Food Chemistry 124 (2011) 767–774

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Influence of the heterogeneity of grape phenolic maturity on wine composition and quality

Nikolaos Kontoudakis [a], Mireia Esteruelas [a], Francesca Fort [a], Joan Miquel Canals [a], Victor De Freitas [b], Fernando Zamora [a,*]

[a] Departament de Bioquímica i Biotecnologia, Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili, Campus de Sescelades, C/Marcel.li Domingo, s/n. 43007 Tarragona, Spain
[b] Centro de Investigaçao em Química, Departamento de Química, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre 687, 4169-007 Porto, Portugal

ARTICLE INFO

Article history:
Received 26 November 2009
Received in revised form 18 May 2010
Accepted 28 June 2010

Keywords:
Phenolic maturity
Heterogeneity
Red wine colour
Phenolic compounds
Anthocyanin

ABSTRACT

Nowadays, consumers demand red wines with deep colour, soft tannins and fruit scents, but these wines can only be obtained from grapes with complete phenolic maturity. Diverse methods have been proposed for measuring phenolic maturity. However, all these methods only provide the average value and do not consider any possible heterogeneity. Throughout ripening, grapes were separated according to their density, which revealed the existence of a large heterogeneity. Grapes at harvest were also separated by density in three groups. The higher the density of the grapes the higher ethanol content, pH, colour intensity, total phenolic index and anthocyanin and proanthocyanidin concentrations, and the lower the titratable acidity and bitterness of the wines. When the grapes were denser the wines were also better balanced in flavour and mouthfeel sensation. These results suggest that grape heterogeneity may influence the final wine composition and quality and therefore it should be considered at harvest.

1. Introduction

One of the major factors affecting red wine quality is the real degree of phenolic maturity in the grapes at harvest time (Ribereau-Gayon, Glories, Maujean, & Dubourdieu, 2006). Most of wine's sensory attributes such as colour, body, and tactile and taste sensations like, astringency and bitterness, are directly associated with the composition of anthocyanins and proanthocyanidins (Arnold & Noble, 1978; Gawel, 1998; Noble, 1990; Vidal et al., 2003) and this composition is strongly affected by ripeness (Canals, Llaudy, Valls, Canals, & Zamora, 2005; Llaudy, Canals, Canals, & Zamora, 2008).

Anthocyanins are only present in grape skins whereas proanthocyanidins are present in skins and seeds (Ribereau-Gayon et al., 2006). Seed proanthocyanidins are made up of (+)-catechin, (−)-epicatechin and (−)-epicatechin-3-gallate (Kennedy, Matthewa, & Waterhouse, 2002; Prieur, Rigaud, Cheynier, & Moutounet, 1994), whereas skin proanthocyanidins have a much lower proportion of (−)-epicatechin-3-gallate and also contain (−)-epigallocatechin (Gonzalez-Manzano, Rivas-Gonzalo, & Santos-Buelga, 2004; Souquet, Cheynier, Brossaud, & Moutounet, 1996).

Molecular sizes and the monomeric composition of proanthocyanidins in particular have a large influence on the sensation of astringency. More specifically, the greater degree of polymerisation and the greater percentage of galloylation, will cause a greater sensation of astringency (Herderich & Smith, 2005; Vidal et al., 2003; Vivas & Glories, 1996).

It is generally considered that ripeness strongly influences the phenolic composition of red wines (Ó-Marques, Reguinga, Laureano, & Ricardo-da-Silva, 2005; Ryan & Revilla, 2003). It has been reported, for example, that insufficiently ripened grapes have a lower extractability of anthocyanins and proanthocyanidins from skins and a higher extractability of proanthocyanidins from seeds (Canals et al., 2005; Peyrot des Gachons & Kennedy, 2003). For this reason, it is generally thought that insufficiently ripened grapes may produce more astringent and bitter wines because their seeds can release a higher amount of proanthocyanidins, which are highly galloylated (Romeyer, Macheix, & Sapis, 1986).

Consequently, for the last two decades winemakers have been very interested in the concept of "Phenolic Maturity". The reason for this is very simple. Consumers demand wines with deep red colour, full body, soft tannins and fruit scents, and this kind of wine can only be obtained from grapes that have reached complete phenolic maturity. If this is not the case, wines may present bitter and astringent sensations and a poor colour.

This interest has led to several investigations to try and find effective methodologies for determining the real level of phenolic ripeness in grapes and thus provide a better criterion for deciding the optimum time of harvest. In fact, various methods for measuring phenolic maturity have been proposed (Celotti, Della Vedova, Ferrarini, & Martinand, 2007; Dupuch, 1993; Glories & Agustin, 1993).

* Corresponding author. Tel.: +34 977 55 86 79; fax: + 34 977 55 86 86.
E-mail address: fernando.zamora@urv.cat (F. Zamora).

0308-8146/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.06.093

Some of these methods can predict reasonably well the phenolic maturity and especially the colour intensity of a wine (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2010). However, all these methods only provide the average value of a representative sample from the whole grape vineyard and do not consider any possible heterogeneity in their degree of maturity.

In a vineyard, grapes do not ripen homogeneously. Each cluster and even each berry matures at different rates through the influence of multiple factors. The location of vines in the vineyard (exposure, altitude, soil composition, temperature, humidity, vine density, etc.), the position of the cluster on the vine and even the position of the berries in the cluster can produce some differences in the ripening rate (Haselgrove et al., 2000; Smart, Robinson, Due, & Brien, 1985). Furthermore, an uneven grape ripeness can affect the quality of the final product. The presence of a non-negligible percentage proportion of unripe berries can considerably increase the appearance of bitter and astringent characters in wine.

