Relationship among Antioxidant Activity, Vasodilation Capacity, and Phenolic Content of Red Wines

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The relationship among antioxidant activity, based on the electron-spin resonance determination of the reduction of Fremy's radical, vasodilation activity, and phenolic content was investigated in 16 red wines. The wines were selected to provide a range of origins, grape varieties, and vinification methods. Sensitive and selective HPLC methods were used for the analysis of the major phenolics in red wine: free and conjugated myricetin, quercetin, kaempferol, and isorhamnetin; (+)-catechin, (−)-epicatechin, gallic acid, *p*-coumaric acid, caffeic acid, caftaric acid, *trans*-resveratrol, *cis*-resveratrol, and *trans*-resveratrol glucoside. Total anthocyanins were measured using a colorimetric assay. The total phenolic content of the wines was determined according to the Folin−Ciocalteu colorimetric assay and also by the cumulative measurements obtained by HPLC. The 16 wines exhibited a wide range in the values of all parameters investigated. However, the total phenol contents, measured both by HPLC and colorimetrically, correlated very strongly with the antioxidant activity and vasodilation activity. In addition, the antioxidant activity was associated with gallic acid, total resveratrol, and total catechin. In contrast, only the total anthocyanins were correlated with vasodilation activity. The results demonstrate that the different phenolic profiles of wines can produce varying antioxidant and vasodilatant activities, which opens up the possibility that some red wines may provide enhanced health benefits for the consumer.

Keywords: *Polyphenols; red wine; HPLC; vasodilation; antioxidant activity; electron spin resonance spectroscopy; Fremy's salt radical*

INTRODUCTION

The ever-increasing human and economic cost of coronary heart disease (CHD) has prompted extensive investigations into the associated risk factors, including alcohol consumption. A number of large-scale epidemiology studies have demonstrated that a moderate consumption of alcohol is associated with reduced mortality and CHD (Klatsky, 1997; Goldberg et al., 1995a). There is evidence that red wine can offer a greater protection than white wine, beer, or spirits (St Leger et al., 1979; Rimm et al., 1996), an association popularized as the "French paradox" (Renaud and de Lorgeril, 1992).

Red wine contains wood- and yeast-derived phenolics in addition to large amounts of phenolic components that originate from grapes, particularly the skins, which are removed during the vinification of white wine (Singleton, 1982). Although structurally diverse, phenolics are classified into two groups—the flavonoids and the nonflavonoids. The flavonoid family includes the flavonols myricetin (**I**; Chart 1), quercetin (**II**), kaempferol (**III**), and isorhamnetin (**IV**), which exist both as aglycons and sugar conjugates; the flavan-3-ols, (+)-catechin (**VI**) and (−)-epicatechin (**VII**); and the anthocyanins such as malvidin-3-glucoside (**VIII**). The nonflavonoids encompass gallic acid (**IX**); hydroxycinnamates, including *p*-coumaric acid (**X**), caffeic acid (**XI**), and caftaric acid (**XII**); and the stilbenes, *trans*-resveratrol (**XIII**), *cis*-resveratrol (**XIV**), and *trans*-resveratrol-*O*-*β*-glucoside (**XV**). A significant proportion of the phenolic content of wine originates from the tannins, which are subdivided into condensed and hydrolyzable tannins. The condensed tannins, known as procyanidins, are oligomers and polymers of (+)-catechin and (−)-epicatechin subunits, whereas the hydrolyzable tannins are based on gallic acid and its derivatives. Red wines

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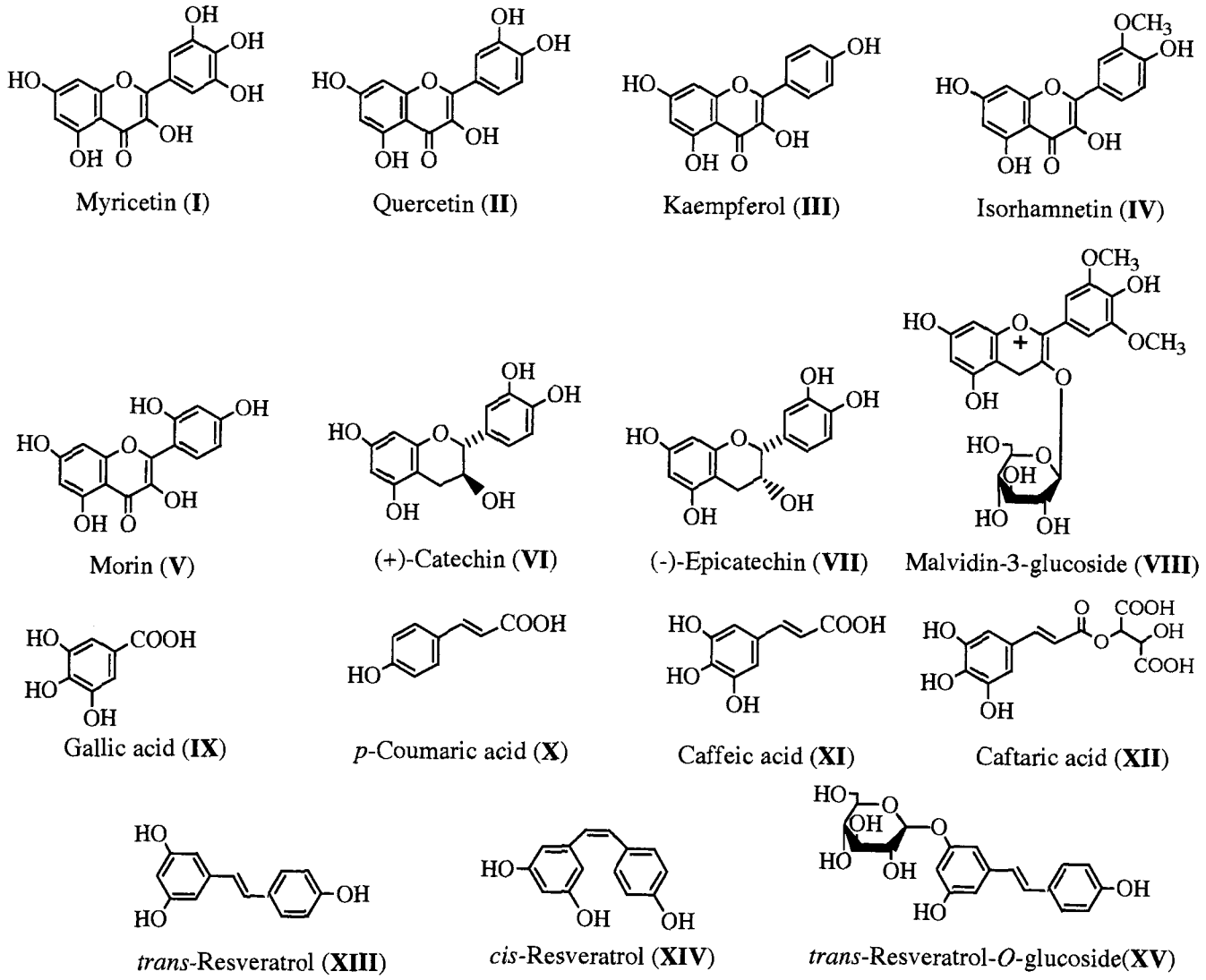
Chart 1. Structures of Myricetin, Quercetin, Kaempferol, Isorhamnetin, Morin, (+)-Catechin, (−)-Epicatechin, Malvidin 3-Glucoside, Gallic Acid, *p*-Coumaric Acid, Caffeic Acid, Caftaric Acid, *trans*-Resveratrol, *cis*-Resveratrol, and *trans*-Resveratrol *O*-β-Glucoside



contain, in total, 1500−2500 mg/L phenolics (Frankel et al., 1993a), although their presence and structures are affected by a number of factors including grape variety, sun exposure, vinification techniques, and aging (Price et al., 1995; McDonald et al., 1998).

Phenolics have a number of important roles to play in viticulture and enology including UV protection, disease resistance, pollination, color, and defense against predation in plants (Harborne, 1992), as well as haze formation, hue, and taste in wines (Singleton, 1982). Red wines do not contain significant amounts of vitamins or selenium, and their protective effects have been ascribed to phenolic components. It has been proposed that they act as antioxidants. The antioxidant capacity of phenolic compounds is essentially due to the ease with which a hydrogen atom from an aromatic hydroxyl group can be donated to a free radical and the ability of the phenolic moiety to support an unpaired electron due to delocalization around the π-electron system (Kanner et al., 1994). Such activity could reduce free radical-mediated oxidation of low-density lipoprotein (LDL) and so decrease artherogenicity (Frankel et al., 1993b, 1995; Fuhrman et al., 1995; Nigdikar et al., 1998). Other mechanisms have been proposed to explain the beneficial effects of red wine in the prevention of CHD including inhibition of platelet aggregation (Gryglewski et al., 1987; Pace-Asiak et al., 1995) and endothelium-dependent relaxation of blood vessels, mediated by the NO-cGMP pathway (Fitzpatrick et al., 1993).

