Anthocyanins, Phenolics, and Color of Cabernet Franc, Merlot, and Pinot Noir Wines from British Columbia†

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Changes in phenolics (anthocyanins, flavonols, tartaric esters, and total phenolics) during ripening of grapes and in phenolics and color during vinification and aging of Cabernet Franc, Merlot, and Pinot Noir wines were studied. Anthocyanins in grape skins showed variations in accumulation pattern, concentration, and distribution depending on variety and to a lesser extent on season. During vinification, colorless phenolics increased during alcoholic fermentation, reached maximum values at pressing, and remained stable during malolactic fermentation and subsequent storage. Anthocyanins and color density, on the other hand, increased during the early stages of alcoholic fermentation, reached maximum values 2−3 days after the start of fermentation, decreased during malolactic fermentation, and slowly declined during subsequent storage. Viticultural practices that increased cluster sun exposure generally led to higher phenolics and color density of wines, whereas changing yeasts used for fermentation had minimal effects.

Keywords: *Wine color; polyphenols; flavonoids; flavonols; anthocyanins; tartaric esters; grapes; polymeric pigments; HPLC analysis; yeast*

INTRODUCTION

Phenolic compounds, especially anthocyanins, flavonols, catechins, and other flavonoids, play a major role in wine quality. They contribute to sensory characteristics of wines, particularly color and astringency, and recently have been demonstrated to have a wide range of biochemical and pharmacological effects, including anticarcinogenic, antiatherogenic, antiinflammatory, antimicrobial, and antioxidant activities (Mazza and Miniati, 1993; Girard and Mazza, 1998).

The anthocyanins that are extracted from the skins of grapes during crushing, pressing, and fermentation are the major components responsible for red wine color. It is generally accepted that the color of red wine changes during maturation and aging due to interactions between anthocyanins and colorless phenolics present in grapes including (+)-catechin, (−)-epicatechin, quercetin, kaempferol, and phenolic acids (Haslam, 1989; Liao et al., 1992; Mazza, 1995). Contact with the oak wood of barrels or wood chips during maturation can result in extraction of additional phenolics from the wood into the wine. In addition to their direct role on color, anthocyanins also contribute to the taste and chemical characteristics of wine because of their interactions with other molecules such as colorless phenolics, polysaccharides, metals, and anthocyanins themselves (Mazza and Brouillard, 1987, 1990; Cai et al., 1990; Liao et al., 1992).

The concentration and composition of phenolics in red wine grapes vary with species, variety, season, and a wide range of environmental and management factors such as climate, soil conditions, canopy management, and crop load (Jackson and Lombard, 1993). The level of solar radiation, for instance, is an important parameter in the red coloration of grapes. Significant differences in anthocyanin concentration between sun-exposed and shaded clusters of Cabernet Sauvignon were found by Crippen and Morrison (1986). More recently, Price et al. (1995) reported that wines made from Pinot Noir clusters highly exposed to the sun had 60% higher anthocyanin concentration than wines from shaded clusters and 14% more than wines from moderately exposed clusters. Similarly, crop level management by cluster thinning and/or by shoot length/density alterations affected phenolics, anthocyanins, and ultimately wine quality (Jackson and Lombard, 1993; Reynolds et al., 1996). The effects of growing conditions on grape quality and yield, therefore, have to be assessed for each variety in a particular growing region.

The role of yeasts in the evolution of phenolic compounds during winemaking and maturation processes has received little attention. However, a recent study by Vasserot et al. (1997) indicated that yeast lees have the capacity to adsorb anthocyanins.

The objectives of this investigation were to (1) examine the changes in phenolics (anthocyanins, tartaric esters, flavonols, and total phenolics) during ripening of grapes and subsequent winemaking and maturation processes for Cabernet Franc, Merlot, and Pinot Noir wines from the Okanagan Valley of British Columbia; (2) determine differences in phenolics and color in Cabernet Franc, Merlot, and Pinot Noir wines produced from grapes subjected to different viticultural treatments; (3) determine the influence of yeast used for fermentation on phenolics and color of Cabernet Franc, Merlot, and Pinot Noir wines; and (4) elucidate possible

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relationships between various families of phenolic compounds and wine color.

MATERIALS AND METHODS

Grape Growing. Cabernet Franc, Merlot, and Pinot Noir grapes (*Vitis vinifera* L.) were grown at Black Sage Vineyards near Osoyoos, BC. All three grape varieties were grown on rootstock S04 with clone 210 for Cabernet Franc, clone 184 for Merlot, and clone 375 for Pinot Noir. Vines were 5−6 years old and oriented east−west. The density of vines was 2865 vines/ha. The bilateral cordon−vertical shoot positioning training system was used. Vines were pruned to 20 buds/vine, except for the 24 shoot treatment. Vines were irrigated overhead, and fertilizer (9−10−12) was applied at a rate of 281 kg/ha. Boron and magnesium were applied as needed. Vine growth was about 4−5 shoot lengths.

In 1996, the vines were maintained under three viticultural treatments: control, cluster thin at veraison, and leaf pull. In 1997, the treatments were increased to five: control, cluster thin at bloom, cluster thin at veraison, 24 shoots, and leaf pull. The cluster thin treatments consisted of removing all secondary clusters by snipping at either bloom or veraison. For the 24 shoot treatment, vines were pruned to 24 buds/vine. The leaf pull treatment consisted of removing basal leaves on the north and south sides of vines by hand at bloom. For the control treatment, no cluster thinning and leaf pulling were done. For each treatment, one row of grapes (100 vines/row for Pinot Noir and Merlot and 93 vines/row for Cabernet Franc) was used.

Grape clusters were randomly sampled every week for 3−6 weeks before harvest. Two bags of 1.4 kg each were collected for each variety and treatment. One bag of grapes was used for soluble solids content, pH, and titratable acidity measurements, and the other bag was frozen at −30 °C for phenolic analysis. At harvest, all the grapes for each treatment (i.e., one row) were picked, and the grapes were weighed to determine the yield.

Vinification Protocol. Grapes were harvested at ∼22 °Brix then processed using a mechanical crusher/destemmer with rotation speed of 30 Hz. Crushed grapes were collected in 40-L primary fermentation pails and enzyme (0.017% Sepazym Red from Keller Mannheim, Germany) and potassium metabisulfite (30 ppm SO_2) were added. For each treatment and variety, replicate trials were produced.

