Extraction of Phenolics and Changes in Antioxidant Activity of Red Wines during Vinification

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The moderate consumption of alcoholic beverages has been associated with protection against the development of coronary heart disease. Although alcohol itself can help prevent coronary heart disease through a number of mechanisms, red wine appears to offer protection above and beyond that attributable to alcohol alone. Red wine is a complex fluid containing grape, yeast, and wood-derived phenolic compounds, the majority of which have been recognized as potent antioxidants. The aim of this study was to investigate the major phenolic contributors to the antioxidant activity of wine. To this end, four wines were followed during the first 7−9 days of vinification. Individual phenolic compounds were quantified by HPLC, and antioxidant activity was determined by electron spin resonance spectroscopy. The extraction of the phenolics was found to be influenced by vinification procedure, grape quality, and grape variety. Although fermenting wines reached a total phenolic content comparable to that of a bottled wine after 9 days of vinification, the antioxidant activity was significantly lower than that of a finished wine. This suggests that the larger polyphenolic complexes and condensation products that appear during aging make a sizable contribution to the overall antioxidant activity of red wines.

Keywords: *Red wine; phenolics; antioxidant activity; vinification*

INTRODUCTION

Red wine is a complex fluid. It contains water, sugars, acids, alcohols, and a wide range of phenolic compounds. The phenolics can be derived from grapes and wood, or they can be metabolites from yeasts. Phenolics are secondary plant metabolites that are distributed throughout the plant kingdom. They have been implicated in a number of varied roles including UV protection, pigmentation, disease resistance, and nodule production (*1*).

Recent years have seen an increased awareness in the importance of diet in the maintenance of health and well being. Much interest has focused on the Mediterranean diet, popularized as the "French Paradox" (*2*). A diet rich in fruit, vegetables, olive oil, and red wine has been shown to help prevent the development of coronary heart disease and some cancers (*3*, *4*). The active components of this diet are believed to include phenolic compounds which act as antioxidants. Phenolic compounds can be classified into two groups: the flavonoids and nonflavonoids. The major $C_6-C_3-C_6$ flavonoids in wine include conjugates of the flavonols, quercetin, and myricetin; the flavan-3-ols (+)-catechin and (−)-epicatechin, and malvidin-3-*O*-glucoside and other anthocyanins. The nonflavonoids incorporate the C_6-C_1 hydroxybenzoic acids, and gallic and ellagic acids; the C_6-C_3 hydroxycinnmates caffeic, caftaric, and *p*-coumaric acids, and the $C_6-C_2-C_6$ stilbenes *trans*-resveratrol, *cis*-resveratol, and *trans*-resveratrol glucoside.

Phenolics contained within the skin, seeds, and flesh of black grapes are extracted into red wines during the processes of vinification. The processes of viticulture and vinification, which vary between countries, regions, and wine-makers, determine the content and profile of phenolic compounds in wine. Vineyard factors such as grape variety, quality, climate, geographical origin, and disease pressure affect the phenolic compounds that accumulate in grapes (*5*−*7*). During vinification the length of skin contact, temperature, and presence of seeds, stems, and enzymes have all been shown to affect the extraction of phenolics into the fermenting juice, which is referred to as the must (*8*−*10*).

This study set out to investigate the influence of four different approaches to vinification on the extraction of grape phenolics into wine. Two varieties of grapes were studied: Cabernet Sauvignon and Merlot. The phenolic contents of grapes and the resultant wine were compared. This provides information on the nature and the extent of the extraction of phenolic compounds from grapes into wine. Measurements were made of changes in the antioxidant activity of wines throughout the study. This enables the relationship between the extraction of individual phenolics into a wine, and any change in the antioxidant activity, to be examined.

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Table 1. Details of Grapes and Wines A−D from Viña San Pedro, Curìco, Chile

wine	grape	comment
A	Cabernet Sauvignon (Basic quality. Supplied to Safeway Stores plc as their own label Chilean Cabernet Sauvignon)	• Large, pale redish-purple grapes. • Grapes collected on 15/3/1999 crushed and de-stemmed. • Juice collected (day 0 sample) then 1 h in the rotor-vat • 16/3/1999 traditional fermentation initiated (day 1). Wine pumped-over twice daily for 15 min. Fermentation in medium-sized steel tanks. Racked and transferred to steel large tanks on 22/3/1999 (day 7). Mixed with other similar wine musts.
B	Merlot (Basic quality. Thermovinified which extracts colour and tannins. Used to add to less tannic wines to ensure vintage to vintage consistency.)	• Large, pale red grapes. • Grapes were collected on 15/3/1999 crushed and de-stemmed. Juice collected (day 0 sample). • Must heated to 60-65 °C in rotor-vat for 1 h. Juice drained. Skins pressed and pressing added back to juice. Pectolytic enzymes added to juice at 7 °C. Stored in medium sized steel tank. • Racked and transferred to large steel tanks on 22/3/1999 (day 7). Mixed with other similar wine musts. • Fermentation initiated after day 9.
C	Cabernet Sauvignon (Resverva quality. This is the highest quality of wine produced by Viña San Pedro. Bottled and sold in Chile under the label 'Cabo de Horno'.)	• Small, dense grapes. Deep red/purple colour. • Grapes collected 15/3/1999. De-stemmed. Placed in vat immediately. • 16/3/1999 juice collected (day 0). • Fermentation initiated 17/3/1999 (day 1). Fermentation in very small concrete tank. Wine mixed using pump out-pump in method 2 x day.
D	Merlot (Varietal quality. Although not top quality, sufficient to be bottled without blending. Produced by Viña San Pedro and supplied to Safeway Stores labelled as '35 Sur Merlot'.)	• Medium-sized grapes. Deep red colour. • Grapes collected 17/3/1999 along with juice (day 0). De-stemmed and placed in rotor-vat for 1 h. • Fermentation initiated 18/3/1999 (day 1). • Fermentation in medium-sized steel tank. Wine pumped-over twice daily for 15 min.

MATERIALS AND METHODS

Chemicals. Quercetin, myricetin, kaempferol, (+)-catechin, (−)-epicatechin, caffeic acid, *p*-coumaric acid, *trans*-resveratrol, ellagic acid, and gallic acid were obtained from Sigma (Poole, Dorset, UK). Isorhamnetin and *trans*-resveratrol-*O*-*β*-glucoside were supplied by Apin (Abingdon, Oxford, UK). *trans*-Resveratrol-*O*-*β*-glucoside was also isolated and crystallized from the root of *Polygonum cuspidatum* by Professor Takao Yokota (Teikyo University, Utsunomiya, Japan). Malvidin-3- glucoside was purchased from Extrasynthase (Lyon, France). *Cis*-resveratrol was obtained by isomerization of *trans*-resveratrol in methanol during 12 h exposure to high white light. Dr. Creina Stockley (Australian Wine Research Institute, Waite campus, Adelaide, Australia) generously provided a sample of caftaric acid.

Methanol (HPLC grade), ethanol, and acetonitrile (HPLC grade) were from Rathburn Chemicals (Walkerburn, UK). Trifluoroacetic acid (TFA), formic acid, aluminum nitrate, and Folin−Ciocalteu's phenol reagent (2.0 N) were supplied by Sigma. Concentrated hydrochloric acid, acetic acid (glacial), and sodium hydroxide (NaOH) were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). All other chemicals and reagents were obtained from Sigma-Aldrich (Poole, Dorset, UK).

Collection of Musts. Samples of musts were collected at Viña San Pedro, Curicó, Chile. Four wines were followed during the first 7 to 9 days of vinification. Samples were collected at the same time each day and processed in the same manner.

Must, 100 mL, was filtered (Whatman 0.7-*μ*m) to remove particulate matter, and 50 mL of ethanol was added to halt fermentation. The liquid was then decanted into 375-mL bottles, purged with carbon dioxide, and corked. Prior to analysis the alcohol was removed by rotary evaporation. The samples were subsequently stored in amber bottles under nitrogen between analyses. Details of the grapes and wines analyzed are given in Table 1. Except when anthocyanins were to be analyzed, samples were untreated prior to analysis. Samples were concentrated for the analysis of anthocyanins. Five mL of sample was dried down using a rotary evaporator with a water bath operating at 35 °C. The sample was redissolved in distilled water, containing 0.5% HCl, to a known volume.

Collection of Grape Samples. Grape samples were collected by randomly selecting fruit from different aspects, clusters, and vines. Samples were weighed and stored at −20 °C prior to transportation to laboratory facilities within the Universidad Catolica, Chile. There they were frozen with liquid nitrogen and packed in dry ice for transport by air to the University of Glasgow, where, upon arrival, the samples were immediately stored at −80 °C.

Preparation of Methanolic Grape Extract. A weighed aliquot of grapes was defrosted at room temperature prior to homogenization with 30 mL of methanol containing 2% formic acid. Samples were centrifuged at 10000*g* for 10 min, and the supernatant was stored at −80 °C until analysis.

HPLC Analysis of Wine Phenolics. Each wine was analyzed for a range of phenolics using the HPLC methods described previously (*11*). These were the free and conjugated flavonols myricetin, quercetin, kaempferol, and isorhamnetin; the flavan-3-ols (+)-catechin and (−)-epicatechin; gallic acid; the hydroxycinnamates caftaric, caffeic, and *p*-coumaric acids; and the stilbenes, *trans*-resveratrol and *trans*-resveratrol-*O*-*β*-glucoside. The free anthocyanins (malvidin-3-glucoside, malvidin-3-acetylglucoside, and malvidin-3-*p*-coumaroylglucoside), in 10-*μ*L volumes of sample, were analyzed on a 250 × 4.6 mm i.d. 4-*μ*m C_{18} Novapak column (Waters, MA) eluted with a 40-min gradient of 5−30% acetonitrile in 5% aqueous formic acid with a flow rate of 1 mL/min. Eluent was then directed to an absorbance monitor operating at 520 nm.

Determination of Total Phenolics. The total phenol contents of the wines were determined using the Folin−Ciocalteu method of Singleton and Rossi (*12*) and also by the summation of the HPLC-derived individual phenolics.

Determination of the Antioxidant Activity. The ability of red wines to reduce the Fremy's salt (potassium nitrosodisulfonate) was measured as described by Gardner et al. (*13*). The wines were diluted to 5% (v/v) with ethanol/water (12:88, v/v). Aliquots of 3-mL each were reacted with an equal volume of 1 mM Fremy radical in ethanol/water (12:88, v/v). The ESR spectra of the low field resonance of the Fremy's radical were obtained after 20 min, by which time the reaction was complete. Signal intensity was obtained by double integration, and the concentration was calculated by comparison with a control reaction using ethanol/water (12:88, v/v) without red wine. Spectra were obtained at 21 °C on a Bruker ECS 106 spectrometer equipped with a cylindrical (TM110 mode) cavity and operating at ca. 9.5 GHz (X-band frequency). The microwave power and modulation amplitude were set at 2 mW and 0.01 mT, respectively.

Statistics. Data are presented as mean values ± standard error (SEM) ($n = 3$). Pearson correlations were used to assess the strength of the association between levels of individual phenolics, total phenolics, and antioxidant activity using Minitab software version 12 (Minitab, Inc., Addison-Wesley Publishing Co., Reading, MA).

