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Antioxidant Activity of South African Red and White Cultivar Wines: Free Radical Scavenging

DALENE DE BEER,† ELIZABETH JOUBERT,*,‡ WENTZEL C. A. GELDERBLOM,§ AND MARENA MANLEY†

Department of Food Science, University of Stellenbosch, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa; Post-harvest and Wine Technology Division, ARC Infruitec-Nietvoorbij (The Fruit, Vine and Wine Institute of the Agricultural Research Council), Private Bag X5026, 7599 Stellenbosch, South Africa; and PROMEC Unit, Medical Research Council, P.O. Box 19070, 7505 Tygerberg, South Africa

The free radical scavenging activity of South African red ($n = 46$) and white ($n = 40$) cultivar wines was determined using 2,2′-azinobis(3-ethylbenzothialozinesulfonic acid) radical cations ($ABTS^{\bullet+}$) and 2,2-diphenyl-1-picrylhydrazyl radicals ($DPPH^{\bullet}$). The total antioxidant activities (TAA) of red and white wines using $ABTS^{\bullet+}$ were 14.916 and 0.939 mM Trolox, respectively, at corresponding total phenol (TP) contents of 2339.0 and 273.8 mg of gallic acid equiv/L. Ruby Cabernet wines had the lowest TAA_{ABTS} (13.177 mM Trolox) of the red wines, whereas the TAA_{ABTS} values of Chardonnay and Chenin blanc wines were the highest (1.060 mM Trolox) and lowest (0.800 mM Trolox) of the white wines. The TAA_{DPPH} values were of the same magnitude as the TAA_{ABTS} values, and similar trends were observed. TAA correlated ($P < 0.001$) with total phenol content of red ($r = 0.935$) and white ($r = 0.907$) wines, as well as flavanol content of red wines ($r = 0.866$) and tartaric acid ester content of white wines ($r = 0.767$). Canonical discriminant analysis using phenolic composition and antioxidant activity was applied to differentiate between red and white cultivar wines.

KEYWORDS: ABTS radical cation; DPPH radical; free radical scavenging; antioxidant; total antioxidant activity; phenolic compounds; polyphenols; anthocyanins; wine

INTRODUCTION

Oxidative stress has been suggested to contribute to free radical-mediated disease such as cancer, arteriosclerosis, ischemic heart disease, and neurodegenerative diseases (*1*−*3*). Wine as a source of dietary antioxidants is receiving more prominence (*4*, *5*) due to the possible link between a moderate intake of red wine and the low incidence of coronary heart disease in the south of France (*6*). Red wine contains phenolic compounds with high in vitro free radical scavenging activity compared to beverages such as beer, tea, and fruit juices (*7*). Although ethanol has a positive effect on lowering blood cholesterol levels (*6*, *8*), the phenolic components are proposed to alleviate coronary heart disease (*9*, *10*). The intake of red wine and foods containing these phenolic compounds is reported to increase the antioxidant content and status of human blood plasma (*11*−*13*).

The principle phenolic compounds in wine include hydroxybenzoic acids, hydroxycinnamic acid derivatives, flavanols, flavonols, and anthocyanins (*14*). Differences in the phenolic content of grape cultivars and vinification techniques contribute to differences in the phenolic composition of wine (*14*). The terroir, which includes soil and climatic conditions, affects the phenolic composition of grapes due to its influence on biochemical synthesis of these compounds (*14*). Fermentation of red wine on the grape seeds and skins allows more extensive extraction of phenolic components such as flavanols, flavonols, and proanthocyanidins than in the case of white wines, for which pomace contact is generally kept to a minimum (*14*, *15*). In addition, white wine contains no anthocyanins as these compounds only occur in the skins of red grapes (*14*).

A few studies have evaluated the effect of vinification techniques (*16*−*19*) and cultivar (*20*, *21*) on the antioxidant activity of different wines. Most of these studies made use of only a very small number of wines. In the only study to date on South African wines, the inhibitory activity of only six red and six white wine fractions on low-density lipoprotein peroxidation was evaluated (*22*). Synthetic radicals, namely, 2,2′-azinobis(3-ethylbenzothialozinesulfonic acid) radical cations ($ABTS^{\bullet+}$) (*4*, *19*), 2,2-diphenyl-1-picrylhydrazyl radicals ($DPPH^{\bullet}$) (*17*, *18*), and *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride radicals (*23*), have been used to determine the antioxidant activity of wine. Standardization of methods has

* Author to whom correspondence should be addressed (telephone +27-21-809-3444; fax +27-21-809-3400; e-mail lizettej@infruit.agric.za).
† University of Stellenbosch.
‡ ARC Infruitec-Nietvoorbij.
§ Medical Research Council.

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not yet been introduced, which makes comparison of wines from different laboratories and countries problematic.

The aim of this study was to determine the free radical scavenging activity, using the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays, of a wide selection of commercially available wines representative of the major red (Pinotage, Cabernet Sauvignon, Merlot, Shiraz, and Ruby Cabernet) and white (Chenin blanc, Sauvignon blanc, and Chardonnay) wine grape cultivars of South Africa. Wines from Colombard, which are used mostly for brandy production, were also included in the study.