Several studies are focused on the influence of grapes harvested at different stages of maturity on wine phenolic composition and quality (Canals et al., 2005; Gambuti, Strollo, Lecce, & Moio, 2007; Llaudy et al., 2008; Pérez-Magarin~o & González-San José, 2006). However, to our knowledge, none of them study the extent and the impact of grape heterogeneity. Since this aspect has not been investigated in detail to date, the aim of this study was to determine the real influence of the heterogeneity in the degree of grape ripeness on wine composition and quality.

2. Materials and methods

2.1. Chemicals

Methanol, acetonitrile, formic acid and acetic acid were HPLC-grade and were purchased from Panreac (Barcelona, Spain). Malvidin-3-*O*-glucoside chloride, (+)-catechin, (−)-epicatechin, (−)-epigallocatechin and (−)-epicatechin-3-*O*-gallate were purchased from Extrasynthès (Genay, France). TSK Toyopearl gel HW-40(S) was purchased from Tosoh (Japan). Ovoalbumin, phloroglucinol and L-ascorbic were purchased from Sigma (Madrid, Spain). The rest of the chemicals were of high purity and were purchased from Panreac (Barcelona, Spain).

2.2. Grapes

This study was carried out with grapes of the cultivar *Vitis vinifera* cv. Cabernet sauvignon from the experimental vineyard at Constantí (AOC Tarragona) which belongs to the Enology Faculty in Tarragona of the Rovira i Virgili University. The grapes were collected in 2007 and 2008 and were only taken from the two central rows of the plot.

2.3. Maturity controls

Six hundred grapes were randomly collected at 1, 3, 5 and 7 weeks after veraison. One hundred berries were used for measuring the sugar content, titratable acidity, pH and weight. Solutions of NaCl of 100–190 g/l were prepared and used to analyse ripening heterogeneity according to the methodology previously described (Fournand et al., 2006). These solutions had densities comprised between 1020 and 1120 mg/ml. One hundred berries were introduced to the less dense solution (1020 mg/ml). The floating berries were considered to have the same density than the solution. They were separated from sank berries and were counted. After that, sank berries were removed and introduced to the following denser solution (1031.1 mg/ml). The same process was repeated with all NaCl solutions. All these measurements were carried out in triplicate.

2.4. Winemaking procedure

Seven weeks after veraison, 70 kg of grapes were harvested and manually destemmed. Grapes were separated into three groups by flotation in two solutions of sucrose of different concentrations. Sucrose solutions of 232 and 244 g/l were used for the 2007 vintage and of 244 and 256 g/l for the 2008 vintage. These solutions presented the following densities at 20 °C: 1090 and 1095 mg/l for the 2007 vintage and 1095 and 1100 mg/l for the 2008 vintage. These solutions were chosen each year to adapt it to the maturity conditions of the vintage.

After that, the berries of each lot were randomly distributed into three groups of four kilogrammes, crushed with a semi-automatic crusher machine (Gual, Villafranca del Penedès, Spain), sulphited (100 mg of $K_2S_2O_5$/l) and introduced in 6 l tanks. All tanks were immediately inoculated with 200 mg/l of selected yeast (DV10, Martin Vialatte, Epernay, France) and maintained at a room temperature of 25 ± 1 °C. All these microvinifications were controlled daily by measuring the temperature and the density of the must. Each day a punch down was carried out to encourage the extraction of phenolic compounds. After 14 days of maceration, the wines were racked, sulphited (100 mg of $K_2S_2O_5$/l) and refrigerated for 3 weeks at 4 °C. After that, wines were decanted and bottled. All the samples were stored at 15 ± 1 °C until the moment of the analysis.

2.5. Standard grape and wine analysis

The analytical methods recommended by O.I.V. (2005) were used to determine sugar content, probable alcoholic degree, titratable acidity and the pH of the grapes and the ethanol content, titratable acidity and pH of the wines.

2.6. Colour parameters

The colour intensity (CI) was estimated using the method described by Glories (1984). The CIELAB coordinates, lightness (L^*), chroma (C^*), hue (h^*), red-greenness (a^*) and yellow-blueness (b^*) were determined according to Ayala, Echávarri, and Negueruela (1997) and the data were processed with the MSCV® software (Ayala, Echávarri, & Negueruela, 2001). The total colour difference (ΔEab^*) between two samples was obtained using the expression: $\Delta Eab^* = [(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2]1/2$ (Pérez-Magariño & González-Sanjose, 2003). All absorbance measurements were taken with a Helios Alpha (Thermo Fisher Scientific Inc., Waltman, MA) UV–vis spectrophotometer using quartz cells of 1 mm path length.

2.7. Anthocyanin analysis

The total anthocyanin content was determined by spectrophotometry using the method described by Niketic-Aleksic and Hrazdrina (1972). Free and combined anthocyanins were calculated using the PVPP index (Glories, 1984). Reversed-phase HPLC analyses of anthocyanins and the anthocyanin-derived pigments vitisin A and vitisin B were carried out with an Agilent 1200 series liquid chromatograph (HPLC–DAD) and an Agilent Zorbax Eclipse XDB-C18, 4.6 × 250 mm 5 μm column (Agilent Technologies, Santa Clara, USA) in accordance with the method described by González-San José, Diez, Santa María, and Garrido (1988). Anthocyanins and anthocyanin-derived compounds were quantified at 520 nm as malvidin-3-glucoside, using malvidin-3-glucoside chloride as an external standard.

