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Electrochemical and Antioxidant Properties of Anthocyanins and Anthocyanidins

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Electrochemical properties of delphinidin, cyanidin, pelargonidin, kuromanin and callistephin were investigated by cyclic and differential pulse voltammetries at different pH values and also in methanol. On the basis of oxidation potentials, the order of antioxidant activity for anthocyanidins is delphinidin > cyanidin > pelargonidin. Oxidation peaks for anthocyanins (kuromanin and callistephin) are shifted to more positive potentials compared to anthocyanidins (delphinidin, cyanidin and pelargonidin). Oxidation peak currents are linearly dependent on the square root of the scan rate, which is typical of a diffusion controlled electrochemical process. Antioxidant activities of the compounds were evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method and they are directly related to their redox potential values. The order of the antioxidant activity is delphinidin > cyanidin > pelargonidin > kuromanin > callistephin.

Keywords
anthocyanins
anthocyanidins
antioxidants
electrochemical properties

INTRODUCTION

Anthocyanins are one of the main classes of flavonoids in red wines. They have been pointed out as the molecular entities that are most likely responsible for what has been described as the »French Paradox«, that is, the lower incidence of coronary atherosclerosis in the French population compared to other Western populations, even though the former consumes a fattier diet.[1] Anthocyanins are representative of plant pigments widely distributed in colored fruits and flowers. They also exhibit antioxidant activities and therefore may contribute to prevention of heart and inflammatory diseases.[2–9] Anthocyanins, and especially anthocyanidins, exhibit a variety of pharmacological properties that render them interesting potential cancer chemopreventive agents.[9–11] Because anthocyanins are widely consumed, finding out additional biological activities related to these compounds would be of great interest.

Differences between the individual anthocyanins lie in the number of hydroxyl groups, the nature and number of sugars attached to the molecule, the position of this attachment, and the nature and number of aliphatic or aromatic acids attached to sugars in the molecule. The aglycones of anthocyanins are called anthocyanidins.

The antioxidant potential of anthocyanins can change in dependence on the substituents.[12,13] Some studies suggest that the glycosidic forms generally display a de-

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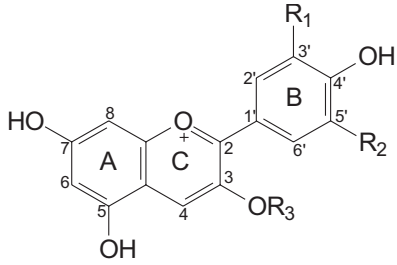
crease in the antioxidant capacity when compared with the corresponding aglycone.[14,15] This may be due to the steric hindrance conferred by the bulky sugars.[16,17]

In this work, we have studied the electrochemical properties of delphinidin, cyanidin, pelargonidin, kuromanin, and callistephin (Figure 1) in a wide range of solution conditions, by cyclic and differential pulse voltammetries. The antioxidant activities of the compounds were determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method and correlated with their oxidation potentials. It is assumed that both the electrochemical oxidation (Fl–OH $\rightarrow$ Fl–O$^{\bullet}$ + e$^-$ + H$^+$) and the antioxidant activity (Fl–OH $\rightarrow$ Fl–O$^{\bullet}$ + H$^{\bullet}$) involve the breaking of the same O–H bond;[18] the lower the oxidation potential, the higher the antioxidant activity of the compound. This suggests that the electrochemical properties of flavonoids may be associated with their biological activities. Hence, we propose that the simple cyclic voltammetry technique can be used to evaluate the biological activity of anthocyanins and anthocyanidins.



	R_1	R_2	R_3
Pelargonidin	H	H	H
Cyanidin	OH	H	H
Delphinidin	OH	OH	H
Kuromanin	OH	H	3-O-glucoside
Callistephin	H	H	3-O-glucoside

Figure 1. Structures of delphinidin, cyanidin, pelargonidin, kuromanin, and callistephin.

EXPERIMENTAL

Reagents

Delphinidin chloride, cyanidin chloride, pelargonidin chloride, kuromanin chloride, and callistephin chloride were purchased from Extrasynthese (Genay, France) and used without further purification.1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Aldrich Chemical Co., HPLC grade methanol from Mallinckrodt Inc. and lithium perchlorate from Acros Organics. All other chemicals were of the highest purity and used as received.