Flavonols, such as quercetin, have been credited with being a major contributor to the antioxidant potential of red wines (Maxwell, 1997). Although members of this family of compounds do exhibit strong antioxidant activity in a variety of systems (Frankel et al., 1993a; Rice-Evans et al., 1995), they are probably not present in sufficient quantities to be considered major determinants of the total antioxidant capacity of red wines (Gardner et al., 1999). It has also been proposed that the stilbene resveratrol plays a key role in the beneficial effects of red wine (Soleas et al., 1997a), and it is known that resveratrol prevents platelet aggregation (Kimura et al., 1985; Pace-Asiak et al., 1995), inhibits the oxidation of LDL (Frankel et al., 1993b), and lessens the risk of cancer (Jang et al., 1997). However, gallic acid, (+)-catechin, (−)-epicatechin, and flavonols (Frankel et al., 1995; Lamuela-Raventós et al., 1993; Goldberg et al., 1995b; McDonald et al., 1998) are all present in red wines in far higher concentrations than resveratrol and exhibit similar, if not greater, antioxidant and antiplatelet aggregation activities than resveratrol

Table 1. Details of Red Wines Analyzed for Their Phenolic Content

	wine	principal grapes	origin	year	price
1	Chilean Cabernet Sauvignon	Cabernet Sauvignon	Lontué, Chile	1997	£3.99
2	Californian oak-aged Cabernet Sauvignon	Cabernet Sauvignon	California	1995	£4.99
3	Young Vatted Cabernet Sauvignon	Cabernet Sauvignon	Sliven, Bulgaria	1996	£3.29
4	Bulgarian matured Cabernet Sauvignon	Cabernet Sauvignon	Svischtov, Bulgaria	1992	£3.29
5	Cono Sur Pinot Noir, 20 barrels	Pinot Noir	Rapel Valley, Chile	1995	£8.99
6	Fetzer Santa Barbara Pinot Noir	Pinot Noir	California	1994	£7.29
7	Domaine Rossignol Trapet, Gevrey Chambertin	Pinot Noir	Burgundy, France	1995	£13.99
8	Villa Montes oak-aged Merlot	Merlot	Curicó, Chile	1994	£4.99
9	Merlot	Merlot	Languedoc, France	1996	£3.49
10	Cosme Palacio y Hermanos Rioja	Tempranillo	Rioja, Spain	1995	£5.99
11	Viña Albali Tempranillo	Tempranillo	Valdepeñas, Spain	1996	£2.99
12	Fetzer Vineyards Zinfandel	Zinfandel	California	1995	£5.99
13	Beaujolais	Gamy	Beaujolais, France	1996	£3.99
14	Domaine Roche Vue, Minervois	Carignon	Aude, France	1995	£3.99
15	Valpolicella	Corvina, Molinara	Veneto, Italy	1996	£3.49
16	Chianti Classico	Sangiovese, Trebbiano	Tuscany, Italy	1995	£5.75

(Frankel et al., 1993a,b, 1995; Vinson et al., 1995; Teissedre et al., 1996).

This paper reports a study with 16 red wines in which antioxidant capacity was measured by electron spin resonance (ESR) spectroscopy and parallel assessments of vasodilation capacity were also carried out. In addition, a variety of high-performance liquid chromatography (HPLC) procedures were used to identify and quantify the phenolic components present in the individual wines, and anthocyanins were determined by using a spectrophotometric method.

MATERIALS AND METHODS

Chemicals. The selected wines (Table 1) were supplied by Safeway Stores plc. Kaempferol, myricetin, quercetin, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, caffeic acid, *p*-coumaric acid, morin (**V**), and gallic acid were obtained from Sigma (Poole, Dorset, U.K.), and isorhamnetin and *trans*-resveratrol-*O*-*β*-glucoside were purchased from Apin (Abingdon, Oxford, U.K.). *cis*-Resveratrol was obtained by isomerization of *trans*-resveratrol in methanol during a 12 h exposure to high white light. Dr. Creina Stockley of the Australian Wine Research Institute generously provided a sample of caftaric acid, and Dr. Rosa Lamuela-Raventós, Universitat de Barcelona, kindly supplied a sample of *Polygonum cuspidatum* root containing a high concentration of *trans*-resveratrol-*O*-*β*-glucoside. Acetylcholine chloride and phenylephrine hydrochloride were both purchased from Sigma. Ethanol (Analar grade) was obtained from BDH (Poole, Dorset, U.K.), methanol and acetonitrile were from Rathburn Chemicals (Walkerburn, U.K.), and all other chemicals and reagents were obtained from Sigma-Aldrich (Poole, Dorset, U.K.).

Measurement of Antioxidant Potential. The ability of red wines to reduce Fremy's salt (potassium nitrosodisulfonate) was measured as described by Gardner et al. (1998). The wines were diluted to 5% (v/v) with ethanol/water (12:88, v/v). Three milliliter aliquots were reacted with an equal volume of 1 mM Fremy radical in ethanol/water (12:88, v/v). The ESR spectra of the low-field resonance of the Fremy's radical were obtained after 20 min, by which time the reaction was complete. Signal intensity was obtained by double integration and concentration calculated by comparison with a control reaction using ethanol/water (12:88, v/v) without red wine. Spectra were obtained at 21 °C on a Bruker ECS 106 spectrometer equipped with a cylindrical (TM110 mode) cavity and operating at ∼9.5 GHz (X-band frequency). The microwave power and modulation amplitude were set at 2 mW and 0.01 mT, respectively.

Assay of Vasodilation Capacity. New Zealand white adult rabbits (∼2.5 kg) were studied. They were killed by sodium pentobarbitone (200 mg/kg), and the thoracic aortas were removed and cleaned of adhering fat and connective tissue. Each aorta was cut into rings (4−5 mm long), suspended from force displacement transducers in 10 mL organ baths, and bathed in Krebs buffer solution (pH 7.4) [composition (mM): NaCl, 118.4; $NaHCO_3$, 25; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 0.6; $CaCl_2$, 2.5; glucose, 11.0; and EDTA, 23.0] at 37 °C. The buffer was bubbled continuously with 16% O_2/5% CO_2 balanced with N_2 to give values similar to those found in vivo. Tension (2 g) was then applied to all rings. Following a 1 h equilibration period the response to 50 mM KCl was determined, followed by wash-out and further equilibration. All tissues were contracted submaximally with phenylephrine (PE; 0.1 μM), and once a stable plateau had been reached, cumulative concentration-dependent response curves (CCRCs) to the wine extracts (1−5000 μg/mL) using various dilutions of these extracts were constructed. Only one wine extract was used for each ring. All drugs and solutions were prepared in distilled water. Extracts were prepared by removing water and alcohol from the wine first by vacuum and then under nitrogen to ensure they were dry. The samples were then diluted to give an initial solution of 500 mg/mL, from which further dilutions were made. Fresh wine extract dilutions were made up daily and used within 24 h. As an index of potency, the pIC_{50} (the concentration in micrograms per milliliter at which each extract caused 50% of maximum vasodilation) for each wine extract was determined using graphical interpolation for each CCRC constructed and expressed as mean ± standard error (SE). Graphically the potency is displayed as $1/pIC_{50} \times 10^3$.

Analysis of Phenolics by HPLC. Red wines were analyzed using a Shimadzu (Kyoto, Japan) LC-10Avp series automated liquid chromatograph comprising an SCL-10Avp system controller, two LC-10ATvp pumps, an SIL-10ADvp autoinjector with sample cooler, a CTO-10Avp column oven operating at 40 °C and linked to a Waters 996 photodiode array (PDA) detector (Waters, Milford, MA), and an RF-10A fluorometer (Shimadzu). Data from both detectors were collected and processed via a Millennium Chromatography Manager (Waters). Sample treatment, HPLC column, solvent conditions, and detector systems used for the different phenolics are summarized below. To optimize resolution, mobile phase conditions for isocratic analyses were designed to provide k' values of ∼4−5 for the compounds of interest. In addition, for each group of compounds, different reverse-phase columns with varying selectivities and polarities were evaluated with red wines to ensure that impurities did not impinge on the homogeneity of quantified peaks.