2.8. Analysis of flavanols

2.8.1. HPLC and HPLC–ESI-MS analysis of catechins and oligomeric proanthocyanidins

Twenty millilitres of wine was extracted thrice with 20 ml of ethyl acetate to analyse the catechins and oligomeric proanthocyanidins. The wine extract was evaporated under vacuum (Buchi rotavoporate, Flawil, Switzerland) and then redissolved with methanol. This wine extract was fractionated through a TSK Toyopearl HW-40(S) gel column (250 mm × 16 mm) using purified methanol as described previously (De Freitas & Glories, 1999). Flow rate was regulated at 0.8 ml/min using a peristaltic pump (Gilson, Middleton, USA). This gave us three fractions of 120 ml each one. The first fraction contained procyanidin monomers, (+)-catechin and (−)-epicatechin, the second fraction contained procyanidin dimers and trimers and the third fraction contained procyanidin trimers and tetramers (De Freitas, Glories, Bourgeois, & Vitry, 1998). All fractions were evaporated and redissolved on 1 ml synthetic solution (12% ethanol, 4 g/l tartaric acid and pH 3.2).

Quantitative analysis was carried out by reverse-phase HPLC (Merck-Hitachi L6200, Darmstadt, Germany) using two Beckman Ultrasphere C18 ODS columns (250 × 4.6 mm, 5 μm) (Beckman Coulter, Fullerton, USA) connected in series and protected with a guard column packed with the same packing. The chromatograms were monitored at 280 nm using a UV detector and in accordance with the method of De Freitas and Glories (1999). The procyanidin monomers and dimers were identified by comparing its retention time with that of the pure compound and by ESI-MS analysis. Trimers and tetramers were identified by ESI-MS analysis.

Mass spectrometry analyses were performed using a Finnigan Surveyor series liquid chromatograph equipped with a Thermo Finnigan (Hypersil Gold) reversed-phase column (150 mm × 4.6 mm, 5 μm, C18) thermostated at 25 °C. The samples were analysed using the same solvents, gradients, injection volume, and flow rate for HPLC analysis. Double-online detection was done by a photodiode spectrophotometer and mass spectrometry. The mass detector was a Finnigan LCQ DECA XP MAX quadrupole ion trap (Finnigan Corp., San Jose, CA) equipped with an atmospheric pressure ionisation (API) source and using an electrospray ionisation (ESI) interface. The vapourizer and the capillary voltages were 5 kV and 4 V, respectively. The capillary temperature was set at 325 °C. Nitrogen was used as both sheath and auxiliary gas at flow rates of 90 and 25, respectively (in arbitrary units). Spectra were recorded in positive ion mode between m/z 250 and 1500.

2.8.2. Analysis of proanthocyanidins following acid-catalysis with phloroglucinol

Acid-catalysis cleavage in the presence of excess phloroglucinol (Kennedy & Jones, 2001) was used to analyse monomeric proanthocyanidin composition and its mean degree of polymerisation (mDP). Ten millilitres of wine was evaporated under a low pressure vacuum (Univapo 100 ECH, Uni Equip, Martinsried, Germany). After that, it was resuspended in 6 ml distilled water and then applied to Set Pak Plus tC18 Environmental cartridges (Waters, Milford, USA) that had been previously activated with 10 ml methanol and 15 ml water. The sample was washed with 15 ml distilled water and then the proanthocyanidins were eluted with 12 ml methanol, immediately evaporated under vacuum and later eluted in 2 ml methanol. Finally, 100 μl of this sample were reacted with 100 μl phloroglucinol solution (0.2 N HCl in methanol, containing 100 g/l phloroglucinol and 20 g/l ascorbic acid) at 50 °C for 20 min. The reaction was stopped by adding 1000 μl of 40 mM aqueous sodium acetate (Kennedy & Jones, 2001). Reversed-phase HPLC analysis (Agilent serie 1200 HPLC–DAD) was carried out according to the method of Kennedy and Jones (2001). The monomers (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-*O*-gallate were identified by comparing its retention time with that of the pure compounds. The phoroglucinol adducts of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin and (−)-epicatechin-3-*O*-gallate were identified by HPLC–TOF analysis. Analyses were performed with Agilent 1200 series HPLC using an Agilent 6210 time of flight (TOF) mass spectrometer equipped with an electrospray ionisation (ESI) system. Elution was carried out under the same HPLC analysis conditions as described by Kennedy and Jones (2001). The capillary voltage was 3.5 kV. Nitrogen was used as both dry gas at a flow rate of 12 l/min at 350 °C and nebulizer gas at 60 psi. Spectra were recorded in positive ion mode between m/z 50 and 2400. This assay was also carried out without addition of phloroglucinol in order to measure the quantity of proanthocyanidin monomers naturally present in the wines.

The number of terminal subunits were considered as the difference between total monomers without phoroglucinol and thus obtained in the analysis performed without phloroglucinol addition. The addition of all phloroglucinol adducts was consider as the extension subunits of the proanthocyanidins. The mean degree of polymerisation (mDP) was calculated by adding terminal and extension subunits (in moles) and dividing by terminal subunits. The total proanthocyanidin concentration was considered as the addition of all terminal and extension subunits. Since acid-catalysis with phloroglucinol is not completely efficient, the real yield of the reaction was measured using a pure B2 proanthocyanidin dimer [(−)-epicatechin-(4 → 8)-(−)-epicatechin]. This yield was used to calculate the total proanthocyanidin concentration.

2.9. Other phenolic compounds

The total phenolic index (TPI) was determined by measuring the absorbance at 280 nm (Ribereau-Gayon et al., 2006). Proanthocyanidin concentration was also estimated by precipitation with methyl-cellulose (Sarneckis et al., 2006).

2.10. Astringency index

Astringency index was estimated using ovoalbumin as a precipitation agent and tannic acid solutions as standards in accordance with in accordance with Llaudy et al.'s method (2004).