Pinot Noir musts were cold soaked at 4 °C for 6 days with one daily punchdown prior to fermentation. All musts were fermented at 25 °C using yeast nutrient (Fermaid, 20 g/100 L) and yeast (20 g/100 L). In 1996, yeast strains used for Pinot Noir included Assmanhausen, Burgundy, EC1118, Fermirouge, RC212, and SIHA8. For Cabernet Franc and Merlot wines, the yeast strains used were BM45, Bordeaux Red, EC1118, ICVD254, L2056, and Redstar. In 1997, RC212, Wädenswil 27, and RA17 yeasts were used for Pinot Noir; EC1118, ICVD254, and L2056 were used for Merlot; and EC1118, Bordeaux Red, and ICVD254 were used for Cabernet Franc. The yeasts were obtained from Lallemand Inc. (Montreal, PQ, Canada). The yeasts were rehydrated according to the manufacturer's recommendations. Fermentation caps were punched down twice daily, and fermentations were monitored daily for temperature and soluble solids content using a hydrometer.

When hydrometer readings reached <0% sugar, pomace was pressed off using a rack and cloth press in 1996 and a bladder press with size 70 spacing (Enorossi Enoagricola Rossi, Italy) in 1997. The pressing procedure consisted of collecting free run wine, gradually increasing pressure to get first press wine, and then further increasing pressure to get hard press wine. Wines were collected in 20-L glass carboys. Leftover wines were collected in 4- and 9-L carboys to be used as top up. Carboys were placed at 20 °C and inoculated with 0.6 g of malolactic culture (Viniflora Oenos, CHR Hansen, Hørsholm, Denmark)/100 L. Malic acid was monitored enzymatically (Boehringer Mannheim Canada, Laval, PQ, Canada) until residual levels reached <30 mg/L, and then 70 ppm SO_2 was added to Pinot Noir wines and 90 ppm SO_2 was added to Merlot and Cabernet Franc wines. The wines were then placed at 0 °C. After 1−2 weeks, the wines were racked and stored at 0 °C for several weeks. About 3−5 weeks prior to bottling, wines were brought up to 15 °C and racked. The pH of Pinot Noir wines was adjusted to 3.6−3.7 prior to bottling with tartaric acid. The SO_2 content of the wines was also measured before bottling, and molecular SO_2 was adjusted to 0.5 mg/L. Free SO_2 was measured by the aeration−oxidation method described in Amerine and Ough (1980). Samples of must and wine were taken for analyses after crushing, after pressing, and at bottling. In 1997, additional samples were taken after cold soaking, during fermentation at ∼10% sugar, and after malolactic fermentation.

Measurement of Soluble Solids Content, pH, and Titratable Acidity. The samples of grapes collected for soluble solids, pH, and titratable acidity measurements were prepared by removing stems and then dividing the grapes into two plastic bags. The grapes were crushed by hand to prevent breakage of seeds. The mashed grapes were placed in a strainer, and juice was collected. Juice samples were analyzed for soluble solids content using a Reichert Abbe Mark II refractometer (AO Scientific Instruments, Buffalo, NY). The pH and titratable acidity were measured using a Metrohm 686 Titroprocessor and 665 Dosimat (Metrohm Ltd., Switzerland). For titratable acidity, 10 mL of sample was titrated with 0.1 N NaOH to an end point of pH 8.1. Titratable acidity was converted to tartaric acid equivalents.

Preparation of Grape Skins for Analyses of Phenolics. Phenolic analyses were done on frozen grapes after removing stems. Three 50-g samples of grapes were randomly selected for each treatment. While the grapes were still frozen, skins were separated from the pulp. Skins were blotted on paper towels to remove any residual pulp. Skins were frozen and then freeze-dried. Both the wet and dry weights were recorded.

A freeze-dried skin sample and 50 mL of 50:1.5:48.5 methanol:formic acid:distilled water were blended for 8 min at low speed in a temperature-controlled (4 °C) Waring blender. The mixture was centrifuged at 10000*g* (9500 rpm) for 10 min at 4 °C. The supernatant was filtered through a 0.45-μm Acrodisc LC PVDF syringe filter (Gelman Sciences, Montreal, PQ) and then stored at −35 °C.

Analyses of Phenolics. A modified version of the Glories' method (Glories, 1979; Romani et al., 1996) was used to estimate the phenolic content of grape skin extract, must, and wine samples. Samples were diluted 1:10 with 10% ethanol. The method consisted of placing 0.25 mL of sample or standard in a test tube and adding 0.25 mL of 0.1% HCl in 95% ethanol and 4.55 mL of 2% HCl. The solution was mixed and allowed to sit for approximately 15 min before reading the absorbance at 280, 320, 360, and 520 nm with a spectrophotometer. The absorbance (A) at 280 nm was used to estimate total phenolic content, $A_{320\ nm}$ was used to estimate tartaric esters, $A_{360\ nm}$ was used to estimate flavonols, and $A_{520\ nm}$ was used to estimate anthocyanins. Standards used were gallic acid in 10% ethanol for total phenolics, caffeic acid in 10% ethanol for tartaric esters, quercetin in 95% ethanol for flavonols, and malvidin-3-glucoside in 10% ethanol for anthocyanins. All standards were obtained from Sigma Chemical Co. (St. Louis, MO) except for malvidin-3-glucoside, which was obtained from Extrasynthese (Genay Cedex, France).

High Performance Liquid Chromatography (HPLC). Analyses of anthocyanins in grape skin extracts and wines were performed using a Waters HPLC system (Mississauga, ON, Canada) equipped with a Waters 990 photodiode array detector. Separation was achieved on a reverse-phase Supelcosil LC-18 column of 25 cm × 2.1 mm i.d. obtained from Supelco, Inc. (Bellefonte, PA). The column temperature was maintained at 25 °C. A flow rate of 0.35 mL/min was used. Solvent A was 5% (v/v) formic acid, and solvent B was methanol. For skin extracts, the following proportions of solvent B were used: 0−5 min, 20%; 5−80 min, 50%; 80−83 min, 100%; 83−85 min, 20%; and 85−89 min, 20%. For wine samples, the following proportions of solvent B were used: 0−5

