Full Paper

Chrysin and (±)-Taxifolin Electrochemical Oxidation Mechanisms

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Abstract
The electrochemical behaviour of the flavone chrysin and the dihydroflavonol (±)-taxifolin was investigated by different electrochemical techniques at different pH values. Dihydroflavonol (±)-taxifolin presented two oxidation peaks. The first electrooxidation reaction of (±)-taxifolin is reversible over the whole pH range. The dependence of peak current on pH showed a maximum around neutral pH values with a decrease in acidic and alkaline media. The influence of the catechol group deprotonation on ring B is related to the electron/proton donating capacity in (±)-taxifolin and to its radical scavenging antioxidant activity. In chrysin, where the ring B has an unsubstituted phenyl, the antioxidant active moiety is in ring A. Chrysin presents an irreversible oxidation peak at higher oxidation potentials than those usually obtained for the catechol moiety ion in ring B and a pH dependent behaviour. The −OH groups on ring A are affected by the 4-keto group as shown by the lower pK_a value of the 7-OH, indicating that the 7-OH group is the more acidic. The 5-OH group is less acid and may be explained by the acidic weakening effect of an intramolecular H-bond between the 5-OH and 4-keto group of taxifolin and chrysin.

Keywords: Chrysin, Taxifolin, Dihydroquercetin, Oxidation, Radical scavenging activity, Flavonoids, Free radicals.

1. Introduction

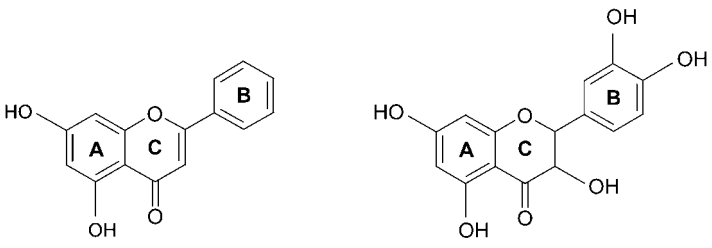
Plants contain phytochemicals with antioxidant properties such as vitamin C, vitamin E, beta-carotene (which the body converts into vitamin A), and also polyphenols (phenolic acids, flavonoids, and tannins) in high concentrations.

The flavonoids constitute a large class of compounds, ubiquitous in plants, containing a number of phenolic hydroxyl groups attached to ring structures, designated A, B and C, conferring antioxidant activity [1–3]. The recent explosion of interest in the bioactivity of the flavonoids of higher plants is due, at least in part, to the potential health benefits of these polyphenolic components of major dietary constituents. It is well-known that diets rich in fruit and vegetables are protective against cardiovascular disease and certain forms of cancer [4].

The biochemical activities of flavonoids and their metabolites depend on their chemical structure and the relative orientation of various moieties in the molecule. Flavonoids are classified according to their chemical structure [5]. From the point of view of free radical chemistry, the antioxidant properties of the flavonoids may be investigated through reversible and irreversible electrode reactions. Their ability to act as chemical defence agents by donating an electron to an oxidant depends critically on the reduction potentials of their radicals. Structure-activity relationship studies of flavonoids have shown that the o-dihydroxy structure in the ring B and the 2,3- double bond in conjugation with the 4-oxo function in the ring C (as in flavones) is essential for effective free radical scavenging activity [6]. The presence of a 3-hydroxyl group in the heterocyclic ring also increases the radical scavenging activity while additional hydroxyl groups at positions 5 and 7 of the ring A appear to be less important [7].

The structures in Scheme 1 represent two flavonoids, chrysin, a dihydroxy flavone and (±)-taxifolin, a pentahydroxyflavanone. Flavones originate from flavanones by 2,3-desaturation, and this oxidation may be considered as a "branch point reaction" in the flavonoid pathway towards to the final flavone and flavonol products. Flavones and flavonols have a plane benzopyranone skeleton whereas the dihydroflavonols have a less plane dihydrobenzopyranone skeleton. Chrysin has a double bond between C-2 and C-3, and the ring B will be coplanar with the rings A and C due to the conjugation. Saturation of this double bond, as occurs in taxifolin, will destroy conjugation and coplanarity [8].

