Response of Grape and Wine Phenolic Composition in *Vitis vinifera* L. cv. Merlot to Variation in Grapevine Water Status

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Grape and wine phenolic composition was monitored over two consecutive seasons in *Vitis vinifera* cv. Merlot following application of irrigation treatments that produced seasonal average stem water potentials ranging between -0.7 MPa and -1.4 MPa. Fresh weight of berries was significantly reduced in response to water deficit, primarily due to decreases in pericarp weight. Increases in the concentration of grape anthocyanins and flavonols in response to water deficit were found when expressed per unit grape berry fresh weight. Skin-derived tannin concentration in grape berries was not affected by the irrigation treatments. The concentration of grape-derived phenolics was monitored during five days of fermentation in a small-lot winemaking experiment. During fermentation, the concentration of anthocyanins and flavonols in wine were highest in the non-irrigated and low-frequency-irrigated treatments, which was reflected in changes in the wine colour of ferments. Finished wines from non-irrigated and low frequency irrigated grapevines showed increases in bisulphite-resistant pigments when compared with those irrigated at a high frequency, but differences in phenolic composition were minor. Increases in bisulphite-resistant pigments were associated with increases in vitisin A and polymeric pigment in the first and second seasons of the study respectively. Ageing of wines for an 18-month period increased bisulphite-resistant pigments, and treatment differences in wine colour density were enhanced, such that increases in both parameters were associated with the non-irrigated and low-frequency-irrigated treatments.

INTRODUCTION

The application of water deficit to grapevines has long been known to affect the development of the grape berry, causing a restriction in pericarp expansion with the production of smaller berries (Roby & Matthews, 2003). This alteration in berry size has been suggested to be due to a limitation in cell wall flexibility, which prevents cell expansion post-veraison, the period during which there is uptake of both water and solutes into grape berry cells (Ojeda *et al*., 1999; Ojeda *et al*., 2001). Studies on the influence of grapevine water deficit on the concentration of skin phenolics in grape berries have produced variable results. Generally, increases in anthocyanin concentration have been observed in berry skins in response to water deficit, as a result of increased anthocyanin biosynthesis, which occurs when the deficit is applied pre-veraison (Castellarin *et al*., 2007). The biosynthetic pathways of other skin phenolics such as tannins (proanthocyanidin or condensed tannin) and flavonols have been shown to be irresponsive to the application of water deficit either pre- or post-veraison (Castellarin *et al*., 2007). Water-deficit application to grapevines therefore frequently results in either minor or no response in terms of grape berry skin tannin (Kennedy *et al.,* 2002; Ojeda *et al.,* 2002; Peterlunger *et al.,* 2005; Kondouras *et al.*, 2009). Where increases in the concentration of skin tannin as a function of berry weight have been observed in response to water deficit, it was evident that this was driven primarily by a restriction in the mesocarp and exocarp, rather than altered biosynthesis (Roby *et al*., 2004). Grape-derived flavonols have been found to either decrease (Kennedy *et al.,* 2002) or increase (Ojeda *et al.,* 2002) in response to water deficit.

The translation of water-deficit-induced changes in berry phenolic composition to wine composition has received limited attention in research. There is evidence that the application of water deficit can significantly affect wine composition with regard to wine colour and sensory properties, with increases in the contribution of bisulphite-resistant pigments to wine colour and increases in 'fruity' aroma notes (Chapman *et al*., 2005; Peterlunger *et al*., 2005; Bindon *et al*., 2008; Chalmers *et al*., 2008). Wine phenolic concentration and composition are strongly influenced by differences in fermentation and winemaking processes, which make comparison between experimental studies difficult. Furthermore, the complexity of the fermentation, winemaking and ageing processes means that the value of grape berry phenolic composition as a marker for

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finished wine phenolics is limited. Some studies have shown minor differences in the composition of berry tannin in response to water deficit, but following vinification, significant increases in wine tannin were found in a water-deficit treatment (Kennedy *et al.,* 2002; Peterlunger *et al*., 2005). These increases in wine tannin in response to water deficit were also associated with increases in wine anthocyanin and flavonols. It is evident from this that the extraction and post-vinification stability of these grape-derived phenolics play a significant role in determining wine composition in addition to berry phenolic composition itself.

In relation to wine colour, the development of stable anthocyanin derivatives such as polymeric pigments and pyranoanthocyanins (e.g. vitisins) is an important stage in ensuring the maintenance of colour during wine ageing (Bakker & Timberlake, 1997; Peng *et al*., 2002; Hayasaka & Kennedy, 2003; Fulcrand *et al.,* 2006; Monagas *et al.,* 2006). There is evidence that altered berry composition resulting from e.g. vigour differences within vineyards can have a significant effect on the development of wine composition and colour. Grapes sourced from low-vigour *Vitis vinifera* cv. Pinot noir vines were shown to have higher levels of skin-derived tannin and anthocyanin than berries from high-vigour vines, but the concentration of seed-derived tannin was unaffected by vigour (Cortell *et al*., 2007 a, b). In that study, regression analysis of wine composition showed a strong relationship between polymeric pigment in wines and measured wine colour density, and increased wine pigmented polymer concentration was positively associated with increased tannin concentration in the berries. Although vigour was not directly associated with water deficit, the low-vigour grapevines produced consistently smaller berries than higher-vigour grapevines (Cortell *et al*., 2007 a, b). However, the question of berry size in relation to wine composition has been shown to produce inconclusive results, such that the relationship between berry size and wine phenolic composition remains unclear (Walker *et al*., 2005; Holt *et al*., 2008). It is therefore necessary to further explore the relationship between fruit and wine composition in relation to specific vineyard parameters.

The aim of the current study was to track the changes in *Vitis vinifera* cv. Merlot fruit phenolic composition produced in response to irrigation treatments to wine phenolic composition for two consecutive seasons. In grapes, the skin anthocyanin, tannin and flavonol concentration and composition was determined by reverse-phase high-performance liquid chromatography (RP-HPLC) following extraction in 50% (v/v) ethanol. In wines, the concentration and composition of anthocyanins, flavonols and tannin during fermentation was monitored for a single season, with a specific focus on exploring the formation of polymeric pigments in both the winemaking and ageing processes. Finally, a more detailed statistical analysis of wine colour and phenolic composition in six-month- and 18-month-aged wines was performed. This is a preliminary study which addresses the broad changes in grape and wine phenolic composition in relation to grapevine water deficit and provides some insight into the factors which might potentially drive the development and maintenance of wine colour.

MATERIALS AND METHODS

Irrigation treatments

The experimental site and design have been published in detail (Myburgh, 2010a). The experimental site was a 15-year-old Merlot/99 Richter vineyard near Wellington in the Western Cape region of South Africa. For the current study, only selected treatments of that experiment were included, and the total irrigation volumes applied during the respective seasons are shown in Table 1. In the 2005/2006 season, a non-irrigated control (T1) was compared to a low-frequency-irrigation treatment (T2) which had 30 mm of irrigation water applied at pea size and véraison, respectively. Grapevines of the T3 treatment were irrigated at a higher frequency, *i.e.* 27 mm irrigation applied at pea size, mid-December, véraison and mid-January, respectively. A further irrigation strategy was also included whereby irrigation was applied *via* subsurface dripper lines in the work rows (T6). For that treatment, a set of alternating work rows received 7 mm irrigation twice a week in the 2005/06 season (Myburgh, 2010a). In the 2006/2007 season, treatments T1, T2 and T6 was as for the previous season, whereas T3 and an additional treatment (T5) received a total of 340 mm of irrigation applied twice a week in the grapevine rows and in the middle of the work rows, respectively. The objective of this irrigation strategy was to maintain the soil water content at 25% plant available water (PAW) depletion (Myburgh, 2010a). A second additional treatment (T4) was included whereby the same irrigation amounts as T2 were applied in the middle of the work rows. All treatments were replicated four times in a randomised block design.

Grapevine water status

Plant water status of the treatments was monitored by stem water potential (Ψ_S) measurements taken at midday, using the pressure chamber technique as outlined in Myburgh (2010a). For each replicate, a fully expanded leaf was chosen from a main shoot, and was covered by a light-reflecting foil bag for an hour prior to measurement of Ψ_S. At midday, selected leaves were detached from the shoot by cutting through the base of the petiole and immediate insertion into the pressure chamber. Water potential readings were recorded when sap was first observed to exude from the cut end of the petiole. Readings of Ψ_S were averaged for the season.

Sample preparation

Two samples of 50 grape berries per replicate were sampled at harvest. The first, fresh sample was lightly pressed by hand in a small plastic bag and the clarified juice used to determine juice total soluble solids (TSS as °B) using an electronic refractometer (Pocket PAL-1, Atago U.S.A. Inc., Bellevue, WA, U.S.A.), and pH at 20˚C (Crison Basic 20, Crison Instruments SA., Barcelona, Spain). For the measurement of titratable acidity (TA) the fresh juice was diluted 1:4 (v/v) in distilled water and frozen at -20°C prior to analysis. Sample dilution before freezing prevents precipitation of acid-salts upon defrosting. TA was measured in defrosted samples at 20°C using an autotitrator (Metrohm 785 DMP Tritino, Metrohm AG, Herisau, Switzerland). The second fifty-berry sample was frozen at -80°C for the later analysis of skin phenolics. Prior to phenolic analysis, frozen berries were weighed and then peeled, with the weight of removed skins and seeds recorded. An estimate of flesh weight was determined by

TABLE 1
Season average stem water potential Ψ_s, berry weight parameters and grape juice composition in response to irrigation treatments T1 to T3 and T6 for the 2005/2006 season and T1 to T6 for the 2006/2007 season (ANOVA; n = 16 in 2005/2006; n = 24 in 2006/2007; ns = not significant; nd = not determined; means followed by different letters in the same row indicate significant differences according to * P=<0.05; ** P = < 0.01; *** P = < 0.001). All samples show comparison at commercial harvest.

	T1	T2	T3	T4	T5	T6	P
2005/2006 season							
Irrigation volume applied (m^3)	0	890	1334			2490	nd
Average Ψ_s (MPa)	-1.44^a	-1.24^b	-0.94^c	-	-	-0.83^d	**
Berry weight (g)	0.90^a	1.02^a	1.18^b	-	-	1.37^c	**
Skin:flesh ratio (F. wt)	0.281^a	0.252^{ab}	0.224^b	-	-	0.191^c	***
TSS (°Brix)	25.75	26.35	25.65	-	-	24.22	ns
Juice pH	3.33	3.42	3.42	-	-	3.47	ns
TA (g/L)	5.40	5.14	5.00	-	-	5.10	ns
2006/2007 season							
Irrigation volume applied (m^3)	0	961	3667	961	3667	2392	nd
Average Ψ_s (MPa)	-1.39^a	-1.33^a	-0.67^c	-1.23^a	-0.69^c	-0.91^b	**
Berry weight (g)	1.14^a	1.20^{ab}	1.49^d	1.30^{bc}	1.46^d	1.36^{cd}	**
Skin:flesh ratio (F. wt)	0.254^a	0.232^{ab}	0.155^d	0.235^a	0.167^{cd}	0.195^{bc}	***
TSS (°Brix)	24.18^a	24.0^{ab}	22.6^{bc}	24.5^a	22.1^c	24.0^{ab}	*
pH	3.27	3.30	3.31	3.30	3.29	3.28	ns
TA (g/L)	8.00^a	8.19^a	6.88^b	8.23^a	7.06^b	6.96^b	***

Treatment description reproduced from Myburgh (2010a). T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T4 = low-frequency irrigation on the work row, T5 = high-frequency irrigation on the work row, T6 = high-frequency irrigation on alternating work rows.

subtraction of fresh skin and seed weight from the berry weight. Skins were then frozen in liquid nitrogen, ground to a fine powder using a mortar and pestle, lyophilised for 48 hours and weighed. Lyophilised skin material was extracted 1:20 (w/v) in 50% (v/v) ethanol on a rotary shaker for two hours in the dark. The extracts were centrifuged for 5 min at 7000 g. An aliquot of supernatant was then analysed by RP-HPLC.