EXPERIMENTAL PROCEDURES

Chemicals. The following chemicals were used: 2,2′-azinobis(3-ethylbenzothialozinesulfonate) diammonium salt (ABTS) and ascorbic acid enzymatic test kit (Boehringer Mannheim GmbH, Mannheim, Germany); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Aldrich Chemical Co., Gillingham, Dorset, U.K.); potassium persulfate ($K_2S_2O_8$), (+)-catechin, and gallic acid (Sigma Chemical Co., St. Louis, MO); Folin−Ciocalteau phenol reagent and quercetin (Merck, Darmstadt, Germany); 4-(dimethylamino)cinnamaldehyde (DAC) and caffeic acid (Fluka AG, Buchs, Switzerland); and methanol (AR) (Riedel-de Häen, AG Seelze-Hanover, Germany). The water was purified and deionized with a Modulab water purification system prior to use (Separations, Cape Town, South Africa).

Wines. Wines produced from five red cultivars (46 wines) of the 1998 vintage and four white cultivars (40 wines) of the 1999 vintage were obtained from a variety of wineries in the Western Cape region, South Africa. Red grape cultivars chosen were Pinotage (unique South African cultivar), Cabernet Sauvignon, Merlot, Shiraz, and Ruby Cabernet comprising 21, 18, 10, 8, and 8%, respectively, of grapes crushed for vinification of red wines in South Africa during the 2001 season (*24*). The white grape cultivars Chenin blanc, Colombard, Sauvignon blanc, and Chardonnay made up 34, 24, 6, and 5%, respectively, of white grapes harvested. Wines in South Africa can be labeled as a single-cultivar wine even though they may contain up to 25% of other cultivar wines (Liquor Products Act No. 60 of 1989). Only red wines were matured in wood to various degrees. Climatic conditions, origin, and vinification techniques of the different wines also differed for the different cultivars.

Sample Preparation. Aliquots of each wine were frozen at −18 °C in plastic screw-top sample holders (40 mL) to preserve the phenolic compounds until analysis. The wines were defrosted and sonicated to dissolve precipitates prior to use.

Determination of the Phenolic Composition of Wine. Wine total phenol content was determined using the Folin−Ciocalteau reagent (*25*). Gallic acid was used as standard, and results are expressed as milligrams of gallic acid equivalents per liter (mg of GAE/L). Possible interference of sulfur dioxide in the determination of total phenols was investigated before final determinations. Ten red and eight white wines were analyzed before and after addition of acetaldehyde (1 g/L) to bind sulfur dioxide 5 min before total phenol analysis (C. Saucier, Université Victor Segalen, Bordeaux, France, personal communication). Anthocyanin content was estimated according to the pH differential method of Burns et al. (*26*). Total, monomeric, and polymeric anthocyanins were quantified as milligrams of malvidin 3-glucoside (Mv-3-glc) equivalents per liter (extinction coefficient, $\epsilon = 28\,000$). Flavanol content of the wines was measured at 640 nm after reaction with the 4-(dimethylamino)cinnamaldehyde reagent (*27*). (+)-Catechin was used as a standard, and the results are expressed as milligrams of catechin equivalents per liter (mg of CE/L). Flavonol and tartaric acid ester contents were estimated by measuring the absorbance of wines at 360 and 320 nm, respectively, after the addition of 2% HCl (*28*). Flavonol and tartaric acid ester contents were expressed as milligrams of quercetin equivalents per liter (mg of QE/L) and milligrams of caffeic acid equivalents per liter (mg of CAE/L), respectively. Red wines were diluted with 10% ethanol before introduction to the assays. Spectrophotometric measurements were performed on a Beckman DU-65 UV−vis spectrophotometer (Beckman, Cape Town, South Africa) using a 1 cm path length quartz cuvette. Data capture software was used for time assays.

Measurement of the $ABTS^{\bullet+}$ Scavenging Activity. The total antioxidant activity (TAA) of wines was determined using the $ABTS^{\bullet+}$ scavenging assay of Re et al. (*29*). An ABTS solution (7 mM) in water was preincubated for at least 12 h with 2.45 mM (final concentration) $K_2S_2O_8$ to produce the radical cation. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of ∼0.7 (± 0.02) at 734 nm; 1 mL of $ABTS^{\bullet+}$ solution was added to 50 μL of diluted wine samples (red wines 50 times and white wines 5 times diluted with 10% ethanol), standard Trolox solution (0−400 μM), or 10% ethanol (control). The absorbance of the mixture was determined after exactly 4 min of incubation at 37 °C.

The concentration of $ABTS^{\bullet+}$ in the control and samples was calculated using the absorbance readings and the extinction coefficient of $ABTS^{\bullet+}$, $\epsilon = 16000$ (*29*). A plot of the remaining $ABTS^{\bullet+}$ concentration against the concentration of Trolox in the standard samples was used to calculate the TAA_{ABTS} of the wines. The antioxidant potency (AP) of the total phenols for each cultivar wine was calculated as the ratio of TAA to total phenols:

$$AP = \frac{TAA}{\text{total phenols}} \times 1000 \quad (1)$$

Measurement of the $DPPH^{\bullet}$ Scavenging Activity. $DPPH^{\bullet}$ scavenging activity was determined according to a modified version of the method of Brand-Williams et al. (*30*): 50 μL of diluted red (0−150 mg of GAE/L) and white (0−250 mg of GAE/L) wine samples, standard Trolox solution (0−400 μM), or 10% ethanol (control) was added to 2 mL of a 3.04×10^{-5} M methanolic solution of $DPPH^{\bullet}$; the absorbance of the reaction mixture at 515 nm was measured at steady state after 2 h of incubation at room temperature (25 °C). Blanks for each sample containing 50 μL of diluted sample and 2 mL of methanol were also incubated to correct for inherent absorbance interference by wines. The concentration of the remaining $DPPH^{\bullet}$ at steady state conditions was calculated using a calibration curve, and the values were plotted on a graph showing $[DPPH^{\bullet}]$ or $\log([DPPH^{\bullet}])$ as a function of the total phenol concentration of the wine sample, from which the EC_{50} value (the total phenol concentration of wine required to scavenge 50% of the initial $DPPH^{\bullet}$ in the reaction mixture) was estimated.

