EFFECTS OF NITROGEN, POTASSIUM FERTILIZER, AND CLUSTERS PER VINE ON ANTHOCYANINS CONTENT IN CABERNET SAUVIGNON WINE

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Abstract

The experiment was carried out in a humid subtropical climate located in the south of China, in Xichang, Sichuan province during the 2005 and 2006 seasons. Eight years old, irrigated Cabernet Sauvignon vines were planted at Xichang Chia Tai Wine & Spirits Co., Ltd. Vine plants were spaced 1.25 m between 2 vines and rows were 2.0 m apart and oriented approximately north/south. The vines were trained in a vertical shoot positioned training system (VSP), and were bilaterally cordon-trained, spur-pruned, and the shoots were vertically positioned upright. The vines were irrigated by drip irrigation. Three different levels of N-K used in this experiment were 0-0, 100-20, and 200-60 g/plant and the 3 different levels of clusters per vine were 10, 20, and 30 clusters per vine. Treatments were applied to a vine plot, each replicated 6 times in a split plot, in which the main plots were for clusters per vine. Vine shoot lengths were maintained at 15 nodes by shoot trimming. It was found that the grape quality highly affected the wine quality. The 2006 pressed and aged wines were a better quality and color than 2005 because of lower yields. Titratable acidity (TA) was decreased by increasing the rate of N-K, but increasing the cluster levels increased the TA in both years. At the rate of N-K 100-20 g/vine the phenolic compounds in the grape and wine were lower than at other rates. For the pressed wine at the rate of N-K 100-20 g/vine there were lower phenolic compounds than at the other rates and increasing the cluster levels increased the degree of red pigment coloration and modified the wine color hue but decreased the total red pigments, total phenolics, and modified wine color density. In aged wine, increasing the cluster levels increased the estimated SO_2 resistant pigment but decreased the wine color density, degree of red pigment coloration, modified wine color density, modified wine color hue, and modified degree of red pigment coloration. Wine anthocyanins content was significantly different between the pressed and the aged wine in both years. The Cabanet Suavignon pressed wine had higher anthocyanins than the aged wine.

Keywords: Anthocyanins, phenolic compounds, pressed wine, aged wine

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Introduction

Anthocyanin profiles of grapes, determined by the relative proportions of the different anthocyanins, are characteristic for each grape variety. Moreover, concentrations of the different compounds can vary significantly within grape cultivars according to environmental conditions, including climate, soil, and vineyard management during the growing season and their extraction during maceration and fermentation (Boulton *et al*., 1996: Kennedy *et al*., 2006). Vineyard management can alter the proportion of pigments in red grapes, while winemaking techniques and wine aging influence the development of subsequent pigments in the wine (Arnous *et al*., 2002). Soil nutritional status affects all parts of the grapevine, from root growth and distribution through to shoot growth and grape composition. The grapevine requires 14 elements from the soil. Six macronutrients are needed in large quantities: Nitrogen (N), Phosphorus (P), Potassium (K), Sulfur (S), Calcium (Ca), and Magnesium (Mg). Eight micronutrients are needed in much smaller amounts: Iron (Fe), Manganese (Mn), Boron (B), Molybdenum (Mo), Copper (Cu), Zinc (Zn), Nickel (Ni), and Chlorine (Cl) (Dalton *et al*., 1988). All must be available as required for optimal vines and fruit. N, K, P, B and Zn are the most common deficiencies in vineyards. Crop level (yield/ vine) is one factor affecting wine grape quality. Since the capacity of a vine to ripen fruit depends largely on the rate of photosynthesis and accumulation of carbohydrates, it follows that a quantitative crop level may be related qualitatively to fruit composition. Of all factors affecting fruit ripening, crop level is the most likely one which growers can manipulate (Winkler *et al*., 1974). Crop level and grapevine light microclimate can be manipulated to influence fruit and wine composition (Chapman *et al*., 2004). Anthocyanins are present in the grape skin of red varieties, while tannins are normally present in the skin, seed, and stem. The concentration of anthocyanins in wines varies greatly according to the age of the wine and the variety. In young wines anthocyanins range from about 100 mg/l to 1500 mg/l (Ribé reau-Gayon *et al*., 2006). Anthocyanins are highly reactive compounds and the concentration decreases rapidly as wine ages and reaches levels of as little as 0-50 mg/l in aged wines (Ribéreau-Gayon *et al*., 2006). The initial color of red wines is mainly due to monomeric anthocyanins extracted from grape skins during maceration and fermentation, principally as flavylium cations (red) and quinoidal anhydro-bases (blue), and to the phenomenon of self-association and copigmentation with other phenols present in wine (i.e., flavanols, flavonols, and hydroxycinnamic acids) (Haslam, 1980). Thus the objective of this research was to find the effect of cultural practices (fertilizer and cluster management) on wine quality.

