COLOUR DYNAMICS IN WINES MADE FROM CO-FERMENTS OF VITIS VINIFERA L. SYRAH AND VIOGNIER

Rod CHITTENDEN[1]*, and Paul A. KILMARTIN[2]
[1] Senior Lecturer Wine Science, Eastern Institute of Technology ,Private bag 1201, Hawkes Bay Mail Centre, Napier, 4142, Hawkes Bay, New Zealand, [2] Director Wine Science, The University of Auckland, Private Bag 92019, Auckland, New Zealand
* Corresponding author [email: rchittenden@eit.ac.nz]

Abstract
Anthocyanin-derived pigment profiles in a series of 100% *Vitis Vinifera* L Syrah and 2, 5, and 10% co-ferments with crushed destemmed *Vitis Vinifera* L Viognier were compared, using spectroscopic, protein precipitation and HPLC methods. The extent of co-pigmentation was highest in the younger wines and declined a month after fermentation and further for the year-old wines. A higher degree of co-pigmentation matched a higher colour density expression. Anthocyanin self association co-pigmentation peaked at day 6 followed by co-pigmentation due to the reaction between anthocyanins and other polyphenolic co-factors peaking at day 12, while polymeric pigment became significant after day 19. The treatments with the higher co-winemaking additions showed the highest % CA colour levels and the fastest rates of % PA and % LPP colour formation. Nevertheless, over the course of the study the control, with Syrah only, was not consistently different in colour enhancement due to co-pigmentation than the Syrah co-fermented with Viognier. The phenolic profile of the added Viognier did not contribute sufficient co-factors to the Syrah ferment to bring about co-pigmentation colour enhancement.
Key words: Co-winemaking; anthocyanin; co-pigmentation; polymeric pigmentation; red wine

INTRODUCTION

Syrah varietal wine is produced in the Northern Rhone within the appellation Cote Rotie, and is often co-fermented with a proportion of Viognier, a practice adopted in other countries including New Zealand. Anecdotally the benefits of co-fermentation include added complexity, better texture, increased aromatic character, improved colour and colour stability leading to enhanced aging ability. However, the colour enhancement expected in co-ferments of Syrah with Viognier has not been confirmed in scientific trials.

Colour is a very important characteristic in defining the quality of red wines. Anthocyanins are the compounds responsible for colour in red wines. They are red and purple in colour, and are influenced by pH and the composition of the medium, and the reactions that they participate in during winemaking affect their stability (Brouillard & Delaporte, 1977; Somers & Evans, 1977; Somers & Evans, 1979). Generally it is accepted that after fermentation, when co-pigmentation assists in raising red colour intensity, the monomeric anthocyanin content decreases by various degradation processes, including oxidation and polymerization with flavan-3-ols, while SO_2 bleaching serves to lessen the colour impact of anthocyanins (Boulton, 2001; Somers et al., 1977; Somers et al., 1979).

Processes in winemaking that enhance colour stability are important to winemakers. Colour stability is improved by minimizing anthocyanin bleaching and by favouring the early formation of stable polymeric pigments that are not susceptible to SO_2 bleaching. Some authors (Boulton, 2001; Brouillard, Mazza, Saad, Albrecht-Gary & Cheminat, 1989; Escribano-Bailon, Dangles & Brouillard, 1996; Liao & Haslam, 1992) have suggested that co-pigmentation of anthocyanins is the first stage towards the formation of more stable polymeric anthocyanins, although the first stage is a readily reversible non-covalent association and the second step involves direct covalent bond making.

The initial co-pigmentation phenomena involve the anthocyanin flavylium cation combining with other anthocyanins (self association) or with colourless phenolic compounds present in the medium (benzoic and cinnamic acid derivatives, tannins, flavones and flavonoids). These interact

via the planar π-electron rich flavylium nucleus of the phenolic structure and form non-covalent bonds upon hydrophobic stacking (Boulton, 2001; Brouillard et al., 1977; Brouillard et al., 1989; Mazza & Brouillard, 1990). Thus the colour is enhanced in intensity (a hyperchromic effect) and the wavelength of maximum absorption shifts to a more bluish hue (a bathochromic shift). Caffeic acid and ferulic acid have been identified as strong co-factors whilst epicatechin and catechin are thought to be weaker co-factors (Eiro & Heinonen, 2002). There have been a number of investigations of co-pigmentation involving the addition of potential co-factors individually to wine and anthocyanin solutions (Baranac, Petranovic & Dimitric-Markovic, 1996; Gomez- Miguez, Gonzalez-Manzano, Escribano-Bailon, Heredia & Santos-Buelga, 2006; Liao et al., 1992; Schwarz, Picazo-Bacete, Winterhalter & Hermosin-Gutierrez, 2005).

More permanent colour stability is derived from the combination of anthocyanins with other polyphenols, including acetaldehyde mediated condensation processes between anthocyanins and catechins (Escribano-Bailon, Alvarez-Garcia, Rivas-Gonzalo, Heredia & Santos-Buelga, 2001), and the pathway involving flavanols and anthocyanins bridged by acetaldehyde is well documented (Alcade-Eon, Boido, Carrau & Rivas- Gonzalo, 2006a; Alcalde-Eon, Escribano-Bailon, Santos-Buelga & Rivas-Gonzalo, 2006b; Duenas, Salas, Cheynier, Dangles & Fulcrand, 2006; Saucier, Little & Glories, 1997; Saucier, Lopes, Mirabel, Guerra & Glories, 2004). In one model solution study of malvidin-3-glucoside and epicatechin, noticeable co-pigmentation was followed by the formation of new acetaldehyde bridged pigment structures after a few months (Mirabel, Saucier, Guerra & Glories, 1999). The colour of these new pigments was less sensitive to pH changes than the initial anthocyanins leading to increased colour stability in aged red wines (Escribano-Bailon et al., 1996).

Lee, Swinny, Asenstorfer & Jones (2004) suggest that the relatively unstable short lived ethyl bridged pigment dimmers undergo cleavage of the ethyl link to give reactive intermediates in the formation of more stable red wine pigments such as vitisin like compounds. It is further suggested that the products of this, e.g. the pyranoanthocyanin Vitisin A, which are resistant to pH (hydration) and bisulfite bleaching but are also stable over time, lead to the changes in wine colour with age (Lee et al., 2004; Sarni-Manchado, Fulcrand, Souquet, Ceynier & Moutounet, 1996). The purple expression of colour changes to more brick red colouration and there is an over all increase in colour stability.