Table 1. Influence of Season and Variety on Total Phenolics, Tartaric Esters, Flavonols, and Anthocyanins in Grapes[a], Must, and Wine[b] from the Okanagan Valley

	sampling date		total phenolics[c] (mg of gallic acid, 280 nm)		tartaric esters[c] (mg of caffeic acid, 320 nm)		flavonols[c] (mg of quercetin, 360 nm)		anthocyanins[c] (mg of malvidin-3-glucoside, 520 nm)		color density (absorbance units)		hue	
variety	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
Cabernet Franc														
grape skin[d]		9/9/97		462 ± 35		75 ± 4		93 ± 7		289 ± 28				
		9/16/97		727 ± 24		123 ± 6		160 ± 12		548 ± 21				
	9/23/96	9/23/97	548 ± 18	820 ± 2	67 ± 2	127 ± 5	82 ± 5	162 ± 11	530 ± 9	799 ± 20				
	9/30/96	9/29/97	631 ± 51	986 ± 29	83 ± 9	152 ± 5	98 ± 13	200 ± 8	701 ± 63	967 ± 31				
	10/7/96	10/7/97	680 ± 23	941 ± 38	90 ± 2	147 ± 4	105 ± 4	179 ± 4	749 ± 28	984 ± 54				
	10/15/96	10/16/97	793 ± 13	1009 ± 44	107 ± 2	153 ± 9	134 ± 2	188 ± 16	826 ± 30	1048 ± 48				
must[e]	10/22/96[f]	10/27/97[f]	163	326	67	194	45	96	8	42	1.163	0.208	2.432	1.172
		10/28/97[g]		592		246		155		324		3.149		0.39
		10/31/97[h]		965		266		181		644		11.732		0.392
wine[e]	11/1/96[i]	11/12/97[i]	982	1193	166	226	128	157	420	469	8.438	9.589	0.473	0.484
		1/7/98[j]		1095		201		121		352		8.42		0.543
	6/1/97[k]	7/23/98[k]	927	1095	165	201	114	123	316	337	5.57	6.061	0.631	0.619
	12/11/97[l]		862		159		98		232		5.511		0.679	
Merlot														
grape skin[d]		9/9/97		633 ± 34		85 ± 7		106 ± 11		485 ± 42				
	9/23/96	9/16/97	777 ± 37	720 ± 43	84 ± 9	97 ± 3	111 ± 14	118 ± 3	708 ± 57	595 ± 38				
	9/30/96	9/23/97	847 ± 111	960 ± 12	104 ± 14	128 ± 4	137 ± 19	152 ± 3	821 ± 99	925 ± 30				
	10/7/96	9/29/97	1128 ± 194	1028 ± 37	132 ± 20	149 ± 6	168 ± 25	182 ± 7	1137 ± 176	1094 ± 47				
	10/15/96	10/7/97	942 ± 28	1002 ± 27	118 ± 3	134 ± 3	150 ± 6	154 ± 5	981 ± 28	1047 ± 37				
must[e]	10/22/96[f]	10/17/97[f]	130	196	40	84	31	47	32	81	0.961	0.284	0.624	0.573
		10/18/97[g]		373		115		80		266		3.747		0.377
		10/27/97[h]		851		194		147		644		11.341		0.382
wine[e]	11/1/96[i]	10/30/97[i]	974	907	147	150	130	113	412	455	7.991	9.17	0.509	0.473
		1/5/98[j]		921		162		110		355		8.753		0.547
	6/1/97[k]	7/24/98[k]	966	916	153	160	128	113	371	338	6.106	6.24	0.673	0.643
	12/11/97[l]		912		162		129		279		6.088		0.733	
Pinot Noir														
grape skin[d]		9/3/97		990 ± 17		104 ± 6		134 ± 8		483 ± 8				
		9/9/97		1006 ± 35		121 ± 16		157 ± 20		607 ± 20				
	9/23/96	9/16/97	806 ± 53	1005 ± 60	81 ± 6	126 ± 4	109 ± 11	171 ± 8	665 ± 85	715 ± 41				
	9/30/96	9/23/97	855 ± 28	1074 ± 30	114 ± 3	132 ± 5	151 ± 1	172 ± 7	657 ± 36	794 ± 33				
	10/7/96	9/29/97	915 ± 7	955 ± 77	102 ± 4	126 ± 9	135 ± 3	167 ± 14	803 ± 9	753 ± 66				
must[e]	10/17/96[f]	10/9/97[f]	209	152	77	84	47	45	78	25	1.324	0.182	0.498	1.064
		10/15/97[g]		442		144		97		200		3.338		0.546
		10/18/97[h]		650		169		109		314		5.377		0.499
wine[e]	10/30/96[i]	10/30/97[i]	748	965	120	127	94	91	340	219	5.698	4.962	0.539	0.652
		12/23/97[j]		913		117		76		166		4.603		0.731
	6/1/97[k]	7/17/98[k]	723	939	121	127	88	86	280	171	3.768	3.323	0.741	0.785
	12/10/97[l]		732		135		90		223		4.242		0.805	

[a] Grapes were grown under typical commercial practices (control treatment). [b] Wines were fermented with EC1118 yeast for Cabernet Franc and Merlot and withRC212 for Pinot Noir. [c] Contents are expressed in mg/kg of grapes or mg/L of must/wine. [d] Values are averages ± standard deviations ($n = 3$). [e] Values are the average of two replicates. [f] At crush. [g] After cold soaking for Pinot Noir or just before adding yeast for Cabernet Franc and Merlot. [h] At approximately 10% sugar during fermentation. [i] After fermentation and following pressing. [j] After malolactic fermentation. [k] At bottling. [l] After approximately 6−7 months of storage.

min, 5%; 5−55 min, 65%; 55−58 min, 100%; 58−60 min, 5%; and 60−64 min, 5%.

A 20-μL sample or standard was injected into the HPLC. Skin extracts were injected directly into the HPLC. Wines were prepared for HPLC injection using the procedure of Gao et al. (1997). Briefly, this procedure consisted of centrifuging the wine at 10000*g* for 10 min at 4 °C. Supernatant (5 mL) was acidified with formic acid to 1.5% and then filtered through a 0.45-μm Acrodisc LC PVDF syringe filter, which was then washed with 1 mL of methanol. The same standards as used for the modified Glories' method were used, except they were prepared in methanolic extract solution or as for the wine samples. The elution profile was monitored at 200−600 nm.

Color Measurement. Must or wine samples were centrifuged at 10000*g* for 10 min at 4 °C and filtered through a 0.45-μm Acrodisc LC PVDF syringe filter. Color was measured on samples diluted 1:10 with pH 3.5 buffer (0.1 M citric acid with 0.2 M Na_2HPO_4). The absorbance of the samples was read at 420, 520, and 700 nm. The color density and the hue/tint were calculated as

$$\text{color density} = (A_{520\text{ nm}} - A_{700\text{ nm}}) + (A_{420\text{ nm}} - A_{700\text{ nm}})$$

and

$$\text{hue/tint} = (A_{420\text{ nm}} - A_{700\text{ nm}})/(A_{520\text{ nm}} - A_{700\text{ nm}})$$

as described by Wrolstad (1976).

