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Electrochemical behaviour and antioxidant capacity of anthocyanins from Chilean red wine, grape and raspberry

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ABSTRACT

The anthocyanin fractions were extracted from Cabernet Sauvignon red wine, skins of *Vitis vinifera* grapes and raspberry fruits (*Rubus idaeus*). In red wine extract, 16 anthocyanins were identified, malvidin-3-O-glucoside being the main anthocyanin, which comprised 53.6% of the total anthocyanin in grape extract. Raspberry extract contained mainly delphinidin-3-O-glucoside and cyanidin-3-O-glucoside. The antioxidant capacity of the extracts was assayed by electrochemical methods. Best resolution of the oxidation peaks for the extracts and diluted wine was obtained by pulse differential voltammetry. The wine diluted 20× presented values of P1 (443 mV) and P2 (676 mV) similar to those corresponding to wine extract, and to the anthocyanin malvidin-3-O-glucoside. The antioxidant capacity of anthocyanins in extracts of wine, grape skin and raspberry fruit was also determined by the Trolox equivalent antioxidant capacity (TEAC) method.

1. Introduction

Red wine is a complex system which contains a large variety of phenolic compounds, such as flavonols, flavan-3-ols, anthocyanins and phenolic acids. Anthocyanins are extracted from red grapes and are largely responsible for the colour exhibited by red wine; they play an important role in the antioxidant activity of red wine (Zoecklein, Fugelsang, Gump, & Nury, 2001). In red wine, anthocyanins interact with phenolics compounds, pyruvic acid and acetaldehyde to give new pigments (Francia-Aricha, Guerra, Rivas-Gonzalo, & Santos-Buelga, 1997; Fulcrand, Bernabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Mateus, Silva, Vercauteren, & De Feitas, 2001). The antioxidative capacity of anthocyanin was studied by different chemical methods. The Folin–Ciocalteu method, the oxygen radical absorbance capacity (ORAC) assay and the Trolox equivalent antioxidant capacity (TEAC) assay are the most considered methods (Prior, Wu, & Schaich, 2005). Kilmartin and Hsu (2001) developed an electrochemical method for the characterization of the antioxidant properties of wine and wine phenolics. Recently, Janeiro and Oliveira Brett (2007) studied the redox behaviour of anthocyanins by voltammetric techniques, they found that all the phenolic hydroxyl groups can be electrochemically oxidized. Da Silva, Ramos Stradiotto, and Oliveira (2008) developed a method for the determination of caffeic acid in red wine by differential pulse voltammetry. Zienlińska, Nagels, and Piskula (2008) determined the antioxidant capacity of quercetin and its glycosides of onion by cyclic voltammetry. Ghiselli, Nardini, Baldi, and Scaccini (1998) reported that among the red wine components, anthocyanins were the most effective in scavenging reactive oxygen species (ROS) and inhibiting lipoprotein oxidation. Anthocyanins comprise around 70% of the total phenols of Italian red wine. In this work we evaluated the electrochemical behaviour of Chilean Cabernet Sauvignon red wine, *Vitis vinifera* grape and raspberry anthocyanin extracts in a model wine solution at pH 3.6, and in acetate buffer.

2. Materials and methods

2.1. Materials

Cabernet Sauvignon red wine vintage 2005 and *V. vinifera* grapes were supplied by a local producer. Mature raspberry fruits (*Rubus idaeus*) were purchased in the local market. All the solvents were reagent grade and/or HPLC grade (Merck, Darmstadt, Germany), all the chemicals were reagent grade (Sigma, St. Louis, MO, USA).

2.2. HPLC analysis

For the high performance liquid chromatography (HPLC) analysis a Waters 600 HPLC chromatograph (Waters, Mildford, MA, USA) equipped with a Waters 2990 diode array detector, and a

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Symmetry C-18 (5 μm) (Waters, Milford, MA, USA) column (3.9 × 150 mm) were used. The solvent system consisted of 1% aqueous formic acid (A) and 1% formic acid in acetonitrile (B). The initial composition of the mobile phase was 95% A and 5% B. With linear gradients the composition changed to 75% A and 25% B within 45 min, and 50% A and 50% B within 60 min. The flow rate was 0.8 ml/min.

