A Standard Red Wine: Monomeric Phenolic Analysis of Commercial Cabernet Sauvignon Wines

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Quantitative data on the concentration of phenolic compounds in a large, representative sample of widely consumed commercial red wines have been lacking. The 21 highest volume Cabernet Sauvignon wines, representing 17% of all red wines sold in US supermarkets (and over two million 9-L cases), were selected based on 1995 WINESCAN data reports. The wines were analyzed using a HPLC method with a ternary solvent system to achieve separation of all monomeric phenols and anthocyanins, and the total phenolic content by Folin-Ciocalteau reagent was determined. The levels of each compound were then averaged using a weighting factor determined by each wine's representative volume to create a weighted average. These results were then compared to five ultra-premium (UP), commercially available Cabernet Sauvignons noted for their aging potential; the UP wines had significantly higher phenolic levels. The wines were also analyzed for their pH, titratable acidity, volatile acidity, free and total sulfur dioxide, alcohol, malic acid, glucose, and fructose using standard methods. Based on this data analysis, a standard Cabernet Sauvignon wine with typical phenolic levels is proposed

KEY WORDS: HPLC, phenolics, flavonoids, anthocyanin, flavonol

With many studies finding positive health benefits for wine drinkers, especially with respect to heart disease [10], and well accepted theories that phenolic antioxidants in wine could be responsible for those effects [8], it has become important to better define the actual phenolic composition of typical, commonly consumed wine. Many recent studies have ascribed specific health-related properties to particular phenolic compounds that are found in wine, including the flavan-3-ols [23] (also found in tea), the flavonols [12], and the anthocyanins [21]. So, it is now particularly pressing to characterize the complex phenolic content of wine with some specificity.

Unfortunately, very little data has been published on the phenolic content of single varietal, commercial or experimentally aged (over 1 year) red wines. The lack of phenolic data on commercial wines could be attributed to the absence of a rapid method of separation by GC or HPLC. In addition, much of the published data on phenolics are reported in relative amounts; thus, it is not possible to ascertain absolute concentrations [14,22]. In 1993, Lamuela-Raventos and Waterhouse developed a ternary HPLC solvent system that effectively separates acidic, neutral and charged phenolics in a single run [13]. This method allows the rapid screening of phenolics in wine.

Most of the published reports on wine phenolics deal with the development of analytical methods for the separation of phenolics or the analysis of grapes and newly fermented musts and wine [22]. Aside from the aspect of methods development, the studies usually analyzed very young wines. Therefore, there is a lack of data on the phenolic content of commercial wines that represent purchases for consumption. This data is essential to evaluating the role that US wine consumption plays in human health.

It is difficult to relate the values given in previous studies to the quantities measured here due to their grouping of phenols, varying methods of analysis, and use of noncommercial wine. One HPLC study analyzed a commercial Bordeaux (1 year old, 85% Cabernet Sauvignon, 15% Merlot) which was found to contain 584 mg/L total anthocyanins and varying levels of catechins and their dimers [3] (Table 1).

Table 1. The flavan-3-ol content of a one-year-old Bordeaux wine [3].

Flavan-3-ol	Concentration (mg/L)
Catechin	94
Epicatechin	48
Procyanidin B1	161
Procyanidin B2	77
Procyanidin B3	47
Procyanidin B4	48

There have been a few studies that have defined phenolics in newly fermented Cabernet Sauvignon wines and young stored wines. Unfortunately, the compounds analyzed in each study were not all the same. These are summarized in Table 2.

In one study important to the composition of commercial wine, Nagel and Wulf [14] studied anthocyanin content changes in aging experimental wines and found that levels drop significantly between fermentation and 240 days. They found that all anthocyanins levels dropped

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Table 2. Phenolic composition of various Cabernet Sauvignon wines in mg/L.

Compound Age of wine	Wine a Young wine	Wine b 9 months	Wine c 2 months	Wine d 10 days	Wine e ~1 year
Gallic acid	23	51			
Caftaric acid	112	113			
Procyanidin B3	3.9	—	9.2	33.3	47
Coutaric glucoside	5	15			
GRP*		3.6	6.5		
Procyanidin B1	27	14.5			161
Coutaric acid	21	25			
Catechin	65	61	26.7	15.3	94
Caffeic acid	8.7	15			
Procyanidin B2	—	12	50.2	17.3	77
Syringic acid	4.9	18			
Epicatechin	34	33.5	17.3	19.3	48
p-coumaric acid	0.9	2.6			
Rutin	24	16.5			
Isoquercitrine	17	—			
Quercetin	Trace	3.8	10.0		

* - Grape reaction product.

Wine a - Gard region of southern France, possibly less than a month [1].

Wine b - Same wine as wine *a*, 9 months old [2]. Note: this paper also has phenolic levels for Carignane, Cabernet Franc, Syrah, Grenache, and Mouvedre wines.

Wine c - Cabernet Sauvignon from Bordeaux region [7].

Wine d - French Cabernet Sauvignon during maceration [3].

Wine e - Bordeaux red wine [3].

up to 97% in approximately eight months. They also found that catechin, caftaric acid and *p*-coutaric acid levels dropped up to 57% in less than seven months. This suggests that the levels of monomeric phenols in commercial wine may be less than might be expected based on the analysis of young wines. The causes for some of these observed effects of aging have been described [6,19,20].

Frankel *et al.*, [9] determined the phenolic levels in three to seven-year-old, commercial Cabernet Sauvignon wines in a study which included the analysis of resveratrol. The levels of each component varied greatly between wines as connoted by the high standard deviations.