Copigmented, Monomeric, Polymeric, and Total Anthocyanins. Copigmented, monomeric, polymeric, and total anthocyanins were determined using a modified method of Boulton (1998). Briefly, this method consisted of adjusting the pH of a wine to 3.6 and then filtering the wine through a 0.45-μm Acrodisc LC PVDF syringe filter. Twenty microliters of 10% (v/v) acetaldehyde was added to 2 mL of prepared wine. The sample was allowed to sit for 45 min at room temperature before measuring $A_{520\text{ nm}}$ (A^{acet}). To another 2 mL of prepared wine, 260 μL of 5% (w/v) SO_2 was added, and $A_{520\text{ nm}}$ was measured (A^{SO_2}). The $A_{520\text{ nm}}$ was also measured for the prepared wine using a 1-mm cuvette. This reading was multiplied by 10 to give the A^{wine}. From these readings, the different forms of anthocyanins were expressed in absorbance units as

$$\text{copigmented anthocyanins} = A^{\text{acet}} - A^{\text{wine}}$$

$$\text{monomeric anthocyanins} = A^{\text{wine}} - A^{SO_2}$$

$$\text{polymeric anthocyanins} = A^{SO_2}$$

$$\text{total anthocyanins} = A^{\text{acet}}$$

The percent distribution of the various forms were calculated as

$$\%\text{ copigmented} = [(A^{\text{acet}} - A^{\text{wine}})/A^{\text{acet}}] \times 100$$

$$\%\text{ monomeric} = [(A^{\text{wine}} - A^{SO_2})/A^{\text{acet}}] \times 100$$

$$\%\text{ polymeric} = [A^{SO_2}/A^{\text{acet}}] \times 100$$

Statistical Analyses. Statistical analyses of the data were done using SAS (SAS Institute Inc., Cary, NC). General linear models procedures were used to determine treatment effects, and Duncan's multiple range tests were used to compare means.

RESULTS AND DISCUSSION

Anthocyanins and Phenolics in Grapes. The changes in concentration of anthocyanins, flavonols, tartaric esters, and total phenolics in Cabernet Franc, Merlot, and Pinot Noir grapes from the 1996 and 1997 vintage seasons are presented in Table 1. All classes of phenolics assayed increased steadily during ripening of grapes. In the skin extracts, anthocyanins were the major class of phenolics present, and their contents showed varietal influence, whereas the contents of the other classes of phenolics were not affected by variety. The content of anthocyanins in grape skin were 753−803 mg/kg for Pinot Noir, 826−1048 mg/kg for Cabernet Franc, and 981−1043 mg/kg for Merlot.

Table 2. Soluble Solids, pH, and Titratable Acidity of Cabernet Franc, Merlot, and Pinot Noir Grapes[a] and Must from the Okanagan Valley during Ripening

	sampling date		soluble solids[b] (°Brix)		pH[b]		titratable acidity[b] (g of tartaric acid/L)	
variety	1996	1997	1996	1997	1996	1997	1996	1997
				Cabernet Franc				
grapes		9/9/97		14		2.93		18.6
		9/16/97		16		2.97		15.8
	9/23/96	9/23/97	17.6	18.4	3.11	3	16.1	15.5
	9/30/96	9/29/97	19.4	20.1	3.2	3.08	14.8	12.4
	10/7/96	10/7/97	20.3	21.3	3.25	3.09	14.6	12
	10/15/96	10/16/97	21.6	22	3.03	3.09	13.6	13.1
must	10/22/96	10/27/97	21.9	22.3	2.97	3.14	13	11.2
				Merlot				
grapes		9/9/97		16		3.06		15.3
	9/23/96	9/16/97	19	16.7	3.26	3.05	11.4	13.7
	9/30/96	9/23/97	20.5	19.4	3.29	3.13	10.9	11.8
	10/7/96	9/29/97	22.2	21.2	3.33	3.15	10.9	11
	10/15/96	10/7/97	22.6	22.2	3.16	3.18	10	10.4
must	10/23/96	10/17/97	23.2	22.4	3.23	3.2	10	10.1
				Pinot Noir				
grapes		9/3/97		16.1		2.83		16.4
		9/9/97		17.4		2.9		12.9
	9/23/96	9/16/97	19.4	19	3.28	3.11	11.3	12
	9/30/96	9/23/97	20.6	20	3.29	3.06	11.5	11.9
	10/7/96	9/29/97	22.4	22.1	3.35	3.13	10.4	11
must	10/17/96	10/9/97	23.5	22.8	3.38	3.08	11.2	11.1

[a] Grapes were grown under typical commercial practices (control treatment). [b] Values are the average of two replicates.

Seasonal differences in anthocyanin content were minimal for Merlot and Pinot Noir grapes. Cabernet Franc grapes had lower levels in 1996 as compared to 1997. The atypical growing season in 1996 as compared to 1997 may have had a greater impact on development of anthocyanins in Cabernet Franc than the other varieties. This may also have resulted in higher titratable acidity of Cabernet Franc grapes in 1996 than 1997 (Table 2). The pattern of accumulation of anthocyanins differed with variety, stabilizing first in Pinot Noir, then in Merlot, and a few days later in Cabernet Franc grapes. This reflects the difference in maturity time for these three grape varieties in the Okanagan Valley of British Columbia, where Pinot Noir is an earlier variety than Merlot and Cabernet Franc. It has been reported that, in grape cell suspensions, acylated anthocyanins peak 6 days after the peaking of nonacylated pigments (Hrazdina et al., 1984). Thus, the fact that Pinot Noir grapes contain only nonacylated anthocyanins (Figure 1, Table 3) may also contribute to the varietal differences with respect to the evolution pattern of anthocyanins during grape ripening.

Because grape sampling started 8−10 days after the onset of veraison, the evolution pattern of anthocyanins could not be accurately related to the end of the berry development phase and the onset of the ripening process. However, the maximum anthocyanin concentration occurred 20−25 days after veraison. At this time, the sugar content was 19−21% (Table 2). These results