RESULTS

Four different wines were followed during the first 7−9 days of vinification (Table 1). Samples of the corresponding grapes were also analyzed. The total phenolic contents and antioxidant activities of the samples are presented in Table 2, and Table 3 summarizes the profile of phenolic families during vinification.

Wine A. This wine was made from relatively large Cabernet Sauvignon grapes and was traditionally fermented on a very large scale in steel vats (Table 1). Because the buyers require a consistent product, the wine is removed from the vats where it was fermented and transferred to an even larger tank where it is mixed with the same type of wine from other vats. This wine has consistently recorded high flavonol levels over a number of years (*7*) and has a higher-than-average total phenolic content and antioxidant capacity (*11*).

Total Phenolics. The total phenol content ranged from 2.8 ± 0.2 mM gallic acid equivalents (GAE) in the juice (day 0) to 8.2 ± 0.1 mM GAE by day 9 (Table 2). The day 9 value is within the range of that found with finished wines (*11*).

Flavonols. Free and conjugated myricetin, quercetin, kaempferol, and isorhamnetin were found in the grapes with a mean total flavonol content of 84.6 ± 3.2 nmol/g grape tissue. The major flavonols in the wine were myricetin and quercetin, however myricetin was not detected in the must until day 2. The total flavonol content increased from 6.5 ± 0.2 μM in the juice to over 90 μM by day 9 (Table 4). Total flavonol levels remained relatively steady from day 5 to day 9.

Flavan-3-ols. Although grapes for wine A contained almost equi-molar levels of (+)-catechin and (−)-epicatechin, (+)-catechin was present in a ca. 2-fold higher concentration in each of the wine samples. In fact (−)-epicatechin was undetected in the juice (day 0) and the day 1 must. Total (+)-catechin and (−)-epicatechin levels ranged from 5.1 ± 0.8 μM in the juice (day 0) to over 100 μM by day 7 (Table 5). Levels of total flavan-3-ols decreased slightly after day 7.

Anthocyanins. Six different anthocyanins were detected in the grapes used to make wine A: delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, malvidin-3-glucoside, malvidin-3-acetylglucoside, and malvidin-3-*p*-coumaroylglucoside. The total anthocyanin content of the grapes was 2470 ± 70 nmol/g (Table 6). Only three anthocyanins could be detected in the fermenting must: malvidin-3-glucoside, malvidin-3-acetylglucoside, and malvidin-3-*p*-coumaroylglucoside (Table 7). Although no anthocyanins could be detected in the juice, by day 9 the maximum levels of 73.7 ± 0.9 μM total anthocyanins were detected.

Gallic acid. Levels of gallic acid increased from 5.4 ± 0.6 μM in the juice (day 0) to a high of 87.6 ± 1.3 μM by day 8 (Table 3). A sharp increase in the content of gallic acid was observed between day 3 and day 4, with values plateauing by day 6.

Hydroxycinnamates. Only caftaric acid and conjugated *p*-coumaric acid were detected in grapes A, with a total content of 345.2 ± 2.9 nmol/g (Table 8). Similar patterns were observed with the wine samples, where only very low levels of free caffeic and *p*-coumaric acids were detected, accounting for less than 5% of the total hydroxycinnamate content at day 9. In all samples, bar

Table 2. Total Phenolic Content and Antioxidant Activity of Wines A−D[a]

sample	Folin−Ciocalteu total phenolics	HPLC-derived total phenolics	ESR-based antioxidant activity
		Wine A	
day 0	2.8 ± 0.2	50.5 ± 2.0	0.12 ± 0.02
day 1	2.9 ± 0.0	85.0 ± 0.5	0.20 ± 0.04
day 2	4.5 ± 0.1	228.9 ± 1.4	0.70 ± 0.22
day 3	7.7 ± 0.2	330.0 ± 4.3	0.82 ± 0.06
day 4	8.4 ± 0.1	571.1 ± 10.9	1.61 ± 0.09
day 5	8.2 ± 0.1	631.8 ± 14.3	1.78 ± 0.03
day 6	9.8 ± 0.3	694.9 ± 2.7	2.21 ± 0.15
day 7	7.9 ± 0.3	538.7 ± 71.7	2.24 ± 0.14
day 8	8.3 ± 0.3	604.4 ± 3.6	2.27 ± 0.00
day 9	8.2 ± 0.1	610.7 ± 1.9	2.01 ± 0.00
		Wine B	
day 0	6.5 ± 0.2	426.1 ± 3.3	1.84 ± 0.20
day 1	9.4 ± 0.1	634.1 ± 3.0	2.74 ± 0.27
day 2	7.1 ± 0.0	524.4 ± 2.0	1.94 ± 0.06
day 3	7.1 ± 0.1	534.9 ± 2.1	2.43 ± 0.11
day 4	8.4 ± 1.0	527.5 ± 2.2	1.93 ± 0.15
day 5	6.5 ± 0.1	473.3 ± 7.6	1.80 ± 0.20
day 6	7.5 ± 0.0	546.1 ± 5.5	2.05 ± 0.11
day 7	6.4 ± 0.1	537.1 ± 0.7	1.87 ± 0.07
day 8	7.3 ± 0.1	515.7 ± 32.4	1.92 ± 0.07
day 9	7.0 ± 0.0	671.0 ± 6.6	1.89 ± 0.00
		Wine C	
day 0	2.1 ± 0.0	303.2 ± 1.4	n.d.
day 1	3.3 ± 0.0	513.5 ± 3.5	0.32 ± 0.12
day 2	5.9 ± 0.2	450.6 ± 1.6	0.67 ± 0.14
day 3	6.8 ± 0.0	549.0 ± 3.7	2.10 ± 0.15
day 4	8.7 ± 0.3	643.3 ± 11.2	2.37 ± 0.13
day 5	9.1 ± 0.2	611.4 ± 9.6	2.58 ± 0.04
day 6	9.5 ± 0.2	873.7 ± 3.0	2.56 ± 0.10
day 7	9.7 ± 0.3	794.1 ± 11.6	3.00 ± 0.08
day 8	10.5 ± 0.3	920.0 ± 21.7	3.64 ± 0.01
		Wine D	
day 0	3.4 ± 0.1	28.2 ± 5.0	0.03 ± 0.00
day 1	4.5 ± 0.1	153.4 ± 21.2	0.85 ± 0.06
day 2	6.1 ± 0.1	284.9 ± 13.7	1.45 ± 0.11
day 3	8.8 ± 0.1	341.4 ± 10.6	2.91 ± 0.17
day 4	11.4 ± 0.3	420.3 ± 5.1	2.75 ± 0.12
day 5	10.9 ± 0.2	547.0 ± 14.7	3.70 ± 0.12
day 6	11.2 ± 0.3	428.0 ± 11.3	2.83 ± 0.10
day 7	11.4 ± 0.1	397.7 ± 9.2	2.95 ± 0.01

[a] Total phenolic content of wine quantified by the Folin−Ciocalteu assay [mM gallic acid equivalents (GAE)] and from HPLC analysis of individual phenolics (μM). Results are expressed as mean values ± SEM, $n = 3$. Antioxidant capacity of wines, measured by ESR spectroscopy, presented as the number of Fremy's radicals reduced by 1 L of wine × 10^{21} ± SEM × 10^{21}. n.d., not detected.

Table 3. Phenolic Profile of Wines A−D during Vinification[a]