In an attempt to advance knowledge of colour development in Syrah wines and in co-ferments with Viognier, the purpose of this present work is to study co-fermentation winemaking and its impact on wine colour, co-pigmentation and colour stability in the wines produced. The co-pigmentation analysis methods of Levengood and Boulton (2004) have been applied, along with HPLC quantification of monomeric anthocyanins, and the polymerized pigment/tannin measures given by the procedure of Harbertson, Picciotto, & Adams (2003).

	Nomenclature
A	Colour due to monomeric anthocyanin
ADWY	Active Dried Wine Yeast
BSA	Bovine Serum Albumin
CA	Colour due to Co-pigmented anthocyanin
CD	Colour density
HPLC	High Performance Liquid Chromatography
LPP	Colour due to Large Polymeric Pigment
MP	Colour due to Monomeric Pigment
PA	Colour due to Polymeric Anthocyanin
S	Control Trial Syrah only
SDS	Sodium Dodecyl Sulfate
SPP	Colour due to Small Polymeric Pigment
SV2	Co-fermentation Trial Syrah + 2% Viognier
SV5	Co-fermentation Trial Syrah + 5% Viognier
SV10	Co-fermentation Trial Syrah + 10% Viognier
SVJ	Co-fermentation Trial Syrah + 10% Viognier equivalent juice only
SVM	Co-fermentation Trial Syrah + 10% Viognier equivalent marc only
TEA	Triethanolamine
TFA	Trifluoroacetic acid

MATERIALS AND METHODS

Grapes and Winemaking procedure

Microvinification trials in triplicate were carried out using clean disease free hand harvested *Vitis vinifera* L. Syrah and Viognier grapes supplied by Trinity Hill Estate from fruit harvested on adjacent company vineyards on the Gimblett Gravels, Hawkes Bay, New Zealand. The harvest dates were 23 March and 6 April 2006, and the maturity levels were 23.7 °Brix and 23.3 °Brix

respectively. Viognier whole bunches were stored at -1°C under cover for the period of time from harvest until fermentation initiated in trials on 7 April 2006.

Winemaking treatments set up as part of the 2006 trial included a control of Syrah only (S), three further treatments of Syrah co-fermented with 2%, 5% and 10% by weight destemmed and crushed Viognier, (SV2, SV5 and SV10), and two treatments of Syrah co-fermented with marc only and juice only, as obtained from 10% by weight Viognier (SVM and SVJ). One further Viognier wine was fermented on skins identically to red wine making methods (V2006). Trials were conducted on 20 kg microvin fermentations in triplicate (except for V2006 where only one wine was produced). Fermentation was carried out in high density food grade polythene 25 L containers. In addition, four further Syrah and three additional Viognier wines were made in single research scale lots in 2007, using fruit sourced from around the Hawkes Bay, to confirm that the phenolic content of the 2006 wines was typical for these wines.

Hand harvest Syrah was destemmed and crushed into a dairy vat using an Enveneto N15T391 destemmer/crusher, homogenised and adjusted for SO_2 with a 50 mg/kg addition. Viognier was destemmed and crushed with a yield of 36.2 kg. 6 kg of must was set aside and the marc and juice from a light pressing of this was used for the SVM and SVJ treatments. 6 kg Viognier must was added to 54 kg Syrah, homogenised and separated into three equal microvin fermentation vessels for SV10. SV5 and SV2 proceeded likewise with 3.0 kg and 1.2 kg Viognier must, and with further 57 kg and 58.8 kg Syrah batches respectively. The remaining 20 kg Viognier was used for the Viognier only trial. All treatments were inoculated at 0.25 g/Kg ADWY (Anchor N96, *Saccharomyces cerevisiae*). The Brix was adjusted to be equal to that of the highest °Brix trial. Nutrient additions of di-ammonium phosphate (DAP) to 300 mg/L in 3 equal steps were made after fermentation started, 1/3 then ½ ways through fermentation. Yeast Superfood at 35 mg/L was added with the first DAP addition. Fermentations were maintained in a heated warm room at 26 to 28°C, plunged twice daily with °Brix, temperature and sensory monitored each time. The total fermentation and maceration time was 15 days. Malolactic fermentation (MLF) culture (Ch Hansen Viniflora oenos, *Oenococcus oenos*) was used to inoculate for MLF as primary fermentation approached dryness. Ferments were drained and pressed off at day 15 using 1.5 bar pressure with a Pillan water bag press. The press and free run fractions were then combined. No oak treatment and no protein fining agents were used. Post malolactic fermentation a level of 25 mg/L free SO_2 was maintained (Aspiration Method). Wines were stored at ambient temperature without ullage in sealed plastic until bottling under screw cap in December 2006.

Analytical methods

Spectrophotometric analyses

A Beckman DU 520 general purpose UV/Vis Spectrophotometer was used for all spectral measures. The % contribution of anthocyanin (A), co-pigmented anthocyanin (CA) and polymeric anthocyanin (PA) were determined following the method of Levengood and Boulton (2004) which is in turn based on the colour measures of Somers and Evans (1977), including the colour density, being the sum of the absorbance values at 520 and 420 nm ($CD = A^{520} + A^{420}$). The preparation of wine samples was standardised so that in all cases a 50 mL sample was centrifuged at 5000 rpm for 3 minutes. The sample was adjusted to pH 3.6 using either 2 M NaOH or HCl as needed followed by 0.45 µm membrane filtering using Sartorius Minisart RC 15 regenerated cellulose single use syringe cartridge filters, prior to the treatment procedures.

The % contribution of monomeric pigment (MP), small polymeric pigment (SPP), long polymeric pigment (LPP) to wine colour and tannin concentrations were determined using the method proposed by Harbertson, Picciotto, & Adams (2003) Bovine serum albumin (BSA No A4503) and (+) –catechin was supplied by Sigma Chemical Co St Louis, MO, USA. Sodium dodecyl sulfate (SDS; lauryl sulfate, sodium salt), triethanolamine (TEA), ferric chloride hexahydrate, potassium metabisulfite were purchased from local laboratory chemical distributors (Merck). Wine was undiluted but adjusted to 25 mg/L free SO_2 prior to the analyses involving BSA tannin precipitation, with centrifuging steps at an Eppendorf Mini Spin Plus. The tannin content was measured in (+)-catechin equivalents.