Apparatus and Measurements

Routine UV-Vis spectra were obtained in 1-cm quartz cells by using a Hewlett-Packard 8453 spectrophotometer. Electrochemical experiments were carried out with a PAR model 273A Potentiostat/Galvanostat. Cyclic and differential pulse voltammetric experiments were performed in a one-compartment cell using a glassy carbon working electrode, a platinum wire auxiliary electrode, and a Ag/AgCl, KCl 3 mol L^{-1} (0.201 *vs* NHE) reference electrode. Cyclic voltamograms were obtained at scan rates ranging from 25 mV s^{-1} to 500 mV s^{-1}. Differential pulse voltammetric experiments were done at a pulse amplitude of 50 mV, pulse width of 70 ms, and scan rate of 10 mV s^{-1}. The glassy carbon working electrode was intensively polished with alumina powder prior to each measurement.

The anthocyanins and anthocyanidins solutions, 0.1 mmol L^{-1}, were prepared from 1 mmol L^{-1} stock solutions in methanol or in buffer solutions; lithium perchlorate was used as the supporting electrolyte in methanol. The pH values of aqueous solutions were buffered at an ionic strength of 0.2 mol L^{-1}, by using Britton-Robinson buffer solutions.[19] The pH measurements were carried out with a Micronal B 474 pH meter instrument.

Evaluation of the Scavenging Activities of Antioxidants by the DPPH Radical Method

An aliquot of methanol solution (0.1 mL) containing different concentrations of standards (1, 2, 4, 6, 10, 15 and 20 μmol L^{-1}) was added to 3.9 mL of DPPH 59.55 μmol L^{-1} in methanol. Reduction of DPPH was followed by monitoring the decrease of absorbance at 515 nm for 5 minutes (time necessary for the reactions to reach a plateau – steady state). A blank solution of DPPH was screened to estimate DPPH decompostion during the measurement time. The exact initial DPPH concentration (c_{DPPH}) in the reaction medium was calculated from the calibration curve with the equation determined by linear regression:

$$A_{515} = 10034 \cdot c_{\mathrm{DPPH}} - 0.01824,\ R = 0.99975$$

For each antioxidant concentration, the amount of the remaining DPPH in percents was calculated as:

$$\mathrm{DPPH}_{\mathrm{remaining}}/\,\% = 100 \cdot [\mathrm{DPPH}]_t / [\mathrm{DPPH}]_{t=0}$$

where $[\mathrm{DPPH}]_t$ is the concentration after 5 minutes of reaction.

The values were plotted *versus* DPPH concentration to obtain the amount of antioxidant needed to reduce the initial DPPH concentration by 50 % (EC_{50}).

RESULTS AND DISCUSSION

Electrochemical Properties of Anthocyanins and Anthocyanidins

Cyclic voltamograms of the compounds studied herein display oxidation peaks and practically no reverse reduction peaks, indicating the occurrence of EC processes. Functional OH groups attached to the antho-