Materials and Methods

Experimental Design

Fertilizer and clusters per vine treatments were applied to a vine plot; each treatment was replicated 6 times in a split plot; the main plot was for cluster numbers and they were arranged in a randomized complete block design. Vine shoot lengths were maintained at 15 nodes by shoot trimming. Three fertilizer treatments and 3 clusters per vine treatments were applied and repeated in each year of the experiment.

Treatments

Fertilizer treatments:

F1 = Control (no fertilizer)

F2 = N-K (100-20 g/vine) split 3 times; the soil application of actual N-K were:- 1st (30-5 g/vine at bud break), 2nd (40-10 g/vine at bloom), and 3rd (30-5 g/vine 30 days after bloom).

F3 = N-K (200-60 g/vine) split 3 times; the soil application of actual N-K were:- 1st (60-20 g/vine at bud break), 2nd (80-20 g/vine at bloom), and 3rd (60-20 g/vine 30 days after bloom).

Urea and potassium sulfate, the sources of N and K, were applied in the rows under the treatment vines and incorporated into the soil.

Clusters per vine treatments:

C1 = 10 clusters per vine

C2 = 20 clusters per vine

C3 = 30 clusters per vine

Clusters per vine treatments were applied at veraison.

Population, Samplings and Location of Research

Vineyard: 8 years old, irrigated Cabernet Sauvignon vines were planted in Xichang, Sichuan province, China (27ºN, 102ºE, and 1650 m above mean sea level) were studied during the 2005 and 2006 seasons at Xichang Chia Tai Wine & Spirits Co., Ltd. Vine plants were spaced at 1.25 m between 2 vines and rows were 2.0 m apart and oriented approximately north/south. Vines were trained in a vertical shoot positioned training system (VSP) and were bilaterally cordon-trained, spur-pruned, and shoots were vertically positioned upright. Vines were irrigated by drip irrigation.

Wine Quality Analyses

After harvest, grape berries of each treatment were mixed, crushed, adjusted to 22ºBrix, and separated to 4 replications. Diammonium phosphate (DAP) was added at 0.30 g/l and each replicate was treated with 50 mg/l of SO_2. It was kept for 6 h and then yeast (Saccharomyces cerevisiae) (Enoferm BDX) was added at 0.36 mg/l. Alcoholic fermentations were carried out in 4 replications in 2-l vessels that were thermostated at 20ºC. Every day the must temperature and ºBrix were measured. The fermenting must was pressed at about 5ºBrix, and Lactic acid bacteria (Leuconostoc oenos) would be added. After malolactic fermentation finished, wines were racked and 75 mg/l of SO_2 was added to the wines which were then bottled. Wine analyses were done for pH, titratable acidity (TA), and phenol composition before bottling (pressed wine) and six months later (aged wine).

A portable pH meter was used to determine pH. Alcohol (Alc), volatile acid (VA), and SO_2 contents were determined by OIV methods (OIV, 1990). TA was determined by titration with 0.1 M of NaOH to a pH 8.2 end point and expressed as g/l of tartaric acid.