HPLC analyses

High Performance Liquid Chromatography (HPLC) of monomeric wine polyphenols was undertaken using a modified HPLC method (Ibern-Gomez, Andres-Lacueva, Lamuela-Raventos & Waterhouse, 2002; Kilmartin, Reynolds, Pagay, Nurgel & Johnson, 2007). Standards of catechin, epicatechin, caffeic acid and quercetin, all of HPLC grade, were obtained from Sigma–Aldrich. Malvidin-3-glucoside of purity higher than 98% was supplied by Extrasynthese (Genay, France). A Shimadzu HPLC was used with online DGU-14A degasser, a SIC-10ADVP auto injector, an SPDM20A Prominence PDA set at wavelengths 280 nm for gallic acid and the flavanols, 320 nm for hydroxycinammates, 360 for flavonols and 520 nm for anthocyanins under the control of a SCL-10AVP System controller. The software used was a LC Solutions Version 1.21 SP.1.

Undiluted samples and standards were filtered through 0.45 µm Satorius RC 15 regenerated cellulose filters (Global Science) and 20 µL of each sample was injected. The column was a Gemini 5 µm packing, C18 110 A^{o} 250 x 4.6 mm with two guard cartridges of identical packing in a CTU-10 ASVP column oven set at 30^{o}C. The solvent was run at 1.0 mL/min, the solvents being **A**, water (HPLC filtered grade) with 0.2% trifluoroacetic acid (TFA 100% BDH Hiper Solvent for HPLC) and solvent **B**, acetonitrile (Merck gradient grade UN 1648 KGaA Dawnstadt, Germany) with 0.2% TFA.

A gradient elution profile with linear gradients between time points was as follows: 10 to 20% B for 12 min, 20 to 40% B for 10 min, 40 to 100% B for 3 min, isocratic 100% B for 9 min, 100 to 10% B for 1 min and isocratic 10% B for 10 min, followed by a cleanup method of 65% acetonitrile running at 0.05 mL/min for 20 min. Stock solutions of standards were made up in 10% ethanol, being catechin (2.0 mg in 10 mL), caffeic acid (2.5 mg in 50 mL), quercetin (1 mg in 10 mL) and malvidin-3-glucoside (1.3 mg in 5 mL). Identification of the phenolic compounds by HPLC is detailed in Table 1.

Statistical analysis

Statistical analysis of variation ANOVA was conducted using Minitab 15.1.0.0. Significant (p<0.05) differences in colorimetric and HPLC wine composition measurements between the means of 3 replicates were identified using Tukeys procedure. Correlation significances (p<0.05) were identified using Pearsons Correlation.

Table 1
Principal phenolic compounds identified in this study by HPLC[a]

Phenolic Compound	***Peak No***	***Retention Time***	***Reference***
Anthocyanins			
Delphinidin-3-glucoside	1	12.4	(Lamuela-Raventos & Waterhouse, 1994)
Cyanidin-3-glucoside	2	14.1	
Petunidin-3-glucoside	3	14.9	(Ibern-Gomez et al., 2002)
Peonidin-3-glucoside	4	16.6	(Wulf & Nagel, 1978)
Malvidin-3-glucoside	5	17.3	Standard
Malvidin-(6-acetyl)-3-glucoside	6	20.8	(Ibern-Gomez et al., 2002)
Malvidin-(6-coumaroyl)-3-glucoside	7	22.8	(Lamuela-Raventos et al., 1994)
Benzoic acids/ Flavan-3-ols			
Gallic Acid	8	5.1	(Ibern-Gomez et al., 2002)
Catechin	9	11.4	Standard
Epicatechin	10	13.9	Standard
Hydroxycinnamates			
Caftaric Acid	11	8.7	(Ibern-Gomez et al., 2002)
2-glutathionyl caftaric acid	12	9.2	(Somers, Verette & Pocock, 1987)
Caffeic Acid	13	13.8	Standard
Flavonols			
Quercetin-3-glucoside	14	19.9	(Ibern-Gomez et al., 2002)
Quercetin	15	26.5	Standard

[a] Standards identified in spiked model wines. Reference identification by measuring/observing variation in UV-Vis absorbance maxima at specific retention times after relative elution orders were determined from literature data.

RESULTS AND DISCUSSION

Spectrographic A,CA, PA and CD

Changes in Colour Density (CD) that took place over the first 42 days of the trials for the Syrah only and co-ferments of Syrah with Viognier are presented in Figure 1. An overall decline in colour density was seen over the first 42 days of the trial, but in an uneven pattern, with higher readings obtained on days 6 and 12 (alcoholic fermentation was complete by day 8, and the wine was pressed off skins on day 15). A progressive lowering of colour density was seen due to the dilution effect of added Viognier in the SV2, SV5 and SV10 trials. On the other hand, very good agreement was obtained for the colour measures across the triplicate ferments.

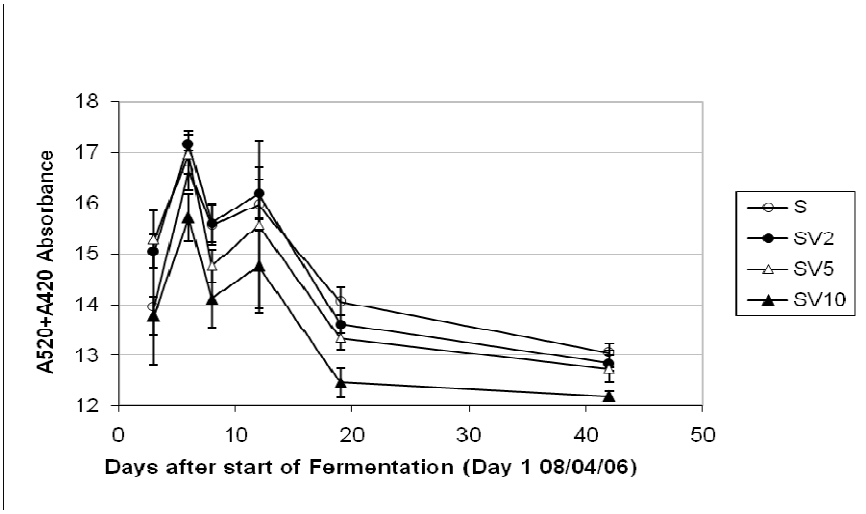

Figure 1.
Wine Colour Density recorded during the first 42 days of the 2006 trials. CD is measured as $A^{520} + A^{420}$ (Somers et al., 1977). Error bars are given for the standard deviation of each set of trial wines (n=3).