Small-scale winemaking

Bunches from the grapevines from each irrigation treatment replicate were harvested, counted and weighed prior to winemaking. In 2005/2006 the date of harvest was on a single date 14/02/2006 when the fruit reached 24°B (commercial harvest). In 2006/2007 harvesting of the treatments was staggered from 31/01/07 in order to allow the treatments to reach a TSS of 24°B. However, two of the treatments, T3 and T5 did not attain this ripeness level and were harvested at 22°B. Fruit harvested from the four vineyard replicates was pooled and divided into three winemaking replicates of 40 kg lots. Grapes were crushed and 50 mg/kg potassium metabisulphite was added to the must. Skin contact was allowed for 1 h before the grapes were inoculated with rehydrated yeast (*Saccharomyces cerevisiae*, VIN13, Anchor Foods, South Africa) at a concentration of 30 g/hL. An addition of 50 g/hL diammonium phosphate was also made at inoculation. Musts were not adjusted for pH. Fermentation was conducted on skins at a fermentation temperature of 25°C and the cap was punched down three times daily. Fifty ml of wine was collected daily from ferments and frozen at -20°C until analysis. Once fermentation had reduced the total soluble solids to below 5°B the wine was pressed off skins at 2 Bar, giving a maceration time of five days. Pressed wine was added to the free-run wine and fermentation continued until the sugar concentration was below 2 g/L. Wines were then racked and the SO_2 concentration adjusted to a total of 85 mg/L and cold stabilised at 0°C for two weeks. The wines did not undergo malolactic fermentation. After cold stabilisation the wine was filtered and bottled into nitrogen-filled bottles at room temperature. The total SO_2 concentration was adjusted at bottling to ensure that it was not less than 85 mg/L. After bottling, wines were stored at 14°C until analysis.

Analysis of wine composition and colour

Finished wines and defrosted wine samples taken during fermentation were centrifuged for 5 min at 7000 *g*. Wine colour was measured spectrophotometrically according to the

standard methods outlined in Iland *et al*. (2000). Wine colour density, hue, bisulphite resistant pigments and degree of pigment colouration (%) were determined at wine pH. Modified measures of wine colour density, hue and degree of pigment colouration (%) were determined at an adjusted wine pH of 3.5 in the presence of acetaldehyde for finished wines, but for ferments acetaldehyde was not included. Modified bisulphite resistant pigments were determined at wine pH 3.5 for both finished wines and ferments. Finished wines were analysed six months after bottling at a commercial laboratory (Integral Laboratory, Paarl, South Africa) for residual sugar (RS) using a Fehlings test, and for % alcohol, malic acid, pH and titratable acidity (TA) using a FOSS WineScan FT120 instrument (Foss Electric, Hillerød, Denmark).

RP-HPLC analysis of grape berry skin extracts and wines

RP-HPLC was performed on a Hewlett Packard Agilent 1100 series HPLC system equipped with a diode array detector (Agilent Technologies, Palo Alto, CA, USA). Data processing was done with Chemstation software (Hewlett Packard, Waldbronn, Germany). Separations were carried out on a polystyrene/divinylbenzene reversed-phase column (PLRP-S, 100Å, 250 × 4.6 mm, 5 µm) protected with a guard cartridge (PLRP-S, 10 × 4.6 mm) with the same packing material (Polymer Laboratories (Ltd), Shropshire, UK). The method was adapted from the method of Peng *et al.* (2002). The mobile phases were: solvent A: 1.5% v/v orthophosphoric acid in de-ionised water and solvent B: 80% acetonitrile, 20% solvent A. A linear gradient was used from A 94%, B 6% at 0 min; to A 69%, B 31% at 73 min; to A 38%, B 62% at 78 min; staying constant for 8 min to 86 min, then returning to the starting conditions in 4 min to 90 min, A 94%, B 6%. A flow rate of 1 mL/min was used and a column temperature of 35°C. The phenolic compounds were identified according to their order of elution, the retention times of commercial standards and with reference to published UV-Vis spectra (Price *et al*., 1995; Peng *et al.,* 2001; Peng *et al.,* 2002; Jeffrey *et al.,* 2008). Phenolics were quantified using external standards: (+)-catechin was obtained from Sigma-Aldrich (St. Louis, USA) and malvidin-3-glucoside and quercetin from Extrasynthése (Genay, France). Dimeric flavan-3-ols and tannins were quantified at 280 nm as mg/L catechin units with a quantification limit of 1.5 mg/L. Flavonol-glycosides and flavonol aglycones were quantified at 360 nm as mg/L quercetin with a quantification limit of 0.05 mg/L. Anthocyanins, polymeric pigments and pyruvic acid adducts of malvidin-3-*O*-glucoside (vitisin A) or malvidin-3-*O*-acetyl-glucoside (vitisin AX) (Bakker & Timberlake, 1997; Fulcrand *et al.,* 1998) were quantified at 520 nm as mg/L malvidin-3-*O*-glucoside with a quantification limit of 1.25 mg/L. The upper limit of detection is defined as a signal-to-noise ratio of 3. The limit of quantification was determined as the smallest area that could be accurately integrated (< 3% standard deviation).

For tannin and polymeric pigments, the peak area at 280 nm and 520 nm respectively, eluting as peak 16 in the original method (Peng *et al*., 2002) has been identified as being polymeric in nature but to date has not been compared with other analytical methods in terms of quantification units. The use of flavan-3-ol monomers as units of quantification has been applied in other methods for tannin analysis. For example, (+)-catechin has been used for quantitation of tannin subunit composition analysis by phloroglucinolysis (Kennedy & Jones, 2001) or (-)-epicatechin for tannin precipitation by methyl cellulose (Sarneckis *et al.,* 2006; Mercurio & Smith, 2008). However, use of the RP-HPLC method adapted from Peng *et al*. (2002) has revealed that the quantification of tannin as catechin equivalents has yielded lower values in comparison with other analytical methods (Bindon, unpublished data). For the current study, it was found that tannin concentration by RP-HPLC as catechin equivalents showed a strong positive relationship by linear regression analysis for two pre-veraison skin tannin extracts (Bindon *et al.,* 2010) of mean degree of polymerisation (mDP) 17 units ($R^2 = 0.994$) and 30 units ($R^2 = 0.995$) respectively (data not shown). While being significantly related, comparison of the quantified amount by the reference RP-HPLC method gave lower values, which was not related to differences in polymer length (mDP). This shows that while the RP-HPLC method can be used to accurately compare tannin concentration between treatments, it provides an underestimation of tannin concentration when determined as catechin equivalents. Since modifications of tannin structure and spectral properties in ripe grape skin and wine might affect their relative peak areas by RP-HPLC, pre-veraison skin tannin was not selected as an alternative standard to catechin for quantification. The tannin concentrations determined by this method are therefore valid for comparison between viticultural treatments only.

Statistical analysis

Experimental data were analysed by means of a one-way analysis of variance (ANOVA) using Statistica 7.1 (StatSoft, Tulsa, OK, USA) and JMP 5.0.1 software (SAS, Cary, NC, USA). Differences between means were determined using a post-hoc Students' t-test. Principle component analysis (PCA) was performed by means of the Unscrambler 9.5 software (CAMO Software Australia and New Zealand, St. Peters, NSW, Australia) using full cross validation.

RESULTS

Grape berry morphology and composition in response to water deficit

A detailed account of this study has already been published and provides extensive information on the irrigation treatment responses in terms of grapevine water status (Myburgh, 2010a), as well as vegetative growth, yield and sensorial wine characteristics (Myburgh, 2010b). A concise summary of the seasonal average Ψ_S in the treatments is therefore reported here to provide clarification of the results which follow. In both 2005/2006 and 2006/2007, significant differences in Ψ_S were found between the irrigation treatments, reported as seasonal average Ψ_S (Table 1). For 2005/2006, average Ψ_S decreased as the water applied to the treatment decreased, with the non-irrigated treatment T1 having the lowest Ψ_S and T6 the highest Ψ_S. The differences in water volumes applied to treatments as irrigation was greater in 2006/2007 than in the former season, producing a wider range of seasonal average Ψ_S for the treatments. Treatments T3 and T5 had higher average Ψ_S than the other treatments, T6 was intermediate, with T1, T2 and T4 having the lowest Ψ_S. The method of water application did not

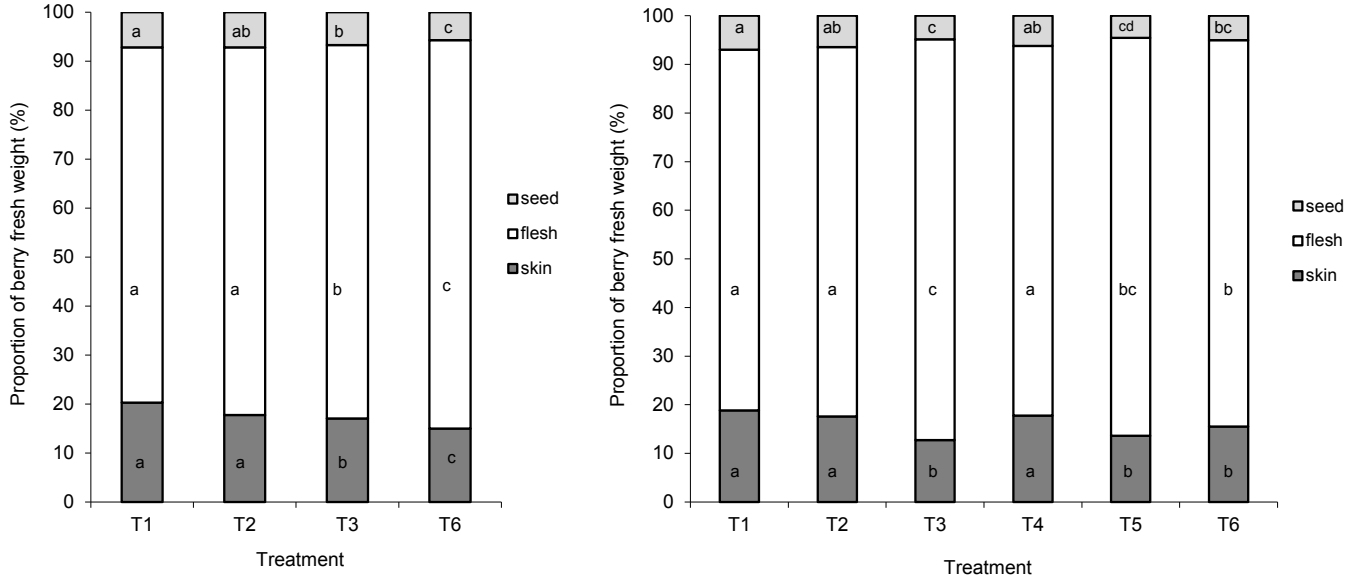

FIGURE 1

Relative proportions of skin, seed and flesh as a function of berry weight in grapes from different irrigation treatments at commercial harvest in the A. 2005/2006 and B. 2006/2007 seasons (ANOVA; n = 16 in 2005/2006 and n = 24 in 2006/2007; different letters indicate significant differences according to P = < 0.05; T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T4 = low-frequency irrigation on the work row, T5 = high-frequency irrigation on the work row, T6 = high-frequency irrigation on alternating work rows).

influence the seasonal average Ψ_S in 2006/2007. Furthermore, in 2006/2007, the low-frequency irrigated treatments T2 and T4 did not have significantly different Ψ_S from the non-irrigated treatment, T1.