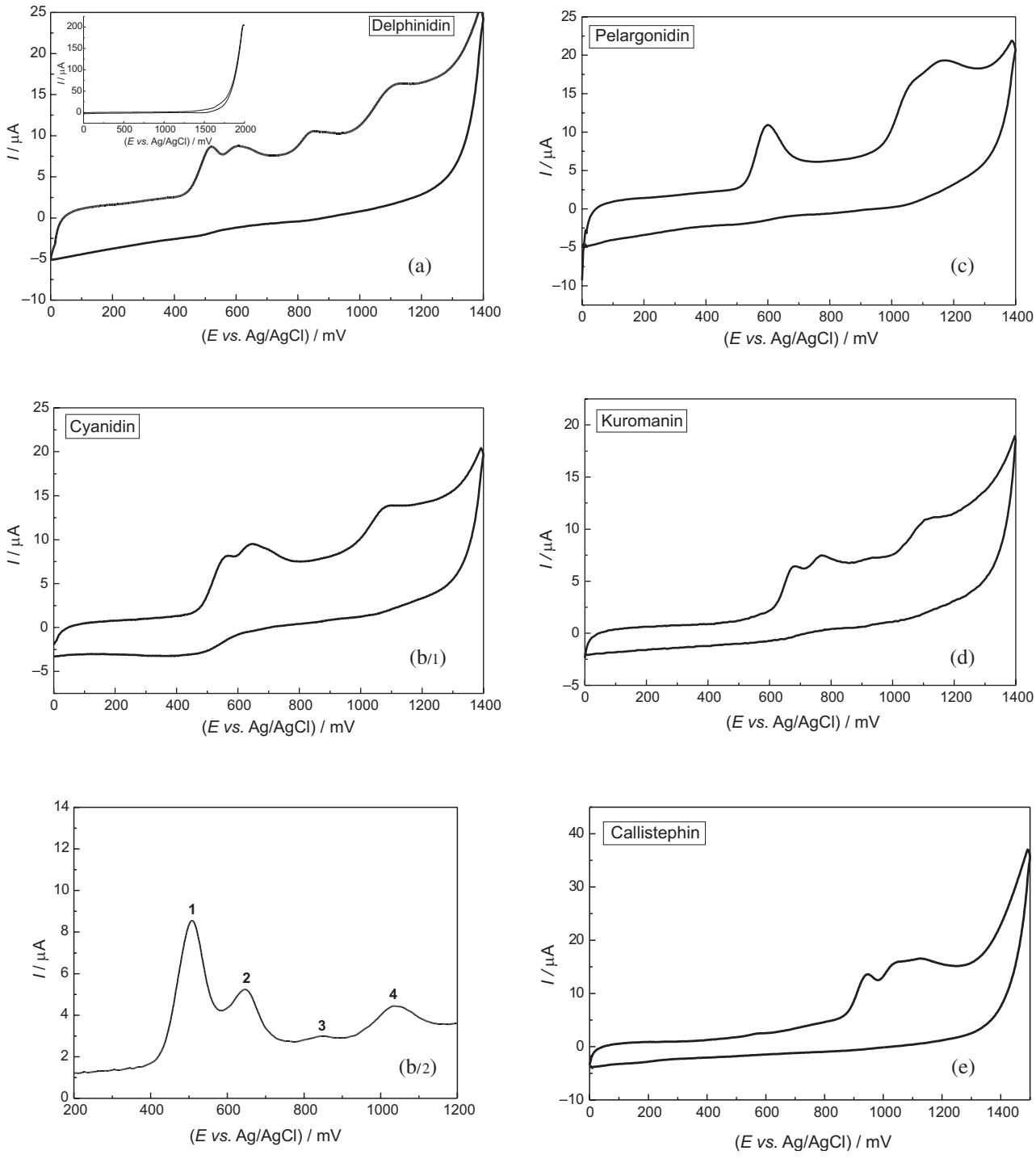


Figure 2. Cyclic voltamograms of a) delphinidin (insert shows the methanol response), b/1) cyanidin, b/2) differential pulse voltamogram of cyanidin, v = 10 mV s^{-1}, c) pelargonidin, d) kuromanin, and e) callistephin; 0.1 mmol L^{-1}, 0.1 mol L^{-1} in methanol; 100 mV s^{-1}, glassy carbon electrode.

cyanin and anthocyanidin ring structures can be electrochemically oxidized. The less positive potential peaks displayed by these flavonoids correspond to oxidations of the more redox-active OH groups of ring B. The potentials become successively more positive for oxidations of the C-3 OH group and of the ring A resorcinol groups.[20] E_{pa} data are shown in Table I. Delphinidin (Figure 2a) displays four oxidation processes; the two less positive peaks correspond to 3',4',5'-OH oxidations, the third process to 3-OH oxidation, and the fourth process to 5,7-OH oxidations. The same processes are observed for cyanidin (Figure

TABLE I. Electrochemical data for anthocyanins and anthocyanidins

Compounds[a]	(E_{pa} *vs* Ag/AgCl) / mV[b]
Delphinidin	519, 608, 844, 1108
Cyanidin	564, 646, 1084
Pelargonidin	598, 1121
Kuromanin	678, 773, 940, 1115
Callistephin	948, 1086

[a]Concentration = 0.1 mmol L^{-1}; [b] in methanol + 0.1 mol L^{-1} lithium perchlorate, ν = 100 mV s^{-1}.