The initial scavenging rate (ISR) for all of the wines was determined during unsteady state conditions. Absorbance was read at 515 nm every 6 s for the first 5 min of the reaction, and the ISR was expressed as the absolute value of the gradient of the line from a plot of $[DPPH^{\bullet}]$ against time for the first minute of the reaction.

The radical scavenging efficiency (RSE), a new parameter combining scavenging activity in terms of the amount of radicals scavenged and ISR, was defined as follows:

$$RSE = \frac{ISR}{EC_{50}} \times 1000 \quad (2)$$

The TAA_{DPPH} and AP_{DPPH} of the total phenols for each cultivar wine were calculated as for the $ABTS^{\bullet+}$ scavenging assay.

Determination of the Contribution of Ascorbic Acid to the Free Radical Scavenging Activity. The ascorbic acid content of white wines was determined with an enzymatic test kit from Boehringer Mannheim (Mannheim, Germany) as some producers add ascorbic acid to white wines to prevent browning.

Due to the antioxidant activity of ascorbic acid, it was necessary to calculate its contribution to the total antioxidant activity of wines using the Trolox equivalent antioxidant capacity (TEAC) of ascorbic acid (*31*). The TEAC of ascorbic acid is 0.99, which means that a 0.99 mM Trolox solution has an equivalent antioxidant activity to a 1 mM ascorbic acid solution. The ascorbic acid contribution to the TAA was calculated as follows:

$$\text{ascorbic acid contribution} = [AA] \times 0.99 \quad (3)$$

where [AA] = ascorbic acid concentration in mM.

Statistical Analysis. All tests were carried out in duplicate (EC_{50} values) or triplicate (all other tests). One-way ANOVA was performed on the means to determine whether they differed significantly. Statistical comparisons between means for cultivar wines were made using

Table 1. Effect of Determination of Total Phenols with and without the Addition of Acetaldehyde

	total phenols	
wine[a]	without acetaldehyde[b]	with acetaldehyde[c]
red	2226.99 a[d] (± 355.52)[e]	2226.99 a (± 362.48)
white	277.45 a (± 58.04)	264.02 a (± 56.22)

[a] Averages for 10 red and 8 white wines, respectively. [b] Total phenol content determined without added acetaldehyde to bind free sulfur dioxide. [c] Total phenol content determined with added acetaldehyde to bind free sulfur dioxide. [d] Averages in a row followed by different letters differ significantly ($P < 0.05$). [e] Standard deviation.

Student's t−LSD test ($P < 0.05$). Canonical discriminant analysis was used to differentiate between cultivars on the basis of phenolic composition [total phenol, free anthocyanin (red wine only), flavanol, flavonol, and tartaric acid ester contents] and antioxidant activity (TAA_{ABTS}, EC_{50}, ISR, RSE, and TAA_{DPPH}). All statistical analyses were carried out using the SAS version 6.12 software package.

RESULTS

Phenolic Composition of Wine. A preliminary investigation was conducted on 10 red and 8 white wine samples to determine the effect of acetaldehyde addition to bind free sulfur dioxide on the apparent total phenol content (**Table 1**). No significant difference between the average total phenol content determined with and without acetaldehyde added was observed for both red and white wines. The total phenol content of wines was therefore determined without the addition of acetaldehyde. The total phenol content of Ruby Cabernet was significantly lower than that of Shiraz and Merlot, whereas its flavanol content was the lowest of all red cultivar wines (**Table 2**). However, its monomeric anthocyanin content was the highest of all red cultivar wines. The red wines did not differ in polymeric anthocyanin content. The flavonol content of Shiraz was significantly higher than that of Cabernet Sauvignon, Ruby Cabernet, and Pinotage, whereas its tartaric acid ester content was significantly higher than that of Ruby Cabernet and Cabernet Sauvignon.

Chardonnay wines had a significantly higher total phenol content than Chenin blanc wines. It had the highest flavonol content, whereas its flavanol content was higher than that of Colombard and Chenin blanc. Chardonnay and Sauvignon blanc had a higher tartaric acid ester content than Chenin blanc.

Contribution of Ascorbic Acid to the Free Radical Scavenging Activity. Most of the white wines (75%) used in the present study tested negative for the presence of ascorbic acid. One Sauvignon blanc (0.348 mM) and one Colombard (0.261 mM) wine contained large amounts of ascorbic acid (**Table 3**), which contributed ca. 30 and 20% to the TAA, respectively. Data for these two wines were omitted from the data set, and the TAA values of other wines were used unadjusted.

Total Antioxidant Activity of Wines Using the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ Scavenging Assays. Except for Ruby Cabernet with a lower average TAA_{ABTS} value than that of Merlot, the TAA_{ABTS} values were not significantly different among the red wine cultivars (**Table 4**). However, the TAA_{DPPH} values of Ruby Cabernet wines were significantly lower than those of Cabernet Sauvignon and Merlot. In the case of EC_{50}, Cabernet Sauvignon with the lowest and Shiraz with the highest value differed significantly. No significant differences were observed among the different red wine cultivars in the ISR, RSE, AP_{ABTS}, and AP_{DPPH} values.