2.11. Sensory analysis

All the wines were tasted by a group of 10 expert enologists from the Rovira i Virgili University 6 weeks after bottling. Dark glasses were used to prevent the influence of colour intensity. Three sensory triangle tests were conducted to compare the three wines in pairs. In all the cases, the first objective was to recognise the different wines and after then indicate which was their favourite and for what reason.

2.12. Statistics

All the physical and chemical data are expressed as the arithmetic average ± of the standard deviation from three replicates. One-factor ANOVA and Scheffe's test were carried out with SPSS software. The level of significance of sensory triangle tests was determined following Jackson's method (2002).

3. Results and discussion

Table 1 shows the evolution of standard parameters of grapes throughout ripening. In both vintages the sugar content, the probable alcoholic degree, the pH and the weight of 100 berries increased and the titratable acidity decreased during ripening as

Table 1
Evolution of standard parameters of grapes throughout ripening.

Parameter	Vintage	Weeks after veraison			
		1	3	5	7
S (g/l)	2007	154.3 ± 3.9a	185.1 ± 0.1b	207.6 ± 1.9c	222.2 ± 1.3d
	2008	176.7 ± 1.7a	190.8 ± 2.3b	197.8 ± 5.6b	206.5 ± 1.0c
PAD (% v/v)	2007	9.17 ± 0.23a	11.0 ± 0.3b	12.3 ± 0.2c	13.2 ± 1.9d
	2008	10.50 ± 0.10a	10.9 ± 0.1b	11.6 ± 0.2c	12.3 ± 0.1d
TA (g/l)	2007	13.34 ± 0.10a	8.95 ± 0.23b	6.90 ± 0.40c	5.65 ± 0.09d
	2008	14.10 ± 0.09a	9.45 ± 0.05b	7.03 ± 0.49c	6.55 ± 0.35c
pH	2007	2.82 ± 0.01a	3.07 ± 0.02b	3.14 ± 0.02c	3.28 ± 0.01d
	2008	2.90 ± 0.02a	3.05 ± 0.02b	3.16 ± 0.02c	3.21 ± 0.01c
W (g)	2007	109.1 ± 1.a	120.4 ± 0.7b	137.6 ± 3.5b	153.6 ± 5.1c
	2008	115.1 ± 3.8a	117.6 ± 3.5ab	124.2 ± 5.8b	127.1 ± 0.5c

All data expressed as the arithmetic average of three replicates ± standard deviation. *S*: sugar concentration, PAD: probable alcoholic degree, TA: titratable acidity, *W*: weight of 100 berries. Statistical analysis: one-factor ANOVA and Scheffe's test (both, $p = 0.05$). Different letter indicates the existence of statistically significant differences between values of the same vintage.

expected. According to these parameters, vintage 2007 reached a greater level of technological maturity than vintage 2008 although both vintages can be considered normal. However, these parameters were measured using an ensemble of 100 berries randomly collected; therefore, only the average value of each parameter is reflected and the berries' heterogeneity is not considered.

To determine the real dispersion of these parameters, several assays were performed using solutions of NaCl of increasing concentration. Separating the grapes that floated from those that sank and counting them made it possible to determine the density distribution of berries throughout the maturation process (Fig. 1). A quick glance at the figure shows a Gaussian bell-shaped distribution in both vintages and at all sampling points. These data confirm that a non-negligible heterogeneity is present from the beginning of the maturation process until the moment of harvest. Consequently, this heterogeneity implies that a considerable percentage of unripe grapes are harvested and introduced into the vinification tanks. Since unripe grapes provide a lower sugar content, higher acidity, fewer anthocyanins and in particular more seed tannins (Llaudy et al., 2008), their presence can increase bitterness and astringency and therefore adversely affect the final quality of wine.

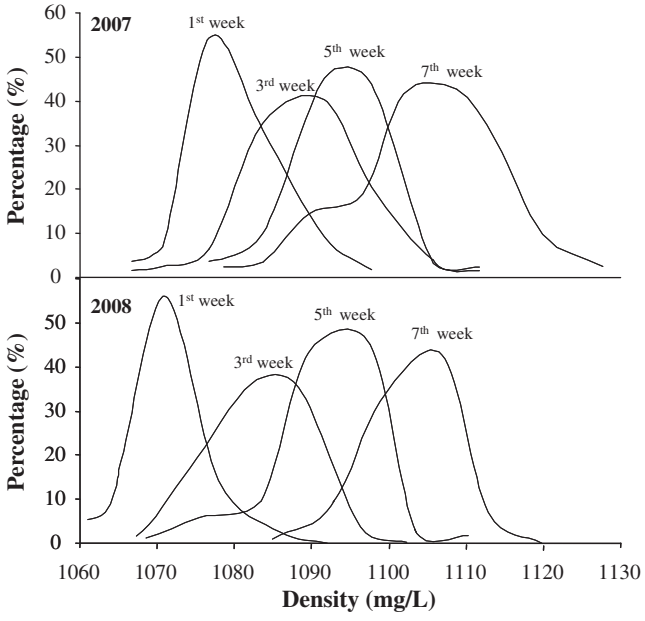

Fig. 1. Distribution of grapes densities throughout ripening.

Table 2 shows the standard parameters of the wines. As was expected, the higher the density of grapes the higher ethanol content and pH of wines. In contrast, the titratable acidity showed the opposite tendency in both vintages. These data confirm that the existing heterogeneity in grape densities also affects the wine composition, at least where the standard parameters are concerned.

Table 3 shows the colour parameters of the wines of both vintages. In general, the wines from the 2007 vintage that have a higher level of technological maturity indicators also presented higher values of colour intensity (CI) and chroma (C^*), and lower luminosity (L^*) values (more dark colour) than those of 2008.

