

The Effect of Half Plunging and No Plunging as Alternative Winemaking Techniques on Phenolic Extraction and Pigment Composition of Wine

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The extraction of phenolics (anthocyanins, total phenolics and tannins) and pigmented tannin evolution in Merlot red wines made using three different winemaking techniques were evaluated over one year. Traditional maceration (TM) (two plunges per day), half plunging (half) and no plunging (NP) methods were compared using triplicate 12 kg vinification. No pre-fermentation maceration occurred and a 15-day fermentation and post-fermentation period was used. The wines were analysed daily during the 15-day maceration period and regularly after pressing during the next 11 months. Phenolic, tannin and colour measurements were analysed using UV/Vis spectral readings processed using the AWRI WineCloud™. At the end of the 15-day maceration, total phenolics, total pigment and free anthocyanins were significantly lower in concentration in the TM treatment compared with NP. The tannin concentration also was significantly lower from days 10 to 12. Pigmented tannin was consistently at higher concentrations in the TM treatment, although not significantly so, during maceration. The significant differences were maintained as the wine developed over the next 11 months; however, the NP trial wine displayed increased pigmented tannin development compared with the TM, being significantly higher in pigmented tannin from about day 90. Higher colour and phenolic extraction in the NP trial led to higher stable colour development in the resulting wines, with the winemaking having an important effect in modifying the pigment profile of the wines. General wine quality parameters did not differ significantly between the three treatments.

INTRODUCTION

Phenolic compounds extracted from grapes are important for their contribution to the quality of red wine colour, mouthfeel and stability. Traditionally, phenolics are extracted during fermentation and maceration, processes usually involving mechanical mixing of the must, including the grape skins and seeds. It is believed that the mixing process during maceration enhances the diffusion and movement of phenolic compounds from the skin and seeds into the juice/wine (Sacchi *et al.*, 2005; Ribereau-Gayon *et al.*, 2006). In a review of the effects of winemaking techniques on phenolic extraction in red wines, Sacchi *et al.* (2005) concluded that fermentation temperature, thermo-vinification, must freezing, saignee, pectolytic enzyme treatments and extended maceration increased phenolic concentration, whereas SO₂ levels and cold soak treatments had little or no lasting effects or led to a decrease in phenolic levels.

Flavanoid phenolic compounds, including anthocyanins, flavan-3-ols and flavonols, are found in the skins and seeds of grape berries. Anthocyanins are fundamental components of young red wine colour and are extracted from the grape

skins, along with flavonols such as quercetin and myricetin, which contribute to wine bitterness. Monomeric flavan-3-ols, such as catechins, are extracted from seeds and polymerise to form proanthocyanidin structures, which contribute to wine astringency (Gawel, 1998; Cheynier *et al.*, 2006; Fulcrand *et al.*, 2006). Extended maceration post-fermentation is often encouraged to enhance the extraction of skin and seed tannins. These tannins, in the presence of high levels of anthocyanins and with controlled introduction of oxygen, lead to the development of ethanal-linked polymerisation structures that incorporate anthocyanins as end units (Es-Safi *et al.*, 1999; Cheynier *et al.*, 2006). These structures are important for stabilising colour in the resulting red wines (Vrhovsek *et al.*, 2002; Sacchi *et al.*, 2005). Traditionally, pump overs, punching down, roto fermenters and air pulsing have been used to encourage the mixing during and after the fermentation maceration time. Punching down and roto treatments yielded the highest total polyphenol concentrations and the pump-over treatment the lowest concentrations in a study by Marais (2003). Temperature, maceration length, pectolytic enzyme addition and alcohol

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concentration are other factors that affect the final phenolic composition of the wine (Gao *et al.*, 1997; Sacchi *et al.*, 2005; De Beer *et al.*, 2006).

Winemakers deeply value coloured and fuller bodied red wines (Lattey *et al.*, 2010). The factors and conditions detailed above are used by winemakers in an attempt to produce wines displaying these desired characteristics. However, grape solid and wine contact time seems to be the main factor involved (Kovac *et al.*, 1992; Gil *et al.*, 2012). Gil *et al.* (2012) and Kovac *et al.* (1992) found that proanthocyanidin extraction from seeds increased throughout maceration. Cerpa-Calderon and Kennedy (2008) found that skin proanthocyanidins from treatments with greater degrees of berry crushing reached higher proportions of total extracted proanthocyanidins earlier than treatments with less berry crushing. They also found that skin anthocyanidins were extracted earlier than seed proanthocyanidins. Casassa *et al.* (2013) discovered that the tannin content of wine produced with extended maceration (30 days' skin contact) was derived mainly from seed tannins, whereas tannins from traditional ten-day skin contact wines had balanced proportions of seed and skin tannins. Anthocyanin concentration was lower in wines with extended maceration because the synthesis of longer polymer pigments during post-fermentation maceration removed monomer anthocyanins faster than in the control. In a qualitative analysis of tannins in grape and seed/skin pomace samples post-maceration, Casassa *et al.* (2013) suggested that a rebinding or incorporation of previously extracted seed tannins into skin tannins occurred during maceration. The opposite process, of skin tannins incorporated into seed tannins, was not observed.

In a comprehensive study of the delayed extraction of anthocyanins and separation of must, Bosso *et al.* (2009) reported that, at racking, the wines produced with the delayed extraction techniques were higher in colour, total and monomeric anthocyanins and total phenolics than the wines produced by traditional red wine maceration techniques. These differences remained after seven months of ageing. In another study on delayed extraction of anthocyanins, by Gonzales-Nevez *et al.* (2010), the pigment in the control wines was higher at all times, but after one year the total pigment content of the wines showed no significant difference between traditional maceration and delayed extraction.

Winemakers may wish to employ red wine maceration techniques that result in higher concentrations of anthocyanins. A panel of experienced wine tasters (winemakers) considered higher quality red wines to be darker in overall colour, bigger in mouthfeel, hotter and astringent (Lattey *et al.*, 2010). Kilmister *et al.* (2014) found that fruit anthocyanin concentration correlated with wine tannin concentration, wine colour and polymeric pigment. They proposed that anthocyanin concentration may be a key component for increasing tannin solubility and extraction into wine, and for the formation of polymeric pigments. This is in agreement with previous suggestions by Singleton and Trousdale (1992). In a sensory and chemical study of phenolic polymers in red wines, Weber *et al.* (2013) reported that astringency in red wines decreased with increasing incorporation of anthocyanins into polymeric structures, and that high molecular weight pigmented polymers evoked the

lowest astringency ratings.

