Phenolics: A Comparison of Diverse Analytical Methods

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Abstract: A variety of phenolic assays were used to evaluate the phenolic content of Californian red and white wines. Conventional methods (Folin-Ciocalteu, RP-HPLC, NP-HPLC, and dimethylaminocinnamaldehyde [DAC] assays) and relatively new methods (tannin and polymeric pigment assay, cyclic voltammetry, and antioxidant assay) were compared. The total phenol contents measured using the Folin-Ciocalteu, RP-HPLC, and cyclic voltammetry methods were strongly correlated ($p < 0.001$, $r > 0.90$). Considering assays measuring the monomeric phenols, the flavan-3-ol content measured using the DAC, RP-HPLC, and NP-HPLC methods were correlated ($p < 0.001$). Anthocyanin content (RP-HPLC) and colored monomer content (NP-HPLC) were also correlated ($r = 0.99$, $p < 0.001$). Tannin and polymeric pigment assays were correlated ($r > 0.9$, $p < 0.001$) to the high molecular-weight polymer (HMWP) and total polymer content using the NP-HPLC method, but not to the LMWP. The small polymeric pigment (SPP) and large polymeric pigment (LPP) measured using the tannin and polymeric pigment assay were both correlated ($r = 0.79$, $p < 0.001$) to the high molecular-weight colored polymers (HMWCP) (NP-HPLC), but neither with the LMWCP. As the sum of SPP and LPP correlated even better with HMWCP ($r = 0.89$, $p < 0.001$), it seems that both these are included in the HMWCP measure. The antioxidant activity of wines correlated ($p < 0.001$) with the total phenol content (using all assays, $r > 0.90$). The identity of the substances quantified in the pigmented tannin assays need clarification.

Key words: antioxidant activity, cyclic voltammetry, Folin-Ciocalteu assay, HPLC, monomeric phenols, polymeric phenols, tannin

Phenolic compounds have an important impact on the organoleptic properties of wines, including color and astringency. They are active antioxidants and are associated with possible health benefits (Kinsella et al. 1993).

Many assays can be used to study the phenolic content and composition of wines. The most common is the total phenol assay, using the Folin-Ciocalteu reagent (Singleton et al. 1999). This assay estimates the content of total phenols by reaction of the hydroxyl groups with a phosphomolybdate reagent. Other spectrophotometric assays that measure total phenol content include permanganate titration, reaction with iron salts, and ultraviolet absorbance.

Identification and quantification of monomeric phenolic compounds are generally achieved using reversed-phase high-performance liquid chromatography (RP-HPLC). The basic method involves separating compounds by polarity with gradients from an aqueous solvent to an organic solvent. Acids are normally used to achieve a low pH. Both reversed-phase silica (C_{18}) (Lamuela-Raventos and Waterhouse 1994) and polystyrene divinylbenzene (Price et al. 1995, Peng et al. 2002) stationary phases can be used.

Assays measuring flavan-3-ols mostly involve reaction with aldehydic reagents such as vanillin or 4-dimethylaminocinnamaldehyde (DAC) in an acidic medium (McMurrough and McDowell 1978). Since the DAC reagent reacts with the aromatic ring on all free *meta*-hydroxyl groups in the A-ring, proanthocyanidins are also included in this measure. Electron-withdrawing functional groups on anthocyanins and flavonols exclude them from this reaction.

Oligomers and polymers present their own challenges because of the high number of different but closely related substances present. Proanthocyanidins can be measured by reaction with the Bate-Smith reagent by cleavage of proanthocyanidin subunits to produce cyanidin (Porter et al. 1986). Protein precipitation of tannins can also be used to quantify the tannin content of wines (Hagerman and Butler 1978). Recently this method was adapted to include measurement of small and large polymeric pigments (SPP and LPP) using bisulfite bleaching (Harbertson et al. 2002).

Normal-phase HPLC (NP-HPLC) methods have also been developed to quantify the polymeric phenolics of wine (Hammerstone et al. 1999). A cacao standard is used and proanthocyanidins up to heptamers can be separated. A method developed by Kennedy and Waterhouse (2000) divides the polymers into low and high molecular-weight polymers (LMWP and HMWP). By monitoring absorbance at 520 nm, the polymeric pigments can also be divided into low and high molecular-weight colored polymers (LMWCP and HMWCP).

Recently, cyclic voltammetry (CV) analysis has been used to measure the redox potential of wine phenolic compounds (Kilmartin et al. 2001, 2002). This relatively new

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technique in wine analysis shows potential to elucidate relationships between the content of specific phenolic compounds and their antioxidant activity (Kilmartin 2001).

The antioxidant activity of wine has been measured using a wide range of assays including free radical scavenging (Pellegrini et al. 2000, De Beer et al. 2003) and lipid peroxidation (Roginsky and Barsukova 2001). Different test systems can measure different aspects of antioxidant activity; systems using a lipid and oxidation initiator occurring in the human body are considered the most biologically relevant (Frankel et al. 1995), but most assays are closely correlated with each other.

The aim of this study was to investigate the phenolic content of a variety of Californian red and white wines using different phenolic assays and to determine relationships between the outcomes. Established methods (Folin-Ciocalteu, RP-HPLC, NP-HPLC, and DAC) were compared to new methods (tannin and polymeric pigment, cyclic voltammetry, and an antioxidant assay).

Materials and Methods

Wines. Red and white wines were selected to represent a broad range of varieties and vintages. Twenty-one red and five white Californian wines were chosen from commercial as well as research wineries (Table 1). Research wines (small-scale fermentation) are usually not aged in oak or fined with protein additions, while commercial wines usually receive oak treatment and could be fined to reduce tannin.

RP-HPLC analysis. Monomeric phenolic compounds were determined in duplicate using the RP-HPLC method of Donovan et al. (1998). A Hewlett-Packard/Agilent model 1100 HPLC (Palo Alto, CA) with a diode array UV-visible detector coupled to HPChemStation software was used. The column was a LiChroSpher C18 (4 x 250 mm, 5-mm particle size) (Merck, Darmstadt, Germany). All wine samples and standards were filtered through 0.45-µm PTFE syringe-tip filters (Gelman Sciences, Ann Arbor, MI) before use. The following standards were used: gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid, and rutin (Sigma, St. Louis, MO) and malvidin-3-glucoside (Mv-3-glc) (Extrasynthese, Genay, France). Compounds were identified by group on the basis of their UV spectra and reported in terms of the related standard compound. These were benzoic acids, hydroxycinnamates, flavan-3-ols, flavonols, and anthocyanins expressed as mg gallic acid equivalents/L (mg GAE/L), mg caffeic acid equivalents/L (mg CAE/L), mg catechin equivalents (mg CE/L), mg rutin equivalents/L (mg RE/L), and mg Mv-3-glc equivalents/L (mg ME/L), respectively. The total area under the chromatograms at 280 nm were integrated and used to calculate the total phenol content expressed as mg GAE/L, probably lower than actual because of the high absorbance of gallic acid.