HPLC analysis of total monomeric anthocyanins (including the 'five' major glucosides plus the acylated and coumaroylated malvidin peaks) was carried out and the results are presented in Figure 2. Again a lower anthocyanin concentrations was seen in the treatments co-fermented with Viognier owing to the dilution affect of the added white wine, all seen against a backdrop of declining monomeric anthocyanin concentrations. The rate of anthocyanin loss occurred at a greater rate than the loss of wine colour seen in Figure 1, suggesting that the anthocyanins were not being degraded only to colourless forms, but were being converted to other coloured species such as the pigmented polymers. Using a similar line of reasoning to that described by Hayasaka and Kennedy (2003) an estimation of polymeric pigmented material and monomeric pigmented material can be made for the time period 7-22 days. This is based on areas of resolved peaks at 520 nm, representing monomeric pigments, and the unresolved base line hump at 520 nm, being a representation of polymeric pigments. The estimate of polymeric pigment is made by subtracting the resolved peak areas from the total elution peak area.

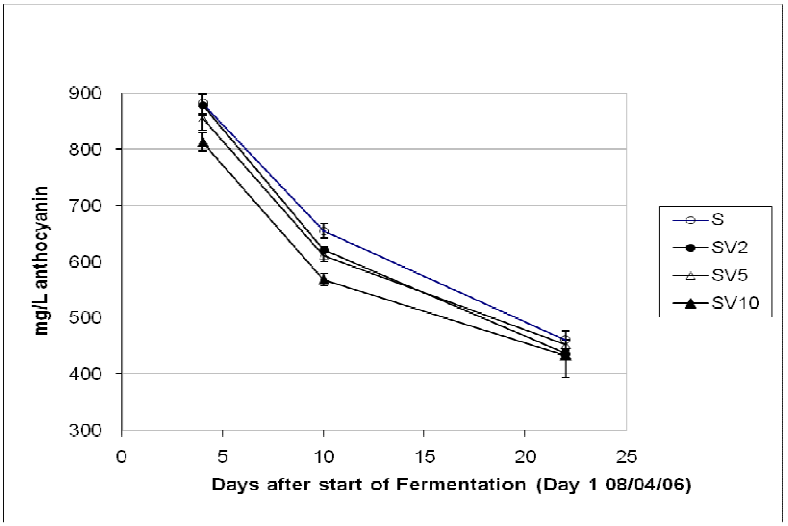

Figure 2
Wine anthocyanin concentration recorded during the first 22 days of the 2006 trials, determined by HPLC and calculated as mg/L malvidin-3-glucoside equivalents. Error bars are given for the standard deviation of each set of trial wines (n=3).

When this is carried out on the HPLC data for analyses on days 7 and 22, the polymeric pigment concentrations for the SV10 treatment can be estimated as: Day 7, 79 ± 0.4 mg/L, and Day 22, 111 ± 2.2 mg/L (malvidin-3- glucoside equivalents). However, by day 22 the anthocyanin concentrations were not significantly different across the treatments, although the SV10 value remained the lowest through to day 134.

It is of interest that during the first 19 days of maceration and immediately post pressing, alternating higher and lower CD values were obtained at the sampling dates for all of the treatments (Figure 1). These were linked closely to the relative A, CA, and PA percentage colour contributions, with a higher colour density obtained when co-pigmentation (CA) was more prominent.

Figures 3A and 3B show the trends in percent contribution to total colour from A, CA and PA during this time for the control, S, and the 10% Viognier treatment (SV10). At day 3 all trials showed higher levels of colour due to A than that due to CA, and low polymeric PA colour. While co-pigmentation was significantly higher ($p < 0.01$) in SV5 (43% (±4)) compared to S (36% (±2)) at this point, the SV10 treatments had similar levels of colour due to CA (37% (±2)) compared with the control S, a trend which continued across the trial.

The further incorporation of A into more densely coloured CA by day 6 resulted in an overall increase in CD, and in some cases, CA was twice that of A. The alcohol level was still relatively low and in this medium the CA are expected to be more stable than in the higher alcohol environments as fermentation proceeded (Boulton, 2001). Anthocyanins were still being extracted and self-association of anthocyanin species was expected to dominate as the extraction of further phenolic co-factors would not yet have reached a maximum (Ribereau-Gayon, 1974).

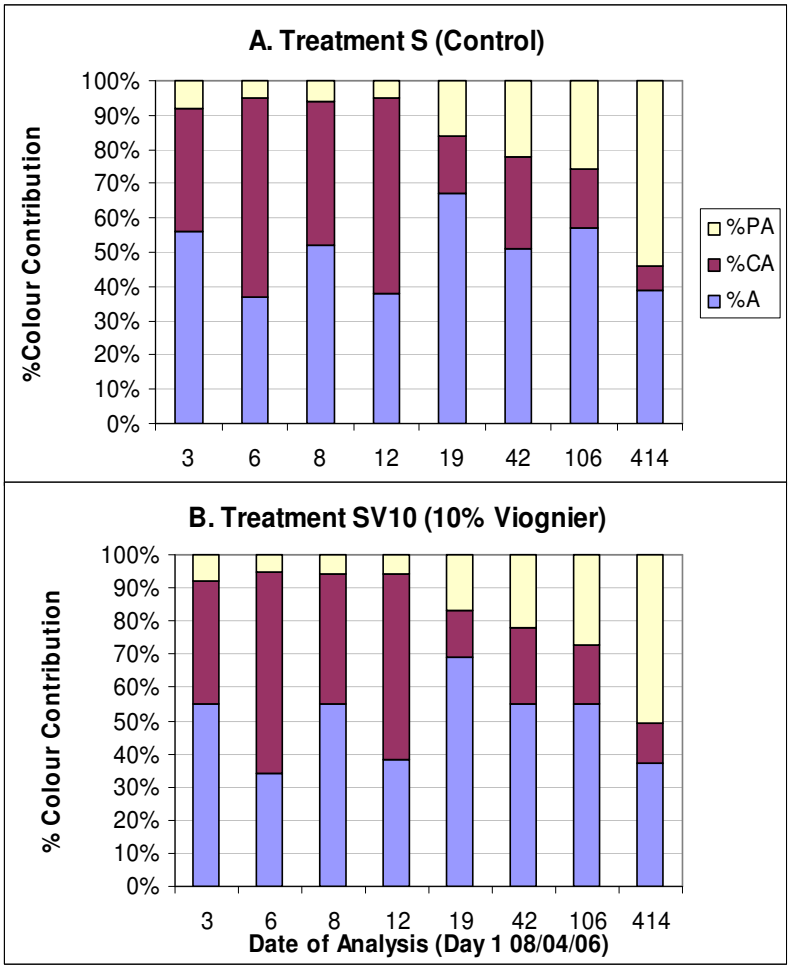

Figure 3
*Percentage of Total Colour in wines due to Monomeric Anthocyanins (A), Co-pigmented Anthocyanins (CA) and Polymeric Anthocyanins (PA) in Control Treatment Syrah S (**A**), and in 10% Viognier Treatment SV10 (**B**).*