2.3. HPLC–MS/MS analysis

HPLC analysis was performed on a Agilent 1100 equipment (Agilent Technologies, Santa Clara, CA, USA). Extracts (20 μl) were injected into a 5 μm C-18 column (250 × 4.6 mm). The mobile phase consisted of 1% formic acid in water (A), and 1% formic acid in acetonitrile (B). The gradient was from 5% to 50% mobile phase B at a flow rate of 0.8 ml/min over a 60 min period. For HPLC/MS analyses, the HPLC apparatus was interfaced to an ESI-IT Esquire 4000 (Bruker Daltonics, Billerica, MA, USA) mass spectrometer equipped with an ionization electrospray chamber. Conditions for the mass spectra analysis in the positive ion mode included a voltage of 4000 V, a nebulising pressure of 35 psi, a drying nitrogen flow of 9.0 ml/min and a temperature of 325 °C. Data were collected on a full scan mode over a mass range of *m/z* 20–2200.

2.4. Extraction of anthocyanins

V. vinifera skins (250 g) were stirred with 500 ml of 1% HCl in methanol at 4° C over one hour, and the mixture was centrifuged for 30 min at 4000*g* and at 25 °C employing a 4000 KS-3000P (Kubota, Tokyo, Japan) centrifuge. The pellet was extracted again with 500 ml of 1% HCl in methanol. The extraction process was repeated four more times. The supernatants were collected and concentrated *in vacuo*, dissolved in 200 ml of distilled water, and sequentially washed with 200 ml of *n*-hexane, chloroform, and ethyl acetate. The aqueous solution was concentrated *in vacuo* and deposited in an Amberlite XAD-7 HP (Sigma, St. Louis, MO, USA) column (200 × 15 mm) and it was eluted with water (250 ml), followed by 250 ml of 1% HCl in methanol. The methanolic fraction was concentrated to a final volume of 50 ml and analysed by HPLC.

Extraction of raspberry was conducted as above, but using 1% trifluoroacetic acid (TFA) in methanol (De Ancos, González, & Cano, 1999).

Cabernet Sauvignon vintage 2005 red wine (750 ml) was concentrated to 250 ml *in vacuo*, and sequentially washed with 200 ml of *n*-hexane, chloroform and ethyl acetate. The resulting aqueous solution was poured onto 1500 ml of ethanol, centrifuged, and the supernatant was concentrated *in vacuo*.

2.5. Colorimetric analysis of anthocyanins

Anthocyanin contents in extracts were determined by the differential pH method, according to Wang and Lin (2000). Absorbance was measured at 700 and 520 nm in a Genesys 5 (ThermoSpectronic, Waltham, MA, USA) spectrophotometer, at pH 1.0 and 3.5. Anthocyanins were quantified as malvidin-3-O-glucoside using the extinction coefficient (ε = 28,000).

2.6. Determination of total phenol content

The total phenol content of red wine was determined using the Folin–Ciocalteu method as described by Singleton and Rossi (1965). Gallic acid was used as standard (Lucerna, Cárdenas, Gallego, & Valcárcel, 2005; Piljac, Martinez, Valek, Stipčevic, & Kovačevic Ganić, 2005).

2.7. Isolation of malvidin 3-O-glucoside

Semi-preparative HPLC separation of the major component of grape extract was performed using a Spherisorb® S10 ODS2 (Waters, Milford, MA, USA) column (10 × 250 mm) and as described in Section 2.2. The compound was analysed by ^{1}H and ^{13}C NMR assignments were performed according to Mas et al. (2000), Hakansson, Pardon, Hayasaka, De Sa, and Herderich (2003), and Tatsuzawa et al. (2009).

^{1}NMR (400 MHz, TFA 10%-CD_3OD): δ (ppm) for malvidin: 8.96 (s, H-4), 6.90 (br d, J = 1.9 Hz, H-6), 7.01 (br d, J = 1.9 Hz, H-8), 7.96 (2H, s, H-2′, 6′), 3.98 (s, 2 *x*-CH3). For β-glucopyranosyl residue: 5.63 (d, J = 7.5 Hz, H-1″), 3.63 (t, J = 8.6 Hz, H-2″), 3.59 (t, J = 8.9 Hz, H-3″), 3.34 (t, J = 8.9 Hz, H-4″), 3.84 (m, H-5″), 4.21 (dd, J = 7.2, 12.2 Hz, H-6″a), 4.46 (dd, J = 2.1, 12.2 Hz, H-6″b).