NP-HPLC analysis. Determination of proanthocyanidins and colored proanthocyanidins was done in duplicate according to the modification of Zimman et al. (2002) to the method of Kennedy and Waterhouse (2000). A Hewlett-Packard model 1090 HPLC (Palo Alto, CA) with diode array UV-visible detector was used. The column was a LiChroSpher Si-60 (4 x 250 mm, 5-mm particle size) (Merck). All wine samples and standards were filtered through 0.45-µm PTFE syringe-tip filters before use. Proanthocyanidins, detected at 280 nm, were divided into three groups: monomers, low molecular-weight proanthocyanidins (LMWP), and high molecular-weight proanthocyanidins (HMWP), based on the retention time of the dimer and tetramer from a cacao bean standard (Rigaud et al. 1993). Concentrations of grouped peaks were calculated as mg epicatechin equivalents/L (mg ECE/L) from (-)-epicatechin as external standard. Colored proanthocyanidins, detected at 520 nm, were quantified using a Mv-3-glc standard (mg ME/L) and peaks were divided as for proanthocyanidins into colored monomers, low molecular-weight colored proanthocyanidins (LMWCP), and high molecular-weight colored proanthocyanidins (HMWCP).

Tannin and polymeric pigment assay. The protein precipitation method of Hagerman and Butler (1978) as modified by Harbertson et al. (2002) was used to determine the tannin content of wines. This method was used in conjunction with bisulfite bleaching to determine the amount of polymeric pigments in the wines as described by Harbertson et al. (2003).

When necessary, wines were diluted with a buffer of 12% aqueous ethanol (v/v) containing 5 g/L potassium bitartrate adjusted to pH 3.3 with HCl. Tannin and polymeric pigment precipitation was carried out using bovine serum albumin (BSA, fraction V powder) (1 mg/mL) in a buffer containing 200 mM acetic acid and 170 mM NaCl adjusted to pH 4.9 using NaOH.

Parallel analysis of polymeric pigments and tannin for each sample was conducted using two 1.5-mL microfuge tubes. The first tube contained 1 mL of the acetic acid/NaCl buffer (without BSA) and 500 µL of the diluted wine. One mL of the mixture was transferred to a cuvette and then 80-µL of 0.36 M potassium metabisulfite was added. After mixing and 10-min incubation the absorbance at 520 nm was determined. This absorbance represents the sum of large and small polymeric pigment in the original wine.

The second tube contained 1 mL of the acetic acid/NaCl buffer with BSA (1 mg/mL) and 500 µL of diluted wine. The mixture was allowed to stand at room temperature for 15 min and then centrifuged for 5 min at 13,500 *g* to pellet the tannin-protein precipitate. One mL of the supernatant was transferred to a cuvette, then 80 µL of 0.36 M potassium metabisulfite was added, and after 10 min the absorbance was determined at 520 nm. This absorbance represents the amount of polymeric pigment that did not precipitate with protein (small polymeric pigment, SPP), and the amount of large polymeric pigment (LPP) was calculated by subtracting the SPP value from the sum described above.

To determine the amount of tannin in the wine, the tannin-protein pellet from tube two was washed with 250 µL of

Table 1 Description of red and white wines investigated.

Sample code	Variety[a]	Vintage	Type[b]	Winery	Appellation/area
Red					
1R	Zinfandel	1989	C	Pedroncelli	Dry Creek Valley, CA
2R	Zinfandel	1994	R	UCDavis	Napa Valley, CA
3R	Barbera	1999	C	Boeger	El Dorado, CA
4R	Pinot noir	1984	R	UCDavis	Oakville, CA
5R	Merlot	1999	C	Beringer	Napa Valley, CA
6R	Carmine	1986	R	UCDavis	Davis, CA
7R	Merlot	1999	C	J. Lohr	Paso Robles, CA
8R	Cab Sauv	1999	R	UCDavis	Oakville, CA
9R	Zinfandel	1999	C	Sierra Vista	El Dorado, CA
10R	Cab Sauv	1998	R	UCDavis	Oakville, CA
11R	Pinot noir	1999	C	Greenwood Ridge	Anderson Valley, CA
12R	Grenache	2001	R	UCDavis	Davis, CA
13R	Barbera	2001	R	UCDavis	Davis, CA
14R	Pinot noir	2000	C	Sanford	Santa Barbara County, CA
15R	Cab Sauv	1977	R	UCDavis	Oakville, CA
16R	Cab Sauv	1999	R	UCDavis	Napa Valley, CA
17R	Pinot noir	1998	C	Fiddlehead	Willamette Valley, OR
18R	Pinot noir	1992	R	UCDavis	Oakville, CA
19R	Zinfandel	1999	C	Fetzer	Mendocino County, CA
20R	Cab Sauv	1984	R	UCDavis	Oakville, CA
21R	Cab Sauv	1973	R	UCDavis	Oakville, CA
White					
1W	Chardonnay	1998	R	R.H. Phillips	Dunnigan Hills, CA
2W	Viognier	2000	C	Sobon Estate	Shenandoah Valley, CA
3W	Chardonnay	2001	R	UCDavis	Davis, CA
4W	Sauv blanc[c]	2000	R	UCDavis	Davis, CA
5W	Sauv blanc	2001	C	Robert Mondavi	Central Coast, CA

[a]Cab Sauv: Cabernet Sauvignon; Sauv blanc: Sauvignon blanc.
[b]C: commercial wine, R: research wine.

the acetic acid/NaCl buffer and then recentrifuged for 1 min at 13,500 g. The wash solution was discarded, then 875 µL of a buffer containing 5% triethanolamine (v/v) and 5% sodium dodecyl sulfate (w/v) was added, and the tube was allowed to stand at room temperature for 10 min. After incubation the tube was vortexed to completely dissolve the tannin-protein precipitate. The solution was allowed to stand at room temperature for 10 min, and then the absorbance (background) at 510 nm was determined. Then 125 µL 10 mM ferric chloride in 10 mM HCl was added and the final absorbance determined after 10-min incubation. The amount of protein precipitable tannin in the sample was calculated as the final absorbance minus the background, and expressed in catechin equivalents by comparison with a standard curve prepared with catechin.

Spectrophotometric analyses. Total phenol content was determined in duplicate using the Folin-Ciocalteu reagent (Singleton et al. 1999). Gallic acid was used as standard and results are expressed as mg GAE/L. The flavan-3-ol content (mostly monomers) of each wine was measured in duplicate at 640 nm after reaction with the 4-dimethylaminocinnamaldehyde (DAC) reagent in a MeOH/HCl solution (75:25 v/v) (McMurrough and McDowell 1978). (+)-Catechin was used as a standard and the results expressed as mg CE/L.

Cyclic voltammetry analysis. A model wine solution (12% ethanol [v/v], 0.033 M tartaric acid adjusted to pH 3.6 with NaOH) was used to make up (+)-catechin standards and to dilute white (10 times) and red (400 times) wines as described previously (Kilmartin et al. 2001). At least three repeat cyclic voltammograms were recorded at a scan rate of 100 mVs^{-1}, using a Bioanalytical Systems (West Lafayette, IN) 100A electroanalyzer and a 3-mm glassy carbon electrode (model M-2012; Bioanalytical Systems), which was freshly abraded before each run on 3-mm alumina powder (PK-4 polishing kit, Bioanalytical Systems). The potentials were recorded against an Ag/AgCl reference electrode (+207 mV versus *she*), and the current was measured relative to a model wine solution blank. The total phenol content for wines was calculated from integrating the area under the peak to 500 mV (Q_{500}), in comparison with the response of catechin standards at 0.01, 0.02, and 0.05 mM (Kilmartin et al. 2002).