In view of this evidence for a key role for phenolics in wine composition and quality, this study aimed to evaluate further the effect of different winemaking techniques on the phenolic and pigment composition of Merlot red wines. The wines were made using three different maceration regimes: traditional maceration, half usual plunging and no plunging at all. Wines were then analysed over an extended period to establish compositional benefits or outcomes.

MATERIALS AND METHODS

Three 12 kg replicates were used for each winemaking procedure. Hawke's Bay Merlot grapes from the 2013 vintage at Esk Valley Estate were destemmed and crushed with an Enoveneta N15T391 (Italy) machine and, after homogenising of the must, divided into nine 15 L capacity plastic fermentation containers with carbon dioxide gas cover (three replicates of three treatments). Must composition analysis was recorded as 23.5°Brix, 4.8 g/L tartaric acid equivalent titratable acidity, and pH 3.43, using classical methods (Iland *et al.*, 1993). No pre-fermentation SO₂ additions were made. Traditional maceration (TM), half plunging (half) and no plunging (NP) were the winemaking techniques compared, with the TM used as the control treatment. Inoculation with Lalvin ICV D254 yeast was carried out at 250 mg/kg, and Lallemand Fermaid A plus diammonium phosphate was added once fermentation started, in three steps of 0.1 g/kg each after a 2°Brix change, and then again at 16°Brix and 12°Brix. Malolactic bacteria were inoculated at the manufacturer's rate using CHR Hansen CH16 with nutrients via Lallemand Optimalo Plus at a rate of 0.1 g/kg when 3°Brix was reached.

The control wines (TM) were made using classical fermentation and maceration on the skins for 15 days, with plunging undertaken as 12 plunges each time, twice per day for each of the 15 days. The temperature of the fermentations was maintained at 26 to 33°C in a temperature-controlled room set at 26°C to maintain realistic commercial fermentation temperatures for the 15 days of maceration. The half plunged wines (Half) were made identically to the TM wines, except the 12 plunges each twice a day did not commence until day 7 and continued for the rest of the 15 days of maceration. The NP wines were also treated identically to the TM wines; however, no plunging took place at all over the entire 15-day maceration period, other than when the addition of nutrients and malolactic bacteria took place. At these points a small plastic tube was carefully inserted through the skin cap to allow the introduction of the additive, followed by careful mixing using a small rod through the tube to allow stirring of the fermenting liquid under the cap. Sampling took place after such stirring, ensuring that a homogenous solution/wine composition was available for extraction.

At the time of pressing, free-run wine was drained through the stainless steel screen of an Enotecnica Pillan (Vincenza, Italy) 20 kg capacity water bladder basket press, followed by pressing to 2 bar pressure, and the press wine was combined with the free run in all trials. Wines were stored in plastic containers until malolactic fermentation was completed, at which time 50 mg/L SO₂ was added following racking. All wines were stored at 13°C in closed plastic 5L

containers, with free SO₂ maintained at 25 mg/L.

Each wine trial and replicate were analysed, using classical methods (Iland *et al.*, 1993) 12 months after vinification, and mean values for each treatment were calculated for alcohol, titratable acidity, pH, volatile acidity, residual sugar, and free and total SO₂.

Wine total phenolics, total tannin, total pigment (total red colour), free anthocyanins and pigmented tannins were evaluated using the Australian Wine Research Institute WineCloud™ (AWRI, 2012) using a Beckman Coulter DU 730 Life Science UV/Vis Spectrophotometer. Analyses were based on 250, 270, 280, 290, 315, 320, 520 and 520 (SO₂ + buffered) nm UV-Vis spectral absorbance readings that used the calibrations and the database maintained by AWRI. This data base is made up of more than 8 000 mainly Australian and New Zealand finished and fermenting wine sample spectral characteristics. The tannin assay is based on the methyl cellulose precipitable (MCP) tannin assay (Sarneckis *et al.*, 2006) and is incorporated into the WineCloud™, along with measurements of wine pigment (total anthocyanins), free (SO₂ bleachable) anthocyanins, pigmented (non-bleachable) tannins and total phenolics. Damberg *et al.* (2012) developed a predictive algorithm based on the MCP method using UV-Vis spectral measurements for calculating tannin concentration. Analytical data developed using this method can be related directly to measurements made using the Adams-Harbertson (A-H) protein precipitation tannin analysis method (Hagerman & Butler, 1978). The MCP and A-H methods have been compared (Harbertson *et al.*, 2003; Mercurio & Smith, 2008; Harbertson & Downey, 2009; Chittenden & Smith, 2011) and the results show that tannin concentrations generated by the MCP method (as utilised by the WineCloud™) are highly correlated with those generated by the A-H assay. The WineCloud™ incorporates the AWRI's standard method (Iland *et al.*, 2004) for the measurement of grape anthocyanins and phenolics. Relative colour and pigment data are distinguished via the WineCloud™ on the basis of these standard methods using the absorbances at the described wavelengths to generate the relative data presented. Algorithms for this purpose are based on results from analyses according to the modified Somers and Evans assay (1977), as reported by Mercurio *et al.* (2007).

The statistical analysis of all the data was expressed as the arithmetic average of three replicates. ANOVA was

conducted using Minitab 15.1.0.0. ANOVA attempts to describe the relationship between several means, providing a measure of difference between treatments within the same characteristics. Tukey's procedure was used to indicate significance at the *p* < 0.05 level.

RESULTS AND DISCUSSION

Table 1 shows the wine composition of the three treatments 11 months after winemaking. Given that each trial used identical homogenised grape must, variations in alcohol, titratable acidity, pH, colour and polyphenol composition of the wines for the three different treatments would have to be due to the different treatments applied.

