *Anal. Chim. Acta* **511** (2004) 71−81.
47. D. Iveković, S. Milardović, M. Roboz, and B. S. Grabarić, *Analyst* **130** (2005) 708−714.
48. A. J. Blasco, M.C. Rogerio, M.C. González, and A. Escarpa, *Anal. Chim. Acta* **539** (2005) 237−244.
49. S. Milardović, D. Iveković, and B. S. Grabarić, *Bioelectrochemistry* **68** (2006) 175−180.
50. L. Campanella, A. Bonanni, E. Finotti, and M. Tomassetti, *Biosens. Bioelectron*. **19** (2004) 641−651.
51. J. Nilsson, D. Pillai, G. Önning, C. Persson, A. Nilsson, and B. Åkesson, *Mol. Nutr. Food Res*. **49** (2005) 239−246.
52. D. Villaño, M. S. Fernández-Pachón, A. M. Troncoso, and M. C. García-Parrilla, *Anal.Chim. Acta* **538** (2005) 391−398.
53. B. Yang, A. Kotani, K. Arai, and F. Kusu, *Anal. Sci*. **17** (2001) 599−604.
54. C. Cren-Olive, P. Hapiot, J. Pinson, and C. Rolando**,** *J. Am. Chem. Soc*. **124** (47) (2002) 14027−14038.
55. S. V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic, and M. G. Simic, *J. Am. Chem. Soc*. **116** (1994) 4846−4851.
56. C. Mielczarek, *Eur. J. Pharm. Sci*. **25** (2005) 273−279.
57. R. van den Berg, G. R. M. M. Haenen, H. van den Berg, and A. Bast, *Food Chem*. **66** (1999) 511−517.
58. K. E. Heim, A. R. Tagliaferro, and D. J. Bobilya, *J. Nutr. Biochem*. **13** (2002) 572−584.
59. A. J. Dugas, J. Castañeda-Acosta, G. C. Bonin, K. L. Price, N. H. Fischer, and G. W. Winston, *J. Nat. Prod*. **63** (2000) 327−331.

SAŽETAK

Elektrokemijska i antioksidacijska svojstva (+)-katehina, kvercetina i rutina

Martina Medvidović-Kosanović, Marijan Šeruga, Lidija Jakobek i Ivana Novak

Zavod za primijenjenu kemiju i ekologiju, Prehrambeno-tehnološki fakultet, Sveučilište J. J. Strossmayera u Osijeku, Kuhačeva 18, P.O. Box 709, HR-31000 Osijek, Hrvatska

Istraživana su elektrokemijska svojstva tri strukturno različita flavonoida ((+)-katehina, kvercetina i rutina) na elektrodi od staklastog ugljika, pri različitim pH vrijednostima, uporabom cikličke, diferencijalne pulsne i pravokutnovalne voltametrije. Utvrđeno je da se u svim istraživanim spojevima oksidira kateholna 3',4'-dihidroksilna skupina na B-prstenu (prvi oksidacijski pik). Taj proces je reverzibilan, ovisi o pH i uključuje izmjenu $2e^-$ i $2H^+$. Produkti elektrokemijske oksidacije svih ispitivanih flavonoida (osobito kvercetina) se snažno adsorbiraju na elektrodnu površinu. U terminima kvantifikacije, vrijednost apsorbancije, proporcionalna koncentraciji preostalog ABTS+•, mjerena je nakon konstantnog vemena reakcije. Rezultati su izraženi kao ekvivalenti Troloksa (TEAC vrijednost), tj. kao koncentracija otopine Troloksa (mmol dm^{-3}), koja ima antioksidacijski kapacitet ekvivalentan vrijednosti određenoj za 1.0 mmol dm^{-3} ispitivane tvari. Utvrđeno je da TEAC vrijednosti ovise o koncentraciji istraživanih spojeva. Aktivnosni slijed ispitivanih, strukturno različitih, flavonoida slijedi niz: kvercetin > (+)-katehin > rutin.