For the current study, a different berry sample set to that published in Myburgh (2010b) was used and data is thus repeated here, although datasets are comparable. Berry weight determined at harvest closely approximated the observed responses of the grapevines in terms of Ψ_S, such that in 2005/2006 significantly smaller berries were found in treatments T1 and T2, compared with T3 and T6 (Table 1). The largest berry weight was found for T6 grapevines. However, no differences were observed for juice composition in terms of TSS, pH or TA in 2005/2006. In 2006/2007, the treatments T3 and T5 that received higher-frequency irrigation than the other treatments had significantly higher berry weight at harvest. This was associated with a slower rate of ripening, which resulted in lower TSS and higher TA values for T3 and T5 at harvest than the other irrigation treatments (Table 1). Differences in TA were not reflected as differences in juice pH which may have been due to differences in the ratio of malate to tartrate, although this was not determined analytically.

A more detailed assessment of the skin, seed and flesh contributions to berry weight revealed that, for both 2005/2006 and 2006/2007, increases in berry weight in response to increased irrigation were associated with a decreasing contribution of skin and seed to berry weight, and proportional increases in flesh weight (Fig. 1). This resulted in a lower skin-to-flesh weight ratio in the berries from the high-frequency irrigated treatments (Table 1). Differences in the skin-to-flesh weight ratio between the non-irrigated treatment T1 and the low-frequency-irrigation treatments were minor.

Grape skin phenolics

The phenolic composition of grape skins at commercial harvest was analysed by RP-HPLC for both the 2005/2006 and 2006/2007 seasons and are expressed both per gram berry fresh weight (Table 2) and per gram skin fresh weight (Table 3). For the 2005/2006 season, when treatments were compared on per gram berry fresh weight, the anthocyanin concentration was the most strongly affected of all the flavonoids studied. Treatment T1 had the highest concentration of total anthocyanin, mono-glucosides, acetyl-glucosides and 3-*p*-coumaryl glucosides; T2 and T3 were intermediate and T6 had the lowest concentration. Of the anthocyanin classes, only the peonidin-glucosides were not significantly influenced by the irrigation treatments. In that season, differences between irrigation treatments were not found on a skin fresh weight basis. In the following 2006/2007 season, grape berry samples from T3 and T5 were at an earlier stage of ripeness (22 °B) whereas T1, T2, T4 and T6 were all taken at 24 °B (Table 1). For comparison of the latter group of treatments at the same sugar ripeness level, a similar response of grape phenolic composition was observed in response to the irrigation treatments, with higher anthocyanin concentration on a per gram berry basis in T1 relative to the other treatments, with T6 having the lowest anthocyanin concentration. For the 2006/2007 season, differences in anthocyanin concentration between T1 and the low-frequency irrigated treatments T2 and T4 were less significant compared with 2005/2006. On a berry fresh weight basis, the low-frequency-irrigation treatment T2 showed reductions in anthocyanin mono-glucosides relative to T1, which resulted in an overall reduction in total anthocyanin,

which was not observed for T4 (Table 2). However, for many of the other anthocyanin classes there were equivalent levels of anthocyanin in T1, T2 and T4. Differences between T2 or T4 and T1 were generally not significant on a skin fresh weight basis. In 2006/2007, treatment effects on anthocyanin concentration were evident on a skin weight basis, such that T6 had a lower skin concentration of anthocyanins than the minimally- (T2, T4) or non-irrigated (T1) treatments at the same sugar ripeness. Compared with T1, T2 and T4, there was a lower skin anthocyanin concentration in treatments T3 and T5, which could potentially be attributed to the slower rate of ripening in those treatments.

For both seasons, significant increases in the concentration per gram berry weight of quercetin-3-*O*-glucuronide in response to the application of water deficit were found, when compared with high-frequency-irrigation treatments, while other measured flavonols did not show significant differences between treatments (Table 2). For 2006/2007, the expression of quercetin-3-*O*-glucuronide concentration on a skin weight basis showed a similar response to that observed for anthocyanins, with differences only evident between T1, T2, T4 and the other irrigation treatments, with T2 having a higher concentration. In both seasons of the study, the concentration of skin-derived

TABLE 2

Concentration of skin-derived phenolics in whole grape berries at commercial harvest in response to irrigation treatment (ANOVA; n = 16 in 2005/2006; n = 24 in 2006/2007; ns = not significant; means followed by different letters in the same row indicate significant differences according to * P = < 0.05; ** P = < 0.01, *** P = < 0.001). All samples show comparison at commercial harvest.

Phenolic compound	Concentration (mg/kg berry fresh weight)											
	2005/2006 season					2006/2007 season						
	T1	T2	T3	T6	P	T1	T2	T3	T4	T5	T6	P
Quercetin-3-glucoside	29.2	25.6	20.5	19.8	ns	41.78	41.50	33.00	38.13	30.69	38.15	ns
Quercetin-3-glucuronide	37.4^{a}	29.7^{ab}	22. 7^{b}	19.2^{b}	*	47.71^{a}	44.55^{a}	25.63^{b}	38.26^{ab}	29.45^{b}	30.99^{b}	**
Quercetin-3-rhamnoside	6.1	5.8	5.0	4.7	ns	5.58	5.22	5.88	5.68	8.41	6.79	ns
Delphinidin-3-glucoside	106.3^{a}	70.7^{b}	64.5^{b}	47.1^{b}	**	175.02^{a}	131.41^{b}	100.50^{bc}	169.43^{a}	82.19^{c}	104.91^{bc}	***
Cyanidin-3-glucoside	16.2	13.1	9.3	8.2	ns	43.45^{ab}	26.38^{c}	30.05^{bc}	41.56^{a}	20.88^{c}	24.15^{c}	**
Petunidin-3-glucoside	86.2^{a}	59.4^{b}	55.6^{b}	40.7^{b}	**	120.00^{a}	92.26^{bc}	68.35^{d}	112.31^{ab}	58.71^{d}	74.37^{cd}	***
Peonidin-3-glucoside	50.8	41.0	39.5	33.4	ns	87.47^{a}	61.64^{bc}	65.41^{bc}	82.72^{ab}	48.73^{c}	60.96^{c}	**
Malvidin-3-glucoside	323.0^{a}	245.2^{b}	218.8^{bc}	167.8^{c}	**	411.97^{a}	319.70^{bc}	223.54^{d}	350.97^{ab}	208.57^{d}	270.10^{cd}	***
Delphinidin-3-acetyl-glucoside	16.7^{a}	12.1^{b}	10.2^{b}	8.8^{b}	*	28.97^{ab}	22.97^{bc}	17.16^{cd}	29.42^{a}	13.90^{d}	18.86^{cd}	***
Petunidin-3-acetyl-glucoside	17.7^{a}	14.0^{ab}	13.0^{bc}	9.8^{c}	**	25.63^{a}	21.21^{b}	14.71^{c}	24.27^{ab}	12.96^{c}	16.77^{c}	***
Peonidin-3-acetyl-glucoside	12.4	11.1	11.2	9.3	ns	21.91^{a}	17.74^{ab}	16.06^{bc}	21.39^{a}	12.45^{c}	17.45^{ab}	**
Malvidin-3-acetyl-glucoside	101.4^{a}	90.6^{ab}	79.7^{b}	62.4^{c}	**	136.41^{a}	115.86^{ab}	78.11^{c}	115.10^{ab}	76.77^{c}	102.77^{b}	***
Delphinidin-3-*p*-coumaroyl-glucoside	14.4	10.8	9.8	7.3	ns	22.98^{a}	19.96^{a}	12.61^{b}	21.26^{a}	12.04^{b}	15.29^{b}	***
Petunidin-3-*p*-coumaroyl-glucoside	14.8^{a}	11.5^{b}	9.8^{bc}	7.1^{c}	**	25.16^{a}	21.35^{ab}	13.60^{bc}	21.50^{a}	14.04^{c}	17.40^{bc}	***
Peonidin-3-*p*-coumaroyl-glucoside	16.9	13.5	13.5	11.5	ns	25.82^{a}	21.39^{abc}	18.29^{cd}	24.97^{a}	14.87^{d}	20.16^{bcd}	**
Malvidin-3-*p*-coumaroyl-glucoside	92.9^{a}	78.9^{ab}	70.0^{bc}	57.7^{c}	*	117.36^{a}	94.98^{ab}	67.80^{c}	94.90^{ab}	66.09^{c}	88.44^{bc}	**
Total anthocyanin	869.6^{a}	671.9^{b}	604.8^{bc}	471.0^{c}	**	1242.15^{a}	966.86^{bc}	726.17^{d}	1109.8^{ab}	642.21^{d}	831.64^{cd}	***
Tannin	644.2	631.0	609.1	553.6	ns	599.17	539.97	551.39	582.27	605.15	674.15	ns

Treatment description reproduced from Myburgh (2010a). T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T4 = low-frequency irrigation on the work row, T5 = high-frequency irrigation on the work row, T6 = high-frequency irrigation on alternating work rows. Total anthocyanin represents the sum of individual anthocyanins.

tannin measured was not significantly affected by the irrigation treatments.

Extraction of phenolics and development of wine colour during fermentation

For 2005/2006, a detailed analysis of the extraction of certain phenolics during the 5-day fermentation period prior to pressing was carried out. For most of the irrigation treatments studied, the flavonol concentrations (Fig. 2) increased in wine during the fermentation period, generally reaching maximum levels between days 3 and 4 of fermentation. Differences in the rate of change in concentration were observed between the irrigation treatments, with T1 and T2 having initially higher levels of flavonols, which either stabilised or decreased by day 5 of fermentation. Treatments T3 and T6 showed a continuous although slight increase in flavonols between days 3 and 5 of fermentation, which reduced treatment differences in final flavonol concentration by day 5. The flavonol quercetin-3-*O*-glucuronide (Fig. 2 B) presented the greatest separation between treatments in terms of concentration on day 5 of fermentation, with the highest levels in T1, and lowest in treatment T6.

The pattern of anthocyanin mono-glucoside concentration evolution during fermentation (Fig. 3) was similar to that for flavonols (Fig. 2). A rapid increase in the concentration of anthocyanin mono-glucosides was observed for the ferments from the water deficit treatments T1 and T2 when compared with a slower rate of increase for T3 and T6. For T1 and T2, maximum wine anthocyanin concentration was evident on day 4 of fermentation and this afterwards declined significantly. However, for T3 and T6, anthocyanin concentration, although lower, continued to increase through day 5 of fermentation. The greatest separation between irrigation treatments in terms of anthocyanin concentration occurred on day 4 of fermentation, with T1 producing the highest concentration of all total anthocyanin, and all anthocyanin monoglucosides except peonidin-3-monoglucoside (Fig. 3 D, F). For these measures, T2 and T3 were intermediate on day 4 of fermentation, and T6 had the lowest concentration. By day 5, although significant treatment differences were retained, the differences between treatments were reduced due to continued increases in anthocyanin concentration in the high-frequency-irrigation treatments T3 and T6.