Wine color properties and total phenolics were determined by spectrophotomeric method (Glories, 1984). The wine color density and hue were determined by measuring absorbance at 420 nm and 520 nm, while total phenolics, total red pigment, and degree of red pigment coloration were determined at 280 nm and 520 nm in HCl solution. The modified wine color density and hue were extracted by CH_3CHO at pH 3.5 then compared absorbance between their 420 nm and 520 nm. Modified degree of red pigment coloration was calculated ratio between absorbance of with CH_3CHO and HCl at pH 3.5 at 520 nm. Estimate of SO_2 resistant pigments was determined by adding sodium metabisulfide solution (SO_2). All measurements were performed in triplicate and quantified as following:

Wine color density (a.u.) $= A_{520} + A_{420}$

Wine color hue $= A_{420}/A_{520}$

Total red pigments (a.u.) $= A^{HCl}{}_{520}$

Degree of red pigment coloration (%)
$= A_{520}/A^{HCl}{}_{520} \times 100$

Modified wine color density (a.u.)
$= (A^{CH_3CHO}{}_{520} + A^{CH_3CHO}{}_{420})_{pH\ 3.5}$

Modified wine color hue
$= (A^{CH_3CHO}{}_{420}/A^{CH_3CHO}{}_{520})_{pH\ 3.5}$

Modified degree of red pigment coloration
$= (A^{CH_3CHO}{}_{520}/A^{HCl}{}_{520})_{pH\ 3.5} \times 100$

Estimate of SO_2 resistant pigments (a.u.)
$= A^{SO_2}{}_{520}$

Total phenolics (a.u.) $= A^{HCl}{}_{280} - 4$

Total anthocyanins were determined using spectrophotometer at 520 and 700 nm with pH differential method (Lee *et al.*, 2005). Anthocyanins content was determined by high performance liquid chromatography (HPLC) methods by modified method of Iland *et al.* (2000). Red wines were centrifuged at 14000 rpm for 10 min and filtrated with a 13 mm, 0.20 μm syringe filter (GAT Asia Limited). Ten microliters were used for HPLC analysis. The gradient cycle consisted of an initial 5 min isocratic segment (solution A, 100%). Then the linear gradient was changed progressively by increasing solution B (100%

CH_3CN) to 10% at 10 min, 15% at 20 min, 20% at 25 min and then increasing solution A to 100% at 30 min. Solution A consisted of 4/4/92 CH_3OH/CH_3CN/87 mmol/L H_3PO_4 in H_2O (v/v/v). Anthocyanins were determined by reverse-phase HPLC using a Luna 5U C18 100A column (4.6 μm, 150 mm) particle diameter 5 μm. A wavelength of 520 nm was used for detection.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) for Windows release 13.0, from SPSS Inc., 2004, was used. As a parametric methodology, variance analysis was used.

Results and Discussion

Wine Quality

Grape quality was highly affected the wine quality. Results from 2 years of experiments, as shown in Table 1, indicated that with increasing N-K levels the wine pH was increased but the wine TA was decreased. Increasing cluster levels decreased the wine pH and volatile acidity (VA) but the wine TA and Alc were increased. That result was the same in both years. Keller *et al.* (1999) reported that high N supplies increased the pH in the juice and wine. Crop thinning may have detrimental effects on wine quality by disturbing the natural balance of the vine and increasing vegetative growth (McDonnell *et al.*, 2008).

Wine Color and Phenolic Compounds

Wine color density decreased from the pressed to the aged wine. The color of the aged wine with the increased cluster levels decreased. Wine color density of the pressed wine in 2005 was lower than in 2006 but in the aged wine in 2005 it was higher than in 2006. In both years, increasing N-K levels and cluster levels seemed to increase the wine color density (Tables 2 and 3). Somers (1998) found that for red wines, differing in age by 2 years, the wine color density values ranged between 3 a.u. and 25 a.u. Almela *et al.* (1999) found that treatment involving the use of more water and fertilizers has no significant improvement on wine color.

Wine color hue increased from the pressed to the aged wine, and in the pressed wine which had increased N-K and cluster levels it decreased (Table 2). An increase in the hue value is expected for a red wine as it ages, described as a shift from purple red via brick red to brown tones for the wine colour.