Total chain-breaking antioxidant capacity. The determination of the chain-breaking antioxidant capacity is based on the capability of wine to inhibit the chain oxidation of methyl linoleate (ML) (Sigma) in micelles under controlled conditions. The protocol for determining antioxidant capacity measured as a number of kinetic chains that can be terminated by a beverage sample, N, expressed in concentration units has been reported in detail (Roginsky and Barsukova 2001, Roginsky 2001). In brief, the testing system was 5 to 10 mM ML, oxidized at 37.0 ± 0.1°C in a micellar solution of 50 mM Triton X-100 (Aldrich, Stenheim, Germany) in a phosphate buffer, pH 7.40 ± 0.02, with 2 to 4 mM 2,2'-azobis(2-amidinopropan) dihydrochloride (AAPH) (Polysciences, Warrington, PA) as an initiator. The kinetics of oxygen consumption during the oxidation of ML was studied using a computerized 5300 Oxygen Biological Monitor (Yellow Springs Instruments, Yellow Springs, OH. The procedure was started with the determination of the rate of free radical generation (initiation), R_{IN}, and the rate of noninhibited chain oxidation, R_0. The second stage was the measurement of N proper. Before each stage, the system was bubbled with pure oxygen for several minutes. R_{IN} was determined by the inhibitor method by using 6-hydroxy-2,2,5,7,8-pentamethylbenzochroman (HPMC) (Sigma) as a reference antioxidant. Then the response to the addition of 5 to 10 µL of wine stock solution (equivalent to 0.4 to 2 µL original wine) in model wine (12% ethanol) was monitored. N was calculated from the induction period, t_{IND}, caused by wine addition using the equation:

$$N = \frac{R_{IN} t_{IND}}{v/V} \qquad (1)$$

where v and V are the volume of the added wine sample and that of the testing system (3.6 mL), respectively.

In turn, t_{IND} was determined by the integration of the $[O_2]$ trace as using the following equation which has been reported previously (Loshadkin et al. 2002):

$$t_{IND} = \int_0^{\infty} \left\{ 1 - (R/R_0)^2 \right\} dt \qquad (2)$$

where R is the rate of the inhibited chain oxidation.

Statistical analysis. Statistical analysis was performed using Statistica data analysis software (version 6; Statsoft, Tulsa, OK). Pearson product moment correlation coefficients were calculated to determine significant correlations between parameters. Significance of differences between means was accepted at a 95% confidence level ($p < 0.05$).

Results and Discussion

RP-HPLC analysis. (-)-Epicatechin could not successfully be quantified in red wine samples because of coelution with anthocyanins. The absorption of coeluting anthocyanins at 280 nm makes quantification of (-)-epicatechin peaks inaccurate. (-)-Epicatechin could not be detected in any of the white wines. Only two red wines, 6R and 21R, did not contain (+)-catechin, while this compound was detected in only one white wine, 3W, at a level of 8.9 mg/L. The (+)-catechin concentration in red wines was between 5.4 and 50.9 mg/L (Table 2).

Benzoic acid peaks were identified by their spectra, which was similar to that of gallic acid with one or two absorption bands between 200 and 300 nm. Benzoic acid peak areas were added together and quantified at 280 nm using gallic acid. The total benzoic acid content of red and white wines ranged from 30.8 to 116.8 and from 10.1 to 43.7 mg GAE/L, respectively (Table 2).

Cinnamic acids and hydroxycinnamates were identified by their peaks at 320 nm, which gave spectra with a double band between 290 and 330 nm. All hydroxycinnamate peak areas were added together and quantified at 320 nm using caffeic acid. In red and white wines, the total hydroxycinnamate content ranged from 12.5 to 105.6 and from 8.4 to 37.3 mg CAE/L, respectively (Table 2).

Anthocyanin content differed greatly among the red wines (3.5 to 427.4 mg/L) (Table 2). All peaks giving characteristic spectra with a narrow band at 270 nm, a broad band at 400 to 600 nm, and a maximum absorbance around 520 nm were tabulated as anthocyanins. Peak areas were summed and quantified as anthocyanins expressed in mg ME/L. Cultivar and age of wines were the factors influencing differences in anthocyanin content. Older wines generally had lower anthocyanin concentration than younger wines. For example, two Cabernet Sauvignon wines of the 1977 and 1973 vintage (15R and 21R) contained 16.2 and 13.6 mg ME/L, while a Barbera wine of the 2001 vintage (13R) contained 427.4 mg ME/L. There was a significant negative correlation between wine vintage and the total anthocyanin ($r = -0.52$, $p < 0.05$) content.

Flavonol peaks giving characteristic spectra with a band between 330 and 400 nm were identified and quantified as mg RE/L from the peak areas at 360 nm. Only two (1W and 2W) of the five white wines contained a measurable amount of flavonols, with total flavonol contents of 4.6 and 5.0 mg RE/L, respectively (Table 2). The total flavonol content of red wines was between 17.2 and 234.8 mg RE/L.

Tannin and polymeric pigment assay. The tannin assay was developed to provide a rapid and easy measurement of the protein-precipitable tannin content of red wines (Harbertson et al. 2002). The assay has recently been extended to include a measurement of the small and large polymeric pigments (SPP and LPP) in red wines based on bisulfite bleaching of pigments before and after protein precipitation of tannin (Harbertson et al. 2003). Thus, the assay is a measure of chemical astringency, detecting only those phenolics that bind to protein, and of the oligomeric proanthocyanindin series that is limited to those with a degree of polymerization greater than four units.

White wines had no measurable amount of tannin when assayed, while red wines contained between 22.6 and 829.0

Table 2 Monomeric phenolic compound contents in red and white wines as determined by RP-HPLC.

Wine[a]	Benzoic acids[b] (mg GAE/L)	Flavan-3-ols[c] (mg/L)	Hydroxycinnamates[d] (mg CAE/L)	Anthocyanins[e] (mg ME/L)	Flavonols[f] (mg RE/L)
Red					
1R	102.1[g]	13.3[h]	83.3[h]	3.5[h]	35.7[h]
2R	38.3	6.0	61.7	36.0	17.2
3R	51.7	18.7	104.3	170.3	122.7
4R	57.8	11.2	76.2	15.6	59.2
5R	81.3	20.3	48.4	114.1	201.7
6R	45.2	nd[i]	40.6	31.5	59.4
7R	116.8	27.2	39.8	96.8	111.1
8R	41.9	5.5	34.4	64.5	89.3
9R	54.3	23.8	90.0	88.0	63.0
10R	30.8	5.4	31.1	41.9	47.6
11R	89.0	50.9	60.0	141.1	42.3
12R	43.1	17.2	105.6	58.9	45.6
13R	49.7	17.6	70.1	427.4	234.8
14R	57.2	25.0	66.3	113.5	118.3
15R	43.4	10.5	28.3	16.2	44.3
16R	89.0	15.8	12.5	74.4	123.2
17R	52.0	33.6	76.2	45.6	83.9
18R	40.4	21.1	86.4	16.9	45.4
19R	91.5	29.0	87.6	211.5	61.6
20R	59.5	6.1	53.2	30.3	77.9
21R	37.4	nd	30.0	13.6	67.2
White					
1W	43.7	nd	39.3	na[j]	5.0
2W	11.5	nd	22.1	na	4.6
3W	12.5	8.86	8.4	na	nd
4W	10.1	nd	25.7	na	nd
5W	10.4	nd	16.7	na	nd

[a]Sample codes as in Table 1.

