Changes in Anthocyanins and Color Characteristics of Pinot Noir Wines during Different Vinification Processes

L. Gao, B. Girard, G. Mazza,* and A. G. Reynolds

Food Research Program, Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia V0H 1Z0, Canada

Simple and polymeric anthocyanins of Pinot noir wines produced by different vinification processes were monitored by high-performance liquid chromatography (HPLC) from the onset of fermentation to bottling. Color characteristics were determined by tristimulus measurement using a spectrophotometer with a color determination and match program. Up to 12 individual polymeric anthocyanins were found. Extraction of monomeric anthocyanins was increased with higher fermentation temperature, but anthocyanin concentration declined gradually after fermentation. In all wines, the dominant type of anthocyanin was malvidin 3-glucoside. In contrast, the content of polymeric anthocyanins increased during fermentation and thereafter, and reached the highest concentrations at bottling. Fermentation temperature was a critical factor for formation of polymeric anthocyanins. The presence of total or individual polymeric anthocyanins was directly related to wine color intensity, and all the polymeric anthocyanins were important to wine color.

Keywords: *Wine color; polymeric anthocyanins; monomeric anthocyanins; flavonoids; fermentation temperature; HPLC analysis; tristimulus analysis*

INTRODUCTION

Anthocyanins are responsible for most color differences between grapes (*Vitis* spp.) and their resulting wines. The amount and composition of anthocyanins in red wine grapes vary greatly with species, cultivar, maturity, season, region, and yield. Within a species, the composition and distribution of anthocyanins are complex and cultivar-specific (Mazza, 1995).

European (*Vitis vinifera* L.) and North American (*V. labrusca* L.) grapes are among many species of grapes grown worldwide. Leading *V. vinifera* red wine cultivars include Cabernet Sauvignon, Merlot, Syrah, and Pinot noir. Delphinidin, cyanidin, petunidin, peonidin, and malvidin 3-monoglucosides are the only anthocyanins found in Pinot noir, while these anthocyanins along with various forms acylated by acetic, coumaric, and caffeic acids are found in Cabernet Sauvignon, Merlot, and Syrah wine/grape varieties (Mazza, 1995).

Maceration, fermentation, and aging conditions affect the composition of wine anthocyanins while the total concentration and composition of anthocyanins affect wine color (Mazza, 1995). Somers and Evans (1979) followed the changes in anthocyanins, total phenolics, and color intensity of heat-treated Syrah grape juice during thermovinification at pH 3.40 and 3.83 and during traditional fermentation on the skins. After fermentation, color intensity had decreased 3- and 5-fold, whereas anthocyanin and total phenolics declined only by 20% and 30% at pH 3.40 and 3.83, respectively. The loss in color intensity was mainly attributed to the effect of ethanol on structures of deeply colored pigment aggregates present in the juice prior to fermentation.

The importance of polymeric anthocyanins is widely accepted (Somers, 1971; Mazza, 1995). Analyses have been generally based on empirical calculations using absorbances at 520 nm and 420 nm (Somers and Evans, 1977; Bakker et al., 1986a; Dallas and Laureano, 1994a). Although the measurements at two wavelengths can be made quickly, the information is limited when compared to the visual spectrum (380−770 nm). The CIELab convention introduced in 1976 has recently been proposed as a better measurement of wine color (Bakker et al., 1986a). The polymeric anthocyanins have been found to coelute with monomeric acylated anthocyanins (McCloskey and Yengoyan, 1981; Bakker, 1986; Roggero et al., 1992). Therefore, the use of a cultivar like Cabernet Sauvignon complicates the study of polymeric anthocyanins by high-performance liquid chromatography (HPLC). During maturation in the presence of oxygen, the redness (A_{520nm}) of a red young wine decreases as the absorbance in the yellow/brown region (near 420 nm) increases. These changes in color characteristics reflect the progressive displacement of grape anthocyanins by more stable polymeric pigments, which account for up to 50% of the color intensity of 1-year-old wines (Somers, 1971). The reactions considered responsible for the formation of these polymeric pigments include: acetaldehyde-mediated condensation, copigmentation, and self-association (Mazza and Miniati, 1993; Mazza, 1995). These reactions lead to color enhancement and pigment complexes less sensitive to pH changes than anthocyanins. Their stability and reactivity in wine can, however, be affected by factors such as temperature, oxygen, SO_2, acetaldehyde, pH, and concentration of pigments and copigments (Mazza, 1995). According to Somers and Evans (1986) and Somers and Pocock (1990), storage temperature is the primary environmental factor influencing changes in the color characteristics of red wines during maturation. However, the influence of vinification conditions on the formation of individual polymeric anthocyanins and their impact on wine color are poorly understood.