An expected increase in wine volatile acidity (VA) due to bacterial action in the undisturbed cap of the Half and NP treatments compared to the TM treatment was not observed, indicating that initial VA production due to cap drying and exposure to air was not an issue. Alcohol and total SO₂ levels were not significantly different for the three treatments. Overall total SO₂ concentrations were high in all treatments due to the inherent difficulties of the large surface-area-to-volume ratios in small-scale wine making. All fermentations went 'dry', with TM showing lower residual sugar than the Half and NP treatments. Both delayed extraction methods produced wines with slightly higher TA and lower pH than the TM trial. Table 1 shows that the TA and pH of the TM treatment were significantly lower compared with NP. This is in agreement with the findings of a study by Bosso *et al.* (2011), in which a trial using mechanical effects on a floating cap by pump overs was compared with a submerged cap. Pump overs led to increased extraction of K⁺, which resulted in more precipitation of potassium bitartrate. The possible increased precipitation of potassium bitartrate in our TM trial resulted in a lower eventual TA compared with the Half and NP treatments. This possible increase in precipitation of potassium bitartrate along with extracted anthocyanins is referred to elsewhere in this discussion.

Tannin concentrations for the treatments are reported in Table 2, and show that tannins increase as ethanol concentration increases over the maceration period, as previously reported by Canals *et al.* (2005).

The NP treatment consistently showed the highest extraction of tannins during this period, although this was

TABLE 1
General composition of Merlot wines at 12 months.

Variety	Winemaking	Titratable acidity	pH	Volatile acidity
Merlot	TM	5.52 b ± 0.06	3.53 b ± 0.01	0.310 a ± 0.012
	Half	5.77 a ± 0.03	3.51 a ± 0.01	0.354 a ± 0.016
	NP	5.75 a ± 0.10	3.51 a ± 0.01	0.354 a ± 0.036
Variety	Winemaking	Alcohol	Residual sugars	Total SO ₂
Merlot	TM	12.8 a ± 0.3	1.0 a ± 0.0	151 a ± 15
	Half	13.0 a ± 0.4	1.8 b ± 0.1	175 a ± 17
	NP	13.1 a ± 0.3	2.0 c ± 0.0	151 a ± 9

Mean values (n = 3) ± standard deviations. Values with the same letter in the same columns mean that there are no statistically significant differences according to a Tukey test (*p* < 0.05). Titratable acidity expressed as g/L tartaric equivalent; volatile acidity as g/L acetic acid equivalent; alcohol as % (v/v); residual sugar in glucose g/L; total SO₂ in mg/L. TM: traditional maceration. Half: half plunged. NP: not plunged.

significant on day 10 and day 12 only. The TM treatment followed similar levels to the NP until day 8, from when it declined steadily, whereas the NP tannin concentration merely flattened at this stage. By day 14 no significant tannin extraction differences between the two trials were observed. Table 3 shows that the extraction of total phenolics and levels in the NP treatment were significantly higher than TM on 11 of the 14 sampling days.

Concentrations in total phenolics followed a similar steady increase, as did tannins, over the first 3 days. From day 5 onwards the NP trial showed higher total phenolics than the TM trial. During this period the rate of increase in total phenolics for both treatments steadied and even showed a slight decline by day 14. This compares with the findings of Marais (2003) and Bosso *et al.* (2011), with similar levelling and declines in total flavonoids and tannins reported during the later stages of maceration. Total pigment levels are shown in Table 4 and demonstrate the classic reported pattern of extraction during maceration, peaking at or about day 6 for both the TM and NP treatments (Ribereau-Gayon *et al.*, 2006; Chittenden, 2008).

However, NP total pigment was significantly higher than that of the TM treatment from day 3 right through to day 14, indicating that the NP regime favoured the extraction of anthocyanins more than the TM trial. Although fermentation temperatures measured as juice/fermenting

liquid temperatures were similar for each treatment over the maceration period, the completely undisturbed cap of the NP trial was at higher cap temperatures than the TM trial for greater periods. Cap temperatures were noted as greater than fermenting juice temperature in each trial, and in the NP trial this higher cap temperature was not "cooled" at each plunging episode as it was in the TM trial. This may have resulted in higher anthocyanin extraction. Gao *et al.* (1997) found that increased fermentation temperatures gave rise to increased monomeric anthocyanin extraction. Very reactive anthocyanins may also be being removed from the TM treatment to a greater extent compared to the NP treatment. This is in agreement with the data in Table 5, which shows the levels of free monomeric anthocyanins during the fermentation maceration period.

Again, the NP trial consistently showed significantly higher levels of free anthocyanins than the TM trial through days 3 to 14. The oxidation of anthocyanins has previously been proposed to be one of the major reasons for the apparent loss of anthocyanins during red wine maceration (Bosso *et al.*, 2009; 2011). Anthocyanins are very reactive phenolic compounds and, as they are extracted during the early aqueous stage of fermentation, they can become oxidised due to catalytic polyphenol oxidase oxidation and coupled oxidation due to O_2 contact with the fermenting must.

Table 6 shows that, during the 14-day maceration period,

TABLE 2
Tannins during maceration.

	Day 1	Day 2	Day 3	Day 4	Day 5
TM	0.49a \pm 0.03	0.72a \pm 0.10	1.03a \pm 0.06	1.39a \pm 0.14	1.64a \pm 0.19
NP	0.47a \pm 0.01	0.53a \pm 0.18	1.15a \pm 0.04	1.42a \pm 0.04	1.92a \pm 0.12
	Day 6	Day 7	Day 8	Day 9	Day 10
TM	2.09a \pm 0.18	2.09a \pm 0.06	2.04a \pm 0.14	1.98a \pm 0.28	1.81b \pm 0.10
NP	2.17a \pm 0.03	2.20a \pm 0.20	2.05a \pm 0.11	2.15a \pm 0.24	2.28a \pm 0.15
	Day 11	Day 12	Day 13	Day 14	
TM	1.78a \pm 0.06	1.82b \pm 0.10	1.76a \pm 0.11	1.83a \pm 0.16	
NP	2.27a \pm 0.17	2.19a \pm 0.20	2.15a \pm 0.17	1.98a \pm 0.13	

Mean values \pm standard deviations. N = 3. Tannins in g/L epicatechin equivalent (AWRI WineCloudTM). Values with the same letter in the same column on the same day mean there are no statistically significant differences according to the Tukey test (p < 0.05). TM: traditional maceration. NP: not plunged.