The existing data on phenolic levels in Cabernet Sauvignon wines vary greatly from study to study. Catechin levels in the above aged wines ranged between 26.7 mg/L and 229 mg/L. There are two studies [2,14] of Cabernet Sauvignon wines as they aged, and the catechin levels remain about the same around 65 mg/L. Epicatechin also had a large range of levels (between 17.3 and 162 mg/L) and appears to remain the same through the first nine months of aging, too. Anthocyanins show significant drops during the first eight months of aging; therefore, the levels expected for aged commercial Cabernet Sauvignon wines are low. But the wide range of levels observed indicates that it is not possible to use these data to ascertain the levels in wine that is commonly consumed.

The purpose of this study is to define a "standard red wine" with regard to phenolic composition and other simple components that may represent what red wine consumers are presently drinking. Since Cabernet Sauvignon is the most common red wine varietal in the international wine market, it was selected for this analysis. The monomeric components were the focus despite the abundance of polymeric phenolics in wine, because, while data specific to phenols is lacking, high molecular weight compounds are not absorbed [15].

Materials and Methods

Wine selection: Nielsen Winescan sales volume data for Cabernet Sauvignon wines purchased through United States retail outlets with price scanners in the year of 1995 were obtained from Gomberg, Fredrickson & Associates, San Francisco, California, and used to select the "high volume" (HV) wines used in this study. Most of the wines were either non-vintage, or from the 1992 or 1993 vintages. The wines were standardized to 750-mL volumes in order to determine the volume each represented in all bottling formats (*i.e.,* 750-mL, 1.5-mL, and 187-mL). Wines that sold in two or more bottling formats were assumed to be from the same blend and grouped together. The volumes for each bottling

Table 3. Label and volume parameters for analyzed wines.

Wine	Vintage	Appellation	Sales vol. (9-L cases)
		High Volume Wines	
1	Non-vintage	California	459202
2	Non-vintage	California	294595
3	1993	California	200283
4	Non-vintage	California	194984
5	Non-vintage	California	184387
6	Non-vintage	California	174850
7	1993	California	125044
8	1993	California	93253
9	1993	California	59343
10	Non-vintage	California	52985
11	Non-vintage	California	36030
12	1993	California	32851
13	Non-vintage	California	31791
14	1993	Central Coast	26492
15	1994	California	23313
16	1992	Columbia Valley	20134
17	1992	Napa Valley	18015
18	1993	Sonoma County	18015
19	1992	Napa Valley	14836
20	1992	Napa Valley	10597
21	1992	Columbia Valley	10597
		Ultra Premium Wines	
1	1993	Rutherford	NA
2	1992	Rutherford	NA
3	1992	Stag's Leap Dist.	NA
4	1992	Napa Valley	NA
5	1992	Sonoma Mountain	NA

Table 4. Solvent gradient used for separation of phenolics on HPLC.

Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)
0	100	0	0
8	92	8	0
20	0	14	86
25	1.5	16.5	82
35	0	21.5	78.5
70	0	50	50
75	100	0	0
80	100	0	0

format were combined and their rank adjusted accordingly. In addition, five "ultra premium" (above $20 per bottle) Cabernet Sauvignons thought to be heavily extracted for increased aging potential were chosen and analyzed for comparison.

Three bottles of each wine were purchased from retail outlets in Davis, California, between 2 February 1996 and 19 February 1996. The wines were purchased in the 750-mL bottle size unless they were only available in formats other than 750 mL. One bottle was used for determining the pH, total acidity, alcohol content, volatile acidity, and free and bound sulfur dioxide. The other two bottles were opened prior to replicate analysis by HPLC, enzymatics, and automated Folin-Ciocalteu colorimetry, placed into three 2-mL HPLC vials and air tight bottles of various sizes and kept in a refrigerator for up to two weeks at 2°C until analyzed. This allowed duplicates for each wine in order to compensate for differing shipping and storage practices. The wines were analyzed between 16 April 1995 and 22 July 1995. The HPLC and Folin Ciocalteu results for the two bottles of each wine were averaged to give one value. Table 3 gives the vintage, appellation, and sales volume for each wine.

Wine production analyses: Standard wine production analyses were performed on each wine at the Buena Vista Winery laboratory in Sonoma, California. The pH was determined by pH meter and titratable acidity by degassing and titration with standardized NaOH [11] . Alcohol was determined by ebulliometer without dilution. Free sulfur dioxide was determined by aeration-oxidation [4], and total sulfur dioxide by aeration-oxidation with heating of the same sample to liberate the bound sulfur dioxide [4]. Total phenolics were determined by automated Folin-Ciocalteu colorimetry [18]. Malic acid, glucose, and fructose concentrations were determined by standard enzymatic analysis from purchased kits produced by Boehringer Mannheim.

Analytical determination of phenolic compounds: A Hewlett-Packard (Palo Alto) model 1090 HPLC system with three solvent pumps and a diode array UV-visible detector coupled with a HP Chem Station was used. A Hewlett-Packard LiChrospher C18 column, 4 mm × 250 mm, 5-µm particle size, kept at 40°C was used as a stationary phase, with a mobile phase flow rate of 0.5 mL/min. Solvent A = 50 m*M* dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid; Solvent B = 20% A with 80% acetonitrile; and Solvent C = 0.2 *M* orthophosphoric acid adjusted with NaOH to pH 1.5. This method is very similar to the one developed by Lamuela-Raventos and Waterhouse [13], except that the solvent gradient was modified for better separation. The modified solvent gradient is given in Table 4. Four wavelengths were monitored: 280 nm for catechins and benzoic acids, 316 nm for hydroxycinnamates, 365 nm for flavonols, and 520 nm for anthocyanins.