sample	total flavonols	total flavan-3-ol	total anthocyanins	gallic acid	total hydroxy-cinnamates	total stilbenes	total HPLC phenolics
			Wine A				
grape[b]	84.6 ± 3.2	943.3 ± 49.2	2470 ± 70.1	27.7 ± 0.7	345.2 ± 2.9	12.3 ± 0.3	3883.1 ± 114.6
day 0	6.5 ± 0.2	5.1 ± 0.8	n.d.	5.4 ± 0.6	33.0 ± 0.1	0.5 ± 0.1	50.5 ± 2.0
day 1	13.9 ± 0.3	4.9 ± 0.3	4.5 ± 0.1	8.2 ± 0.0	51.2 ± 0.4	2.2 ± 0.1	85.0 ± 0.5
day 2	25.1 ± 0.4	16.3 ± 0.6	28.2 ± 0.2	22.4 ± 0.2	129.7 ± 0.8	5.5 ± 0.1	228.9 ± 1.4
day 3	37.9 ± 0.8	48.4 ± 1.4	26.8 ± 0.2	28.3 ± 0.2	180.8 ± 0.4	4.8 ± 0.2	330.0 ± 4.3
day 4	73.9 ± 3.4	56.8 ± 0.8	29.7 ± 2.5	60.3 ± 0.3	336.9 ± 3.3	7.0 ± 0.1	571.1 ± 10.9
day 5	98.0 ± 10.2	73.1 ± 3.9	36.0 ± 0.8	72.7 ± 0.5	335.7 ± 0.9	8.0 ± 0.5	631.8 ± 14.3
day 6	88.5 ± 1.5	80.6 ± 0.8	27.5 ± 0.2	79.8 ± 0.5	401.2 ± 4.2	9.9 ± 0.2	694.9 ± 2.7
day 7	83.7 ± 7.0	102.6 ± 0.5	28.8 ± 0.3	80.8 ± 0.4	265.7 ± 0.8	5.5 ± 0.5	538.7 ± 71.7
day 8	86.70 ± 3.1	97.8 ± 1.2	35.8 ± 1.5	87.6 ± 1.3	282.4 ± 1.4	7.5 ± 0.9	604.4 ± 3.6
day 9	91.9 ± 2.2	86.7 ± 2.1	73.7 ± 0.9	80.7 ± 1.4	271.4 ± 0.8	6.7 ± 0.3	610.7 ± 1.9
			Wine B				
grape[b]	93.3 ± 3.5	746.7 ± 8.8	1000.0 ± 17.3	19.3 ± 0.8	154.6 ± 4.2	34.3 ± 1.6	2048 ± 23.4
day 0	28.2 ± 0.9	63.5 ± 0.7	n.d.	11.3 ± 0.3	284.4 ± 1.7	38.3 ± 1.8	426.1 ± 3.3
day 1	37.2 ± 1.8	79.2 ± 1.1	n.d.	28.3 ± 0.7	412.5 ± 2.6	78.0 ± 2.8	634.1 ± 3.0
day 2	32.9 ± 0.2	80.6 ± 1.3	1.2 ± 0.1	15.6 ± 0.3	334.8 ± 1.1	58.6 ± 2.6	524.4 ± 2.0
day 3	33.5 ± 0.3	78.4 ± 1.9	n.d.	16.0 ± 0.6	313.9 ± 1.1	94.2 ± 1.6	534.9 ± 2.1
day 4	38.2 ± 1.6	62.2 ± 1.9	9.2 ± 1.1	19.8 ± 0.3	333.6 ± 1.5	64.4 ± 0.6	527.5 ± 2.2
day 5	25.3 ± 0.3	96.1 ± 1.2	5.2 ± 0.0	14.1 ± 0.3	280.2 ± 0.8	59.1 ± 0.6	473.3 ± 7.6
day 6	36.8 ± 0.6	87.5 ± 1.6	8.5 ± 0.6	15.6 ± 0.2	334.5 ± 2.2	71.8 ± 0.0	546.1 ± 5.5
day 7	32.4 ± 1.2	102.1 ± 0.3	7.3 ± 0.4	13.4 ± 0.6	303.6 ± 1.9	78.1 ± 0.6	537.1 ± 0.7
day 8	65.1 ± 1.3	99.5 ± 0.6	8.4 ± 0.3	14.0 ± 0.3	290.7 ± 1.0	71.1 ± 0.4	515.7 ± 32.4
day 9	69.4 ± 5.4	93.0 ± 1.3	20.6 ± 0.4	14.2 ± 0.3	357.6 ± 2.0	118.3 ± 0.8	671.0 ± 6.6
			Wine C				
grape[b]	143.9 ± 7.4	576.7 ± 14.5	3296.7 ± 52.4	21.5 ± 0.2	368.3 ± 42.2	6.8 ± 0.3	4413.9 ± 79.5
day 0	5.7 ± 0.1	3.8 ± 0.0	4.9 ± 0.0	2.6 ± 0.4	102.5 ± 0.2	2.0 ± 0.1	303.2 ± 1.4
day 1	53.0 ± 1.2	9.0 ± 0.2	29.6 ± 0.2	7.3 ± 0.2	74.4 ± 0.4	2.1 ± 0.0	513.5 ± 3.5
day 2	53.0 ± 0.6	14.1 ± 0.2	23.6 ± 0.1	20.6 ± 0.5	227.8 ± 0.8	4.4 ± 0.1	450.6 ± 1.6
day 3	110.1 ± 5.8	24.9 ± 0.2	62.3 ± 4.1	32.0 ± 0.9	326.7 ± 4.2	5.7 ± 0.3	549.0 ± 3.7
day 4	132.8 ± 10.5	43.9 ± 0.2	85.2 ± 4.9	38.8 ± 0.1	302.6 ± 1.4	9.1 ± 0.7	643.3 ± 11.2
day 5	164.7 ± 11.4	49.6 ± 0.2	58.7 ± 3.1	48.9 ± 0.5	409.7 ± 2.0	9.2 ± 0.3	611.4 ± 9.6
day 6	205.5 ± 12.3	58.2 ± 0.4	209.9 ± 11.7	53.8 ± 1.0	386.1 ± 2.5	11.7 ± 0.4	873.7 ± 3.0
day 7	193.3 ± 10.1	82.5 ± 0.5	145.0 ± 2.1	63.0 ± 0.8	425.2 ± 0.4	6.6 ± 0.5	794.1 ± 11.6
day 8	213.3 ± 0.5	95.3 ± 0.4	239.1 ± 25.7	63.7 ± 0.4	386.7 ± 4.9	13.8 ± 0.1	920.0 ± 21.7
			Wine D				
grape[b]	327.9 ± 6.7	1076.7 ± 8.8	2206.7 ± 18.6	40.0 ± 0.9	323.0 ± 5.6	24.3 ± 1.2	3998.6 ± 30.0
day 0	16.5 ± 0.4	7.7 ± 0.3	0.8 ± 0.1	6.3 ± 0.3	99.4 ± 0.4	1.6 ± 0.0	28.2 ± 5.0
day 1	47.4 ± 1.8	34.3 ± 0.8	37.4 ± 2.9	9.9 ± 1.2	151.3 ± 0.9	3.6 ± 0.1	153.4 ± 21.2
day 2	85.9 ± 5.5	51.6 ± 0.7	57.2 ± 11.1	29.3 ± 1.1	220.8 ± 0.8	5.8 ± 0.3	284.9 ± 13.7
day 3	137.8 ± 8.3	40.7 ± 0.6	42.7 ± 4.4	40.6 ± 0.8	262.3 ± 1.5	4.0 ± 0.1	341.4 ± 10.6
day 4	214.2 ± 3.5	68.7 ± 0.4	27.3 ± 1.9	62.2 ± 1.1	365.4 ± 1.3	6.6 ± 0.2	420.3 ± 5.1
day 5	166.1 ± 2.8	82.6 ± 0.5	n.d.	59.2 ± 1.6	325.3 ± 0.3	5.1 ± 0.4	547.0 ± 14.7
day 6	191.3 ± 7.2	92.7 ± 0.9	30.9 ± 1.9	60.2 ± 1.3	296.8 ± 1.8	5.3 ± 0.2	428.0 ± 11.3
day 7	171.9 ± 2.0	83.6 ± 0.5	30.0 ± 4.0	65.7 ± 1.1	258.4 ± 0.7	6.5 ± 0.5	397.7 ± 9.2

[a] Grape data expressed as nmol/g grape tissue ± SEM ($n = 3$), and wine data expressed as μM ± SEM ($n = 3$). n.d., not detected. [b] Total flavonols, free and conjugated myricetin, quercetin, kaempferol and isorhamnetin; total flavan-3-ols, (+)-catechin and (−)-epicatechin; total anthocyanins, delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, malvidin-3-acetylglucoside, malvidin-3-*p*-coumaroyl glucoside; total hydroxycinnamates, caffeic, caftaric, and *p*-coumaric acids; total stilbenes, *trans*-resveratrol and *trans*-resveratrol glucoside.

the juice (day 0), total *p*-coumaric acid was present in higher levels than total caffeic acid. Maximum levels of 401.2 ± 4.2 μM were reached by day 6, and then fell to around 270 μM by day 9.

Stilbenes. Only *trans*-resveratrol and its glucoside were detected in grapes A, with *trans*-resveratrol glucoside contributing 78% of the total stilbene content (Table 9). Levels of *trans*-resveratrol in wine ranged from 0.1 ± 0.0 μM in the juice (day 0) to a maximum of 1.8 ± 0.1 μM in the fermenting wines. The glucoside was the major stilbene present in the wine samples. Maximum levels of 8.2 ± 0.2 μM were obtained by day 6 from a minimum of 0.4 ± 0.1 μM in the juice at day 0.

Wine B. This wine underwent thermovinification treatment whereby it was heated to over 60 °C for 1 h. It did not undergo fermentation until after day 9 when no further samples could be collected. Thermovinification allows much of the color to be quickly extracted from the grapes and into the wine. In a poor vintage such wines can be added to those wines which do not have sufficient body. This will add color and tannins, and help improve the original wine. Thermovinified wines are not bottled without prior blending with wines produced with traditional fermentation.

Total Phenolics. The total phenolic content of wine B varied randomly from 6.5 ± 0.2 mM GAE on day 0 to 7.0 ± 0.0 mM on day 9 (Table 2). The phenolic content of wine B on day 0 was significantly higher than that of the other wines at a similar stage. This is attributed to the high temperature during thermovinification, which encourages the early extraction of phenolics. Although the wine did not undergo fermentation, its final total phenolic content is similar to that of wine A.

Flavonols. The Merlot grapes contained free myricetin and free and conjugated quercetin, kaempferol, and isorhamnetin. Grapes contained an average of 93.3 ±

Table 4. Flavonol Content of Wines A to D[a]