TABLE 3
Total phenolics during maceration.

	Day 1	Day 2	Day 3	Day 4	Day 5
TM	37.39a \pm 1.10	46.84a \pm 3.29	48.16b \pm 2.43	54.40b \pm 4.16	57.75b \pm 7.27
NP	37.30a \pm 0.49	42.04a \pm 4.50	58.64a \pm 3.29	63.30a \pm 2.22	74.64a \pm 2.47
	Day 6	Day 7	Day 8	Day 9	Day 10
TM	67.19b \pm 2.90	63.78b \pm 1.85	61.44b \pm 4.08	65.83a \pm 4.00	61.63b \pm 1.76
NP	77.11a \pm 0.62	74.64a \pm 3.48	72.10a \pm 1.64	73.67a \pm 3.65	76.54a \pm 1.75
	Day 11	Day 12	Day 13	Day 14	
TM	61.31b \pm 0.42	62.37b \pm 1.82	60.93b \pm 2.47	63.23b \pm 3.08	
NP	75.54a \pm 3.95	75.12a \pm 4.95	74.78a \pm 2.33	70.19a \pm 2.33	

Mean values \pm standard deviations. N = 3. Total phenolics in AU (AWRI WineCloudTM). Values with the same letter in the same column on the same day mean there are no statistically significant differences according to the Tukey test (p < 0.05). TM: traditional maceration. NP: not plunged.

TABLE 4

Total pigments during maceration.

	Day 1	Day 2	Day 3	Day 4	Day 5
TM	21.71a ± 0.85	31.40a ± 2.16	30.60b ± 1.36	32.13b ± 2.83	32.11b ± 4.60
NP	21.81a ± 0.72	28.25a ± 3.28	37.91a ± 2.77	38.73a ± 1.53	44.25a ± 1.90
	Day 6	Day 7	Day 8	Day 9	Day 10
TM	35.77b ± 1.46	31.35b ± 1.11	29.02b ± 2.21	29.63b ± 2.00	26.04b ± 0.95
NP	43.74a ± 0.38	39.51a ± 2.71	36.58a ± 0.98	36.53a ± 1.76	37.59a ± 0.98
	Day 11	Day 12	Day 13	Day 14	
TM	25.11b ± 0.33	25.50b ± 1.26	23.73b ± 0.93	24.48b ± 1.69	
NP	35.63a ± 2.28	35.75a ± 2.89	35.02a ± 1.88	32.25a ± 1.01	

Mean values ± standard deviations. N = 3. Total pigment in AU (AWRI WineCloud™). Values with the same letter in the same column on the same day mean there are no statistically significant differences according to the Tukey test (p < 0.05). TM: traditional maceration. NP: not plunged.

TABLE 5

Free anthocyanins during maceration.

	Day 1	Day 2	Day 3	Day 4	Day 5
TM	20.71a ± 0.82	30.07a ± 2.05	28.79b ± 1.36	30.31b ± 2.80	30.22b ± 4.45
NP	20.59a ± 0.71	26.93a ± 3.27	35.93a ± 2.50	36.68a ± 1.48	41.78a ± 1.93
	Day 6	Day 7	Day 8	Day 9	Day 10
TM	33.35b ± 1.38	29.04b ± 1.05	26.72b ± 2.12	27.00b ± 1.97	23.64b ± 0.86
NP	41.40a ± 0.38	37.27a ± 2.45	34.48a ± 1.00	34.33a ± 1.63	35.23a ± 0.95
	Day 11	Day 12	Day 13	Day 14	
TM	22.52b ± 0.30	22.81b ± 1.10	21.14b ± 0.95	21.43b ± 1.39	
NP	33.19a ± 2.14	33.16a ± 2.63	32.41a ± 1.89	29.54a ± 0.97	

Mean values ± standard deviations. N = 3. Free anthocyanins in AU (AWRI WineCloud™). Values with the same letter in the same column on the same day mean there are no statistically significant differences according to the Tukey test (p < 0.05). TM: traditional maceration. NP: not plunged.

levels of polymerised pigment (pigmented tannin) did not differ significantly between the NP and TM trials.

Some differences occurred at day 5, when the NP trial showed a significantly higher pigmented tannin level than the TM trial. This difference was not maintained and, by day 14, TM showed higher pigmented tannin levels than NP, although the difference was not significant. Early oxygen exposure results in polyphenol oxidase enzymatic oxidation in the fermenting must, but more importantly, mechanical cap management such as pumping over and plunging (as with the TM treatment) can give rise to higher oxygen exposure of the bulk of the must compared with the no plunging technique, giving rise to the production of greater amounts of H₂O₂ (Waterhouse & Laurie, 2006). This strong oxidant is capable of reacting with all oxidisable substrates in solution. One such reaction leads to ethanol being oxidised to ethanal (acetaldehyde). Thus the treatment with plunging, TM, would logically have a higher production of H₂O₂ than the NP treatment, and a result of this is demonstrated in the higher rate of polymerisation expressed as pigmented tannins found during the maceration period (although only significant on day 9). This agrees with the findings of Bosso *et al.* (2011), who found a submerged cap (SC) maceration initially showed lower acetaldehyde concentrations than the floating cap (FC) that experienced pump overs. However, as maceration time proceeded they found that the trend for acetaldehyde production was similar in the two trials. The early pump overs in the Bosso *et al.* (2011) trial were

conducted in the absence of air, whereas in our study all the plunging was conducted with aeration. This may have caused a higher oxygen uptake in the TM trial compared with the NP trial, which did not occur in the FC trial. This led to the higher level of pigmented tannins during maceration for the TM compared with the NP, which is similar to the findings when comparing FC with SC (Bosso *et al.*, 2011).

The results found in this study, in which the TM trial has lower total phenolics, tannin and anthocyanin concentrations compared with the NP trial throughout much of the maceration period, are similar to those of the Bosso *et al.* (2011) study, which found that FC maceration resulted in lower extracted anthocyanins than SC maceration. However, the FC trial involved pump overs, whereas our NP trial involved no mechanical cap disturbance. The result is that the NP trial would have extracted less K⁺ from the skins compared with the TM treatment, leading to a decreased loss of anthocyanins due to anthocyanins being incorporated and lost in potassium bitartrate precipitation. Bosso *et al.* (2011) also found the FC with pump over had lower tannin extraction and total flavonoids overall at the end of maceration than the SC with fewer pump overs, again a similar finding to that of the current study.