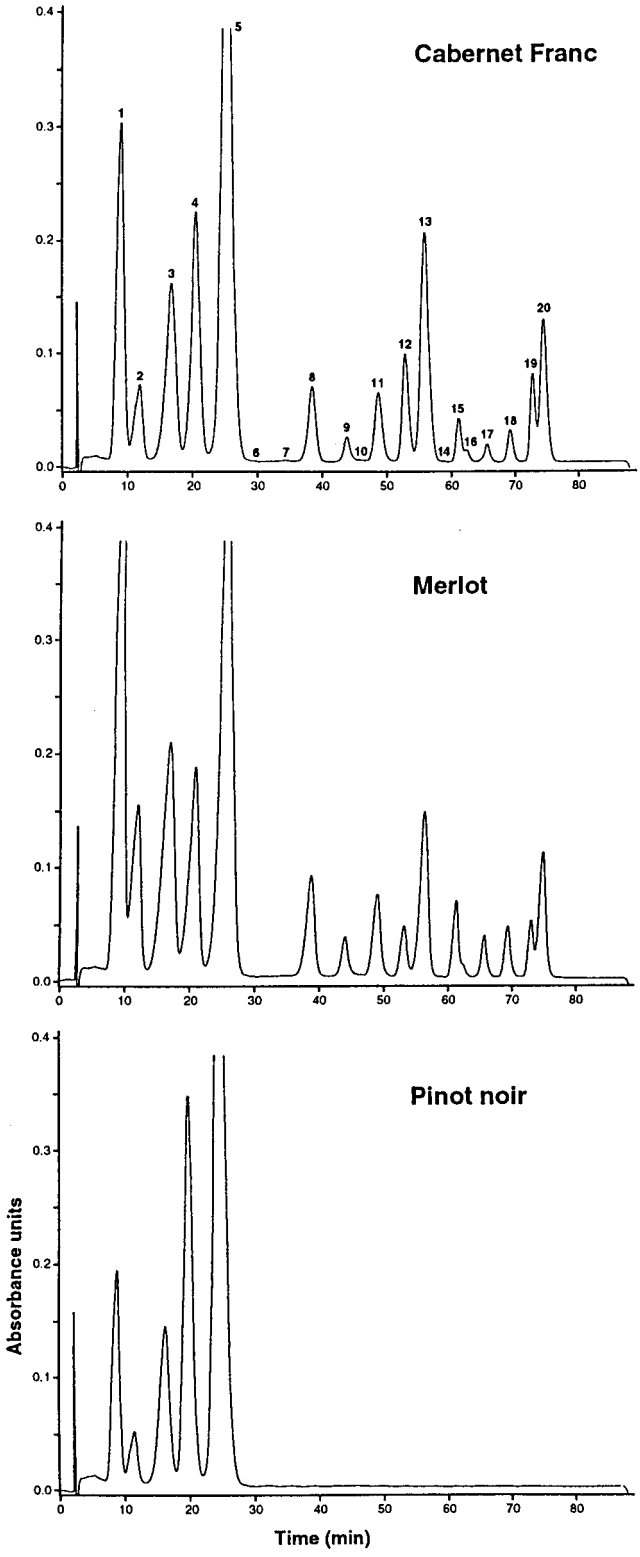

Figure 1. HPLC chromatograms of anthocyanins in 1997 Cabernet Franc, Merlot, and Pinot Noir grape skin extracts monitored at 525 nm. (Refer to Table 3 for peak numbers.)

were comparable to results reported by Somers (1976) and Roggero et al. (1986) for grapes grown in South Australia and Cotes du Rhone, respectively.

Figure 1 shows typical HPLC chromatograms of anthocyanins in Cabernet Franc, Merlot, and Pinot Noir grape skin extracts. Peaks 1−5 represent the 3-glucosides of the five anthocyanidins found in grapes. Delphinidin, cyanidin, petunidin, peonidin, and malvidin

Table 3. Content and Distribution of Anthocyanins in Cabernet Franc, Merlot, and Pinot Noir Grape Skin Extracts[a]

		content (mg of malvidin 3-monoglucoside/kg of grapes)						distribution (%)					
		Cabernet Franc[c]		Merlot[c]		Pinot Noir[c]		Cabernet Franc[c]		Merlot[c]		Pinot Noir[c]	
peak[b]	anthocyanin	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
1	delphinidin 3-monoglucoside	115.7 ± 4.4	105.0 ± 2.8	250.6 ± 12.2	173.0 ± 12.8	105.6 ± 10.2	62.5 ± 1.7	15.1 ± 0.6	12.8 ± 0.3	26.0 ± 1.3	19.0 ± 1.4	14.5 ± 1.4	10.4 ± 0.3
2	cyanidin 3-monoglucoside	53.8 ± 0.4	29.9 ± 0.8	87.5 ± 7.0	59.4 ± 4.7	24.1 ± 1.3	21.1 ± 0.8	7.0 ± 0.1	3.7 ± 0.1	9.1 ± 0.7	6.5 ± 0.5	3.3 ± 0.2	3.5 ± 0.1
3	petunidin 3-monoglucoside	73.1 ± 2.7	78.8 ± 1.7	125.6 ± 4.8	110.0 ± 7.1	104.5 ± 7.6	68.7 ± 1.5	9.5 ± 0.4	9.6 ± 0.2	13.0 ± 0.5	12.1 ± 0.8	14.4 ± 1.0	11.4 ± 0.2
4	peonidin 3-monoglucoside	106.2 ± 1.2	87.4 ± 1.0	74.2 ± 4.0	80.9 ± 2.9	114.2 ± 1.2	146.2 ± 8.6	13.8 ± 0.2	10.7 ± 0.1	7.7 ± 0.4	8.9 ± 0.3	15.7 ± 0.2	24.3 ± 1.4
5	malvidin 3-monoglucoside	188.3 ± 1.7	263.1 ± 2.1	203.0 ± 2.5	248.2 ± 9.4	378.5 ± 24.5	303.5 ± 9.1	24.5 ± 0.2	32.1 ± 0.3	21.0 ± 0.3	27.3 ± 1.0	52.1 ± 3.4	50.4 ± 1.5
6	unknown	4.3 ± 1.4	3.0 ± 0.4	3.4 ± 0.2	3.1 ± 0.5			0.6 ± 0.2	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.1		
7	unknown	3.6 ± 0.3	3.7 ± 0.1	3.5 ± 0.7	3.4 ± 0.5			0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.1		
8	delphinidin 3-monoglucoside-acetate	31.5 ± 1.2	25.6 ± 0.3	46.5 ± 1.5	34.2 ± 2.9			4.1 ± 0.2	3.1 ± 0.0	4.8 ± 0.2	3.8 ± 0.3		
9	cyanidin 3-monoglucoside-acetate	14.0 ± 0.3	8.1 ± 0.2	15.4 ± 0.9	11.6 ± 1.0			1.8 ± 0.0	1.0 ± 0.0	1.6 ± 0.1	1.3 ± 0.1		
10	unknown	1.6 ± 0.2	1.9 ± 0.1	2.1 ± 0.2	2.0 ± 0.4			0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0		
11	petunidin 3-monoglucoside-acetate	23.0 ± 0.6	22.8 ± 0.5	29.2 ± 0.6	25.4 ± 2.1			3.0 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	2.8 ± 0.2		
12	peonidin 3-monoglucoside-acetate	35.2 ± 0.9	29.7 ± 1.3	12.7 ± 0.2	14.1 ± 0.8			4.6 ± 0.1	3.6 ± 0.2	1.3 ± 0.0	1.6 ± 0.1		
13	malvidin 3-monoglucoside-acetate	55.4 ± 0.8	76.8 ± 1.5	43.6 ± 1.3	56.1 ± 3.1			7.2 ± 0.1	9.4 ± 0.2	4.5 ± 0.1	6.2 ± 0.3		
14	unknown	0.8 ± 0.2	1.1 ± 0.2	1.3 ± 0.2	1.3 ± 0.1			0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0		
15	delphinidin 3-monoglucoside-*p*-coumarate	8.3 ± 0.2	10.1 ± 0.4	16.6 ± 0.6	16.5 ± 1.0			1.1 ± 0.0	1.2 ± 0.0	1.7 ± 0.1	1.8 ± 0.1		
16	peonidin 3-monoglucoside-caffeoate	1.6 ± 0.3	2.6 ± 0.2	2.1 ± 0.3	3.1 ± 0.4			0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0		
17	cyanidin 3-monoglucoside-*p*-coumarate	5.1 ± 0.4	4.3 ± 0.2	9.5 ± 0.5	8.7 ± 0.5			0.7 ± 0.1	0.5 ± 0.0	1.0 ± 0.1	1.0 ± 0.1		
18	petunidin 3-monoglucoside-*p*-coumarate	5.5 ± 0.3	7.7 ± 0.2	9.4 ± 0.1	11.7 ± 0.4			0.7 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.3 ± 0.0		
19	peonidin 3-monoglucoside-*p*-coumarate	17.4 ± 0.2	18.4 ± 1.0	8.8 ± 0.5	12.0 ± 0.4			2.3 ± 0.0	2.2 ± 0.1	0.9 ± 0.1	1.3 ± 0.1		
20	malvidin 3-monoglucoside-*p*-coumarate	23.0 ± 1.4	39.9 ± 1.5	20.6 ± 0.6	35.1 ± 1.2			3.0 ± 0.2	4.9 ± 0.2	2.1 ± 0.1	3.9 ± 0.1		
		767	819	965	909	726	602						