By day 8 the alcoholic fermentation was complete, and CA (39-46%) was less stable at the higher alcohol levels, giving some reversion back to the less intensely coloured A (49-53%) forms (Hermosin Gutierrez, Sanchez-Palomo Lorenzo & Vicario Espinosa, 2005). This was seen in the overall lower CD and CA results (Figures 1, 3A and 3B)

During the higher alcoholic maceration time from day 8 to 12, low molecular weight polyphenols (potential co-factors) are expected to be extracted at relatively higher rates than anthocyanins (Ribereau-Gayon, 1974). By day 12 the fraction of colour due to CA (46-59%) had again become the major contributor to increased CD (Day 8: 14-15.8, Day 12: 14.8-16.8 AU) in all trials agreeing with the findings of Lorenzo, Pardo, Zalacain, Alonso, & Salinas (2005). Anthocyanins are expected to continue to be extracted (albeit more slowly than earlier in the fermentation) and to undergo co-pigmentation in the form of co-factor-anthocyanin structures, but more slowly than earlier at lower alcohol concentrations. Boulton (2001) suggests that continued co-pigmentation allows extraction of more anthocyanins over this period of time.

After day 12 the CA colour fraction began to decrease in all trials resulting in a corresponding lower CD, while the PA colour fraction began to increase (Figures 3A and 3B). The decrease in CD was due to a lowering of the CA contribution to colour and an increase in the PA contribution (Brouillard et al., 1977). The fraction of colour provided by A (Day 19, 62-70%) became the major contributor to the young wines' colour at this point as the co-pigments were beginning to dissociate (CA Day 19, 13-22%). At day 42 the % PA (Day 12, 5.2-5.8%, Day 42, 21.4-22.8%) colour contribution had increased further and the CD (Day 12, 14.8-16.8, Day 42, 12.2-13.1AU) continued to decrease, while the % CA colour for all trials in fact increased a little compared to day 19.

By day 414 PA was the dominant provider of colour (about 50%), and while the polymeric pigments are more stable and less susceptible to bleaching, they are expected to be less colored than A and CA (Boulton, 2001; Brouillard & Dangles, 1994; Hermosin Gutierrez et al., 2005). Note that the % colour due to A, CA and PA is not a measure quantitatively of the concentrations of each of these pigment types as each species exhibits different colour densities, both through photochromic and bathochromic shifts (CA > A > PA) (Boulton 2001). In other words, for any given percentage contribution to colour it would take more PA to make this contribution than A or CA. Overall there was a strong negative correlation between CD and % PA over the first 106 days of the trials ($p < 0.05$), but this correlation had ceased by day 414 (data not shown here). CD relates to the concentrations of red and yellow/brown pigments. As PA are not as intensely red coloured as A (Mirabel et al., 1999), the observation that as polymerization of pigment took place, a decreased CD, is to be expected.

What was clear from the CA data was that S, the control with no Viognier, was not significantly different to SV2, SV5 and SV10 over the course of the study in these colour parameters. On the other hand, wines that produced higher CA at days 6 and 12, led to wines with higher % PA colour at day 106. This linear trend is shown in Figure 4. These wines appeared to show a faster conversion of CA→PA, and may support a mechanism based upon the process A→ CA→ PA (Mirabel et al., 1999). The treatments with the higher whole berry additions of Viognier during fermentation, SV5 and SV10, tended to show the highest CA levels at days 6 to 12 and fastest ultimate PA formation.

Turning to the SVJ and SVM wines (the Viognier juice only, or Viognier marc only treatments), the trend in higher % CA (on days 6 and 12) and lower % CA (on days 8 and 19) was also observed (Table 2), similar to the results presented in Figure 3. However, the CA values for the SVJ wines did not vary as widely as the SV10 treatments, with the SVM and S treatments giving more intermediate results. The other trials were somewhere in between and closer to SV10, although with these lesser Viognier addition treatments (2 to 5%) the effect was masked by variations between ferments.

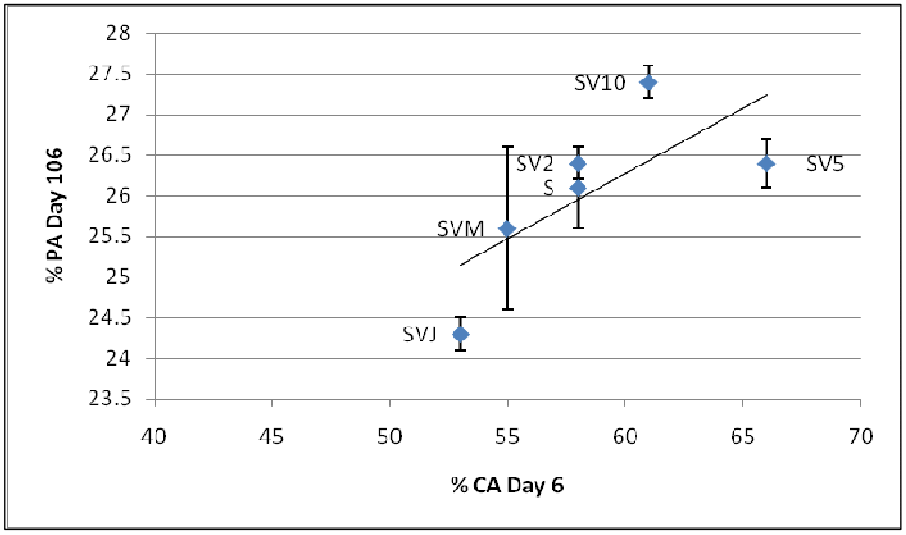

Figure 4
Comparison of % PA colour contribution at day 106 with % CA colour contribution at day 6, for individual trial wines.

It would seem that SVJ had less co-factor and anthocyanin available to form co-pigments than SV10, while in SV10 the % CA declined the most over this time period (6 to 19 days). Co-factors continued to be extracted up until pressing (day 15), allowing co-pigment formation to continue and keep up with losses due to pigment polymerization. With SVJ, co-factor extraction did not continue at as fast a rate as in SV10, and polymerization could more than keep up with co-pigmentation rates, while S, SV2, SV5 and SVM were somewhere in between these two extremes (Table 2). At day 12 the SVJ trial had a significantly lower ($p<0.001$) colour fraction due to CA (46%) than all of the other trials, and a lower PA fraction of colour. This perhaps indicates that the lower availability of potential co-factors was slowing co-pigmentation at this point. Again Boulton suggests that the concentration of co-factors is critical to the formation of CA. The SVJ treatment showed the slowest rate of CA decrease and the lowest PA colour fraction, and this continued to day 42. By day 106 analysis showed significantly higher PA fractions in all trials that contained Viognier skins compared with the SVJ treatment.