[b]mg gallic acid equivalents/L; [c]mg catechin equivalents/L; [d]mg caffeic acid equivalents/L; [e]mg malvidin-3-glucoside equivalents/L; [f]mg rutin equivalents/L; [g]average SD within 10%; [h]average SD within 5%; [i]nd: not detected; [j]na: not applicable.

mg CE/L (Table 3). A wide range of SPP and LPP contents was also observed for red wines. SPP and LPP contents were between 0.235 and 0.949 and between 0.146 and 1.249 absorbance units, respectively. Older red wines (1989 vintage and older) tended to have more LPP than SPP, while for young wines (younger than 1999 vintage and some wines between 1992 and 1999) this trend was reversed. Only LPP showed significant correlation with wine age, with a correlation coefficient of 0.56 ($p < 0.01$).

NP-HPLC analysis. The NP-HPLC method separates the phenolics in wine on the basis of their molecular weights. In this study, a division of the compounds based on retention time (RT) of a cacao bean standard were made to quantify monomers (RT < RT of dimer), LMWP (RT > RT of dimer and < RT of tetramer), and HMWP (RT > RT of tetramer).The same division was made for colored polymers using the areas at 520 nm in the NP-HPLC chromatograms of the red wines. Red wines contained monomers, representing mostly (+)-catechin and (-)-epicatechin, of between 297.6 and 957.1 mg ECE/L. LMWP and HMWP contents ranged from 194.5 to 579.5 and from 657.2 to 2486.6 mg ECE/L (Table 3), respectively. In white wines, the monomer content was between 90.5 and 283.8 mg ECE/L, with between 38.3 and 95.1 mg ECE/L and between 32.7 and 131.4 mg ECE/L for LMWP and HMWP, respectively (Table 3). The total polymer concentration of red and white wines ranged from 901.3 to 2974.2 and from 71.0 to 226.6 mg ECE/L, respectively. Both monomer and LMWP contents of red wines decreased with wine age, as illustrated by the correlation coefficient between red wine vintage and both monomers (r = -0.68, $p < 0.001$) and LMWP (r = -0.70, $p < 0.001$).

In red wines, between 8.9 and 296.8 mg ME/L colored monomers were observed with between 336.5 and 770.1 mg ME/L total colored polymers (Table 3). The total colored polymers consisted of between 13.2 and 110.0 mg ME/L LMWCP and between 58.3 and 278.7 mg ME/L HMWCP. In general, older wines contained more polymers and colored polymers and less monomers and colored monomers than young wines. However, only the colored monomer (r = -0.58, $p < 0.01$) and LMWCP (r = -0.60, $p < 0.01$) contents of red

Table 3 Flavan-3-ols and proanthocyanidin contents in red and white wines.

	DAC assay	Tannin assay			NP-HPLC							
Wine[a]	Flavan-3-ols (mg CE/L)[b]	Tannin[c] (mg CE/L)	SPP[d] (AU)	LPP[e] (AU)	Monomers[f] (mg ECE/L)	LMWP[g] (mg ECE/L)	HMWP[h] (mg ECE/L)	Total polymers[f] (mg ECE/L)	Colored monomers[i] (mg ME/L)	LMWCP[j] (mg ME/L)	HMWCP[k] (mg ME/L)	Total colored polymers[i] (mg ME/L)
Red												
1R	77.7[l]	626.0[l]	0.278[l]	0.482[l]	549.3[m]	312.8[m]	2095.5[l]	2408.3[m]	8.9[n]	13.2[n]	102.0[n]	115.1[n]
2R	25.5	22.6	0.466	0.156	375.7	244.1	657.2	901.3	31.6	31.6	80.3	111.9
3R	73.5	46.9	0.561	0.210	750.7	453.9	961.1	1414.9	120.1	64.1	120.1	184.2
4R	54.9	611.3	0.291	0.529	519.1	254.8	1754.3	2009.2	10.7	15.6	111.8	127.4
5R	135.8	574.8	0.636	0.490	691.6	570.7	2211.8	2782.5	101.9	73.3	218.9	292.3
6R	15.3	372.3	0.440	0.625	324.2	196.3	1305.1	1501.4	11.1	30.7	171.5	202.2
7R	148.8	849.0	0.640	0.785	711.0	543.2	2431.0	2974.2	92.9	66.5	229.0	295.5
8R	50.2	464.7	0.949	0.706	494.8	316.8	1627.2	1944.0	61.5	57.9	278.7	336.5
9R	114.6	630.0	0.571	0.562	649.3	487.8	2062.9	2550.7	67.1	51.2	199.6	250.8
10R	47.9	230.6	0.868	0.472	486.0	275.5	1261.0	1536.5	50.0	40.9	176.8	217.7
11R	119.0	357.7	0.251	0.146	676.6	425.1	1267.2	1692.3	95.2	29.4	88.4	117.8
12R	59.3	307.9	0.297	0.214	449.9	285.7	914.2	1199.9	52.1	37.5	108.3	145.8
13R	82.2	271.8	0.347	0.189	957.1	517.6	1249.4	1767.0	296.8	110.0	172.9	282.9
14R	102.1	215.9	0.434	0.230	879.0	454.9	1166.4	1621.2	102.6	47.4	133.2	180.5
15R	83.4	746.4	0.638	1.249	297.6	249.7	2486.6	2736.2	22.1	31.5	256.7	288.2
16R	111.3	658.8	0.613	0.785	553.4	406.7	2240.2	2646.9	62.7	53.7	265.8	319.5
17R	96.2	310.7	0.287	0.268	655.2	410.6	1356.5	1767.0	38.5	32.5	97.7	130.2
18R	69.3	80.3	0.235	0.176	423.4	255.6	852.0	1107.6	18.9	18.8	58.3	77.1
19R	134.4	644.9	0.378	0.364	841.4	579.5	2056.8	2636.2	132.4	53.9	119.8	173.7
20R	38.1	656.4	0.642	1.154	470.9	252.8	2216.0	2468.8	16.4	33.0	250.3	283.4
21R	16.6	309.6	0.496	0.652	344.6	194.5	1429.2	1623.7	13.7	20.0	129.3	149.2
White												
1W	2.0	nd[o]	na[p]	na	283.8	95.1	131.4	226.6	na	na	na	na
2W	3.8	nd	na	na	152.5	84.3	93.8	178.1	na	na	na	na
3W	nd	nd	na	na	162.0	50.2	43.2	93.4	na	na	na	na
4W	nd	nd	na	na	90.5	38.3	32.7	71.0	na	na	na	na
5W	1.3	nd	na	na	154.4	55.8	57.3	113.0	na	na	na	na

[a]Sample codes as in Table 1. [b]catechin equivalents/L; [c]mg catechin equivalents/L; [d]small polymeric pigment content (absorbance units); [e]large polymeric pigment content (absorbance units); [f]mg epicatechin equivalents/L (mg ECE/L); [g]low molecular-weight polymers (mg ECE/L); [h]high molecular-weight polymers (mg ECE/L); [i]mg malvidin-3-glucoside equivalents/L (mg Mv-3-glc/L); [j]low molecular-weight colored polymers (mg Mv-3-glc/L); [k]high molecular-weight colored polymers (mg Mv-3-glc/L); [l]average SD within 5%; [m]average SD within 10%; [n]average SD within 15%; [o]nd: not detected; [p]na: not applicable.

wines were significantly correlated to wine age. An important consideration is that the original monomer content of different wines is different and that will lead to differences in monomer and polymer content with aging.