Figs 1 to 5 show the continued development of the five analysis parameters over the next 11 months and also include data for a middle treatment of partially delayed extraction called Half, where plunging was not started until day 6 of the 14-day maceration period. Tannin, total phenolics, total

TABLE 6
Pigmented tannins during maceration.

	Day 1	Day 2	Day 3	Day 4	Day 5
TM	0.60b ± 0.02	0.80a ± 0.07	1.08a ± 0.07	1.09a ± 0.03	1.14b ± 0.10
NP	0.73a ± 0.01	0.79a ± 0.01	1.18a ± 0.17	1.23a ± 0.07	1.48a ± 0.02
	Day 6	Day 7	Day 8	Day 9	Day 10
TM	1.45a ± 0.05	1.39a ± 0.05	1.38a ± 0.06	1.58b ± 0.04	1.44a ± 0.08
NP	1.41a ± 0.05	1.34a ± 0.16	1.26a ± 0.05	1.32a ± 0.14	1.42a ± 0.06
	Day 11	Day 12	Day 13	Day 14	
TM	1.55a ± 0.02	1.61a ± 0.10	1.55a ± 0.05	1.83a ± 0.18	
NP	1.46a ± 0.09	1.56a ± 0.16	1.56a ± 0.01	1.63a ± 0.07	

Mean values ± standard deviations. N = 3. Pigmented tannins in AU (AWRI WineCloud™). Values with the same letter in the same column on the same day mean there are no statistically significant differences according to the Tukey test (p < 0.05). TM: traditional maceration. NP: not plunged.

pigment and pigmented tannin were significantly higher in the NP treatment compared with the other two treatments over much of the post-maceration wine ageing period, such that at day 341, all of the measured analytes were at significantly higher concentrations in the NP wine. In all cases except for pigmented tannins, the intermediate of half plunging contained the middle concentration of measured phenolic compounds and was significantly higher than the TM treatment.

Total phenolic concentrations for all three treatments levelled off after maceration, as would be expected, with no further extraction possible (Fig. 1). Within this pool of total phenolics, tannin levels continued to rise until points between 90 and 137 days and then became steady for all three treatments (Fig. 2). The increases in tannin over this time are due to ongoing phenolic polymerisation processes in each of the three treatments. Overall, the total phenolic level for each treatment does not change significantly from day 50 to 200 (Fig. 1), but the proportion of phenolics existing as di- and tri-anthocyanidins and short polymeric pigments (SPP) (Harbertson *et al.*, 2003) has increased, giving rise to the higher tannin concentrations shown in Fig. 2. Note that the WineCloud™ tannin analysis result is based on the MCP (Mercurio & Smith, 2008) assay and removes more tannin material from the wine than the A-H assay, which does not precipitate the di- and tri-proanthocyanidins and SPP with the bovine protein BSA. This would indicate that polymerisation rates slowed or that competing tannin-removal reactions became significant compared to competing polymerisation reactions. The depolymerisation of ethanal-bridged tannins and the dehydration of an ethanal-flavan-3-ol adduct formed during ethanal-flavan-3-ol condensation, leading to p-vinylphenols and then to more complex compounds in which the ethanal bridges are rearranged, will contribute to lower concentrations of quantifiable tannins (Drinkine *et al.*, 2007). Pigmented tannin concentrations continued to rise until day 341 in all three treatments (Fig. 5), with the NP treatment showing the greatest increase. The formation of pigmented tannin in the NP trial increased at the end of maceration and the concentration became greater than that found in the other two trials between days 70 and 90.

Total pigment concentrations can also be interpreted as a crude measure of wine colour, and Fig. 3 shows a slow but

steady decline from days 5 and 6 for all treatments until after day 225, when the decline almost ceased. Colour stability for each of the three treatments is similar, as can be seen by the very slight negative gradient over the period from day 223 to day 341, in Fig. 3. What this indicates is that the colour stability of the NP treatment is of the same order as that for the TM treatment. If the free anthocyanin concentrations are considered, a similar pattern is evident, where Fig. 4 shows that free anthocyanins in each of the three treatments peak at days 5 and 6; with the Half and TM peaking at lower levels than the NP, but all the treatments steadily declining in free anthocyanins from then on. These results are similar to the overall trends reported in a trial by Perez-Magarino and Gonzalez-San Jose (2004). The rate of decline is similar for all three treatments, again indicating similar colour stability conditions in the treatments between day 223 to day 341. This is also supported by the results of Bosso *et al.* (2009), in which a delayed extraction regime consisting of one short pump over per day until 6% alcohol was reached during fermentation, and then pump overs twice a day, led to significantly higher total anthocyanins and free anthocyanins than was determined in their conventional maceration trial. They reported similar results for total phenolics and proanthocyanidins, where the delayed extraction trial had significantly higher concentrations at racking compared to the conventional maceration reported in our study. Our results at seven months are similar to those of Bosso *et al.* (2009), who found that total anthocyanins, proanthocyanins, total phenolics and free anthocyanins remained significantly higher in concentration in the delayed extraction trial compared with conventional maceration. They explain the greater extraction as being due to the higher temperature and pH in the cap, giving rise to higher natural pectolytic enzyme activity in the cap, resulting in the higher polyphenolic content in the delayed extraction trial at racking. However, the Bosso *et al.* (2009) study reports that, at seven months, the delayed extraction trials had lower values of non-SO₂ bleachable pigments (pigmented tannin) compared with the conventional maceration treatment, and this is in contrast to our findings, shown in Fig. 5.