Compounds found in the study were identified by comparison to standards as well as by comparison with standards previously characterized on the HPLC system by both relative elution times and spectral matching. Standards for each phenolic class were prepared from commercial sources. Gallic acid was used for the gallates, caftaric acid for the cinnamates, rutin for the flavonols, catechin for the flavan-3-ols, and malvin-3,5-diglucoside for the anthocyanins. The response factor for each of the standards was used to calculate the concentrations of all compounds in that class.

Results and Discussion

Twenty phenols were identified and quantified in each of the wines in this study. These include six cinnamates, two gallates, five flavonols, four flavan-3-

Table 5. Compounds identified and analyzed in study.

Compound	Retention time (minutes)	Wavelength monitored (nm)
Cinnamates		316
cis-Caftaric acid	20.53	
trans-Caftaric acid	22.7	
cis-Coutaric acid	28.1	
trans-Coutaric acid	29.0	
Caffeic acid	30.4	
Coumaric acid	39.0	
Gallates		280
Gallic Acid	15.0	
Syringic Acid	32.2	
Flavonols		365
Flavonol 1	40.8	
Flavonol 2	46.3	
Flavonol 3	51.8	
Myricitin	52.7	
Quercetin	61.1	
Flavan-3-ols		280
Catechin	26.7	
Procyanidin B2	30.0	
Epicatechin	32.8	
Anthocyanins		520
Malvidin-3-glucoside	40.2	
Malvidin-3-Glu-Ac	50.5	
Malvidin-3-glu-*p*-coum	56.8	

ols, and three anthocyanins. Table 5 shows which compounds were quantified, their retention times, and the wavelength at which each was monitored.

The amount of individual phenolic compounds in each wine was then taken from the average of the two analyses. However, in a few cases, only one of the duplicate wines contained a specific compound. In those cases, the amount observed in the one sample was small, and it was assumed that the absence in the other analysis was due to baseline distortion or insufficient separation, and thus the well-quantified peak was used to represent the level in both duplicates. The average variability in the phenolic peak areas between replicate wine bottles was approximately 15%. It might be expected that compounds with hydrolyzable functional groups would have higher variability, such as the hydroxycinnamates or glycosides, but that was not the case; all classes of substances had similar overall variability.

In calculating the "standard" levels of each component, it is important to note that the two highest volume wines represent 36% of the total sample volume of the high-volume wine set and the six highest volume wines represent over 72% of the sample volume. Since the average value which is used to determine the standard is based on a volume-weighted average, these top wines have the most influence in determining averages.

Since the method used to quantify the phenolics in this paper is relatively new, the results from previous reports may not be fully correlative. Previous studies used colorimetric procedures and HPLC programs other than the one used in this study. Also, the older papers rarely deal with individual phenolics, instead they deal with groups of phenolics. In 1982, Singleton [16] acknowledged that there was very little data on the values of single phenolic components in wine and emphasized the importance of developing new methods to quantify each in order to better understand their roles in wine. Singleton [17] also gave a simplified approximation of typical young wines phenol content in mg/L GAE. Again, this does not quantify individual phenols, only groups.

Defining the phenolic standard: Several assumptions must be made in order to define a "standard red wine" because of the wide range of wine styles available to consumers. Cabernet Sauvignon was chosen because it represents the largest percentage of single varietal labeled red wines on the market (Nielsen Winescan). By limiting the wines to a single varietal, it is possible to limit fluctuations in style and composition. Of course, these wines could contain as much as 25% of wines other than Cabernet Sauvignon under US labeling laws. The wines chosen also needed to be widely available and represent a large percentage of the Cabernet Sauvignon and red wine markets. The

Table 6. Standard chemistry and enzymatic results for high volume wines.

Wine	pH	TA (mg/100mL)	VA (g/100mL)	Alcohol (vol. %)	Free SO_2 (ppm)	Tot. SO_2 (ppm)	Phenols (GAE)	Malic acid (g/L)	Glucose (g/L)	Fructose (g/L)
1	3.55	0.63	0.027	11.7	11	63	1512	0.961	3.394	3.423
2	3.65	0.57	0.028	12.4	15	66	1467	1.188	2.856	2.679
3	3.66	0.58	0.055	12.6	21	71	1876	0.080	0.180	0.089
4	3.42	0.66	0.065	12.0	11	98	2089	0.730	0.428	0.425
5	3.60	0.57	0.036	12.3	20	94	1688	0.362	0.362	0.153
6	3.62	0.60	0.033	12.6	26	101	1751	0.713	0.306	0.203
7	3.54	0.66	0.040	13.0	27	94	1943	0.552	0.518	0.390
8	3.48	0.63	0.045	11.9	28	74	1746	0.283	1.223	1.051
9	3.52	0.61	0.048	12.4	21	98	2465	0.117	0.325	0.183
10	3.45	0.62	0.048	12.4	28	118	1889	0.262	0.798	0.951
11	3.36	0.63	0.037	12.2	19	124	1983	0.543	1.182	1.204
12	3.58	0.61	0.056	13.4	23	86	2516	0.147	0.287	0.127
13	3.49	0.60	0.034	12.1	17	119	1785	0.201	0.213	0.142
14	3.56	0.62	0.050	12.8	15	81	2151	0.184	0.988	0.974
15	3.54	0.56	0.033	12.6	15	78	1489	0.386	0.360	0.177
16	3.63	0.60	0.055	13.1	9	45	2559	0.176	0.165	0.093
17	3.44	0.63	0.061	13.2	15	69	2438	0.193	0.115	0.114
18	3.52	0.60	0.052	13.6	23	74	2051	0.061	0.383	0.263
19	3.45	0.63	0.055	13.2	15	65	2168	0.065	0.373	0.375
20	3.55	0.59	0.054	14.0	17	79	3435	0.133	0.258	0.154
21	3.58	0.62	0.053	13.4	15	59	2975	0.112	0.351	0.224
Weighted average	3.55	0.61	0.040	12.3	18	81	1784	0.631	1.440	1.361
High	3.66	0.66	0.065	14.0	28	124	3435	1.188	3.394	3.423
Low	3.36	0.56	0.027	11.7	9	45	1467	0.061	0.115	0.089
t-test	0.786	0.0378	0.011	0.00013	—	—	0.0023	0.305	0.356	0.355