The objectives of this investigation were the following: (1) to study the extraction and retention of monomeric anthocyanins and the formation of polymeric anthocyanins in Pinot noir grapes subjected to three vinification treatments; (2) to compare the effects of the

* Author to whom correspondence should be addressed [fax (250) 494-0755; Internet MAZZAG@EM.AGR.CA].

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Table 1. Physical and Chemical Characteristics of Must and Wines of the Three Vinification Treatments

analysis	treatment 1	treatment 2	treatment 3
must composition[a]			
soluble solids (°Brix)	22.1a[b]	22.0a	22.0a
titratable acidity (g/L)	11.2a	12.5a	9.2b
pH	3.25b	3.32a	3.26b
wine composition			
titratable acidity (g/L)	8.8a	8.9a	7.7b
pH	3.40b	3.53ab	3.60a
ethanol (% v/v)	10.8b	11.0b	14.3a

[a] Musts of treatments 1 and 2 were the juices of freshly crushed grapes; must of treatment 3 was the combined free run and press fractions. [b] Means within rows followed by the same letter are not significantly different, $p \leq 0.05$.

Table 2. Vinification Protocol for Treatments 1 and 2

step	operation
1	crushing/destemming of grapes
2	addition of potassium metabisulfite, 40 mg of SO_2/L
3	inoculation with Prise de Mousse (Lalvin EC1118), 10 g/hL
4	fermentation at 20 °C (treatment 1) or 30 °C (treatment 2)
5	punching cap twice a day
6	pressing after cap disintegration at day 7 (treatment 1), or day 5 (treatment 2), and both maturing at 15 °C
7	racking at week 1, week 2, and 1st month
8	addition of bentonite, 1 g/L
9	cold stabilization at 0−1 °C, racking after 2 months
10	addition of potassium metabisulfite (50 mg of SO_2/L)
11	filtration: AøY AN pad filter followed by 0.45 μM pleated cartridge
12	bottling after 7 months

processing conditions on the formation of polymeric anthocyanins and wine color and; (3) to elucidate possible relationships between various anthocyanins and wine color characteristics. This work complements a concomitant study on the influence of thermal vinification on aroma constituents and sensory descriptors of Pinot noir wines (Girard et al., 1997).

MATERIALS AND METHODS

About 360 kg of Pinot noir grapes (*V. vinifera* L.) was harvested at commercial maturity [22.1 °Brix; 11.9 g/L titratable acidity (TA); 3.29 pH] on October 19, 1993, at a commercial vineyard near Kelowna, British Columbia. This vineyard had been used for a viticultural trial between 1990 and 1993 (Reynolds et al., 1994), and was well-known for producing high-quality fruit. The grapes were crushed in a Garolla-type crusher−destemmer, collected into 20 L food-grade plastic pails, and subdivided into nine 40 kg lots to provide three replicates of three vinification treatments.

Must samples (50 mL) were collected from each treatment replicate to determine soluble solids (°Brix), TA, pH, and total anthocyanins (Amerine and Ough, 1980). Ethanol was determined using a Hewlett-Packard 5700 gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a Porapak Q-100 (3.2 mm × 2 m SS) column. Other conditions of operation were the following: N_2 carrier gas flow rate, 20 mL/min; oven temperature, 175 °C; injector temperature, 200 °C; and detector temperature, 200 °C. Wine samples (2 mL) were pipetted into 100 mL volumetric flasks and made up to volume with 0.2% 2-propanol as the internal standard. Injection volume was 1 μL. The results of these analyses are tabulated in Table 1.