sample	free myricetin	conj myricetin	total myricetin	free quercetin	conj quercetin	total quercetin	free kaempf	conj kaempf	total kaempf	free isorham	conj isorham	total isorham	total flavonol
						Wine A							
grape	16.3 ± 1.4	13.3 ± 1.8	29.6 ± 3.1	2.9 ± 0.3	43.6 ± 0.3	46.5 ± 0.3	0.8 ± 0.1	6.9 ± 0.2	7.8 ± 0.1	0.8 ± 0.1	n.d.	0.8 ± 0.1	84.6 ± 3.2
day 0	n.d.	n.d.	n.d.	0.2 ± 0.0	5.8 ± 0.2	6.0 ± 0.2	0.3 ± 0.0	n.d.	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	6.5 ± 0.2
day 1	n.d.	n.d.	n.d.	0.2 ± 0.0	11.5 ± 0.2	11.8 ± 0.2	0.3 ± 0.0	1.0 ± 0.1	1.3 ± 0.1	0.1 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	13.9 ± 0.3
day 2	n.d.	n.d.	2.8 ± 0.2	2.3 ± 0.0	16.0 ± 0.6	18.3 ± 0.6	1.1 ± 0.0	1.2 ± 0.1	2.3 ± 0.1	0.3 ± 0.0	1.4 ± 0.1	1.7 ± 0.0	25.1 ± 0.4
day 3	1.2 ± 0.0	9.8 ± 0.0	11.0 ± 0.1	3.1 ± 0.1	18.0 ± 0.4	21.1 ± 0.6	0.7 ± 0.0	2.9 ± 1.1	3.5 ± 0.1	0.4 ± 0.0	1.9 ± 0.1	2.3 ± 0.1	37.9 ± 0.8
day 4	1.7 ± 0.2	24.0 ± 1.0	25.7 ± 1.0	5.9 ± 0.3	29.7 ± 1.6	35.6 ± 1.6	1.3 ± 0.0	6.3 ± 0.5	7.6 ± 0.5	0.6 ± 0.0	4.3 ± 0.4	4.9 ± 0.4	73.9 ± 3.4
day 5	2.0 ± 0.1	35.2 ± 3.1	37.3 ± 3.0	5.9 ± 0.1	38.1 ± 0.5	44.0 ± 4.9	1.5 ± 0.1	8.1 ± 1.3	9.6 ± 1.2	0.7 ± 0.0	6.5 ± 1.0	7.2 ± 1.0	98.0 ± 10.2
day 6	4.0 ± 0.1	33.1 ± 1.4	37.0 ± 1.3	10.1 ± 0.2	28.1 ± 0.5	38.2 ± 0.3	2.2 ± 0.0	5.6 ± 0.2	7.8 ± 0.2	0.9 ± 0.1	4.6 ± 0.1	5.5 ± 0.1	88.5 ± 1.5
day 7	4.2 ± 0.1	35.5 ± 3.9	39.8 ± 3.9	6.8 ± 0.2	28.3 ± 2.6	35.1 ± 2.5	0.8 ± 0.0	3.8 ± 0.4	4.6 ± 0.3	0.7 ± 0.0	4.6 ± 0.1	4.2 ± 0.3	83.7 ± 7.0
day 8	3.8 ± 0.4	34.9 ± 19	38.7 ± 2.2	8.0 ± 0.5	30.0 ± 1.6	38.0 ± 1.3	1.0 ± 0.1	4.0 ± 0.2	5.0 ± 0.2	0.7 ± 0.0	4.3 ± 0.6	5.0 ± 0.7	86.7 ± 3.1
day 9	7.6 ± 0.2	46.8 ± 0.9	54.8 ± 1.0	9.4 ± 0.0	20.3 ± 0.6	29.7 ± 0.6	1.5 ± 0.2	2.6 ± 0.0	4.2 ± 0.1	1.1 ± 0.1	3.2 ± 0.2	3.2 ± 1.2	91.9 ± 2.2
						Wine B							
grape	11.1 ± 0.6	n.d.	11.1 ± 0.6	5.1 ± 0.2	61.9 ± 2.7	66.9 ± 2.8	1.3 ± 0.0	10.1 ± 0.6	11.3 ± 0.6	1.1 ± 0.3	6.1 ± 0.3	7.2 ± 0.4	93.3 ± 3.5
day 0	4.6 ± 0.2	n.d.	4.6 ± 0.2	5.1± 0.1	15.2 ± 0.7	20.3 ± 0.7	1.3 ± 0.0	1.0 ± 0.1	2.3 ± 0.1	1.1 ± 0.0	n.d.	1.1 ± 0.1	28.2 ± 0.9
day 1	7.8 ± 0.2	n.d.	7.8 ± 0.2	6.2 ± 0.1	18.4 ± 1.5	24.6 ± 1.5	1.4 ± 0.0	1.7 ± 0.3	3.1 ± 0.3	1.4 ± 0.0	0.4 ± 0.2	1.8 ± 0.2	37.2 ± 1.8
day 2	6.9 ± 0.2	n.d.	6.93± 0.2	6.0 ± 0.1	17.1 ± 0.1	23.0 ± 0.0	1.3 ± 0.1	1.6 ± 0.1	1.3 ± 0.0	1.2 ± 0.0	0.5 ± 0.0	1.7 ± 0.0	32.9 ± 0.2
day 3	6.0 ± 0.5	n.d.	6.02± 0.2	5.7 ± 0.2	18.1 ± 0.4	23.8 ± 0.2	1.3 ± 0.0	1.8 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	n.d.	1.7 ± 0.1	33.5 ± 0.3
day 4	8.7 ± 0.5	n.d.	8.7 ± 0.5	12.6 ± 0.2	11.7 ± 0.9	24.3 ± 1.0	3.7 ± 0.2	n.d.	3.4 ± 0.2	1.8 ± 0.2	0.1 ± 0.1	1.9 ± 0.1	38.2 ± 1.6
day 5	5.2 ± 0.3	n.d.	5.2 ± 0.3	4.8 ± 0.0	21.0 ± 4.0	16.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.5	2.4 ± 0.0	1.8 ± 0.0	n.d.	1.6 ± 0.0	25.3 ± 0.3
day 6	7.5 ± 0.9	n.d.	7.5 ± 0.9	5.9 ± 0.7	19.8 ± 0.5	25.0 ± 0.5	1.1 ± 0.2	2.2 ± 0.0	3.1 ± 0.0	2.2 ± 0.2	0.1 ± 0.0	2.1 ± 0.1	36.8 ± 0.6
day 7	6.1 ± 0.1	n.d.	6.1 ± 0.1	5.2 ± 0.0	17.0 ± 0.9	22.1 ± 0.9	1.1 ± 0.0	1.4 ± 0.1	2.5 ± 0.2	1.2 ± 0.0	0.5 ± 0.1	1.6 ± 0.1	32.4 ± 1.2
day 8	4.7 ± 0.2	13.7 ± 0.5	18.4 ± 0.4	2.8 ± 0.1	43.1 ± 1.0	36.6 ± 0.9	0.5 ± 0.1	5.1 ± 0.3	5.6 ± 0.2	2.0 ± 0.1	2.2 ± 0.1	4.2 ± 0.1	65.1 ± 1.3
day 9	4.4 ± 0.1	14.9 ± 3.1	19.3 ± 3.2	3.9 ± 0.1	36.2 ± 1.9	40.1 ± 1.8	0.6 ± 0.0	5.1 ± 0.4	5. 7 ± 0.4	2.3 ± 0.0	2.1 ± 0.3	4.4 ± 0.3	69.4 ± 5.4
						Wine C							
grape	14.6 ± 0.3	41.4 ± 2.8	61.0 ± 5.3	3.8 ± 0.1	51.3 ± 0.4	56.6 ± 1.5	1.3 ± 0.0	11.2 ± 0.3	12.9 ± 0.4	1.0 ± 0.0	12.1 ± 0.0	13.4 ± 0.4	143.9 ± 7.4
day 0	n.d.	n.d.	n.d.	0.6 ± 0.1	4.0 ± 0.0	4.6 ± 0.2	0.2 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	5.7 ± 0.1
day 1	n.d.	n.d.	22.8 ± 1.1	1.4 ± 0.1	20.3 ± 0.2	21.7 ± 0.4	0.4 ± 0.0	4.1 ± 0.2	4.5 ± 0.2	0.3 ± 0.0	3.8 ± 0.1	4.0 ± 0.1	53.3 ± 1.2
day 2	1.8 ± 0.1	21.4 ± 1.0	23.2 ± 1.0	3.0 ± 0.1	18.2 ± 0.6	21.2 ± 0.5	1.0 ± 0.0	3.5 ± 0.1	4.5 ± 0.1	0.5 ± 0.0	3.6 ± 0.0	4.1 ± 0.0	53.0 ± 0.6
day 3	8.0 ± 1.1	53.7 ± 2.6	61.7 ± 2.0	5.5 ± 0.5	28.6 ± 2.4	34.0 ± 2.2	1.4 ± 0.1	6.2 ± 0.7	7.6 ± 0.7	0.8 ± 0.1	6.1 ± 0.8	6.8 ± 0.8	110.1 ± 5.8
day 4	11.6 ± 0.6	66.9 ± 6.9	78.5 ± 7.5	7.7 ± 0.3	30.8 ± 2.2	38.5 ± 2.3	1.8 ± 0.1	6.7 ± 0.5	8.6 ± 0.6	1.3 ± 0.0	6.0 ± 0.4	7.3 ± 0.4	132.8 ± 10.5
day 5	11.1 ± 0.2	87.5 ± 6.9	98.6 ± 7.0	8.0 ± 0.0	38.6 ± 3.3	46.6 ± 3.3	2.0 ± 0.0	8.4 ± 0.8	10.4 ± 0.8	1.2 ± 0.0	7.9 ± 0.4	9.1 ± 0.4	164.7 ± 11.4
day 6	8.9 ± 0.7	114.4 ± 7.3	123.3 ± 8.0	6.9 ± 0.1	51.8 ± 3.4	5.8 ± 3.3	1.7 ± 0.1	10.8 ± 0.6	12.4 ± 0.7	1.3 ± 0.0	9.7 ± 0.5	11.0 ± 0.5	205.5 ± 12.3
day 7	10.0 ± 0.2	108.0 ± 5.6	118.2 ± 5.3	7.3 ± 0.1	46.8 ± 3.2	54.1 ± 3.2	1.6 ± 0.0	9.7 ± 0.8	11.3 ± 0.8	1.1 ± 0.0	8.6 ± 0.8	9.8 ± 0.8	193.3 ± 10.1
day 8	16.7 ± 0.4	117.6 ± 0.4	134.4 ± 0.2	10.1 ± 0.2	47.3 ± 0.2	57.4 ± 0.1	2.1 ± 0.0	9.4 ± 0.1	11.5 ± 0.1	1.4 ± 0.1	8.7 ± 0.1	10.1 ± 0.2	213.3 ± 0.5
						Wine D							
grape	29.1 ± 3.3	59.9 ± 3.7	89.0 ± 2.6	9.6 ± 0.1	151.4 ± 4.5	161.0 ± 4.6	3.4 ± 0.0	40.1 ± 0.9	43.4 ± 0.9	1.5 ± 0.1	33.0 ± 0.8	34.4 ± 0.8	327.9 ± 6.7
day 0	n.d.	n.d.	n.d.	2.0 ± 0.0	11.2 ± 0.4	13.2 ± 0.4	1.3 ± 0.0	0.3 ± 0.0	1.7 ± 0.0	0.5 ± 0.0	1.1 ± 0.0	1.6 ± 0.0	16.5 ± 0.4
day 1	1.7 ± 0.1	16.2 ± 1.5	18.0 ± 1.5	2.0 ± 0.0	21.1 ± 0.1	23.0 ± 0.2	0.4 ± 0.0	3.0 ± 0.0	3.4 ± 0.0	0.4 ± 0.0	2.6 ± 0.1	3.0 ± 0.1	47.4 ± 1.8
day 2	2.5 ± 0.0	36.8 ± 2.6	39.3 ± 2.6	3.5 ± 0.0	32.2 ± 2.2	35.7 ± 2.2	0.6 ± 0.0	5.3 ± 0.3	5.9 ± 0.3	0.5 ± 0.0	4.5 ± 0.5	5.0 ± 0.5	85.9 ± 5.5
day 3	7.4 ± 0.2	64.4 ± 3.8	71.8 ± 4.0	6.4 ± 0.1	43.0 ± 2.5	49.4 ± 2.7	1.3 ± 0.0	7.6 ± 0.7	8.9 ± 0.7	0.8 ± 0.0	6.9 ± 0.9	7.7 ± 0.9	137.8 ± 8.3
day 4	6.7 ± 0.5	111.8 ± 1.5	118.5 ± 1.8	8.9 ± 0.3	61.8 ± 0.9	70.7 ± 1.2	1.9 ± 0.1	11.0 ± 0.3	12.9 ± 0.3	1.0 ± 0.0	11.1 ± 0.2	12.1 ± 0.2	214.2 ± 3.5
day 5	14.5 ± 0.3	81.6 ± 1.8	96.1 ± 1.6	10.7 ± 0.2	43.0 ± 0.9	53.7 ± 0.8	1.8 ± 0.0	7.2 ± 0.2	8.9 ± 0.2	1.1 ± 0.0	8.2 ± 0.4	9.4 ± 0.4	166.1 ± 2.8
day 6	7.3 ± 0.2	98.3 ± 3.5	105.6 ± 3.5	9.0 ± 0.1	52.9 ± 2.3	61.9 ± 2.3	1.6 ± 0.0	8.3 ± 0.7	9.9 ± 0.6	1.3 ± 0.0	12.6 ± 0.8	13.9 ± 0.8	191.3 ± 7.2
day 7	6.7 ± 0.2	83.5 ± 4.0	93.5 ± 2.4	9.3 ± 0.0	48.6 ± 2.5	57.9 ± 2.5	2.0 ± 0.0	8.1 ± 0.4	10.1 ± 0.4	1.3 ± 0.0	9.2 ± 0.6	10.6 ± 0.6	171.9 ± 2.0

[a] Data expressed as nmol/g grape tissue ± SEM, $n = 3$; wine data expressed as μM ± SEM, $n = 3$; n.d., not detected; conj, conjugated; kaempf, kaempferol; isorham, isorhamnetin.