Table 2
Trends in percentage colour due to Co-pigmented Anthocyanins (% CA[b]) during the first 19 days.

Trial	Day6	Day 8	Day 12	Day 19 PostP[c]
S	58	42	57 bc[a]	17 ab
SV10	61	39	56 bc	14 a
SVM	55	44	50 ab	15 ab
SVJ	53	46	46 a	22 b
	n.s.	n.s.	p<0.001	p<0.05

[a]Within column values followed by different letters are significantly different at the confidence level indicated.
[b]CA measured as % colour due to Co-pigmented Anthocyanins (Levengood et al., 2004)
[c]PostP Post pressing

Spectrographic MP, SPP, LPP and Tannin

The Adams/Harbertson protein precipitation method for Monomeric Pigment (MP), Short Polymeric Pigment (SPP) and Long Polymeric Pigment (LPP) gave results that reinforced the spectroscopic Boulton analyses described above. Figure 5 shows the trends in percent colour contribution by MP, SPP and LPP during days 26 to 414 for treatments S and SV10. Between days 26 to 69, the treatments with higher levels of Viognier skin contact during co-fermentation, SV5 and SV10 had significantly higher ($p < 0.05$) colour fractions due to LPP than the control S.

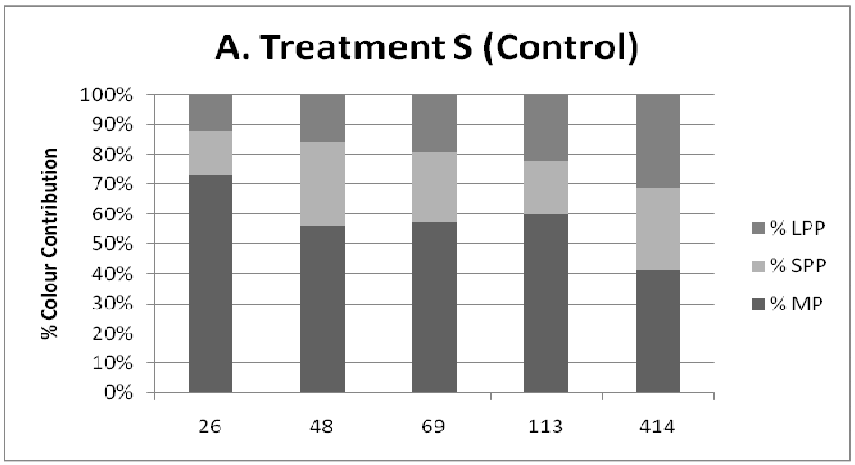

Figure 5
*Percentage of total colour in wines due to Monomeric Pigments (MP), Short Polymeric Pigments (SPP) and Long Polymeric Pigments (LPP) in Control Treatment Syrah S (**A**), and 10% Viognier treatment SV10 (**B**)*

Table 3 shows that SV10 had a significantly higher LPP to tannin ratio than the control (S) during this 26 to 69 day period. Given that the LPP represents a more stable pigment arrangement for colour expression (Escribano-Bailon et al., 1996), SV10 was well advanced in developing stable colour pigmentation. The LPP/Tannin ratio is suggested as a measure of wine astringency and the treatment displaying a higher ratio (SV10) would be expected to be less astringent to the mouth feel than the treatment displaying a lower ratio (S), since grape anthocyanins and polymeric pigments are not typically perceived as bitter or astringent (Gawel, 1998; Singleton & Trousdale, 1992; Vidal, Francis, Noble, Kwiatkowski, Cheynier & Waters, 2004). Again it should be noted that although the difference is measurable it may not be great enough to show up as an astringency difference in sensory analyses.

Trial	Day 26	Day48	Day 69	Day 113	Day 414
S	.027a	.035a	.037a[a]	.052	.070b
SV2	.026a	.037a	.039ab	.052	.062ab
SV5	.039c	.041ab	.038a	.052	.061ab
SV10	.034bc	.045b	.045b	.048	.070b
SVM	.031b	.035a	.039ab	.049	.057a
SVJ	.031b	.038b	.043b	.049	.067b
	P<0.001	P<0.01	P<0.01	n.s.	P<0.01

Table 3
Ratio Long Polymerized Pigment to Tannin (LPP/T)[b] for the 2006 Trial Wines

[a]Within column values followed by different letters are significantly different at the confidence level indicated.
[b]LPP measured as % Colour due to Long Polymerized Pigment AU
[b]T Tannin measured in mg/L catechin equivalent (Harbertson et al., 2003)

By day 48, S and SV2 were beginning to produce more polymeric pigment but mainly in the SPP stage, with 28% (±0.6) and 27% (±0.5) of total colour expression respectively in this form compared to SV10 25% (±0.2) ($p < 0.001$). By day 414 SV10 still had the highest ratio of LPP/SPP and of LPP/Tannin (Table 3). However, the S treatment was also high in these ratios, somewhat unexpectedly. Given that these treatments represent very different Viognier processing methods it seems that 14 months after inoculation, other variables are having more control on the speed of pigment polymerization than any effect the Viognier co-fermentation might have had earlier in the process.

The protein precipitable tannin concentration in the SVM treatment was the highest for all trials at all analysis dates (466 (±24) to 521 (±27) mg/L catechin equivalents). This is the trial that had 10% equivalent Viognier skins and seeds only in the co-fermentation. It appears that the tannin extraction for this trial was greater than the other trials, even compared to SV10 which had an equivalent amount of Viognier skins and seeds but also contained the juice. This is consistent with an explanation that the LPP/Tannin ratio for SVM was lower than for other Viognier containing trials (Table 3), and indicates that the presence of Viognier juice as well as skins means that more

anthocyanin can be extracted because of the higher juice to red skin ratio. As a result more anthocyanin was available for LPP formation, less tannin was extracted (compared to Viognier skins only being present), leading to the LPP/Tannin ratio being higher in the treatments with Viognier juice as well as skins. From this, it could be expected that the wines with whole berry Viognier co-ferments would be less astringent than the co-ferment with Viognier skins only (Gawel, 1998; Singleton et al., 1992; Vidal et al., 2004).