Vinification treatment 1 was a conventional on-skin fermentation at 20 °C; treatment 2 was carried out at 30 °C (Table 2), and treatment 3 consisted of passive dejuicing (free run) of each of the three replicates, and high-temperature/short-time (HTST) treatment of the dejuiced grapes at 90−95 °C for 1 min using a Cherry-Burell Thermutator scraped surface heat exchanger (Model SWB-324, Cedar Rapids, IA). The heated mash from treatment 3 was then pressed on a hydraulic rack and cloth press, and the cake was reextracted by introduction of the free run juice. The free run and pressed

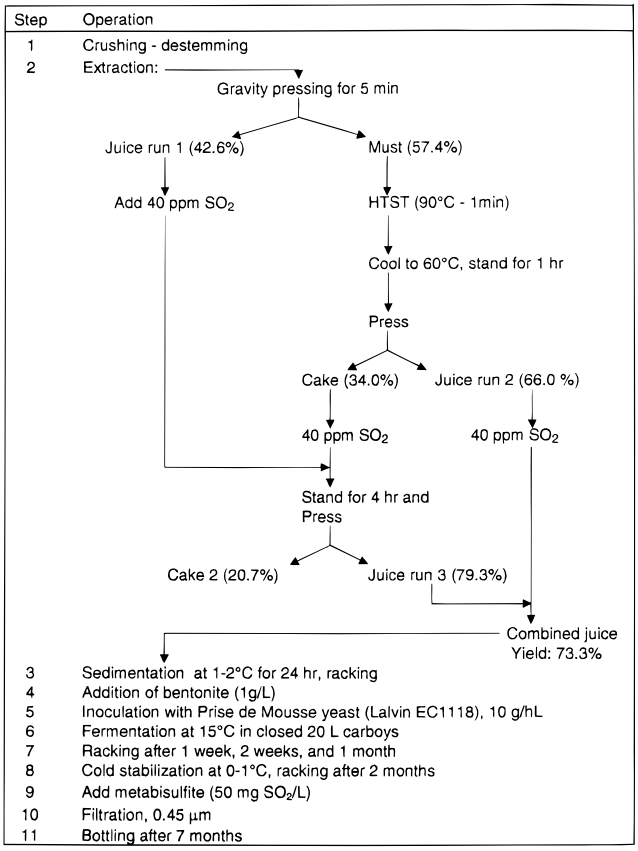

Figure 1. Schematic diagram of vinification protocol for treatment 3.

fractions were combined within replicates thereafter for inoculation and fermentation, which was carried out in glass at 15 °C (Figure 1). Treatment 3 was intended to improve the extraction of anthocyanin pigments and other phenolics of grapes, and possibly increase wine color. All fermentations were carried out to dryness using Prise de Mousse yeast (*Saccharomyces bayanus*; EC 1118, Lallemand Inc., Montreal).

Temperature and °Brix were monitored periodically during the fermentations. Wine samples (250 mL) were collected from all of the treatment replicates at the completion of fermentation. Additional samples of whole berries (500 g), must, and wines (250 mL/treatment replicate) were retained for subsequent anthocyanin analysis. These samples were stored at −35 °C and analyzed by HPLC.

Sample Preparation and Anthocyanin Analysis. Grape skin samples (5 g) were extracted in a temperature-controlled (15 °C) blender with 50 mL of methanolic solvent consisting of 1:1 (v/v) aqueous formic acid (3%)/methanol. The resulting slurry was filtered through a 0.45 μm hydrophilic Durapore membrane filter (Millipore, Bedford, MA), and the filtrate was left for 2 h at room temperature before HPLC analysis.

Must and wine samples were centrifuged at 10000*g*, 4 °C, for 10 min. An aliquot of the supernatant (2 mL × 2) was taken and used directly for CIELab color measurement. Another 20 mL aliquot was acidified by addition of formic acid to 1.5%. Five milliliters of the resulting sample was filtered through a 0.45 μm hydrophilic Durapore membrane which was subsequently washed with 1 mL of methanol. Twenty microliters of the resulting filtrate was injected into HPLC for analysis.

High-Performance Liquid Chromatography (HPLC). Analysis for anthocyanin content and composition was carried out as described by Gao and Mazza (1994) using a Hewlett Packard 1090 Series II Liquid Chromatograph [Hewlett Packard (Canada) Ltd., Richmond, BC] equipped with a diode array detector. Separation was achieved on a reverse-phase minibore column (Supelcosil LC-18, 250 × 2.1 mm, 5 μM; Supelco, Inc., Bellefonte, PA). The solvents used were 5% (v/v) formic acid (solvent A) and methanol (solvent B). The flow rate was