Cyclic voltammetry analysis. Cyclic voltammetry plots for three red and two white wines are shown in Figures 1A and 2. Red wines gave two broad anodic peaks around 400 and 660 mV, while white wines only gave one anodic peak around 400 mV. The peak at 660 mV in red wine voltammograms is mainly due to the anthocyanins. The appearance of current at 480 mV as observed for 13R and 2W can be associated with the quercetin glycosides, while the current at 300 mV as observed for 15R can be associated with compounds with a triphenols group on the flavonoid B-ring (pyrogallols), such as myricetin and (epi)gallocatechin. The first derivative of the cyclic voltammograms for three red wines are shown in Figure 1B, where it is clear that wine 15R contains more pyrogallol groups than the other wines. This seems to be the case for all but one Cabernet Sauvignon wine. When considering the ratio between the first derivative at 350 mV to that at 300 mV (which accentuates the peak or shoulder at 300 mV) the average value for the Cabernet Sauvignon wines is 0.90; the average is 1.55 for all the wines. Interestingly, Pinot noir wines show an average value of 2.00 for this ratio. The ratio between the first derivative at 350 mV and that at 300 mV for catechin (containing no pyrogallol group) is 7.61, which seems to indicate that Pinot noir wines are dominated by catechol-containing polyphenols, while in Cabernet Sauvignon wines the (epi)gallocatechins are more prevalent. The average content of catechol-containing polyphenols was higher in Pinot noir wines: the (+)-catechin content of Pinot noir and Cabernet Sauvignon wines was 28.36 and 7.22 mg/L, respectively, while the average total hydroxycinnamate content (expected to be dominated by caffeic acid derivatives) of these cultivars was 76.3 and 31.6 mg CAE/L, respectively.

The difference between the anodic peak potential and the half-peak potential ($E_{p,a}$ - $E_{p/2}$) is used to estimate the peak width, which indicates the broadness of the anodic peak. For red wines, this value was between 46 and 81 mV, while for white wines it was between 44 and 63 mV (Table 4). A distinct difference can be seen in the peak width of Pinot noir (average = 56 mV) and Cabernet Sauvignon (average = 70 mV), which can be ascribed to the fact that Pinot noir wines are mainly dominated by catechol-containing polyphenols, with Cabernet Sauvignon containing more pyrogallol groups, broadening the peak to lower potentials.

The anodic peak current ($I_{p,a}$) gives an indication of the total catechol and pyrogallol-containing polyphenols. For red wines diluted 400 times and white wines diluted 10 times the $I_{p,a}$ ranged from 0.41 to 1.77 and from 0.68 to 1.92 µA, respectively (Table 4). Pinot noir and Cabernet Sauvignon wines have average $I_{p,a}$ values of 0.96 and 0.78 µA, respectively, again highlighting the difference in catechol-containing groups.

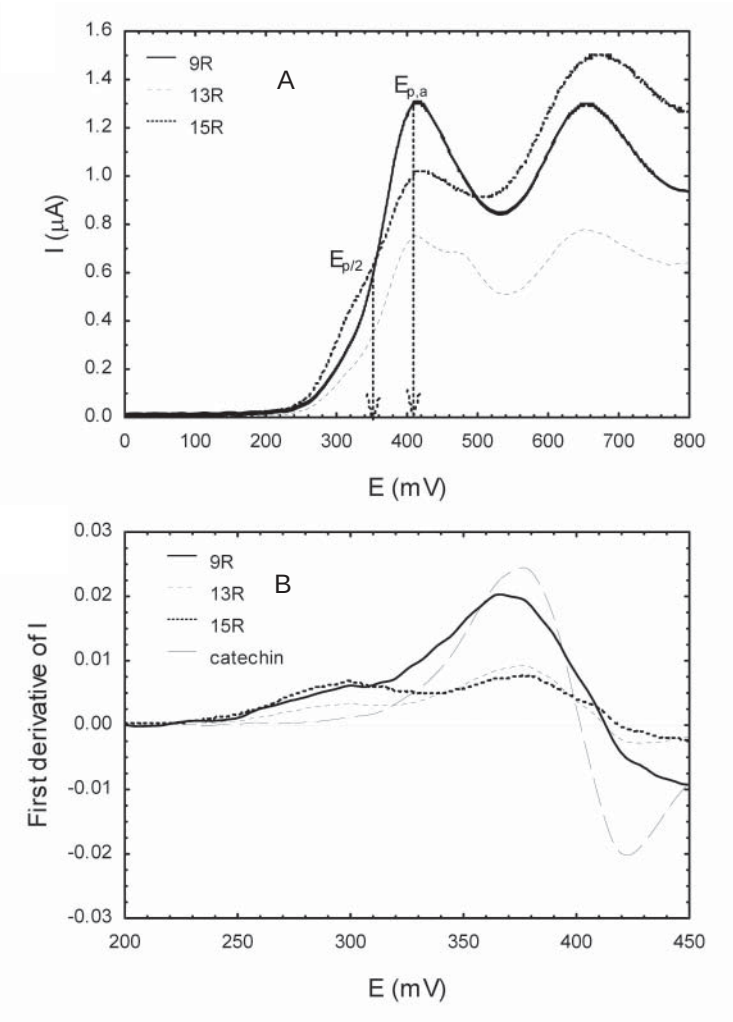

Figure 1 (**A**) Cyclic voltammograms of three red wines (9R, 13R, 15R). (**B**) The first derivative plot of the cyclic voltammograms with that of catechin added.

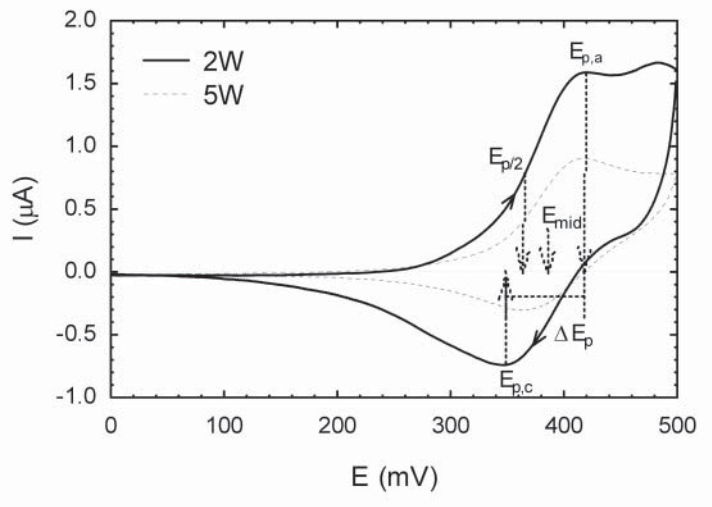

Figure 2 Cyclic voltammograms of two white wines (2W, 5W).