Table 1. Influence of fertilizers (N-K) and number of clusters on wine characteristic (averaged 2 years data)

Treatments	pH	TA (g/l)	Alc (%)	VA (g/l)
Fertilizer (N-K)				
F1	3.59[a]	6.78[a]	11.19[a]	0.63[a]
F2	3.62[b]	6.61[b]	11.12[a]	0.64[a]
F3	3.63[b]	6.31[a]	11.11[a]	0.63[a]
No. of clusters				
C1	3.63[b]	6.46[a]	10.89a	0.68[b]
C2	3.62[b]	6.46[a]	11.29[b]	0.61[ab]
C3	3.59[a]	6.79[b]	11.24[b]	0.60[a]
Mean	3.61	6.57	11.14	0.63

In a column each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Alcalde-Eon *et al.* (2006) reported that, as wine became older, the percentages of anthocyanins decreased slightly, whereas that of the anthocyanin-derived pigments increased, particularly the compounds providing the wine with orange hues (pyranoanthocyanins). Pyranoanthocyanins are generated by reaction between monomeric anthocyanins and wine

Table 2. Influence of fertilizers (N-K) and number of clusters on wine color characteristics (averaged 2 years data)

Treatments	Wine color density (a.u.)		Wine color hue		Degree of red pigment coloration (%)	
	Pressed	Aged	Pressed	Aged	Pressed	Aged
Fertilizer (N-K)						
F1	4.80[a]	4.70[ab]	0.71[b]	0.76[a]	17.53[a]	29.93[a]
F2	4.81[a]	4.57[a]	0.69[ab]	0.78[a]	17.14[a]	26.67[a]
F3	5.45[b]	5.38[b]	0.67[a]	0.73[a]	18.92[a]	35.76[a]
No. of clusters						
C1	4.83[a]	5.41[b]	0.71[b]	0.74[a]	16.38[a]	34.72[b]
C2	5.09[a]	5.06[ab]	0.68[a]	0.73[a]	18.69[b]	34.62[ab]
C3	5.13[a]	4.17[a]	0.68[a]	0.80[a]	18.51[b]	23.02[a]
Mean	5.02	4.88	0.69	0.76	17.86	30.79

In a column each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 3. Influence of fertilizers (N-K) and number of clusters on wine color characteristics of pressed wine

Treatments	Wine color density (a.u.)		Wine color hue		Degree of red pigment coloration (%)	
	2005	2006	2005	2006	2005	2006
Fertilizer (N-K)						
F1	4.361[a]	5.242[a]	0.616[b]	0.798[a]	16.678[a]	18.378[a]
F2	4.398[a]	5.214[a]	0.601[ab]	0.783[a]	16.011[a]	18.267[a]
F3	4.916[b]	5.979[b]	0.587[a]	0.744[a]	16.756[a]	21.078[a]
No. of clusters						
C1	4.276[a]	5.392[a]	0.606[a]	0.817[b]	14.600[a]	18.167[a]
C2	4.654[b]	5.440[a]	0.596[a]	0.756[a]	16.844[b]	20.533[a]
C3	4.744[b]	5.603[a]	0.602[a]	0.753[a]	18.000[b]	19.022[a]
Mean	4.558	5.478	0.601	0.775	16.482	19.241

In a column each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

compounds having a polarised double bond, as do some secondary yeast metabolites (pyruvic acid), 4-vinylphenols, 8-vinylflavanols, and hydroxycinnamic acids (Fulcrand *et al.*, 1998, Hayasaka and Asenstorfer, 2002, and Mateus *et al.*, 2003). In young wines, the hue was 0.4 to 0.5 which increased to 0.8 to 0.9 in aged red wines (Somers and Verette, 1988). Delgado *et al.* (2006) reported that low application rates of N caused a major increase in the hue and yellow component but did not modify color intensity. But the highest rate of N increased color intensity as well as the red and blue hues and K, by itself, reduced the red hue and increased the yellow component.

Degree of red pigment coloration was increased from the pressed to the aged wine. Increasing cluster levels increased the value in the pressed wine but decreased it in the aged wine (Table 2).

Estimated SO_2 resistant pigments increased from the pressed to the aged wine, and were highest at the highest levels of N-K for both the pressed and aged wine; higher cluster numbers increased the levels for the aged wine but not for the pressed wine (Table 4). It is well known that polymeric pigments are more stable to SO_2 bleaching than monomeric pigments (Versari *et al.*, 2008).