An overall view of these data indicates that the density of the grapes significantly influences the wine colour. Specifically, the higher the density of grapes the higher the CI, C^* and red-greenness (a^*). On the other hand, L^* had the opposite tendency for both years. The other CIELAB coordinates, hue (h^*) and yellow-blueness (b^*) did not show uniform behaviour. On one hand, h^* tended to increase with the density of the grapes in the 2007 wines whereas it tended to decrease in the 2008 wines. On the other hand, b^* also tended to increase with the density of the grapes in the 2007 wines whereas it was erratic in the 2008 wines.

Table 4 presents the total colour differences (ΔEab^*) amongst wines of the same vintage. The human eye can generally distinguish two colours when $\Delta Eab^* \geqslant 1$ (Pérez-Magariño & González-Sanjose, 2003). However, it is also generally accepted that tasters can only distinguish the colour of two wines through the glass when $\Delta Eab^* \geqslant 5$ units (Pérez-Magariño & González-Sanjose, 2003). In fact, the differences that can be distinguished by the human eye also depend on the colour intensity, because the discriminating capacity becomes less accurate when colour perception reaches the saturation level. In our experimental conditions, the three wines obtained for both vintages all had ΔEab^* differences greater than 5 units. Therefore, the existing colour differences

Table 2
Wine parameters.

Parameter	Vintage	Low density	Medium density	High density
Ethanol (% v/v)	2007	12.2 ± 0.1a	13.2 ± 0.1b	14.2 ± 0.1c
	2008	11.0 ± 0.1a	12.7 ± 0.2b	13.7 ± 0.2c
TA (g of tartaric acid/l)	2007	5.90 ± 0.35a	5.85 ± 0.30a	5.35 ± 0.09b
	2008	6.17 ± 0.15[a]	6.00 ± 0.10ab	5.93 ± 0.06b
pH	2007	3.56 ± 0.02a	3.61 ± 0.03ab	3.66 ± 0.003b
	2008	3.18 ± 0.01a	3.30 ± 0.02b	3.35 ± 0.01c

All data are expressed as the arithmetic average of three replicates ± standard deviation. TA: titratable acidity. Statistical analysis: one-factor ANOVA and Scheffe's test (both, $p = 0.05$). Different letter indicates the existence of statistically significant differences between values of the same vintage.

amongst the wines obtained from grapes of different densities are great enough to be detected by the human eye even when the wines come from grapes of relatively close densities. These data confirm that the existing heterogeneity in the maturity of the grapes has a real impact on wine colour because the presence of less dense grapes may affect the visual quality of wine.

Table 3
Colour parameters.

Parameter	Vintage	Low density	Medium density	High density
CI	2007	17.13 ± 0.40a	19.62 ± 1.01b	21.80 ± 0.73c
	2008	9.17 ± 0.49a	12.05 ± 1.13b	14.80 ± 1.29c
C^*	2007	67.71 ± 0.22a	68.88 ± 0.21b	69.60 ± 0.25c
	2008	48.13 ± 2.70a	54.43 ± 2.13b	59.02 ± 1.39c
L^*	2007	40.90 ± 0.95a	38.53 ± 1.14b	36.30 ± 0.56c
	2008	57.35 ± 1.63a	48.83 ± 3.05b	42.83 ± 2.29c
h^*	2007	11.87 ± 0.71a	15.22 ± 1.96b	19.27 ± 1.23c
	2008	14.51 ± 1.31a	9.38 ± 1.32b	8.61 ± 1.60b
a^*	2007	65.26 ± 0.38a	66.44 ± 0.46b	66.59 ± 0.13b
	2008	46.57 ± 2.33a	53.69 ± 2.30b	58.34 ± 1.21c
b^*	2007	13.93 ± 0.78a	18.08 ± 2.32b	22.94 ± 1.56c
	2008	11.09 ± 0.05a	8.84 ± 0.89b	9.84 ± 0.58c

All data are expressed as the arithmetic average of three replicates ± standard deviation. CI: colour intensity, C^*: chroma, L^*: luminosity, h^*: hue, a^*: red-greeness, b^*: yelow-blueness. Statistical analysis: one-factor ANOVA and Scheffe's test (both, $p = 0.05$). Different letter indicates the existence of statistically significant differences between values of the same vintage.

Table 4
Total colour differences (ΔEab^*) amongst wines.

	Low	Medium	High
Low	–	4.9 ± 1.7	10.1 ± 1.1
Medium	10.1 ± 1.2	–	5.5 ± 0.9
High	17.5 ± 0.5	7.76 ± 1.2	–

All data are expressed as the arithmetic average of three replicates ± standard deviation. TA: titratable acidity. Values in dark grey correspond to 2007 vintage. Values in clear grey correspond to 2008 vintage.

Table 5
Spectrophotometric analysis of anthocyanins.

Parameter	Vintage	Low density	Medium density	High density
Free anthocyanins (mg/l)	2007	559 ± 26a	801 ± 10b	806 ± 23b
	2008	283 ± 15a	474 ± 58b	666 ± 54c
Combined anthocyanins (mg/l)	2007	165 ± 9a	221 ± 21b	302 ± 34c
	2008	133 ± 11a	177 ± 3b	212 ± 17c
Total anthocyanin (mg/l)	2007	725 ± 35a	1021 ± 11b	1108 ± 49c
	2008	415 ± 25a	651 ± 61b	878 ± 38c

All data are expressed as the arithmetic average of three replicates ± standard deviation. TA: titratable acidity. Statistical analysis: one-factor ANOVA and Scheffe's test (both, $p = 0.05$). Different letter indicates the existence of statistically significant differences between values of the same vintage.

