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Phenolic compounds and total antioxidant potential of commercial wines

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Abstract

Growing evidence of the role of free radicals and antioxidants in health and ageing has focussed great interest on these compounds. The relationship between the total antioxidant potential and the phenolic content of commercial wines was evaluated. A close relationship between total phenolic content and total antioxidant potential for all wines was observed. Capillary zone electrophoresis showed that, in red wines, gallic acid was the highest of the phenolic acids and (+)-catechin and (−)-epicatechin were the next most abundant phenolics. Also, these compounds were strictly correlated with the total antioxidant potential of wines. Total antioxidant potential, by bleaching of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cations, using gallic acid as standard, could be a practical and simple measurement to evaluate the characteristics of different wines. Furthermore, capillary electrophoresis is a powerful and high-performing tool for evaluating principal antioxidant wine components.

Keywords: Wines; Antioxidants; Phenolic compounds; Capillary zone electrophoresis; CZE

1. Introduction

Free radicals are extremely harmful to living organisms in that they attack different constituents of the cell, which leads to acceleration of the ageing process and sometimes, even, cell destruction or, if the DNA is affected, irreversible malfunctions (De Gaulejac, Glories, & Vivas, 1999). Growing evidence of the role of free radicals and antioxidants in health and ageing has focussed great interest on these compounds. Fruits and vegetables are natural sources of vitamins and antioxidants. Vitamins C, E and the various carotenoids are ubiquitous in plants. Flavonoids and other phenolic compounds found in plants have received much attention in the prevention of human degenerative diseases (Aruoma, 1996).

Phenolic compounds are responsible for some of the major organoleptic properties of wines, in particular colour and astringency. Wine phenolic composition depends on the grapes used to make the wine and on the vinification conditions (Cheynier, Hidalgo Arellano, Souquet, & Moutounet, 1997). Polyphenolic components of wine fall into one of two major classes. Non-flavonoids comprise hydroxybenzoates and hydroxycinnamates. Flavonoids include flavonols (e.g. quercetin, myricetin), flavan-3-ols (e.g. catechin and epicatechin), as well as polymers of the latter, defined as procyanidins, and anthocyanins that are the pigments responsible for the colour of red wines; collectively they are 20-fold higher in red than in white wine (Soleas & Goldberg, 1999).

The flavonoid content of red wine has been suggested as an explanation of the "French paradox", i.e. the fact that French people have low incidence of coronary heart disease, despite having a diet high in fat and being heavy smokers (Aruoma, 1996). The mechanism of this

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protective action of the flavonoids is a subject of considerable debate. As polyphenolic compounds, flavonoids have the ability to act as antioxidants by a free radical-scavenging mechanism (with the formation of less reactive flavonoid phenoxyl radicals) and metal ion chelation (Arora, Nair, & Strasburg, 1998; Lodovici, Guglielmi, Casalini, Meoni, Cheynier, & Dolara, 2001). A wide range of studies has shown the antioxidative properties of these compounds in protection against arteriosclerosis and coronary heart disease (Estruch, 2000; Santos-Buelga & Scalbert, 2000; Stupans, Kirlich, Tuck, & Hayball, 2002; Sun, Simonyi, & Sun, 2002; Visioli, Borsani, & Galli, 2000). Other effects include modulation of eicosanoid synthesis toward a more antiatherogenic pattern and inhibition of tumour growth in vitro and in human cancer patients (Soleas, Dam, Carey, & Goldberg, 1997).

The identification of the active phenolic compound or the phenol class that is responsible for red wine's antioxidant properties has raised much interest (Kerry & Abbey, 1997). These antioxidant properties provide a rationale for exploring the polyphenol content of commercial wines to define those that are specially abundant in these desirable compounds and to stimulate the development of enological techniques for their enrichment (Soleas, Dam et al., 1997).

Total phenols and polyphenols are usually quantified by employing Folin-Ciocalteu's reagent. This procedure is also employed in the wine industry, where gallic acid is usually selected as a standard. On the other hand, total antioxidant activity (TAA) values are measured from induction times in free radical mediated processes and/or from the bleaching of stable free radicals, such as 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)-derived radical cation ($ABTS^{\bullet+}$) (Campodonico, Barbieri, Pizarro, Sotomayor, & Lissi, 1998).