Table 7. Proposed winemaking chemistry ranges for a standard wine and averages for high volume and UP wines.

Component	Standard range	High volume wines Weighted average	UP wines Average
pH	3.45 - 3.65	3.55	3.52
TA (g/100 mL)	0.56 - 0.66	0.61	0.65
VA (mg/100 mL)	0.030 - 0.060	0.040	0.061
Free SO_2 (mg/L)	10 - 30	18	No data
Total SO_2 (mg/L)	60 - 100	81	No data
Alcohol (v/v%)	11.5 - 13.0	12.3	14.1
Tot. phenols (mg/L GAE)	1700 - 1900	1784	2942
Glucose (g/L)	Dependent	1.44	0.35
Fructose (g/L)	on	1.36	0.26
Malic acid (g/L)	style	0.63	0.20

wines for this study were selected based on Nielsen Winescan information provided by Gomberg, Fredrickson & Associates. Nielsen Winescan tracks the volume of specific items sold through supermarkets with price scanners. The 21 highest unit volume Cabernet Sauvignon wines were selected from 1995 Nielsen Winescan data for the United States and used in this study. They represent a volume of 2 081 596 nine-liter cases and 17% of all red wines purchased in Winescan supermarkets in 1995. When the phenolic data for the wines were determined, typical levels for each component were found by taking a weighted average based on its wines sales volume. The weighting factor is found by dividing each wine's representative volume by the total sales volume of the overall sample.

Winemaking analyses: The standard wine industry analyses are given as a supplement to the phenolic standard to ensure that the composition of the standard is well defined in terms of common wine parameters. Since it would be difficult to obtain the precise averages when attempting to produce a "standard" wine, the proposed standard is described in terms of ranges. The range is given along with the specific weighted average value to make production easier and to provide for variances due to vintage, style and origin. Results for the standard chemistry and enzymatic analysis are shown in Table 6. Table 7 contains the proposed ranges for the conventional chemistries of the standard wine. These data result in a standard Cabernet Sauvignon having the following parameters: pH 3.55 (3.45 - 3.65), titratable acidity 0.61 (0.56 - 0.66) g/100 mL, volatile acidity 0.040 (0.030 - 0.060) mg/100 mL, alcohol 12.3% (11.5% - 13.0%) v/v, free sulfur dioxide 18 (10 - 30) mg/L, total sulfur dioxide (70 -100) 81 mg/L, and total phenols 1784 (1700 - 1900) gallic acid equivalents by Folin-Ciocalteu. The levels of sugars and malic acid (in the typical range of table wine composition) depend on the style of winemaking, and varied widely in our sample; thus, we do not set values for these components in the definition of the standard.

The standard chemistry results for the ultra-premium (UP) wines differ from the high-volume wine results (Table 8). The free and total sulfur dioxide levels were not determined due to equipment difficulties. Student's t-test was performed to determine if the standard chemistry values for the high volume wines and UP wines come from different populations. It was found that the titratable acidity, volatile acidity, alcohol, and total phenol content were different and had less than a 4% chance of coming from the same populations (Table 6). The average pH and titratable acidity levels for the UP wines were 3.51 and 0.65 g/100 mL. The total phenols level of 2942 GAE supports the assumption that these wines are more heavily extracted or are produced from grapes that have higher levels of extractable phenols. This level is 65% higher than the high volume wines. The average enzymatic levels of 0.20 g/L malic acid, 0.35 g/L glucose and 0.26 g/L fructose suggest that the UP wines went through more complete malolactic and alcoholic fermentations. The alcohol content of 14.1% for the UP wines suggests that the grapes used to make the wines were picked at a higher sugar content, possibly to get riper flavors.

Phenolic analysis: The results of the phenolic analysis for the high volume wines are found in Tables 9 and 10. The weighted averages for each component are the levels proposed for the standard Cabernet Sauvignon wine. The anthocyanins levels for the top 10 wines were omitted from the standard due to difficulties in calibration. Therefore, the anthocyanin levels for the high volume wines are based on the bottom 11 wines. The results of the phenolic analysis of the UP wines are seen in Tables 11 and 12.

Table 8. Standard chemistry and enzymatic results for ultra-premium wines.