The formation of the more stable coloured adduct of tannin-bound polymeric pigment (Fig. 4 A) reflected the rate of increase in tannin concentration (Fig. 4 B) during fermentation. The treatment differences between the rate of increase in tannin concentration were not as clear as for anthocyanin, but on day 4 of fermentation, T1 and T2 had both higher polymeric pigment and tannin concentrations than T3 and T6. By day 5, differences in tannin concentration were not evident due to stabilisation of the tannin concentration in T1 and T2, whilst T3 and T6 increased from day 4 to 5 of fermentation. On day 5 of fermentation, minor differences in polymeric pigment between treatments were seen, with only T2 having a higher concentration than T6.

The wine colour density and modified wine colour density were similar during the first five days of fermentation (Fig. 5 A, B) and both closely reflected the changes which were observed in free monomeric anthocyanins (Fig. 3). Maximum wine colour density, under wine pH and an adjusted pH of 3.5, reached maximum levels on day 4 of fermentation for T1 and T2, decreasing or stabilising afterwards for each treatment respectively. On the other hand, both measures of wine colour density increased after day 4 for T3 and T6, such that by day 5 the differences between treatments were reduced. The development of bisulphite-resistant pigments followed a similar pattern to that of polymeric pigment (Figs. 4 A, 6 C). For all treatments, bisulphite-resistant pigments increased during fermentation and higher measures were evident for T1 and T2 when compared with the wines from the high-frequency

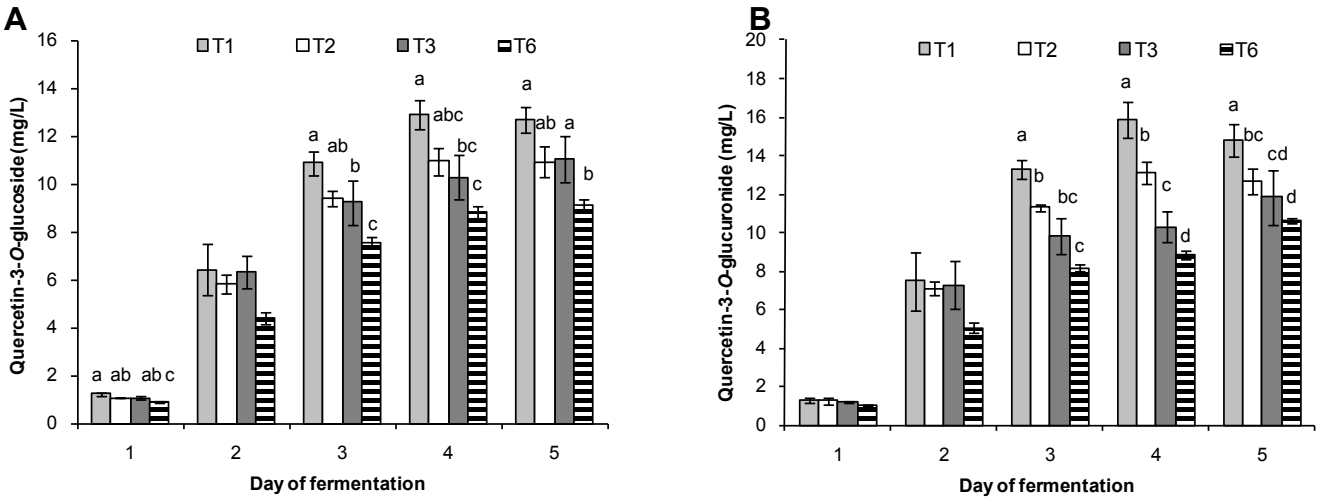

FIGURE 2

Extraction of the flavonols A. quercetin-3-*O*-glucoside and B. quercetin-3-*O*-glucuronide, during days 1 to 5 of fermentation for the 2005/2006 season. Wines were made from grapes of irrigation treatments T1 to T3 and T6 at commercial harvest (ANOVA; n = 12; data represent mean ± S.E. of the mean; different letters indicate significant differences between treatments within a single day according to P = < 0.05; T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T6 = high-frequency irrigation on alternating work rows).

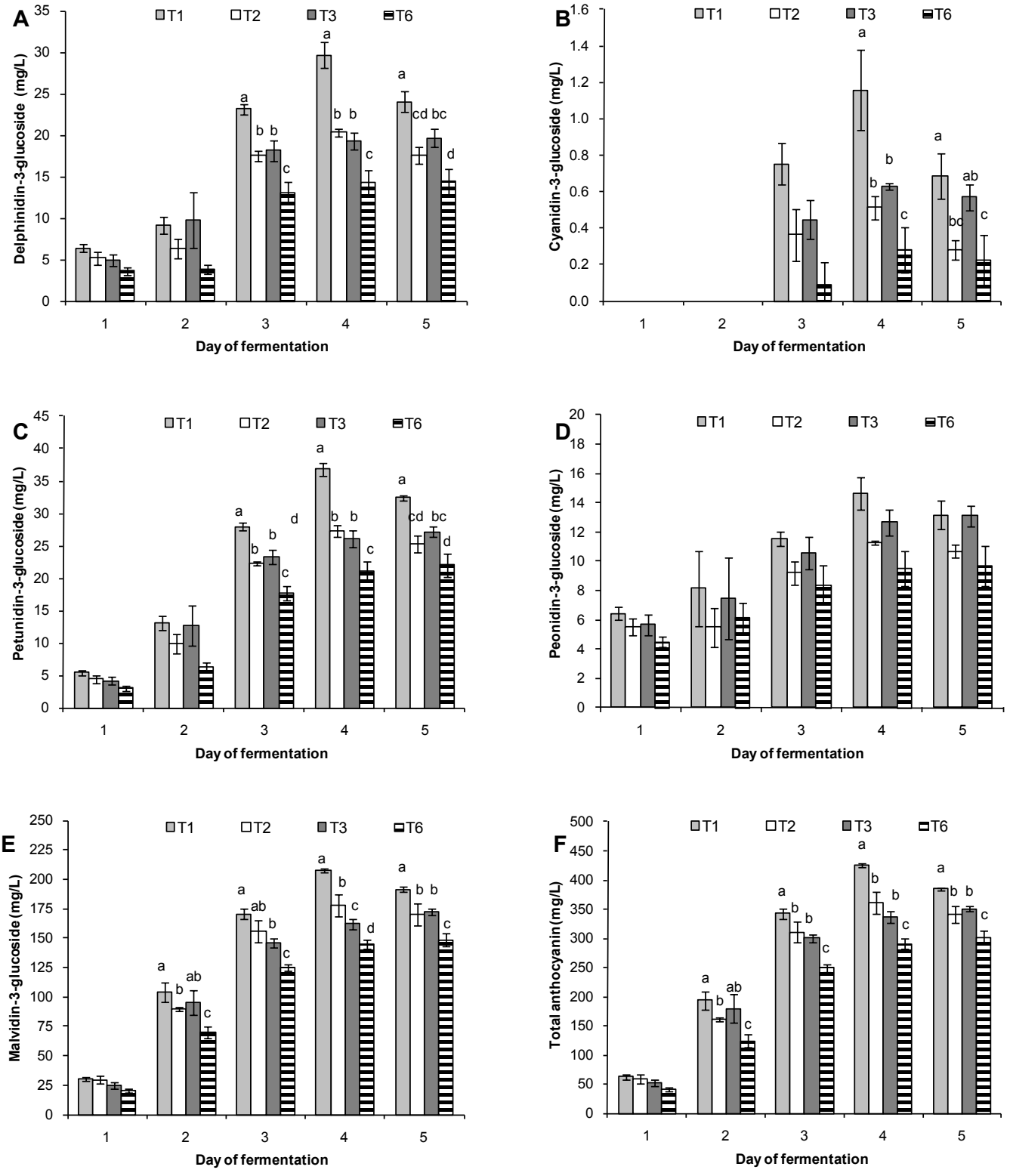

FIGURE 3

Extraction of the anthocyanin monoglucosides A. delphinidin-3-glucoside, B. cyanidin-3-glucoside, C. petunidin-3-glucoside, D. peonidin-3-glucoside, E. malvidin-3-glucoside and F. total anthocyanin during days 1 to 5 of fermentation for the 2005/2006 season. Wines were made from grapes of irrigation treatments T1 to T3 and T6 at commercial harvest (ANOVA; n = 12; data represent mean ± S.E. of the mean; different letters indicate significant differences according to P = < 0.05; T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T6 = high-frequency irrigation on alternating work rows).

irrigated treatments T3 and T6. On day 5, treatment differences in bisulphite-resistant pigments showed higher values for T2 over the other treatments (Fig. 5 C). The assay of total red pigments, which represents both free monomeric anthocyanin and anthocyanin adducts under acidic pH had a less clear response in terms of treatment differences than the other wine colour measures (Fig. 5 D). Generally, the measure of total red pigments increased throughout fermentation for T1, T3 and T6, but decreased for T2 after day 3. By day 5 of fermentation, the lowest measure of total red pigments was found for T6 relative to the other treatments, which were not significantly different.

Composition and colour of finished wines

The wines from the 2005/2006 season showed small differences in alcohol concentration, although these were not significantly different. This reflects the fact that the treatments did not show differences in their rate of berry ripening and final TSS attained (Table 4). Larger differences were observed for TA, with T6 having higher concentrations of both measurements compared with the non-irrigated control. The opposite relationship was observed for wine malic acid, with T6 having a higher concentration than the other treatments, which may reflect a higher contribution of tartaric acid to TA, although this was not determined in this study. The pH of the wines was not

TABLE 3

Concentration of skin-derived phenolics in grape berry skins at commercial harvest in response to irrigation treatment (ANOVA; n = 16 in 2005/2006; n = 24 in 2006/2007; ns = not significant; means followed by different letters in the same row indicate significant differences according to * P = < 0.05; ** P = < 0.01, *** P = < 0.001). All samples show comparison at commercial harvest.