Table 5 shows the anthocyanin concentration, measured by spectrophotometry, of the wines of both vintages. Parallel to what was observed in the colour, the total anthocyanin concentration of wines from the 2007 vintage was also higher than that of 2008, reconfirming the greater maturity of this vintage. In both years, the density of the grapes also played a key role in the anthocyanin concentration of the wines. The higher the grape density, the higher the anthocyanin concentration in the wine. This behaviour was similar for free anthocyanins and also for anthocyanins combined to flavanols. The higher anthocyanin concentration found in denser grapes can mainly due to the higher concentration of anthocyanins in the skins and also maybe to the higher ethanol concentration of these grapes which exerts a significant effect on anthocyanin extraction during winemaking (Canals et al., 2005).

Anthocyanins were also analysed by HPLC (Table 6) and the overall results were similar to those obtained by spectrophotometry, although the values were significantly lower. This is because spectrophotometric analysis overestimates the total anthocyanin concentration because it also detects other pigments (Rivas-Gonzalo, Gutierrez, Hebrero, & Santos-Buelga, 1992), whereas HPLC only detects free anthocyanins (Rivas-Gonzalo, 2003). Furthermore, conversion of spectrophotometric data to anthocyanin concentration is necessarily imprecise, because many different pigments showing different extinction coefficients contribute to the absorbance.

The total anthocyanin concentration measured by HPLC was also higher in wines from 2007 than in those from 2008 when wines of same grape density were compared, confirming again the greater maturity of this vintage. As with spectrophotometry, HPLC also showed that the density of the grapes had a large influence on wine anthocyanin concentration. The higher the density of the grapes, the higher the anthocyanin concentration of the wines. A similar tendency was observed in anthocyanidin-3-monoglucosides and in acetylated and coumarylated anthocyanins as well as in derived pigments such as vitisin A and vitisin B. Together these results indicate that the density of the grapes greatly affects the concentration of the principal molecules responsible for wine colour. In particular, the presence of a high proportion of less dense grapes in the whole harvest can lower the anthocyanin concentration below expected levels, which corroborate again that the grape heterogeneity may be more important than is commonly thought.

Table 7 shows the results for total phenolic compounds, proanthocyanidins, measured by means of a precipitation with methyl-cellulose, and astringency. The total phenolic indexes (TPI) and proanthocyanidin concentration of 2007 wines were once more greater than those of 2008 when similar densities were

Table 6
HPLC analysis of anthocyanins and anthocyanin-derived pigments.

Parameter	Vintage	Low density	Medium density	High density
Anthocyanidin-3-monoglycosides (mg/l)	2007	194.3 ± 3.7a	229.4 ± 15.1b	242.1 ± 13.9b
	2008	72.4 ± 4.1a	173.2 ± 34.9b	228.2 ± 18.6c
Acetylated anthocyanins (mg/l)	2007	61.7 ± 1.3a	68.0 ± 3.9b	70.2 ± 4.1b
	2008	27.5 ± 0.9a	68.1 ± 14.9b	91.5 ± 6.1c
p-Coumaroyl anthocyanins (mg/l)	2007	12.0 ± 3.8a	16.9 ± 0.4b	20.7 ± 0.7c
	2008	4.2 ± 0.1a	12.5 ± 3.6b	19.0 ± 1.7c
Total free anthocyanins (mg/l)	2007	275.4 ± 1.8a	313.4 ± 19.5b	351.0 ± 18.7b
	2008	104.2 ± 4.2a	253.8 ± 53.4b	338.7 ± 26.1c
Vitisin A (mg/l)	2007	0.10 ± 0.01a	0.15 ± 0.01b	0.21 ± 0.04c
	2008	10.20 ± 0.65a	11.53 ± 0.56ab	13.97 ± 1.56b
Vitisin B (mg/l)	2007	3.17 ± 1.50a	5.14 ± 0.73ab	5.49 ± 0.68b
	2008	2.04 ± 0.41a	3.28 ± 0.37b	4.36 ± 0.84b

All data are expressed as the arithmetic average of three replicates ± standard deviation. TA: titratable acidity. Statistical analysis: one-factor ANOVA and Scheffe's test (both, $p = 0.05$). Different letter indicates the existence of statistically significant differences between values of the same vintage.

Table 7
Total phenolic compounds, proanthocyanidins and related parameters.

Parameter	Vintage	Low density	Medium density	High density
TPI	2007	39.9 ± 2.0a	49.1 ± 1.6b	51.8 ± 1.3b
	2008	35.4 ± 1.8a	40.8 ± 2.7b	49.9 ± 3.4c
Condensed tannins (mg epicatechin/l)	2007	992 ± 130a	1113 ± 241a	1571 ± 350a
	2008	887 ± 66a	836 ± 50a	1322 ± 216b
Astringency index (g of tannic acid/l)	2007	0.159 ± 0.009a	0.202 ± 0.005b	0.231 ± 0.004c
	2008	0.086 ± 0.003a	0.096 ± 0.013a	0.142 ± 0.030b

All data are expressed as the arithmetic average of three replicates ± standard deviation. TPI: total phenol index. Statistical analysis: one-factor ANOVA and Scheffe's test (both, $p = 0.05$). Different letter indicates the existence of statistically significant differences between values of the same vintage.

Table 8
HPLC analysis of total proanthocyanidins and related parameters, following acid-catalysis in the presence of excess of phloroglucinol for the wines of 2008.