Higher pigmented polymer concentrations leading to increased colour stability have been claimed by Bosso *et al.* (2009) as the consequence of increased oxygen contact

Figure 1. Total Phenolics

n=3. Error bars ± 1 Std Dev

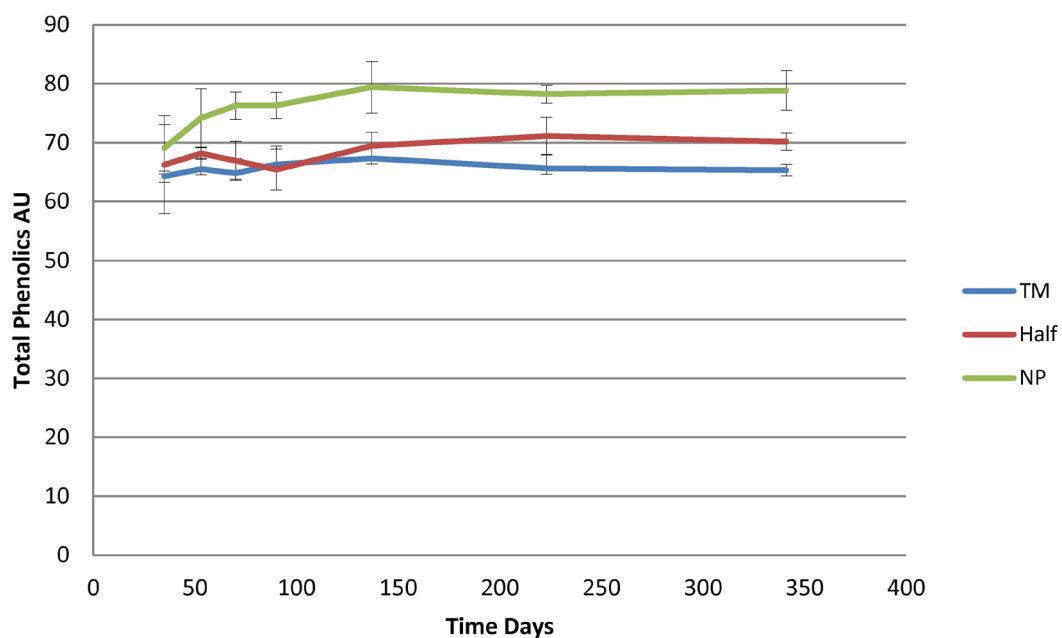


FIGURE 1

Total phenolic contents of the three winemaking procedures post-maceration, beginning at day 35, expressed in absorbance units. TM: traditional maceration. Half: half plunged. NP: not plunged.

Figure 2. Tannin

n=3. Error bars ± 1 Std Dev

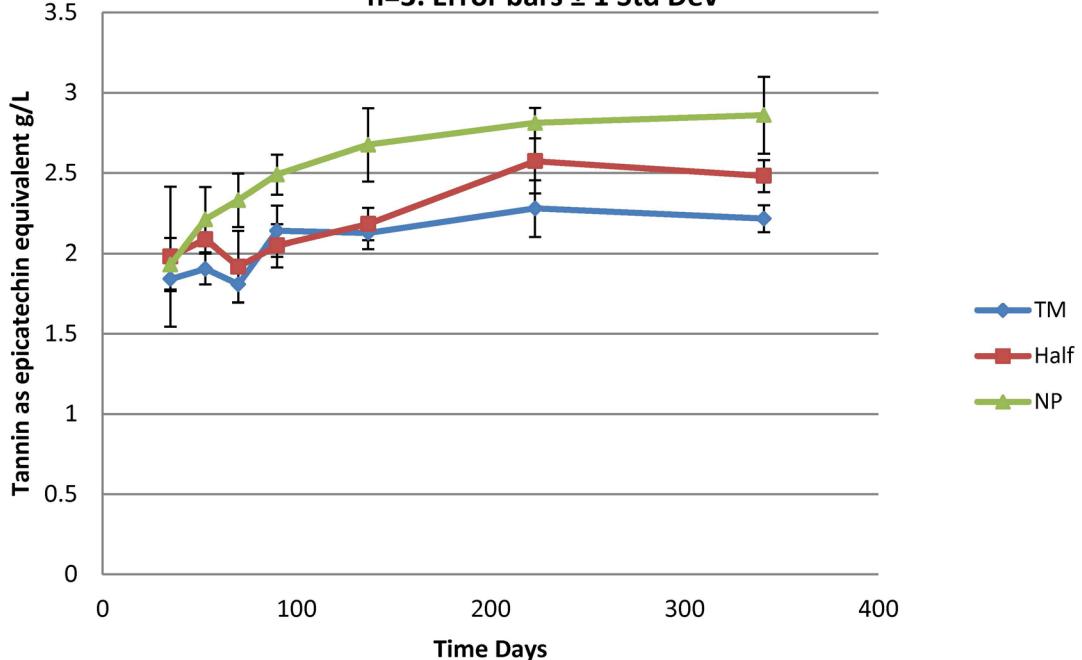


FIGURE 2

Tannin contents of the three winemaking procedures post-maceration, beginning at day 35, expressed as g/L epicatechin equivalents. TM: traditional maceration. Half: half plunged. NP: not plunged.

during fermentation. The improved extraction and retention of free anthocyanin monomers during fermentation and maceration in our 12 kg NP trial compared to the TM trial led to higher concentrations of free anthocyanins during wine ageing. This led to increased anthocyanin-tannin polymerisation reactions in the NP trial and therefore higher pigmented tannin concentrations, improving colour stability. Kilmister *et al.* (2014) explain the notion of an optimum ratio of anthocyanin to tannin in wine for polymeric pigment formation, or long-term colour stability. They also reported that higher anthocyanin concentrations in wines at pressing resulted in wines with higher pigmented polymer concentrations after ageing. Work by Oberholster *et al.* (2009) and Singleton and Trousdale (1992) also supports this interpretation. However, in a 2010 study, Gonzales-Neves *et al.* found that, in 70 kg treatments, TM wines showed the highest anthocyanin content at devatting and that differences were attenuated over time, so that total pigment content of the wines after one year of ageing showed no statistical difference to wines obtained by delayed extraction. They concluded that the pigment profile of the wines depended mainly on age rather than on differences determined by the alternative winemaking techniques evaluated. However, it should be noted that their delayed extraction involved pump overs, which may have influenced the result.

