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# Phenolic Contents and Antioxidant Activities of Major Australian Red Wines throughout the Winemaking Process

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Three Australian red wine types (Shiraz, Cabernet Sauvignon, and Merlot) were analyzed for antioxidant activity and a range of phenolic component contents using various spectral methods. More than half of the total phenolic compounds were tannins, whereas monomeric anthocyanins and flavonols were present in much lesser amounts (<10%). The evolution of phenolic contents and the respective antioxidant activities in wine samples from all stages of winemaking showed progressive changes toward those of commercial wines. The antioxidant activity of the wines in DPPH and ABTS assays was positively correlated with total phenolic contents and tannins. Comparisons of the three wine varieties based on their individual phenolic component groups and antioxidant activities showed limited differences between the different varieties. However, when all of the variables were combined in a principal component analysis, variety differentiation was observed. The three varieties of red wines all contained similar and high concentrations of antioxidants despite differences in grape variety/maturity and winemaking process, suggesting that related health benefits would accrue from all of the red wines studied.

**KEYWORDS: Red wine; phenolic contents; anthocyanins; tannins; flavonols; antioxidant activity**

## INTRODUCTION

There have been extensive reports on the positive effect of plant-based foods and beverages in reducing the risk of chronic diseases such as cancer, cardiovascular diseases, diabetes, hypertension, and many other aging-related diseases (*1*). Much of the protective effects of these foods and beverages have been attributed to their phenolic compounds. In their natural form, phenolic compounds provide an array of functions such as protection against environmental stress (e.g., excess UV-B rays), herbivores, and pathogens, often through their antioxidant and metal chelation properties. Thus, there is a strong possibility that consuming these plants as food or drink may extend their protective effects toward humans.

The presence of antioxidant phenolic compounds in wines, especially red wines, is well established (*2*−*8*). Quick and simple in vitro methods such as ABTS [2,2′-azinobis (3-ethylbenzothiazol-6-sulfonic acid) diammonium salt] (*2*−*5*, *8*) and DPPH (2,2-diphenyl-1-picrylhydrazyl) (*3*, *8*) assays are commonly used for antioxidant quantification in wine samples. Other methods include FRAP (ferric reducing/antioxidant power) (*2*, *6*), ORAC (oxygen radical scavenging activity) (*2*, *3*), and ESR (electron spin resonance) (*7*, *8*) assays. Total phenolic content in wine is often determined using the Folin−Ciocalteu method (*3*−*5*, *7*, *8*).

Red wine contains up to 3000 mg/L of phenolic compounds (*5*), which can be grouped into hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols, anthocyanins, stilbenes, and tannins. Most wines that are available for consumption are those that have undergone an elaborate process of winemaking or vinification, which includes crushing of grape berries, alcoholic (primary) and malolactic acid fermentation of the juice, aging of the wine in oak barrels or in steel tanks, and finally bottling. Each of the winemaking stages involves various chemical changes, which ultimately modify the phenolic composition and antioxidant activities of the wine (*7*, *9*, *10*). Previous studies in this area investigated only part and not the whole of the winemaking process, such as the fermentation (*7*) or maturation and aging stages (*9*, *10*).

Three major red wine varieties, namely, Shiraz, Cabernet Sauvignon, and Merlot, were studied and compared with a white wine variety, Chardonnay. These three varieties are the most common dark-skinned *Vitis vinifera* grape varieties used for the production of red wines in Australia, accounting for 83.9% of red wine grapes produced in Australia (*11*). The first aim of the present study was to follow the changes in individual phenolic component classes (monomeric and polymeric anthocyanins, tannins, flavonols) and antioxidant activity of wines from the start (crush) until the end (bottle) of the winemaking process, over a span of almost 2 years. Sourcing samples directly from wineries as well as retail products provided a realistic data set, based on commercial winemaking processes. The second aim was to use phenolic compound profiles to identify relationships between antioxidant activity, grape cultivar, and the winemaking process using both winemaking and commercial samples.

## MATERIALS AND METHODS

**Wine Samples.** Commercial red wines (var. Shiraz, Cabernet Sauvignon, and Merlot) produced in Australia were purchased from local

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bottle shops. Red wine samples (var. Shiraz and Cabernet Sauvignon) from different stages of winemaking were kindly donated in 2006 by Sirromet (Mount Cotton, Queensland) and Ballandean Estate (Stanthorpe, Queensland) wineries. Unfortunately, in 2006, unblended Merlot wine was not produced at these two wineries due to the Merlot harvest yield being insufficient. The samples (200 mL) were collected during crushing (harvest day), after alcoholic fermentation (5−16 days postharvest), after malolactic fermentation (45−51 days postharvest), after oak aging (430−470 days postharvest), and from the bottle (589−622 days postharvest).

**Chemicals.** Folin−Ciocalteu reagent, sodium carbonate anhydrous, sodium acetate, methyl cellulose, ammonium sulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazol-6-sulfonic acid) diammonium salt (ABTS), and potassium persulfate were purchased from Sigma-Aldrich (St. Louis, MO) and were of analytical grade. Malvidin-3-glucoside chloride, malvidin-3-*O*-galactoside chloride, delphinidin-3-*O*-glucoside chloride, quercetin-3-*O*-galactoside, procyanidin B1, and procyanidin B2 were purchased from Extrasynthese (Genay, France); all other phenolic standards were purchased from Sigma-Aldrich. Other chemicals and solvents not specified above were all of analytical grade and obtained from local suppliers. The water used in all analyses was triply deionized and then further deionized using a Milli-Q water system from Millipore Corp. (Bedford, MA).

**Determination of Total Phenolics.** The total phenolic content in red wine samples was determined as gallic acid equivalents (GAE) using the Folin−Ciocalteu method (*12*).

**Determination of Monomeric Anthocyanins.** Monomeric anthocyanin content was measured by the pH differential method as described by Giusti and Wrolstad (*13*). The total monomeric anthocyanin concentration was expressed in malvidin-3-glucoside equivalents (mg of MGE/L). Molar absorbance value ($\varepsilon$) for malvidin-3-glucoside was obtained from the literature (28000 L/cm·mg) (*14*).

**Determination of Polymeric Anthocyanins.** The contribution of polymerized anthocyanins toward the total pigment color was determined by measuring the remaining absorbance after the addition of a bleaching agent (sodium bisulfite) to wine samples. The assay used was as described by Giusti and Wrolstad (*13*).