The aim of this work was to evaluate the relationship between the total antioxidant potential and the total phenol content of commercial wines, determined by bleaching of pre-formed ABTS radical cations and Folin-Ciocalteu's reagent, respectively. To identify phenolic compounds responsible for the antioxidant activity and to obtain a phenolic profile content of such wine, a very high-performing analytical technique was used, i.e. capillary zone electrophoresis. High efficiency and the capacity to resolve a very complex matrix of natural compounds, namely wine, were the reasons for the choice of capillary electrophoresis for the purpose (Pazourek, Gonzalez, Revilla, & Havel, 2000). The useful application of capillary electrophoresis for wine and polyphenols analyses has already been well demonstrated (Chu, O'Dwyer, & Zeece, 1998; Mellenthin & Galensa, 1999). The calibration and recovery data used now for the technique are shown in Table 1.

2. Materials and methods

2.1. Wines

The different commercial types of red, rosé and white wines, and their vintages, variety, and region of production are listed in Tables 2 and 3. The samples (50 ml aliquots of freshly opened wine bottles) were immediately analysed for total phenols and total antioxidant potential, and the extractions were carried out in the dark and under nitrogen atmosphere.

Table 1
Calibration and recovery data by capillary zone electrophoresis

Substance	Regression equation[a]	λ_{Abs}	Correlation coefficient	Recovery (%)	L.O.D. (ng/ml)
Tyrosol	$y = 5.25\cdot10^{-4}x - 2.49\cdot10^{-1}$	206	1.000	100	200
cis-Resveratrol	$y = 1.15\cdot10^{-4}x + 1.52\cdot10^{-1}$	206	1.000	100	18
trans-Resveratrol	$y = 1.03\cdot10^{-3}x - 6.05\cdot10^{-1}$	312	0.998	100	50
(+)-Catechin	$y = 1.13\cdot10^{-4}x + 2.21\cdot10^{-1}$	206	0.999	76	33
(−)-Epicatechin	$y = 7.31\cdot10^{-5}x + 5.01\cdot10^{-1}$	206	0.999	57	44
Hydroxytyrosol	$y = 1.39\cdot10^{-4}x - 3.46\cdot10^{-2}$	206	1.000	50	100
Sinapic acid	$y = 3.84\cdot10^{-4}x + 2.58\cdot10^{-1}$	217	0.998	67	149
Epicatechin gallate	$y = 1.61\cdot10^{-4}x + 2.34$	206	0.999	77	65
Syringic acid	$y = 2.47\cdot10^{-4}x - 6.80\cdot10^{-2}$	206	0.998	96	52
o-Coumaric acid	$y = 9.00\cdot10^{-5}x - 9.75\cdot10^{-2}$	217	1.000	100	25
p-Coumaric acid	$y = 1.04\cdot10^{-4}x - 1.26\cdot10^{-1}$	206	1.000	100	25
Vanillic acid	$y = 2.30\cdot10^{-4}x - 2.33\cdot10^{-1}$	206	0.999	83	30
Gentisic acid	$y = 1.01\cdot10^{-4}x + 2.39\cdot10^{-1}$	206	1.000	31	81
p-Hydroxybenzoic acid	$y = 1.02\cdot10^{-4}x + 4.11\cdot10^{-1}$	206	0.999	56	45
Salicylic acid	$y = 7.47\cdot10^{-5}x - 1.64\cdot10^{-1}$	206	0.999	84	30
Caffeic acid	$y = 3.29\cdot10^{-4}x + 4.89\cdot10^{-1}$	217	0.999	70	286
Gallic acid	$y = 7.43\cdot10^{-5}x + 3.27\cdot10^{-1}$	217	0.999	100	25
Protocatechuic acid	$y = 6.45\cdot10^{-5}x - 4.07\cdot10^{-3}$	206	0.999	22	114

[a] x is the peak area and y is the concentration in μg/ml.