^{13}C NMR (100 MHz, TFA 10%–CD3OD): δ (ppm) for malvidin: 163.1 (C-2), 148.4 (C-3), 135.6 (C-4), 158.1 (C-5), 102.6 (C-6), 169.6 (C-7), 94.6 (C-8), 160.6 (C-9), 113.4 (C-10), 118.7 (C-1′), 112.5 (C-2′), 144.3 (C-3′), 144.5 (C-4′), 149.8 (C-5′), 112.9 (C6′), 56.3 (–CH_3). For glucopyranosyl residue: 102.3 (C-1″), 75.0 (C-2″), 77.4 (C-3″), 71.4 (C-4″), 76.0 (C-5″), 64.5 (C-6″).

2.8. Trolox equivalent antioxidant capacity (TEAC) assay

The antioxidant activity was determined with ABTS radical cation according to the procedure described by Re et al. (1999). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as antioxidant standard. Assay was conducted in triplicate. Results are presented as mean ± standard error followed by Student's *t*-test. Differences were considered to be statistically significant if $P < 0.05$.

2.9. Voltammetric experiments

Electrochemical measurements were performed on a CHI 104 Scanning Electrochemical microscope, CHI 900B (CH Instruments, Austin, TX, USA) at room temperature. The working electrode was glassy carbon, the counter electrode was a platinum wire, and the reference electrode was Ag/AgCl. Prior to each measurement the working electrode was polished with 0.3 μm alumina powder. A wine model solution consisting of 0.1 M NaCl in 12% aqueous ethanol was used. Malvidin-3-O-glucoside and the anthocyanin extracts were dissolved in the model wine solution in the concentration range of 0.2–1.0 mg/ml. The pH was adjusted to 3.6 with 1 M NaOH and the solution was purged with nitrogen for 20 min. Electrochemical measurements were conducted also in pH 3.6 acetate-acetic acid buffer prepared by mixing 10.9 ml of 0.1 M sodium acetate and 89.1 ml 0.1 M acetic acid, and in pH 3.6 acetate-acetic acid buffer solution containing 12% ethanol (10.9 ml of 0.1 M sodium acetate and 89.1 ml 0.1 M acetic acid containing 13.5% ethanol). Linear voltammetry measurements were performed at a scan rate of 5 mV/s in the range −0.2 to 1.0 (vs Ag/AgCl). The cyclic voltammograms were acquired in the range of 0 to +0.8 mV at a scan rate of 100 mV/s at 1 mV intervals. Ascorbic acid (0.05–0.1 mg/ml) was used as reference substance. Differential pulse voltammetry measurements were conducted with a pulse amplitude of 50 mV and a pulse width of 50 ms. Experiments were run in triplicates.

3. Results and discussion

3.1. Anthocyanins characterization

The total content of anthocyanins, and of oligomeric anthocyanins in extracts of Cabernet Sauvignon red wine, grape and rasp-

Table 1
Contents of phenols and anthocyanins in red wine, grape skins and raspberry fruits extracts.

Extract	Phenol	Anthocyanins		
		Total	Oligomeric	Free
Red wine[a]	110.23 ± 1.43	70.50 ± 4.50	32.43 ± 2.50	38.07
Grape skin[b]	144.09 ± 1.84	80.21 ± 3.85	7.08 ± 1.70	73.13
Raspberry[c]	285.01 ± 2.64	120.05 ± 1.86	14.56 ± 1.80	105.49

[a] Phenol content expressed as milligrams of gallic acid equivalent per 100 ml of wine, anthocyanin content expressed as milligrams of malvidin-3-O-glucoside per 100 ml of wine.
[b] Phenol content expressed as milligrams of gallic acid equivalents per 100 g of fresh weight, anthocyanin content expressed as milligrams of malvidin-3-O-glucoside per 100 g of fresh weight.
[c] Phenol content expressed as milligrams of gallic acid equivalent per 100 g of fresh weight, anthocyanins content are expressed as milligrams of cyanidin-3-O-glucoside per 100 g of fresh weight.