Table 4 Cyclic voltammetry parameters for red and white wines.

Wine[a]	$I_{p,a}$ (μA)[b]	E_{mid} (mV)[c]	$E_{p,a}$ (mV)[d]	$E_{p,a2}$ (mV)[d]	$E_{p,a} - E_{p/2}$ (mV)[e]	$I_{p,c}/I_{p,a}$[f]
Red						
1R	0.94	373	414	663	59	0.65
2R	0.41	378	410	680	60	0.59
3R	0.84	379	409	650	56	0.65
4R	0.93	379	420	668	63	0.66
5R	1.77	375	419	673	63	0.62
6R	0.57	372	416	700	62	0.54
7R	1.63	376	416	674	57	0.65
8R	0.73	358	411	638	75	0.56
9R	1.18	378	417	651	57	0.64
10R	0.60	367	409	660	59	0.58
11R	0.97	377	408	655	52	0.73
12R	0.91	377	402	648	51	0.69
13R	0.70	379	412	653	54	0.60
14R	1.14	376	410	662	60	0.67
15R	0.89	357	419	673	81	0.66
16R	1.30	374	413	665	62	0.57
17R	0.99	401	427	668	46	0.43
18R	0.77	379	408	675	57	0.69
19R	1.44	374	410	663	57	0.67
20R	0.70	356	413	710	67	0.63
21R	0.46	356	417	720	77	0.61
White						
1W	1.92	397	442	na[g]	63	0.51
2W	1.59	383	419	482	53	0.47
3W	0.99	401	427	na	46	0.43
4W	0.68	402	422	na	44	0.46
5W	0.91	390	417	na	49	0.33

[a]Sample codes as in Table 1; [b]anodic peak current; [c]potential midway between anodic and cathodic peak potential; [d]first and second anodic peak potential; [e]difference between anodic peak potential and potential at half peak height; [f]ratio of cathodic peak current to anodic peak current; [g]na: not applicable.

Given the variable broadness of the peaks, total phenol content can be estimated using the charge passed to 500 mV (Q_{500}). The total phenol content measured using cyclic voltammetry (TP_{CV}) for red wines was between 752 and 3949 mg CE/L, while for white wines it was between 53 and 177 mg CE/L (Table 5). As in the case of TP_{FC}, TP_{CV} did not correlate with wine vintage ($r = 0.35$, $p = 0.124$). The main factors that would increase the calculated TP_{CV} are increased height and breadth of the anodic peak, with higher values for $E_{p,a} - E_{p/2}$ and $I_{p,a}$. Interestingly enough, both these parameters correlated significantly with wine vintage, although for $E_{p,a} - E_{p/2}$ the correlation is positive ($r = 0.695$, $p < 0.001$) and for $I_{p,a}$ the correlation is negative ($r = -0.44$, $p < 0.05$).

The reversibility of the oxidation reaction is indicated by the value of $I_{p,c}/I_{p,a}$, with a value of 1 corresponding to total reversibility. The reversibility of the white wines (0.33 to 0.51) was significantly less than that of the red wines (0.43 to 0.73) (Table 4). This is due to the higher concentration in red wines of compounds giving high reversibility, compared to that of the white wines. For example, red wines contain more flavan-3-ols, with reversibility of almost 1.0, than white wines. In addition, white wines contain mostly hydroxycinnamates, with lower reversibility (Kilmartin et al. 2002).

The formal potential can be estimated using potential midway between the anodic and cathodic peaks (E_{mid}) or the potential midway between the half-peak potential ($E_{p/2}$) and the anodic peak potential, namely $(E_{p,a} + E_{p/2})/2$. These measures are useful for ranking the reductive strength of the major wine polyphenols. Both of these measures were significantly higher for white wines than for red wines. The E_{mid} for red and white wines ranged from 356 to 401 and from 383 to 402 mV, respectively, while their $(E_{p,a} + E_{p/2})/2$ values ranged from 374 to 404 and from 393 to 411 mV, respectively. The generally lower values in the case of red wines are due to higher concentrations of flavan-3-ols, in comparison to the hydroxycinnamates, which dominate the phenolic content of white wines. E_{mid} of the flavan-3-ols (+)-catechin and (-)-epicatechin (360 to 370 mV) is lower than that of the caffeic acid-type hydroxycinnamates (400 to 410 mV) (Kilmartin et al. 2002).

Spectrophotometric analyses. Monomeric flavan-3-ols determined using the DAC reagent were significantly higher for red (range = 15.3 to 148.8 mg CE/L) than white (range = 1.3 to 3.8 mg CE/L) wines, although only three white wines (1W, 2W, and 5W) had measurable amounts of flavan-3-ols using this method (Table 3). Proanthocyanidins also react with the DAC reagents, although to a much lesser degree than monomeric flavan-3-ols (McMurrough and McDowell 1978). Flavan-3-ols were also negatively correlated with red wine vintage ($r = -0.57$, $p < 0.01$).

Total phenols measured using the Folin-Ciocalteu reagent (TP_{FC}) differed significantly between red (range = 689 to 2616 mg GAE/L) and white (range = 99 to 333 mg GAE/L) wines (Table 5). Total phenols measured from the total area of the RP-HPLC chromatogram at 280 nm (TP_{HPLC}) were usually less than the Folin-Ciocalteu determination. TP_{HPLC} for red wines ranged from 554 to 1366 mg GAE/L and for white wines from 63 to 401 mg GAE/L (Table 5). No significant correlation was observed between red wine vintage and TP_{FC} content ($r = 0.12$, $p = 0.61$), although phenolic compounds in wine are degraded as aging takes place. A possible explanation is that many monomeric phenols react to form polymers which may retain hydroxyl groups that can still react with the Folin-Ciocalteu reagent.

Total chain-breaking antioxidant activity. All of the wines displayed a pronounced ability to retard methyl linoleate oxidation (Figure 3). The key feature of the $[O_2]$ traces observed after adding wine was that, during the first part of the induction period, R decreased with time (an increase in inhibition) and then increased after the $[O_2]$ trace went through an inflection point. This tendency was more

Table 5 Total phenol content and antioxidant activity of red and white wines.