Table 2
Contents of total phenolics and TAP values of wine samples

Colour, country	Vintage	Main variety	Total phenolics (mg l^{-1})[a]	TAP (mg l^{-1})[a]
White, Argentine	–	Chardonnay	216	103
White, Brazil	1998	Blended	347	172
White, Brazil	1997	Riesling	353	165
White, Brazil	1998	Cabernet Blanc	256	121
Red, Brazil	1996	Blended	1947	808
Red, Brazil	1996	Pinot	1984	807
Red, Chile	1996	Cabernet Sauvignon	2133	859
Red, Portugal	–	Blended	1615	556
Average white wines			293	140
Average red wines			1920	758

[a] Values are expressed as mg of gallic acid equivalents (GAE) l^{-1}.

2.2. Total phenolic content

The total phenolic contents of the wine samples were determined with the Folin-Ciocalteu reagent, using gallic acid as standard. To 1000 μl of wine sample (adequately diluted), 250 μl of carbonate–tartrate solution (200 g of Na_2CO_3 and 12 g of $Na_2C_4H_4O_6{\cdot}2H_2O$ in 1 l of distilled water) and 25 μl of Folin-Ciocalteu's reagent were added. The absorbance of the sample was measured at 700 nm after 30 min of reaction. The results were expressed as mg of gallic acid equivalents (GAE) l^{-1}.

2.3. Assay of polyphenols

2.3.1. Chemicals

Boric acid, sodium hydroxide and diethyl ether were obtained from Pro Analysis Merck (Darmstadt, Germany); methanol and hydrochloric acid were bought from Carlo Erba (Milan, Italy). All solutions were prepared with deionised water (Milli-Q, Millipore, MA, USA) and filtered with 0.2 μm Minisart filters from Sartorius (Göttingen, Germany). Drying procedure of the extraction step was performed on Safe-Lock 2.0-ml vials (Eppendorf-Netheler-Hinz-Gmbh, Hamburg, Germany).

2.3.2. Sample preparation

For the liquid/liquid extraction, 1 ml of red wine (2 ml in case of white or rosé wine) was extracted with 1 ml (2 ml for white or rosé wine) of diethyl ether (twice). The organic phases were completely dried in the dark under nitrogen flux and resuspended with 100 μl of methanol (10%) in electrophoretic buffer. Electrophoretic buffer composition was phosphate 25 mmol l^{-1}, borate 10 mmol l^{-1}, at pH 8.8. This buffer was obtained by mixing solutions of H_3BO_3 (100 mmol l^{-1}) and Na_2HPO_4 (100 mmol l^{-1}), and NaOH (2 mol l^{-1}) to reach the desired pH value.

Table 3
Contents of total phenolics and TAP values of Italian wine samples

Wines (samples)	Vintage	Main variety	Total phenolics (mg l^{-1})[a]	TAP (mg l^{-1})[a]
White (1)	1998	Greco di Tufo	854	178
White (2)	1997	Pinot Grigio	439	129
White (3)	1998	Verdicchio	610	152
Rosé (4)	1998	blended	1304	284
Red (5)	1998	Barbera	3314	625
Red (6)	1998	Montepulciano	4177	868
Red (7)	1996	blended	3791	805
Average white wines			634	153
Average red wines			3760	766

[a] Values are expressed as mg of gallic acid equivalents (GAE) l^{-1}.

2.3.3. Capillary electrophoresis procedure

Capillary zone electrophoresis was employed to quantify the polyphenols listed in Table 4 according to Rossi, Di Tommaso, and Rotilio (1998). Capillary electrophoresis analyses were performed using a P/ACE 5500 (Beckman Instruments Inc., Fullerton, CA, USA), equipped with a diode-array detector, and elaborated with Beckman P/ACE Station 5000 software, on an Epson Endeavor XL personal computer. The column used was an uncoated fused silica capillary tube of 75 μm i.d. (Beckman) with effective and total lengths of 50 and 57 cm, respectively. Electrophoretic analyses were performed at an applied voltage of 15 kV at 20 °C. Moreover, the silica column was pre-rinsed with bidistilled water (1.5 min) and separation buffer (1.5 min), and after each cycle the column was rinsed with a solu-