Parameter	Low density	Medium density	High density
Total proanthocyanidins (mg/l)	639.1 ± 92.5a	715.4 ± 168.6ab	1006.2 ± 191.9b
mDP	3.70 ± 0.22a	4.71 ± 0.48b	4.91 ± 0.25b
(+)-Catechin (%)	16.8 ± 0.4a	13.3 ± 1.7b	12.1 ± 0.4b
(−)-Epicatechin (%)	62.0 ± 0.2a	61.8 ± 1.6a	60.6 ± 0.9a
(−)-Epigallocatechin (%)	18.2 ± 0.8a	21.7 ± 1.0b	24.1 ± 0.9c
(−)-Epicatechin gallate (%)	2.9 ± 0.1a	3.2 ± 0.2a	3.2 ± 0.2a
Total proanthocyanidins (μmol/l)	585 ± 115a	572 ± 59a	683 ± 101a
Molecular weight average (Da)	1100 ± 66a	1403 ± 145b	1466 ± 76b

All data are expressed as the arithmetic average of three replicates ± standard deviation. mDP: mean degree of polymerisation. Statistical analysis: one-factor ANOVA and Scheffe's test (both, $p = 0.05$). Different letter indicates the existence of statistically significant differences.

compared. Again it was confirmed that the maturity of the 2007 vintage was higher. In this case the trends were also very clear and indicated that the density of the grapes had a strong influence on the final quality of wine. Both the IPT and proanthocyanidin concentration of the wine increased significantly with the density of the grapes. As with the anthocyanins, there are two causes for this. First, denser grapes have a higher proanthocyanin accumulation and second, more these compounds are extracted due to the higher ethanol concentration in the wines obtained from these grapes (Canals et al., 2005). The astringency of the wines was also measured and presented behaviour that was parallel to that of the proanthocyanidins.

Table 8 shows the results of the analysis of wine proanthocyanidins obtained by acid-catalysis in the presence of excess phloroglucinol (2008 vintage only). The results of the proanthocyanidin concentration measured by this method do not exactly match those obtained by precipitation with methyl-cellulose. However, the tendencies observed by both methods are very similar, the proanthocyanidin concentration of the wines being higher when the density of the grapes was greater. Moreover, when the density of the berries increased, the proportion of (+)-catechin significantly decreased and the proportion of (−)-epigallocatechin significantly increased. Meanwhile, the proportions of the other two monomers, (−)-epicatechin and (−)-epicatechin-3-*O*-gallate remained constant. Since epigallocatechin is only present in skin proanthocyanidins (Souquet et al., 1996) these data indicate that the contribution of skins to the wine proanthocyainidin concentration increases when the grapes are denser and therefore riper. It seems therefore that denser grapes provide more proanthocyanidins which would produce more tannic wines with a greater capacity for ageing. In contrast, less dense grapes would produce slight wines without capacity for ageing. Hence, the grape heterogeneity can affect these attributes.

The mean degree of polymerisation (mDP) of proanthocyanidins of the wines obtained with the grapes of medium and high density present similar values. However, the mDP of the wine obtained with berries of low density was significantly lower. This data confirms that the degree of polymerisation of proanthocyanidins is higher when the grapes are riper. Also it would suggest that the lower density grapes mainly provide seed proanthocyanidins since the mDP of skin proanthocyanidins are higher than the mDP of seed proanthocyanidins (Prieur et al., 1994; Souquet et al., 1996). These results agree with those of Kennedy et al. (2002) who have found that the degree of proanthocyanidin polymerisation increases with maturity.

Acid-catalysis in the presence of excess phloroglucinol also allows the molar concentration and consequently the molecular weight average of proanthocyanidins to be obtained. As was expected, the molecular weight average followed a similar trend to the mDP. However, the proanthocyanidin molar concentration surprisingly did not vary significantly amongst the wines made from grapes of different densities. This fact indicates that all the grapes, regardless of their density, release a similar number of proanthocyanidin molecules during winemaking. Therefore, the increase observed in the proanthocyanidin concentration, expressed in mg/l, is only to the result of an increase in the degree of proanthocyanidin polymerisation and not to an increase in the number of molecules. These data also justify why the wines from denser grapes presented higher astringency. According to Vidal et al. (2003) astringency augments when the degree of proanthocyanidin polymerisation increases. Given that wines made with high density grapes presented higher proanthocyanin concentration and that their proanthocyanins also presented a higher mDP, it is logical that their astringency was greater too.

Table 9 presents the HPLC analysis of flavanols (catechins and oligomers). Also in this case, the density of the berries significantly affected the concentration of proanthocyanidin monomers and oligomers. The total monomer concentration increased when the density of the grapes was higher. This augmentation mainly occurs due to the increase of (+)-catechin concentration whilst (−)-epicatechin concentration remained at similar levels. On the other hand, the wine concentration of proanthocyanidin dimers also increased when the grapes were denser. However, this increased was only due to dimers B1 and in particular B3 whereas other dimers such as B4, B5 and B6 present similar values in all wines. The trimers and tetramers followed a similar profile to the dimers. The denser

Table 9
HPLC analysis of flavanols (catechins and proanthocyanidin oligomers) for the wines of 2008.