Phenolic compound	Concentration (mg/kg skin fresh weight)											
	2005/2006 season					2006/2007 season						
	T1	T2	T3	T6	P	T1	T2	T3	T4	T5	T6	P
Quercetin-3-glucoside	144.33	137.58	119.73	135.26	ns	273.40	335.30	254.23	287.56	222.17	245.34	ns
Quercetin-3-glucuronide	184.68	158.92	132.67	127.97	ns	316.57^{a}	361.14^{a}	197.51^{c}	289.74^{ab}	212.86^{bc}	199.76^{c}	**
Quercetin-3-rhamnoside	29.94	31.16	29.12	31.69	ns	36.17	41.61	45.79	42.70	64.95	43.78	ns
Delphinidin-3-glucoside	524.30	379.86	376.79	331.35	ns	1132.69^{ab}	1050.97^{b}	787.50^{c}	1283.71^{a}	597.20^{d}	676.00^{cd}	***
Cyanidin-3-glucoside	79.86	70.20	54.40	59.07	ns	276.41^{ab}	210.63^{bc}	234.77^{b}	315.39^{a}	151.45^{c}	155.03^{c}	**
Petunidin-3-glucoside	425.53	319.42	324.91	283.69	ns	780.50^{ab}	739.91^{b}	535.61^{c}	850.81^{a}	426.79^{d}	479.60^{cd}	***
Peonidin-3-glucoside	250.37	219.98	230.79	235.49	ns	562.49^{ab}	494.08^{bc}	514.06^{b}	626.87^{a}	353.28^{d}	391.75^{cd}	**
Malvidin-3-glucoside	1596.25	1320.36	1278.37	1156.78	ns	2713.93^{a}	2579.31^{a}	1747.80^{b}	2661.26^{a}	1515.57^{b}	1740.68^{b}	***
Delphinidin-3-acetyl-glucoside	82.47	64.91	59.81	61.58	ns	187.80^{b}	184.41^{b}	133.97^{c}	222.87^{a}	101.01^{d}	121.54^{cd}	***
Petunidin-3-acetyl-glucoside	87.34	75.36	76.05	68.80	ns	167.18^{a}	171.13^{a}	115.16^{b}	183.96^{a}	94.15^{b}	108.14^{b}	***
Peonidin-3-acetyl-glucoside	61.30	59.69	65.23	64.67	ns	141.73^{ab}	143.12^{ab}	126.39^{bc}	162.01^{a}	90.22^{d}	112.26^{cd}	**
Malvidin-3-acetyl-glucoside	500.94	488.09	465.41	426.17	ns	902.38^{a}	941.45^{a}	608.87^{b}	873.10^{a}	557.01^{b}	662.04^{b}	***
Delphinidin-3-*p*-coumaroyl-glucoside	70.88	58.34	57.40	50.78	ns	150.30^{a}	161.14^{a}	98.43^{b}	161.20^{a}	87.29^{b}	98.60^{b}	***
Petunidin-3-*p*-coumaroyl-glucoside	73.24	62.00	57.01	49.17	ns	165.84^{a}	172.47^{a}	106.12^{b}	163.18^{a}	101.73^{b}	112.20^{b}	***
Peonidin-3-*p*-coumaroyl-glucoside	83.53	72.50	79.01	80.52	ns	168.46^{ab}	172.11^{ab}	143.23^{bc}	188.97^{a}	107.73^{d}	129.73^{cd}	***
Malvidin-3-*p*-coumaroyl-glucoside	459.13	425.16	408.75	392.92	ns	777.86^{a}	769.72^{a}	525.19^{c}	721.12^{ab}	478.49^{d}	569.25^{cd}	**
Total anthocyanin	4295.13	3615.88	3533.93	3260.98	ns	8127.57^{a}	7790.45^{a}	5677.09^{b}	8414.45^{a}	4661.94^{b}	5356.82^{b}	***
Tannin	3178.84	3387.71	3567.33	3723.20	ns	3900.11	4337.31	4371.20	4427.26	4431.43	4347.08	ns

Treatment description reproduced from Myburgh (2010a). T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T4 = low-frequency irrigation on the work row, T5 = high-frequency irrigation on the work row, T6 = high-frequency irrigation on alternating work rows. Total anthocyanin represents the sum of individual anthocyanins.

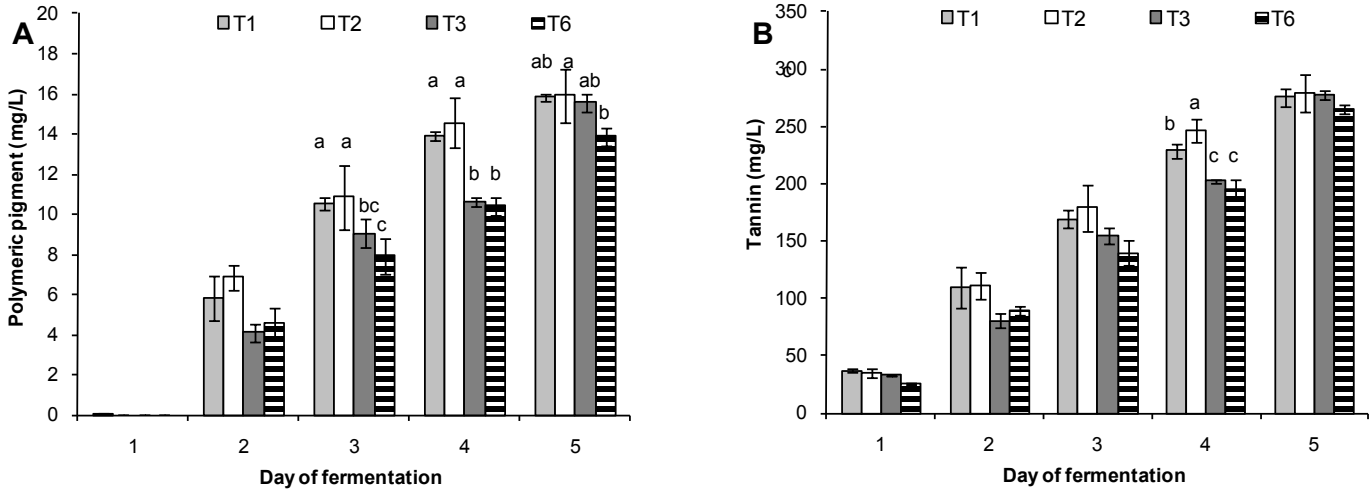

FIGURE 4

Accumulation of A. polymeric pigment and B. tannin during days 1 to 5 of fermentation for the 2005/2006 season. Wines were made from grapes of irrigation treatments T1 to T3 and T6 at commercial harvest (ANOVA; n = 12; data represent mean ± S.E. of the mean; different letters indicate significant differences according to P = < 0.05; T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T6 = high-frequency irrigation on alternating work rows).

TABLE 4

General wine analysis of 6-month-old wines made from grapes of different irrigation treatments at commercial harvest (ANOVA; n = 12 in 2005/2006; n = 18 in 2006/2007; ns = not significant; nd = not detected; means followed by different letters in the same row indicate significant differences according to * P = < 0.05; ** P = < 0.01, *** P = < 0.001).

	2005/2006 season					2006/2007 season						
	T1	T2	T3	T6	P	T1	T2	T3	T4	T5	T6	P
Alcohol (%)	16.32	16.01	15.56	14.54	ns	16.45^{a}	15.92^{a}	13.83^{b}	16.18^{a}	12.99^{b}	13.74^{b}	**
Residual sugar (g/L)	1.20^{ab}	1.34^{a}	1.08^{b}	0.81^{c}	**	1.74	1.23	1.33	1.62	1.14	1.43	ns
Titratable acidity (g/L)	5.88^{a}	5.33^{b}	5.32^{b}	5.05^{b}	*	5.61	5.37	5.23	5.23	5.22	4.99	ns
pH	3.56	3.65	3.68	3.77	ns	3.52	3.63	3.71	3.68	3.58	3.63	ns
Malic acid (g/L)	0.97^{a}	0.90^{a}	1.05^{ab}	1.19^{b}	**	1.29	1.39	1.24	1.23	1.01	1.18	ns

Treatment description reproduced from Myburgh (2010a). T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T4 = low-frequency irrigation on the work row, T5 = high-frequency irrigation on the work row, T6 = high-frequency irrigation on alternating work rows.

significantly different between treatments in that season. In the second season of the study, significant differences were observed between ripening rates and final TSS concentration in the fruit (Table 1), which was reflected as lower alcohol levels for T3 and T5 relative to the non-irrigated and low-frequency-irrigation treatments T1, T2 and T4 (Table 4). Although T6 was harvested at a TSS of 24 °B, the final alcohol concentration of the wines was also lower relative to T1, T2 and T4. Differences in pH, malic acid and TA between treatments were not evident in the wines for 2006/2007. It should be noted that a discrepancy was observed in the alcohol levels for both seasons studied, which would have been expected on the basis of grape juice TSS levels (Table 1) for the treatments and the final ethanol concentration in the wines, which may reflect increases in must sugars derived from grape solids during vinification.

Analysis of the phenolic composition of the six-month-old finished wines for both 2005/2006 and 2006/2007 showed minor differences between treatments (Table 5). In both seasons, only quercetin-3-*O*-glucuronide showed a significant response, which reflected the differences in both grape composition (Table 2) and changes in concentration during fermentation (Fig. 2 B). For the finished wines from the 2005/2006 season, T1 had the highest concentration of quercetin-3-*O*-glucuronide, with T2 and T3 as intermediate, and T6 having the lowest level. For 2006/2007, there were no significant differences between the non-irrigated treatment T1 and the low-frequency irrigated treatments T2 and T4 in the concentration of quercetin-3-*O*-glucuronide. However, these three treatments all had significantly higher concentrations than T3, T5 and T6. For the 2005/2006 wines, the anthocyanin derivative vitisin A reflected the responses observed for grape berry anthocyanin concentration between irrigation treatments, with T1 having higher levels with concentrations lowering in descending order from T2 to T3 and to T6 (Table 5). In 2005/2006, wine tannin

TABLE 5
Concentration of skin-derived phenolics in 6-month-old wines made from grapes of different irrigation treatments at commercial harvest (ANOVA; n = 12 in 2005/2006; n = 18 in 2006/2007; ns = not significant; nd = not detected; means followed by different letters in the same row indicate significant differences according to * P = < 0.05; ** P = < 0.01, *** P = < 0.001).