Flavonols. Free and conjugated myricetin, quercetin, kaempferol, and isorhamnetin were analyzed in samples with a morin internal standard, before and after acid hydrolysis (McDonald et al., 1998) using a 150 × 3.0 mm i.d., 4 μm C_{18} Genesis column (Jones Chromatography, Mid-Glamorgan, U.K.) eluted at a flow rate of 0.5 mL/min with a 20 min gradient of 20−40% acetonitrile in water adjusted to pH 2.5 with trifluoroacetic acid (TFA). After passing through the PDA detector operating at 365 nm, the column eluate was mixed with methanolic aluminum nitrate in 7.5% acetic acid pumped at a flow rate of 0.5 mL/min and fluorescent flavonol complexes were detected with a fluorometer (excitation = 425 nm, emission = 480 nm) as described by Aziz et al. (1998). Sample volumes analyzed were equivalent to 4.6 μL of wine.

Stilbenes. *trans*- and *cis*-resveratrol in 10 μL volumes of red wine were analyzed on a 250 × 4.6 mm i.d., 5 μm ODS-Hypersil (Shandon, Astmoor, U.K.) column, packed in-house and eluted at a flow rate of 1 mL/min with 25% acetonitrile in water adjusted to pH 1.5 with TFA using a PDA detector at 307 nm. *trans*-Resveratrol-*O*-β-glucoside was analyzed under similar conditions except that the mobile phase was 17% acetonitrile in water adjusted to pH 1.5 with TFA.

Gallic Acid. The gallic acid contents of 5 μL volumes of red wines were analyzed on a 150 × 3.0 mm i.d., 4 μm C_{18} Genesis column (Jones Chromatography) eluted at a flow rate of 1.0 mL/min with 2% methanol in water adjusted to pH 1.5 with TFA using a PDA detector at 280 nm.

Hydroxycinnamates. Five microliter volumes of samples were analyzed before and after alkaline hydrolysis, which was used to cleave conjugated caffeic acid and *p*-coumaric acid. Five microliter volumes of hydrolysate are equivalent to 1.67 μL of wine. This was achieved by mixing 1 mL of red wine and 1 mL of 4 N NaOH in a 3 mL glass V-vial, which was incubated in darkness at room temperature for 2 h before being acidified with 1 mL of 6 M HCl. The method was adapted and optimized from that of Rapisarda et al. (1998). Samples were analyzed on a 150 × 3.0 mm i.d., 5 μm C_{18} Nemesis column (Phenomenex, Macclesfield, U.K.) eluted at a flow rate of 1 mL/min with either 5 or 9% acetonitrile in water adjusted to pH 1.5 with TFA and a PDA detector operating at 313 nm. Caffeic acid and caftaric acid were analyzed with 5% acetonitrile, whereas a 9% acetonitrile mobile phase was used for free *p*-coumaric acid as well as caffeic acid and *p*-coumaric acid released by alkaline hydrolysis. Using a 9% acetonitrile mobile phase, ferulic and sinapic acids could also be separated but were not found in detectable levels in the wines under study.

(+)-Catechin and (−)-Epicatechin. A 150 × 4.6 mm i.d., 5 μm C_{18} Luna column (Phenomenex, Macclesfield, U.K.) eluted at a flow rate of 1 mL/min with 10% acetonitrile in water adjusted to pH 1.5 with TFA was used to analyze the (+)-catechin and (−)-epicatechin contents of 5 μL volumes of red wines. (+)-Catechin and (−)-epicatechin were detected with a fluorometer operating at excitation = 280 nm and emission = 310 nm (Arts and Hollman, 1998) and by absorbance at 280 nm. The method also allowed the separation of epigallocatechin, epigallocatechin gallate, and epicatechin gallate. These compounds could be detected only by using absorbance at 280 nm and were not found in detectable levels in the wines analyzed.

Colorimetric Analysis of Anthocyanins. The anthocyanin content of red wines was estimated using a pH shift method adapted from Ribéreau-Gayon and Stonestreet (1965). Two test tubes were set up, each containing 1 mL of wine and 1 mL of 0.1% concentrated HCl in 95% ethanol. Ten milliliters of 2% concentrated HCl (pH 0.6) was added to one tube and 10 mL of pH 3.5 buffer (300 mL of 0.2 M Na_2HPO_4 and 700 mL of 0.1 M citric acid, adjusted to pH 3.5 with 0.1 M citric acid) to the other. Absorbance was read at 700 nm to allow for correction of the haze and then at 520 nm for anthocyanin determination. Anthocyanins were quantified as malvidin-3-glucoside equivalents, the major anthocyanin in red wine, using the extinction coefficient $\epsilon = 28000$. At pH <1 anthocyanins are found entirely in their red flavylium form, allowing the determination of the total anthocyanins. However, at pH 3.5 the flavylium form of the anthocyanin is primarily in equilibrium with the colorless carbinol; therefore, absorbance is due to polymeric anthocyanins or interfering brown substances. The difference in absorbance between pH <1 and pH 3.5 is due to the free anthocyanin content.

Determination of Total Phenol Content. The total phenol contents of the wines were determined using the Folin−Ciocalteu method of Singleton and Rossi (1965).

Statistics. Data are presented as mean values ± standard error (SEM) ($n = 3$). A matrix plot was used to graphically represent the data obtained. Some relationships between results were apparently nonlinear; therefore, nonparametric Spearman rank correlations were used to assess the strength of the association between them using Minitab software, version 12 (Minitab Inc., Addison-Wesley Publishing Co., Reading, MA).

Table 2. Antioxidant Activity, Vasodilation Activity, and Phenolic Content of Red Wines

wine	ESR-based antioxidant activity[a]	vasodilation activity[b]	Folin−Ciocalteu total phenolics[c]	HPLC total phenolics[d]
1	6.99 ± 0.17	39 ± 8	10.48 ± 0.20	1.01 ± 0.02
2	5.99 ± 0.18	188 ± 64	10.16 ± 0.06	1.06 ± 0.02
3	9.29 ± 0.27	9 ± 3	18.6 ± 0.10	1.66 ± 0.01
4	6.16 ± 0.23	127 ± 27	11.1 ± 0.12	0.95 ± 0.00
5	7.27 ± 0.27	46 ± 6	13.28 ± 0.09	1.62 ± 0.02
6	6.60 ± 0.13	239 ± 51	11.78 ± 0.03	1.12 ± 0.00
7	8.03 ± 0.06	28 ± 7	15.74 ± 0.06	1.33 ± 0.10
8	5.98 ± 0.08	46 ± 6	10.39 ± 0.06	0.87 ± 0.02
9	7.98 ± 0.23	28 ± 6	14.55 ± 0.06	1.37 ± 0.01
10	7.49 ± 0.25	37 ± 5	14.24 ± 0.03	1.23 ± 0.01
11	6.60 ± 0.15	50 ± 10	12.06 ± 0.03	1.20 ± 0.01
12	6.37 ± 0.08	54 ± 10	11.55 ± 0.03	1.17 ± 0.0
13	4.13 ± 0.13	256 ± 123	6.47 ± 0.03	0.92 ± 0.01
14	8.29 ± 0.13	21 ± 4	17.39 ± 0.09	2.03 ± 0.02
15	4.45 ± 0.02	380 ± 142	7.72 ± 0.09	0.84 ± 0.00
16	7.05 ± 0.09	27 ± 6	13.50 ± 0.06	1.23 ± 0.01

[a] Antioxidant capacity of red wines, measured by ESR spectroscopy, presented as the number of Fremy's radicals reduced by 1 L of wine × 10^{21}. [b] Vasodilation capacity expressed as concentration of wine extract required to give 50% maximal contraction of aortic rings, pIC_{50}. [c,d] Total phenol content of red wine determined by using the Folin−Ciocalteu method (mM gallic acid equivalents GAE) and from HPLC analysis of individual phenolics (mM). All data expressed as mean values ± SE.

RESULTS

Antioxidant Activity. The ability of the 16 wines to reduce the Fremy's salt free radical in the ESR-based antioxidant assay was assessed, and values ranging from 4.13 × 10^{21} to 9.29 × 10^{21} radicals reduced/L were obtained (Table 2). This compares with a range of 6.59 × 10^{21} to 8.55 × 10^{21} radicals/L for 7 red wines studied previously (Gardner et al., 1999). In this chemical model system, Beaujolais (wine 13) and Valpolicella (wine 15) showed the lowest activities, whereas Bulgarian Young Vatted Cabernet Sauvignon (wine 3) and Minervois (wine 14) were ranked first and second, respectively.