With regard to the white wines, only Chardonnay with the highest TAA_{ABTS} value differed from Chenin blanc with significantly lower values (**Table 4**). The TAA_{ABTS} values of Colombard and Sauvignon blanc were, however, not significantly lower than that of Chardonnay and also not significantly higher than that of Chenin blanc. A similar pattern was obtained

Table 2. Phenolic Composition of Different South African Cultivar Wines

wine	total phenols[a]	monomeric anthocyanins[b]	polymeric anthocyanins[b]	flavanols[c]	flavonols[d]	tartaric acid esters[e]
red						
Pinotage	2342.4 ab[f] (± 411.4)[g]	128.12 b (± 39.16)	83.59 a (± 17.51)	239.07 ab (± 62.41)	66.62 bc (± 24.02)	240.64 ab (± 32.28)
Cabernet Sauvignon	2344.4 ab (± 262.8)	115.28 b (± 31.74)	82.65 a (± 14.98)	247.63 a (± 54.51)	50.78 c (± 27.98)	188.19 c (± 33.09)
Merlot	2498.8 a (± 410.7)	130.62 b (± 15.02)	90.24 a (± 24.03)	265.82 a (± 70.02)	88.60 ab (± 24.84)	252.63 ab (± 46.64)
Shiraz	2412.4 a (± 450.6)	138.31 b (± 23.06)	85.01 a (± 15.82)	253.16 a (± 60.75)	99.58 a (± 37.45)	271.81 a (± 48.54)
Ruby Cabernet	2016.0 b (± 364.2)	168.21 a (± 39.85)	85.17 a (± 27.12)	189.14 b (±38.49)	66.52 bc (± 33.89)	214.75 bc (± 47.36)
av	2339.0	133.48	85.24	241.63	74.63	234.42
white						
Chenin blanc	242.0 b (± 52.4)	na[h]	na	3.59 c (± 1.76)	6.34 b (± 2.94)	35.27 b (± 8.39)
Colombard	268.4 ab (± 53.8)	na	na	5.51 bc (± 2.30)	7.85 b (± 5.84)	39.29 ab (± 13.90)
Sauvignon blanc	266.7 ab (± 31.2)	na	na	6.98 ab (± 2.76)	6.72 b (± 2.92)	46.11 a (± 8.02)
Chardonnay	292.7 a (± 28.7)	na	na	9.52 a (± 4.37)	12.11 a (± 3.30)	46.36 a (± 4.97)
av	273.8	na	na	6.23	8.22	41.77

[a] Total phenol content expressed as mg of gallic acid equiv/L. [b] Monomeric anthocyanin content expressed as mg of malvidin 3-glucoside equiv/L. [c] Flavanol content expressed as mg of catechin equiv/L. [d] Flavonol content expressed as mg of quercetin equiv/L. [e] Tartaric acid ester content expressed as mg of caffeic acid equiv/L. [f] Averages in a column followed by different letters differ significantly ($P < 0.05$). Data for red and white wines were analyzed separately. [g] Standard deviation. [h] Not applicable.

Table 3. Ascorbic Acid Content and Contribution to Total Antioxidant Activity of White Wines

wine	ascorbic acid content[a]	ascorbic acid contribution[b]	% of total activity[c]
Chardonnay 1	0.012	0.012	1.31
Chardonnay 7	0.017	0.017	1.41
Colombard 1	0.017	0.017	1.69
Colombard 3	0.043	0.046	5.33
Colombard 8	0.261	0.274	19.59
Chenin blanc 2	0.096	0.101	8.15
Chenin blanc 6	0.011	0.011	1.34
Chenin blanc 10	0.080	0.084	6.87
Sauvignon blanc 3	0.059	0.062	5.76
Sauvignon blanc 5	0.015	0.016	1.31
Sauvignon blanc 8	0.348	0.366	29.87

[a] Millimolar. [b] Contribution of ascorbic acid to the total antioxidant activity as mM Trolox equiv measured using the $ABTS^{\bullet+}$ scavenging assay. [c] Percent of total antioxidant activity contributed by ascorbic acid.

for the TAA_{DPPH} value, although this assay could also distinguish between Chardonnay and Colombard. The AP_{ABTS} was significantly higher for Chardonnay than for Chenin blanc, whereas the AP_{DPPH} values of both Chardonnay and Sauvignon blanc were significantly higher than that of Colombard. Chardonnay wines had a lower EC_{50} value than Chenin blanc and Colombard wines. Chardonnay also exhibited a higher average RSE value than Chenin blanc. Of the white cultivar wines, Colombard displayed a higher ISR than both Sauvignon blanc and Chenin blanc, but it was not significantly higher than that of Chardonnay.

Correlation Analysis of Data. The TAA_{DPPH} values correlated well with the TAA_{ABTS} values for red ($r = 0.768$, $P < 0.001$) and white ($r = 0.769$, $P < 0.001$) wines, although two different free radicals were scavenged. A highly significant ($P < 0.001$) correlation was obtained for TAA_{ABTS} values of red wines ($r = 0.935$) and white wines ($r = 0.907$) with their total phenol content. With respect to the individual phenolic groups, the flavanol content of red wines ($r = 0.866$) and the tartaric acid ester content of white wines ($r = 0.767$) correlated ($P < 0.001$) with the TAA_{ABTS} values. Correlations of polymeric anthocyanins ($r = 0.54$) and tartaric acid esters ($r = 0.50$) in red wine and flavonols ($r = 0.62$) in white wine with TAA_{ABTS} were weaker, but still significant ($P < 0.001$). The monomeric anthocyanin content of red wines showed no correlation ($P = 0.706$) with TAA_{ABTS} values ($r = 0.057$). The same trends in correlation of antioxidant activity with phenolic groups were also observed for TAA_{DPPH} values (data not shown).