Table 5. (+)-Catechin and (−)-Epicatechin Content of Wines A−D[a]

sample	(+)-catechin	(−)-epicatechin	total	(+)-cat: (−)-epi ratio
		Wine A		
grape	483.4 ± 10.2	457.8 ± 41.7	943.3 ± 49.2	1.1
day 0	5.1 ± 0.8	n.d.	5.1 ± 0.8	n.d.
day 1	4.9 ± 0.2	n.d.	4.9 ± 0.3	n.d.
day 2	12.6 ± 0.3	3.7 ± 0.3	16.3 ± 0.6	3.4
day 3	34.2 ± 1.0	14.3 ± 0.5	48.4 ± 1.4	2.4
day 4	40.0 ± 0.6	16.9 ± 0.3	56.8 ± 0.8	2.4
day 5	51.0 ± 2.7	21.8 ± 1.2	73.1 ± 3.9	2.4
day 6	56.5 ± 0.4	24.1 ± 0.8	80.6 ± 0.8	2.3
day 7	66.9 ± 0.2	35.7 ± 0.5	102.6 ± 0.5	2.0
day 8	64.2 ± 1.0	33.6 ± 0.46	97.8 ± 1.2	1.9
day 9	8.3 ± 1.2	28.5 ± 0.9	86.7 ± 2.1	2.0
		Wine B		
grape	382.7 ± 7.7	457.8 ± 41.7	746.7 ± 8.8	1.0
day 0	45.7 ± 0.1	17.7 ± 0.6	63.5 ± 0.7	2.6
day 1	55.2 ± 0.8	24.0 ± 0.3	79.2 ± 1.1	2.3
day 2	55.2 ± 1.0	25.4 ± 0.4	80.6 ± 1.3	2.2
day 3	54.6 ± 1.7	23.8 ± 0.4	78.4 ± 1.9	2.3
day 4	44.9 ± 1.1	17.3 ± 0.8	62.2 ± 1.9	2.6
day 5	63.1 ± 1.0	33.0 ± 0.3	96.1 ± 1.2	1.9
day 6	59.5 ± 1.0	28.0 ± 0.7	87.5 ± 1.6	2.1
day 7	66.2 ± 0.6	35.9 ± 0.5	102.1 ± 0.3	1.8
day 8	65.5 ± 0.4	36.4 ± 2.5	99.5 ± 0.6	1.8
day 9	60.9 ± 0.7	32.1 ± 0.7	93.0 ± 1.3	1.9
		Wine C		
grape	300.9 ± 8.4	276.4 ± 6.5	576.7 ± 14.5	1.1
day 0	3.8 ± 0.0	n.d.	n.d.	n.d.
day 1	7.9 ± 0.2	1.0 ± 0.1	3.8 ± 0.0	7.7
day 2	11.1 ± 0.2	3.0 ± 0.2	9.0 ± 0.2	3.7
day 3	19.4 ± 0.1	5.4 ± 0.3	14.1 ± 0.2	3.6
day 4	32.8 ± 0.2	11.1 ± 0.3	24.9 ± 0.2	2.9
day 5	35.6 ± 0.1	14.1 ± 0.3	49.6 ± 0.2	2.5
day 6	40.4 ± 0.4	17.9 ± 0.3	58.2 ± 0.4	2.3
day 7	48.6 ± 0.2	33.9 ± 0.5	82.5 ± 0.5	1.4
day 8	69.1 ± 0.6	26.2 ± 0.5	95.3 ± 0.4	2.6
		Wine D		
grape	634.8 ± 14.3	443.4 ± 4.4	1076.7 ± 8.8	1.4
day 0	7.7 ± 0.3	n.d.	7.7 ± 0.3	n.d.
day 1	27.0 ± 0.4	7.4 ± 0.7	34.3 ± 0.8	3.7
day 2	38.1 ± 0.4	13.6 ± 0.4	51.6 ± 0.7	2.8
day 3	31.9 ± 0.5	8.8 ± 0.2	40.7 ± 0.6	3.6
day 4	53.7 ± 0.4	15.0 ± 0.1	68.7 ± 0.4	3.6
day 5	64.1 ± 0.2	18.5 ± 0.4	82.6 ± 0.5	3.5
day 6	72.1 ± 0.6	20.6 ± 0.5	92.7 ± 0.9	3.5
day 7	64.9 ± 0.4	17.7 ± 0.5	83.6 ± 0.5	3.7

[a] Data expressed as nmol/g grape tissue ± SEM, $n = 3$; wine data expressed as μM ± SEM, $n = 3$; n.d., not detected.

3.5 nmol/g total flavonols (Table 4). Free and conjugated quercetin accounted for over 60% of the total flavonol content of the grapes. Quercetin was also the major flavonol extracted into wine; myricetin, kaempferol, and isorhamnetin were found in relatively low levels. The total flavonol concentration remained very steady from the juice, day 0, (28.2 ± 0.9 μM) to day 7 (32.4 ± 1.2 μM). The samples taken at day 8 and day 9 had significantly higher total flavonol levels of 65.1 ± 1.3 and 69.4 ± 5.4 μM and contained conjugated myricetin which was not present in the grapes and had not previously been detected in the must.

Flavan-3-ols. Equimolar concentrations of (+)-catechin and (−)-epicatechin were found in grapes B, with an average total concentration of 746.7 ± 8.8 μmol/g. In wine samples, however, (+)-catechin was found in levels almost 2-fold higher than (−)-epicatechin. Total flavan-3-ol levels increased slightly from 63.5 ± 0.7 μM in the juice (day 0) to between 90 and 100 μM by days 7, 8, and 9 (Table 5). On average, levels of (+)-catechin and (−)-epicatechin did not vary greatly over the 9 days of sampling.

Anthocyanins. Although they were present in only low levels, seven anthocyanins were found in grapes B (delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, malvidin-3-acetylglucoside, and malvidin-3-*p*-coumaroylglucoside). The total anthocyanin content of the grapes was found to be approximately 1000 ± 17.3 nmol/g (Table 6). This is 2- to 3-fold lower than levels in the other grapes investigated. It was noted that these grapes were relatively large and fleshier than the other grapes analyzed, i.e., they had a lower ratio of skin to volume. As anthocyanins are found within the skin, the presence of considerable flesh had a diluting influence. Very low levels of anthocyanins were found in all the samples of wine B. No anthocyanins could be detected until day 2, and maximum levels reached only 20.6 ± 0.4 μM total anthocyanins (Table 7). Once again, only the malvidin conjugates could be detected in the wine samples.

Gallic Acid. Little gallic acid is extracted with thermovinification. The gallic acid content remains under 20 μM (with the exception of day 1), which is comparable to the content of gallic acid at day 3 of wine A (Table 3).

Hydroxycinnamates. Grapes B contained the conjugates of caffeic and *p*-coumaric acids, with a total hydroxycinnamate content of 154.6 ± 4.2 nmol/g. No free caffeic acid was detected in any of the wine samples, and free *p*-coumaric acid was always less than 2% of the total *p*-coumaric acid quantified. Very high levels of total hydroxycinnamates were found in the juice (day 0): 284.4 ± 1.7 μM (Table 8). Although levels fluctuated throughout the sampling period, the final concentration of total hydroxycinnamates reached only 357.6 ± 2.0 μM. Conjugated *p*-coumaric acid was the major hydroxycinnamate present, with total caffeic acids contributing on average only one-third of the total hydroxycinnamate content.

Stilbenes. High levels of *trans*-resveratrol glucoside were found in grapes B (31.8 ± 1.5 nmol/g), with *trans*-resveratrol present on average at a concentration of only 2.4 ± 0.1 nmol/g (Table 9). Similarly high levels were observed with the fermenting musts. Levels of *trans*-resveratrol ranged from 2.8 ± 0.1 μM in the juice (day 0) to 6.4 ± 0.1 μM by day 8. Apart from a rogue point on day 3, *trans*-resveratrol appears to be steadily extracted into the wine.

Wine C. The grapes used for wine C were characterized by being very small in size, with a deep purple hue. They had been grown on old vines and in an area of the vineyard known to produce grapes with a concentrated flavor. Because of the high quality of the Cabernet Sauvignon grapes, this wine was to be made into a premium reserva wine. This means that it would be aged for three years, at least a year of which must take place in oak. Vinification took place in small concrete vats that would allow more contact between the skins and the juice. To further encourage this mixing of the skins and the juice the wine was pumped-over. In this case the wine was completely emptied from the vat, allowing the skins to fall to the bottom. The wine was then added back, spraying onto the top of the skins.

Total Phenolics. The phenolic content of the wines increased steadily from 2.1 ± 0.0 mM GAE on day 0 to 10.5 ± 0.3 mM GAE on day 8. Although the rate of increase began to decrease around day 4, the total

Table 6. Total Anthocyanin Content of Grapes A−D[a]

grape	anthocyanin 1	2	3	4	5	6	7	total anthocyanins
A	210.3 ± 11.8	16.4 ± 1.2	137.7 ± 6.2	n.d.	1275.5 ± 49.3	743.8 ± 10.4	355.3 ± 5.1	2470.0 ± 70.1
B	98.6 ± 0.0	61.5 ± 0.7	74.4 ± 1.7	118.7 ± 4.4	406.2 ± 1.2	166.1 ± 15.7	166.1 ± 1.4	1000.0 ± 17.3
C	277.6 ± 6.7	n.d.	201.1 ± 3.4	n.d.	1716.0 ± 28.5	927.5 ± 14.2	421.4 ± 5.4	3296.7 ± 52.4
D	304.5 ± 3.2	117.4 ± 1.1	214.1 ± 4.9	297.0 ± 1.2	859.1 ± 12.2	268.6 ± 1.5	306.5 ± 1.4	2206.7 ± 18.6

[a] Data expressed as nmol anthocyanin/g grape fresh weight. Anthocyanin 1, delphinidin-3-glucoside; anthocyanin 2, cyanidin-3-glucoside; anthocyanin 3, petunidin-3-glucoside; anthocyanin 4, peonidin-3-glucoside; anthocyanin 5, malvidin-3-glucoside; anthocyanin 6, malvidin-3-acetylglucoside; anthocyanin 7, malvidin-3-*p*-coumaroyl glucoside. n.d., not detected.

Table 7. Free Anthocyanin Content of Wines A−D[a]