**Determination of Total Tannins.** The total tannins in wines were determined according to the methyl cellulose precipitation (MCP) method of Sarneckis et al. (*15*). Briefly, 900 $\mu$L of 0.04% methyl cellulose (w/v in Milli-Q water) was added to 100 $\mu$L of wine sample and mixed thoroughly. The mixture was allowed to stand at room temperature for 2−3 min, after which saturated ammonium sulfate solution (600 $\mu$L) and Milli-Q water (1.425 mL) were added. The mixture was again mixed and allowed to stand at room temperature for 10 min. The precipitate formed was separated from the solution by centrifugation for 5 min at 2500*g* using an Eppendorf 5702 R centrifuge (Hamburg, Germany). The supernatant was transferred carefully into a quartz cuvette, and the absorbance was read against a blank at 280 nm. The blank sample was prepared similarly, using 14% ethanol instead of a wine sample. A control sample was prepared for each sample, which underwent the same procedure, except that the methyl cellulose was replaced by Milli-Q water. Tannin concentration was expressed in epicatechin equivalents (mg of EE/L) and calculated from an epicatechin calibration curve prepared from the absorbance readings of epicatechin solution (2.5−150 mg/L in Milli-Q water) at 280 nm. The calculations used were

$$\mathrm{Abs_{tannin}} = \mathrm{Abs_{control}} - \mathrm{Abs_{supernatant}}$$

$$\text{tannin concentration (mg of EE/L)} = [\text{tannin}] \times \mathrm{DF}$$

where [tannin] is the tannin concentration calculated from the epicatechin calibration curve and DF is the dilution factor (DF = 40).

**Determination of Total Flavonols.** Total flavonol content was estimated using a modification of the method of Popova et al. (*16*). A wine sample (100 $\mu$L), diluted 1:2 with 14% ethanol, was introduced into a 1.5 mL disposable cuvette and 900 $\mu$L of aluminum chloride ($AlCl_3$) solution (1% in Milli-Q water) added. Because wine gives some background absorbance values at 432 nm, parallel control samples were prepared in which 900 $\mu$L of Milli-Q water was added to the 100 $\mu$L of diluted wine sample in place of the $AlCl_3$ solution. The mixtures were vortexed and left to stand for 10 min at room temperature. Following this, the absorbance at 432 nm was read against a blank, which was prepared similarly but with the wine sample replaced with Milli-Q water. Final flavonol absorbance was calculated by subtracting the value of $\mathrm{Abs_{control}}$ from the $\mathrm{Abs_{sample}}$. The concentration of total flavonols was estimated from a calibration curve, constructed by plotting known concentrations of quercetin (25−200 mg/L in 14% ethanol) in the sample−$AlCl_3$ mixture, against their absorbance at 432 nm.

**Determination of DPPH Radical Scavenging Activity.** The DPPH assay was modified from the method of Brand-Williams et al. (*17*). On the day of analysis, a purple DPPH radical solution (25 mg/L) was prepared in methanol, and the red wine samples were diluted in 14% ethanol to 5, 10, 15, 20, and 30% (v/v). In a 1.5 mL disposable cuvette, the prepared DPPH (975 $\mu$L) solution was added to each diluted wine sample (25 $\mu$L). The solutions were vortexed, the cuvettes were sealed using Parafilm and incubated at 30 °C for 30 min, and the absorbance at 515 nm ($\mathrm{Abs_{sample}}$) was read against a blank (methanol). The initial absorbance (prior to incubation) of 14% ethanol (25 $\mu$L) added to the DPPH solution (975 $\mu$L) was used as a reference absorbance ($\mathrm{Abs_{control}}$). The percentage of DPPH radical remaining after 30 min of incubation was calculated as follows:

$$\%\ \mathrm{DPPH_{rem}} = \frac{\mathrm{Abs_{sample}}\ \text{at}\ t = 30\ \text{min}}{\mathrm{Abs_{control}}\ \text{at}\ t = 0\ \text{min}} \times 100$$

A plot of % $\mathrm{DPPH_{rem}}$ value versus wine concentration (%) was constructed, and the concentration of wine required to reduce the original DPPH radical to half was estimated. This value is called the "effective concentration" or $EC_{50}$ value. The antioxidant capacity of a sample was expressed as a function of the wine concentration (%, v/v).

**Determination of ABTS Radical Scavenging Activity.** The method for measuring the antioxidant activity of wine samples was modified from the original method of Re et al. (*18*). The colored (dark green) ABTS radical cation stock solution was prepared by incubation of 5 mL of ABTS solution (7 mM in Milli-Q water) with 88 $\mu$L of potassium persulfate (140 mM in Milli-Q water) for 12−16 h at room temperature (23 °C). ABTS radical solution was prepared fresh on the day of analysis by diluting the stock solution (1:88 ratio) with phosphate buffer, pH 7.4 (75 mM), to an absorbance of 0.70 (±0.02) at 734 nm. Wine samples were diluted using 14% ethanol to 0.5, 1.0, 1.5, and 2.0% (v/v). In a 1.5 mL disposable cuvette, 900 $\mu$L of the ABTS radical cation solution was added to 10 $\mu$L of wine sample and mixed. The absorbance of each sample was measured against a blank (phosphate buffer) at 734 nm after incubation at 37 °C for 30 min. The antioxidant activity of the sample was determined on the basis of its $EC_{50}$ value (%, v/v), similar to the method used in the DPPH assay.

Similar DPPH and ABTS procedures were also used to determine the $EC_{50}$ value of Trolox, a water-soluble vitamin E analogue. On the basis of the $EC_{50}$ value of Trolox, the antioxidant activity of the wine samples was expressed as millimolar Trolox equivalents.

**Statistical Analysis.** Unless otherwise stated, all of the experimental results were expressed as mean ± standard deviation of three determinations. A one-way ANOVA was performed on the means to determine whether they differed significantly. $P$ values of $<0.05$ were regarded as significant. The degree of linear relationship between two variables was measured using the Pearson product moment correlation coefficient ($r$). Variables with $r$ values close to 0 indicate no linear relationship, whereas $r$ values that are close to 1 suggest a strong linear relationship. Principal component analysis (PCA) based on a correlation matrix was performed to simplify the data set and also to investigate if the parameters were able to classify the wines according to their varieties. All statistical analysis was done using Minitab 15 (Minitab Inc.).

## RESULTS AND DISCUSSION

**Phenolic Composition of Commercial Wines. Table 1** lists the concentrations of different phenolic classes as determined by the various spectral methods. The red wine samples (Shiraz, Cabernet Sauvignon, and Merlot) contained 4−5 times more phenolic compounds than the white wine sample (Chardonnay), as expected (**Table 1**). On average, Merlot contained slightly higher phenolic levels than Cabernet Sauvignon, which was slightly higher than Shiraz, but the range was large for each of the varieties, resulting in no significant differences ($P > 0.05$) in terms of phenolic composition among the three wine varieties. In addition,