berry were determined by differential pH. It can be seen in Table 1 that the complex anthocyanin content is higher in red wine than in grapes, indicating that in the young wine reaction of anthocyanins with other components present in the solution has taken place. Also, the total phenolic contents of the extracts and red wine, determined by Folin–Ciocalteu method are presented in Table 1. The levels of total phenol in wines determined according to the Folin–Ciocalteu method are not absolute measurements of the amounts of phenolic materials but are in fact based on their chemical reducing capacity relative to an equivalent reducing capacity of gallic acid (Frankel, Waterhouse, & Teissedre, 1995). Additionally, the amounts of phenolic materials vary considerably in different types of wine, depending on the grape variety, environmental factors in the vineyard, and wine processing techniques. The concentrations of total phenol determined by the Folin–Ciocalteu method varied from 1100 to 4000 mg/l GAE, averaging 2567 mg/l GAE, for the red wines (Piljac et al., 2005).

The anthocyanin content in grape skin is similar to those reported by Ryan and Revilla (2003) for Cabernet Sauvignon grapes from Toledo, Spain. Burns et al. (2000) reported the total anthocyanins content in Chilean Cabernet Sauvignon red wine (325.7 μM malvidin 3-glucoside equivalents), being the highest value among 16 red wines of different origins. They informed that polymeric anthocyanins were present in all wines in larger amounts than free

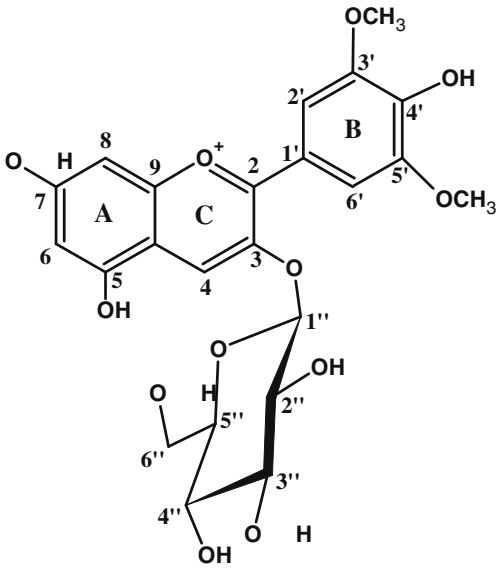

Fig. 1. Structure of malvidin-3-O-glucoside.

anthocyanins. Total anthocyanin content in grape skin is similar to those reported for Spanish Cabernet Sauvignon grapes (Ryan & Revilla, 2003). In the case of raspberry extract the anthocyanin and total phenolic contents are higher than those reported by Wang and Lin (2000) for different varieties of ripe fruits. The analysis of wine, grape and raspberry extracts was conducted by the combination of HPLC with diode array detection and HPLC coupled to electrospray ionization-mass spectrometry. HPLC analysis of the methanolic extracts revealed the presence of more compounds in wine than in the grape extract. The anthocyanins in wine extracts were identified in the mass spectra by the molecular ions, and the fragment peaks corresponding to the aglycones, according to Giusti, Rodríguez-Sanoa, Griffin, and Wrolstad (1999), García-Beneytez, Cabello, and Revilla (2003) and Cho, Howard, Prior, and Clark (2004). As expected the major anthocyanin in red wine extract (Table 2) was malvidin-3-O-glucoside (Fig. 1) which accounted for half of the total anthocyanins. This anthocyanin was isolated by semi-preparative HPLC and identified by ^{1}H and

Table 2
Anthocyanins identified by HPLC–MS in red wine extract.