sample	malvidin-3-glucoside	malvidin-3-acetyl glucoside	malvidin-3-*p*-coumaroyl glucoside	total
		Wine A		
day 0	n.d.	n.d.	n.d.	n.d.
day 1	3.1 ± 0.0	1.4 ± 0.1	n.d.	4.5 ± 0.1
day 2	20.4 ± 0.5	6.8 ± 0.1	0.9 ± 0.1	28.2 ± 0.2
day 3	19.7 ± 0.1	6.5 ± 0.1	0.6 ± 0.0	26.8 ± 0.2
day 4	20.6 ± 1.5	7.8 ± 0.8	1.2 ± 0.2	29.7 ± 2.5
day 5	23.8 ± 0.5	9.1 ± 0.4	3.2 ± 0.1	36.0 ± 0.8
day 6	19.8 ± 0.3	6.3 ± 0.2	1.4 ± 0.1	27.5 ± 0.2
day 7	21.0 ± 0.2	6.8 ± 0.3	0.9 ± 0.2	28.8 ± 0.3
day 8	26.5 ± 1.0	7.9 ± 0.3	1.4 ± 0.2	35.8 ± 1.5
day 9	50.0 ± 0.4	18.1 ± 0.3	5.6 ± 0.2	73.7 ± 0.9
		Wine B		
day 0	n.d.	n.d.	n.d.	n.d.
day 1	n.d.	n.d.	n.d.	n.d.
day 2	0.6 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	1.2 ± 0.1
day 3	n.d.	n.d.	n.d.	n.d.
day 4	5.0 ± 0.5	2.2 ± 0.2	2.1 ± 0.5	9.2 ± 1.1
day 5	3.2 ± 0.1	1.1 ± 0.1	0.9 ± 0.0	5.2 ± 0.0
day 6	5.2 ± 0.3	1.8 ± 0.1	1.5 ± 0.3	8.5 ± 0.6
day 7	4.6 ± 0.1	1.5 ± 0.1	1.2 ± 0.2	7.3 ± 0.4
day 8	4.9 ± 0.2	1.8 ± 0.1	1.7 ± 0.0	8.4 ± 0.3
day 9	12.4 ± 0.2	4.8 ± 0.1	3.4 ± 0.1	20.6 ± 0.4
		Wine C		
day 0	2.7 ± 0.1	1.9 ± 0.0	0.3 ± 0.0	4.9 ± 0.0
day 1	19.6 ± 0.2	8.7 ± 0.1	1.2 ± 0.0	29.6 ± 0.2
day 2	15.7 ± 0.2	6.9 ± 0.2	1.0 ± 0.0	23.6 ± 0.1
day 3	42.8 ± 2.4	15.6 ± 1.1	3.9 ± 0.6	62.3 ± 4.1
day 4	57.0 ± 2.3	22.7 ± 1.3	5.5 ± 1.4	85.2 ± 4.9
day 5	39.5 ± 2.0	15.2 ± 0.8	4.1 ± 0.3	58.7 ± 3.1
day 6	132.5 ± 6.6	58.3 ± 3.6	19.1 ± 1.7	209.9 ± 11.7
day 7	91.8 ± 1.5	39.9 ± 0.6	13.3 ± 0.4	145.0 ± 2.1
day 8	149.9 ± 14.2	64.2 ± 7.6	24.9 ± 4.0	239.1 ± 25.7
		Wine D		
day 0	0.5 ± 0.1	0.4 ± 0.0	n.d.	0.8 ± 0.1
day 1	23.8 ± 1.3	11.5 ± 1.2	2.1 ± 0.5	37.4 ± 2.9
day 2	35.3 ± 5.7	16.8 ± 3.5	5.1 ± 2.0	57.2 ± 11.1
day 3	29.6 ± 2.8	9.9 ± 0.9	3.1 ± 0.7	42.7 ± 4.4
day 4	17.6 ± 1.2	6.9 ± 0.6	2.8 ± 0.2	27.3 ± 1.9
day 5	n.d.	n.d.	n.d.	n.d.
day 6	76.6 ± 5.4	7.7 ± 0.3	2.7 ± 0.3	30.9 ± 1.9
day 7	20.5 ± 1.5	7.8 ± 1.1	3.0 ± 0.5	30.0 ± 4.0

[a] Results are expressed as μM ± SEM, $n = 3$; n.d., not detected.

phenolic content had not yet plateaued by the end of the sampling period.

Flavonols. The small dense Cabernet Sauvignon berries had a mean total flavonol content of 143.9 ± 7.4 nmol/g grape tissue, almost 1.5 times higher than that observed with grapes A and B. Although no myricetin was detected in the juice (day 0), it was rapidly extracted from the grapes into the wine. The total flavonol content of the wine increased from 5.7 ± 0.1 μM in the juice to over 200 μM in the must by day 6, at which point the levels remained steady (Table 4). On average, myricetin accounted for over half of the grape total flavonol content. Although quercetin was responsible for just under half of the grape total flavonol content, it reached levels of only around a quarter of the total flavonol content of the wine from day 6 onward.

Flavan-3-ols. Grapes C had an average of 576.7 nmol/g total flavan-3-ols, with a ratio of (−)-epicatechin to (+)-catechin of approximately 1:1 (Table 5). The flavan-3-ol content is significantly lower than those observed for grapes A, B, and D. Maximum flavan-3-ol levels in wine C were found to be similar to those of wines A, B, and D. A maximum of 95.3 μM total flavan-3-ols was recorded on day 8, after an almost linear extraction from the juice over the sampling period. (+)-Catechin was present, on average, in a 2- to 3-fold excess compared with (−)-epicatechin.

Anthocyanins. As with the Cabernet Sauvignon grapes A, not all of the expected anthocyanins could be detected in grapes C. Five anthocyanins were quantified (delphinidin-3-glucoside, petunidin-3-glucoside, malvidin-3-glucoside, malvidin-3-acetylglucoside, and malvidin-3-*p*-coumaroylglucoside), with a total anthocyanin content of 3296.7 ± 52.4 nmol/g (Table 6). This was the highest concentration of anthocyanins of the grapes analyzed. This is most likely to be attributable to the high skin-to-volume ratio of the small, dense berries. Compared with levels of the other wines analyzed, high levels of anthocyanins were extracted into the wine. Although levels of anthocyanins fluctuated during the extraction period, the maximum total anthocyanin content of 239.1 ± 25.7 μM was recorded on day 8 (Table 7).

Gallic Acid. The content of gallic acid increased at a steady rate from 2.6 ± 0.4 μM in the juice to 63.7 ± 0.4 μM by day 8 (Table 3). The gallic acid content began to plateau by days 7 and 8.

Hydroxycinnamates. The total hydroxycinnamate content of grapes C was found to be, on average, 368.3 ± 42.2 nmol/g (Table 8). In contrast to results from the other grapes, 45% of the total *p*-coumaric acid was found as the free aglycone. This pattern was not observed in the wine samples where free caffeic and *p*-coumaric acids only contributed around 2% of the total hydroxycinnamates present. The total hydroxycinnamate content of the wine samples increased from 102.5 ± 0.2 μM in the juice (day 0) to 425.2 ± 0.4 μM by day 8. As with the other wines, *p*-coumaric acid was the major hydroxycinnamate present.

Stilbenes. Trans-resveratrol was not detected in grapes C, and *trans*-resveratrol glucoside was found at a concentration of only 6.8 nmol/g (Table 9). *trans*-Resveratrol was found in very low levels in wine C. It was undetected in the juice and day 1 samples, and reached a maximum of only 2.1 ± 0.1 μM on day 8. The glucoside, however, was steadily extracted into the wine. Levels of 2.0 ± 0.1 μM were found in the juice (day 0) and they increased to 11.7 ± 0.3 μM by day 8.

Wine D. The grapes used to make wine D were of a deep red hue and were medium-sized. They were of sufficient quality to merit a "varietal" status. They did not require blending prior to bottling. The wine underwent a traditional fermentation after the grapes were

Table 8. Hydroxycinnamate Content of Grapes and Wines A−D[a]

sample	free caffeic acid	caftaric acid	total caffeic acid	free *p*-coumaric acid	conj. *p*-coumaric acid	total *p*-coumaric acid	total hydroxycinnamates
				Wine A			
grape	n.d.	195.8 ± 1.0	195.8 ± 1.0	n.d.	149.3 ± 2.3	149.3 ± 2.3	345.2 ± 2.9
day 0	n.d.	18.2 ± 0.3	21.6 ± 0.2	0.7 ± 0.1	10.7 ± 0.3	11.4 ± 0.2	33.0 ± 0.1
day 1	1.0 ± 0.0	10.1 ± 0.0	19.2 ± 0.2	2.0 ± 0.1	30.0 ± 0.7	32.0 ± 0.6	51.2 ± 0.4
day 2	2.3 ± 0.0	18.7 ± 0.1	49.2 ± 0.2	3.4 ± 0.2	77.1 ± 0.7	80.5 ± 0.7	129.7 ± 0.8
day 3	8.3 ± 0.2	61.5 ± 0.5	87.7 ± 0.1	5.2 ± 0.1	87.9 ± 0.3	93.1 ± 0.3	180.8 ± 0.4
day 4	12.1 ± 0.2	131.9 ± 0.7	150.9 ± 2.3	4.1 ± 0.1	181.7 ± 1.8	185.9 ± 1.0	336.9 ± 3.3
day 5	9.9 ± 0.1	112.1 ± 0.6	143.2 ± 0.5	4.0 ± 0.0	188.8 ± 1.2	192.8 ± 1.2	335.7 ± 0.9
day 6	13.0 ± 0.1	141.7 ± 0.4	160.1 ± 1.5	4.5 ± 0.1	236.6 ± 3.4	241.1 ± 3.3	401.2 ± 4.2
day 7	11.6 ± 0.2	114.0 ± 0.9	101.3 ± 0.2	3.5 ± 0.0	160.9 ± 0.7	164.4 ± 0.6	265.7 ± 0.8
day 8	11.7 ± 0.0	107.6 ± 0.1	113.2 ± 0.3	5.1 ± 0.5	164.1 ± 1.2	169.2 ± 1.7	282.4 ± 1.4
day 9	10.8 ± 0.1	104.4 ± 0.6	108.2 ± 0.8	3.6 ± 0.1	159.4 ± 0.8	163.3 ± 0.8	271.4 ± 0.8
				Wine B			
grape	n.d.	79.8 ± 3.0	79.8 ± 3.0	n.d.	74.8 ± 1.3	74.8 ± 1.3	154.6 ± 4.2
day 0	n.d.	49.8 ± 0.3	95.0 ± 0.6	1.8 ± 0.0	187.6 ± 1.1	189.4 ± 1.1	284.4 ± 1.7
day 1	n.d.	73.0 ± 0.1	142 0 ± 0.6	2.4 ± 0.1	268.1 ± 2.6	270.5 ± 2.6	412.5 ± 2.6
day 2	n.d.	59.4 ± 0.3	119 1 ± 0.4	1.5 ± 0.0	214.3 ± 1.1	215.8 ± 1.1	334.8 ± 1.1
day 3	n.d.	55.3 ± 0.4	105.9 ± 0.4	1.8 ± 0.0	206.3 ± 1.2	208.0 ± 1.2	313.9 ± 1.1
day 4	n.d.	47.9 ± 0.2	80.7 ± 0.3	4.7 ± 0.0	248.1 ± 1.8	253.1 ± 1.6	333.6 ± 1.5
day 5	n.d.	58.4 ± 0.1	107.2 ± 0.5	1.1 ± 0.0	172.0 ± 0.8	173.1 ± 0.8	280.2 ± 0.8
day 6	n.d.	62.6 ± 0.2	123 6 ± 1.2	1.3 ± 0.0	209.7 ± 1.6	211.0 ± 1.6	334.5 ± 2.2
day 7	n.d.	54.2 ± 0.1	110.3 ± 0.3	0.9 ± 0.0	192.5 ± 2.1	193.4 ± 2.2	303.6 ± 1.9
day 8	n.d.	57.4 ± 0.1	105.5 ± 0.4	1.6 ± 0.1	183.6 ± 1.4	185.9 ± 1.0	290.7 ± 1.0
				Wine C			
grape	n.d.	189.0 ± 21.5	189.0 ± 21.5	80.4 ± 0.3	100.1 ± 22.5	179.4 ± 20.7	368.3 ± 42.2
day 0	2.0 ± 0.1	31.0 ± 0.2	76.9 ± 0.4	2.0 ± 0.0	23.7 ± 0.5	25.6 ± 0.5	102.5 ± 0.2
day 1	n.d.	4.5 ± 0.1	20.6 ± 0.2	1.1 ± 0.1	52.7 ± 0.4	53.8 ± 0.3	74.4 ± 0.4
day 2	n.d.	31.7 ± 0.1	80.4 ± 0.3	1.9 ± 0.1	145.4 ± 0.8	147.4 ± 0.8	227.8 ± 0.8
day 3	1.2 ± 0.2	52.4 ± 0.0	115.0 ± 0.6	2.0 ± 0.0	209.8 ± 3.7	211.7 ± 3.7	326.7 ± 4.2
day 4	1.5 ± 0.1	77.9 ± 0.4	104.4 ± 0.4	2.6 ± 0.0	195.6 ± 1.6	198.1 ± 1.6	302.6 ± 1.4
day 5	5.4 ± 0.1	92.2 ± 0.3	138.1 ± 0.1	3.5 ± 0.0	268.1 ± 2.1	271.6 ± 2.1	409.7 ± 2.0
day 6	4.8 ± 0.2	82.1 ± 2.2	125.1 ± 0.3	3.0 ± 0.1	258.0 ± 2.8	261.0 ± 2.1	386.1 ± 2.5
day 7	4.3 ± 0.2	81.5 ± 0.9	129.9 ± 0.3	2.7 ± 0.0	292.6 ± 0.1	295.4 ± 0.1	425.2 ± 0.4
day 8	3.5 ± 0.3	81.5 ± 1.6	111.0 ± 1.4	2.7 ± 0.0	272.9 ± 3.5	275.7 ± 3.5	386.7 ± 4.9
				Wine D			
grape	n.d.	171.9 ± 4.0	171.9 ± 4.0	n.d.	151.1 ± 2.0	151.1 ± 2.0	323.0 ± 5.6
day 0	n.d.	12.9 ± 0.1	41.5 ± 0.3	2.4 ± 0.1	55.5 ± 0.3	57.8 ± 0.2	99.4 ± 0.4
day 1	n.d.	24.2 ± 0.0	60.2 ± 0.7	4.0 ± 0.2	87.1 ± 0.2	91.1 ± 0.2	151.3 ± 0.9
day 2	1.3 ± 0.1	35.5 ± 1.7	79.6 ± 0.2	3.6 ± 0.1	137.7 ± 0.6	141.2 ± 0.7	220.8 ± 0.8
day 3	1.7 ± 0.2	42.0 ± 2.2	81.2 ± 0.4	5.9 ± 0.1	177.3 ± 1.2	183.2 ± 1.1	262.3 ± 1.5
day 4	3.4 ± 0.5	46.7 ± 1.0	97.2 ± 0.4	5.9 ± 0.0	262.4 ± 0.9	268.3 ± 0.9	365.4 ± 1.3
day 5	4.1 ± 0.1	45.0 ± 0.3	83.8 ± 0.3	8.0 ± 0.1	233.4 ± 0.2	241.4 ± 0.1	325.3 ± 0.3
day 6	5.0 ± 0.3	44.0 ± 0.2	77.7 ± 0.5	6.0 ± 0.1	213.1 ± 1.3	219.1 ± 1.3	296.8 ± 1.8
day 7	5.8 ± 0.6	51.7 ± 4.0	67.9 ± 0.2	5.1 ± 0.2	185.5 ± 0.5	190.5 ± 0.5	258.4 ± 0.7