Wine[a]	TP_{FC}[b] (mg GAE/L)	TP_{HPLC}[c] (mg GAE/L)	TP_{CV}[d] (mg CE/L)	N[e] (mM)
Red				
1R	1854[f]	1084[g]	2011[g]	19.80[h]
2R	689	554	752	7.86
3R	1155	880	1672	15.58
4R	1655	943	1941	20.44
5R	2410	1241	4819	29.33
6R	980	678	1165	11.99
7R	2616	1364	3949	28.17
8R	1193	838	1655	12.84
9R	2152	1175	2372	24.41
10R	1162	750	1366	11.99
11R	1693	933	1890	20.08
12R	996	706	1738	18.55
13R	1597	1011	1335	17.13
14R	1711	943	2504	17.78
15R	2379	1122	2099	27.88
16R	2361	1181	3157	27.92
17R	1563	953	1180	17.77
18R	930	722	1573	15.60
19R	2457	1175	3336	24.66
20R	1897	1074	1493	20.61
21R	1002	747	941	11.60
White				
1W	333	401	177	2.71
2W	276	63	167	1.72
3W	205	321	79	0.96
4W	99	209	53	0.53
5W	220	265	85	1.12

[a]Sample codes as in Table 1; [b]measured with Folin-Ciocalteu assay; [c]measured with RP-HPLC method; [d]measured with Q_{500} recorded during CV analysis; [e]number of terminated chains measured using the total chain-breaking antioxidant assay; [f]average SD within 15%; [g]average SD within 10%; [h]average SD within 5%.

pronounced for the aged wines than for younger wines. Possible explanations for such unexpected shape of the $[O_2]$ traces have been discussed previously (Roginsky and Barsukova 2001, Roginsky 2003, Roginsky et al. 2003).

N for red wines varies between 7.86 mM and 29.33 mM, while for white wines it ranges from 0.53 to 2.74 mM (Table 5). Changes in N with the variety are not pronounced. The average values of N for the different cultivars were found to be (number of samples in brackets): Cabernet Sauvignon (6), 18.81 ± 6.66 mM; Pinot noir (5), 17.93 ± 1.59 mM; Zinfandel (4), 19.13 ± 5.69 mM; Barbera (2), 16.36 ± 0.78; and Merlot (2), 28.75 ± 0.58 mM. It may be that the difference in N between various varieties does not exceed the variation of N within a certain variety, with the exception of Merlot, for which N was significantly higher than the average values of N for any other variety. However, the number of the samples studied in this work did not allow a statistically significant conclusion. Previous studies of French (Landrault et al. 2001) and South African (De Beer et al. 2003) red wines showed more distinct variation of the antioxidant activity with variety. It is likely that the wide range of ages of each variety and the differences in source led to such high variability that varietal differences are difficult to distinguish.

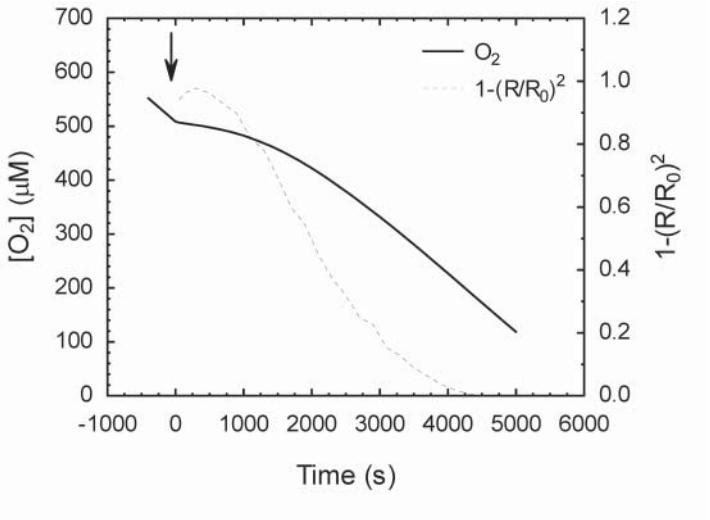

Figure 3 The effect of 0.75 μL red wine 5R on the kinetics of oxygen consumption during the oxidation of 5 mM methyl linoleate in 0.05 M Triton 100-X induced by 4 mM AAPH. The arrow shows the moment of wine addition.

Similar to the total phenols determined by the Folin-Ciocalteu assay, the antioxidant activity expressed as N does not show a significant correlation with red wine vintage ($r = 0.13$, $p = 0.58$).

Comparison of assays measuring total phenol content. The total phenol content of red and white wines was measured using the Folin-Ciocalteu assay (TP_{FC}), as well as the total area under the RP-HPLC chromatogram at 280 nm (TP_{HPLC}) and the total charge passed to 500 mV using CV (TP_{CV}). The advantages of the Folin-Ciocalteu assay are that it is reproducible, its basis is understood, and its applicability has been verified (Singleton et al. 1999). However, differences in reactivity between different phenols (Singleton et al. 1999) make it difficult to compare samples with greatly different phenolic composition. The same type of problem occurs when using UV absorption to measure total phenol content, as individual phenols differ greatly in absorbance maxima as well as molar absorbance. In addition, other components of wine may absorb at 280 nm, causing interference. The disadvantage of using cyclic voltammetry to measure total phenols using Q_{500} is that it only reflects the total content of phenolic compounds containing pyrogallol, gallate, and catechol groups in monomers, oligomers, or polymers, such as flavanols, proanthocyanidins, flavonols, and phenolic acids. The major wine anthocyanins and major part of white wine phenols, for example, only produce anodic peaks at potentials higher than 500 mV and are therefore not included in this measure. Quantification of these phenols at higher potentials is complicated by changes that occur at the electrode surface due to the accumulation of the products of polyphenol oxidation. Cyclic voltammetry is

a very rapid analysis compared with the total phenol and RP-HPLC methods, although some experience is required to ensure reproducible and consistent results.

As the Folin-Ciocalteu assay is the most widely used and accepted for determining the total phenol content of wine, the other two measures will be evaluated against it. The TP_{FC} content of red wines correlated significantly ($p < 0.001$) with both TP_{HPLC} ($r = 0.97$) and TP_{CV} ($r = 0.81$) contents. For white wines the correlation coefficient of TP_{FC} content with TP_{HPLC} content was 0.92 ($p < 0.05$), while no significant ($p \geq 0.05$) correlation was observed between TP_{FC} and TP_{CV} content. The low number of white wine samples may have affected the ability to observe a significant correlation. Although good correlation was observed between total phenol measures, the absolute values differ as different characteristics of phenols are used to estimate their total content.

Comparison of assays measuring monomeric phenolic content. The flavan-3-ols were measured using the DAC assay as well as the RP-HPLC and NP-HPLC methods. The RP-HPLC method did not consistently quantify epicatechin, and epicatechin gallate was not quantified. The major disadvantage with the DAC and NP-HPLC measures is that flavan-3-ols are not being measured exclusively. The DAC assay is sensitive to terminal flavan-3-ol units on proanthocyanidins, while in NP-HPLC the "monomer" peak also includes many nonflavanol monomers. There are several advantages of the DAC method; it is rapid and easy to perform and the interpretation of results is simple. On the other hand, it is difficult to measure catechin and epicatechin using RP-HPLC because of coelution with other phenolic compounds.

Only the data for red wines are considered in this regard as the flavan-3-ol content of most white wines was not measurable using the DAC assay. The flavan-3-ol content measured using the DAC reagent correlated ($p < 0.001$) moderately well with the (+)-catechin content ($r = 0.71$) measured using the RP-HPLC method, as well as the monomer ($r = 0.68$) content (consisting of mostly (+)-catechin and (-)-epicatechin) measured using the NP-HPLC method. Considering the high proportion of flavan-3-ol content of the total polymers detected by the NP method, it is surprising that the DAC assay has such a low correlation ($r = 0.62$, $p = 0.005$) with it. It is also notable that the DAC assay does not correlate well with any of the other assays, its strongest relationship being with the LMWP content ($r = 0.87$, $p < 0.001$) and the sum of monomer and LMWP contents ($r = 0.79$, $p < 0.001$).