The mechanism of action as antioxidants seems to involve the ability of phenols to scavenge radicals by an H-atom, or electron transfer process in which the phenol is converted



Flavone: Chrysin *Flavanone*: (±)-taxifolin

Scheme 1. Chemical structure of the flavone: chrysin (5,7-dihydroxyflavone) and dihydroflavonol: (±)-taxifolin (3,3′,4′,5,7-pentahydroxyflavanone).

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into a phenoxyl radical resulting in the formation of the semiquinone, which can donate a further electron to form the quinone, Scheme 2 [7, 9]. Electrochemical measurements lead to physicochemical parameters for antioxidants, which help in evaluating their antioxidative abilities [10–12], and understanding their reaction mechanisms.

In this study the electrochemical mechanism of oxidation of the flavone chrysin and the dihydroflavonol (±)-taxifolin was investigated, in a wide range of solution conditions, using cyclic, differential pulse and square-wave voltammetry. Information on the oxidation mechanisms obtained from results at different pH may play a crucial role in understanding antioxidant activity.

2. Experimental

Flavonoids were used as received without further purification. The flavonoids chrysin (5,7-dihydroxyflavone) and (±)-taxifolin (3,3′,4′,5,7-pentahydroxyflavanone) of 85% purity and the compounds resorcinol and catechol were from Sigma-Aldrich, Madrid, Spain, and all other reagents were Merck analytical grade. All solutions were made up using ultrapure water obtained from a Millipore Milli-Q purification system (resistivity = 18 MΩ cm). The 10 mM stock solutions of both compounds were prepared in 100% ethanol.

All experiments were carried out at room temperature (ca. 22 ± 1 °C) and in the presence of dissolved oxygen. Solutions of buffer supporting electrolyte of ionic strength 0.2 M were used in all experiments (Table 1).

Table 1. Supporting electrolyte buffer solutions.

Supporting electrolyte solutions	pH
0.2 M KCl + 0.2 M HCl	1.1
0.2 M KCl + 0.2 M HCl	2.1
0.2 M NaOAc + 0.2 M HOAc	3.4
0.2 M NaOAc + 0.2 M HOAc	4.1
0.2 M NaOAc + 0.2 M HOAc	5.2
0.2 M Na_2HPO_4 + 0.2 M NaH_2PO_4	6.0
0.2 M Na_2HPO_4 + 0.2 M NaH_2PO_4	7.0
0.2 M Na_2HPO_4 + 0.2 M NaH_2PO_4	8.5
2.0 M NH_3 + 2 M NH_4Cl	9.4
0.2 M KCl + 0.2 M NaOH	12.0
0.2 M KCl + 0.2 M NaOH	12.8

The pH measurements were carried out with a Crison GLP 21 pH-meter.

Electrochemical experiments were done using an Autolab PGSTAT 10 running with GPES (General Purpose Electrochemical System) version 4.9, Eco-Chemie, Utrecht, The Netherlands. Voltammetric curves were recorded at room temperature using a three-electrode system in a small volume electrochemical cell of capacity 2 mL (Cypress System, Inc., USA). The working electrode was a glassy carbon (GCE) mini-electrode of 1.5 mm diameter; Ag/AgCl (saturated KCl) was used as a reference electrode and platinum wire as a counter electrode. In this work, all potentials were reported vs. Ag/AgCl (sat KCl) electrode. The glassy carbon working electrode was polished with diamond spray (6 and 1 μm).

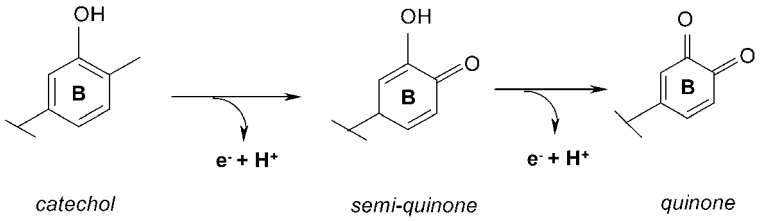
Cyclic voltammograms were performed at scan rates of 25, 50, 100 mV s^{-1}. Differential pulse voltammetry conditions used were pulse amplitude 50 mV, pulse width 70 ms, and scan rate of 5 mV s^{-1}. Square-wave voltammetry conditions were frequency 13, 25 and 50 Hz, amplitude 50 mV, and potential increment 2 mV (effective scan rates were 25, 50 and 100 mV s^{-1}, respectively).