[a] Skins were removed from grapes grown under control viticultural treatment and harvested on 10/7/96 and 10/7/97 for Cabernet Franc, on 10/15/96 and 10/7/97 for Merlot, and on 10/7/96 and 9/29/97 for Pinot Noir. [b] Peak numbers correspond to peaks indicated in Figure 1. [c] Values are means ± standard deviations, $n = 3$.

Table 4. Copigmented, Monomeric, Polymeric and Total Anthocyanins, in 1996 and 1997 Cabernet Franc, Merlot, and Pinot Noir Wines with Different Viticultural Treatments at Bottling Time

	Cabernet Franc		Merlot		Pinot Noir	
property[a]	1996	1997	1996	1997	1996	1997
			Control			
copigmented anthocyanins (absorbance units)	2.08	2.35	2.55	2.15	1.74	0.98
monomeric anthocyanins (absorbance units)	1.58	1.54	1.70	1.57	0.93	0.81
polymeric anthocyanins (absorbance units)	1.18	1.29	1.04	1.20	0.70	0.7
total anthocyanins (absorbance units)	4.84	5.18	5.29	4.92	3.37	2.49
% copigmented anthocyanins	43	45.4	48.3	43.6	51.6	39.4
% monomeric anthocyanins	32.6	29.6	32	32	27.5	32.5
% polymeric anthocyanins	24.5	25	19.7	24.4	20.9	28.1
			Cluster Thin at Veraison			
copigmented anthocyanins (absorbance units)	1.87	2.59	2.64	2.15	1.90	1.47
monomeric anthocyanins (absorbance units)	1.49	2.04	1.89	1.57	1.01	1.30
polymeric anthocyanins (absorbance units)	1.19	1.64	1.05	1.20	0.84	1.00
total anthocyanins (absorbance units)	4.55	6.26	5.57	4.92	3.74	3.77
% copigmented anthocyanins	41.2	41.3	47.3	43.6	50.7	39
% monomeric anthocyanins	32.7	32.6	33.9	32	26.9	34.4
% polymeric anthocyanins	26.1	26.1	18.8	24.4	22.4	26.6
			Leaf Pull			
copigmented anthocyanins (absorbance units)	2.39	2.41	3.04	2.43	1.92	1.07
monomeric anthocyanins (absorbance units)	1.78	1.90	2.34	1.93	1.00	0.97
polymeric anthocyanins (absorbance units)	1.20	1.52	1.24	1.33	0.81	0.88
total anthocyanins (absorbance units)	5.36	5.82	6.63	5.69	3.73	2.91
% copigmented anthocyanins	44.5	41.3	45.9	42.7	51.3	36.8
% monomeric anthocyanins	33.2	32.6	35.4	33.9	26.9	33.2
% polymeric anthocyanins	22.3	26.1	18.7	23.4	21.8	30

[a] Values are the average of two replicates.

3-glucosides were the only anthocyanins found in Pinot Noir grapes, which completely lacked acylated anthocyanins. Peaks 8, 9, 11, and 13 were identified as the 3-monoglucoside-acetates; peaks 15 and 17−20 were identified as the monoglucoside-coumarates; peak 16 was identified as peonidin 3-monoglucoside-caffeoate; and peaks 6, 7, 10, and 14 were not identified (Table 3). Both Cabernet Franc and Merlot grape skins contained the same anthocyanins, but their distributions were different. The distributions also varied slightly depending on season.

Anthocyanins, Phenolics, and Color of Wine. During vinification, total phenolics, tartaric esters, and flavonols increased during alcoholic fermentation, reached maximum values at pressing, and remained stable following malolactic fermentation and subsequent storage (Table 1). Anthocyanins and color density, on the other hand, increased during the initial stages of alcoholic fermentation, reached maximum values 2−3 days after the start of fermentation, and slowly decreased following malolactic fermentation and subsequent storage (Table 1). Hue values moved in the opposite direction to color density. During bottled storage in the 1996 vintage, anthocyanins slowly decreased probably due to polymerization reactions. The changes in anthocyanins in musts and wines were comparable to those reported by Leone et al. (1984), who studied the evolution of anthocyanins in Troia grape must and wine produced by conventional fermentation methods. In their study, the anthocyanin content reached its maximum on the second and third day of fermentation.