**Table 1.** Phenolic Composition of Australian Red Wines[a]

| wine sample | | phenolic composition | | | | | AOX (mM Trolox equiv) | |
|---|---|---|---|---|---|---|---|---|
| code | vintage (state) | TPC (mg/L) | TT (mg/L) | TMA (mg/L) | PAF (%) | TF (mg/L) | DPPH assay | ABTS assay |
| S1 | 2004 (SA) | 2510 | 1726 | 327 | 53 | 22.51 | 13.95 | 17.12 |
| S2 | 2004 (VIC) | 2023 | 1211 | 243 | 51 | 7.53 | 11.16 | 14.51 |
| S3 | 2005 (NSW) | 1893 | 1219 | 299 | 41 | 27.61 | 10.80 | 13.06 |
| S4 | 2004 (WA) | 2443 | 1222 | 259 | 51 | 6.21 | 12.53 | 12.77 |
| S5 | 2002 (WA) | 2048 | 1216 | 119 | 60 | 20.29 | 12.09 | 12.53 |
| S6 | 2003 (NSW) | 1737 | 1037 | 86 | 61 | 27.61 | 10.43 | 13.49 |
| S7 | 2004 (NSW) | 2109 | 1024 | 289 | 48 | 4.07 | 13.26 | 14.62 |
| S8 | 2002 (QLD) | 2134 | 1507 | 97 | 70 | 16.26 | 13.72 | 14.21 |
| S9 | 2002 (QLD) | 1650 | 1331 | 71 | 70 | 13.21 | 11.54 | 24.25 |
| S10 | 2005 (QLD) | 2030 | 1312 | 177 | 48 | 28.35 | 15.76 | 24.81 |
| S11 | 2005 (QLD) | 2131 | 1414 | 215 | 52 | 22.51 | 17.82 | 24.58 |
| pooled means | | 2064 ± 258 b | 1293 ± 203 a | 198 ± 93 a | 55 ± 9.4 a | 17.8 ± 9.0 a | 13.01 ± 2.24 b | 16.90 ± 5.06 a |
| % CV | | 12.5 | 15.7 | 47.0 | 17.1 | 50.6 | 17.3 | 29.9 |
| CS1 | 2004 (WA) | 2340 | 1066 | 182 | 51 | 13.24 | 15.29 | 16.48 |
| CS2 | 2004 (SA) | 2150 | 1233 | 191 | 48 | 6.63 | 14.30 | 15.93 |
| CS3 | 2004 (NSW) | 2739 | 1832 | 249 | 47 | 9.59 | 17.78 | 14.53 |
| CS4 | 2004 (NSW) | 2322 | 1457 | 267 | 48 | 17.13 | 15.15 | 16.48 |
| CS5 | 2004 (VIC) | 2585 | 1964 | 208 | 56 | 15.93 | 13.99 | 19.20 |
| CS6 | 2003 (NSW) | 2513 | 1428 | 118 | 47 | 1.48 | 16.61 | 18.41 |
| CS7 | 2003 (NSW) | 3503 | 2261 | 160 | 67 | 6.54 | 21.39 | 18.90 |
| CS8 | 2004 (VIC) | 2473 | 1276 | 246 | 56 | 14.97 | 15.12 | 19.10 |
| CS9 | 2005 (QLD) | 1836 | 1058 | 189 | 55 | 10.16 | 16.75 | 22.18 |
| CS10 | 2005 (QLD) | 2073 | 1541 | 189 | 54 | 17.41 | 15.29 | 16.48 |
| CS11 | 2004 (QLD) | 1673 | 1115 | 89 | 69 | 5.72 | 14.30 | 15.93 |
| pooled means | | 2382 ± 490 ab | 1476 ± 395 a | 190 ± 54.0 a | 54 ± 7.6 a | 10.80 ± 5.32 b | 15.90 ± 2.27 a | 18.85 ± 2.99 a |
| % CV | | 20.6 | 26.8 | 28.4 | 14.1 | 49.2 | 14.3 | 15.9 |
| M1 | 2005 (SA) | 1886 | 789 | 211 | 39 | 4.16 | 10.75 | 16.61 |
| M2 | 2004 (NSW) | 2137 | 964 | 85 | 54 | 0.00 | 10.67 | 17.72 |
| M3 | 2004 (NSW) | 2587 | 1346 | 107 | 68 | 8.93 | 14.09 | 15.24 |
| M4 | 2004 (SA) | 2758 | 1522 | 155 | 61 | 11.48 | 17.05 | 14.38 |
| M5 | 2004 (SA) | 3610 | 1785 | 123 | 73 | 8.93 | 19.50 | 13.34 |
| M6 | 2003 (NSW) | 2604 | 1251 | 137 | 67 | 0.00 | 16.82 | 15.27 |
| M7 | 2003 (VIC) | 2261 | 1175 | 101 | 69 | 6.26 | 13.91 | 17.23 |
| M8 | 2004 (NSW) | 2684 | 1229 | 122 | 66 | 0.00 | 18.30 | 19.74 |
| M9 | 2005 (QLD) | 2131 | 1750 | 163 | 63 | 32.06 | 15.78 | 29.40 |
| pooled means | | 2518 ± 506 a | 1312 ± 333 a | 134 ± 38.4 b | 62 ± 10.2 a | 7.98 ± 10.02 b | 15.21 ± 3.12 ab | 17.66 ± 4.80 a |
| % CV | | 20.1 | 25.4 | 28.6 | 16.4 | 125.6 | 20.5 | 27.2 |
| C1 | 2004 (NSW) | 502 | ND | ND | ND | ND | 1.47 | 2.05 |
| C2 | 2005 (SA) | 388 | ND | ND | ND | ND | 1.61 | 1.41 |
| pooled means | | 445 ± 80 c | ND | ND | ND | ND | 1.54 ± 0.10 c | 1.73 ± 0.45 b |
| % CV | | 18.0 | NA | NA | NA | NA | 6.5 | 26.2 |

[a] Values are expressed as mean ± standard deviation of $n = 3$ experimental replicates. Pooled means in a column followed by different letters differ significantly ($P < 0.05$). ND, not detected; NA, not applicable; TPC, total phenolic content (gallic acid equivalent); TT, total tannins (epicatechin equivalent); TMA, total monomeric anthocyanins (malvidin-3-glucoside equivalent); PAF, polymeric anthocyanin fraction; TF, total flavonols (quercetin equivalent); WA, Western Australia; SA, South Australia; VIC, Victoria; NSW, New South Wales; QLD, Queensland; S, Shiraz; CS, Cabernet Sauvignon; M, Merlot; C, Chardonnay. Antioxidant activity (AOX) was expressed as mM Trolox equivalents per 100% wine (mM TE/wine).

comparison of the wines based on their geographical source (Australian state) and vintage years showed no significant differences ($P > 0.05$), most probably due to the limited number of samples for each group. The results obtained for the wine phenolic composition were comparable to those reported elsewhere for the same variety of wines from France (*5*), South America (*4*), and Spain (*9*). With values of >2000 mg of GAE/L, a glass of red wine (~100 mL) can provide a total phenolic amount equivalent to approximately half a liter (480 mL) of red grape juice.