Total red pigments increased from the pressed to the aged wine (Table 4). Versari *et al.* (2008) suggested that the polymerization of colored phenolics might occur in a similar way in the different cultivars, the age of wine probably being the factor making more of a difference. In the press wine, the increasing N-K level increased the red pigment but the increasing cluster levels decreased the red pigment. In the aged wine, the increasing N-K level had no affect but the highest level of cluster numbers gave the highest red pigment.

Total phenolics were decreased which increasing cluster level of pressed wine but aged wine had no affect (Table 4).

Modified wine color density of both the pressed and aged wine was increased by the increasing N-K levels from 5.21 to 5.80 a.u. and 5.22 to 5.97 a.u., respectively, and was decreased by the increasing cluster levels for the aged wine from 5.92 to 5.24 (Table 5).

Table 4. Influence of fertilizers (N-K) and number of clusters on wine color characteristics (averaged 2 years data)

Treatments	Treatments Estimated SO_2 resistant pigments (a.u.)		Total red pigments (a.u.)		Total phenolics (a.u.)	
	Pressed	Aged	Pressed	Aged	Pressed	Aged
Fertilizer (N-K)						
F1	0.89^{ab}	1.37^{ab}	16.29^{a}	10.12^{a}	24.62^{a}	20.49^{a}
F2	0.85^{a}	1.23^{a}	16.74^{ab}	10.89^{a}	24.75^{a}	20.64^{a}
F3	0.95^{b}	1.88^{b}	17.49^{b}	10.24^{a}	25.72^{a}	20.59^{a}
No. of clusters						
C1	0.89^{a}	0.94^{a}	17.52^{b}	10.27^{ab}	26.09^{b}	20.48^{a}
C2	0.91^{a}	1.65^{b}	16.53^{ab}	09.67^{a}	25.19^{ab}	19.90^{a}
C3	0.89^{a}	1.89^{b}	16.47^{a}	11.32^{b}	23.81^{a}	21.33^{a}
Mean	0.90	1.49	16.84	10.42	25.03	20.57

In a column each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Modified wine color hue was not significantly different for the N-K levels in both years; but for the pressed wine it increased from 0.62 to 0.65, while on the other hand for the aged wine it decreased from 0.65 to 0.61 with the increasing cluster levels (Table 5).

Modified degree of red pigment coloration for the aged wine decreased with the increasing cluster levels from 38.22% to 30.12%, but there was no effect on the pressed wine (Table 5).

In the 2005 pressed wine, increasing N-K levels and cluster levels increased the wine color density. Increasing N-K levels decreased the wine color hue. The degree of red pigment coloration was increased by the increasing cluster levels (Table 6). Estimated SO_2 resistant pigments seemed to increase with the increasing N-K levels. Total red pigment was increased by the increasing N-K levels and was decreased by the increasing cluster levels. The increasing cluster levels decreased the total phenolics. However, in the 2006 pressed wine was not significantly different in all the parameters (Table 7).

In the 2005 aged wine, increasing N-K and cluster levels decreased the wine color hue (Table 8). Increasing cluster levels decreased the estimated SO_2 resistant pigments. In 2006, increasing cluster levels decreased the wine color density and the degree of red pigment coloration. Total red pigment and total phenolics increased with the increasing cluster levels from 9.156 to 11.367 a.u. and 19.189 to 22.189 a.u., respectively, in the 2006 aged wine (Table 9).