Anthocyanins and phenolics by HPLC

HPLC determination of potential co-factor levels revealed that caffeic acid, caftaric acid, quercetin-3-glucoside and epicatechin concentrations in treatments were on occasions significantly different. However, these differences appeared to be random and not related to Viognier co-fermentation treatments. By day 134 SV10 catechin concentration had dropped from 107 (±3) mg/L on day 22 (Table 3) to 95 (±8) mg/L, compared to S which had increased from 105 (±1) mg/L to 108 (±9) mg/L and after 370 days (33 (±1) mg/L) was significantly lower than S (39 (±1) mg/L). This is consistent with polymerization in SV10 taking place at a faster rate than in S. Apart from SV10; catechin concentrations did not decrease from 22 days to 134 days but had reduced to one third of their 22 day level by 370 days. The steady or even increasing concentration of catechin during days 22 to 34 need not be an indication that they were inactive in polymerization processes. Singleton and Trousdale (1983) explain that monomeric flavan-3-ols can be released from their galloylated precursors and hence polymerization reactions involving catechin could still be occurring even if their concentration seemed to be remaining constant.

Table 4 shows a comparison of the phenolics concentrations in selected 2006 trial wines and in further trial wines produced the following year. It is important to note that the concentrations of the flavonols quercetin and quercetin-3-glucoside were not particularly high in the Viognier samples, and considerably lower than in the Syrah trial wines.

Table 4

Comparison of the 2006 trial wines with selected 2007 trial wines for phenolic concentrations at an equivalent stage (2006 at day 22, 2007 at day 32)

Trial[b]	Catechin[c]	Epicatechin[c]	Caffeic Acid[d]	Quercetin-3-glucoside[e]	Quercetin[e]	Gallic[c] Acid
S 2006	105[a]	157	20 (30)[f]	17	18	181
SV10 2006	107	225	21 (32)	15	17	190
S 2007	185	81	15 (37)	28	36	144
SV10 2007	186	87	11 (33)	26	37	150
V 2006	56	61	1 (19)	5	5	Not Determined
V 2007	38-60	40-78	1 (16-32)	7-15	4-11	Not Determined

[a]All values determined by HPLC. [b]Abreviations S = Syrah only. SV10 = Syrah co-fermented with 10% Viognier. V = Viognier wine (fermented as for red wine trials)
[c]Catechin, epicatechin and gallic Acid calculated as mg/L catechin equivalents.
[d]Caffeic Acid calculated as mg/L caffeic Acid
[e]Quercetin-3-glucoside and quercetin calculated as mg/L quercetin equivalents.
[f]Bracketed smaller font numbers in the caffeic acid column represent the sum of caftaric acid, GRP, and caffeic acid in mg/L caffeic acid equivalents

The flavonols are known to be important compounds for the development of co-pigmentation colour enhancement, and we would only expect a noticeable increase in co-pigment colour if a white grape juice was able to provide high levels of co-factors of this sort to a red wine lacking in these compounds. Instead the Syrah wines in this study had quite high concentrations of flavonol compounds, which raises questions about the origin of any colour enhancement that has been attributed to Syrah/Viogner coferments in the past. It is reasonable to conclude that in both of the years' trials analysed in this study, the Viognier was not a source of extra potential co-

factors for co-fermentation and polymerization of pigment, and instead would have had a diluting effect and decrease potential co-factor concentrations.

In conclusion the Syrah/Viognier co-winemaking trials showed that co-fermentation only leads to minor changes in co-pigmentation and subsequent pigment polymerization. Over the course of the study the control, with Syrah only, was not consistently different in colour enhancement due to co-pigmentation than the co-fermented trials with 2, 5, and 10% Viognier. The treatments with the higher whole berry co-winemaking additions showed the highest % CA colour levels and the fastest rates of % PA and % LPP colour formation, but the differences between treatments were not great. The results showed that SV10, the 10% Viognier trial, during the early stages of winemaking, did express more advanced polymeric pigment levels compared with the control and this process of pigment polymerization does involve the formation of co-pigmented anthocyanin prior to polymerization. However, the existence of a single co-factor in Viognier that would accelerate pigment polymerization in Viognier co-fermented Syrah was not identified in this study, and the concentrations of important co-factor classes, such as the flavonols, were decreased, rather than increased, through the addition of Viognier juice to a Syrah ferment.

Acknowledgements
This research was supported by Trinity Hill Winery, Hawkes Bay, New Zealand, and by The Faculty of Science and Technology, Eastern Institute of Technology, Hawkes Bay, New Zealand.

Literature Cited

Alcade-Eon, C., Boido, E., Carrau, E., D., & Rivas- Gonzalo, C. (2006). Pigment profiles in monovarietal wines produced in Uruguay. *American Journal of Enology and Viticulture*, *57*(4), 449-459.

Alcalde-Eon, C., Escribano-Bailon, T., Santos-Buelga, C., & Rivas-Gonzalo, C. (2006). Changes in the detailed pigment composition of red wine during maturity and ageing A comprehensive study. *Analytica Chimica Acta 563*, 238-254.

Baranac, J. M., Petranovic, N. A., & Dimitric-Markovic, J. M. (1996). Spectrophotometric study of anthocyanin copigmentation reactions. *Journal of Agricultural and Food Chemistry*, *44*, 1333-1336.

Boulton, R. (2001). The co-pigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture*, *52*, 67-87.

Brouillard, R., & Dangles, O. (1994). Anthocyanin molecular interactions: the first step in the formation of new pigments during wine aging? *Food Chemistry*, *51*, 365 371.

Brouillard, R., & Delaporte, B. (1977). Chemistry of anthocyanin pigments. 2. Kinetic and thermodynamic study of proton transfer, hydration, and tautomeric reactions of malvidin 3-glucoside. *Journal of the American Chemistry Society*, *99*, 8461-8468.

Brouillard, R., Mazza, G., Saad, Z., Albrecht-Gary, A. M., & Cheminat, A. (1989). The copigmentation reaction of anthocyanins: A microprobe for the stuctural study of aqueous solutions. *Journal of The American Chemical Society*, *111*, 2604-2610.

Duenas, M., Salas, E., Cheynier, V., Dangles, O., & Fulcrand, H. (2006). UV-Visible spectroscopic investigation of the 8, 8-methylmethine catechin-malvidin 3-glucocide pigments in aqueous solution: Structural transformations and molecular complexation with chlorogenic acid. *Journal of Agricultural and Food Chemistry*, *54*, 189-196.