Tannin makes up >50% of the phenolic compounds in the red wine samples (**Table 1**). Red or black grapes contain about 1−2 mg of tannins per berry, with an equal fraction in the seeds and skin of Cabernet Sauvignon grapes and more in the seeds of Shiraz (75% of total) (*19*). The total amount of tannins measured in the present study was slightly lower than that reported by Mercurio et al. (*20*), using the same assay method. Although the average tannin contents of the three different varieties were not significantly different from each other ($P > 0.05$), these results followed a trend similar to earlier studies (*16*, *21*), with the highest tannin content found in Cabernet Sauvignon, followed by Merlot, and last by Shiraz. Apparently, different methods could also affect the amount of measurable tannins in wine.

**Table 2.** Comparison of Anthocyanin Contents in Grape Berries, Skins, and Wines

| total anthocyanins | wine variety | | | analytical method | ref |
|---|---|---|---|---|---|
| | Shiraz | Cabernet Sauvignon | Merlot | | |
| in berry (mg/kg of berries) | 2337 | 2150 | 1145 | HPLC-DAD | *28* |
| | 1065 | 1242 | 879 | HPLC-DAD | *24* |
| | 1514 | 1381 | 1142 | spectral | *23* |
| | 1521 | 1339 | 1160 | HPLC-DAD | *23* |
| in skin (mg/kg of lyophilized skin) | 43200 | 29600 | 46000 | spectral | *22* |
| | 27900 | 41300 | 65100 | HPLC-DAD | *22* |
| in skin (mg/kg of fresh skin) | 8222 | 6191 | 4777 | HPLC-DAD | *24* |
| in wine (mg/L) | 198 | 190 | 134 | spectral | present result[a] |
| | | 125 | 110 | spectral | *30* |
| in wine (mg/kg of grapes) | 692 | 568 | 497 | spectral | *23* |
| | 491 | 411 | 378 | HPLC-DAD | *23* |

[a] Total anthocyanin values were based on the total monomeric anthocyanin concentrations.

Harbertson et al. (*19*), who used a protein precipitation method on California red wines, reported much lower tannin contents (~600 mg/L). This difference can also be influenced by the variability (% CV) between different samples within the same variety, as shown in the present study (**Table 1**). Oak tannins are commonly added into the wine by the wineries and may have some contribution to the total tannin levels. However, their direct contribution is limited because the present MCP method (*15*) is aimed at estimating grape-based tannins.

The results showed that the concentrations of total monomeric anthocyanin in Merlot wines were significantly lower ($P < 0.05$) than those in the other two wine varieties (**Table 1**). Anthocyanins are present in small amounts in red wine (< 200 mg/L), when compared to the levels of tannins, but play a vital role in giving red wine its characteristic color. Anthocyanins are located in the vacuole of the outer layer of grape skin and extracted into the wine during the maceration and fermentation processes. The amount of extractable anthocyanins depends on the amount available in the grape skin, which to some extent is governed by the grape cultivar (**Table 2**). As reported by Arnous and Meyer (*22*), Merlot skin contains higher anthocyanin contents than Cabernet Sauvignon and Shiraz grapes. However, not all are extractable during conventional winemaking (ref *23* and the present study) or liquid-alcoholic extraction methods (*23*, *24*). Instead, most wines, fresh skin, and fresh berries from Merlot have lower anthocyanin contents than those from Cabernet Sauvignon and Shiraz (**Table 2**). Studies on different grape skins (*25*, *26*) suggested that the low anthocyanin extractability from Merlot skin may be caused by its higher level of skin cell wall material (firmer and thicker skin) and higher contents of polysaccharides and lignin.

More than 50% of the pigment color in wine comes from the polymerized anthocyanins (**Table 1**). High proportions of polymerized anthocyanins are common in aged red wines (*27*), as supported in the present study. Although not statistically significant ($P > 0.05$), Merlot wines appeared to contain more polymerized anthocyanin fractions compared to Shiraz and Cabernet Sauvignon wines. This difference may also partially explain the lower total monomeric anthocyanins in Merlot wines, as more anthocyanins are present in their polymeric forms.

Flavonols formed a very small fraction of the total wine phenolic content in the present study (<30 mg/L). Depending on variety, red-skinned grapes typically contain 3−80 mg of flavonols/kg of fresh grape skin, whereas white-skinned varieties contain much less (*28*). However, the final concentration in wine depends on the winemaking style, sample preparation prior to analysis, and the method of determination. The content of flavonols in Cabernet Sauvignon in this study (10.8 mg/L) was slightly lower than those reported by Castillo-Munoz et al. (*29*) for Spanish red wines, who employed HPLC as a means of separating and detecting individual flavonols. Although highly specific, the HPLC method requires reference standards for the identification and quantification of each individual flavonol. Cliff et al. (*30*) estimated the amount of flavonols directly by their absorbance values at 360 nm. This may lead to overestimation as there are other phenolic compounds (e.g., cinnamic acids, anthocyanins) that also have some absorbance at 360 nm. Thus, it is unsurprising that Cliff et al. (*30*) reported 3 times higher values of total flavonols (50−60 mg/L) than the data of the present study for their Cabernet Sauvignon and Merlot wines. A more specific spectral method of flavonol estimation is by complex formation with aluminum chloride, which was used in this study. This method has been used for many other types of samples such as honey, propolis (*16*), and flowers (*31*) and is rather specific to flavonols, because the aluminum complexation requires a 4-keto group and at least one neighboring (3- or 5-) hydroxyl group, which are common features of flavonols. Similar features are also present in flavones and flavanones, but these compounds are not common in red wines. The weakness of this method, when applied to red wine, is the presence of a reddish hue (background color) at the analytical wavelength (432 nm) in addition to the yellow color developed by the complex formation. The present study attempted to normalize this background color by subtracting a wine blank, but may also have removed some of the complex absorbance, hence underestimating the actual flavonol content.

Approximately 40% of the total phenolics in the red wine sample were not accounted for in the component groups that were determined as described above. On the basis of other studies (*5*, *7*, *9*, *10*), the remaining phenolics are likely to be hydroxybenzoic (e.g., gallic acid) and hydroxycinnamic acids (e.g., caftaric and caffeic acids) and, at a much lower level, stilbenes (e.g., resveratrol).