Compound	t_R (min)	%	Anthocyanins	(M^+) *m/z*	MS^2 fragment ions ($M^+ - X$) *m/z*	MS^3 fragment ions ($MS^{2+} - X$) *m/z*
1	19.6–20.4	5.3	Delphinidin-3-O-glucoside	465	303	257
			Malvidin-3-O-glucoside-catechin	781	619	
2	21.3–21.6	4.0	Peonidin-3-O-glucoside-4-vinylphenol	579		
3	23.8–25.2	4.5	Dimer malvidin-3-O-glucoside-petunidin-3-O-glucoside	971		
4	27.8–29.3	53.2	Malvidin-3-O-glucoside	493	331	
5	34.9–35.3	5.9	Dimer malvidin-3-O-glucoside-delphinidin-6-O-coumaroyl-3-O-glucoside	1104		
6	38.1–38.3	1.6	Malvidin-3-O-glucoside-4-vinyl-epicatechin	805		
7	39.4–40.9	9.1	Malvidin-6-O-acetyl-3-O-glucoside	535	331	
			Malvidin-6-O-coumaroyl-3-O-glucoside–catechin	927		
			Dimer malvidin-3-O-glucoside	985	823	661
8	46.3–46.9	1.5	Malvidin-6-O-p-coumaroyl-3-O-glucoside-pyruvate	708		
9	47.7–48.0	2.1	Dimer malvidin-3-O-glucoside-petunidin-6-O-coumaroyl-3-O-glucoside	1116		
10	48.3–48.9	3.9	Malvidin-6-O-p-coumaroyl-3-O-glucoside	639	331	316
11	50.3–50.6	6.6	Malvidin-3-O-glucoside-4-vinylphenol	609	447	431
12	51.2	–	Malvidin-6-O-acetyl-3-O-glucoside-epicatechin	847	685	531
13	51.7–52.0	1.6	Malvidin-6-O-acetyl-3-O-glucoside–gallocatechin	839	677	659
14	53.2–53.4	0.7	Malvidin-3-O-diglucoside	655	331	
			Malvidin-6-O-acetyl-3-O-glucoside-4-vinyl-epicatechin	926		
			Malvidin-6-O-acetyl-3-O-glucoside-4-vinyl-diepicatechin	1094		

Table 3
Voltammetry data obtained in model wine solution (12% ethanol, 0.1 M NaCl, and added NaOH to pH 3.6) for red wine, grape skin and raspberry extracts, diluted wine and malvidin-3-O-glucoside.

	Voltammetry					
	Linear sweep		Cyclic		Differential pulse	
	P1 (mV)	P2 (mV)	P1 (mV)	P2 (mV)	P1 (mV)	P2 (mV)
Grape extract	494	720	420	658	464	646
Raspberry extract	470	590	416	553	538	751
Red wine extract	538	723	437	686	435	657
Wine diluted 20×	475	675	491	–	443	666
Malvidin-3-O-glucoside	635	754	384	691	483	698

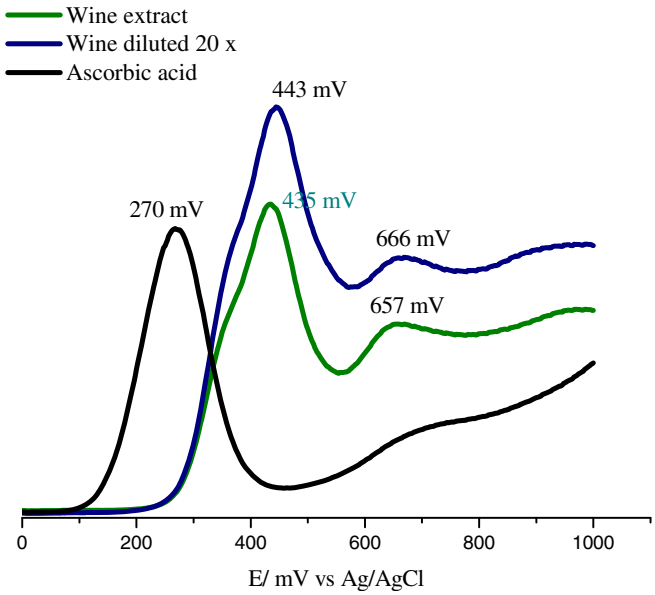

Fig. 2. Differential pulse voltammograms of red wine extract, diluted wine 20× and ascorbic acid in model wine solution (12% ethanol, 0.1 M NaCl and added NaOH to pH 3.6). Scan rate: 5 mV/s.