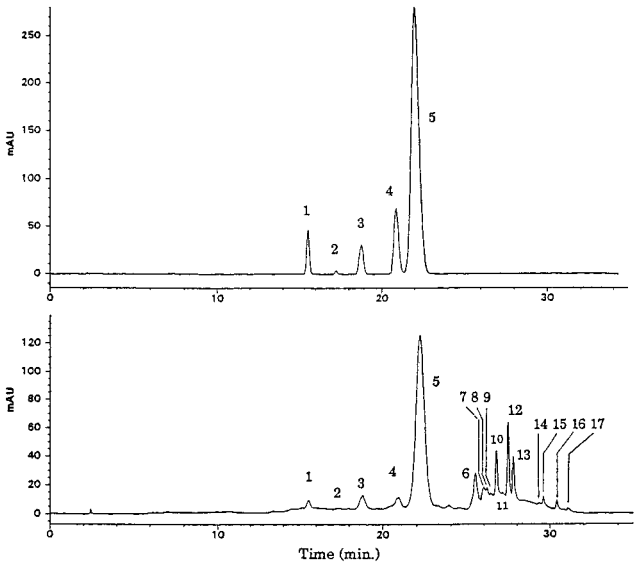

Figure 2. HPLC chromatograms at 525 nm of Pinot noir grape skin extract (top) and wine (bottom) produced by vinification treatment 2 (see Table 2). Peaks 1−5 are 3-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, respectively. Peaks 6−17, polymeric anthocyanins. Injection volume: 20 μL.

at 0.350 mL/min, with a linear gradient profile consisting of solvent A with the following proportions (v/v) of solvent B: 0−2.5 min, 5% B; 2.5−3 min, 5−17% B; 3−10 min, 17−19% B; 10−12 min, 19−28% B; 12−22 min, 28−34% B; 22−28 min, 34−70% B; 28−29 min, 70−100% B; 29−31 min, 100% B; 31−32 min, 100−5% B; 32−35 min, 5% B. The column temperature was maintained at 25 ± 0.1 °C by circulating water from a water bath through the column chamber. Twenty microliters of wine or juice sample was injected into the HPLC for analysis. The elution was monitored at 200−700 nm. Quantitation of anthocyanins was based on peak areas at 525 nm and calculated as cyanidin 3-glucoside which was used as the standard.

Color Determination. Tristimulus analysis was carried out on a Beckman DU 640 Spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). The $L^*a^*b^*$ values of the wine samples were determined in a 1 cm cuvette with a color determination and match program (CIE, E308, Illuminant C). Hue angle (h, in degrees) was calculated from $h = \arctan(b^*/a^*)$.

Pinot noir wines (1991 and 1992 vintages) used for the analysis of the relationship between color and polymeric anthocyanins in aged wines were made from grapes harvested from commercial vineyards near Kelowna, British Columbia. The wines selected had pH values between 3.59 and 3.61 so that color difference due to pH was minimized. Wine had consistently been stored at 10 °C.

Statistical analysis was carried out using the SAS REG Procedure, and means were compared by Scheffe's test (Version 1993, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Changes in Anthocyanin Composition. Typical HPLC chomatograms of Pinot noir must and wine are shown in Figure 2. Peaks 1−5, identified as 3-glucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, were present in grape, must, and wine. Peaks 6−17, which eluted later than any of the monomeric anthocyanins, probably because of their higher hydrophobicity, were present only in the wine samples. 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 from 400 to 600 nm with a shoulder between 440 and 480 nm. All wines, irrespective of the vinification protocol, showed qualitatively the same chromatographic profile.

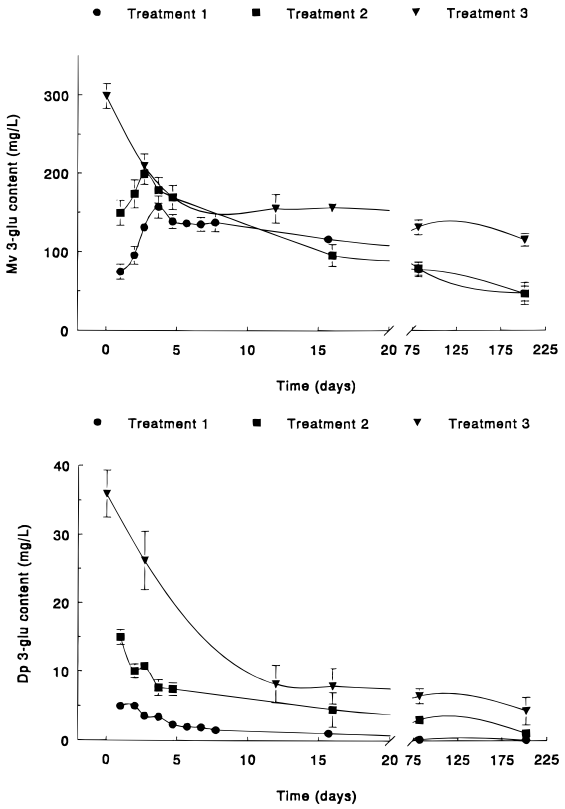

Figure 3. Changes in malvidin 3-glucoside (top) and delphinidin 3-glucoside (bottom) in Pinot noir wines produced by three vinification protocols. (Fermentation time was 3 days for treatments 1 and 3, and 5 days for treatment 2.)