Data on wine phenolic composition data are growing (*4*, *5*, *9*), but little information is known about Australian wines, except for their tannin contents (*15*, *20*). LC-MS profiling was done on these samples and will be reported elsewhere (*32*). In all of the phenolic compounds measured, Australian wines are similar to wines of the same variety from other countries. The lack of significant differentiation among the three red wine varieties (Shiraz, Cabernet

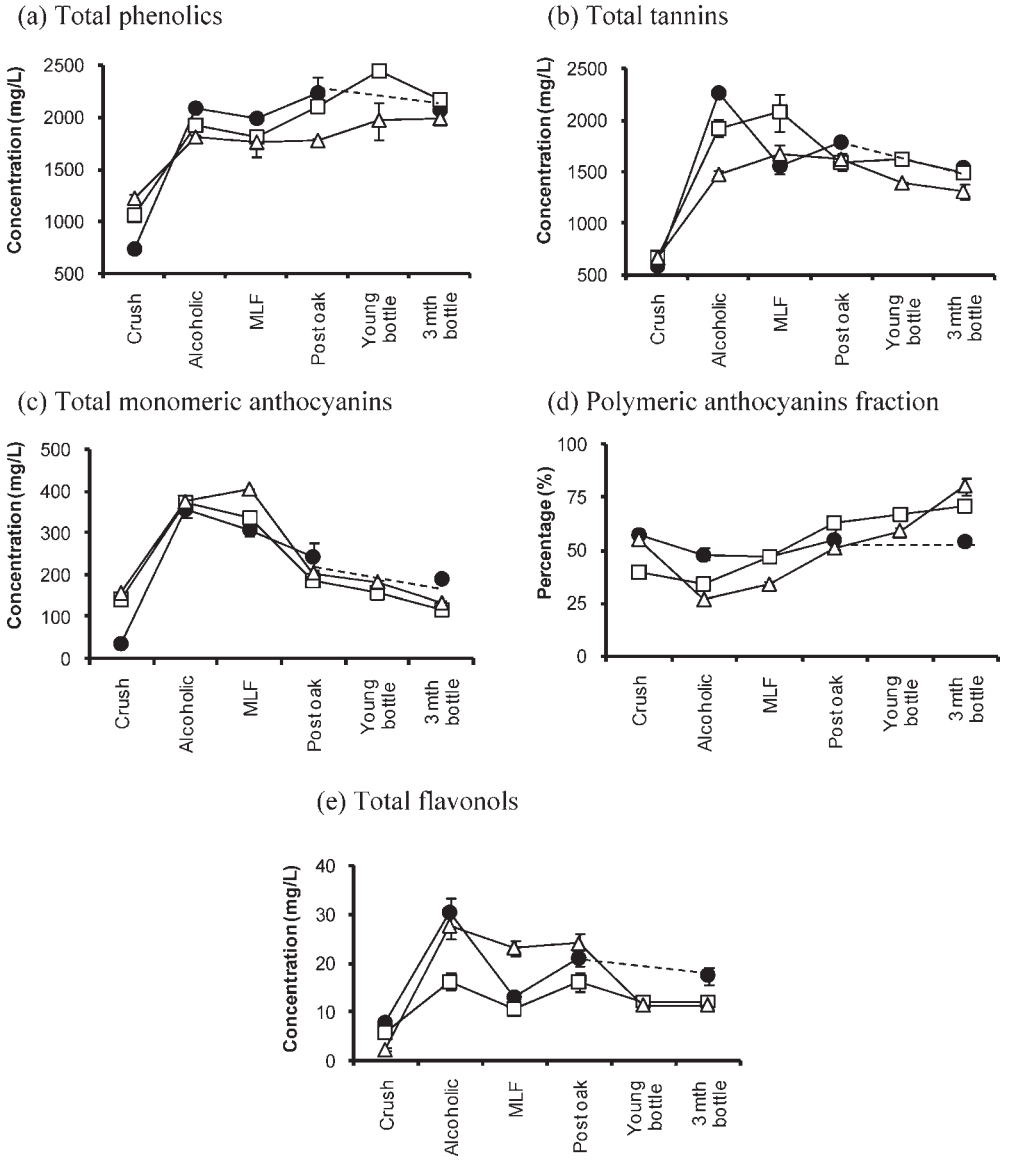


**Figure 1.** Phenolic composition of red wines from different stages of winemaking: (●) Sirromet Cabernet Sauvignon; (□) Ballandean Cabernet Sauvignon; (△) Ballandean Shiraz. Each data point represents the mean of triplicate determinations of one field sample. Standard deviation (SD) error bars represent method replications.

Sauvignon, and Merlot) in terms of phenolic composition and phenolic classes could be due to the variability of the samples (% CV > 10%). This is not surprising, because the tested wines were sourced from a range of Australian winery regions and vintages from 2002 to 2006. Nevertheless, the present results suggest that each of these three wine varieties provide a rich source of phenolic compounds.

**Phenolic Composition of Wines from Different Stages of Winemaking. Figure 1** shows that the three red wine variety types are similar to each other in terms of phenolic changes throughout the winemaking process. Three general trends were observed. The first trend was for total phenolic contents (**Figure 1a**) to have a low value at the crush stage, followed by a rapid increase during fermentation (alcoholic and MLF) until the initial bottle aging stage, after which a slight decrease occurred as the wine aged in the bottle. Because this study was not replicated throughout the winemaking process, the unexpected increase in the total phenolic content for the BCS from the malolactic fermentation to the bottling stage (**Figure 1a**) cannot be confirmed as being a real increase. Similarly, the unexpected drop in the total phenolic content of the BCS and SCS during the 3 months in the bottle may not be real, but a lack of replication here precludes a more definitive statement being made. The second trend was observed in total tannins (**Figure 1b**), total monomeric anthocyanins (**Figure 1c**), and total flavonols (**Figure 1e**); the concentrations of each increased rapidly during the early stage of fermentation and then gradually decreased as the wine aged. For the tannins of the BCS and SCS, an unexpected increase in the total tannin content was observed (**Figure 1b**), but this may not be a real change because replication of this process was not carried out. The third trend was for the percentage of polymeric anthocyanins (**Figure 1d**), which showed a slight drop after the crush and then a gradual increase with each subsequent stage thereafter.

The phenolic content for the crush samples was lower than those for the rest of the winemaking samples, most likely because the phenolic compounds had not been extracted fully from the skin, seeds, and stems. In this study, the total amount of phenolics in crush samples for SCS was about 700 mg of GAE/L, which is similar to those found in commercial grape juice (*33*) and slightly higher than found in Chardonnay (**Table 1**). For BCS and BS

wines, the total phenolic contents were much higher (~1000 mg of GAE/L). Burns et al. (*7*) reported a range between 357 and 1105 mg/L for phenolics present in Merlot and Cabernet Sauvignon grape juice with higher extraction efficiency at higher extraction temperature (60 °C for 1 h). However, for the wine samples in the present study, both wineries used a traditional fermentation technique; that is, grape berries were crushed and juice was extracted at room temperature while the fermentation tank was maintained at 21 °C. Thus, the difference most likely lies within the grape berry itself. Judging from the appearance of the berries, it is possible that Ballandean Estate harvested their grapes at a later maturity stage than Sirromet. Ballandean grape berries were softer than Sirromet berries, and the pulp appeared colored, suggesting leakage of phenolics (anthocyanins) from the skin into the pulp of the grape. Firmer grape skin is associated with lower anthocyanin extractability (*25*). This was confirmed by the lower concentrations of monomeric anthocyanins in the juice of Sirromet samples (**Figure 1c**). This, in effect gave a lower total phenolic content in SCS sample at crush.