Table 4
Total amount (mg l^{-1})[a] of substances in the analysed Italian wines

Substances	Wines (samples)						
	1	2	3	4	5	6	7
Tyrosol (1)	1.1	2.3	3.0	5.0	5.9	5.9	6.0
cis-Resveratrol (2)	0.3	0.2	0.2	1.2	2.4	3.5	2.6
trans-Resveratrol (3)	0.3	0.2	0.1	0.8	2.3	3.0	2.2
(+)-Catechin (4)	4.9	0.6	0.1	3.5	13.8	14.0	15.2
(−)-Epicatechin (5)	2.8	0.3	0.6	3.3	11.0	13.7	10.7
Hydroxytyrosol (6)	2.7	1.9	1.6	6.1	9.6	0.5	5.9
Sinapic acid (7)	0.1	0.2	0.4	0.5	0.9	1.7	2.4
Epicatechin Gallate (8)	1.3	ND	1.2	ND	3.9	8.1	4.8
Syringic acid (9)	0.1	ND	0.2	1.5	2.2	3.0	3.9
o-Coumaric acid (10)	ND	0.7	0.3	0.8	0.2	0.3	0.4
p-Coumaric acid (11)	ND	1.0	0.8	2.7	2.3	2.9	1.0
Vanillic acid (12)	0.1	0.1	0.6	0.3	2.0	2.1	2.1
Gentisic acid (13)	ND	0.2	0.3	0.2	0.9	0.9	1.0
p-Hydroxybenzoic acid (14)	0.2	ND	0.2	0.7	1.0	1.3	0.7
Salicylic acid (15)	0.4	0.2	1.0	0.3	0.4	0.5	0.5
Caffeic acid (16)	2.2	0.5	1.1	5.0	3.6	2.7	2.7
Gallic acid (17)	2.2	3.5	0.6	14.3	54.8	58.3	55.2
Protocatechuic acid (18)	1.1	0.1	0.4	2.4	2.6	7.2	5.1

ND, not detected.
[a] Any concentration value is the mean of three measures.

tion of HCl (0.1 mol l^{-1}) (1.5 min), NaOH (0.1 mol l^{-1}) (1.5 min) and bidistilled water (1.5 min). Samples were hydrodynamically injected at $3.45 \cdot 10^3$ Pa pressure for 7 s. Calibration curves were obtained by hydrodynamic injection of concentrations, from 1 to 50 mg l^{-1}, of each compound (Sigma, Italy) for 7 s, at a pressure of $3.45 \cdot 10^3$ Pa. For qualitative peak recognition spectra were obtained by diode-array detection (DAD) and, in order to improve method sensitivity, peak integration was calculated at different wavelengths for each compound. DAD parameters, calibration and recovery data are shown in Table 1.

2.4. Total antioxidant potential (TAP)

The total antioxidant potential was measured by bleaching of ABTS radical cations. ABTS (Sigma) radical cations were prepared by incubation of 150 μmol l^{-1} (50 ml) with 2 mol l^{-1} potassium persulphate (1.25 ml) for 2 h at 50 °C in phosphate buffer, pH 7.0 (0.02 mol l^{-1}) (Campodonico et al., 1998). To 996 μl of the ABTS radical cation, 4 μl of the wine sample, adequately diluted, were added. The absorbance of the sample was measured after 15 min at 734 nm. Gallic acid was used as standard and the results were expressed as mg of gallic acid equivalents (GAE) l^{-1}.

3. Results

3.1. Total phenolic content and total antioxidant potential

The wine samples were tested for total phenolic content and total antioxidant potential in two sets of analyses. In the first set of analyses, five wine samples (three white and two red) from Brazil, one (red) from Chile, one (red) from Portugal and one (white) from Argentina were tested. The content of total phenols varied from 1615 to 2133 mg l^{-1}, averaging 1920 mg l^{-1}, for the red wines and from 216 to 353 mg l^{-1}, averaging 293 mg l^{-1}, for the white wines (Table 2). In the second set of analyses, eight Italian wines samples were tested (four red wines, three white wines, and one rosé wine). The content of total phenols varied from 3314 to 4177 mg l^{-1}, averaging 3760 mg l^{-1}, for the red wines, and from 439 to 854 mg l^{-1}, averaging 634 mg l^{-1}, for the white wines. A value of 1304 mg l^{-1} for the rosé wine was found (Table 3).