Delphinidin, cyanidin, petunidin, peonidin, and malvidin 3-monoglucosides were the only anthocyanins found in Pinot noir grape, which is consistent with published reports (Mazza, 1995) indicating that Pinot noir does not contain acylated anthocyanins. This relatively simpler anthocyanin composition provided an advantage for resolution of polymeric from monomeric anthocyanins in the Pinot noir wines.

The changes in malvidin 3-glucoside (Mv3Glu) and delphinidin 3-glucoside (Dp3Glu) in musts and wines produced by three vinification protocols are shown in Figure 3. The concentrations of individual monomeric anthocyanins were affected by fermentation treatments. Malvidin 3-glucoside, which is the major anthocyanin of Pinot noir grapes, was extracted from the grape skins into the must wine much more rapidly in treatment 2 (30 °C, max 200 mg/L at day 3) than treatment 1 (20 °C, max 157 mg/L at day 4). This difference was presumably due to the higher fermentation temperature used in treatment 2, which facilitated the extraction of anthocyanins. The levels of Mv3Glu in wines dropped rapidly in the later part of fermentation (7 and 5 days for treatments 1 and 2, respectively; see Table 2), and this trend continued albeit gradually during aging. However, Mv3Glu concentration in wines from treatment 2 dropped more rapidly so that the final residual level (46.6 mg/L) at bottling did not differ from that of wines in treatment 1 (46.4 mg/L).

The initial free run juice from treatment 3 did not contain appreciable amounts of anthocyanins. The heat-treated pressed fraction, however, contained a very high concentration of Mv3Glu (599 mg/L). Rinsing the resulting press cake with the free run juice produced a juice containing ca. 127 mg/L Mv3Glu. Prior to fermen-

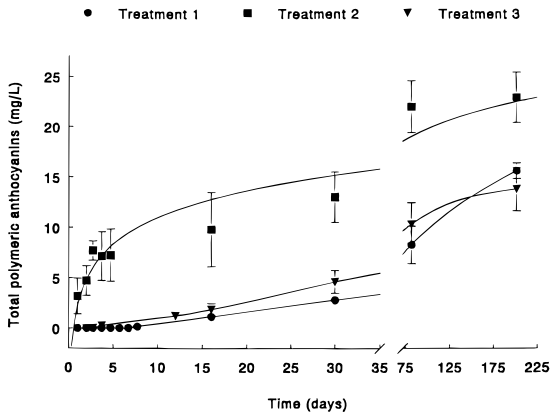

Figure 4. Changes in polymeric anthocyanins in Pinot noir wines produced by three vinification protocols. Concentration expressed as cyanidin 3-glucoside.

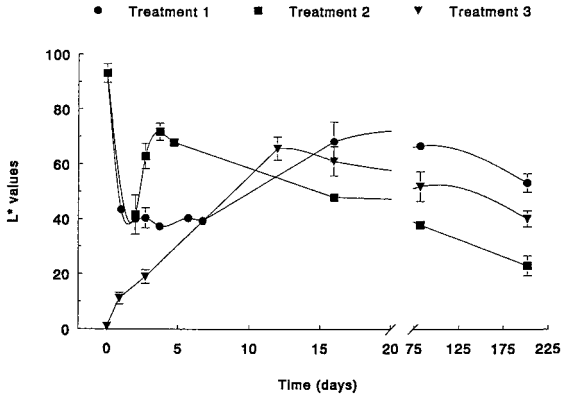

Figure 5. Changes of CIELab *L** values of Pinot noir wines produced by three vinification protocols.

tation, the treatment 3 must (free run + press fraction) had the highest amount of extracted anthocyanins. The Mv3Glu concentration dropped rapidly from the initial 299 mg/L to 209 mg/L after 2 days of fermentation, representing a 30% decrease. The rate of decrease slowed after fermentation (7 days), and the pigment reached a final concentration of 114 mg/L at bottling. The concentration of this anthocyanin in wines of treatment 3 was still much higher (ca. 2.5 times) than in wines produced by the other two protocols.