During the fermentation process, the skin, seed, and stem of the grape are mixed with the juice, and more phenols are extracted into the juice. For the Sirromet winery, the initial fermentation process (alcoholic) lasted for 5 days and for Ballandean Estate, it was 20 days. The fermentation process produces ethanol, which aids in the extraction of phenolic compounds from the grape skin, seed, and stem. As a result, the total phenolic content doubled during the fermentations (alcoholic fermentation and MLF). This explanation also extends to total tannins, total monomeric anthocyanins, and total flavonols. The increase was previously recorded by Burns et al. (*7*), with the phenolic level peaking after 6−7 days, depending on grape and winemaking styles. As the wine aged, the level of total phenolic compounds increased gradually and then stabilized when the wines were bottled. The individual changes for each phenolic class differ slightly, with a gradual decrease occurring for the total tannins, total monomeric anthocyanins, and total flavonols.

As shown in **Table 1**, tannin is the largest single group of wine phenolics. Condensed tannins originate mainly from the seed, skin, and stem of the grape, whereas hydrolyzable tannins are from oak wood and/or nut. It is a common practice to add oak tannins to wine at various stages of winemaking. Sirromet added about 100 mg/L of oak tannins to their wine (J. Ferguson, personal communication). Ballandean Estate, on the other hand, added much more, with a total of 274 mg/L of oak tannins added to their Cabernet Sauvignon wine and a total of 540 mg/L to their Shiraz wine (D. Rhymer, personal communication). The amount of tannins added varies with the seasons to complement the available chemicals, especially phenolic compounds in the crush and wine. Fruits with fuller flavor require more tannin for their aging process (D. Rhymer, personal communication), with these tannins being added at the crush and both before and at the end of the fermentation process. Tannins act to stabilize red wine color and to improve the body of the wine, by forming polymers with the anthocyanins and other copigments (*34*). In addition, tannins are added to precipitate any remaining proteinaceous material in the wine (e.g., yeast). Polymer formation with anthocyanins and proteins resulted in a reduced concentration of tannins at the end of the oak aging process. A filtration process using 0.8−1 $\mu$m pore diameter filters eliminates any large particulates from the oak sample prior to bottling (D. Rhymer, personal communication), thus reducing the amount of tannins recovered in the bottled sample. Apart from that, tannins (e.g., ellagitannin) are also extracted from the oak into the wine during oak aging (*35*). As shown in **Figure 1b**, the amount of tannins increased after oak aging of the SCS wines. An opposite effect was observed for the BCS and BS wines, for which oak aging resulted in a decreased concentration of tannin (**Figure 1b**). This is despite the fact that more oak tannins were added to these latter wine samples. It is possible that the additional tannins were added to compensate for a lower tannin concentration in those wine samples at the start of the fermentation process. The MCP method, which was used to estimate total tannins in this study, although relatively simple and reported to be robust, still suffers from some limitations. Sarneckis et al. (*15*) cautioned against extending the applicability of the method to nongrape tannins. This may explain the small effect on Ballandean wines when oak tannins were added.

Anthocyanin concentration increases with the onset of ripening (veraison) (*36*). Using a fast extraction protocol, Jensen et al. (*23*) reported averages of 1555 and 1766 mg/L of anthocyanins in Cabernet Sauvignon and Shiraz juice, respectively. However, a typical winemaking process extracts only a fraction of this amount, which was also reported by Jensen et al. (*23*) in their wine samples. In the present study, <200 mg/L was reported, with BCS and BS crush samples having more anthocyanins than the SCS crush sample. As discussed earlier, this may be due to the different winemaking practices of the two wineries, such as harvesting the grapes at different maturity stages. Moreover, the grape berries may have different characteristics and thus affect their anthocyanin contents.

The total monomeric anthocyanin in finished red wine was much lower than that recorded during fermentation. In this study, approximately one-third (117 and 134 mg/L in BCS and BS wines, respectively) or half (189 mg/L in SCS wine) of the monomeric anthocyanins at fermentation were measured in the final red wine. During the winemaking process, changes in temperature, pH, and formation of other compounds (e.g., ethanol, oxygen, other phenolics etc.) may lead to pigment degradation and/or copigmentation. Copigmentation can be desirable as it helps to improve the color stability (*37*) and taste of the finished wine (e.g., reduced astringency) (*38*). In the present study, polymerization was detected as an increase in the polymeric anthocyanin levels in the aged wines (**Figure 1d**). The opposite effect was observed for the monomeric anthocyanin levels, suggesting that the polymeric fraction was formed from the monomeric anthocyanins through copigmentation reactions.

In addition, the results for the final bottled wine samples (**Figure 1**) were compared with the values measured in this study for commercial wine samples (**Table 1**). Except for the polymeric anthocyanins, all of the other phenolic categories were within the range determined for the commercial samples. Polymeric anthocyanin fraction contents in BCS and BS wine samples were slightly higher (2−10% more) than those for the commercial wine samples. When the individual commercial samples were checked (**Table 1**), some wines from Ballandean Estate (S9, CS11, M9) had higher than average polymeric anthocyanin fractions (>60%) for all three grape varieties, suggesting that the winemaking technique used by the winery may be responsible.

**Antioxidant Activity of Commercial Wine Samples.** The antioxidant activities of the wines were determined using both the ABTS and DPPH methods. Both antioxidant assays showed similar results, with Cabernet Sauvignon being the most efficient antioxidant wine, followed by Merlot and Shiraz, and finally by Chardonnay. The difference in Trolox values using the two different methods was to be expected as each assay responds to different concentrations of antioxidants.

As shown in **Table 1**, the antioxidant activity of white wine was significantly lower ($P < 0.05$) than the rest of the wine samples, showing $^1/_{10}$ of the antioxidant activity recorded in red wines. Apart from the quantity, the type of the phenolic compounds present in the wine also determines the overall antioxidant effect.

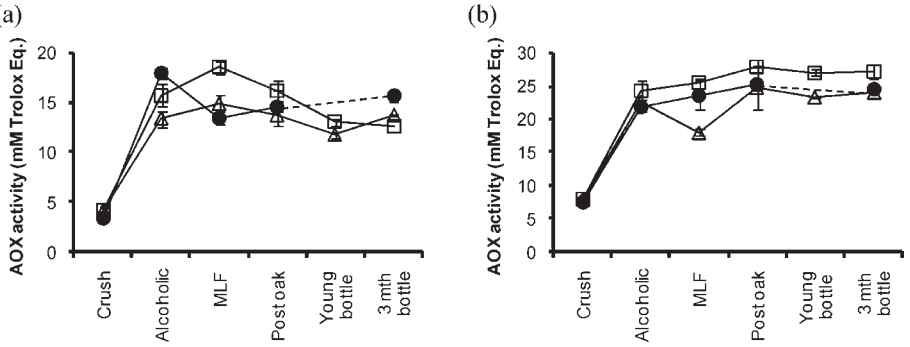


**Figure 2.** Antioxidant activities of wines as determined with (**a**) DPPH assay and (**b**) ABTS assay: (●) Sirromet Cabernet Sauvignon; (□) Ballandean Cabernet Sauvignon; (△) Ballandean Shiraz. Each data point represents the mean of triplicate determinations of one field sample. Standard deviation (SD) error bars represent method replications.