3. Results and Discussion

The flavonoid structure presents functional OH groups attached to ring structures that can be electrochemically oxidized, Scheme 1. Electrochemical studies revealed that the catechol group in ring B, Scheme 2, is more easily oxidizable than the resorcinol group in ring A, and on ring B the most oxidizable phenolic function is the more basic site [13]. The pK_a values were assigned to ring A and ring B of both compounds following references [14, 15]. The oxidation mechanisms of a dihydroflavonol, (±)-taxifolin, and a flavone, chrysin, were studied by different electrochemical techniques. The reproducibility was tested in repeated measurements and a detection limit of 1 μM was found.

3.1. Electrochemical Oxidation of (±)-Taxifolin

The cyclic voltammogram in pH 7.0 buffer of (±)-taxifolin showed two oxidation peaks associated with the oxidation centres present in the molecule: a reversible peak 1, confirmed in the second scan by reversing the potential scan just before peak 2, and an irreversible peak 2, Figure 1.



Scheme 2. Oxidation mechanism of catechol moiety.

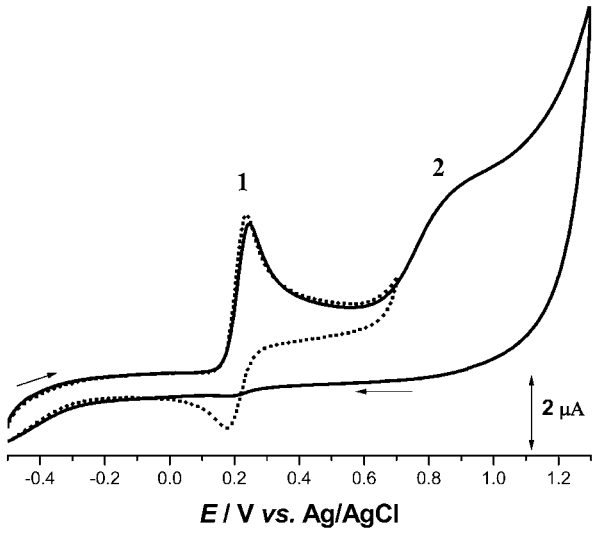


Fig. 1. Cyclic voltammograms of 1 mM (±)-taxifolin in pH 7.0 phosphate buffer. Scan rate 25 mV s^{-1}.

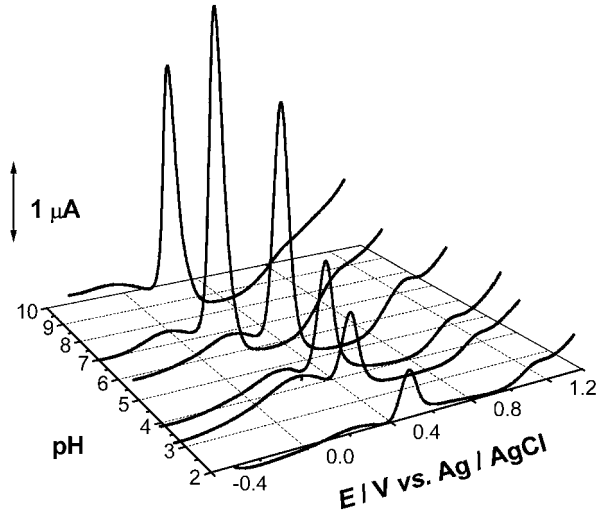


Fig. 3. 3D plot of differential pulse voltammograms of 1 mM (±)-taxifolin as a function of pH. Scan rate 5 mV s^{-1}.