The changes in anthocyanins in musts and wines from treatments 1 and 2 were similar to those reported by Leone et al. (1984), who studied the evolution of anthocyanins in Troia 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, which was similar to the behavior of our samples from treatment 2 but about 1 day earlier than treatment 1.

Polymeric Anthocyanins and Wine Color. Polymeric anthocyanins possess a relatively high hydrophobicity, and, therefore, a high methanol concentration was required to elute them from the HPLC column. The amounts of these pigments were likely underestimated because their extinction coefficients in the high methanol eluent are expected to be lower than in the high formic acid eluent where the standard cyanidin 3-glucoside eluted. Nevertheless, it was valid to compare the relative concentration of the respective pigments between wines from different treatments. In addition, the method of analysis used makes it possible to examine the potential contribution of each polymeric anthocyanin to wine color.

The rate of formation of the polymeric anthocyanins showed marked differences between the treatments (Figure 4). In treatment 1, there were essentially no polymeric anthocyanins formed during fermentation. After fermentation, polymeric anthocyanins began to form gradually and reached ca. 15 mg/L at bottling. The formation rate of polymeric anthocyanins in treatment 3 was similar to that of treatment 1 although it started a few days earlier (fourth day of fermentation). It parallelled the formation of polymeric anthocyanins in treatment 1. At the end of fermentation, the concentration of polymeric anthocyanins (15 mg/L) was the same as in treatment 1. In contrast to the previous two protocols, treatment 2 wines started to produce polymeric anthocyanins almost immediately following the initiation of fermentation and at a considerably higher rate than wines from the other two treatments. The wines continued to form polymeric anthocyanins after the completion of fermentation although at a much lower rate than during fermentation. The final concentration of polymeric anthocyanins reached 22 mg/L, about 50% higher than those from treatments 1 and 3.

The formation of individual polymeric anthocyanins followed a similar curve in each vinification protocol. Individual anthocyanins showed similar trends over time to those of total polymeric anthocyanins (data not shown). However, the concentration of individual pigments varied. The rates of formation in treatment 1 wines were all similar among the major polymeric peaks 6, 10, and 12. The level of peak 6 anthocyanin was the lowest relative to other peaks in treatment 3 and the highest in treatment 2 wines.

These results suggest that fermentation temperature was a major factor in the formation of polymeric anthocyanins. Must pH may also have an effect, as suggested by Dallas and Laureano (1994b). The highest formation rate of polymeric anthocyanins in our study was observed during fermentation of treatment 2 wines, which utilized a relatively high fermentation temperature. Although treatment 3 used the lowest fermentation temperature (15 °C), the heat treatment enhanced the extraction of juice and anthocyanins. This may explain the onset of polymeric anthocyanin formation early in the fermentation (Figure 4).

The visual perception of red table wine color is a result of color display by both monomeric anthocyanins extracted from the grape skin during fermentation and polymeric anthocyanins formed during fermentation and aging of the wine (Somers, 1971; Timberlake, 1981; Mazza, 1995). In the initial stages of fermentation in treatments 1 and 2, the must displayed very little red color (Figure 5) as both monomeric (Figure 3) and polymeric (Figure 4) anthocyanins were at low levels. Color intensity (as reflected by a low *L**) increased after fermentation in all treatments (Figure 5), and paralleled the concentration of polymeric anthocyanins (Figure 4). The change in darkness intensity during fermentation and the few weeks following fermentation was notable (Figure 5) because of the rapidly changing concentrations of monomeric (Figure 3) and polymeric anthocyanins (Figure 4).

The results of the tristimulus measurements showed further the effect and importance of fermentation temperature on the color intensity of the wines. At the onset of fermentation in treatments 1 and 2, the lightness of the must decreased rapidly from approximately 95 to about 40 (Figure 5) as a result of anthocyanin extraction from the skins (Figure 3). The lightness of wines in treatment 2 increased considerably from 40 on the third day to about 70 on the fifth day of fermentation while wine lightness for treatment 1