**Table 3.** Correlations of Parameters Measured in 48 Wine Samples[a]

| | TPC | TT | TMA | PAF | TF | $DPPH_{Trolox\ equiv}$ |
|---|---|---|---|---|---|---|
| TT | **0.541 (0.000)** | | | | | |
| TMA | 0.001 (0.992) | **0.453 (0.001)** | | | | |
| PAF | **0.327 (0.023)** | 0.011 (0.940) | **−0.730 (0.000)** | | | |
| TF | −0.132 (0.371) | **0.406 (0.004)** | **0.406 (0.004)** | −0.248 (0.089) | | |
| $DPPH_{Trolox\ equiv}$ | **0.756 (0.000)** | **0.730 (0.000)** | 0.254 (0.082) | 0.130 (0.350) | 0.140 (0.341) | |
| $ABTS_{Trolox\ equiv}$ | 0.082 (0.572) | **0.527 (0.000)** | 0.192 (0.192) | 0.106 (0.475) | **0.380 (0.008)** | **0.475 (0.001)** |

[a] Values are expressed as Pearson correlation coefficient ($r$) with $P$ value in parentheses. Values in bold are significantly different at $P < 0.05$. TPC, total phenolic content; TMA, total monomeric anthocyanins; PAF, polymeric anthocyanin fraction; TT, total tannins; TF, total flavonols.

As an example, whereas the total phenolic content for Chardonnay was 25% less than that found for the red wines, the antioxidant activity for Chardonnay was far lower (90% less) than for the red wines. Tedesco et al. (*6*) reported that the anthocyanin fraction of red wine is more effective in protecting red blood cells against oxidative damage than the other types of phenolic compounds of red wine. Another study (*39*) showed that the radical scavenging capacity of anthocyanin increases with increasing pH. This is particularly important when referring to their physiological antioxidant capacity, because the pH of the human digestive system ranges from pH 1 in the stomach to pH 8 in the large intestine and pH 7.4 in the blood. Tannins, another important group, are powerful antioxidants (*40*) that contribute toward the antioxidant activity of red wine as more than half of the phenolic compounds in the present wine samples are tannin-based (**Table 1**).

The spectral assays in the present study quantified 58−73% of total phenolics in red wine on the basis of their total tannins, total monomeric anthocyanins, and total flavonols contents, with a high number of the phenolic groups in these compounds proven to possess strong antioxidant activities. Thus, these assays provide a good measure of the major phenolic antioxidants in red wines. The absence of detectable anthocyanins and tannins in the white wine samples partly explains their low antioxidant activities and also suggests that there are other unidentified groups of phenolic compounds which possess some antioxidant properties. It is highly likely that these compounds include simple phenolic acids such as gallic acid, caffeic acid, coumaric acid, and related compounds.

**Antioxidant Activity of Wines from Different Stages of Winemaking.** The effects of the different stages of the winemaking process on the antioxidant activities of the wines are summarized in **Figure 2**. Both DPPH and ABTS assays gave similar trends, with very low antioxidant activity at the beginning of the winemaking process (crushing), high activity during the fermentation process (alcoholic fermentation and/or MLF), and stable or slightly decreasing values during the aging period (oak and bottle aging). All three samples gave very similar values for each stage, with the % CV (measure of variability) ranging from 7.5 to 16.9% for the DPPH assay and from 2.7 to 17.2% for the ABTS assay. All of the parameters for the winery samples were within the range of antioxidant activity values of commercial wines (**Table 1**).

The pattern observed for the antioxidant activity of each wine sample closely resembles those recorded for the total phenolic content and total tannin content (**Figure 1**), further confirming the positive correlation between these parameters. Thus, most of the antioxidant changes occurring during the winemaking process are likely to be explained by the changes in their phenolic composition. For example, it is apparent that the increased extraction of phenolic compounds from the skin, stem, and seed material during maceration and fermentation increased the antioxidant capacity of the wine dramatically. Following fermentation, the antioxidants were maintained during the aging process, possibly through the stabilization of the phenolic compounds.

**Correlation Analysis.** Detailed $r$ and $P$ values for all pairs of variables in all red wine samples are shown in **Table 3**. Of the four classes of phenolic compounds, only total tannins and polymeric anthocyanin fractions have weak but significant positive correlation ($r = 0.327-0.541$; $P \leq 0.023$) with the total phenolic contents. Having contributed more than half (~1300 mg/L) of the total phenolic compounds in wine, the correlation between total tannin and total phenolics was expected. The low concentrations of total monomeric anthocyanins (<200 mg/L) and total flavonols (<20 mg/L) in comparison to the total phenolic contents (>2000 mg/L) resulted in insignificant correlations with the total phenolic contents in wine. In relation to this, the correlation between polymeric anthocyanin percentage and total phenolic content was more evident than those of the monomeric anthocyanins, because aged wines contain more polymeric anthocyanins than monomeric anthocyanins.

For antioxidant activities (Trolox Equivalents), both DPPH and ABTS assays gave significantly positive correlations ($r > 0.527$;

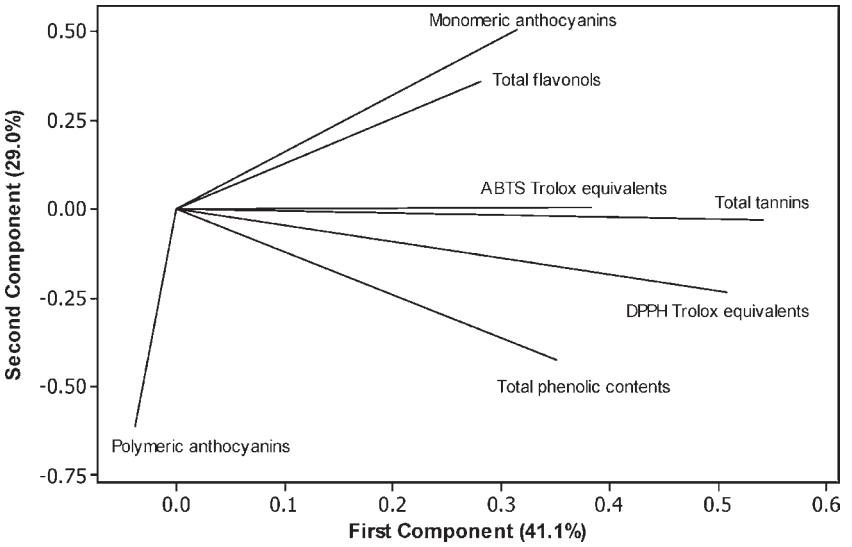


**Figure 3.** Principal component loading plot from phenolic composition and antioxidant activities of 53 red wine samples.