Wine	pH	TA (g/100 mL)	VA (mg/100 mL)	Alcohol (vol. %)	Tot. phenols (GAE)	Malic acid (g/L)	Glucose (g/l)	Fructose (g/L)
1	3.46	0.73	0.060	13.8	2407	0.145	0.337	0.247
2	3.64	0.58	0.065	14.9	3267	0.254	0.270	0.147
3	3.45	0.66	0.061	14.0	3124	0.207	0.412	0.366
4	3.56	0.58	0.048	14.1	2402	0.138	0.266	0.147
5	3.50	0.71	0.069	13.5	3510	0.277	0.451	0.388
Average	3.52	0.65	0.61	14.1	2942	0.204	0.347	0.259
High	3.64	0.73	0.069	14.9	3510	0.277	0.451	0.388
Low	3.45	0.58	0.048	13.5	2402	0.138	0.266	0.147

Table 9. Non-flavonoid levels for high volume wines.

Wine	*cis*-Caftaric	*trans*-Caftaric	*cis*-Coutaric	*trans*-Coutaric	Caffeic	Coumaric	Total cinnamates	Galic acid	Syringic acid	Total gallates
1	13.30	18.15	5.68	10.66	5.85	2.86	**56.50**	18.55	—	**18.55**
2	11.39	28.98	6.60	16.03	7.81	4.47	**75.30**	21.67	3.47	**25.14**
3	12.04	17.28	4.20	8.04	4.50	9.93	**55.97**	18.24	5.63	**23.87**
4	10.96	10.35	3.41	5.39	5.74	5.48	**41.33**	35.94	6.79	**42.74**
5	11.74	14.45	3.87	6.20	9.08	9.81	**55.14**	25.64	4.70	**30.34**
6	12.42	19.66	5.29	8.47	6.56	6.24	**58.64**	27.99	4.63	**32.62**
7	8.30	23.93	5.98	14.88	5.02	10.33	**68.45**	31.98	5.20	**37.17**
8	13.36	18.25	4.47	10.84	6.63	10.54	**64.09**	26.50	9.42	**35.91**
9	9.65	26.01	4.05	15.94	15.43	15.62	**86.70**	40.47	5.88	**46.35**
10	12.69	16.37	3.50	11.34	8.20	14.65	**66.76**	32.23	5.40	**37.63**
11	11.53	14.14	3.74	7.24	6.97	6.95	**50.56**	34.79	6.46	**41.25**
12	4.21	28.86	4.64	14.25	14.37	13.39	**79.72**	45.26	6.47	**51.72**
13	5.45	8.03	2.56	4.29	—	7.41	**27.74**	23.49	4.58	**28.06**
14	9.44	24.58	4.08	12.86	10.87	10.23	**72.05**	36.11	9.32	**45.43**
15	5.97	22.95	5.73	8.74	9.41	24.97	**77.77**	28.51	5.40	**33.92**
16	5.69	28.40	5.07	14.12	11.34	11.23	**75.86**	35.37	6.16	**41.54**
17	9.41	36.99	6.34	23.86	8.77	10.76	**96.14**	36.22	6.46	**42.68**
18	—	19.57	3.50	9.21	11.11	18.42	**61.80**	23.70	7.28	**30.99**
19	4.91	35.25	5.85	22.61	10.35	15.41	**94.38**	38.46	5.35	**43.81**
20	7.50	68.53	8.89	42.63	18.94	11.62	**158.11**	55.27	3.73	**58.99**
21	11.10	56.21	7.89	26.38	11.88	15.19	**128.65**	22.24	3.63	**25.86**
Wt'd Avg.	11.31	20.12	5.02	10.89	7.07	7.50	**61.92**	25.97	4.23	**30.20**
Str. Avg.	9.55	25.57	5.02	14.00	9.44	11.22	**73.89**	31.36	5.80	**36.88**
Std. Dev.	2.99	14.45	1.57	8.87	3.72	5.11	**28.72**	9.23	1.62	**9.94**
High	13.36	68.53	8.89	42.63	18.94	24.97	**158.11**	55.27	9.42	**58.99**
Low	4.21	8.03	2.56	4.29	4.50	2.86	**27.74**	18.24	3.47	**18.55**
Top 8	11.95	19.20	5.13	10.29	6.41	6.29	**59.28**	24.13	3.87	**27.99**
Bot 13	8.21	24.63	4.45	13.81	10.27	13.37	**74.76**	34.93	5.99	**40.93**
% diff.	-5.63	4.61	-2.32	5.51	9.30	16.09	**4.26**	7.10	8.57	**7.30**

The phenolic levels given for the standard generally agree with levels found in published data. The isomeric forms of caftaric acid were not distinguished in previous literature, but the combined value of 31.43 mg/L found in this study is below published values. The same is true for the combined value of the coutaric isomers at 15.91 mg/L. The caffeic acid level of 7.07 mg/L falls within the range of published data, especially Frankel *et al* [9]. The coumaric acid level is higher at 7.50 mg/L than the published data. These differences are no doubt due to the continuing process of ester hydrolysis where the tartrate esters are being hydrolyzed to the free acids, so these wines, which had been aged for a couple of years, had more of the free acids. The gallic acid level at 25.97 mg/L is at the lower range found in the literature search, which is between 23 and 126 mg/L. The same is true for syringic acid, which has a level of 4.23 mg/L in the standard.