Vasodilation Activity. The vasodilation activity of the wines was also assessed, and the pIC_{50} values were determined (Table 2). Although all showed activity, a varying response was observed across the range of wines. The young vatted Bulgarian Cabernet Sauvignon (wine 3) and the Minervois (wine 14) were once again found to be the most active, whereas Beaujolais (wine 13) and Valpolicella (wine 15) exhibited the lowest activity.

Analysis of Individual Phenolic Compounds. To investigate the phenolic content of the red wines in detail, samples were analyzed using a number of HPLC systems custom designed for the different categories of phenolic components. Anthocyanins were measured using a spectrophotometric method. The data obtained were as follows.

Flavonols. The flavonols were analyzed by gradient RP-HPLC with detection at A_{365nm}, as used in previous studies with fruits, vegetables (Crozier et al., 1997), and wines (McDonald et al., 1998). However, in this instance an additional, on-line postcolumn derivatization step was used to provide sensitive and selective detection of flavonols (Aziz et al., 1998). The value of this procedure can be seen by comparing the absorbance and fluorescence traces obtained with an aliquot of an acid-

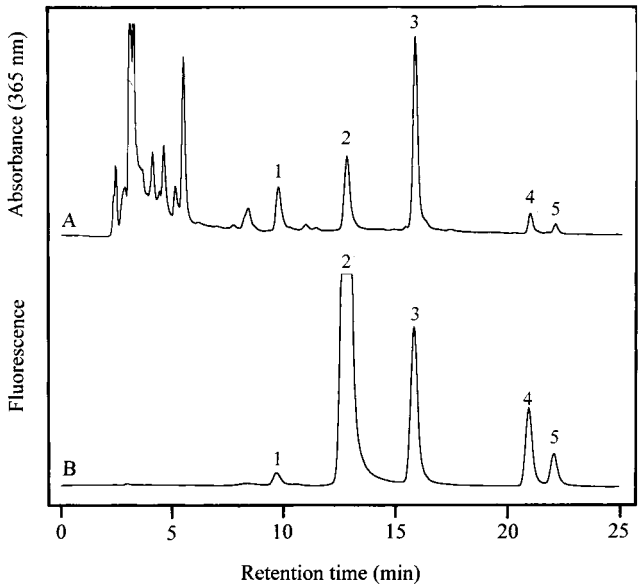


Figure 1. Gradient reversed-phase HPLC analysis of free and conjugated flavonols in wine 14, a 1995 French Minervois: column, 150 × 3.0 mm i.d., 4 μm Genesis C_{18} at 40 °C; mobile phase, 20 min gradient of 20−40% acetonitrile in water adjusted to pH 2.5 with TFA; flow rate, 0.5 mL/min; samples, extract aliquot equivalent to 4.6 μL of wine after acid hydrolysis; detection, (A) absorbance at 365 nm and (B) fluorescence (excitation = 425 nm, emission = 480 nm) after postcolumn derivatization with methanolic aluminum nitrate. Peaks: (1) myricetin, (2) morin (internal standard), (3) quercetin, (4) kaempferol, and (5) isorhamnetin.

hydrolyzed sample of wine 14, the 1995 Minervois, which contains myricetin, quercetin, kaempferol, and isorhamnetin, as well as morin, which was added as an internal standard (Figure 1).

The flavonol contents of the 16 red wines are presented in Table 3. All of the wines contained free and conjugated myricetin, quercetin, kaempferol, and isorhamnetin in various concentrations and with different aglycon/conjugate ratios. As in our previous study with red wines (McDonald et al., 1998), there are >10-fold differences in total flavonol contents, with values ranging from 17.6 μM in the 1992 Bulgarian Cabernet Sauvignon (wine 4) to >187 μM in the Chilean Cabernet Sauvignon (wine 1) and Pinot Noir (wine 5) and Minervois (wine 14). The slightly higher flavonol levels observed in the present study are attributable to the additional detection of isorhamnetin and kaempferol with postcolumn derivatization and fluorescence detection.

Resveratrol. Information on the levels of *cis*- and *trans*-resveratrol and *trans*-resveratrol-*O*-β-glucoside in the wines under study is presented in Table 4. *cis*-Resveratrol-*O*-β-glucoside was not detected in any of the wines analyzed. Typical HPLC traces obtained in the analysis of the stilbenes in wine 14 are illustrated in Figure 2. Most wines contained much higher levels of the aglycons than the conjugate, although high concentrations of the glucoside were present in the French Gevrey Chambertin (wine 7) and Minervois (wine 14). Total resveratrol content ranged >20-fold from 4.3 μM in wine 2, a Californian Cabernet Sauvignon, to 87.9 μM in the conjugate-rich, Pinot Noir-based Gevrey Chambertin (wine 7). Wines 5 and 6, Pinot Noirs from California and Chile, also contained high total levels of resveratrol.

Table 3. Free and Conjugated Flavonol Contents of Red Wines[a]

wine	free M	conj M	total M	free Q	conj Q	total Q	free K	conj K	total K	free I	conj I	total I	total flavonols	% free
1	6.0 ± 0.2	51.0 ± 3.3	55.1 ± 4.3	11.9 ± 0.1	87.8 ± 11.2	95.8 ± 14.3	2.3 ± 0.0	7.3 ± 0.3	8.8 ± 0.7	2.3 ± 0.1	25.8 ± 2.8	27.4 ± 3.5	187.1 ± 19.1	12.0
2	12.5 ± 0.5	16.6 ± 1.6	29.1 ± 1.0	26.8 ± 0.8	41.1 ± 3.3	67.9 ± 2.6	3.8 ± 0.1	1.5 ± 0.5	5.4 ± 0.4	6.1 ± 0.2	7.0 ± 1.8	13.1 ± 1.6	115.5 ± 4.6	42.6
3	8.5 ± 0.1	12.4 ± 0.4	20.9 ± 0.3	3.5 ± 0.1	13.2 ± 0.7	16.7 ± 0.8	1.7 ± 0.0	0.7 ± 0.2	2.3 ± 0.2	0.3 ± 0.0	13.4 ± 0.9	13.6 ± 0.9	53.5 ± 1.7	26.2
4	2.3 ± 0.1	3.5 ± 0.2	5.8 ± 0.3	1.8 ± 0.1	3.2 ± 0.1	5.0 ± 0.1	nd	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	6.3 ± 0.1	6.6 ± 0.1	17.6 ± 0.5	25.0
5	18.1 ± 0.1	13.8 ± 1.5	32.5 ± 0.6	36.6 ± 1.6	51.2 ± 0.2	88.6 ± 2.1	2.7 ± 0.1	2.7 ± 0.2	5.4 ± 0.3	6.8 ± 0.1	54.2 ± 3.6	61.1 ± 3.5	187.6 ± 7.9	34.2
6	8.4 ± 0.4	8.8 ± 1.0	17.2 ± 0.6	17.1 ± 1.4	29.9 ± 2.5	47.1 ± 1.6	1.8 ± 0.0	1.6 ± 0.1	3.4 ± 0.2	2.4 ± 0.1	30.5 ± 0.9	33.0 ± 0.9	100.7 ± 2.4	29.5
7	3.2 ± 0.2	9.1 ± 0.6	12.4 ± 0.4	21.2 ± 0.5	20.0 ± 0.2	41.7 ± 0.1	2.1 ± 0.1	0.3 ± 0.0	2.5 ± 0.0	1.8 ± 0.0	16.8 ± 0.1	18.6 ± 0.1	75.2 ± 0.5	37.6
8	10.7 ± 0.7	22.7 ± 4.8	27.6 ± 0.8	11.0 ± 0.6	28.8 ± 5.5	34.6 ± 0.7	1.7 ± 0.1	1.5 ± 1.0	2.4 ± 0.2	2.8 ± 0.1	12.1 ±2.8	13.6 ± 1.6	78.2 ± 3.9	35.5
9	6.4 ± 1.4	12.8 ± 2.9	19.2 ± 3.1	32.8 ± 3.8	29.2 ± 4.9	62.0 ± 5.1	4.0 ± 0.3	0.9 ± 0.3	4.8 ± 0.1	4.4 ± 0.5	16.9 ± 0.9	21.3 ± 1.1	107.3 ± 9.2	44.4
10	20.7 ± 1.1	18.4 ± 5.0	39.1 ± 5.5	3.5 ± 0.1	20.3 ± 3.7	23.9 ± 3.8	1.8 ± 0.0	1.8 ± 0.6	3.6 ± 0.6	0.3 ± 0.0	17.6 ± 3.1	17.9 ± 3.1	84.5 ± 11.9	31.1
11	17.1 ± 0.4	9.0 ± 1.8	26.1 ± 1.4	15.0 ± 0.1	15.3 ± 1.4	30.2 ± 1.5	0.9 ± 0.0	0.7 ± 0.1	1.6 ± 0.1	3.2 ± 0.0	11.4 ± 0.8	14.6 ± 0.8	72.5 ± 3.7	49.9
12	7.6 ± 0.1	4.5 ± 0.7	12.1 ± 0.7	7.4 ± 0.2	13.3 ± 1.1	20.8 ± 1.0	1.6 ± 0.0	0.6 ± 0.1	2.1 ± 0.1	0.7 ± 0.0	5.0 ± 0.4	5.7 ± 0.4	40.7 ± 1.7	42.5
13	3.4 ± 0.1	4.9 ± 0.4	8.3 ± 0.5	6.8 ± 0.2	9.2 ± 1.2	16.0 ± 1.3	0.2 ± 0.0	0.6 ± 0.1	0.7 ± 0.1	1.2 ± 0.0	8.7 ± 0.9	9.9 ± 1.0	34.9 ± 2.7	33.2
14	20.7 ± 4.4	30.9 ± 8.6	51.7 ± 4.3	41.9 ± 4.6	62.8 ± 14.7	104.7 ± 10.5	4.5 ± 0.2	3.2 ± 1.1	7.6 ± 0.9	7.1 ± 0.6	24.3 ± 6.1	31.4 ± 5.6	195.4 ± 21.2	38.0
15	3.1 ± 0.6	8.8 ± 1.2	12.0 ± 0.6	2.3 ± 0.2	16.6 ± 1.7	18.9 ± 1.5	1.1 ± 0.0	1.0 ± 0.1	2.0 ± 0.2	0.2 ± 0.0	16.0 ± 1.6	16.2 ± 1.6	49.1 ± 3.8	13.6
16	4.9 ± 1.3	17.0 ± 1.4	21.9 ± 0.5	20.9 ± 2.5	36.1 ± 2.4	57.0 ± 1.5	1.6 ± 0.1	0.8 ± 0.1	2.4 ± 0.0	0.5 ± 0.0	7.6 ± 0.2	8.1 ± 0.2	89.4 ± 1.9	31.2