The first set of analyses showed TAP, values ranging from 556 to 859 mg l^{-1}, averaging 758 mg l^{-1} for red wines and from 103 to 172 mg l^{-1}, averaging 140 mg l^{-1} for white wines (Table 2). The second set of analyses showed TAP values ranging from 625 to 868 mg l^{-1}, averaging 766 mg l^{-1} for red wines and from 129 to 178 mg l^{-1}, averaging 153 mg l^{-1} for white wines. The value for the rosé wine was 284 mg l^{-1} (Table 3).

3.2. Assay of polyphenols by capillary zone electrophoresis

The concentrations of 18 polyphenols (listed in Table 4) were measured by capillary zone electrophoresis in the Italian wines listed in Table 3. The results are shown in Table 4. A representative electropherogram is shown in Fig. 1. In red wines, gallic acid was the highest of the polyphenols, ranging from 54.8 to 58.3 mg l^{-1}. The rosé wine presented a value of 14.3 mg l^{-1}. The highest level of this component in white wines was 3.5 mg l^{-1}. (+)-Catechin and (−)-epicatechin were the next most abundant phenolics, ranging from 13.8 to 15.2 mg l^{-1} and 10.7 to 13.7 mg l^{-1} in red wines, respectively. (−)-Epicatechin levels in all red wines were lower than the (+)-catechin levels. Tyrosol was around 6.0 mg l^{-1} in red wines and varied from 1.1 to 3.0 mg l^{-1} in white wines. The value in the rosé wine was 5.0 mg l^{-1}. The highest level of caffeic acid was found in the rosé wine

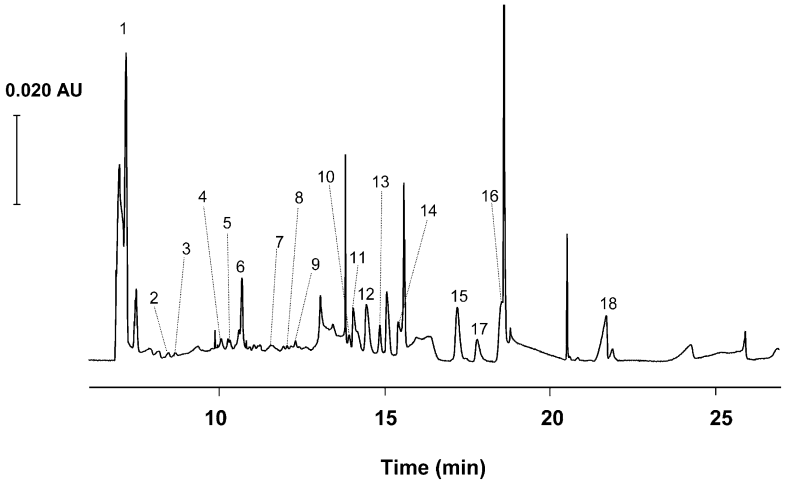

Fig. 1. Electropherogram of a white wine extract. For peak identification, see Table 4.

(5.0 mg l^{-1}), followed by red wines (range from 2.7 to 3.6 mg l^{-1}). In one red Montepulciano wine, sample 6, the concentration of hydroxytyrosol was very low (0.5 mg l^{-1}). The level of this component in the other red wines ranged from 5.9 to 9.6 mg l^{-1}. The rosé wine presented a value of 6.1 mg l^{-1} and in white wines it ranged from 1.6 to 2.7 mg l^{-1}. Epicatechin gallate and protocatechuic acid presented values of 3.9–8.1 and 2.6−7.2 mg l^{-1} in red wines, respectively. Smaller amounts of the following compounds were found in the wines: *cis*-resveratrol (<3.5 mg l^{-1}), *trans*-resveratrol (<3.0 mg l^{-1}), sinapic acid (<2.4 mg l^{-1}), syringic acid (<3.9 mg l^{-1}), *o*-coumaric acid (<0.8 mg l^{-1}), *p*-coumaric acid (<2.9 mg l^{-1}), vanillic acid (<2.1 mg l^{-1}), gentisic acid (<1.0 mg l^{-1}), *p*-hydroxybenzoic acid (<1.3 mg l^{-1}), salicylic acid (<1.0 mg l^{-1}).