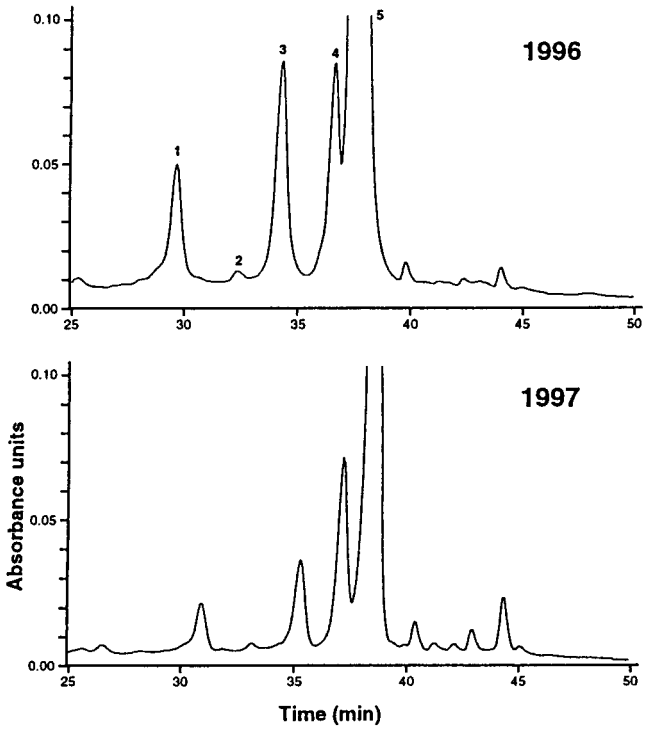

Figure 2. HPLC chromatograms of anthocyanins in Pinot Noir wines from 1996 and 1997 monitored at 525 nm. Wines were produced from grapes grown under typical commercial practices (control treatment). (Refer to Table 3 for peak numbers.)

Polymeric Pigments and Wine Color. Figure 2 shows HPLC chromatograms of Pinot Noir wine from the 1996 and 1997 vintages at bottling time. The 1997 wines contained several peaks in addition to the 3-glucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin (peaks 1−5). These peaks eluted later than the 3-glucosides and were present to a lesser extent in the 1996 wine. The spectral characteristics of the latter peaks were similar to those of monomeric anthocyanins with a sharp absorption peak at 280 nm, and a relatively wide band at 400−600 nm with a shoulder at 440 and 460 nm. These spectral properties together with their chromatographic characteristics clearly indicated that they were polymeric anthocyanins as previously noted by Gao et al. (1997). The presence of acylated anthocyanins in Cabernet Franc and Merlot

Table 5. Influence of Viticultural Treatment on the Phenolic Composition and Color of 1996 and 1997 Cabernet Franc, Merlot, and Pinot Noir Wines from the Okanagan Valley at Bottling Time

		total phenolics[a] (mg of gallic acid/L, 280 nm)		tartaric esters[a] (mg of caffeic acid/L, 320 nm)		flavonols[a] (mg of quercetin/L, 360 nm)		anthocyanins[a] (mg of malvidin-3-glucoside/L, 520 nm)		color density (absorbance units)		hue	
variety	treatment	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
Cabernet Franc[b]	control	927	1095	165	201	114	123	316	337	5.57	6.061	0.631	0.619
	cluster thin at veraison	924	1340	158	238	110	158	286	367	5.337	7.554	0.632	0.627
	leaf pull	1028	1273	191	226	138	159	363	352	6.161	7.222	0.623	0.619
	cluster thin at bloom		1278		210		134		336		7.309		0.621
	24 shoots		1225		207		135		313		6.268		0.507
Merlot[b]	control	966	916	153	160	128	113	371	338	6.106	6.24	0.673	0.643
	cluster thin at veraison	1002	980	159	171	129	129	413	375	6.59	7.133	0.68	0.65
	leaf pull	1113	1082	189	185	164	143	454	387	7.708	7.325	0.663	0.646
	cluster thin at bloom		942		170		122		383		6.872		0.641
	24 shoots		994		172		126		369		7.03		0.641
Pinot Noir[c]	control	657	939	117	127	87	86	273	171	3.794	3.323	0.728	0.785
	cluster thin at veraison	727	1133	124	157	93	115	295	255	4.189	4.847	0.714	0.744
	leaf pull	712	963	130	140	96	107	291	198	4.008	3.844	0.718	0.784
	cluster thin at bloom		996		138		94		202		3.705		0.772
	24 shoots		1062		147		107		231		4.185		0.777

[a] Values are the average of two replicates. [b] Wines for both vintage years were fermented with EC1118 yeast. [c] Wines for the 1996 and 1997 vintage years were fermented with EC1118 and RC212 yeast, respectively.

Table 6. Influence of Viticultural Treatment on the Phenolic Skin Composition of 1996 and 1997 Cabernet Franc, Merlot, and Pinot Noir grapes from the Okanagan Valley

		total phenolics[a] (mg of gallic acid, 280 nm)		tartaric esters[a] (mg of caffeic acid, 320 nm)		flavonols[a] (mg of quercetin, 360 nm)		anthocyanins[a] (mg of malvidin-3-glucoside, 520 nm)	
variety	treatment	1996	1997	1996	1997	1996	1997	1996	1997
Cabernet Franc	control	737 b	975 c	99 c	150 b	120 c	183 b	787 b	1016 c
	cluster thin at veraison	754 b	1084 b	106 b	177 a	136 b	219 a	735 c	1085 b
	leaf pull	828 a	988 c	118 a	177 a	153 a	214 a	866 a	994 c
	cluster thin at bloom		1151 a		170 a		208 a		1162 a
	24 shoots		1125 ab		179 a		215 a		1105 ab
Merlot	control	1035 a	1015 c	125 b	141 c	159 b	168 c	1059 a	1071 c
	cluster thin at veraison	1075 a	1061 bc	134 ab	160 b	165 b	198 b	1135 a	1098 bc
	leaf pull	1074 a	1116 a	146 a	182 a	195 a	228 a	1101 a	1151 ab
	cluster thin at bloom		1103 ab		160 b		194 b		1165 a
	24 shoots		1093 ab		158 b		193 b		1135 ab
Pinot Noir	control	885 b	1015 b	108 b	129 b	143 b	169 b	730 b	774 b
	cluster thin at veraison	944 ab	1100 a	108 b	133 ab	145 b	175 ab	836 a	852 a
	leaf pull	1002 a	1013 b	121 a	137 a	167 a	184 a	854 a	739 b
	cluster thin at bloom		934 c		115 c		142 c		662 c
	24 shoots		1008 b		119 c		147 c		743 b

[a] Values are averages of data combined from the last two sampling weeks before harvest in mg/kg of grapes ($n = 6$). For Cabernet Franc, combined dates used were 10/7/96 and 10/15/96 for 1996 and were 10/7/97 and 10/15/97 for 1997. For Merlot, combined dates used were 10/7/96 and 10/15/96 for 1996 and were 9/29/97 and 10/7/97 for 1997. For Pinot Noir, combined dates used were 9/30/96 and 10/7/96 for 1996 and were 9/23/97 and 9/29/97 for 1997. Values with the same letters within a column block are not significantly different for $p \leq 0.05$.

wines made it difficult to determine the existence of polymeric anthocyanins in these wines by HPLC. A spectrophotometric method was therefore used to determine the content of polymeric anthocyanins in the wines.