Canonical Discriminant Analysis. Canonical discriminant analysis was used to provide a clear graphical presentation of the differences between red and white cultivar wines. The parameters used in the canonical discriminant analysis were total phenol, free anthocyanin (red wine only), flavanol, flavonol, and tartaric acid ester contents, as well as TAA_{ABTS}, EC_{50}, ISR, RSE, and TAA_{DPPH}.

For the red wines (**Figure 1**), the tartaric acid ester and flavonol contents, as well as the EC_{50} value, had a positive correlation with the first canonical variable (explaining 53.8% of variation). The total phenol content, flavanol content, and TAA_{DPPH} exhibited a negative correlation with the second canonical variable (explaining 31.5% of variation), whereas the monomeric anthocyanin content was positively correlated. Ruby Cabernet wines were differentiated from the other cultivar wines, although two data points of Pinotage overlapped. Cabernet Sauvignon and Shiraz could also be differentiated from each other and from Ruby Cabernet. The flavanol and flavonol contents were positively correlated with the first canonical variable (explaining 58.8% of variation) for the white wines with the EC_{50} being positively correlated with the second canonical variable (explaining 32.7% of variation) (**Figure 2**). The Chardonnay, Sauvignon blanc, and Colombard wines could be differentiated from each other, whereas data points for Chenin blanc wines were scattered among those of other white cultivar wines. When data for Chenin blanc wines were removed from

Table 4. Free Radical Scavenging Activity of Different South African Red and White Cultivar Wines

wine	TAA_{ABTS}[a]	AP_{ABTS}[b]	EC_{50}[c]	ISR[d]	RSE[e]	TAA_{DPPH}[a]	AP_{DPPH}[b]
red							
Pinotage	15.286 ab[f] (± 2.964)[g]	6.52 a (± 0.32)	67.88 ab (± 12.90)	0.0340 a (± 0.0058)	0.507 a (± 0.072)	11.913 ab (± 2.355)	5.11 a (± 0.72)
Cabernet Sauvignon	15.073 ab (± 1.392)	6.45 a (± 0.38)	63.56 b (± 14.52)	0.0321 a (± 0.0070)	0.516 a (± 0.096)	12.390 a (± 3.243)	5.24 a (± 0.92)
Merlot	15.757 a (± 2.412)	6.32 a (± 0.30)	70.58 ab (± 11.08)	0.0336 a (± 0.0043)	0.480 a (± 0.043)	12.133 a (± 2.462)	4.84 a (± 0.37)
Shiraz	14.851 ab (± 2.617)	6.18 a (± 0.31)	78.09 a (± 10.88)	0.0349 a (±0.0056)	0.455 a (± 0.095)	11.517 ab (± 2.586)	4.76 a (± 0.52)
Ruby Cabernet	13.177 b (± 2.742)	6.53 a (± 0.57)	73.86 ab (± 9.96)	0.0341 a (± 0.0031)	0.466 a (± 0.051)	9.510 b (± 2.058)	4.73 a (± 0.72)
av	14.916	6.39	70.60	0.0337	0.486	11.608	4.95
white							
Chenin blanc	0.800 b (± 0.251)	3.25 b (± 0.39)	157.54 a (± 28.27)	0.0232 b (± 0.0039)	0.150 b (± 0.026)	0.544 b (± 0.200)	2.22 ab (± 0.54)
Colombard	0.896 ab (± 0.255)	3.30 ab (± 0.44)	160.81 a (± 15.39)	0.0268 a (± 0.0038)	0.169 ab (± 0.033)	0.532 b (± 0.191)	1.95 b (± 0.34)
Sauvignon blanc	0.919 ab (± 0.175)	3.43 ab (± 0.43)	133.52 b (± 14.40)	0.0231 b (0.0023)	0.175 ab (± 0.027)	0.631 ab (± 0.171)	2.37 a (± 0.59)
Chardonnay	1.060 a (± 0.140)	3.62 a (± 0.30)	127.15 b (± 7.22)	0.0243 ab (± 0.0033)	0.192 a (± 0.028)	0.719 a (± 0.096)	2.46 a (± 0.27)
av	0.939	3.40	143.84	0.0240	0.170	0.626	2.27

[a] TAA (total antioxidant activity) as mM Trolox equiv measured using the $ABTS^{\bullet+}$ or $DPPH^{\bullet}$ scavenging assay. [b] AP (antioxidant potency) = TAA (mM Trolox) × 1000/total phenols (mg of gallic acid equiv/L). [c] EC_{50} = total phenol concentration of wine in mg of gallic acid equiv/L required to obtain 50% scavenging. [d] The ISR (initial scavenging rate) of wines was estimated as the negative of the slope of the line for $[DPPH^{\bullet}]$ against time for the first minute of the reaction. [e] RSE (radical scavenging efficiency) = ISR × 1000/EC_{50}. [f] Averages in a column followed by different letters differ significantly ($P < 0.05$). Data for red and white wines were analyzed separately. [g] Standard deviation.