Wine Anthocyanins

The pressed wine had a higher anthocynins content than the aged wine (Table 10). The pressed and aged wine were not significantly different in wine anthocyanins content for the N-K levels but increasing the cluster levels seemed to increase the wine anthocyanins content of the pressed wine (Table 10), and the 20 clusters level had the highest. Malvidin (Mv) was the most abundant anthocyanins in both the pressed and aged wines (Tables 11 and 12). In the 2005 pressed wine was higher

Table 5. Influence of fertilizers (N-K) and number of clusters on wine color characteristics (averaged 2 years data)

Treatments	Modified wine color density (a.u.)		Modified wine color hue		Modified degree red pigment coloration (%)	
	Pressed	Aged	Pressed	Aged	Pressed	Aged
Fertilizer (N-K)						
F1	5.21[a]	5.22[a]	0.63[a]	0.64[a]	19.79[a]	33.85[a]
F2	5.21[a]	5.37[a]	0.64[a]	0.64[a]	19.41[a]	31.83[a]
F3	5.80[b]	5.97[b]	0.64[a]	0.64[a]	20.38[a]	39.46[a]
No. of clusters						
C1	5.57[a]	5.92[b]	0.62[a]	0.65	19.82[a]	38.22[b]
C2	5.33[a]	5.40[a]	0.64[ab]	0.66[b]	19.84[a]	36.79[ab]
C3	5.32[a]	5.24[a]	0.65[b]	0.61[a]	19.93[a]	30.12[a]
Mean	5.41	5.52	0.64	0.64	19.86	35.05

In a column each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 6. Influence of fertilizers (N-K) and number of clusters on wine color characteristics of pressed wine

Treatments	Wine color density (a.u.)		Wine color hue		Degree of red pigment coloration (%)	
	2005	2006	2005	2006	2005	2006
Fertilizer (N-K)						
F1	4.361[a]	5.242[a]	0.616[b]	0.798[a]	16.678[a]	18.378[a]
F2	4.398[a]	5.214[a]	0.601[ab]	0.783[a]	16.011[a]	18.267[a]
F3	4.916[b]	5.979[b]	0.587[a]	0.744[a]	16.756[a]	21.078[a]
No. of clusters						
C1	4.276[a]	5.392[a]	0.606[a]	0.817[b]	14.600[a]	18.167[a]
C2	4.654[b]	5.440[a]	0.596[a]	0.756[a]	16.844[b]	20.533[a]
C3	4.744[b]	5.603[a]	0.602[a]	0.753[a]	18.000[b]	19.022[a]
Mean	4.558	5.478	0.601	0.775	16.482	19.241

In a column each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 7. Influence of fertilizers (N-K) and number of clusters on wine color characteristics of pressed wine

Treatments	Estimated SO_2 resistant pigment (a.u.)		Total red pigment (a.u.)		Total phenolics (a.u.)	
	2005	2006	2005	2006	2005	2006
Fertilizer (N-K)						
F1	0.872[ab]	0.908[a]	16.356[a]	16.222[a]	25.344[a]	23.889[a]
F2	0.831[a]	0.864[a]	17.244[b]	16.244[a]	25.611[a]	23.889[a]
F3	0.931[b]	0.973[a]	18.644[c]	16.333[a]	25.956[a]	25.478[a]
No. of clusters						
C1	0.864[a]	0.914[a]	18.278[b]	16.767[a]	27.933[c]	24.244[a]
C2	0.883[a]	0.930[a]	17.367[a]	15.700[a]	26.433[b]	23.944[a]
C3	0.887[a]	0.901[a]	16.600[a]	16.333[a]	22.544[a]	25.067[a]
Mean	0.878	0.915	17.415	16.266	25.637	24.419

In a column each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 8. Influence of fertilizers (N-K) and Number of clusters on wine color of aged wine

Treatments	Wine color density (a.u.)		Wine color hue		Degree of red pigment coloration (%)	
	2005	2006	2005	2006	2005	2006
Fertilizer (N-K)						
F1	5.617[a]	3.777[a]	0.638[b]	0.872[a]	38.989[a]	20.867[a]
F2	5.437[a]	3.706[a]	0.643[b]	0.924[a]	32.522[a]	20.822[a]
F3	6.432[b]	4.322[a]	0.584[a]	0.878[a]	42.411[a]	29.111[a]
No. of clusters						
C1	6.066[a]	4.756[b]	0.663[b]	0.821[a]	36.400[a]	33.044[b]
C2	5.996[a]	4.127[b]	0.639[b]	0.816[a]	44.189[a]	25.056[ab]
C3	5.424[a]	2.922[a]	0.563[a]	1.038[a]	33.333[a]	12.700[a]
Mean	5.829	3.935	0.622	0.891	37.974	23.600