4. Discussion

Much attention has been focussed on the protective biochemical function of naturally occurring antioxidants in biological systems and on the mechanism of their action (Kanner, Frankel, Granit, German, & Kinsella, 1994). Despite much interest in the antioxidant activity of red wine, it is uncertain which of the phenols exhibit the greatest antioxidant effect (Kerry & Abbey, 1997). Most aromatic plant acids exist as derivatives of benzoic acid or cinnamic acid. The existing methods used for the analysis of aromatic plant acids are generally high-performance liquid chromatography, thin-layer chromatography and gas chromatography. In this work we have used capillary zone electrophoresis with diode array detection. This is a very efficient method because its high-resolution separation, simplicity of operation, versatility and sensitivity (Gu, Chub, O'Dwyer & Zeece, 2000; Hiermann & Hadl, 1998; Kulomaa, Siren, & Riekkola, 1997), and the diode-array detector, make it a very powerful tool in qualitative and quantitative determination of natural substances (Da Costa, Horton, & Margolis, 2000).

The amounts of phenolic materials vary considerably in different types of wine, depending on the grape variety, environmental factors in the vineyard, and the wine processing techniques (Frankel, Waterhouse, & Teissedre, 1995). Our results confirm a variation in phenolic content among wine samples tested. These results are in agreement with the available literature (Campodonico et al., 1998; Frankel et al., 1995; Hurtado, Caldú, Gonzalo, Ramon Mínguez, & Fiol, 1997; Kanner et al., 1994; Sato, Ramarathnam, Suzuki, Ohkubo, Takeuchi, & Ochi, 1996; Simonetti, Pietra, & Testolin, 1997). The presence of high concentrations of gallic acid in red wines would be expected since this phenolic acid is principally formed by hydrolysis of flavonoid gallate esters, which are largely absent in white wines, due to the lack of skin extraction (Frankel et al., 1995). Soleas, Dam et al. (1997) presented data for concentrations of 15 polyphenolics in a range of white and red Canadian wines. They observed that, in white wines, caffeic acid was the highest in Chardonnay and Vidal wines and *p*-coumaric acid in those from Seyval Blanc. The individual phenolic acids demonstrated a similar pattern among all of the red wines analysed, with gallic acid being the highest and the caffeic acid the second highest. Pinot Noir wines were highest in catechin, epicatechin and polydatin concentrations.

The TAP of wine samples was determined by bleaching of pre-formed ABTS radical cations. The addition of free radical-scavengers to a solution containing ABTS-derived radical cations leads to a decrease in the absorbance of the sample at 734 nm that is proportional to the size of the wine aliquot (Campos, Escobar, & Lissi, 1996). In general, the measurement of antioxidant ability uses standard solutions of antioxidant compounds, such as Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) or ascorbic acid. We have used gallic acid as standard, considering that this compound is generally present in high concentrations in red wines. In order to see the response of the Folin and ABTS methods to different compounds, Campodonico et al. (1998) tested several mono and polyphenols. The results showed that both methods were able to titrate a variety of phenols and it is noteworthy that the response of different phenolic groups may differ by a factor up to nearly 2. In the case of Trolox and propyl gallate (gallic acid propyl ester), the responses were very similar, considering that Trolox is a mono phenol and propyl gallate is a tri-phenol. Bohm (2000) compared three common methods to measure the antioxidant action (TEAC, TRAP and LDL oxidation assays) using four standard antioxidants (gallic acid, uric acid, Trolox and ascorbic acid). The results were not comparable. Whereas gallic acid was the strongest antioxidant in any test, ascorbic acid showed less activity in the TRAP and the LDL oxidation assays, but higher activity in the TEAC assay.