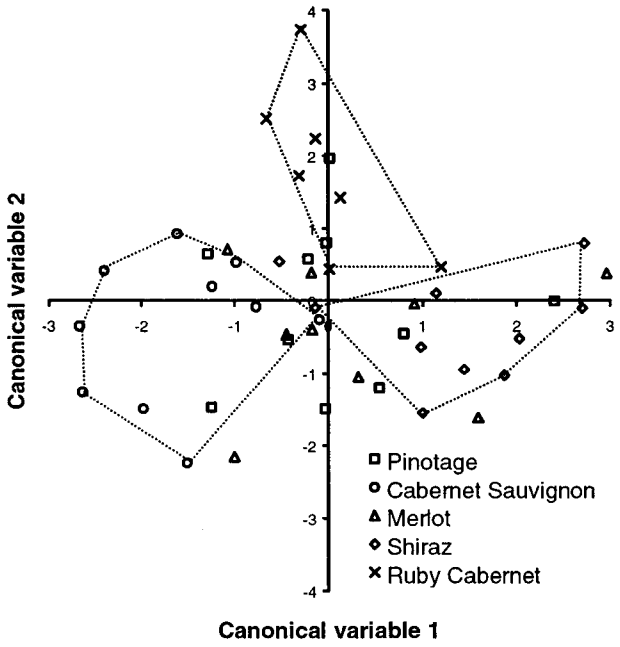

Figure 1. Canonical discriminant analysis plot for red wines using the following parameters: total phenol, monomeric anthocyanin (red wine only), flavanol, flavonol, and tartaric acid ester contents, as well as TAA_{ABTS}, EC_{50}, ISR, RSE, and TAA_{DPPH}.

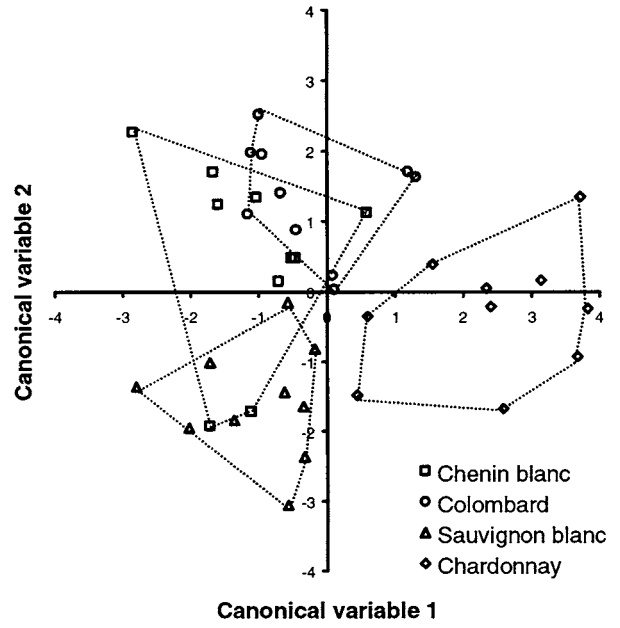

Figure 2. Canonical discriminant analysis plot for white wines using the following parameters: total phenol, monomeric anthocyanin (red wine only), flavanol, flavonol, and tartaric acid ester contents, as well as TAA_{ABTS}, EC_{50}, ISR, RSE, and TAA_{DPPH}.

the data set, good differentiation of the three remaining white cultivar wines was still obtained.

Stepwise variable selection was also used to attempt better differentiation of cultivar wines. In the case of red wines, total phenol, tartaric acid ester, and monomeric anthocyanin content were selected for the first and second canonical variables, explaining 64.5 and 34.7% of the variation, respectively. For the white wines, the selected variables were tartaric acid ester, flavanol, and flavonol content, as well as EC_{50}, with the first and second canonical variables explaining 66.6 and 29.8% of the variation, respectively. Using this technique, less differentiation was obtained than when all parameters were used (data not shown). In the case of the red cultivar wines, Ruby Cabernet could only be differentiated from Shiraz, whereas Shiraz and Cabernet Sauvignon were differentiated from each other. In the case of white cultivar wines, Chenin blanc could not be differentiated from Colombard or Sauvignon blanc wines.

DISCUSSION

Phenolic Composition of Wine. The influence of sulfur dioxide on the total phenol assay has been reported previously (*32*, *33*). In the present study, however, this influence has been found to be minimal (**Table 1**). A possible explanation could be the manner in which samples were prepared. As the samples were frozen until analyses and sonication of the defrosted samples was used to dissolve any precipitates present, it is possible that free sulfur dioxide in the wines may have been released from the wine into the atmosphere. The average total phenol content of red wine was ~8 times higher than that of white wine (**Table 2**). The reason for this is the presence of anthocyanins in red wine and also the better extraction of the other phenolic compounds from the grape pomace during the fermentation of red wine on the skins and seeds (*14*, *15*). High standard deviations were observed for the different phenolic groups monitored for the same cultivar. This is attributed to differences in climate (*34*), soil type (*35*), vinification techniques (*14*), and time of wood maturation (only for red wines) (*36*) as they were purchased from different areas and wineries around the Western Cape. The climatic conditions of the different areas vary from cool to warm during the growth and ripening seasons.

Contribution of Ascorbic Acid to the Free Radical Scavenging Activity. The ascorbic acid content of white wines (**Table 3**) was determined as it is often added to prevent unacceptable oxidative browning or pinking reactions in white wines. As ascorbic acid has a high antioxidant activity in both the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging assays (*29*, *30*), the TAA of the wines with added ascorbic acid could therefore not be solely attributed to their phenolic content. Although Saucier and Waterhouse (*32*) found no synergistic effect when ascorbic acid was added to a (+)-catechin solution, the contribution of ascorbic acid to the total phenol content might not be additive as wine is a complex mixture of different phenolic compounds and other constituents. For the purpose of this study, the data pertaining to the two Sauvignon blanc and Colombard wines, containing a high amount of ascorbic acid, were therefore removed from the data set and TAA values for other wines were used unadjusted, as ascorbic acid content of remaining wines contributed <10% to the total antioxidant activity.