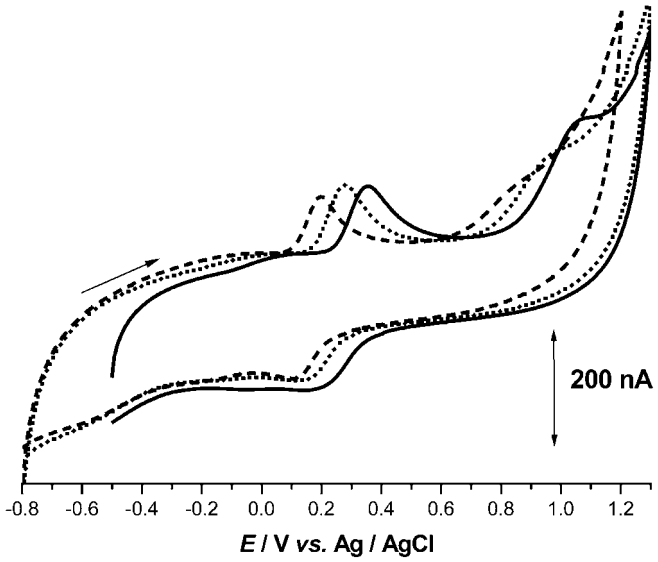


Fig. 2. Cyclic voltammograms of 1 mM (±)-taxifolin in: (——) pH 6.0 phosphate buffer; (·····) pH 7.0 phosphate buffer; (----) pH 8.5 phosphate buffer. Scan rate 1 V s^{-1}.

The reduction peak corresponds to the reduction of the oxidation products formed in peak 1.

Some information on the mechanism of polyphenol oxidation was provided by comparing the oxidation potentials at different pH, Figure 2, which showed that an increase of pH is associated with a decrease of the oxidation potentials. The reversibility of peak 1 of (±)-taxifolin was observed over the whole pH range studied although the current was higher and the separation between oxidation and reduction peaks was smaller at pH 8.5.

Considering the reversible reaction represented by peak 1 of (±)-taxifolin a value of $E_{p,c} - E_{p/2,c} = 75$ mV at 298 K was obtained [19]. This indicated that one electron is involved in the first oxidation reaction. This approach is valid only for reversible systems; modifications for irreversible and quasi-reversible systems have been described in detail [20].

A differential pulse voltammetric study of (±)-taxifolin was performed over a wide pH range from 1.1 to 12.5, Figure 3. Peaks 1 and 2 were observed at all pHs providing information on the mechanism of (±)-taxifolin oxidation and the dependence on pH. The results confirmed that the two (±)-taxifolin oxidation peaks correspond to the two oxidizable moieties. Peak 2 could be identified as the 5,7-dihydroxyl moiety in ring A (resorcinol moiety).

The dependence of the current of peak 1 vs. pH of (±)-taxifolin, Figure 3, is very high when compared with the small current of peak 2, which is in agreement with higher radical scavenging activity corresponding to the oxidation of the catechol moiety. The oxidation current is strongly dependent on pH and is higher at neutral pH values corresponding to the hydroxyl moiety deprotonation, meaning that after deprotonation electron transfer becomes easier, and the mechanism of radical scavenging antioxidant activity of (±)-taxifolin in the neutral form is increased.

A differential pulse voltammogram of (±)-taxifolin is compared with those obtained for resorcinol and catechol, Figure 4. This confirms that oxidation peak 1 corresponds to the catechol group on the ring B. The oxidation peak 2 does not occur at the same potential as the resorcinol molecule but this could be explained by the acid weakening effect of the intramolecular H-bond between the 5-OH and 4-keto group of (±)-taxifolin.