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 9. Influence of fertilizers (N-K) and number of clusters on wine SO_2 resistant, red pigment, and total phenolics of aged wine

Treatments	Estimated SO_2 resistant pigment (a.u.)		Total red pigment (a.u.)		Total phenolics (a.u.)	
	2005	2006	2005	2006	2005	2006
Fertilizer (N-K)						
F1	1.701[ab]	1.034[a]	09.900[a]	10.344[a]	20.567[a]	20.411[a]
F2	1.564[a]	0.897[a]	10.756[a]	10.211[a]	21.000[a]	20.278[a]
F3	2.376[a]	1.374[a]	11.578[a]	09.733[a]	20.200[a]	20.978[a]
No. of clusters						
C1	2.150[a]	1.620[a]	11.389[a]	09.156[a]	21.778[a]	19.189[a]
C2	2.304[a]	0.992[a]	09.567[a]	09.767[a]	19.511[a]	20.289[a]
C3	1.187[a]	0.693[a]	11.278[a]	11.367[a]	20.478[a]	22.189[a]
Mean	1.880	1.102	10.745	10.096	20.589	20.556

In a column each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

in delphinidin (Dp) and petunidin (Pt), but lower cyanidin (Cy) and Mv than the 2006 pressed wine. In the 2005 aged wine was higher in Cy but lower in Dp, Pt, peonidin (Pn) and Mv than the 2006 aged wine. The ratio between Mv and Pn was 25.1 and 19.2 in the pressed and aged wine, respectively (Table 13). There were the same results with many experimental studies (Revilla *et al.*, 2001; Monagas *et al.*, 2003). The ratio between malvidins and peonidins (Mv/Pn), which is related to the flavonoid-3'-hydroxylase (FH) and o-dihydroxyphenyl-O-methyltransferase (MT) enzyme activities in plants (Roggero *et al.*, 1986) was lower for Graciano (4.6 and 5.1, respectively, after 1.5 and 12 months in

Table 10. Influence of fertilizers (N-K) and number of clusters on wine total anthocyanins content (mg/ml)

Fertilizers	Pressed	Aged
Fertilizer (N-K)		
F1	0.1944[a]	0.1327[a]
F2	0.2002[a]	0.1536[a]
F3	0.1761[a]	0.1319[a]
No. of clusters		
C1	0.1661[a]	0.1414[a]
C2	0.2251[b]	0.1259[a]
C3	0.1795[ab]	0.1508[a]
Mean	0.1902	0.1394

In a column each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 11. Influence of fertilizers (N-K) and number of clusters on wine anthocyanin contents of pressed wine (mg/ml)

Treatments	Dp	Cy	Pt	Pn	Mv
Fertilizer (N-K)					
F1	0.0112[a]	0.0187[a]	0.0030[a]	0.0066[a]	0.1551[a]
F2	0.0104[a]	0.0185[a]	0.0019[a]	0.0054[a]	0.1640[a]
F3	0.0108[a]	0.0152[a]	0.0027[a]	0.0063[a]	0.1411[a]
No. of clusters					
C1	0.0098[a]	0.0117[a]	0.0026[a]	0.0057[a]	0.1363[a]
C2	0.0116[a]	0.0276[b]	0.0023[a]	0.0051[a]	0.1785[a]
C3	0.0109[a]	0.0131[a]	0.0027[a]	0.0075[b]	0.1453[a]
Mean	0.0108	0.0175	0.0025	0.0061	0.1534

In a column each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

bottle) than for Tempranillo (57.6 and 77.2) and Cabernet Sauvignon (39.2 and 51.3) (Monagas *et al*., 2003). In young red wine, some researchers believe that the enhanced perception of wine color is considered to be influenced by copigmentation and self-association reactions of anthocyanins with other polyphenols (Asen *et al*., 1972). In aged red wine, the concentration of malvidin 3-glucoside and other anthocyanins decreases to almost negligible amounts (Schwarz *et al*., 2003; Somers, 2003; Hermosin Gutierrez *et al*., 2005). At wine pH (pH 3.5), the less colored quinoidal based forms and a complex mixture of the neutral and anionic species of malvidin 3-glucoside are the predominant forms (Asenstorfer *et al*., 2003). Hermosin Gutierrez *et al*. (2005) found that the fraction of red color due to copigmented anthocyanins ranged from 32% to 43% at the end of the alcoholic fermentation, and this decreased to 20–34% after 3 months of ageing when a