The flavonoid levels for the standard generally fell within the lower ranges of published data. Myricetin and quercetin at 9.00 mg/L and 9.61 mg/L, respectively, for the standard were in close agreement with Frankel *et al* [9] at 9.0 and 8.4 mg/L. The catechin and epicatechin levels at 29.0 mg/L and 22.6 mg/L, respectively were at the low end of the range found in the literature, but were much less than the average level found by Frankel *et al.* [9] at 169 mg/L and 81.7 mg/L. The standard levels of the only oligomers studied are 19.4 mg/L procyanidin B2 and 24.0 mg/L for procyanidin B3 and these levels also fell within the lower ranges of previous studies. The malvidin derivative levels were higher than the values found in the literature. Nagel and Wulf [14] found levels in their 240 day old Cabernet Sauvignon wine for malvidin-3-glucoside, malvidin-3-glucoside-acetate, and malvidin-3-glucoside-*p*-coumarate of 10.3 mg/L, 3.7 mg/L, and 0.5 mg/L, respectively, which are much lower than the standards at 66.5 mg/L, 17.2 mg/L, and 5.1 mg/L.

In order to define a standard wine which may become a target wine for experimental purposes, we propose that the phenolic compounds be grouped by class in order to make it feasible to make such a wine. Table 14 gives the ranges for each group of phenols that should be achieved in order to create a wine representative of the standard. Following these criteria, as well as the ranges given for the standard chemistry, four of the wines analyzed in this study [3,5,7,10] are representative of the standard.

Table 10. Flavonoid levels for high volume wines.

Wine	Flavonol 1	Flavonol 2	Flavonol 3	Myre-cetin	Quer-cetin	Total flavonols	Pro B3	Catechin	Pro B2	Epi-catechin	Total catechins	Mal-3-glu	Mal-3-al-3-glu-Ac	Mal-3-glu-*p*-c	Total malvidins
1	10.41	7.49	4.01	3.50	0.92	**26.33**	19.28	22.15	19.75	16.43	**77.62**	—	—	—	—
2	20.85	16.73	7.79	8.35	3.92	**57.63**	20.07	22.74	15.48	14.51	**72.80**	—	—	—	—
3	11.07	7.05	5.79	8.27	12.32	**44.51**	24.19	20.78	24.82	27.23	**97.02**	—	—	—	—
4	24.79	12.16	17.35	13.59	13.24	**81.12**	31.48	34.21	12.33	24.86	**102.88**	—	—	—	—
5	12.70	8.45	4.82	8.20	12.94	**47.10**	23.10	24.43	20.70	20.70	**88.93**	—	—	—	—
6	13.32	9.43	5.15	4.59	6.73	**39.23**	26.30	34.49	23.69	24.37	**108.85**	—	—	—	—
7	12.66	8.27	5.54	7.75	13.17	**47.39**	26.77	30.99	25.34	27.81	**110.91**	—	—	—	—
8	12.65	7.43	4.91	7.35	8.81	**41.16**	21.26	31.81	25.92	48.34	**127.32**	—	—	—	—
9	13.97	5.89	5.57	14.07	14.17	**53.68**	44.06	34.37	19.75	22.36	**120.54**	—	—	—	—
10	12.76	8.27	6.40	14.06	10.78	**52.27**	22.83	24.67	19.46	25.45	**92.42**	—	—	—	—
11	27.46	12.67	11.34	10.58	20.98	**83.03**	30.09	31.77	17.66	24.38	**103.90**	67.65	17.93	7.50	**93.09**
12	11.93	6.55	6.39	15.58	9.69	**50.15**	33.25	33.91	21.65	25.31	**114.12**	91.56	23.91	7.79	**123.26**
13	20.88	6.37	10.57	16.74	11.78	**66.34**	12.17	25.87	35.47	19.76	**93.28**	64.10	16.15	6.04	**86.29**
14	26.42	14.26	17.46	28.62	28.22	**114.97**	21.91	27.82	18.40	29.08	**97.20**	63.59	15.03	—	**78.62**
15	14.81	3.78	10.79	16.37	11.90	**57.64**	23.73	31.80	—	25.94	**81.48**	102.93	28.22	8.38	**139.52**
16	26.52	20.01	8.94	14.30	29.08	**98.86**	34.64	41.59	18.68	36.69	**131.61**	29.22	9.28	—	**38.50**
17	51.06	45.49	22.92	28.03	51.64	**199.13**	22.91	26.43	16.73	21.86	**87.92**	40.27	9.31	—	**49.59**
18	16.24	4.60	13.71	29.28	16.18	**80.01**	24.23	28.93	—	29.96	**83.12**	89.92	21.43	8.70	**120.05**
19	34.46	27.09	19.61	34.73	44.64	**160.54**	19.29	26.63	—	16.98	**62.90**	49.71	11.74	6.29	**67.75**
20	81.16	60.66	25.45	25.54	63.01	**255.82**	35.28	40.73	—	18.01	**94.02**	53.12	14.43	7.34	**74.90**
21	54.13	36.97	12.45	12.36	30.75	**146.66**	32.06	42.85	—	38.05	**112.95**	31.48	9.75	—	**41.22**
												Bottom 11			
Wt'd Avg.	16.19	10.55	7.44	9.00	9.61	**52.78**	24.02	26.95	19.40	22.62	**93.00**	66.49	17.19	5.12	**88.80**
Str. Avg.	24.30	15.70	10.81	15.33	19.76	**85.88**	26.14	30.43	15.99	25.62	**98.18**	62.14	16.11	4.73	**82.98**
Std. Dev.	17.93	14.97	6.39	8.89	16.16	**59.12**	7.14	6.32	10.26	7.93	**17.82**	24.65	6.29	3.83	**33.85**
High	81.16	60.66	25.45	34.73	63.01	**255.82**	44.06	42.85	35.47	48.34	**131.61**	102.93	28.22	8.70	**139.52**
Low	10.41	3.78	4.01	3.50	0.92	**26.33**	12.17	20.78	12.33	14.51	**62.90**	29.22	9.28	6.04	**38.50**
Top 8	14.71	9.89	6.73	7.15	7.33	**45.82**	23.13	26.11	20.01	22.12	**91.37**				
Bot 13	23.34	13.72	10.90	17.98	20.70	**86.65**	28.36	31.05	16.46	25.09	**100.96**				
% Diff	9.09	6.18	9.57	20.54	23.73	**13.19**	3.71	3.12	-3.12	2.24	**1.76**				