Phenolic compound	Concentration in wine (mg/L)											
	2005/2006 season					2006/2007 season						
	T1	T2	T3	T6	P	T1	T2	T3	T4	T5	T6	P
Quercetin-3-glucoside	9.67	7.92	8.44	6.45	ns	15.08^{ab}	17.51^{a}	12.15^{bc}	15.85^{ab}	6.84^{c}	6.50^{c}	*
Quercetin-3-glucuronide	16.25^{a}	13.57^{b}	12.63^{bc}	10.50^{c}	**	21.69^{a}	22.81^{a}	12.52^{b}	21.80^{a}	8.43^{b}	14.22^{b}	**
Quercetin-3-rhamnoside	3.75	3.79	3.86	3.21	ns	5.91^{ab}	6.81^{a}	3.86^{bc}	6.51^{a}	3.50^{c}	6.47^{a}	*
Quercetin	4.17	3.85	4.38	4.71	ns	4.74	6.47	7.90	4.40	4.73	8.88	ns
Kaempherol	1.09	1.09	0.95	1.03	ns	0.35	0.87	0.44	0.67	0.57	1.34	ns
Delphinidin-3-glucoside	16.63	12.79	14.51	10.78	ns	11.64	13.47	9.36	11.90	10.87	9.74	ns
Cyanidin-3-glucoside	0.59	0.26	0.53	0.23	ns	1.94	2.13	1.43	2.09	1.66	1.42	ns
Petunidin-3-glucoside	22.34	18.34	20.77	17.02	ns	15.15	17.71	12.69	15.22	14.84	13.52	ns
Peonidin-3-glucoside	9.90	8.12	9.64	8.06	ns	11.00	13.19	8.67	12.26	10.20	9.60	ns
Malvidin-3-glucoside	128.54	123.90	131.53	119.24	ns	91.48	104.91	75.02	85.99	87.18	91.09	ns
Delphinidin-3-acetyl-glucoside	3.42	2.95	3.62	3.81	ns	4.11	4.38	3.46	3.96	4.15	3.81	ns
Petunidin-3-acetyl-glucoside	4.61	4.83	5.47	5.07	ns	4.55	5.36	3.84	4.48	4.48	4.24	ns
Peonidin-3-acetyl-glucoside	4.25^{a}	4.37^{a}	5.63^{b}	5.67^{b}	*	5.29	6.73	4.91	5.72	5.77	6.04	ns
Malvidin-3-acetyl-glucoside	40.05	44.06	45.53	42.92	ns	30.14	37.01	24.24	28.15	28.08	32.06	ns
Delphinidin-3-*p*-coumaroyl-glucoside	0.94	0.82	0.90	0.75	ns	1.78	1.91	1.50	1.61	1.56	1.76	ns
Petunidin-3-*p*-coumaroyl-glucoside	2.00	1.61	1.76	1.64	ns	2.23	2.40	1.96	2.14	1.95	2.34	ns
Peonidin-3-*p*-coumaroyl-glucoside	1.90	1.76	2.34	2.21	ns	2.78	3.35	2.80	2.84	3.09	3.49	ns
Malvidin-3-*p*-coumaroyl-glucoside	16.18	16.94	18.38	17.11	ns	12.50	14.74	10.84	10.96	12.54	14.89	ns
Total anthocyanin	251.35	240.76	257.40	234.52	ns	194.58	227.29	160.73	187.32	186.38	194.00	ns
Vitisin A	1.17^{a}	0.75^{bc}	0.84^{abc}	0.50^{c}	*	1.99	1.92	1.74	2.33	1.55	1.98	ns
Vitisin AX	0.47	0.33	0.34	0.58	ns	1.49	1.52	1.23	1.64	1.17	1.43	ns
Polymeric pigment	18.33	17.52	19.55	15.69	ns	20.87^{ab}	22.10^{ab}	16.85^{bc}	25.54^{a}	10.47^{c}	17.49^{b}	**
Procyanidin B1	21.83^{a}	26.20^{a}	23.75^{a}	15.43^{b}	**	28.23^{ab}	nd	21.17^{bc}	28.45^{a}	nd	21.41^{c}	*
Tannin	266.33	242.09	263.88	231.75	ns	346.27^{a}	357.02^{a}	283.98^{ab}	352.80^{a}	203.30^{b}	301.30^{ab}	*

Treatment description reproduced from Myburgh *et al.* (2010a). T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T4 = low-frequency irrigation on the work row, T5 = high-frequency irrigation on the work row, T6 = high-frequency irrigation on alternating work rows. Total anthocyanin represents the sum of individual anthocyanins.

TABLE 6
Wine colour measurements in six-month-old wines made from grapes of different irrigation treatments at commercial harvest (ANOVA; n = 12 in 2005/2006; n = 18 in 2006/2007; ns = not significant; nd = not detected; means followed by different letters in the same row indicate significant differences according to * P = < 0.05; ** P = < 0.01, *** P = < 0.001).

Wine Colour Component	2005/2006 season					2006/2007 season						
	T1	T2	T3	T6	P	T1	T2	T3	T4	T5	T6	P
Wine colour density (wine pH)	8.37	6.55	6.51	5.93	ns	8.0^{a}	8.0^{a}	6.30^{ab}	9.08^{c}	5.35^{b}	6.42^{ab}	**
Wine colour hue (wine pH)	0.60	0.70	0.71	0.77	ns	0.68	0.64	0.73	0.76	0.67	0.62	ns
Degree of red pigment colouration (%) (wine pH)	21.16	14.31	14.79	22.56	ns	21.03	23.41	24.54	22.33	21.17	37.54	ns
Estimate of SO_2 resistant pigments (wine pH)	1.76^{a}	1.63^{a}	1.56^{ab}	1.04^{b}	*	2.97^{a}	3.04^{a}	1.92^{b}	3.46^{a}	1.38^{b}	1.97^{b}	***
Total phenolics (A_{280} HCl)	38.15	33.07	36.20	32.83	ns	38.08	38.18	32.29	46.7	37.14	32.29	ns
Total red pigments (A_{520} HCl)	25.45^{a}	27.17^{a}	25.65^{a}	15.08^{b}	***	26.97^{a}	27.10^{a}	21.78^{ab}	20.50^{ab}	16.50^{b}	16.16^{b}	*
Modified wine colour density (pH 3.5)	11.98	11.53	12.10	9.60	ns	10.32^{ab}	10.77^{ab}	7.82^{ab}	11.11^{a}	7.39^{b}	8.62^{ab}	*
Modified wine colour hue (pH 3.5)	0.49	0.49	0.46	0.49	ns	0.56	0.55	0.59	0.56	0.52	0.55	ns
Modified degree of red pigment colouration% (pH 3.5)	31.93^{a}	25.44^{ab}	29.28^{ab}	46.43^{c}	***	24.90^{ab}	26.23^{ab}	22.77^{a}	35.28^{c}	29.51^{bc}	34.53^{c}	***
Modified estimate of SO_2 resistant pigments (pH 3.5)	1.91^{a}	1.65^{ab}	1.77^{ab}	1.09^{b}	*	3.08^{ac}	2.43^{ab}	1.88^{bd}	3.56^{c}	1.19^{d}	2.00^{bd}	***

Treatment description reproduced from Myburgh (2010a). T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T4 = low-frequency irrigation on the work row, T5 = high-frequency irrigation on the work row, T6 = high-frequency irrigation on alternating work rows. Modified wine colour density and hue are at from wines at adjusted pH 3.5 in the presence of acetaldehyde.

was not significantly different between irrigation treatments. For 2006/2007, no differences in vitisin concentrations were found between treatments, but significant differences in polymeric pigment and tannin concentrations were observed. For polymeric pigment, the low-frequency-irrigation treatment T4 had the highest concentration and the high-frequency-irrigation treatment T5 the lowest. For wine tannin, T1, T2 and T4 had higher levels than T3 and T6, which were intermediate, with T5 having the lowest concentration.

For the 2005/2006 season, no significant differences were observed in the wine colour density and modified wine colour density (pH 3.5 with acetaldehyde) of the six-month-aged wines (Table 6). For the 2006/2007 season, six-month aged wines had higher wine colour density and modified wine colour density for the low frequency irrigated treatment T4, and the lowest values for the high-frequency irrigated treatment T5 (Table 6). For both vintages, differences between treatments were most evident for bisulphite-resistant pigments, with the non-irrigated and low-frequency-irrigation treatments having higher levels than the other irrigation treatments. The adjustment of wine pH to 3.5 minimised these differences as modified bisulphite-resistant pigments. The estimate of total red pigments showed similar, small differences between irrigation treatments for both seasons of the study. In 2005/2006, wine from T6 had lower total red pigments than wines from the other irrigation treatments, and in 2006/2007 wines from T5 and T6 were recorded lowest in this measure. For other measures of wine colour, neither season showed significant differences between irrigation treatments (Table 6).

Assay of wine colour measurements in the wines made from the 2005/2006 season after 18 months of bottle ageing showed significant differences between treatments (Table 7). Significant differences were observed between treatments for wine colour density and bisulphite-resistant pigments (at wine pH and pH 3.5), with T1 having higher values for both than T6. For the latter measure, there were no significant differences between T1 and T2, or T3. For wine hue at wine pH, T6 had a higher value than T1. However, similar to the results observed for six-month-aged wines, modified wine colour density showed no significant differences between treatments, indicating that either pH differences or bisulphite bleaching of pigments may have contributed to the observed differences under wine conditions. Despite spectrophotometric differences in wine colour, the 18-month-aged wines from 2005/2006 showed no differences between treatments for phenolic composition by RP-HPLC (data not shown). In order to explain the observed changes in wine colour measures between treatments, a comparison of the % change of coloured pigments was made between the six-month and 18-month wines from 2005/2006 (Fig. 6). In general, all treatments showed significant increases in the concentration of vitisin A and vitisin AX. For vitisin A, no differences between treatments were found, but for vitisin AX, significant increases were found for T2 and T3 after 12 months' ageing, compared with wines from the other irrigation

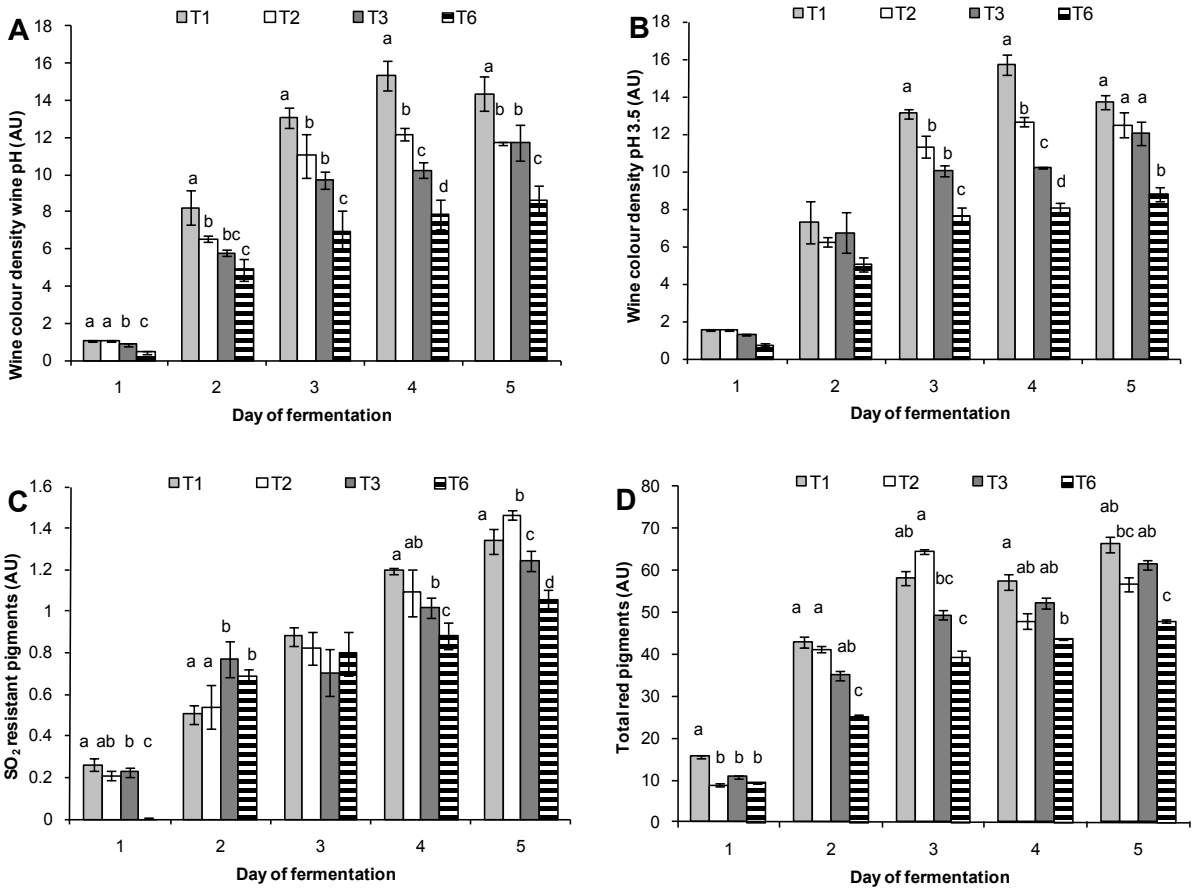

FIGURE 5

Development of wine colour measured at wine pH during days 1 to 5 of fermentation for the 2005/2006 season where A. Wine colour density at wine pH; B. Wine colour density at pH 3.5; C. SO_2 resistant pigments; and D. Total red pigments. Wines were made from grapes of irrigation treatments T1 to T3 and T6 at commercial harvest (ANOVA; n = 12; data represent mean ± S.E. of the mean; different letters indicate significant differences according to P = < 0.05; T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T6 = high-frequency irrigation on alternating work rows).