Eiro, M. J., & Heinonen, M. (2002). Anthocyanin color behaviour and stability during storage: Effect of intermolecular copigmentation. *Journal of Agricultural and Food Chemistry*, *50*, 7461-7466.

Escribano-Bailon, T., Alvarez-Garcia, M., Rivas-Gonzalo, J. C., Heredia, F. J., & Santos-Buelga, C. (2001). Color and stability of pigments derived from the acetaldehyde-mediated condensation between Malvidin 3-*O*- glucoside and (+)-catechin. *Journal of Agricultural and Food Chemistry*, *49*, 1213-1217.

Escribano-Bailon, T., Dangles, O., & Brouillard, R. (1996). Coupling reactions between flavylium ions and catechin. *Phytochemistry.*, *41*, 1583-1592.

Gawel, R. (1998). Red wine astringency: a review. *Australian Journal of Grape and Wine Research*, *4*, 74-95.

Gomez- Miguez, M., Gonzalez-Manzano, S., Escribano-Bailon, M. T., Heredia, F. J., & Santos-Buelga, C. (2006). Influence of different phenolic co-pigments on the color of malvidin 3-glucoside. *Journal of Agricultural and Food Chemistry*, *54*, 5422-5429.

Harbertson, J. F., Picciotto, E. A., & Adams, D. O. (2003). Measurement of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. *American Journal of Enology and Viticulture*, *54*, 301-306.

Hayasaka, Y., & Kennedy, J. A. (2003). Mass spectrometric evidence for the formation of pigmented polymers in red wine. *Australian Journal of Grape and Wine Research*, *9*, 210-220.

Hermosin Gutierrez, I., Sanchez-Palomo Lorenzo, E., & Vicario Espinosa, A. (2005). Phenolic composition and magnitude of copigmentation in young and shortly aged red wines made from the cultivars, Cabernet Sauvignon, Cencibel, and Syrah. *Food Chemistry*, *92*, 269-283.

Ibern-Gomez, M., Andres-Lacueva, C., Lamuela-Raventos, R. M., & Waterhouse, A. L. (2002). Rapid HPLC analysis of phenolic compounds in red wines. *American Journal of Enology and Viticulture*, *53*(3), 218-221.

Kilmartin, P. A., Reynolds, A. G., Pagay, V., Nurgel, C., & Johnson, R. (2007). Polyphenol content and browning of Canadian Icewines. *Journal of Food Agriculture and the Environment*., *5*, 52-57.

Lamuela-Raventos, R. M., & Waterhouse, A. L. (1994). A direct HPLC separation of wine phenolics. *American Journal of Enology and Viticulture*, *45*, 1-5.

Lee, D. L., Swinny, E. E., Asenstorfer, R. E., & Jones, G. P. (2004). Factors affecting the rate of red wine pigments. In: A. L. Waterhouse, & Kennedy, J. A., *Red wine color: Revealing the mysteries.* (pp. 125-142). Washington, DC.: American Chemical Society.

Levengood, J., & Boulton, R. (2004). The variation in color due to copigmentation in young cabernet sauvignon wines. In: A. L. Waterhouse, & Kennedy, J. A., *Red wine color: Revealing the mysteries* (pp. 35-52). Washington, DC: American Chemical Society.

Liao, H., & Haslam, E. (1992). Polyphenol interactions. Anthocyanins: Co-pigmentation and color changes in red wines. *Journal of the Science of Food and Agriculture*, *59*, 299-305.

Lorenzo, C., Pardo, F., Zalacain, A., Alonso, G. L., & Salinas, M. R. (2005). Effect of red grapes co-winemaking in polyphenols and color of wines. *Journal of Agricultural and Food Chemistry*, *53*, 7609-7616.

Mazza, G., & Brouillard, R. (1990). The mechanism of copigmentation of anthocyanins in aqueous solutions. *Phytochemistry*., *29*, 1097-1102.

Mirabel, M., Saucier, C., Guerra, C., & Glories, Y. (1999). Copigmentation in model wine solutions: Occurrence and relation to wine aging. *American Journal of Enology and Viticulture*, *50*, 211-218.

Ribereau-Gayon, P. (1974). Anthocyanins of grapes and wines. In: P. Markakis, *Anthocyanins as food colors* (pp. 209-244). New York: Academic Press.

Sarni-Manchado, P., Fulcrand, H., Souquet, J.-M., Ceynier, V., & Moutounet, M. (1996). Stability and color of unreported wine anthocyanin-derived pigments. *Journal of Food Science*, *61*, 938-941.

Saucier, C., Little, D., & Glories, Y. (1997). First evidence of acetaldehyde-flavonol condensation products in red wine. *American Journal of Enology and Viticulture*, *48*, 369-373.

Saucier, C., Lopes, P., Mirabel, M., Guerra, C., & Glories, Y. (2004). Tannin-anthocyanin interactions: Influence on wine color. In: A. L. Waterhouse, & Kennedy, J. A., *Red wine color: Revealing the mysteries.* (pp. 265-274). Washington, DC.: American Chemical Society.

Schwarz, M., Picazo-Bacete, J. J., Winterhalter, P., & Hermosin-Gutierrez, I. (2005). Effects of copigments and grape cultivar on the color of red wines fermented after addition of copigments. *Journal of Agricultural and Food Chemistry*, *53*, 8372-8381.

Singleton, V. L., & Trousdale, E. K. (1983). White wine phenolics: Varietal and processing differences as shown by HPLC. *American Journal of Enology and Viticulture*, *34*, 27-34.

Singleton, V. L., & Trousdale, E. K. (1992). Anthocyanin-tannin interactions explaining differences in polymeric phenols between white and red wines. *American Journal of Enology and Viticulture*, *43*, 63-70.

Somers, T. C., & Evans, M. E. (1977). Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO2, "chemical age.". *Journal of Agricultural and Food Chemistry*, *28*, 279-287.

Somers, T. C., & Evans, M. E. (1979). Grape pigment phenomena: Interpretation of major colorloss during vinification. *Journal of Agricultural and Food Chemistry*, *30*, 623-633.

Somers, T. C., Verette, E., & Pocock, K. F. (1987). Hydroxycinnamate esters of *Vitis vinfera*: Changes during white vinification, and effects of exogenous enzymic hydrolysis. *Journal of the Science of Food and Agriculture*, *40*, 67-87.

Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V., & Waters, E. (2004). Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Analitica Chimica Acta*, *513*, 57-65.

Wulf, L. W., & Nagel, C. W. (1978). High -pressure liquid chromatographic separation of anthocyanins of vitis vinifera. *American Journal of Enology and Viticulture*, *29*, 42-49.