remained low during the fermentation period. The higher fermentation temperature used in treatment 2 caused a more rapid decrease in monomeric anthocyanins. At this stage, the concentration of polymeric anthocyanins was low and did not exert a large effect on the color intensity of wines. However, at the end of fermentation and following the initial maturation period, wines from treatment 2 became steadily darker and were the darkest ($L^* = 25$) at bottling time. In contrast, the color intensity after fermentation in treatment 1 decreased for another 2 weeks until the L^* value reached a peak (ca. 70), which was comparable to that obtained by the end of treatment 2 fermentation. Thereafter, L^* values of wines from treatment 1 paralleled those of treatment 2 wines. In general, it appears that the higher temperature in treatment 2 (30 °C) caused an increase in L^* values beginning at the fermentation stage. This effect manifested itself later in wines from treatment 1 fermented at a lower temperature. The fermentation temperature seems to have also been responsible for the higher concentration of polymeric anthocyanins and slightly lower concentration of monomeric anthocyanins in treatment 2 than in treatment 1 wines (Figure 4). Further work is required to determine whether the earlier peak of L^* values in treatment 2 wines is a result of faster maturation than in treatment 1 wines.

The changes in color intensity of wines from treatment 3 were simpler than the changes which occurred in the L^* values of the wines produced by the other two treatments. The initial must was dark at the start of fermentation ($L^* \approx 0$) as a result of the high concentration of monomeric anthocyanins. During the course of fermentation, the lightness gradually increased until it reached about 65 at day 12. This was followed by a gradual decrease which was similar to that observed in the other wines. The color intensity of the wines from treatment 3 reached an L^* value of 40 at bottling, which was between those of the other two wines.

The final ranking of treatment 3 wines resulted from a collective color display by monomeric and polymeric anthocyanins. Although the polymeric anthocyanin content was lower than that in treatment 2 wines and similar to that in treatment 1 wines, the monomeric anthocyanin concentration in treatment 3 wines was much higher (by 85%) than in treatment 2 wines.

The level of polymeric anthocyanins formed in these wines was a critical factor for the wine color intensity. The rapid formation of polymeric anthocyanins as it occurred in treatment 2 (Figure 4) indicates that the wines proceed through a dynamic transition period from monomeric to polymeric anthocyanins, which results in a temporary loss of color produced by monomeric anthocyanins. This transition phase was slower in the wines from the two treatments fermented at lower temperatures. Gomez-Cordoves and Gonzalez-SanJose (1995) also noticed a rapid decrease in color intensity of wines in the initial stage of winemaking.

These results suggested that the rate and degree of polymeric anthocyanins formation were at least partially temperature dependent. Thus, treatment 2 wines contained the highest polymeric anthocyanins because the fermentation temperature was the highest. Although treatment 3 wines contained a high concentration of monomeric anthocyanins (assumed to be the reactants for polymerization), relatively low concentrations of polymeric anthocyanins were produced. This is consistent with the observation of Somers and Evans (1986), who found that temperature had a major influence on the formation of polymeric wine color as

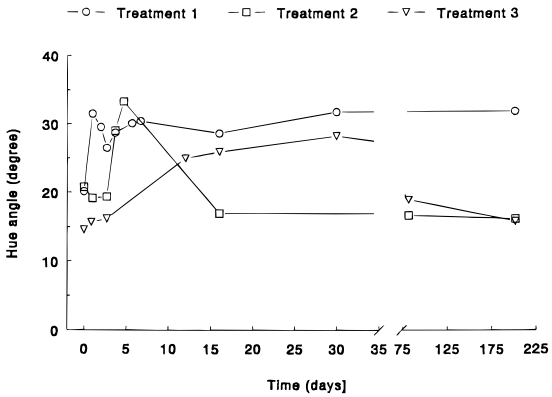

Figure 6. Changes of hue angle values of Pinot noir wines produced by three vinification protocols.

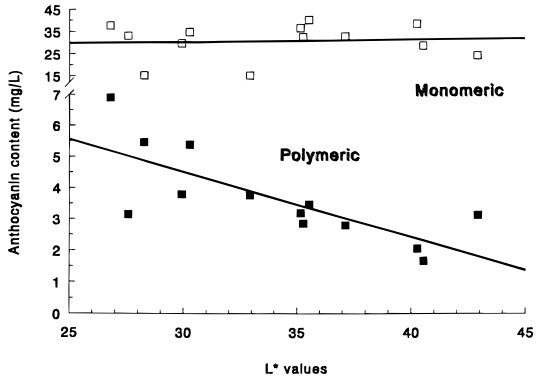

Figure 7. Relationship between monomeric and polymeric anthocyanin contents and color intensity of 13 Pinot noir wines.

measured by the absorbance at 520 nm in the presence of SO_2. Dallas and Laureano (1994b) confirmed a positive effect of higher temperature on the formation of polymeric color.