Results from the analysis of 18 wines for copigmented, monomeric, polymeric, and total anthocyanins are presented in Table 4. Both the 1997 Pinot Noir and Merlot wines had a higher proportion of polymeric anthocyanins than their 1996 counterparts. The results for the Pinot Noir wines confirm the observations noted by HPLC. Gao et al. (1997) reported that fermentation temperature is a critical factor in the formation of polymeric anthocyanins in Pinot Noir wines produced by different vinification processes. Other factors known to affect the progressive displacement of anthocyanins by polymeric pigments include pH, acetaldehyde, sulfur dioxide, oxygen, and concentration of molecules with the ability to act as copigment (Mazza and Miniati, 1993). Thus, observed differences in polymeric pigments may reflect variations in fermentation and/or storage conditions between vintage seasons.

Phenolic Composition and Viticultural Treatment. In both seasons, cluster thin at veraison and leaf pull treatments generally resulted in higher wine phenolic contents than in controls (Table 5). However, there were varietal differences. Leaf pull led to higher phenolics and color density in Merlot wines than cluster thin at veraison. For Pinot Noir, cluster thin at veraison gave higher values than leaf pull. For Cabernet Franc in 1997, cluster thin resulted in higher values than leaf pull; but in 1996, cluster thin resulted in lower values than for both control and leaf pull treatments. The additional treatments of cluster thin at bloom and 24 shoots in 1997 also resulted in higher phenolics and color density than for the control in Merlot and Pinot Noir wines but not for Cabernet Franc. The variations in results for Cabernet Franc suggest that other factors besides viticultural treatment can have a significant influence on grape development in this variety.

In 1997, yields were determined for the various treatments. For all varieties, the control treatment gave the highest yields. Yields for cluster thin at bloom ranged from 81 to 92% of the control, cluster thin at

Table 7. Influence of Yeast Strain on the Phenolic Composition of 1996 and 1997 Pinot Noir, Merlot and Cabernet Franc Wines from the Okanagan Valley at Bottling Time

		total phenolics[b] (mg of gallic acid/L, 280 nm)		tartaric esters[b] (mg of caffeic acid/L, 320 nm		flavonols[b] (mg of quercetin/L, 360 nm)		anthocyanins[b] (mg of malvidin-3-glucoside/L, 520 nm)		color density (absorbance units)		hue	
variety[a]	yeast	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
Cabernet Franc	BM45	975		176		121		331		5.888		0.62	
	Bordeaux Red	915	1068	159	199	112	120	304	328	5.073	5.736	0.639	0.617
	EC1118	927	1095	165	201	114	123	316	337	5.57	6.061	0.631	0.619
	ICVD254	926	1189	165	203	114	124	315	323	5.268	5.941	0.638	0.648
	L2056	933		166		115		316		5.183		0.635	
	Redstar	1098		194		133		349		5.798		0.628	
Merlot	BM45	986		157		130		363		6.037		0.673	
	Bordeaux Red	1101		167		137		407		6.488		0.672	
	EC1118	966	916	153	160	128	113	371	338	6.106	6.24	0.673	0.643
	ICVD254	1111	976	166	163	133	113	395	322	6.306	6.091	0.664	0.658
	L2056	1031	885	164	153	136	109	395	312	6.36	5.957	0.67	0.653
	Redstar	1013		160		131		390		5.84		0.68	
Pinot Noir	Assmanhausen	723		123		90		292		3.971		0.746	
	Burgundy	750		126		93		282		3.927		0.78	
	EC1118	657		117		87		273		3.794		0.728	
	Fermirouge	734		127		94		309		4.142		0.711	
	RA17		864		119		83		158		3.142		0.795
	RC212	723	939	121	127	88	86	280	171	3.768	3.323	0.741	0.785
	SIHA8	801		136		99		329		4.365		0.723	
	Wad27		753		108		76		139		2.958		0.789

[a] Wines were made from grapes grown under typical commercial practices (control treatment). [b] Values are the average of two replicates.

veraison ranged from 63 to 73%, leaf pull ranged from 89 to 92%, and 24 shoots ranged from 73 to 89%.

Variations in copigmented, monomeric, polymeric, and total anthocyanins (Table 4) for the different treatments paralleled those noted in Table 5. The proportions of polymeric anthocyanins were similar for all treatments within a season, which indicated that all wines were subjected to similar conditions during vinification.

The results demonstrate that cluster sun exposure can lead to higher levels of phenolics and color density in red wines. Differences in phenolics and color noted in the wines corresponded to differences noted for grape skin extracts (Tables 1 and 6). Grapes containing high levels of phenolics therefore yielded wines richer in health-promoting phenolics and with improved color characteristics. However, yields decreased when treatments that led to increased phenolics were used.

Phenolic Composition and Yeasts. Table 7 presents the results on the effect of yeast strain used on the phenolic composition and color of Cabernet Franc, Merlot, and Pinot Noir wines at bottling time. The yeasts used for fermentation did not affect wine properties noticeably, except for Pinot Noir wines. With the Pinot Noir wines, Wädenswil 27 yeast gave lower color density and phenolic content than the other yeasts tested. This yeast fermented at a slower rate, which may have led to the differences.

CONCLUSIONS

Phenolics, mainly anthocyanins in Cabernet Franc, Merlot, and Pinot Noir grape skins, increased steadily and then stabilized during ripening. The content, distribution, and accumulation of anthocyanins in grape skins was largely influenced by variety. During vinification, colorless phenolics increased during alcoholic fermentation, reached maximum values at the time of pressing, and remained stable during malolactic fermentation and subsequent storage. Anthocyanins and color intensity, on the other hand, increased during the early stages of alcoholic fermentation, reached maximum values 2−3 days after the start of the fermentation, and decreased during malolactic fermentation and subsequent storage. Polymeric anthocyanins were present in higher proportion in 1997 wines than 1996. Differences in grape phenolics were transferred into wines. For each variety, colorless phenolics, anthocyanins, and color density in red wines were influenced by cluster sun exposure and season, but yeast used for fermentation had minimal effect.

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