[a] Data expressed as nmol/g grape tissue ± SEM, $n = 3$; wine data expressed as μM caffeic, caftaric, or *p*-coumaric acids ± SEM, $n = 3$; conj., conjugated; n.d., not detected.

Table 9. *trans*-Resveratrol and *trans*-Resveratrol Glucoside Content of Grapes and Wines A−D[a]

	wine A		wine B		wine C		wine D	
sample	*t*-resveratrol	*t*-resveratrol glucoside	*t*-resveratrol	*t*-resveratrol glucoside	*t*-resveratrol	*t*-resveratrol glucoside	*t*-resveratrol	*t*-resveratrol glucoside
grape	2.2 ± 0.2	9.7 ± 0.5	2.4 ± 0.1	31.8 ± 1.5	n.d.	6.8 ± 0.3	n.d.	24.0 ± 1.2
day 0	0.1 ± 0.0	0.4 ± 0.1	2.8 ± 0.1	35.5 ± 1.7	n.d.	2.0 ± 0.1	1.6 ± 0.0	1.6 ± 0.0
day 1	0.3 ± 0.0	2.0 ± 0.0	4.2 ± 0.1	73.8 ± 2.7	n.d.	2.1 ± 0.0	2.8 ± 0.1	2.8 ± 0.1
day 2	0.8 ± 0.1	4.7 ± 0.1	4.4 ± 0.1	54.2 ± 2.6	0.2 ± 0.0	4.2 ± 0.1	4.4 ± 0.2	4.4 ± 0.2
day 3	0.6 ± 0.1	4.2 ± 0.1	6.6 ± 0.1	87.6 ± 1.7	0.4 ± 0.1	5.3 ± 0.2	3.8 ± 0.1	3.8 ± 0.1
day 4	0.9 ± 0.0	6.1 ± 0.0	3.6 ± 0.3	60.7 ± 0.9	0.9 ± 0.2	8.2 ± 0.5	6.3 ± 0.2	6.3 ± 0.2
day 5	1.5 ± 0.3	6.5 ± 0.3	4.2 ± 0.2	54.8 ± 0.5	0.6 ± 0.1	8.8 ± 0.5	4.4 ± 0.4	4.4 ± 0.4
day 6	1.8 ± 0.1	8.2 ± 0.2	4.7 ± 0.2	67.1 ± 0.2	1.5 ± 0.4	10.2 ± 0.0	4.9 ± 0.2	4.9 ± 0.2
day 7	1.4 ± 0.2	4.0 ± 0.3	5.0 ± 0.4	73.1 ± 0.3	0.7 ± 0.0	5.9 ± 0.4	5.8 ± 0.5	5.8 ± 0.5
day 8	1.4 ± 0.3	6.1 ± 0.6	5.8 ± 0.2	65.3 ± 0.6	2.1 ± 0.2	11.7 ± 0.3	-	-
day 9	1.1 ± 0.1	5.6 ± 0.3	6.4 ± 0.1	112.0 ± 0.9	-	-	-	-

[a] Data expressed as nmol/g grape tissue ± SEM, $n = 3$; wine data expressed as μM *trans*-resveratrol ± SEM, $n = 3$; n.d., not detected.

extracted in the rotor-vat to maximize the color and tannins of the wine.

Total Phenolics. Of the four wines followed, wine D recorded the highest total phenolic content of 11.4 ± 0.1 mM GAE on day 7. The total phenolic content of the wine had begun to reach a steady level around day 4.

Flavonols. The total flavonol content of the Merlot grapes used for this wine was 327.9 ± 6.7 nmol/g (Table 4), 4-fold higher than that found in grapes A and B, and 2-fold higher than that in grapes C. Although the grapes contained twice the flavonol content of grapes C, the final total flavonol content of the wine was 171.9

± 2.0 μM, slightly less than the final value recorded for wine C. Once again the major flavonol in the wine was myricetin, ranging from being undetected in the juice (day 0) to accounting for over 50% of the total flavonols by day 7. The maximum flavonol content occurred at day 4 and remained relatively steady from that point onward.

Flavan-3-ols. Total flavan-3-ol levels of 1076.7 ± 8.8 nmol/g were recorded in grape D, with (+)-catechin present in excess of (−)-epicatechin (Table 5). This pattern continued, with (+)-catechin found in the wines at levels over 3-fold higher than the levels of (−)-epicatechin. Maximum flavan-3-ol levels of 92.7 ± 0.9 μM were attained on day 6, nearly 78% of which was due to (+)-catechin. Compared to wines A and C, quite high levels of flavan-3-ols were extracted into wine D during the first 2 days of vinification. However, although grapes D had higher flavan-3-ol levels than the others grapes, the maximum levels attained in the must were similar to those found in wines A, B, and C.

Anthocyanins. Once again, seven anthocyanins could be quantified in the Merlot grapes D (delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, malvidin-3-acetylglucoside, and malvidin-3-*p*-coumaroylglucoside (Table 6)). Total anthocyanin levels of 2206.7 ± 18.6 nmol/g were recorded, and malvidin-3-glucoside contributed around 37% of this total. An unusual extraction profile was observed with wine D anthocyanins. Maximum total anthocyanin levels were obtained by day 2 (57.2 ± 11.1 μM), and then decreased to only 30.0 ± 4.0 μM by day 7 (Table 7). Anthocyanin values are not reported for day 5 because of sample deterioration.

Gallic Acid. Although wine D reaches a maximum gallic acid concentration similar to that of the other wines, it is attained at an earlier stage (Table 3). By day 4 gallic acid had reached levels of over 60 μM. This is compared to only 6.3 ± 0.3 μM in the juice (day 0).

Hydroxycinnamates. Free hydroxycinnamic acids were not detected in grapes D. Conjugated *p*-coumaric acids were responsible for over 80% of the total hydroxycinnamate content. In the wine samples, the total hydroxycinnamic content ranged from 99.4 ± 0.4 μM in the juice (day 0) to a maximum of 365.4 ± 1.3 μM by day 4 (Table 8). At this point, the hydroxycinnamate content decreased to 258.4 ± 0.7 μM by day 7. Once again, conjugated *p*-coumaric acids were the major hydroxycinnamates present, with levels of total caffeic acids remaining relatively steady over the sampling period.

Stilbenes. Although *trans*-resveratrol glucoside was found in grapes D at levels of approximately 24.0 ± 1.2 nmol/g, the aglycon was not detected (Table 9). Very low levels of *trans*-resveratrol were detected in wine D; on average they were less than 1μg/g. *trans*-Resveratrol glucoside was steadily extracted into the wine. From 1.6 ± 0.0 μM in the juice (day 0), levels reached 5.8 ± 0.5 μM by day 8.

Changes in Antioxidant Activity During Vinification. In addition to analyzing the phenolic content of each sample, the ESR-derived antioxidant capacity was also determined. The correlation between the antioxidant activity and the extraction of each phenolic was assessed statistically using Pearson correlations. As with the analyses of bottled wines, a very close relationship was observed between the Folin−Ciocalteu total phenolic content and the antioxidant activity of each wine (Figure 1).

Wine A. The ESR-based antioxidant activity rose concomitantly with the total phenol content (Figure 1A) from 0.12 ± 0.2 × 10^{21} Fremys radicals reduced per L on day 0 to 2.0 ± 0.0 × 10^{21} Fremys radicals reduced on day 9; however, the antioxidant activities of the unfinished wines are up to 4-fold less than that of a finished wine, 4.5 to 9.3 × 10^{21} (*11*). The antioxidant activity peaks on day 6, along with the total phenol content, derived by either the Folin−Ciocalteu assay or HPLC. This wine was racked on day 7 and moved to a larger mixing vat. The decrease in total phenolic and antioxidant activity at this point could be due to dilution with less well-extracted wines. The antioxidant activity was very highly significantly or highly significantly correlated with all of the individual phenolic families (data not shown) with the exception of the total anthocyanins ($r_p = 0.674$, $p = 0.033$).