Total Antioxidant Activity of Wines Using the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ Scavenging Assays. The higher TAA_{ABTS} and TAA_{DPPH} values ("as-is" basis) for red wines than for white wines were consistent with their higher total phenol content (**Table 4**). However, according to AP_{ABTS} and AP_{DPPH} the potency of red wine total phenols was twice that of white wines. The average EC_{50} value for the red wines was ~50% less than that of the white wines. Red wine phenolic constituents not only scavenged more $DPPH^{\bullet}$ than those of white wine, but the ISR for $DPPH^{\bullet}$ of red wine was also higher. This is in agreement with a study by Sánchez-Moreno et al. (*37*), indicating that red wine scavenged $DPPH^{\bullet}$ more quickly than white wine. The average RSE of red wine was almost 3 times as much as that of white wine due to its higher scavenging activity and ISR. Sánchez-Moreno et al. (*37*) also found that red wine has a much higher antiradical efficiency than white wine. They defined antiradical efficiency as the ratio of EC_{50} to the time required to completely scavenge $DPPH^{\bullet}$ at the EC_{50} concentration. These

data imply that the red wine total phenols are more effective free radical scavengers than those of white wine. This could be attributed to a concentration effect of specific phenolic groups, the presence of individual phenolic compounds with high potency, and/or synergistic interactions of specific combinations of phenolic compounds.

The higher concentration of monomeric anthocyanins in Ruby Cabernet did not compensate for its lower flavanol and total phenol contents in terms of total antioxidant activity compared to the other red cultivar wines. This can be explained by the relative efficacy of flavanols and anthocyanins in the $ABTS^{\bullet+}$ scavenging assay. (+)-Catechin and (−)-epicatechin have TEAC values of 2.4 and 2.5 mM Trolox, respectively, whereas malvidin 3-glucoside (the major anthocyanin in red wine) has a TEAC value of 1.78 mM Trolox (*31*). With regard to the white cultivar wines, the higher total phenol, flavanol, flavonol, and tartaric acid ester content of Chardonnay explains its higher total antioxidant activity compared to Chenin blanc.

The lack of differentiation among red cultivar wines in the expression of total antioxidant activity (TAA) on a similar total phenol basis (AP_{ABTS} and AP_{DPPH}) could be due to small differences in the efficacy of the total phenols of the different cultivars. Although the RSE value in the present study takes into account both the EC_{50} with significant differences between cultivars and the ISR, no significant differences in RSE were found between the different red cultivar wines. The higher AP_{ABTS} value of Chardonnay compared to Chenin blanc and the higher AP_{DPPH} and EC_{50} values of Chardonnay and Sauvignon blanc compared to Colombard indicate a higher efficacy of their total phenols, possibly due to differences in flavanol and tartaric acid ester contents. The similar patterns of the RSE and EC_{50} values indicate that the concentration of the phenolic compounds needed to scavenge 50% of $DPPH^{\bullet}$ was the most important factor determining the RSE of white wines and not the ISR.

The high ISR of Colombard compared to Sauvignon blanc and Chenin blanc could not be explained by the concentration of the individual phenolic groups alone, as they were similar. On the other hand, the ISR values of Chardonnay and Chenin blanc did not differ, although they represent the respective high and low values for the different phenolic groups. Therefore, the contribution of individual phenolic compounds of high potency or interactions of combinations of phenolic compounds could be of importance, and further study on this aspect is needed.

The total antioxidant activities for both red and white wines were of the same order of magnitude using the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging assays when expressed in terms of Trolox. Similar trends were observed for these two assays in terms of differences among cultivars. However, some minor differences existed that could be of importance in the application of the two methods in subsequent studies. Compared to TAA_{DPPH}, TAA_{ABTS} could not differentiate between the antioxidant activity of Cabernet Sauvignon and Ruby Cabernet. No difference in terms of discrimination between cultivar wines exists between the assays with regard to the antioxidant potency of the red wine total phenols. In the case of white wines, both the TAA_{DPPH} and AP_{DPPH} distinguished between Chardonnay and Colombard, whereas no effect could be observed using the $ABTS^{\bullet+}$ scavenging assay. The differences in differentiation obtained with these methods cannot be attributed to the reaction medium as both of these assays are carried out in an alcoholic medium (ethanol and methanol for the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging assays, respectively). The size and accessibility of the radical center of the synthetic radicals involved with the phenolic compounds may play a role in the ability to discriminate between wines (*38*).