The higher pigmented tannin concentrations in the NP trial compared to TM can also be explained by co-pigmentation. The earlier observed higher concentration of free anthocyanins and total phenolics in the NP treatment led to higher co-pigmentation rates during maceration, and subsequently to higher levels of pigmented tannins. This is consistent with Boulton (2001), who cites views that co-pigmentation formation can increase the rates of

polymerisation, leading to pigmented tannins. Further support for this view can be found in studies by Mirabel *et al.* (1999) and Eiro and Heinonen (2002).

A further suggestion is that the NP treatment led to lower cell wall material suspension due to less mechanical breakdown of flesh and skin compared with the TM trial with plunging twice a day. This resulted in higher net extractions in the NP trial due to more adsorption of extracted proanthocyanidins onto cell wall material, followed by settling, in the TM treatment. This is supported by the report of Bindon *et al.* (2010), who cite work by Cerpa-Calderon & Kennedy (2008) that showed that the degree of berry crushing significantly altered skin proanthocyanidin extraction, with suspended cell wall material adsorbing the extracted proanthocyanidins.

Identical commercial, non-replicated 4 t trials were carried out during the same 2013 vintage on the same batch of Merlot grapes at Esk Valley Estate. Samples were collected and analysed by the same methods used for the microvinifications. The results showed that, after 90 days, comparable levels of tannin, pigmented tannin and total pigment were found in the resulting commercial trial wines. During maceration the extraction of these analytes showed similar trends between the NP and TM treatments and did not contradict the microvinification results over this time (see Tables 7, 8 and 9).

In an unpublished non-replicated commercial 2 t trial carried out by Musther (2010), using Merlot fruit processed at Mission Estate, Hawke's Bay. The no plunging regime resulted in wines with greater 520 nm absorbance, 280 nm absorbance and 520 nm absorbance after SO₂ bleaching, compared with a regime of one plunge per day during maceration.

Figure 3. Total Pigment- Colour

n=3. Error bars \pm 1 Std Dev

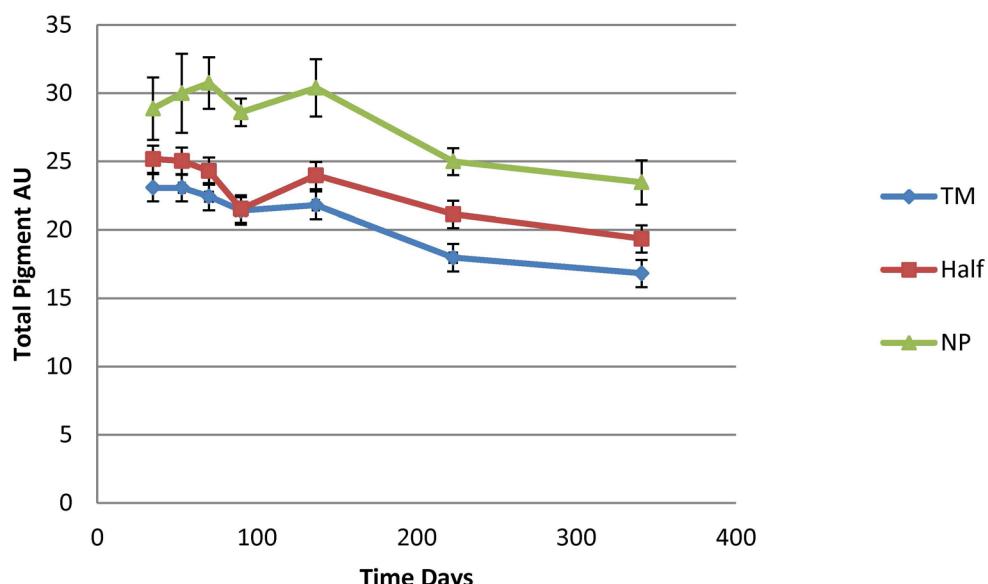


FIGURE 3

Total pigment content of the three winemaking procedures post-maceration, beginning at day 35, expressed in absorbance units. TM: traditional maceration. Half: half plunged. NP: not plunged.

TABLE 7

Commercial trial tannin – first 90 days.

	Day 5	Day 6	Day 7	Day 8	Day 9
TM	1.06	1.72	1.67	1.97	1.77
NP	1.01	0.74	1.3	1.55	1.31
	Day 10	Day 11	Day 12	Day 13	Day 14
TM	1.86	2.15	1.98	1.63	2.09
NP	1.53	1.35	1.80	1.67	1.91
	Day 53	Day 90			
TM	1.82	2.19			
NP	1.76	2.19			

N = 1. Tannins in g/L epicatechin equivalent (AWRI WineCloud™)

TABLE 8

Commercial trial pigmented tannins – first 90 days.

	Day 5	Day 6	Day 7	Day 8	Day 9
TM	0.66	1	0.82	0.74	0.97
NP	0.56	0.44	0.61	0.8	0.9
	Day 10	Day 11	Day 12	Day 13	Day 14
TM	0.98	1.04	1.05	0.85	1.22
NP	0.93	0.9	0.84	0.78	0.94
	Day 53	Day 90			
TM	1.83	2.27			
NP	1.78	2.11			

N = 1. Pigmented tannins in AU (AWRI WineCloud™).

TABLE 9

Commercial trial total pigment – first 90 days.

	Day 5	Day 6	Day 7	Day 8	Day 9
TM	31.11	39.58	34.78	35.29	34.99
NP	31.21	24.73	34.17	36.31	33.71
	Day 10	Day 11	Day 12	Day 13	Day 14
TM	34.02	35.39	33.86	28.66	32.54
NP	35.24	32.84	34.53	32.38	33.71
	Day 53	Day 90			
TM	29.02	28.86			
NP	30.19	30.24			

N = 1. Total pigment in AU (AWRI WineCloud™)

CONCLUSIONS

The extraction of phenolics (anthocyanins, total phenolics and tannins) and pigmented tannin evolution were significantly different in the winemaking processes studied here and varied as a function of time. At the end of maceration, total phenolics, total pigment and free anthocyanins were significantly lower in concentration in the TM treatment compared with the NP treatment. The tannin concentration also was lower, significantly so over days 10 to 12. Pigmented tannin was consistently at higher concentrations in the TM treatment, although not significantly so during maceration. The implication here is that the mechanical disruption of the fermenting wine cap extracted less phenolic material than no mechanical disruption of the cap at all. Development

over the next 11 months showed a continuation of these differences, although pigmented tannin levels in the NP trial increased at a greater rate than in the TM wine, to the extent that significantly higher stable colour was found in the NP wine from about day 90 onwards.