Square-wave voltammetry was also used since it has the advantage of greater speed of analysis, lower consumption of electroactive species in relation to differential pulse voltammetry, and reduced problems with blocking of the electrode surface [19, 20]. Square-wave voltammetry showed similar results as differential pulse and cyclic voltammetry, i.e., oxidation peaks 1 and 2 for (±)-taxifolin. It showed the appearance of significantly lower currents due

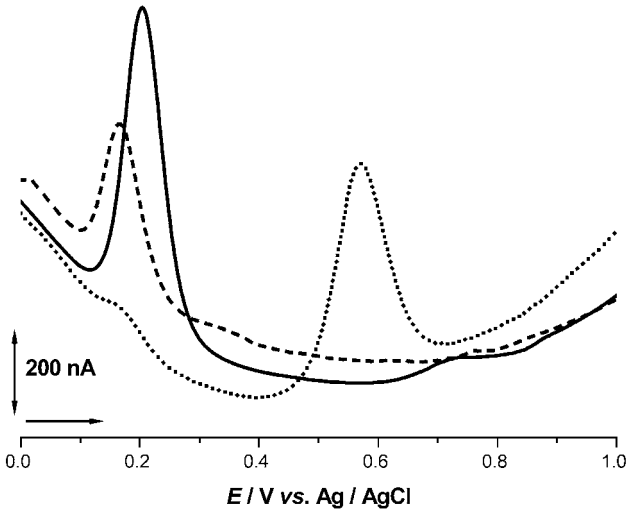


Fig. 4. Differential pulse voltammograms in pH 7.0 phosphate buffer of 10 μM (----) catechol, (·····) resorcinol and (——) (±)-taxifolin. Scan rate 5 mV s^{-1}.

to adsorption on the second scan, together with a decrease in peak current in more acidic and in more alkaline media. The square wave voltammetry conditions chosen, an effective scan rate of 50 mV s^{-1}, led to well-defined voltammograms, Figure 5.

3.2. Electrochemical Oxidation of Chrysin

The cyclic voltammograms in pH 7.0 buffer for (±)-taxifolin and chrysin are shown in Figure 6. (±)-taxifolin showed a reversible peak 1 at +0.26 V, and an irreversible peak 2 at +0.90 V. The reduction peak potential was at +0.20 V. The cyclic voltammogram of chrysin showed just one irreversible peak at +1.0 V, Figure 6; this peak has a similar potential to peak 2 of (±)-taxifolin. This means that in the flavonoids the oxidation peak localized at ~ +1.0 V at pH 7.0 corresponds to the oxidation of the 5,7-dihydroxyl moiety at ring A. This was observed in previous studies by cyclic voltammetry with other flavonoids [16–18].

A differential pulse voltammetric study of chrysin was also performed over a wide pH range from 1.1 to 12.5. In agreement with the previous results (±)-taxifolin presented two oxidizable moieties and has two oxidation peaks whereas chrysin always showed just one peak, Figure 7. Peak 2 of (±)-taxifolin is coincident with the chrysin oxidation peak, since this molecule presents a unique oxidizable moiety at ring A. The peak 2 of (±)-taxifolin could be identified as 5,7-dihydroxyl moiety at ring A (resorcinol moiety).

Differential pulse voltammograms of resorcinol and chrysin are compared in Figure 8. The difference in oxidation potentials has the same explanation as that for the (±)-taxifolin.

In the differential pulse voltammetry of chrysin and (±)-taxifolin over the pH range 1.1 to 12.5 the slope of the E_p vs.