[a] Data are expressed as μM ± SE (n = 3). M, myricetin; Q, quercetin; K, kaempferol; I, isorhamnetin; nd, not detected; % free, free flavonols as % of total; conj, conjugated.

Table 4. Resveratrol and Gallic Acid Content of Red Wines[a]

	wine	year	*trans*-resveratrol	*cis*-resveratrol	*trans*-resveratol glucoside	total resveratrol	gallic acid
1	Chilean Cabernet Sauvignon	1997	2.1 ± 0.3	2.5 ± 0.5	2.8 ± 0.3	7.4 ± 0.6	130.8 ± 4.9
2	Californian oak-aged Cabernet Sauvignon	1995	2.3 ± 0.1	2.0 ± 0.1	nd	4.3 ± 0.1	167.1 ± 0.6
3	Young Vatted Cabernet Sauvignon	1996	29.4 ± 0.6	22.8 ± 0.7	8.3 ± 0.2	60.5 ± 1.1	416.6 ± 4.0
4	Bulgarian Matured Cabernet Sauvignon	1992	27.9 ± 0.3	8.2 ± 0.2	3.1 ± 0.0	39.2 ± 0.5	344.7 ± 0.7
5	Cono Sur Pinot Noir, 20 barrels	1995	39.0 ± 2.2	28.1 ± 0.4	7.8 ± 1.6	74.9 ± 2.9	205.2 ± 2.6
6	Fetzer Santa Barbara Pinot Noir	1994	46.3 ± 2.6	32.7 ± 0.7	nd	79.0 ± 3.1	282.9 ± 1.7
7	Domaine Rossignol Trapet, Gevrey Chambertin	1995	30.4 ± 0.8	27.1 ± 0.4	30.4 ± 0.8	87.9 ± 2.1	300.7 ± 4.1
8	Villa Montes oak-aged Merlot	1994	2.1 ± 0.1	6.6 ± 0.2	nd	8.7 ± 0.1	45.9 ± 1.7
9	Merlot	1996	22.8 ± 2.2	22.1 ± 1.1	7.0 ± 0.4	51.9 ± 2.1	300.0 ± 1.8
10	Cosme Palacio y Hermanos Rioja	1995	15.1 ± 0.2	11.3 ± 0.4	2.9 ± 0.2	29.3 ± 0.4	145.1 ± 0.5
11	Viña Albali Tempranillo	1996	9.1 ± 0.3	6.0 ± 0.1	7.1 ± 0.2	22.2 ± 0.5	225.8 ± 1.4
12	Fetzer Vineyards Zinfandel	1995	5.7 ± 0.0	4.6 ± 0.3	2.6 ± 0.1	12.9 ± 0.4	245.8 ± 1.7
13	Beaujolais	1996	11.0 ± 0.5	9.9 ± 0.1	1.3 ± 0.2	22.2 ± 0.5	89.1 ± 0.4
14	Domaine Roche Vue, Minervois	1995	18.6 ± 0.8	7.1 ± 0.8	18.6 ± 0.8	44.3 ± 0.9	274.4 ± 4.1
15	Valpolicella	1996	9.2 ± 0.9	4.4 ± 0.1	9.2 ± 0.9	22.8 ± 1.9	147.4 ± 2.6
16	Chianti Classico	1995	9.1 ± 0.8	4.7 ± 0.7	9.1 ± 0.8	22.9 ± 1.0	335.5 ± 2.4

[a] Data are expressed as μM ± SE (n = 3). nd, not detected.

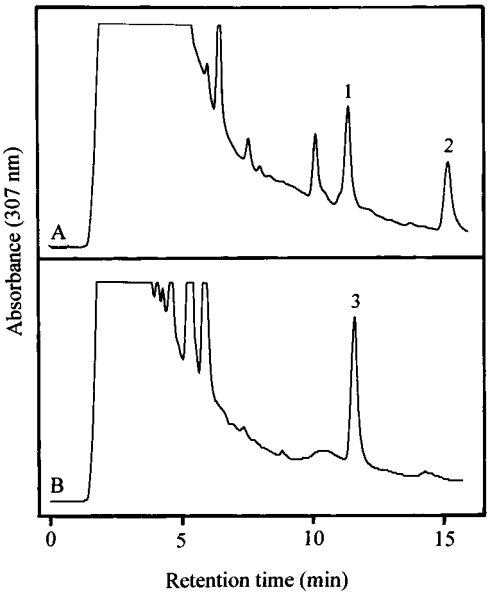


Figure 2. Reversed-phase HPLC analysis of *trans*- and *cis*-resveratrol and *trans*-resveratrol-*O*-β-D-glucoside in 10 μL aliquots of wine 14, a 1995 French Minervois: (A) analysis of *trans*-reseveratrol (1) and *cis*-resveratrol (2) using a 250 × 4.6 mm i.d., 5 μm ODS-Hypersil column eluted with 25% acetonitrile in water adjusted to pH 1.5 with TFA at 1 mL/min and detection at 307 nm; (B) analysis of *trans*-resveratrol-*O*-β-glucoside (3) using a 250 × 4.6 mm i.d., 5 μm ODS-Hypersil column eluted with 17% acetonitrile in water adjusted to pH 1.5 with TFA and detection at 307 nm.

Gallic Acid. A typical trace illustrating HPLC analysis of gallic acid in wine 14 is presented in Figure 3A. The levels of gallic acid varied almost 10-fold from 416.6 μM in wine 3, a 1996 Bulgarian Cabernet Sauvignon, to 45.9 μM in wine 8, a Chilean Merlot (Table 4).

Hydroxycinnamates. Quantitative estimates of caftaric acid, caffeic acid, *p*-coumaric acid, and conjugated *p*-coumaric acid are presented in Table 5, and an HPLC trace illustrating the analysis of the hydroxycinnamates is shown in Figure 3B. Wine 13, a Beaujolais produced by light extraction of Gamay grapes, contained by far the highest concentration of caftaric acid, 331.8 μM. Wine 14, the Minervois, contained the highest levels of both free and conjugated *p*-coumaric acid, 210.6 and 462.2 μM, respectively. As a consequence, the overall concentration of hydroxycinnamates in the Minervois, 903.4 μM, was ~2-fold higher than the levels detected in any of the other wines. With the exception of the Minervois, the total hydroxycinnamate content did not vary greatly in the wines that were investigated (Table 5). Unlike the skin-derived stilbenes and flavonols, the hydroxycinnamates are located primarily in the flesh of the grape and as such are found in comparable levels in both red and white wines.