Table 12. Influence of fertilizers (N-K) and number of clusters on wine anthocyanin contents of aged wine (mg/ml)

Treatments	Dp	Cy	Pt	Pn	Mv
Fertilizer (N-K)					
F1	0.0152[a]	0.0026[a]	0.0028[a]	0.0059[a]	0.1062[a]
F2	0.0176[a]	0.0032[a]	0.0027[a]	0.0053[a]	0.1247[a]
F3	0.0168[a]	0.0028[a]	0.0026[a]	0.0061[a]	0.1035[a]
No. of clusters					
C1	0.0168[a]	0.0027[a]	0.0028[a]	0.0057[a]	0.1135[a]
C2	0.0145[a]	0.0026[a]	0.0029[a]	0.0053[a]	0.1005[a]
C3	0.0183[a]	0.0033[a]	0.0024[a]	0.0063[a]	0.1206[a]
Mean	0.0165	0.0029	0.0027	0.0058	0.1115

In a column each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 13. Years effects on wine anthocyanins content of pressed and aged wine (mg/ml)

Anthocynins	Pressed wine		Aged wine	
	2005	2006	2005	2006
Dp	0.0193[b]	0.0022[a]	0.0141[a]	0.0190[b]
Cy	0.0024[a]	0.0325[b]	0.0033[b]	0.0025[a]
Pt	0.0030[b]	0.0020[a]	0.0023[a]	0.0031[b]
Pn	0.0061[a]	0.0060[a]	0.0054[a]	0.0061[b]
Mv	0.1258[a]	0.1809[b]	0.0986[a]	0.1244[b]
Sum	0.1566	0.2236	0.1237	0.1551

In each wine each type of anthocyanins, means followed by a common letter are not significantly different at the 5% level by DMRT.

decrease in both monomeric anthocyanin and flavonol concentrations was also observed, and after 9 months of ageing the amounts of remaining monomeric anthocyanins were 40% and 38% for Syrah and Cencibel wines, respectively, whereas Cabernet Sauvignon wines only retained 32% of the monomeric anthocyanins initialy found at the end of alcoholic fermentation. Pinheiro *et al.* (2009) reported that a reduction of 59.83% in the content of anthocyanins, in relation to the initial time of storage, was verified. Gambelli and Santaroni (2004) indicated that high amounts of malvidin-3-glucoside are still present in aged wine. Petunidin was determined only in young wines. Peonidin and cyaniding decreased during ageing. The delphinidin content was lower than peonidin and cyaniding.

Conclusions

The 2 years experimental data showed similar results, that increasing N-K levels increased wine pH, wine color density (pressed wine), total red pigment (pressed wine) and modified wine color density (pressed and aged wine) but decreased wine TA and wine color hue. Increasing the cluster numbers increased wine TA, degree of red pigment coloration (pressed wine), estimated SO_2 resistance (aged wine) and modified wine color hue (pressed wine) but decreased wine pH, VA, wine color density (aged wine), wine color hue (pressed wine), degree of red pigment coloration (aged wine), total red pigment (pressed wine) and total phenolics (pressed wine), and modified wine color density for both the pressed and aged wine. The modified wine color hue (aged wine), and modified degree of red pigment coloration (aged wine) were also decreased when the cluster numbers were increased. Wine anthocyanins content was significantly different between the pressed and aged wine in both years. The main wine anthocynin of the pressed and aged wine was Mv. All these data suggested that the changes in the anthocyanins content of wines in relation to the anthocyanins content of the grape berry probably took place during winemaking and wine ageing. This could result in the existing differences: rate of anthocyanins degradation, or polymerization during winemaking and wine ageing. The answers to all these questions need further research to understand the differences between each of the anthocyanins of grapes and corresponding wines.

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