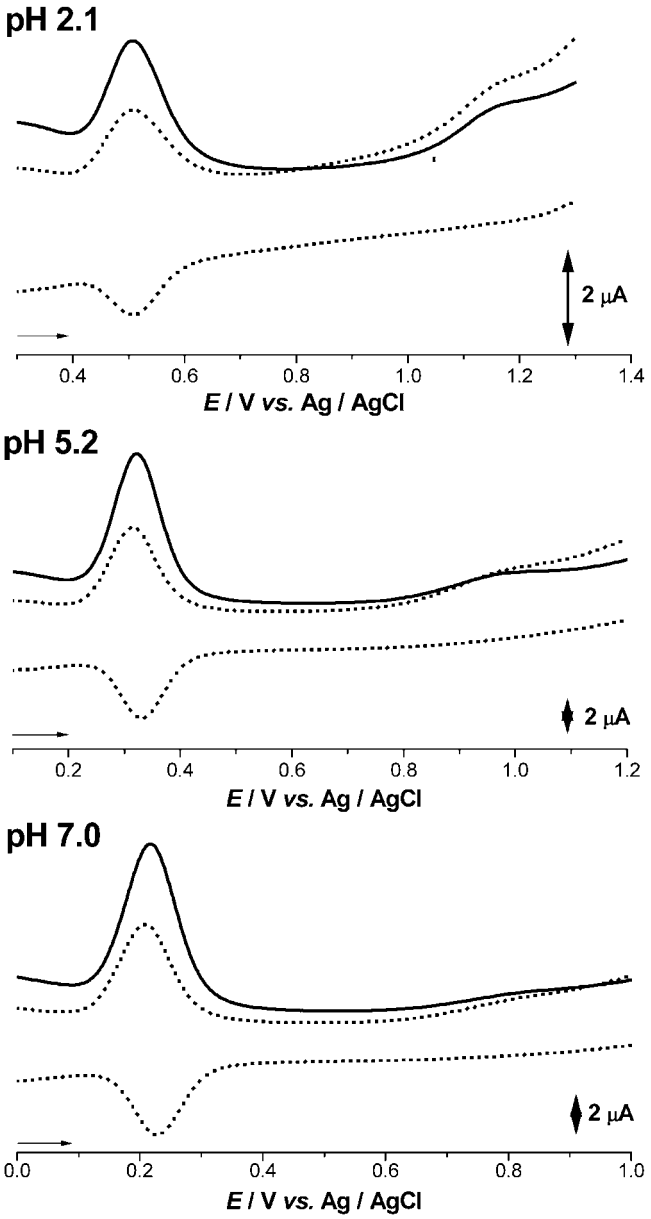


Fig. 5. Square-wave voltammograms of 1mM (±)-taxifolin at different pHs. Frequency 25 Hz, amplitude 50 mV, effective scan rate 50 mV s^{-1}.

pH plot is 59 mV per pH unit for (±)-taxifolin peak 1 over the whole pH range studied, Fig. 9. This peak corresponds to the 3′,4′-dihydroxyl moiety at ring B (catechol moiety) of (±)-taxifolin. The plot shows not only electrons but also protons are released from the molecule over the whole pH range [12].

In the plot of E_p vs. pH, Figure 9, the same behaviour was observed for the oxidation peak of chrysin and peak 2 of (±)-taxifolin, enabling confirmation of the proposed mechanism. The plot showed that the reaction is pH dependent until pH ~ 10 and independent for higher pHs for both. These peaks are associated with the moiety in the resorcinol ring A, which has two hydroxyl groups. The number of electrons involved was determined by the peak width at half

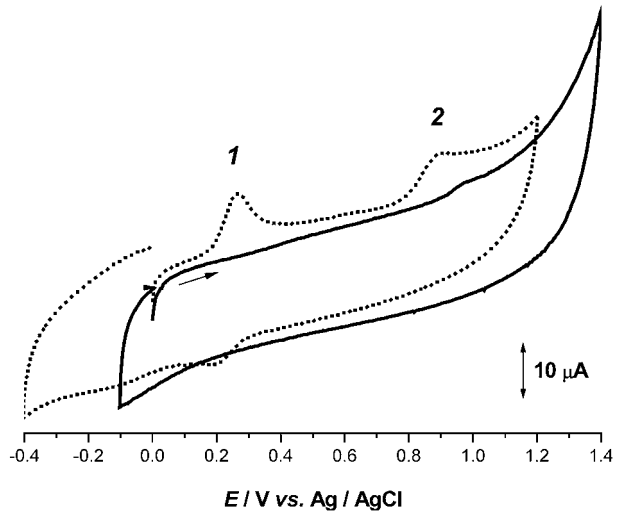


Fig. 6. Cyclic voltammograms in pH 7.0 phosphate buffer of 10 μM: (·····) (±)-taxifolin and (——) chrysin. Scan rate 2 V s^{-1}.