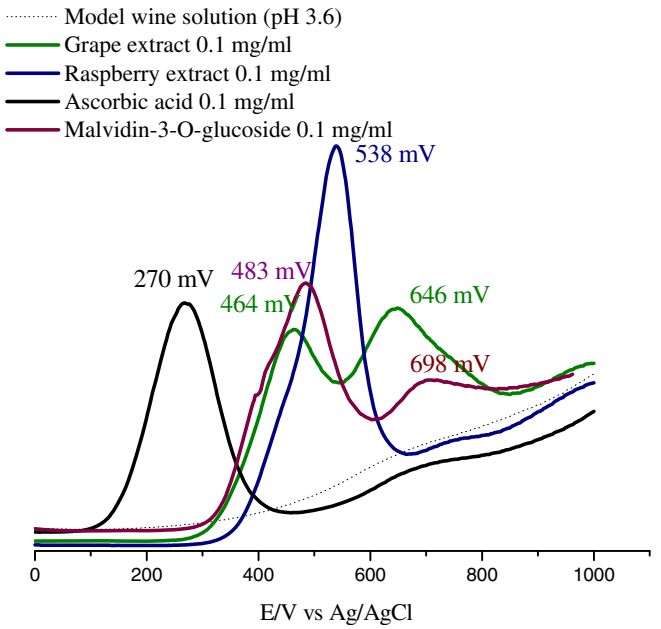

Fig. 3. Differential pulse voltammograms of malvidin-3-Oglucoside, grape extract, raspberry extract, and ascorbic acid in model wine solution (12% ethanol, 0.1 M NaCl, and added NaOH to pH 3.6). Scan rate: 5 mV/s.

^{13}C NMR spectra. The amount of anthocyanins derivatives in red wine found by HPLC–MS analysis is close to that found by the differential pH method. Ten anthocyanins were identified by HPLC–MS in grape skin extracts, malvidin-3-O-glucoside being the major component (53.6%). For comparison, the analysis of anthocyanin extract of raspberry fruits was included in this work. Unexpectedly, the raspberry extract contains only four anthocyanins, being delphinidin-3-glucoside (39.6%) and cyanidin-3-O-glucoside (35.4%) the major components. Its composition is quite different from those reported for Spanish raspberry; according to De Ancos et al. (1999) the anthocyanins composition was characteristic for each cultivar. They found that cyanidin-3-glucoside was the major component (96.08–122.88 mg/100 g) in two cultivars and cyanidin-3-sophoroside (63.86–21.91 mg/100 g) in other three cultivars. The latter anthocyanin was also the major component (673 mg/100 g) of the anthocyanin extract of raspberry studied by Ogawa et al. (2008).

3.2. Electrochemical behaviour of anthocyanins

The linear voltammetry curve of a 0.1 mg/ml solution of malvidin-3-O-glucoside in acetate buffer (pH 3.6) presented two peaks at 490 and 674 mV (Table 3). The first peak (P1) is attributed to the oxidation of OH of B-ring, the second oxidation peak (P2) may be due to 5,7-dihydroxyl moiety of A-ring, although is shifted to lower potential in relation to the value (880 mV) reported by Janeiro and Oliveira Brett (2007). Experiments were conducted also in model wine solution (pH 3.6) the first peak P1 in the oxidation of malvidin-3-O-glucoside is shifted to 635 mV, while P2 appeared at 754 mV. It is noteworthy the shift of P1 to higher potentials value in ethanol, which indicates that this solvent is interacting with the compound, stabilizing the phenolic group of ring A. The shift of P2 is smaller which may indicate that ring B is less exposed to interaction with the solvent. In acetate buffer containing 12% ethanol the value of P1 is similar to that obtained in aqueous solution, indicating that no interaction with ethanol occurred. The linear voltammetry curve of grape skin extract in buffer acetate presents peaks P1 and P2 at lower values than malvidin-3-O-glucoside, which may be due to the presence of other anthocyanins in the extract. The linear sweep voltammograms of grape extract in wine model solution is similar to the one taken in pH 3.6 buffer acetate containing 12% ethanol. Also, in this case the solvent displaces the oxidation potentials to more positive values. In order to compare with red wine, the linear voltammograms of extracts were taken in model wine solutions, Table 3 summarises the voltammetry data obtained.

Cyclic voltammograms of malvidin-3-O-glucoside and wine extract in pH 3.6 model wine solution presented values of oxidation peaks similar to those obtained for grape extract in pH 3.6 buffer acetate containing 12% ethanol (P1 = 424 mV, and P2 = 677 mV) and in model wine solution (Table 3).