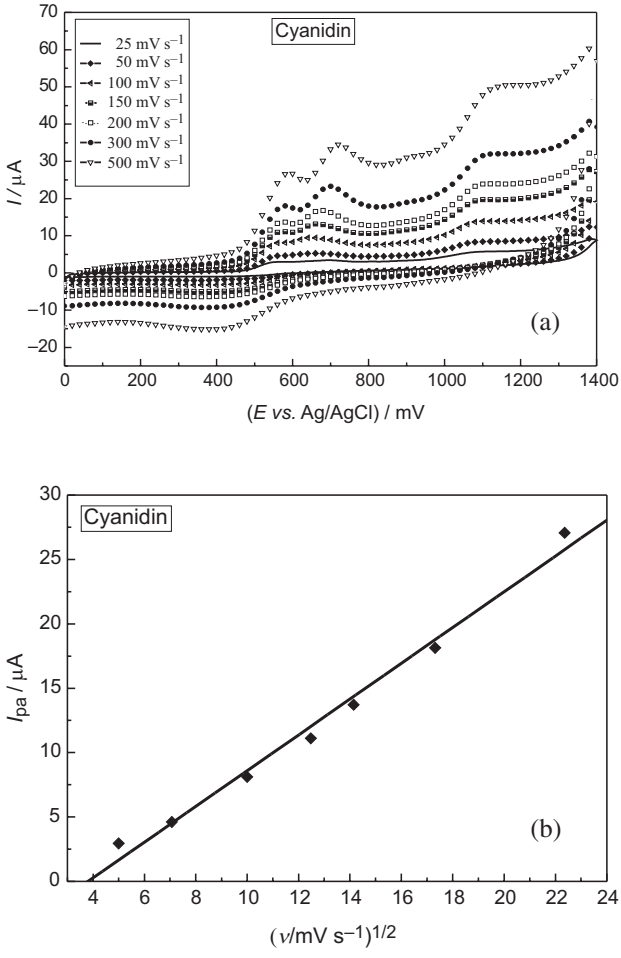


Figure 3. a) Cyclic voltamograms of cyanidin at various scan rates. b) Plot of the first anodic peak currents (I_{pa}) *vs.* the square root of scan rates ($\nu^{1/2}$) 0.1 mmol L^{-1}, 0.1 mol L^{-1} lithium perchlorate in methanol, glassy carbon electrode.

2b/1), except for the fact that the two less positive peaks correspond to 3',4'-OH oxidations. Figure 2b/2 shows the differential pulse voltamogram for cyanidin in which the four processes can be clearly observed. As for pelargonidin (Figure 2c), the broad wave between +1.0 V and +1.3 V corresponds to oxidations of the 3-OH and resorcinol groups.

The presence of sugar moieties at position 3 of kuromanin and callistephin results in a shift of oxidation peaks to more positive potentials (Figures 2d, 2e). This effect is probably due to a loss of coplanarity of ring B with respect to the rest of the molecule, which causes a decrease in conjugation. Concerning cyanidin and pelargonidin, the 3-OH moiety interacts with ring B through a hydrogen bond with 6'-hydrogen, thus maintaining ring B in the same plane as rings A and C.[21–23]

We have previously shown that there is a relationship between the antioxidant activity and the cyclic voltammetric oxidation peak potential[23,24] in the case of flavonoids. On the basis of electrochemical data, the order of antioxidant activity for anthocyanidins is: delphinidin > cyanidin > pelargonidin. It is well known that the number of hydroxyl substituents in ring B affects the E_{pa} values: the larger the number of hydroxyl groups, the lower the oxidation potential.[24] The increasing order for the less positive E_{pa} values observed for anthocyanidins follows the same increasing order of their first pK_a values.[25]

In the range of 25 mV s^{-1} to 500 mV s^{-1}, it can be seen that the oxidation peak potentials shift to more positive values and the peak currents increase with increasing scanning rate, for both the anthocyanins and the anthocyanidins. This indicates that EC mechanisms take place (Figure 3a shows this behaviour for cyanidin). The anodic peak currents (I_{pa}) increase linearly with the square root of the scan rate (Figure 3b; correlation coefficient, r = 0.99374), which is typical of a diffusion controlled electrochemical process.