Catechins. The concentrations of (+)-catechin and (−)-epicatechin are presented in Table 6, and a typical HPLC trace upon which these estimates are based is illustrated in Figure 3C,D. Note that fluorescence detection provided greater sensitivity and selectivity than the more traditional absorbance at 280 nm. Although the method also separates epigallocatechin, epigallocatechin gallate, and epicatechin gallate, only (+)-catechin and (−)-epicatechin were present in the wines in detectable quantities. The highest total catechin concentrations, 645.6 and 637.5 μM, were found in wines 5 and 7, Pinot Noirs from Chile and France, respectively, which also contained high levels of total resveratrol. Wine 10, a Spanish Rioja, contained the lowest amount of total catechins, 172.6 μM, albeit only 3.5-fold less than the highest value in wine 5. (+)-Catechin was invariably present in larger amounts than (−)-epicatechin, with ratios ranging from 2.7 in the Chilean Pinot Noir, wine 5, to 16.0 in wine 14, the Minervois. The catechin levels presented in Table 6 are in keeping with the findings of Goldberg et al. (1998), who also found highest concentrations in wines made from Pinot Noir grapes, which appear to be constitutively higher in (+)-catechin and (−)-epicatechin than other grape varieties.

Anthocyanins. Polymeric anthocyanins were present in all wines in larger amounts than free anthocyanins with ratios varying from 1.4 to 6.1. The highest total anthocyanin concentrations, 325.5 and 308.8 μM malvidin-3-glucoside equivalents, were detected in wine 1, the Chilean Cabernet Sauvignon, and wine 14, the Minervois, respectively. The lowest level, 101.5 μM, was observed in wine 7, the Gevrey Chambertin Pinot Noir (Table 6).

Total Phenolic Content of Red Wines. The total phenolic content of the 16 red wines determined by using the Folin−Ciocalteu colorimetric method are presented in Table 2. There are almost 3-fold differences in the levels present in the different wines, with concentrations ranging from 6.47 to 18.6 mM of gallic acid equivalents (GAE). These figures, corresponding to

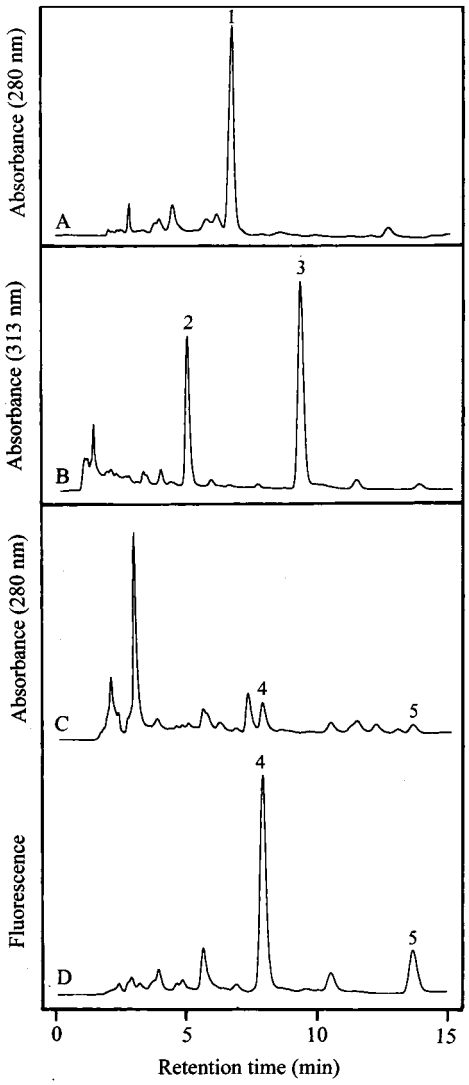


Figure 3. Reversed-phase HPLC analysis of gallic acid, hydroxycinnamates, (+)-catechin, and (−)-epicatechin in wine 14, a 1995 French Minervois: (A) analysis of gallic acid (1) in 5 μL of wine using a 150 × 3.0 mm i.d., 4 μm Genesis C_{18} column eluted at a flow rate of 1.0 mL/min with 2% methanol in water adjusted to pH 1.5 with TFA and detection at 280 nm; (B) analysis of caffeic acid (2) and *p*-coumaric acid (3) after alkaline hydrolysis using a 150 × 3.0 mm C_{18}, 5 μm Nemesis column eluted at a flow rate of 1.0 mL/min with 9% acetonitrile in water adjusted to pH 1.5 with TFA and detection at 313 nm (5 μL injection of hydrolysate is equivalent to 1.67 μL of wine); (C) analysis of (+)-catechin (4) and (−)-epicatechin (5) in 5 μL wine using a 150 × 4.6 mm i.d., 5 μm C_{18} Luna column eluted at a flow rate of 1 mL/min with 10% acetonitrile in water adjusted to pH 1.5 with TFA and detection at 280 nm; (D) same as (C) but with fluorescence detection (excitation = 280 nm, emission = 310 nm).

1100−3165 mg/L GAE, are comparable with values obtained for red wines by other investigators (Frankel et al., 1995; Sato et al., 1996; Ritchey and Waterhouse, 1999). In the current study phenolic-rich wines included the 1996 Young Vatted Bulgarian Cabernet Sauvignon (wine 3), the 1995 Minervois (wine 14), and the 1995 Gevrey Chambertain Pinot Noir (wine 7). Lowest concentrations were detected in wine 13, the 1996 Beaujolais, and the 1996 Valpolicella (wine 15).

A second method was used to assess the total phenolic content of the red wines. This involved combining the figures obtained from the HPLC-based analyses of flavonols, hydroxycinnamates, (+)-catechin, (−)-epicatechin, *cis*-resveratrol, *trans*-resveratrol, *trans*-resveratrol-*O*-β-glucoside, and gallic acid as well as anthocyanin values obtained with the colorimetric assay. The figures based on this method of assessment of the total phenolic content are presented in Table 2. Although showing a similar trend, they are ∼10-fold lower than those obtained with the Folin−Ciocalteu assay. There are two likely reasons for this discrepancy. First, the analysis of the individual components in the wines did not include the condensed tannins, the oligomers and polymers of (+)-catechin and (−)-epicatechin, so their contribution to the total phenolic content of the wines was not determined using the HPLC methods. However, according to Soleas et al. (1997b) and Singleton (1982), these components comprise only ∼20% of the total phenolics in red wines. Second, and probably the main cause of the difference between figures obtained by the two methods, is the fact that the Folin−Ciocalteu method does not provide a specific assay for phenolics as it reacts positively with many easily oxidizable nonphenolic compounds present in red wines and other matrices (Singleton, 1982). In addition, as different phenolics have widely varying reaction stoichiometries, expressing the Folin−Ciocalteu results as GAE may cause an overestimation in the total phenolic content of the wines.

Relationship among Antioxidant Activity, Vasodilation Activity, and Phenolics. The statistical significance of the relationships among antioxidant activity, vasodilation capacity, and the total phenolic contents of the red wines (see Table 2) was analyzed using nonparametric Spearman rank correlation and Minitab software. The ESR-based antioxidant potentials were found to correlate strongly with the Folin−Ciocalteu estimates of total phenol content ($r_S = 0.96$ and $p < 0.001$). The total phenolic content based on HPLC analyses also correlated with the antioxidant potential ($r_S = 0.94$ and $p < 0.001$). The pIC_{50} values for the wines were closely associated with their total HPLC-derived phenolic content ($r_S = -0.811$, $p < 0.001$) and the Folin−Ciocalteu estimate of phenolic content ($r_s = -0.862$, $p < 0.001$). The vasodilation activity of the wines was found to correlate very strongly with their antioxidant activity as determined by the ESR method ($r_S = -0.883$, $p < 0.001$). Figure 4 demonstrates the relationship between the ESR-based antioxidant activity, the vasodilation activity, and the Folin−Ciocalteu- and HPLC-derived phenolic contents of the wines. This close association between the ESR chemical model system and an ex vivo biological assay suggests that the chemical method has biological relevance.