Tables 2 and 3 show that red wines presented a substantial antioxidant capacity. The red Chilean wine tested showed a very high effect of free radical scavengers, which is in agreement with Campos and Lissi (1996). A very close relationship between total phenolic content and total antioxidant potential, for all wines, was observed in both sets of results [$r = 0.9878$ (Fig. 2a) and $r = 0.992$ (Fig. 2b)]. These results are in agreement with other reports in the literature (Campodonico et al., 1998; Fogliano, Verde, Randazzo, & Ritieni, 1999; Henn & Stehle, 1998; Sánchez-Moreno, Larrauri, & Saura-Calixto, 1999a; Sato et al., 1996; Simonetti et al., 1997). Total antioxidant potential also correlated with the concentrations of gallic acid ($r = 0.9572$, Fig. 3a), (−)-epicatechin ($r = 0.9583$, Fig. 3b) and (+)-catechin

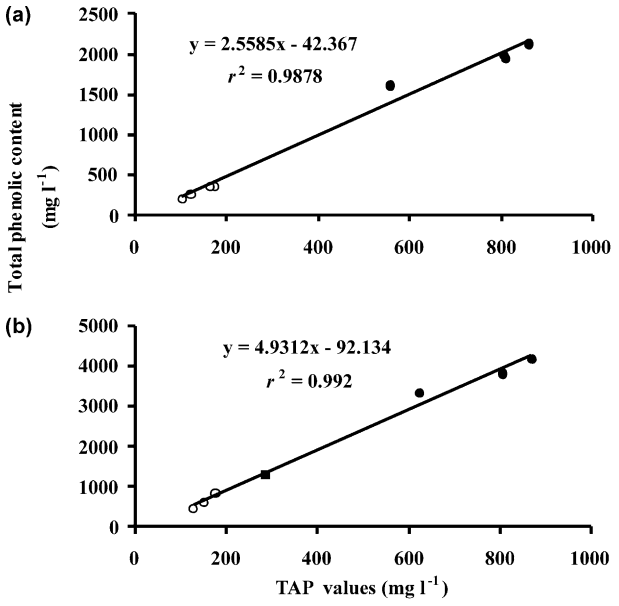

Fig. 2. Relationship between total phenolic contents and TAP values of wines listed in Table 2 (a) and Table 3 (b). (○) white wine; (■) rosé wine; (●) red wine.

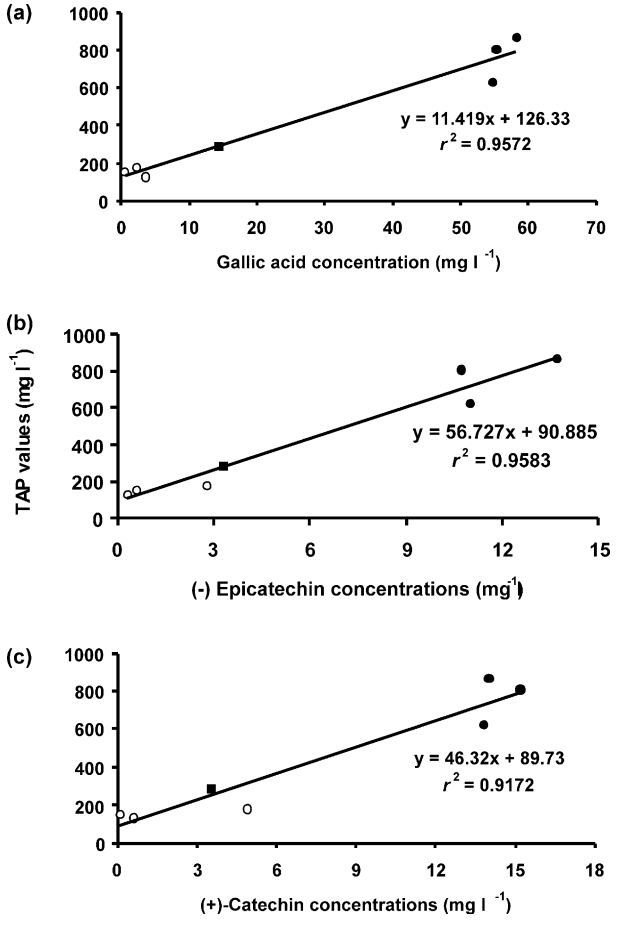

Fig. 3. Relationship between gallic acid (a), (−)-epicatechin (b) and (+)-catechin (c) concentrations and TAP values of wines.