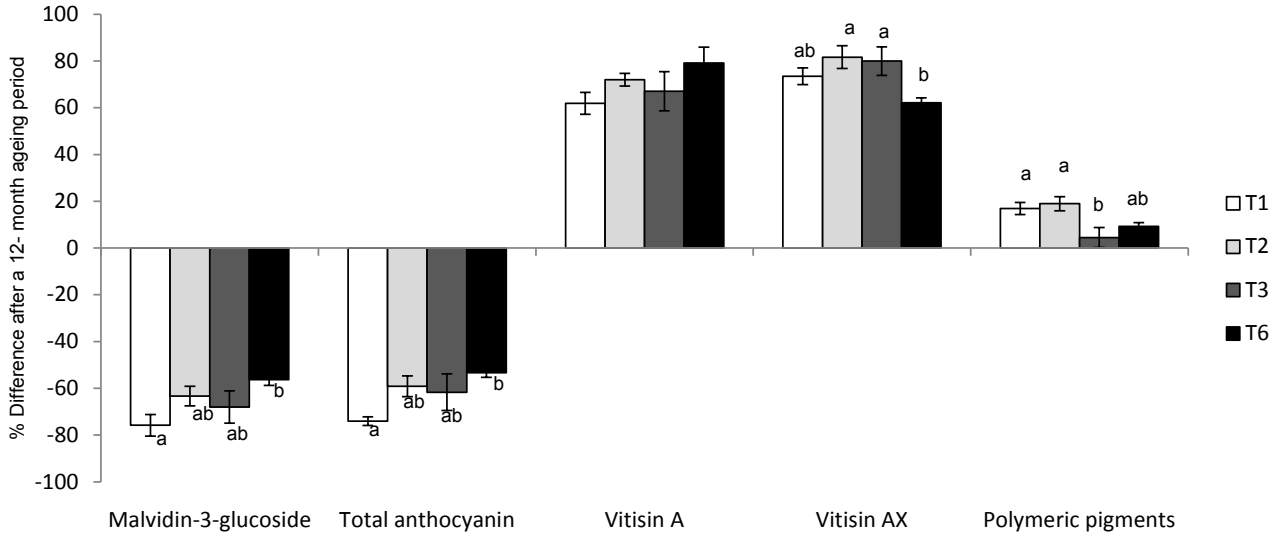

FIGURE 6

Changes in anthocyanin and anthocyanin derivatives in 2005/2006 Merlot wines following a 12-month ageing period from six months post-fermentation to 18 months (Students' t-test; n = 12; data represent mean ± S.E. of the mean; different letters indicate significant differences according to P = < 0.05; T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T6 = high-frequency irrigation on alternating work rows).

treatments. On the other hand, smaller increases in polymeric pigments were found after ageing than for vitisins, which were greatest in the low-frequency-irrigated treatment T2 and the non-irrigated treatment. Conversely, decreases in monomeric anthocyanin after 18 months from the analysis taken at six months were found for all treatments (Fig. 6), and these were greater for treatments T1 and T2 than for the high-frequency irrigation treatments. It is evident from this analysis that increases in polymeric pigment in the low-frequency-irrigation treatments may be related to the significant differences in bisulphite-resistant pigments between irrigation treatments in the 18-month-aged wines.

The use of PCA allowed examination of this complex data set, where differences between treatments were related to

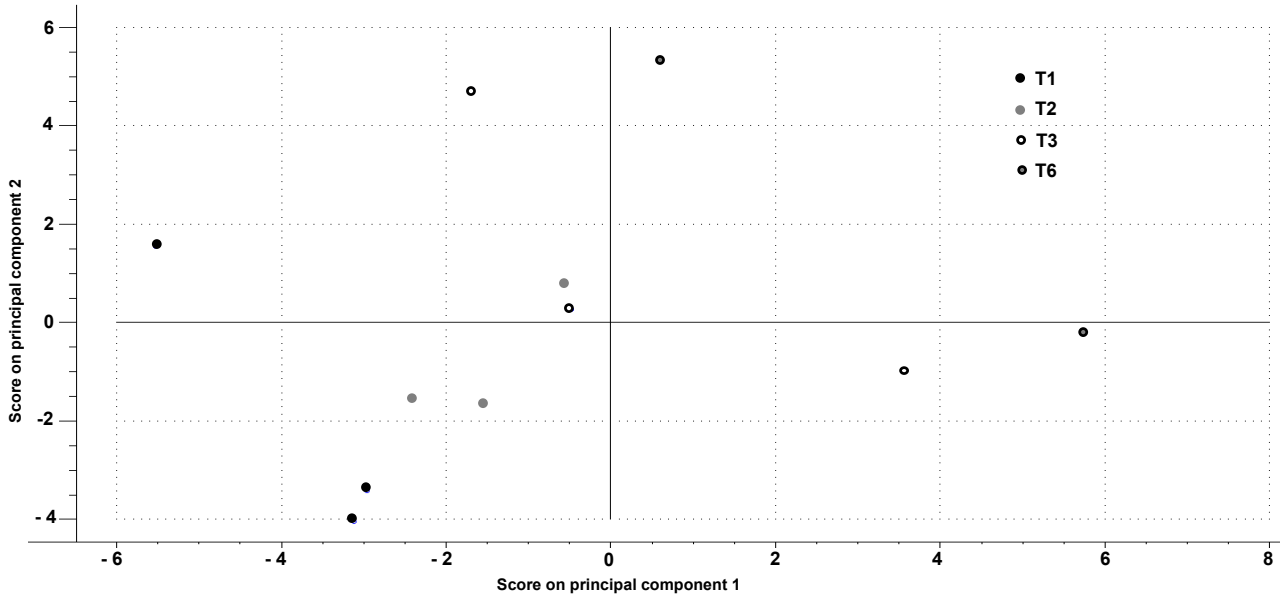

FIGURE 7

Plot of PCA analysis scores for two principal components (PC1 and PC2) of 18-month-old 2005/2006 wines from irrigation treatments using 28 wine analysis variables. T1 = non-irrigated; T2 = low-frequency irrigation on the vine row; T3 = high-frequency irrigation on the vine row; T6 = high-frequency irrigation on alternating work rows. Separation on PC1 and PC2 correspond to 46% and 24% of the dataset variance respectively.

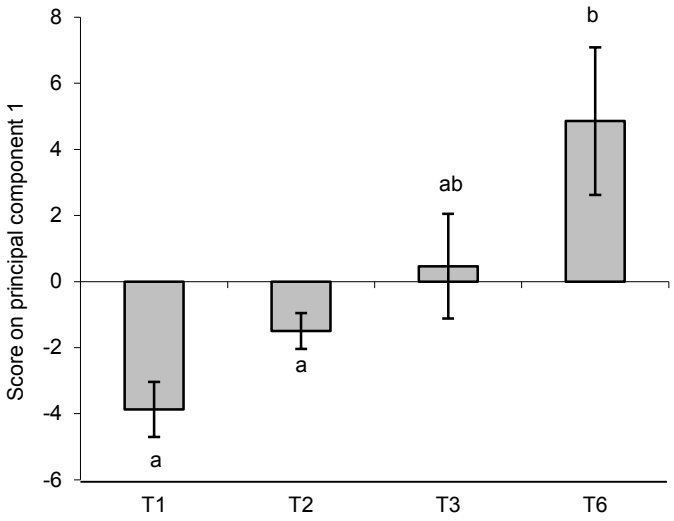

FIGURE 8

PCA separation of 18-month-old 2005/2006 wines from irrigation treatments according to their score on principal component 1, which describes 46% of the dataset variance. T1 = non-irrigated; T2 = low-frequency irrigation on the vine row; T3 = high-frequency irrigation on the vine row; T6 = high-frequency irrigation on alternating work rows (scores for each treatment were compared by one-way ANOVA where n = 12; different letters indicate significant differences according to P = < 0.05).

interactions between several, rather than individual, parameters. This analysis was initially performed using the whole dataset to determine the parameters which most strongly separated the treatments. Measures which did not contribute significantly to the model were removed for the final PCA. A significant separation of the treatments for wine compositional data by PCA was found only for the first principal component (PC1) which described 46% of the dataset variance (Figs 7 and 8). Interpretation of the dataset using this model provided a separation between treatments T1 and T2 against T6. The scores for the treatments on PC1 showed a negative correlation of T1 and T2 with PC1 and a positive correlation of T6. A histogram of the loadings of the wine compositional parameters which describe PC1 (Fig. 9) shows that co-correlation exists between wine colour measures, bisulphite-resistant pigments and the phenolic parameters tannin, polymeric pigment, vitisins and flavonols, while a negative relationship was found for free anthocyanin. The inclusion of parameters such as alcohol and TA showed that these were significant (Fig. 9), such that higher TA and alcohol contributed positively to the model, co-correlating with the wine colour measurements. It is therefore evident that, for the current results, 'matrix effects' related to differences in wine composition other than phenolics may contribute significantly to wine colour.

TABLE 7

Wine colour measurements in 18-month-old wines made from grapes of different irrigation treatments at commercial harvest in the 2005/2006 season (ANOVA; n = 12; ns = not significant; nd = not detected; means followed by different letters in the same row indicate significant differences according to * P = < 0.05; ** P = < 0.01, *** P = < 0.001).

Wine Colour Component	T1	T2	T3	T6	P
Wine colour density (wine pH)	8.06[a]	6.98[ab]	6.86[ab]	5.49[b]	*
Wine colour hue (wine pH)	0.64[a]	0.69[ab]	0.70[ab]	0.77[ba?]	**
Degree of red pigment colouration (%) (wine pH)	24.34[a]	18.68[ab]	20.24[ab]	14.48[b]	*
Estimate of SO_2 resistant pigments (wine pH)	2.89[a]	2.61[a]	2.36[a]	1.61[b]	***
Total phenolics (A_{280} HCl)	37.81[a]	29.5[b]	36.53[ab]	36.74[ab]	*
Total red pigments (A_{520} HCl)	15.28	12.59	12.63	14.04	ns
Modified wine colour density (pH 3.5)	10.04	9.41	9.56	8.53	ns
Modified wine colour hue (pH 3.5)	0.59	0.59	0.57	0.58	ns
Modified degree of red pigment colouration% (pH 3.5)	41.76	47.14	48.23	38.28	ns
Modified estimate of SO_2 resistant pigments (pH 3.5)	2.97[a]	2.63[a]	2.38[ab]	2.05[b]	**

Treatment description reproduced from Myburgh (2010a). T1 = non-irrigated, T2 = low-frequency irrigation on the vine row, T3 = high-frequency irrigation on the vine row, T4 = low-frequency irrigation on the work row, T5 = high-frequency irrigation on the work row, T6 = high-frequency irrigation on alternating work rows. Modified wine colour density and hue are at from wines at adjusted pH 3.5 in the presence of acetaldehyde.