pH Dependence on the Oxidation Potentials of Anthocyanins and Anthocyanidins

Some information on the oxidation mechanism of anthocyanins and anthocyanidins could be provided by comparing oxidation potentials at different pH values. It can be observed that, in all cases, oxidation potentials of the investigated anthocyanins and anthocyanidins shift toward lower values when the pH is increased, indicating that at higher pH, the compounds are easily oxidized and their antioxidant activity is increased. This happens because higher pH values facilitate deprotonation of the hydroxyl groups. Figure 4a shows this behaviour for kuromanin. Plots of E_{pa} values *versus* pH lead to straight lines with slopes of – 0.062, – 0.059, – 0.067, – 0.053, and – 0.063 V/pH for delphinidin, cyanidin, pelargonidin, kuromanin, and callistephin, respectively (Figure 4b shows the plot for kuromanin). These values are very close to the Nernstian prediction of – 0.059 V/pH units, which indicates that electron oxidations accompanied by the corresponding proton dissociations occur.[26–28]

In aqueous solution, the nature of voltammetric peaks shows a linear relationship between the anodic peak current (I_{pa}) and the square root of the potential

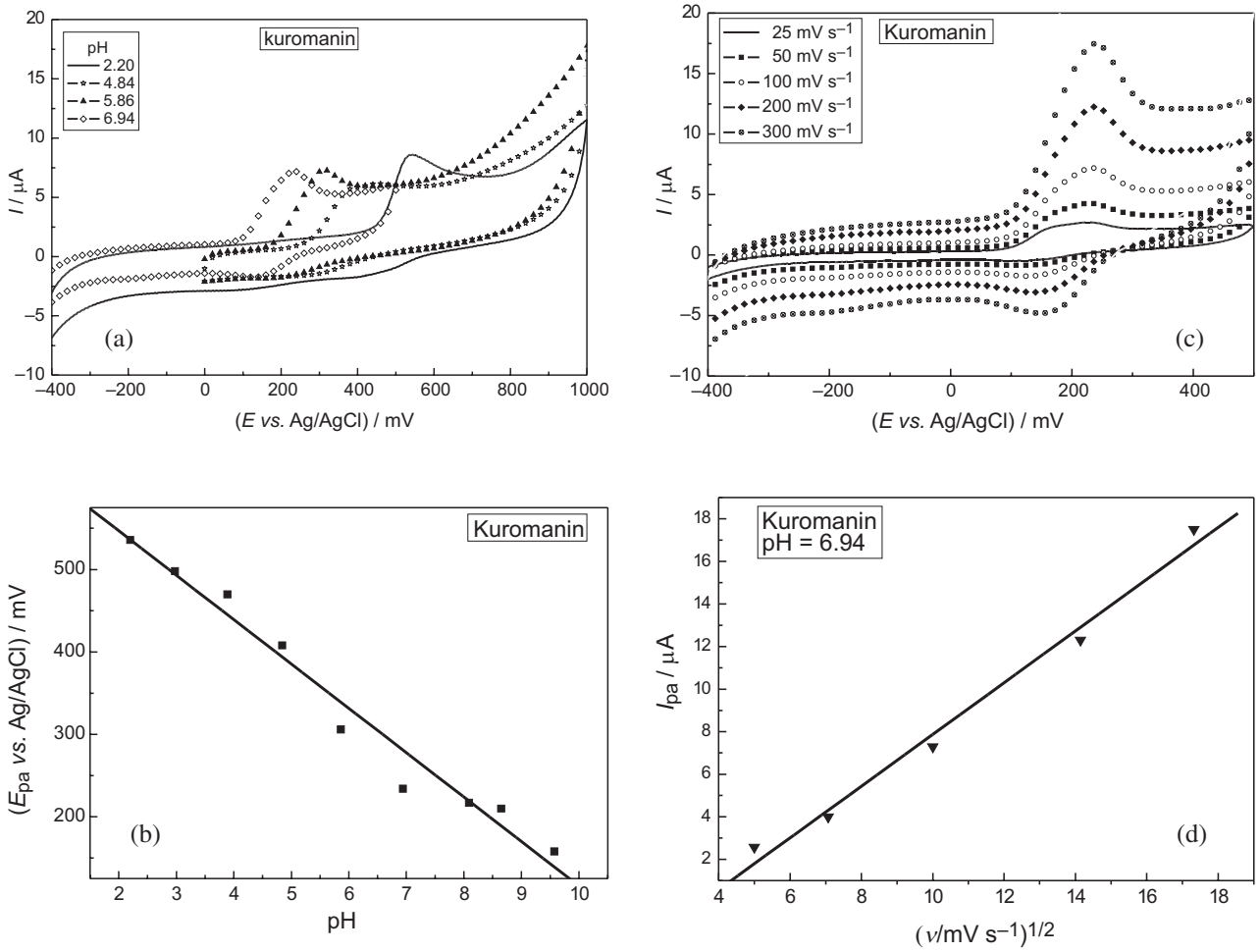


Figure 4. a) Cyclic voltamograms of kuromanin 0.1 mmol L^{-1} in Britton-Robinson buffer, ν = 100 mV s^{-1}. b) Plot of E_{pa} *vs.* pH for kuromanin. c) Cyclic voltamograms of kuromanin at various scan rates, pH = 6.94. d) I_{pa} *versus* $\nu^{1/2}$, for kuromanin, pH = 6.94.

sweep rate ($\nu^{1/2}$), shown in Figures 4c and 4d (correlation coefficient, r = 0.99361) for kuromanin.

Antioxidant Activities of the Compounds; DPPH Radical Scavenging Method

When monitored by UV-Vis spectroscopy, the reaction between an antioxidant and DPPH shows a very quick decay in absorbance (λ_{max} = 515 nm in methanol), which reaches a virtually constant value after 5 minutes. The fast decay essentially refers to the abstractions of the most labile H atoms from the antioxidants (3'-,4'- and 5'-positions).

TABLE II. EC_{50} data for anthocyanins and anthocyanidins

Compounds	EC_{50}
Delphinidin	3.74
Cyanidin	4.85
Pelargonidin	5.25
Kuromanin	7.29
Callistephin	10.9