The hue angle of the wines varied among treatments (Figure 6). In the early stages of fermentation, treatment 1 wines had a relatively high hue angle of ca. 30° while treatment 3 wines had low hue angles of ca. 15°. Treatment 2 wines had high hue angles during fermentation. In the late stages of fermentation and thereafter, the hue angle of treatment 1 wines increased slightly. This behavior was consistent with that observed by Bakker et al. (1986b), who reported that the hue angle of Tinta Barroca Port wines continuously increased during aging. However, in our study, the hue angles for treatment 2 and 3 wines displayed different trends. In treatment 2 wines, the hue angle rapidly decreased from as high as 30° to about 16° at day 15, and remained at this level until bottling. For treatment 3 wines, the hue angle increased from the onset of fermentation to about 28° at day 30, and declined to the same level as wines from treatment 2 at bottling. Therefore, the hue of wines from treatment 1 was slightly less reddish than wines from treatments 2 and 3. Again, this is likely due to the fact the treatments 2 and 3 underwent relatively higher temperature conditions.

Relationship between Wine Color and Content of Polymeric Anthocyanins. Figure 7 shows the relationships between the amount of polymeric and monomeric anthocyanins and the color intensity values as measured by L^* of 12 wines of similar pH values (pH 3.59−3.61), all of which were fermented at 30 °C in 1991 and 1992. The level of polymeric anthocyanins was directly related to the darkness of the wines, while the

Table 3. Relationships between Various Anthocyanin Peaks and the Color Intensity of 13 Pinot Noir Wines

peak	relationship of peak area with color intensity	$r^{2\,a}$
total polymeric	$=1361.8 - 27.4L^*$	0.7827^{SSS}
peak 6	$=189.7 - 2.1L^*$	0.2997^{S}
peak 10	$=106.6 - 2.1L^*$	0.7597^{SSS}
peak 12	$=138.3 - 2.4L^*$	0.4171^{SS}
total monomeric	$=3406.9 + 5.4L^*$	0.0015
Cn3-G	$=315.5 - 2.6L^*$	0.0421
Pn3-G	$=328.0 - 1.7L^*$	0.015
Mv3-G	$=2763.3 + 9.7L^*$	0.0063

[a] S, $P < 0.1$; SS, $P < 0.05$; SSS, $P < 0.0001$.

amount of monomeric anthocyanins in these wines was not correlated to the intensity of wine color. This may be a result of several factors. First, at natural wine pH, the percentage of colored (flavylium and quinonoidal) forms of anthocyanins existing in wines is relatively low (Mazza and Miniati, 1993). The amount of monomeric anthocyanins remaining in wines was only a fraction of the initial amount obtained in the early stages of fermentation (Figure 3). The monomeric fraction thus contributed little to the color of these Pinot noir wines.

Table 3 shows linear regressions of the amount of anthocyanins with the lightness of the wines. As opposed to the monomeric anthocyanins, all the polymeric peaks contributed to wine color. More wine samples need be tested to confirm this apparent relationship.

CONCLUSIONS

HPLC analysis revealed that as many as 12 individual polymeric anthocyanins were present in Pinot noir wines. Total or individual polymeric anthocyanins were directly related to the color intensity of the wines, and all polymeric anthocyanins were important contributors to wine color. Fermentation conditions had a profound influence on monomeric and polymeric anthocyanins. High extraction of monomeric anthocyanins from the skin was enhanced by an elevated fermentation temperature and an HTST treatment. However, the fermentation temperature was found to be the critical factor in the generation of polymeric anthocyanins. High concentrations of monomeric anthocyanins obtained by treatment 3 did not produce wines of higher color intensity, and, therefore, this process seems to be a relatively minor factor for enhancing wine color intensity. Fermentation temperature of 30 °C favored both the formation of polymeric anthocyanins and a darker wine. After fermentation, the content of individual monomeric anthocyanins declined gradually while those of polymeric anthocyanins increased. The dominant monomeric anthocyanin was malvidin 3-glucoside. At bottling, residual monomeric anthocyanins were only a fraction of the initial concentration, and the total polymeric anthocyanin content reached its highest levels. Further work is required to elucidate the chemical nature of the individual polymeric anthocyanins, and the mechanisms for their formation during vinification.

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