Attempts to correlate statistically the levels of specific phenolics in the red wines (Tables 3−6) with antioxidant activity and vasodilation capacity were less successful. Although correlations with antioxidant activity were detected with gallic acid ($r_S = 0.56$, $p = 0.024$), total resveratrol ($r_S = 0.61$, $p = 0.013$), and total (+)-catechin and (−)-epicatechin ($r_S = 0.60$, $p = 0.014$), the other individual correlations were lower (total hydroxycinnamates, $r_S = 0.26$, $p = 0.341$; total flavonols, $r_S = 0.45$, $p = 0.08$; total anthocyanins, $r_S = 0.35$, $p = 0.182$). Likewise, although the pIC_{50} values of the wines were closely correlated with their total HPLC-derived phenolic contents ($r_S = -0.811$, $p < 0.001$), this association was not evident with individual phenolics with the sole exception of total anthocyanins ($r_S = -0.52$, $p = 0.038$).

Table 5. Hydroxycinnamate Content of Red Wines[a]

	wine	year	caftaric acid	free caffeic acid	free *p*-coumaric acid	conj *p*-coumaric acid	total hydroxy-cinnamates
1	Chilean Cabernet Sauvignon	1997	111.2 ± 0.7	23.0 ± 0.7	23.9 ± 2.3	25.5 ± 0.8	183.6 ± 4.3
2	Californian oak-aged Cabernet Sauvignon	1995	42.9 ± 1.7	32.3 ± 0.6	131.9 ±0.6	35.9 ± 0.7	243.0 ± 2.5
3	Young Vatted Cabernet Sauvignon	1996	95.7 ± 0.5	13.3 ± 0.4	115.6 ± 0.4	14.8 ± 0.4	239.4 ± 9.6
4	Bulgarian matured Cabernet Sauvignon	1992	82.9 ± 1.6	15.3 ± 0.7	71.6 ± 0.6	17.0 ± 0.8	186.8 ± 0.8
5	Cono Sur Pinot Noir, 20 barrels	1995	188.0 ± 0.5	50.6 ± 0.3	32.7 ± 4.7	56.2 ± 0.3	327.5 ± 4.3
6	Fetzer Santa Barbara Pinot Noir	1994	49.5 ± 2.7	94.2 ± 0.2	33.9 ± 0.3	131.6 ± 1.8	309.2 ± 2.2
7	Domaine Rossignol Trapet, Gevrey Chambertin	1995	89.8 ± 1.9	106.4 ± 7.0	31.7 ± 1.2	25.7 ± 1.4	253.6 ± 7.7
8	Villa Montes oak-aged Merlot	1994	17.4 ± 0.4	74.4 ± 0.5	14.7 ± 0.2	197.6 ± 0.7	304.1 ± 1.1
9	Merlot	1996	110.0 ± 0.4	24.1 ± 0.1	16.1 ± 0.5	188.2 ± 0.5	338.4 ± 1.2
10	Cosme Palacio y Hermanos Rioja	1995	128.5 ± 0.9	47.6 ± 0.8	23.7 ± 0.2	319.2 ± 1.0	519.0 ± 1.9
11	Viña Albali Tempranillo	1996	117.9 ± 0.4	6.6 ± 0.2	33.9 ± 0.3	131.6 ± 1.8	290.0 ± 2.2
12	Fetzer Vineyards Zinfandel	1995	94.4 ± 1.2	101.0 ± 0.3	54.6 ± 0.2	201.5 ± 1.6	451.5 ± 3.0
13	Beaujolais	1996	331.8 ± 9.8	28.9 ± 0.4	26.4 ± 0.2	32.1 ± 0.4	419.2 ± 9.3
14	Domaine Roche Vue, Minervois	1995	189.7 ± 2.2	40.9 ± 0.3	210.6 ± 0.3	462.2 ± 1.8	903.4 ± 5.5
15	Valpolicella	1996	110.5 ± 0.1	11.1 ± 0.2	74.7 ± 0.7	52.8 ± 1.9	249.1 ± 1.6
16	Chianti Classico	1995	121.8 ± 0.9	30.6 ± 0.3	21.8 ± 0.8	142.0 ± 0.8	316.2 ± 1.1

[a] Data are expressed as μM ± SE ($n = 3$). Caftaric acid quantified as caffeic acid equivalents. Conj, conjugated.

Table 6. Flavan 3-ol and Anthocyanin Contents of Red Wines[a]

	wine	year	(+)-catechin	(−)-epi-catechin	total catechins	(+)-cat./(−)-epi ratio	free antho-cyanins	polymeric pigments	total antho-cyanins
1	Chilean Cabernet Sauvignon	1997	239.8 ± 16.4	23.8 ± 0.7	263.6 ± 16.3	10.1	72.0	253.7	325.7
2	Californian oak-aged Cabernet Sauvignon	1995	270.3 ± 16.6	47.4 ± 2.0	317.7 ± 15.6	5.7	75.0	135.3	210.3
3	Young Vatted Cabernet Sauvignon	1996	468.1 ± 7.8	130.5 ± 2.0	598.6 ± 8.3	3.6	82.7	202.3	285.0
4	Bulgarian matured Cabernet Sauvignon	1992	188.9 ± 0.6	59.4 ± 3.0	248.3 ± 3.7	3.2	15.5	94.7	110.2
5	Cono Sur Pinot Noir, 20 barrels	1995	472.6 ± 4.5	173.0 ± 5.5	645.6 ± 10.1	2.7	72.4	110.6	183.0
6	Fetzer Santa Barbara Pinot Noir	1994	233.2 ± 1.3	91.5 ± 0.8	324.7 ± 0.6	2.5	25.3	83.9	109.2
7	Domaine Rossignol Trapet, Gevrey Chambertin	1995	490.5 ± 8.5	147.0 ± 8.1	637.5 ± 16.5	3.3	26.1	75.4	101.5
8	Villa Montes oak-aged Merlot	1994	186.6 ± 7.0	36.6 ± 1.3	223.2 ± 7.6	5.1	80.2	154.2	234.4
9	Merlot	1996	296.8 ± 1.2	83.8 ± 0.9	380.6 ± 2.1	3.5	56.1	135.0	191.1
10	Cosme Palacio y Hermanos Rioja	1995	151.0 ± 2.0	21.6 ± 1.0	172.6 ± 1.3	7.0	112.7	163.3	276.0
11	Viña Albali Tempranillo	1996	299.3 ± 4.6	69.1 ± 2.2	368.4 ± 5.8	4.3	90.9	135.3	226.2
12	Fetzer Vineyards Zinfandel	1995	202.7 ± 0.9	53.5 ± 1.2	256.2 ± 1.4	3.8	41.2	121.4	162.6
13	Beaujolais	1996	193.8 ± 1.9	30.6 ± 1.1	224.4 ± 3.0	6.5	49.3	84.9	134.2
14	Domaine Roche Vue, Minervois	1995	284.5 ± 1.1	17.8 ± 1.5	302.3 ± 2.6	16.0	87.3	221.2	308.5
15	Valpolicella	1996	198.6 ± 2.2	48.9 ± 1.1	247.5 ± 3.3	4.1	52.7	74.5	127.2
16	Chianti Classico	1995	242.2 ± 2.3	58.4 ± 3.8	300.6 ± 6.0	4.1	46.3	120.8	167.1

[a] Data for (+)-catechin and (−)-epicatechin expressed as μM ± SE ($n = 3$). (+)-cat./(−)-epi ratio, ratio of (+)-catechin to (−)-epicatechin. Anthocyanin data expressed as μM malvidin 3-glucoside equivalents.

DISCUSSION

The varying capacities of the 16 red wines to act both as ex vivo vasodilators and as in vitro antioxidants appear to be associated with the phenolic content of the wines, whether determined by the Folin−Ciocalteu assay or by the summation of the levels of individual phenolics analyzed primarily by HPLC. These relationships become more evident in Figure 5 in which the vasodilation pIC$_{50}$ figures are plotted as inverse values $\times 10^3$ and the wines are ranked to visualize the concomitant reductions in vasodilation and antioxidant activity that are paralleled by decreasing phenolic content. Although wines all showed activity, there is a large spread in the antioxidant capacity of the individual wines and likewise in their ability to relax precontracted aorta. The phenolic-rich Bulgarian Young Vatted Cabernet Sauvignon (wines 3) has high antioxidant and vasodilation capacities, whereas at the other end of the scale the low phenolic content of the Beaujolais (wine 13) is characterized by markedly lower vasodilation and antioxidant activities. The Bulgarian Young Vatted Cabernet Sauvignon (wine 3) underwent extensive skin extraction using a rotary extractor during vinification, facilitating the release of more phenolics than would have otherwise been possible using more traditional methods. In contrast, the Beaujolais (wine 13) was produced conventionally from thin-skinned Gamy grapes that undergo carbonic maceration and are, therefore, only lightly extracted.