Figs. 2 and 3 illustrate differential pulse voltammograms for diluted wine, malvidin-3-O-glucoside and the extracts. These voltammograms present a better resolution of the oxidation peaks than the linear sweep and cyclic voltammograms. The results were compared with those obtained for the oxidation of ascorbic acid (270 mV), a known antioxidant. The wine diluted 20× present values of P1 and P2 similar to those corresponding to wine extract, and at lower potential than the anthocyanin malvidin-3-O-glucoside (Table 3). Raspberry extract which mainly contained delphinidin-3-O-glucoside and cyanidin-3-O-glucoside showed one oxidation peak at higher potentials than wine, and wine and grape extracts. Delphinidin 3-O-glucoside differs from malvidin-3-O-glucoside that is not methylated at positions 3′ and 4′ in ring B, while cyanidin-O-glucoside carries only OH groups in position 3′ and 4′. According to Janeiro and Oliveira Brett (2007), the first oxidation process for the latter, corresponded to the oxidation of the hydroxyl group on C4′.

3.3. TEAC scavenging activity

The antioxidant capacity of anthocyanins in extracts of wine, grape skin and raspberry fruit was also determined by the TEAC method based on the inhibition by antioxidants of the absorbance of the radical cation ABTS. The value expressed as mM Trolox for wine (23.6 ± 0.56) is similar to those reported by Rivero-Pérez, Muñiz, and González-Sanjosé (2007) for Spanish red wines; the total phenolic content is also similar. Values found for grape and raspberry extracts (4.4 ± 0.08, and 4.8 ± 0.09, respectively) are higher than those reported in the literature for anthocyanins isolated from grape skin (Muselik, García-Alonso, Martín-López, Žemlička, & Rivas-Gonzalo, 2007), and from red cabagge, black currant, roselle and chokeberry (Degenhardt, Knapp, & Winterhalter, 2000).

4. Conclusions

Cabernet Sauvignon red wine contains more anthocyanins than *V. vinifera* grape skin. The presence of complex anthocyanins in the former indicates that the condensation process has already taken place in the young wine. The results obtained in this work by electrochemical techniques indicate that pulse differential voltammetry is a good technique for the characterization of the antioxidant properties of red wines. Good results are obtained simply by dilution of wine samples without the presence of buffers.

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References

Burns, J., Gradner, P. T., O'Neil, J. O., Crawford, S., Morecroft, I., & McPhail, D. B. (2000). Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. *Journal of Agricultural and Food Chemistry, 48*, 220–230.

Cho, M. J., Howard, L. R., Prior, R. L., & Clark, J. R. (2004). Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture, 84*, 1771–1782.

Da Silva, L. F., Ramos Stradiotto, N., & Oliveira, H. P. (2008). Determination of caffeic acid in red wine by voltammetric method. *Electroanalysis, 20*, 1252–1258.

De Ancos, B., González, E., & Cano, M. P. (1999). Differentiation of raspberry varieties according to anthocyanin composition. *Zeitschrift fur Lebensmittel-Unterssuchung und -Forschung A., 208*, 33–38.

Degenhardt, A., Knapp, H., & Winterhalter, P. (2000). Separation and purification of anthocyanins by high speed countercurrent chromatography and screening for antioxidant activity. *Journal of Agricultural and Food Chemistry, 48*, 338–343.

Francia-Aricha, E. M., Guerra, M. T., Rivas-Gonzalo, J. C., & Santos-Buelga, C. (1997). New anthocyanins pigments formed alter condensation with flavanols. *Journal of Agricultural and Food Chemistry, 45*, 2262–2266.

Frankel, E. N., Waterhouse, A. L., & Teissedre, P. L. (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *Journal of Agricultural and Food Chemistry, 43*, 890–894.

Fulcrand, H., Bernabdeljalil, C., Rigaud, J., Cheynier, V., & Moutounet, M. (1998). A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry, 47*, 1401–1407.

García-Beneytez, E., Cabello, F., & Revilla, E. (2003). Analysis of grape and wine anthocyanins by HPLC–MS. *Journal of Agricultural and Food Chemistry, 51*, 5622–5629.