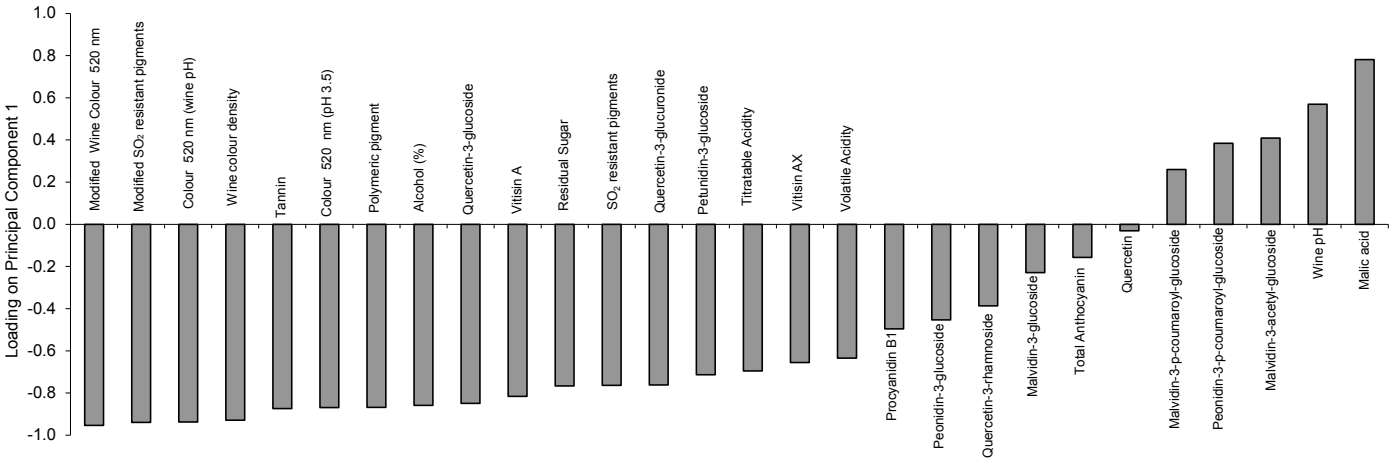

FIGURE 9

Loadings of variables used to describe differences between 18-month-old 2005/2006 wines from irrigation treatments using PCA analysis. According to the PCA, principal component 1 describes 46% of the dataset variance.

DISCUSSION

Relationships between grape and wine phenolic composition

Previous studies on grapevines have shown that decreases in berry weight as a result of water deficit were caused by the decrease in pericarp volume due to restricted cell expansion, and that cell multiplication was not significantly affected (Ojeda *et al*., 1999; 2001). This is consistent with the results of the current study, which found that berry weight decreased with water deficit, and skin-to-flesh weight ratio increased, indicating that relative skin weight was not decreased. In the current study, the concentration of phenolic compounds was expressed in terms of skin fresh weight and per kg fresh berry weight in order to provide an indication as to whether changes in phenolic compounds occurred as a result of changes in biosynthesis or were related to altered berry size. In both seasons, changes in the concentration of phenolics in berries appeared to be related to changes in berry weight, with greater differences observed in response to the irrigation treatments on a fresh berry weight basis than on a skin weight basis. In general, the results reflect the changes observed for berry weight and skin-to-berry weight ratios between treatments. However, there was an exception with respect to anthocyanin concentration per kg berry fresh weight which was higher in T1 than in T2 in 2005/2006, despite there being no significant difference in either skin:flesh or skin:berry weight ratios, which may reflect differences in phenolic extractability. Nevertheless, these observations indicate that, for this experiment, changes in phenolic concentration were not strongly associated with changes in biosynthesis in response to the irrigation treatments. For the second season of the study, differences in the concentration of phenolics on a skin weight basis appear to be more strongly associated with delayed ripening as a result of the irrigation application. An assumption, therefore, is that grapes produced either by no or by minimal irrigation would have a higher concentration of phenolics, namely anthocyanins, than those undergoing high-frequency irrigation, if they are harvested at the same time rather than at the same TSS. This may have implications for phenolic extraction during vinification. However, the poor relationship between the phenolic composition of grapes produced by deficit irrigation and that of the finished wines shows that this relationship is more complex than simply that of concentration. Studies have shown that a strong relationship between grape phenolic composition and wine concentration and composition exists under some conditions (Peterlunger *et al*., 2005; Cortell *et al*., 2005; Kondouras *et al*., 2006; Cortell *et al*., 2007a, b; Ristic *et al*., 2010) while in others it is evident that it is influenced by additional unknown factors (Kennedy *et al*., 2002; De Beer *et al*., 2006; Ristic *et al*., 2007; Bindon *et al*., 2008; Jensen *et al.,* 2008; Holt *et al.,* 2008). Studies in model systems have shown that grape skin cell wall structure may have a significant relationship with the prediction of anthocyanin extractability (Ortega-Regules *et al.,* 2006). Model extraction studies using solvents which differ in their solubility for phenolics have shown that restriction in extractability of phenolics, particularly anthocyanins and tannins, can occur in grapes produced under conditions of water deficit (Sivilotti *et al*., 2005). It was proposed that limited anthocyanin extractability in a weak solvent, which simulates later stages of fermentation, may have been due to a tighter berry cell-wall structure in grape skins from water-stressed grapevines. In the present study, a study of phenolic extraction showed that treatment differences in the rate of extraction of anthocyanin and flavonols during the initial stages of fermentation closely approximated the differences in concentration of these compounds between the grape sources, but that this relationship broke down as fermentation progressed, with extraction slowing in deficit-irrigation ferments while continuing in ferments from the high-frequency-irrigated fruit sources. While this might be as a result of differences in phenolic extraction, it might also reflect differences in the rate of the formation of phenolic derivatives or phenolic oxidation. Since treatment differences in wine phenolic composition were minimal at the end of fermentation and even further reduced after six months and 18 months of wine ageing, it is evident that the translation of analytical information from grape to wine is complex. The lack of a strong correlation in the results of this study from grape to wine highlights the need for a greater understanding of factors affecting phenolic extractability under varying viticultural growing conditions and management practices. When looking exclusively at grape phenolic composition from the viticultural perspective, a further important aspect which will contribute to wine colour formation and stabilisation is anthocyanin copigmentation and adduct formation with yeast metabolites and other phenolics, which are dependent upon their relative proportions (Schwarz *et al.,* 2005). The increased concentration of the flavonol quercetin-3-*O*-glucuronide in deficit-irrigation treated grapes and wines could have contributed to the stabilisation of anthocyanin in copigment complexes early in vinification, leading to the formation of stable bisulphite-resistant adducts as the wine aged (Schwarz *et al.,* 2005). It is of interest to note that this flavonol has recently been identified and quantified in red wines, and has been shown to occur in more significant quantities than other flavonols (Jeffery *et al.,* 2008).

Phenolic composition and the development of wine colour

The process of vinification facilitates both the rapid, partial extraction of grape phenolics into dilute alcohol solution, but low pH and the presence of yeast-derived metabolites also initiates their conversion into derivatives, formation of non-covalent associations and oxidative degradation (Cheynier *et al*., 2006). Therefore, any investigation of the vinification process provides a limited snapshot of the many reactions under way at a point in time. A key process taking place during vinification and wine ageing that was investigated in the present study is the development of bisulphite-resistant pigments. In aqueous solution, the flavylium structure of anthocyanins reacts with bisulphite ions, rendering a colourless form, which is a similar reaction to the formation of the hydrated hemiketal form that occurs at pHs higher than 2 (Cheynier *et al.*, 2006). Pigments resistant to this hydration reaction are anthocyanin derivatives such as vitisins and polymeric pigments. The present study showed small differences in the concentration of bisulphite-resistant pigments, which was increased in the wines of treatments receiving no or minimal irrigation. The associated changes in specific wine phenolic compounds were unclear; in the first season estimated bisulphite-resistant pigments reflected changes in wine vitisin A concentrations whereas, in the second season, there was a stronger relationship with wine polymeric pigment concentration due to a greater variability in wine tannin concentration between treatments. The ageing process gave

additional complexity to the interpretation of the data, such that greater losses of monomeric anthocyanin and increases in polymeric pigments were associated with the deficit-irrigation treatments, while vitisin A was increased for all treatments. It would therefore appear that a higher concentration of grape-derived anthocyanin might initially contribute to accelerated formation of bisulphite-resistant pigments, but that loss of free anthocyanin is enhanced over time. The potential exists that the loss of free anthocyanin from these treatments reflects its partial conversion to stable anthocyanin derivatives, which, in the data presented, was only confirmed by increases in polymeric pigment, but not in either of the vitisins studied.

The PCA of the 18-month-aged wines from the first season indicated that, despite minimal treatment differences in wine phenolic composition, there was significant co-correlation of both vitisin and polymeric pigment concentration with wine colour density and bisulphite-resistant pigments, while free monomeric anthocyanin was poorly correlated. Therefore, although seasonal variation in wine phenolic composition was found in the current study, the literature indicates that both vitisin and polymeric pigment concentration are likely to strongly contribute to wine colour at wine pH (Bakker & Timberlake, 1997, Malien-Aubert *et al,*. 2002). However, the estimate of bisulphite-resistant pigments reflects a greater variety of anthocyanin derivatives than those determined in the present study (Hea *et al*., 2006; Fulcrand *et al.,* 2006). The conditions which influence the formation of bisulphite-resistant pigments are both phenolic composition and wine pH, with lower wine pHs facilitating aldehyde polycondensation and the cleavage of inter-flavan bonds of the flavan-3-ols (Fulcrand *et al.,* 2006). The possibility that differences in wine pH in the wines produced from the different irrigation treatments in 2005/2006 might also have contributed to the formation of bisulphite-resistant pigments cannot be overlooked. Other studies have shown increases in bisulphite-resistant pigments in wines produced from deficit-irrigation treatments that were not clearly related to either grape or wine phenolic composition (Bindon *et al*., 2008; Chalmers *et al,*. 2008). This study has been unable to distinguish the observed differences in wine colour between treatments during fermentation and through an 18-month-ageing period from unknown 'matrix' effects. Since differences in TA and malic acid concentration were observed for the wines in the first season of the study, which were tracked though fermentation and ageing, it is not possible to exclude the contribution of these variables in the development of wine colour.

It is evident from the results of the present study that the factors affecting phenolic extraction, and the processes of their conversion both during fermentation and wine ageing are complex, thus it is not possible to draw direct relationships between grape phenolic composition and the resultant wine composition in this case. However, the data show that increases in the concentration of grape anthocyanins related to water deficit can be associated with increases in the colour and bisulphite-resistant pigments of aged wines.

CONCLUSIONS

This study has demonstrated that the application of water deficit can significantly influence the concentration of skin-derived anthocyanins and flavonols in grapes, primarily through an alteration in skin to flesh ratio. However, the process of fermentation which involves both phenolic extraction, conversion and potentially significant losses of extracted phenolics is a complex process, with anthocyanin extraction limited by unknown factors, possibly cell wall composition and integrity. As a result, a strong correlation between grape and wine phenolic composition was not observed, but increases in grape anthocyanin concentration were associated with the formation of higher levels of bisulphite-resistant pigments in wines. This has implications for the promotion of the long-term stability of colour in aged wines. Future research which aims to characterise grape compositional responses to water deficit should include factors which might potentially affect extraction, namely the influence of grape cell wall composition on phenolic extractability.

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