TAA values of South African wines (red = 9.2−19.5 mM Trolox, white = 0.5−1.4 mM Trolox) analyzed in this study were in the range obtained for wines from other wine-producing countries such as France (red = 9.6−29.9 mM Trolox, white = 1.7−3.7 mM Trolox) (*39*), Italy (red = 6.1−19.8 mM Trolox, white = 0−3.6 mM Trolox) (*4*, *23*), Canada (red = 7.5−28.6 mM Trolox) (*40*), and Spain (red = 14 mM Trolox, white = 0.8 mM Trolox) (*41*). Differences between TAA values reported for wines from different countries could be due not only to environmental factors and technological aspects related to the vinification process, but also to differences in the experimental procedure used for measuring the antioxidant activity. These include differences in radical generation methods and reaction time. Radical generation strategies differ, that is, generation of radicals in the presence of antioxidant samples using ferrylmyoglobin (*4*, *23*, *39*, *40*) or pregeneration of radicals using chemical oxidants (*41*) such as used in the present study. When the radical cations are generated enzymatically in the presence of the antioxidant sample, more than one effect could account for the antioxidant activity measured (*42*, *43*). Antioxidant molecules could then scavenge radical cations or inhibit the generation of radical cations by inhibition of the enzyme, causing overestimation of antioxidant activity. Another factor is that different reaction times are used such as 1 min (*23*), 3 min (*4*, *40*), 4 min (as in the present study) (*39*), or 6 min (*41*). These factors stress the importance of a standardized method for the measurement of antioxidant activity of wines to allow reliable comparison of data generated in different laboratories. In the present study, the two free radical scavenging methods used to measure the antioxidant activity of wines gave similar results. The $DPPH^{\bullet}$ scavenging assay was, however, more effective for the differentiation between different cultivar wines in this study. Due to practical advantages such as assay time and ease of automation, more researchers currently use the $ABTS^{\bullet+}$ scavenging assay to determine the free radical scavenging activity of wines and foods. This assay would, therefore, be best suited for standardization for use in screening large numbers of samples for comparison between countries and laboratories. The use of a standardized method to compare wines from different countries is needed before the reliable use of antioxidant activity as a quality parameter for wines becomes feasible.

Correlation Analysis of Data. Total phenol content was the best predictor for TAA_{ABTS} values of red and white wines, whereas the flavanol content of red wines and the tartaric acid ester content of white wines appear to have the most predictive value for TAA_{ABTS} when phenolic groups are considered. In previous studies, the antioxidant activity of wine, utilizing the low-density lipoprotein (*44*, *45*) and free radical scavenging (*4*, *23*) assays, was also found to correlate with total phenol and flavanol contents of red wine. Although monomeric anthocyanins are effective free radical scavengers in the $ABTS^{\bullet+}$ scavenging assay (*31*), no correlation with TAA_{ABTS} values was found. Landrault et al. (*39*) found no correlation between the $ABTS^{\bullet+}$ scavenging activity of 34 red wines and their individual anthocyanin contents. However, it was shown that anthocyanins correlated with the inhibition of low-density lipoprotein (LDL) oxidation of red wines (*44*, *46*) and grape extracts (*45*). It is necessary to keep in mind that differences in the hydrophobic/hydrophilic nature of the environment could play a role in the measurement of antioxidant activity.

The lack of correlation of monomeric anthocyanin content with TAA value and the importance of flavanols in contributing to the TAA for red wines explains the low TAA values for Ruby Cabernet, which has a high anthocyanin content but a low flavanol content. On the basis of the correlations obtained, the difference in TAA_{ABTS} between Chardonnay and Chenin blanc could be mostly attributed to the contribution of flavonols and tartaric acid esters with a lesser contribution by flavanols. The low correlation coefficients for flavanols and flavonols in white wines could be attributed to a concentration effect as low amounts were found. Another factor to consider is that the estimation of phenolic group contents is based on a similar basic structure, although substitution patterns may differ. The specific substitution patterns of individual compounds are closely related to antioxidant activity (*31*), but do not affect the estimation of phenolic group content to the same extent (*25*).

Canonical Discriminant Analysis of Data. Discriminant analysis has been used previously to discriminate between cultivar wines on the basis of pigments and flavonoid compounds (*20*), as well as volatile components (*21*). In the present study, canonical discriminant analysis, using different parameters for phenolic composition and antioxidant activity, discriminated between certain red and white cultivar wines. Ruby Cabernet could be clearly distinguished from the other red cultivar wines as could be expected from its phenolic profile (**Figure 1**). However, the possible addition of up to 25% of other cultivar wines could contribute to the relatively poor differentiation of the other red cultivar wines. With regard to the white cultivar wines, Chenin blanc could not be clearly distinguished from Colombard and Sauvignon blanc, although their phenolic compositions differ (**Figure 2**). Data points for Chardonnay and Chenin blanc were, however, separated, as can be expected from their substantially different phenolic compositions and antioxidant behaviors.

In conclusion, the present study indicates differences in phenolic composition and antioxidant activity of different cultivar wines despite differences in vinification techniques and climatic conditions. In addition, the total antioxidant activity of wines correlated well with their phenolic contents and compositions, whereas different phenolic groups were not equal contributors to the total antioxidant activity. Different combinations of phenolic compounds and/or synergistic interactions between them are likely to affect the outcome when the free radical scavenging activities of different cultivar wines are compared. Future research should include investigation of the effect of different individual phenolic compounds on the antioxidant activity of wines.

ABBREVIATIONS USED

$ABTS^{\bullet+}$, 2,2′-azinobis(3-ethylbenzothialozinesulfonic acid) radical cation; $DPPH^{\bullet}$, 2,2-diphenyl-1-picrylhydrazyl radical; TAA, total antioxidant activity; GAE, gallic acid equivalents; Mv-3-glc, malvidin 3-glucoside; CE, catechin equivalents; QE, quercetin equivalents; CAE, caffeic acid equivalents; AP, antioxidant potency; EC_{50}, the total phenol concentration of wine required to scavenge 50% of the initial $DPPH^{\bullet}$ in the reaction mixture; ISR, initial scavenging rate; RSE, radical scavenging efficiency; TEAC, Trolox equivalent antioxidant capacity; LDL, low-density lipoprotein.

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