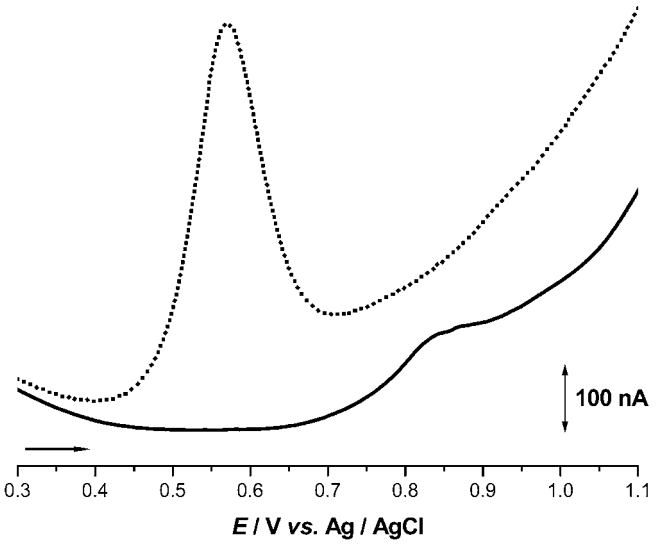


Fig. 8. Differential pulse voltammograms in pH 7.0 phosphate buffer of 10 μM (·····) resorcinol and (——) chrysin. Scan rate 5 mV s^{-1}.

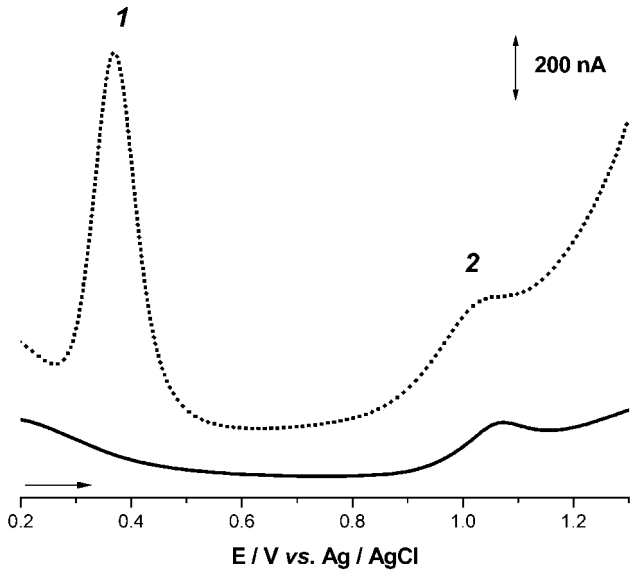


Fig. 7. Differential pulse voltammograms in pH 4.1 acetate buffer of 1mM: (·····) (±)-taxifolin and (——) chrysin. Scan rate 5 mV s^{-1}.

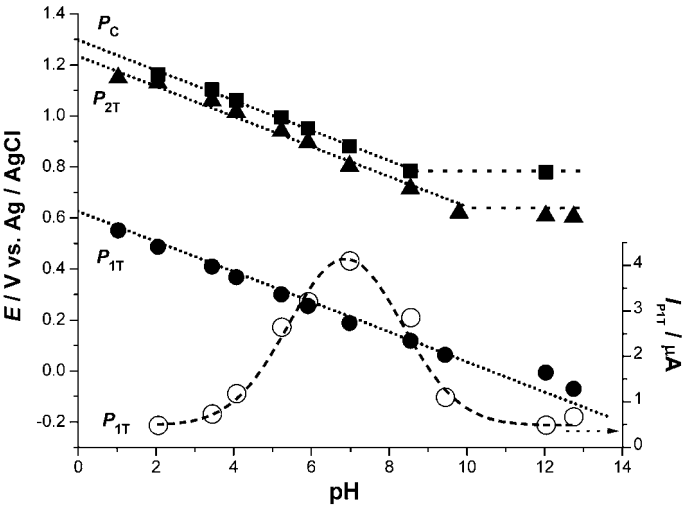


Fig. 9. Dependence with pH, for chrysin and (±)-taxifolin, in buffer supporting electrolyte, of oxidation potentials: E_p of (●–P_{1T}) peak 1 and (▲–P_{2T}) peak 2 of (±)-taxifolin and (■–P_c) peak of chrysin; and peak current: I_p of (○–P_{1T}) peak 1 of (±)-taxifolin vs. pH.

height, using the differential pulse voltammograms [19]. The value of $W_{1/2}$ obtained was 95 mV for chrysin and 100 mV for peak 2 of (±)-taxifolin, thus indicating that one electron is involved, and hence one proton also. The electron is from the OH groups of the ring A, which are affected by the 4-keto group as shown by the lower pK_a value of the 7-OH, indicating that the 7-OH group is the more acidic [14]. The 5-OH group is less acid because of the already mentioned acid weakening effect of an intramolecular H-bond between the 5-OH and 4-keto group of (±)-taxifolin.