Ghiselli, A., Nardini, M., Baldi, A., & Scaccini, C. (1998). Antioxidant activity of different phenolic fractions separated from an Italian red wine. *Journal of Agricultural and Food Chemistry, 46*, 361–367.

Giusti, M. M., Rodríguez-Sanoa, L. E., Griffin, D., & Wrolstad, R. E. (1999). Electrospray and tandem mass spectroscopy as tools fro anthocyanin characterization. *Journal of Agricultural and Food Chemistry, 47*, 4657–4664.

Hakansson, A. E., Pardon, K., Hayasaka, Y., De Sa, M., & Herderich, M. (2003). Structure and colour properties of new red wine pigments. *Tetrahedron Letters, 44*, 4887–4891.

Janeiro, P., & Oliveira Brett, A. M. (2007). Redox behaviour of anthocyanins present in *Vitis vinifera* L. *Electroanalysis, 19*, 1779–1786.

Kilmartin, P. A., & Hsu, C. F. (2001). A cyclic voltammetry method suitable for characterizing antioxidant properties of wine and wine phenolics. *Journal of Agricultural and Food Chemistry, 49*, 1957–1965.

Lucerna, R., Cárdenas, S., Gallego, M., & Valcárcel, M. (2005). Autoanalyzer for continuos fractionation and quantitation of the polyphenols contents in wines. *Journal of Chromatography A, 1081*, 127–131.

Mas, T., Susperregui, J., Berké, B., Chèze, C., Moreau, S., Nuhrich, A., & Vercauteren, J. (2000). DNA triples stabilization property of natural anthocyanins. *Phytochemistry, 53*, 679–687.

Mateus, N., Silva, A. M. S., Vercauteren, J., & De Feitas, V. (2001). Occurrence of anthocyanins derived pigments in red wines. *Journal of Agricultural and Food Chemistry, 49*, 4836–4840.

Muselik, J., García-Alonso, M., Martín-López, M. P., Žemlička, M., & Rivas-Gonzalo, J. C. (2007). Measurement of antioxidant activity of wine catechins, procyanidins, anthocyanins and pyranoanthocyanins. *International Journal of Molecular Sciences, 8*, 797–809.

Ogawa, K., Sakakibara, H., Iwata, R., Ishii, T., Sato, T., & Goda, T. (2008). Anthocyanin composition and antioxidant activity of the crowberry (*Empetrum nigrum*) and other berries. *Journal of Agricultural and Food Chemistry, 56*, 4457–4462.

Piljac, J., Martinez, S., Valek, L., Stipčevic, T., & Kovačevic Ganić, K. (2005). Phenolic content and antioxidant activity of Croatian wines. *Food Technology and Biotechnology, 43*, 271–276.

Prior, R., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Re, R., Pellegrini, N., Protegente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 9/10*, 1231–1237.

Rivero-Pérez, M. D., Muñiz, P., & González-Sanjosé, M. L. (2007). Antioxidant profile of red wines evaluated by total antioxidant capacity, scavenger activity, and biomarkers of oxidative stress methodologies. *Journal of Agricultural and Food Chemistry, 55*, 5476–5483.

Ryan, J.-M., & Revilla, E. (2003). Anthocyanin composition of Cabernet Sauvignon and Tempranillo grapes at different stages of ripening. *Journal of Agricultural and Food Chemistry, 51*, 3372–3378.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagent. *American Journal of Enology and Viticulture, 16*, 144–158.

Tatsuzawa, F., Saito, N., Mikanagi, Y., Shinoda, K., Toki, K., & Shigihara, A. (2009). An unusual acylated malvidin 3-glucoside from flowers of *Impatiens textori* Miq. (Balsaminaceae). *Phytochemistry, 70*, 672–674.

Wang, S. Y., & Lin, H.-S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and development stage. *Journal of Agricultural and Food Chemistry, 48*, 140–146.

Zienlińska, D., Nagels, L., & Piskula, M. K. (2008). Determination of quercetin and its glucosides in onion by electrochemical methods. *Analytica Chimica Acta, 617*, 22–31.

Zoecklein, B. W., Fugelsang, K. C., Gump, B. H., & Nury, F. S. (2001). *Análisis y producción de vino*. Zaragoza, Spain: Acribia.