There is a large discrepancy in the total phenols by HPLC and total phenols by Folin-Ciocalteu. The total phenols by HPLC for the standard is 307.3 mg/L compared to 1784 mg/L GAE by Folin-Ciocalteu. The explanation for this difference is that the HPLC method only quantified 20 compounds that were visible using this method (the identified components do represent approximately 90% of the visible peaks in the chromatogram) and it does not quantify high molecular weight polymers. The difference is due to these high molecular weight components, but for the reasons mentioned above, they are less important to quantify for the purposes of understanding the health effects of wine.

Weighted averages of the top eight wines and bottom 13 wines were determined in order to see what influence the bottom 13 wines had on the weighted average for all 21 wines. The top eight wines were chosen because they represented over 83% of the total

Table 11. Non-flavonoid values for ultra-premium wines.

	cis-Caftaric	*trans*-Caftaric	*cis*-Coutaric	*trans*-Coutaric	Caffeic	Coumaric	Total cinnam-ates	Gallic acid	Syringic acid	Total gallates
Wine 1	11.28	31.68	4.41	15.05	—	11.71	**74.13**	40.76	5.43	**46.19**
Wine 2	8.64	47.30	5.53	24.87	25.63	16.88	**128.85**	43.53	5.17	**48.70**
Wine 3	10.92	32.35	4.67	15.74	18.23	16.24	**98.15**	46.87	5.69	**52.56**
Wine 4	8.63	44.70	7.26	23.71	9.37	11.08	**104.75**	32.63	6.00	**38.63**
Wine 5	9.73	30.89	3.85	14.07	18.78	10.18	**87.51**	51.65	7.03	**58.68**
UP Average	9.84	37.38	5.14	18.69	18.00	13.22	**98.68**	43.09	5.86	**48.95**
UP Std. Dev	1.24	7.94	1.33	5.17	6.67	3.11	**20.46**	7.12	0.72	**7.45**
High	11.28	47.30	7.26	24.87	25.63	16.88	**128.85**	51.65	7.03	**58.68**
Low	8.63	30.89	3.85	14.07	9.37	7.59	**74.13**	18.00	4.64	**38.63**

Table 12. Flavonoid values for ultra-premium wines.

Wine	Flavonol 1	Flavonol 2	Flavonol 3	Myrecetin	Quercetin	Total flavonols	Pro B	Catechin	Epicatechin	Total catechins	Mal-3-glu	Mal-3-glu-Ac	Mal-3-glu-*p*-coum
Wine 1	43.05	29.67	21.39	37.46	54.63	**186.20**	26.25	33.55	32.25	**92.05**	74.60	21.80	8.77
Wine 2	50.69	46.30	23.94	47.21	70.20	**238.34**	25.02	29.21	11.48	**65.72**	71.98	21.83	8.61
Wine 3	47.01	33.52	18.61	33.30	49.11	**181.56**	35.07	40.76	32.59	**108.42**	71.07	19.78	8.29
Wine 4	40.84	39.10	20.68	42.84	61.17	**204.64**	16.56	20.38	13.33	**50.26**	50.90	13.18	
Wine 5	58.83	30.72	17.66	64.52	29.94	**201.67**	40.26	41.08	17.21	**98.56**	101.49	23.39	8.76
UP avg.	48.09	35.86	20.46	45.06	53.01	**202.48**	28.63	33.00	21.37	**83.00**	74.01	19.99	8.61
UP Std. Dev	7.09	6.89	2.47	12.09	15.10	**22.33**	9.24	8.65	10.29	**24.19**	18.04	4.02	0.22
High	58.83	46.30	23.94	64.52	70.20	**238.34**	40.26	41.08	32.59	**108.42**	101.49	23.39	8.77
Low	40.84	29.67	17.66	33.30	29.94	**181.56**	16.56	20.38	11.48	**50.26**	50.90	13.18	8.29

sample volume. It was found that the top eight weighted average differed by less than 10% for 14 of 17 phenolics (the malvidin derivatives were not included due to incomplete sampling). The only phenolics that varied more than 10% were coumaric acid, myricetin, and quercetin. Thus, the wines that represented 80% of the sample volume resulted in an average fairly comparable to a standard that represents the entire volume of a sample (Table 9).

Table 13. Direct comparison of phenolic levels in the high volume and ultra premium wines (mg/L).