Square-wave voltammetry showed similar results concerning oxidation peaks 1 and 2 for (±)-taxifolin and one irreversible oxidation peak for chrysin.

The flavonoids investigated exhibit either one or two oxidation peaks. Moreover, (±)-taxifolin and chrysin adsorbed strongly on the electrode surface and the final oxidation product blocks the electrode surface, as demonstrated by the rapid decrease of the oxidation peaks on repeated cycling.

The (±)-taxifolin oxidation processes proceed in a cascade mechanism and are related with the substituent group in ring B and the resorcinol group in ring A which all present electroactivity, and the oxidation potentials are identified with the oxidation peaks 1 and peak 2, described previously. The first oxidation occurs at low positive

potentials that implicates a higher radical scavenging activity and is a reversible reaction. The hydroxyl group oxidised next was shown to undergo an irreversible oxidation reaction. Chrysin exhibited only one irreversible oxidation peak.

Chrysin, with a double bond between C-2 and C-3, on ring B will have a planar structure together with rings A and C due to conjugation. Saturation of this double bond occurs in (±)-taxifolin, which will not enable conjugation and coplanarity. The acid strengthening effect of the 4-keto group of (±)-taxifolin has no effect on the OH groups of ring B as shown by the pK_a. This confirms that ring A and ring B of this flavonoid are unconjugated. Only the OH groups of ring A are affected by the 4-keto group as shown by the lower pK_a value of the 7-OH, indicating that the 7-OH group is the most acidic. The 5-OH group is less acid and this may be explained by the acid weakening effect of an intramolecular H-bond between the 5-OH and 4-keto group of (±)-taxifolin.

The oxidation current of (±)-taxifolin peak 1 is higher at neutral pH values corresponding to the hydroxyl moiety deprotonation, meaning that after deprotonation electron transfer becomes easier, and the mechanism of radical scavenging antioxidant activity of (±)-taxifolin in the neutral form is increased.

Previous research showed that the pH-dependent effect on hydroxyflavone antioxidant activity is mainly due to an increased radical scavenging ability of the flavonoids upon their deprotonation. Because deprotonation generally enhances the antioxidant action of the hydroxyflavones and because only the ionisation potential (IP), and not the bond dissociation energies (BDE) of the hydroxyflavones becomes significantly lower upon deprotonation, it can be concluded that electron donation is the dominant mechanism of antioxidant action of hydroxyflavones after deprotonation. Upon deprotonation the radical scavenging capacity increases because electron and not proton donation becomes easier. This implies that not only the ease of radical scavenging but also the mechanism of antioxidant activity may change upon deprotonation, and electron donation may be more important for flavonoid antioxidant action at physiological pH [21].

4. Conclusions

The oxidation mechanisms of a dihydroflavonol, (±)-taxifolin, and a flavone, chrysin, have been studied by different electrochemical techniques. The oxidation process of the dihydroflavonol (±)-taxifolin is complex, pH-dependent, the first step is reversible, (±)-taxifolin is strongly adsorbed on the electrode surface and the final product is not electroactive and blocks the electrode surface. The first electrooxidation reaction of (±)-taxifolin is reversible over the whole pH range. A dependence of peak current on pH was observed showing a maximum around neutral pH values and decreasing in acidic and alkaline media. The influence of catechol group deprotonation is related to the electron/proton donating capacity in (±)-taxifolin and to its radical scavenging antioxidant activity. The oxidation process of chrysin occurs at higher potentials, has only one step and is also pH-dependent.

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