Lower extraction in the TM wines was partially due to increased exposure to oxygen during mechanical cap management, but also due to the increased disruption of berry solids, leading to more adsorption, oxidation and potassium bitartrate precipitation removing portions of the extracted phenolics. Higher anthocyanin and total phenolic concentrations in the NP wine may have led to higher co-pigmentation rates, which in turn led to greater pigmented

Figure 4. Free Anthocyanins

n=3. Error bars ± 1 Std Dev

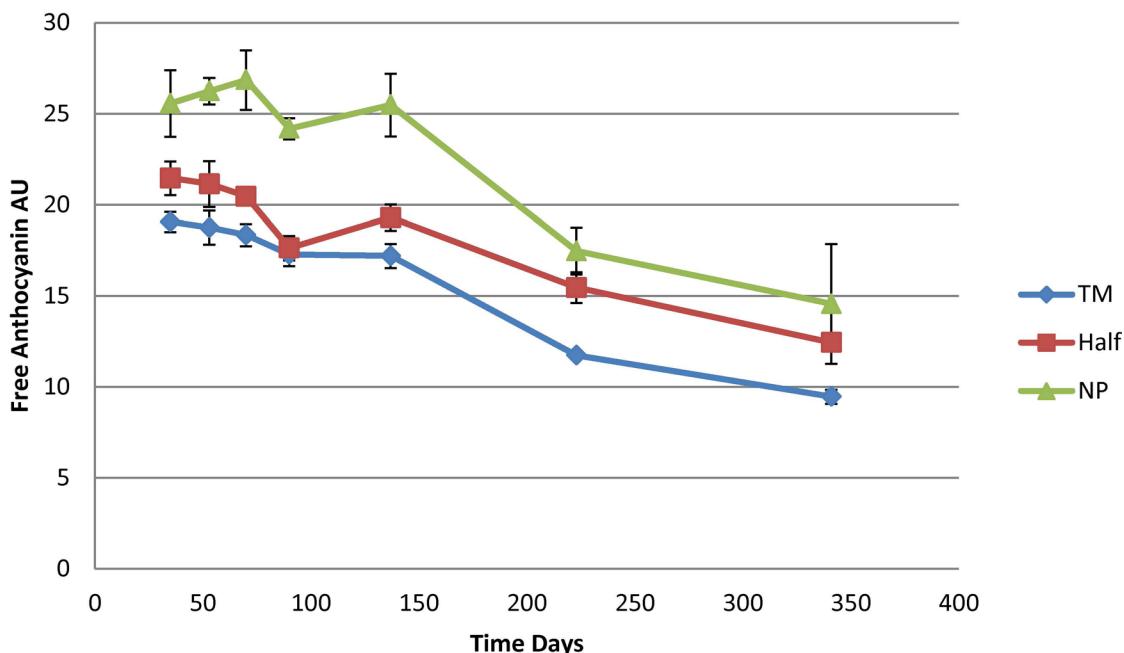


FIGURE 4

Free anthocyanin content of the three winemaking procedures post-maceration, beginning at day 35, expressed as absorbance units. TM: traditional maceration. Half: half plunged. NP: not plunged.

Figure 5. Pigmented Tannin

n=3. Error bars ± 1 Std Dev

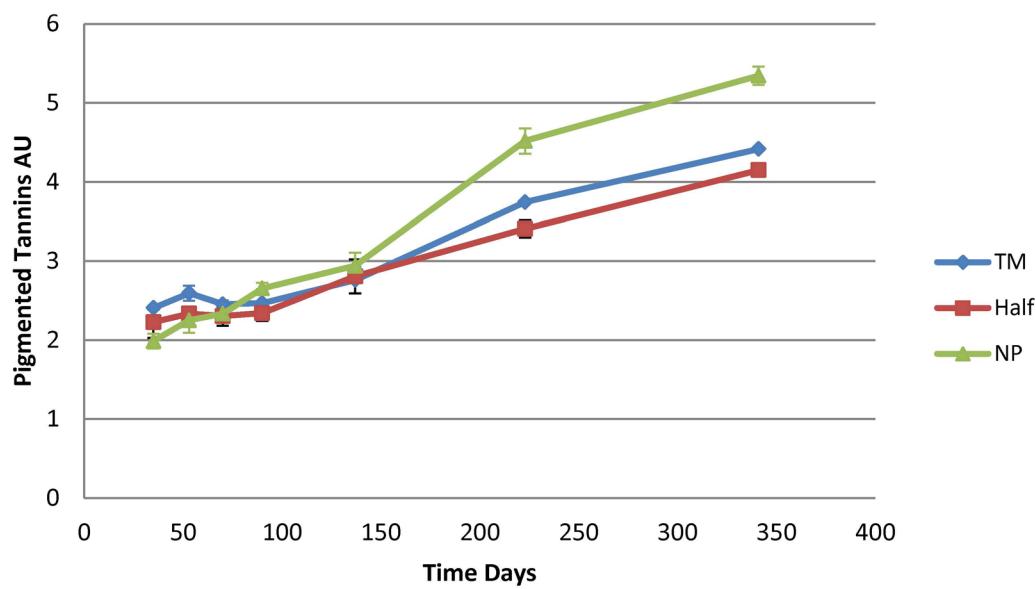


FIGURE 5

Pigmented tannin content of the three winemaking procedures post-maceration, beginning at day 35, expressed as absorbance units. TM: traditional maceration. Half: half plunged. NP: not plunged.

tannin development in the NP wine.

This study shows that the small-scale NP treatment produced Merlot wines with more colour and more stable colour after one year compared with the TM wine.

Winemaking resources are in high demand during vintage time. More efficient use of labour and resources would be enabled by reducing and even eliminating the need for red wine cap management during maceration. The wines

produced would also exhibit greater colour stability after one year. Further study on a commercial scale and with longer ageing time will help confirm these conclusions.

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