Wine B. Although the phenolic profile of wine B was erratic, the antioxidant activity was significantly correlated with the Folin−Ciocalteu derived total phenol content ($r_p = 0.720$, $p = 0.019$). This relationship is illustrated in Figure 1B. Even though this wine did not undergo alcoholic fermentation, its final total phenolic content and antioxidant activity are similar to those of wine A (Table 2). Very few significant relationships were observed between the phenolic components and antioxidant activity of wine B. The ESR antioxidant activity and the Folin−Ciocalteu total phenol content were both highly significantly correlated to the gallic acid content of the wine ($r_p = 0.811$, $p = 0.004$ and $r_p = 0.946$, $p = 0.000$, respectively).

Wine C. The antioxidant activity of wine C increased steadily throughout the sampling period. Both the final Folin−Ciocalteu total phenol content and antioxidant activity were significantly higher than those achieved for wines A and B (Figure 1C). The antioxidant activity was undetectable at day 0, but rose to 3.6 ± 0.0 × 10^{21} Fremys radicals reduced per L wine, while the Folin−Ciocalteu total phenolic content increased from 2.1 ± 0.0 mM GAE to 10.5 ± 0.3 mM GAE (Table 2). The antioxidant activity was at least highly significantly correlated to all of the major phenolic families. Similar close relationships were found with Folin−Ciocalteu and HPLC total phenolic contents and each individual family (data not shown).

Wine D. The highest Folin−Ciocalteu total phenol content was found in wine D, 11.4 ± 0.1 mM GAE, however the corresponding antioxidant activity was only 2.9 ± 0.0 × 10^{21} Fremys radicals reduced per L (Figure 1D). Indeed, the relationship between the total phenolic content and the antioxidant activity was not as close as was found with the other traditionally fermented wines, A and C ($r_p = 0.939$, $p = 0.001$). The antioxidant activity appeared to plateau around day 3, with day 5 recording an aberrant value. Indeed, the anthocyanin data for this time point were abnormal and were omitted. The antioxidant activity was significantly correlated with total flavan-3-ols, flavonols, gallic acid, and total hydroxycinnamates. Similar patterns were found with both Folin−Ciocalteu and HPLC total phenolics and the individual phenolics.

DISCUSSION

The phenolic content of the wine samples appeared to vary with vinification approach and grape quality and variety. Two major approaches to vinification were investigated: traditional and thermovinification. Com-

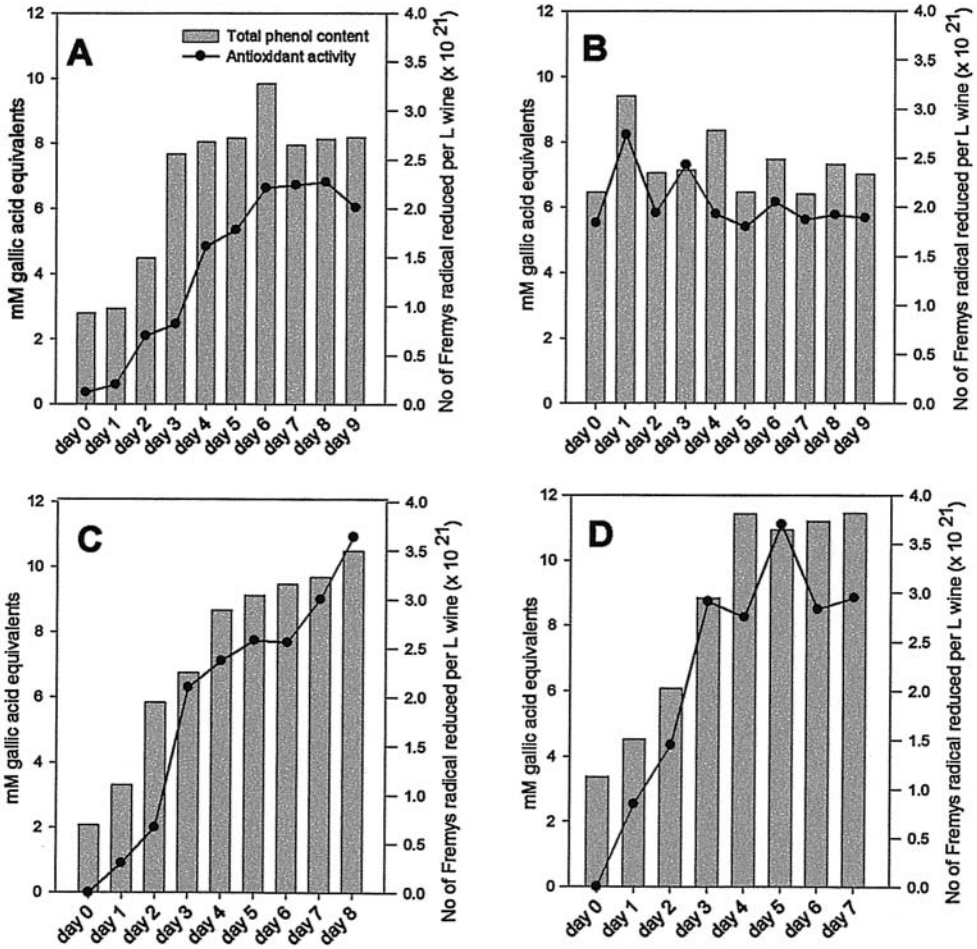

Figure 1. Comparison of the extraction of total phenols and antioxidant activity in wines A−D. Total phenol content determined by Folin−Ciocalteu assay with results expressed as mM gallic acid equivalents (GAE). Antioxidant activity determined as the number of Fremy's radicals reduced $\times$ 10^{21} per L of must.

pared with the traditional approach, the thermovinified wine was heated to over 60 °C for 1 h and did not immediately undergo alcoholic fermentation. At increased temperatures phenolics are more efficiently extracted into wine from grapes (*8*). Both *trans*-resveratrol and its glucoside were present in the thermovinified wines at a higher proportion than in traditionally vinified wines (Table 9). The absence of alcohol in the thermovinified wine also affected the extraction of phenolics. The flavonol myricetin was not well extracted from grapes into thermovinified wine. In the other wines analyzed myricetin was the major flavonol present, compared with quercetin in the thermovinified wine.

Grape quality is also an important determinant of the eventual phenolic content of a wine. With increasing quality grapes become smaller and more concentrated in terms of flavor and phenolic content. A high-quality grape has a higher ratio of skin to volume compared with that of a lower-quality grape. Because of the small size of the high-quality grapes a much greater volume of these grapes is required in order to make the same volume of wine as would be produced from lower-quality fleshier grapes. Wines made from high-quality grapes have a higher content of skin-derived phenolics than those made from the more diluted, lower-quality, grapes.

It is well-established that different varieties of fruits and vegetables have varying phenolic profiles (*14*), and grapes are no exception. A comparison of Merlot and Cabernet Sauvignon wines found that, irrespective of quality, the anthocyanins found in the two wines were different. Anthocyanin profiles are used in the authentication of wines and other anthocyanin-containing products (*15*). Likewise, Pinot Noir grapes are known to be constituitively higher in catechins than other varieties (*16*).

The antioxidant activity of a wine is due to polyphenolic compounds (*17*−*20*). Removing polyphenols by precipitating them with insoluble polyvinylpolypyrrolidone (PVPP) abolishes the antioxidant activity of a wine (*18*). Studies to identify the particular phenolics responsible for the antioxidant activity have highlighted the anthocyanin and proanthocyanidin classes (*19*, *21*). This current study found that using traditional fermentation (wines A, C, and D) the content of the majority of phenolic families was correlated with the ESR-derived antioxidant activity. Contrary to other studies (*21*), total anthocyanin levels were not found to be related to the antioxidant activity in wines A and D. The extraction of anthocyanins was rather erratic, and it may be that this obscured a relationship that might have been apparent at a later date. Previous investigations of finished wines found that there was no correlation between antioxidant activity and spectral anthocyanin content (*11*). However, a more recent study observed a significant relationship between the polymeric pigment content and the antioxidant activity of New World wines ($r_s = 0.52$, $p = 0.014$) (*22*).

Total flavan-3-ols, (+)-catechin, and (−)-epicatechin were correlated to antioxidant activity in each of the traditionally fermented wines (A, C, and D). This is in

line with other work that has shown that the catechin/proanthocyanidin fraction of a wine is responsible for its antioxidant activity (*19*).

Throughout this study the antioxidant activity of samples has been assessed using electron spin resonance spectroscopy. Unlike many antioxidant activity assays, this method can be used with turbid and colored samples. The application of this approach has been tested by parallel analysis with the common FRAP (ferric reducing potential) assay. A previous study of 22 red wines (*22*) has shown that, although they are assessing the ability of the wine to act as an antioxidant by two different mechanisms, the results obtained from the ESR and FRAP methods are closely correlated ($r_s = 0.95$, $p < 0.001$).

Although the fermenting wines attained a Folin−Ciocalteu total phenolic content comparable with that of a finished wine, the final antioxidant activity was significantly lower than that of a finished wine. Finished wines ranged from 4.5 to 9.3×10^{21} Fremys radicals reduced per L of wine (*11*), compared with final values of 2.0, 1.9, 3.6, and 3.0×10^{21} Fremys radicals reduced per L of wine for wines A, B, C, and D respectively (wine C corresponds to the Chilean Cabernet Sauvignon noted in a previous study to have an antioxidant activity of ca. 7.0×10^{21} Fremys radicals reduced). This anomaly has been attributed to the young chemical age and maturity of the fermenting wines. Although the samples may have their full complement of phenolics, they lack the complexes and condensation products that appear over time (*23*). These larger complexes may contribute significantly to the antioxidant activity of a finished wine.

There has been conflicting evidence on the effect of aging on the antioxidant activity of wines. As wine ages anthocyanins and other compounds complex, and with the proanthocyanidins they contribute to the formation of tannins (*23*). A study of aging in Spanish red wines found that older wines had a greater antioxidant activity (*24*). This was attributed to the increase in tannins during aging. Indeed, an increase in the degree of polymerization of polyphenols from grape seed extracts has resulted in an increase in their superoxide radical scavenging activity (*25*). However, a comparison of young Italian wines and their aged counterparts found that the young wines made with carbonic maceration, and to be consumed within three months, had a higher antioxidant activity than the wine made for aging (*26*). There was no difference in the total phenol or flavan-3-ol content of the wines, and the authors speculated that the phenolics lose antioxidant activity as they age. However, the young and aged wines were vinified differently and may have had significantly altered phenolic profiles, which would also contribute to differences in their antioxidant activity.

The twin studies of viticulture and vinification may be selectively invoked and manipulated to produce wines with high antioxidant activity. It is likely that, given the extensive selective breeding/cloning of grape varieties, it should be possible to isolate a variety that is naturally higher in a particular phenolic that has been identified as an important antioxidant or as a building block in a more structurally complex antioxidant. Likewise, it is already well-established that the processes of vinification have a significant influence on the nature and content of phenolics in wine. With the recent controversy and public fear in Europe and the UK over genetic modification of foods, such approaches could yield the same eventual result but without the public hysteria.

Although a significant proportion of the antioxidant activity of a wine may be attributed to large complexes such as the condensed tannins, little information is available on the extent of their absorption and bioavailability. Unfortunately, there is no correlation between high antioxidant activity and high bioavailability. Further work in the field of absorption and bioavailability of tannins is required to assess the nutritional benefits of increasing their content in a wine.

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