The DPPH scavenging activities, evaluated by the EC_{50} data (Table II), reveal the following order of effectiveness for antioxidant activities of the compounds: delphinidin > cyanidin > pelargonidin > kuromanin > callistephin. In general, the larger the number of hydroxyl groups in the flavonoid structures, the lower the EC_{50} values. The higher antioxidant activity of delphinidin may be attributed to the highly significant contribution of the 3', 4', 5'-OH groups on the B-ring.

CONCLUSIONS

The results obtained in this work show that the pyrogallol group is more easily oxidized than the catechol group, which in turn is more easily oxidized than the resorcinol group.

Although the presence of glycosides at position 3 stabilizes[29] these compounds, it clearly leads to a reduction in their antioxidant activities.

Deprotonation of the hydroxyl groups in ring B is related to the electron/proton donating capacity of antho-

cyanins and anthocyanidins and to their radical scavenging antioxidant activity.

Evidence presented herein and in previous works suggests that the electrochemical parameters typical of anthocyanins and anthocyanidins are a very good indication of their antioxidant activity. Therefore, cyclic voltammetry can be a useful and simple method to monitor the antioxidant properties of anthocyanins and anthocyanidins and to estimate the antioxidant activity of these compounds, ubiquitous in food and medicinal plants.

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SAŽETAK

Elektrokemijska i antioksidacijska svojstva antocijanina i antocijanidina

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Elektrokemijska svojstva delfinidina, cijanidina, pelargonidina, kuromanina i kalistefina istražena su cikličkom i diferencijalnom pulsnom voltametrijom u vodenim otopinama različitih pH vrijednosti i u metanolu. Na osnovu potencijala oksidacije zaključeno je da se antioksidacijska aktivnost antocijanidina smanjuje redom: delfinidin > cijanidin > pelargonidin. Do oksidacije antocijanina (kuromanin i kalistefin) dolazi kod višeg potencijala nego oksidacija antocijanidina (delfinidin, cijanidin i pelargonidin). Vršne struje oksidacije linearno ovise o korijenu brzine promjene potencijala, što dokazuje da su elektrodne reakcije kontrolirane difuzijom. Antioksidacijska aktivnost navedenih spojeva procijenjena je metodom vezivanja radikala koristeći 1,1-difenil-2-pikrilhidrazil. Aktivnost opada s porastom potencijala oksidacije. Antioksidacijska aktivnost opada redom: delfinidin > cijanidin > pelargonidin > kuromanin > kalistefin.