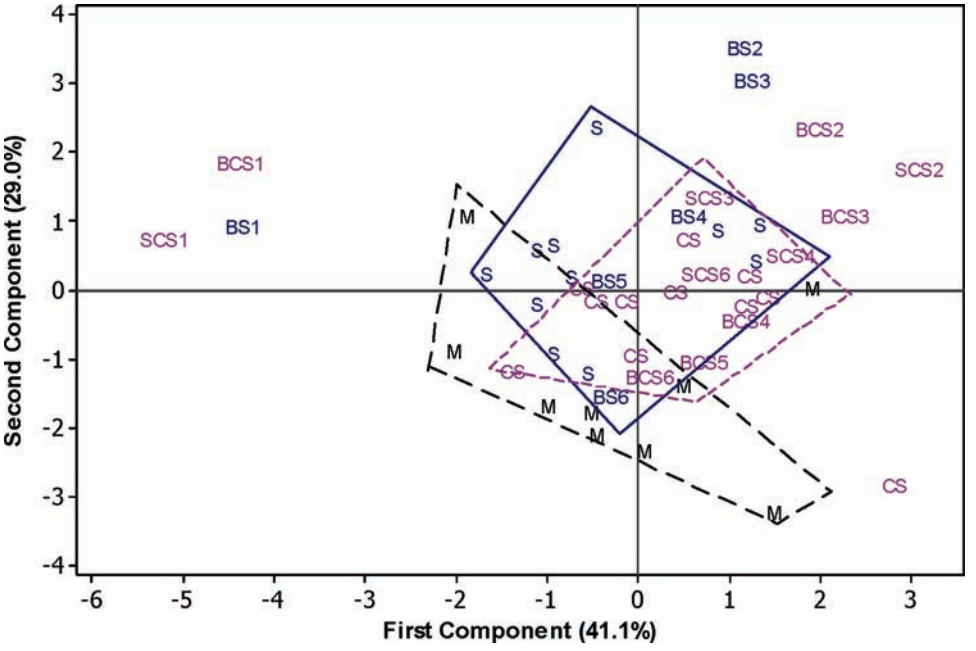


**Figure 4.** Principal component scores from phenolic composition and antioxidant activities of red wines (commercial and winemaking process). CS, commercial Cabernet Sauvignon; S, commercial Shiraz; M, commercial Merlot; BS, Ballandean Shiraz; SCS, Sirromet Cabernet Sauvignon; BCS, Ballandean Cabernet Sauvignon. Numerical code for winery samples SCS, BCS, and BS: 1, crush; 2, primary fermentation; 3, MLF; 4, oak; 5, bottled (0 months); 6, bottled (3 months). Varietal cluster representations: long-dash line, Merlot; solid line, Shiraz; short-dash line, Cabernet Sauvignon.

$P = 0.000$) with the total tannin contents, whereas only the DPPH assay gave positive correlation ($r = 0.756$; $P = 0.000$) with the total phenolic contents. In addition, the ABTS assay gave a weak positive correlation ($r = 0.380$; $P = 0.008$) with the total flavonol contents. The positive correlation between the antioxidant activities and phenolic contents are expected due to the strong antioxidant activities of these phenolic compounds. Except for total flavonols, the correlations between antioxidant activities determined by the ABTS assay with different types of phenolic compounds were weaker compared to those shown by the DPPH assay, suggesting that there are different relative reactivities of phenolic compounds toward different antioxidant assays. The medium in which the reactions occur is also important. The DPPH assay was conducted in mainly methanolic solution, whereas the ABTS assay was conducted in a controlled pH condition at pH 7.4, similar to that of blood. Thus, the results suggest that wine phenolics may not be as effective a source of antioxidants at physiological pH as would be anticipated from a DPPH assay.

**Principal Component Analysis (PCA).** The multivariate analysis PCA was performed on both commercial and winery (different stages of winemaking) samples totaling 48 samples and 7 variables to provide partial visualization of the data set in reduced dimension (2-D). The first three principal components (PC) with eigenvalues of >1, explained 85.3% of the total variance. The loading plot of the first two components (explaining 70.1% of the total variance) is shown in **Figure 3**. The first PC accounted for 41.1% of the variance and correlated positively with all of the variances except for the polymeric anthocyanin fraction. Of the positive correlations, the total tannins and the antioxidant

activity based on the DPPH assay had higher weightings in this component. The second PC (29.0%) correlated positively with the total monomeric anthocyanins and total flavonols and negatively with the rest of the variances. However, total tannins and antioxidant activity based on the ABTS assay did not have weighting in this component, whereas higher weightings were observed for the total monomeric anthocyanins, polymeric anthocyanin fractions, and total phenolic contents.

In **Figure 4**, when the scores of each wine samples were examined in a two-dimensional plot of the first two principal components, some loose clustering of wine samples based on varieties was observed. By referring to the principal component loading and score plots (**Figures 3** and **4**), Merlot wines appeared in the positive part of PC1 and the negative part of PC2, due to their low total monomeric contents, higher fractions of polymeric anthocyanins, and higher total phenolic contents. A Cabernet Sauvignon cluster appeared mostly in the positive part of PC1, with some samples (negative PC2) having high total phenolic contents, high polymeric anthocyanin fractions, and stronger antioxidant activities, whereas some samples (positive PC2) had higher monomeric anthocyanin and total flavonol contents. Shiraz samples appeared to scatter around the base of both components, which suggests that the wine variety cannot be distinguished fully by the measured variances. The presence of several outliers clearly shows that some wine samples do not conform to the others in terms of phenolic composition and antioxidant activities. For example, the Merlot wine located on the bottom far right of the plot is a sample that has a very high total phenolic content (3610 mg/L) compared to other Merlot samples (1886−2756 mg/L).

Plotting the scores according to their sources (commercial or winery samples) draws some distinction between the commercial and winery wine samples. Both Shiraz (BS) and Cabernet Sauvignon (BCS and SCS) samples appeared mostly on the positive side of PC2 (**Figure 4**). The crush samples are located at the far left, indicating the weak influence of phenolic components and antioxidant activities. During fermentation and oak aging stages, total flavonols and monomeric anthocyanins appear to have more influence (samples in the top right plot), and as the samples were bottled, the samples join the commercial cluster (toward axis and the negative side of PC2). The "migration" of samples as a function of the stage in the winemaking process can be followed by the progressive numbers tagged to the sample plot. This shows that most of the wines share common properties once they are bottled.

As discussed earlier, other factors such as seasonal variety, exact wine age, winemaking practices, and wine regional source are likely to play important roles in defining the phenolic content and the resulting antioxidant properties of the finished wine. As these factors were not controlled in this study, tight clustering would not be expected in PC plots. Nevertheless, the evidence of some variety-based clustering in this study proves that simple spectral-based determinations of phenolic content and antioxidant profiles are potentially sufficient to discriminate between wine varieties. By adding more samples to these data, the tightness of the clustering may be improved.

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