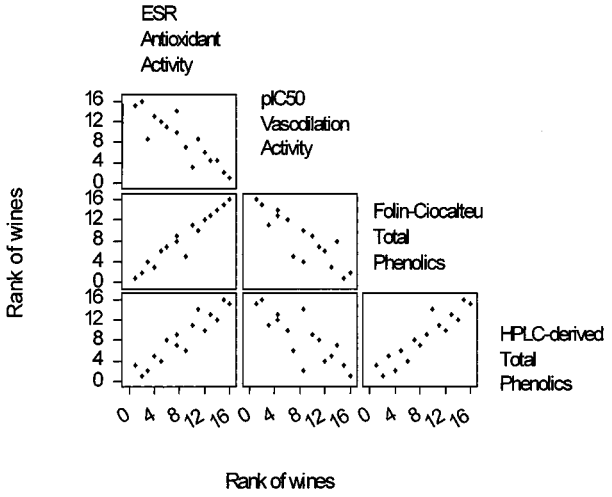


Figure 4. Matrix plot derived from Spearman rank correlations, highlighting the relationships between ESR-based antioxidant activity, vasodilation activity, and total phenolics. Antioxidant activity was determined as the number of Fremy's radicals reduced per liter of wine $\times 10^{21}$; vasodilation activity was expressed as the concentration in μg/mL at which each extract caused 50% maximal vasodilation, pIC$_{50}$; total phenolic content was determined by Folin−Ciocalteu colorimetric assay and HPLC.

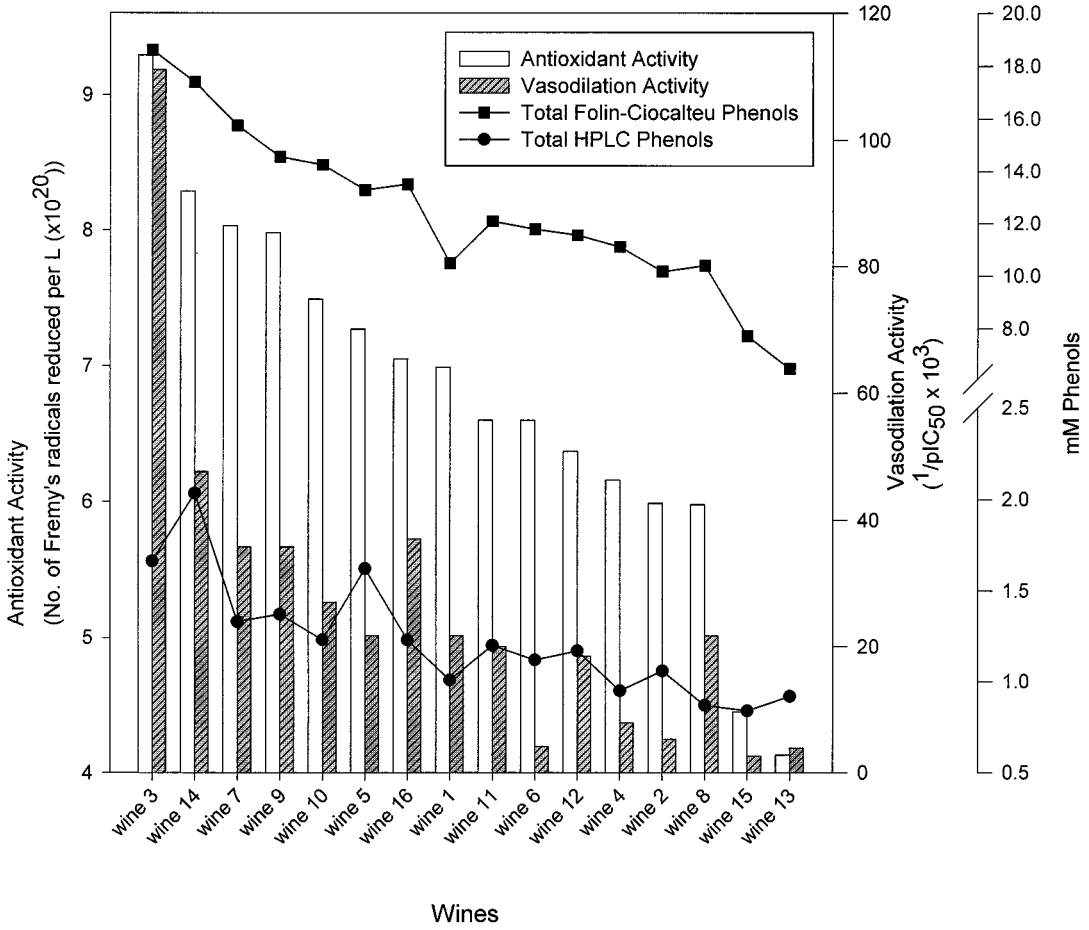


Figure 5. Relationship between antioxidant activity, vasodilation activity, and phenolic content of red wines. Antioxidant activity was determined as the number of Fremy's radicals reduced per liter of wine $\times 10^{21}$; vasodilation activity was expressed as $1/p\mathrm{IC}_{50} \times 10^{21}$, where $p\mathrm{IC}_{50}$ is the concentration in μg/mL of wine extract at which there is 50% maximal contraction of aortic rings. Total phenolic content was determined by two methods, the Folin−Ciocalteu colorimetric assay [results expressed as mM gallic acid equivalents (GAE)] and HPLC (results expressed as mM of individual phenolics as presented in Tables 2−6). All data are expressed as mean values ± SE.

A number of studies have previously investigated the vasodilation activity of a range of plant extracts, including red wine, grape juice, and grape skin extract (Fitzpatrick et al., 1993, 1995; Andriambeloson et al., 1998). There is clear evidence that the most active compounds are skin-derived, supported by the low vasodilation activity of white wine as opposed to red wine, for which there is extensive grape skin extraction. Recent studies have attempted to identify the vasoactive agent. Investigations using the flavonols apigenin, isorhamnetin, kaempferol, luteolin, myricetin, quercetin, and rutin showed that apart from rutin they all induced significant relaxation of rat pulmonary arteries (MacLean et al., 1997). In contrast, Andriambeloson et al. (1998) found that only the anthocyanin and oligomeric condensed tannin containing fractions of red wine showed the same activity as the original red wine polyphenolic fraction. The present study found that although there is a strong correlation between vasodilation activity and total phenolics quantified either by the Folin−Ciocalteu assay or by HPLC, the only correlation with a phenolic family was with the total anthocyanins.

Although a substantial amount of work has been carried out into the action of phenolics using in vitro systems, until recently there has been a dearth of information on their absorption. However, there is now a significant body of evidence supporting the theory that the flavonol conjugates are preferentially absorbed and that the nature of the conjugation may be important (Hollman et al., 1995). In studies on the absorption and excretion of flavonols after onion consumption, it has been shown that quercetin-4′-glucoside and isorhamnetin-4′-glucoside accumulate in human plasma and are excreted in urine to a much greater extent than their aglycons (Aziz et al., 1998; Aziz, 2000). Conjugated quercetin can be similarly detected in human plasma after the consumption of red wine (Crozier et al., 1999). A recent study has demonstrated the presence of catechin metabolites in human plasma after red wine consumption. Although free (+)-catechin and its metabolite, 3′-*O*-methylcatechin, were detected, their levels were low compared to those of (+)-catechin sulfate and/or glucuronide conjugates (Donovan et al., 1999). Anthocyanins also appear to be absorbed as anthocyanin-like compounds have been detected in human urine after consumption of red wine (Lapidot et al., 1998). In addition to flavonoids, the bioavailability of other wine phenolics is now coming under investigation, and it has been demonstrated with a model rat system that resveratrol accumulates in plasma after ingestion of the stilbene (Bertelli et al., 1996; Juan et al., 1999). As with other dietary compounds, extensive postconsumption metabolism of phenolics is possible, and it may be, in some instances, that metabolites rather than the parent

compound are responsible for the advantageous effects of red wine.

It seems likely that the beneficial effects of red wine are due to the cumulative effects of several phenolics rather than one individual compound. Identification of the bioavailable antioxidant and/or vasodilatant agents will open up the possibility of identifying wines that are rich in these components and, which, as a consequence, may provide enhanced protection against CHD. This could also be achieved by making wines from grapes that have been genetically modified to produce high levels of the active phenolics in both the flesh and the skin.

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