Compound	HV weighted average	Ultra-premium average	% Difference	t-test result
cis-Caftaric acid	11.31	9.84	-13	0.837
trans-Caftaric acid	20.12	37.38	86	0.093
cis-Coutaric acid	5.02	5.14	2	0.869
trans-Coutaric acid	10.89	18.69	72	0.271
Caffeic acid	7.07	19.24	172	0.00131
Coumaric acid	7.50	13.22	76	0.413
Total cinnamates	**61.91**	**103.5**	**67**	**0.083**
Gallic acid	25.97	43.09	66	0.014
Syringic acid	4.23	5.73	35	0.930
Total gallates	**30.2**	**48.82**	**62**	**0.018**
Flavonol 1	16.19	48.09	197	0.0078
Flavonol 2	10.55	35.86	240	0.0083
Flavonol 3	7.44	20.46	175	0.0032
Myricitin	9.00	45.06	401	0.0000017
Quercetin	9.61	53.01	452	0.00033
Total Flavonols	**52.79**	**202.5**	**284**	**0.00026**
Procyanidin B3	24.02	28.63	19	0.512
Catechin	26.95	33.00	22	0.453
Procyanidin B2	19.40	—	NA	
Epicatechin	22.62	21.37	-6	0.318
Total flavan-3-ols	**73.59**	**83.00**	**13**	**0.929**
Malvidin-3-glu	66.49	74.01	11	
Malvidin-3-glu-ac	17.19	19.99	16	
Malvidin-3-glu-*p*-coum	5.12	8.61	68	
Total malvidin derivatives	**88.8**	**100.9**	**14**	

The levels of phenolics are generally higher in the UP wines compared to the HV wines. With the exception of *cis*-caftaric acid and epicatechin, all the phenolic levels in the ultra premium wines were between 2% and 452% higher than the high volume wines (Table 13). The catechins and the malvidin derivatives are only about 15% higher in the UP wines compared to the high volume wines as a group. The nonflavonoids levels in the UP wines are generally higher than the levels found in the standard. As groups, the gallates are 62% higher, and the cinnamates are 67% higher. The flavonols have the largest difference in levels with the UP wines having between 175% and 452% more of each flavonol than the high volume wines.

Student's t-test was applied to determine if each phenolic component analyzed was significantly different between the high volume wines and the UP wines. The two samples were assumed to be normally distributed with the same variance. All the flavonols along with gallic and caffeic acids were found to have less than a two percent probability of coming from the same population. The rest of the compounds were found to have greater than an 8% probability of coming from the same population. The malvidin derivatives were not analyzed due to the missing data for the top 10 wines. As groups, total gallates and total flavonols were found to have less than a 2% probability of coming from the same population.

The high levels of flavonols in the ultra premium wine suggest that different maceration techniques were used, leading to a more efficient recovery in the wine or, more likely, that the grapes used for these wines were different. If the latter is true, the level of flavonols in grapes may be a useful marker for Cabernet Sauvignon wine grape quality as well as wine quality. The production of these compounds in Pinot noir has been attributed to sun exposure [24], and the cultivation practices in the vineyard areas that produced the UP wines (trellised vines, leaf removal) would have resulted in more sun exposure.

It should be noted that the numbered flavonols quantified here are certainly glycosides of kaempferol, quercetin and myricetin , but due to the large number of these components in grapes [5] and the lack of stan-

Table 14. Standard ranges for phenolic groups (mg/L).

Group	Range	Weighted average
Cinnamates	40 - 80	61.9
Gallates	20 - 40	30.2
Flavonols	40 - 80	52.8
Catechins	75 - 115	93.0
Malvidins	60 - 110	88.8

dards, it was not possible to specifically identify each compound. The UP wines had a total flavonol content of 202.5 mg/L compared to the HV wines which had 52.8 mg/L.

The levels of total phenols quantified by HPLC are 307.3 mg/L for the standard and 540.4 mg/L for the UP wines. This is a difference of 76%. Most of the variation between the HV wines and UP wines is due to the flavonols which contribute over 150 mg/L of the 213 mg/L difference. There are also differences in the ratios between the *cis-* and *trans-* forms of caftaric and coutaric acids. For the HV wines, the average cis/trans caftaric ratio is 0.49 with a high of 1.06 and a low of 0.11. The average *cis-*/*trans*-coutaric ratio is 0.43 with a high of 0.66 and a low of 0.21. The coutaric isomer ratios have a narrower range than the caftaric isomer ratios. For the ultra premium wines, the average cis/*trans*-caftaric ratio is 0.28 with a high of 0.36 and a low of 0.18. The average cis/trans coutaric ratio is also 0.28 with a high of 0.31 and a low of 0.22.

Conclusions

This is the first time the phenolic content of commercial red wines has been measured in a sample determined by actual sales volume in order to gather data on wine actually consumed. The resulting standard is based on an average of ordinary wine parameters as well as classes of phenolic compounds as measured by RP-HPLC. Thus the standard is complex, and it may prove difficult to produce such a wine on demand, although we found that four of the wines [3,5,7,10] analyzed in this set met the standard.

This standard does define a benchmark for comparing specific wines that are used in experiments to investigate the health effects of wine. If a particular wine-drinking population exhibits a notable trait, a comparative analysis of the local wines consumed may help reveal the cause of those traits in terms of particular components. Such tests help define the role wine may play in human health by providing the information needed to understand the effects of wine at a molecular level. This is of particular importance because the phenolic composition of wine is both complex and variable. Such analyses of wines consumed by populations noted for their paradoxically low disease rates that are attributed to wine consumption would be of particular interest.

While the composition of the widely consumed (US) commercial Cabernet Sauvignon wine is well defined here, additional investigation into different grape varieties, vintage years, regions, and wine types are necessary in order to fully define the typical phenolic content of commercial "red" wine.

Finally, the question of flavonol or quercetin level as an indicator of Cabernet Sauvignon wine quality deserves further investigation.

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