($r = 0.9172$, Fig. 3c). According to Frankel et al. (1995), the relative antioxidant activity of 20 selected California wines correlated with total phenol contents of wines ($r = 0.94$) and with the concentrations of gallic acid ($r = 0.92$), catechin ($r = 0.75$), myricetin ($r = 0.70$), quercetin ($r = 0.68$), caffeic acid ($r = 0.63$), rutin ($r = 0.50$), epicatechin ($r = 0.45$), cyanidin ($r = 0.43$), and malvidin 3-glucoside ($r = 0.38$). Their work also showed that the antioxidant activity of different commercial wines toward low density lipoprotein oxidation is not a property of a single phenolic compound and that this activity is widely distributed among the phenolic phytochemical constituents. Sánchez-Moreno, Larrauri, and Saura-Calixto (1999b) observed that the free radical-scavenging activity of gallic acid was the highest; tannic acid, caffeic acid, quercetin, 3-tertiary-butyl-4-hydroxyanisole (BHA) and rutin activities were intermediate and ferulic acid, DL-α-tocopherol and resveratrol were the lowest.

Soleas, Tomlinson, Diamandis, and Goldberg (1997) analysed the concentrations of 17 phenolic constituents in a red wine by a number of multiple regression models for their contribution to total antioxidant status. On the basis of single analysis of each phenolic in the wine matrix, only seven were significantly correlated with total antioxidant status of the wine sample; the highest values for r were observed for vanillic and gallic acids. With statistical modelling, utilizing both linear and non-linear approaches to predict the total antioxidant status of wine samples from their polyphenol content, the best results were obtained with vanillic acid, *trans*-polydatin, catechin, *m*-coumaric acid, epicatechin, quercetin, *cis*-polydatin and *trans*-resveratrol. Although syringic and gallic acids were significantly correlated with total antioxidant status in a univariate analysis, they do not contribute to a statistical description of this parameter with the eight constituents already identified. Ghiselli, Nardini, Baldi, and Scaccini (1998) showed that the protective effect of wine is mainly due to the anthocyanic fraction (quantitatively the more abundant phenolic subclass in red wine), although their results do not exclude the possibility of a synergistic action among the different classes of polyphenols. Also, the anthocyanic fraction showed a high free radical scavenging power in relation to the other tannic fractions (De Gaulejac et al., 1999). Kerry and Abbey (1997) observed that the antioxidant property of red wine is due predominantly to monomeric catechins, procyanidins, monomeric anthocyanidins and phenolic acids. Simonetti et al. (1997) suggested that absorption and metabolism studies should be preferably focussed upon gallic acid derivatives and flavonols, since they are the most significant phenols in red wines. Kondo, Kurihara, Miyata, Suzuki, and Toyoda (1999) investigated the antioxidative effects and mechanisms of catechins using liquid chromatography/mass spectrometry, spectrophotometric analyses, and

semiempirical molecular orbital calculations. The authors observed that (−)-epicatechin would be gently converted to an anthocyanin-like compound. According to the mechanisms, the compound produced from (−)-epicatechin by radical oxidation can also function as an antioxidant. Valls-Belles, Muniz, Gonzalez, Gonzalez-Sanjose and Beltran (2002) showed that epicatechin is an effective antioxidant; it completely prevents tert-butylhydroperoxide-induced oxidation and thereby prevents subsequent lipid peroxidation.

Capillary zone electrophoresis, coupled with a diode array detector, has been shown to be a powerful tool, able to resolve a complex matrix such as wine; furthermore a sample pre-concentration step improves method sensitivity, allowing determinations at sub-ppm level.

5. Conclusion

A positive correlation between the total antioxidant potential of wine and the gallic acid, (−)-epicatechin, (+)-catechin concentrations and total phenol content has been demonstrated in the present study. Total antioxidant potential, by bleaching of ABTS radical cations and using gallic acid as standard, could be a practical and simple measurement to evaluate the characteristics of different wines. Capillary zone electrophoresis could be a powerful tool to analyse polyphenol contents of white and red wines, with an opportune sample pre-concentration step.

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