Anthocyanin content was measured using RP-HPLC and the colored monomer content calculated using NP-HPLC. The advantage of RP-HPLC is that individual anthocyanins can be quantified, while with NP-HPLC the monomeric anthocyanin peaks are not separated. However, the NP-HPLC method is adequate when only a total measure of monomeric anthocyanins is needed.

The assumption that the colored monomer content mostly consists of monomeric anthocyanins is confirmed by the high correlation ($r = 0.99$, $p < 0.001$) between anthocyanin content by RP-HPLC and colored monomer content by NP-HPLC. The very high correlation ($p < 0.001$) between malvidin-3-glucoside with total anthocyanin content ($r = 0.99$) and colored monomer by NP-HPLC ($r = 0.97$) demonstrates that malvidin-3 glucoside is an excellent marker of relative anthocyanin content in wines. By comparison, quercetin is not as well correlated with total flavonol content, ($r = 0.82$, $p < 0.001$).

Comparison of assays measuring polymeric phenolic content. The polymeric phenolic content of wines was estimated using the NP-HPLC method and tannin and polymeric pigment assay. NP-HPLC is the most common method for polymer quantification, as polymers for different molecular mass can be separated. The use of a cocoa bean standard which contains a mixture of polymers of differing molecular weights makes this a useful technique when information on the size distribution of polymers is needed. When only an estimation of total polymer content is needed, the tannin assay could be preferred as this assay is relatively rapid and inexpensive.

For the purpose of this comparison, only data from the red wines will be used as the amount of tannin in white wines was below the detection threshold of the tannin assay. White wines also contain no pigments. Tannin measured using the tannin assay correlates ($p < 0.001$) well with HMWP ($r = 0.96$) and total polymer ($r = 0.93$) content measured using NP-HPLC. Given the greater degree of correlation of tannins to HMWP than total polymer, it can be concluded that LMWP measured using NP-HPLC is not measured during the tannin assay, corroborating Adams and Harbertson's (1999) report that dimeric and trimeric proanthocyanidins are not precipitated in the tannin assay.

When considering colored polymer content, both SPP ($r = 0.79$) and LPP ($r = 0.79$) contents measured using the tannin assay correlate ($p < 0.001$) well with HMWCP content. Neither SPP nor LPP content correlated significantly ($p \geq 0.05$) with LMWCP. The total polymeric pigment measured using the tannin assay also correlates ($p < 0.001$) well with HMWCP ($r = 0.89$), as well as total colored polymer ($r = 0.77$) contents measured using NP-HPLC. From these results it seems that both the SPP and LPP in the tannin assay is measured as HMWCP in the NP-HPLC method and that the polymeric pigments with less than four subunits are not detected in the tannin and polymeric pigment assay.

Comparison of antioxidant activity with phenolic content. The antioxidant activity of wines expressed as N correlates well with total phenol content measured using the Folin-Ciocalteu method for both red ($r = 0.94$, $p < 0.001$) (Figure 4) and white wines ($r = 0.94$, $p < 0.05$). The correlation may be improved significantly to $r = 0.98$ ($p < 0.001$) when wines 12R and 18R are excluded from the correlation for red wines. Even taking both red and white wines into

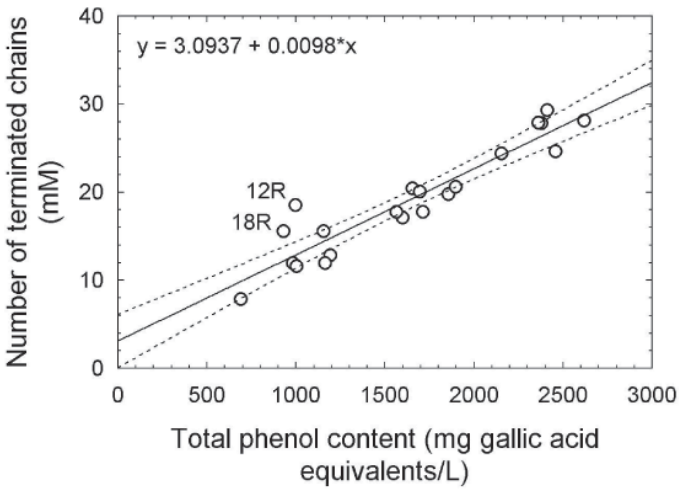

Figure 4 Relationship of antioxidant activity with total phenol content measured using the Folin-Ciocalteu assay.

consideration, a good correlation (r = 0.97, $p < 0.001$) is observed. The total chain-breaking antioxidant activity also correlates ($p < 0.001$) well with TP_{HPLC} (r = 0.96) and TP_{CV} (r = 0.90) for all wines. The total phenol content of white (Baderschneider et al. 1999) and red (Frankel et al. 1995, Landrault et al. 2001, De Beer et al. 2003) wines has previously been shown to correlate well with their antioxidant activity in several test assays. Correlations could not be calculated for white wines since not all wines contained appreciable amounts of all the monomeric phenols quantified.

Conclusions

Considering the complexity of phenolics, it would not be practical to seek a single best assay to characterize the phenolic composition of a wine sample, as the broad diversity of phenolics with regard to molecular weight and other properties defies the utility of a single measurement. Choosing an appropriate phenolic assay depends on what information is required. For example, a total phenol measured by the Folin-Ciocalteu assay is useful to determine the approximate actual mass of phenolic material present, but this amount may be unrelated to the sensory property of astringency, or to color, each so important to wine. The very lack of strong correlations between some of the fairly general phenolic assays, such as Folin, tannin, and DAC, not only demonstrates that these methods are measuring different properties, but also, with a diverse set of wines, these properties are not correlated with each other. This contradicts an expectation that wines usually have similar proportions of tannins or flavan-3-ols. On the other hand, the strong correlation between the Folin and RP-HPLC totals suggests that the total area measured by the LC method is in fact including much of the oligo- and polymeric flavan-3-ols, substances that are evidenced only by a "drifting" baseline during the analysis.

More specific tests, such as the tannin assay and the NP-HPLC method, do show similar responses, as expected. However, is important to note the differences in response in order to understand how the parameters relate to each other. For example, the HMWP content (NP-HPLC) correlates well to the tannin content, while the LMWP content is not included in the tannin. With pigments, the SPP and LPP components are both included in the HMWCP content (NP-HPLC), while the LMWCP content is not measured in the tannin assay. To resolve the basis of these differences, it would be most useful to know the structures of the colored components that are observed by these assays.

Cyclic voltammetry provides qualitative and semiquantitative information about the most easily oxidizable polyphenols. It appears that to have a better correlation with the standard total phenolic assay, it may be necessary to find a means to overcome the technical difficulties in quantifying total current to higher voltages. In that case a rapid assay for total phenolics would be available—one that generated negligible chemical waste.

Because so many phenolic substances are so complex, the complete characterization of the phenolic content of a wine is not yet possible. Too many unidentified compounds still remain, especially with the polymeric fraction. Depending on the needs of an experiment, using some combination of assays is currently the best approach to properly characterize the phenolic composition of a sample.

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