Parameter	Low density	Medium density	High density
(+)-Catechin (mg/l)	377.8 ± 24.5a	522.4 ± 54.4b	671.2 ± 2.5c
(−)-Epicatechin (mg/l)	415.0 ± 42.7a	444.9 ± 23.4a	533.0 ± 91.1a
Catechins (mg/l)	792.8 ± 66.6a	967.3 ± 77.8b	1152.8 ± 24.1c
B1 (mg/l)	11.4 ± 2.9a	32.2 ± 3.2b	58.9 ± 3.4c
B3 (mg/l)	67.7 ± 11.1a	103.6 ± 16.0b	104.9 ± 25.7c
B4 (mg/l)	13.8 ± 0.9a	12.1 ± 1.9a	13.0 ± 2.0a
B5 (mg/l)	3.4 ± 0.1a	3.3 ± 0.5a	3.0 ± 0.4a
B6 (mg/l)	48.6 ± 1.6a	49.0 ± 1.9a	50.3 ± 2.0a
Dimers (mg/l)	132.1 ± 9.7a	196.1 ± 16.6b	230.5 ± 27.4b
Trimers (mg/l)	39.2 ± 1.3a	41.7 ± 16.0ab	67.1 ± 10.2b
Tetramers (mg/l)	8.6 ± 0.7a	11.5 ± 1.1b	15.0 ± 0.1c
Oligomers (mg/l)	179.9 ± 10.1a	252.7 ± 3.3b	312.7 ± 35.3c
Catechins + oligomers (mg/l)	1005.2 ± 20.1a	1220.0 ± 74.5b	1468.3 ± 73.5c

All data are expressed as the arithmetic average of three replicates ± standard deviation. TA: titratable acidity. Statistical analysis: one-factor ANOVA and Scheffe's test (both, $p = 0.05$). Different letter indicates the existence of statistically significant differences.

the grapes, the higher their concentration. This increase in oligomers agrees with the previously mentioned results concerning the mDP.

The sensory triangular tests were carried out by comparing in pairs the three wines obtained from the different density grapes. Only 5 of 10 tasters were able to differentiate between the high density wines and the medium density wines. This result was not statistically significant ($p > 0.05$). In contrast, all the tasters distinguished the low density wine when it was compared with the medium or high density wine, being the results statistically significant ($p < 0.05$). All these sensory analyses were done in dark glasses to prevent the tasters from being influenced by the colour. Therefore, these differences must be attributed to flavours and/or mouth sensation and not wine colour. In all cases, the tasters preferred wines from denser grapes. The reason given was that these wines had a higher fruit and floral scents. Also these were better balanced as they were sweeter, less acidic and less bitter than the low density wines.

4. Conclusions

It can be concluded that the heterogeneity of the grapes at the moment of the harvest is more important that it is usually thought. The ensemble of all the grapes always has a proportion of very well ripened grapes, another proportion of sufficiently ripened grapes and finally a proportion of less ripened grapes. This fact must be taken into account because the presence of less ripened grapes can affect seriously the final composition and consequently the quality of the wine. These less ripened grapes diminish the final ethanol content, pH, anthocyanin concentration, colour intensity, total phenolic index and proanthocyanidin concentration and increase titratable acidity. Moreover, these lower density grapes contribute less polymerised proanthocyanidins, lower proportions of (−)-epigallocatechin and higher proportions of (+)-catechin. These data suggest that lower density grapes release more seed proanthocyanidins than skin proanthocyanidins. Finally, the sensory comparison of wines, leaves no doubt, since the wine obtained from lower density grapes was always recognised in all the triangle trials and was considered as less balanced and bitterer. Further research is needed to determine how this heterogeneity can be measured easily and quickly. Only in this way will winemakers have the tools which enable them to take the heterogeneity of the grapes into account in their maturity controls and even to consider the possibility of applying a sorting table for eliminating the unripe grapes.

Acknowledgements

We thank CICYT (AGL2007-66338) and CDTI (Project CENIT Demeter) for financial support.

References

Arnold, R., & Noble, A. C. (1978). Bitterness and astringency of grape seed phenolics in a model solution. *American Journal of Enology and Viticulture, 29*, 150–152.
Ayala, F., Echávarri, J. F., & Negueruela, A. I. (1997). A new simplified method for measuring the colour of wines I. Red and rose wines. *American Journal of Enology and Viticulture, 48*, 357–363.
Ayala, F., Echávarri, J. F., & Negueruela, A. I. (2001). <http://www.unizar.es/negueruela/MSCVes.zip>.
Canals, R., Llaudy, M. C., Valls, J., Canals, J. M., & Zamora, F. (2005). Influence of ethanol concentration on the extraction of colour and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *Journal of Agricultural and Food Chemistry, 53*, 4019–4025.
Celotti, E., Della Vedova, T., Ferrarini, R., & Martinand, S. (2007). The use of reflectance for monitoring phenolic maturity curves in red grapes. *Italian Journal of Food Science, 19*, 91–100.
De Freitas, V., & Glories, Y. (1999). Concentration and compositional changes of procyanidins in grape seeds and skin of white *Vitis vinifera* varieties. *Journal of the Science of Food and Agriculture, 79*, 1601–1606.
De Freitas, V. A. P., Glories, Y., Bourgeois, G., & Vitry, C. (1998). Characterisation of oligomeric and polymeric procyanidins from grape seeds by liquid secondary ion mass spectrometry. *Phytochemistry, 49*(5), 1435–1441.
Dupuch, V. (1993). Appréciation de la metière phénolique des vins rouges; application à la détermination de la date de récolte. Actes du Colloque ''Journé technique du CIVB" 21 janvier 1993, Bordeaux (pp. 62–69).
Fournand, D., Vicens, A., Sidhoum, L., Souquet, J. M., Moutounet, M., & Cheynier, C. (2006). Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. *Journal of Agricultural and Food Chemistry, 54*, 7331–7338.
Gambuti, A., Strollo, D., Lecce, L., & Moio, L. (2007). The effect of grape ripeness stages on polyphenol content of aglianico (*Vitis vinifera* L.) wine aged for two years. *Acta Horticulturae, 754*, 449–456.
Gawel, R. (1998). Red wine astringency: A review. *Australian Journal of Grape and Wine Research, 4*, 74–98.
Glories, Y. (1984). La couleur des vins rouges. 1e're partie: Les equilibres des anthocyanes et des tannins. *Connaisance de la Vigne et du Vin, 18*, 195–217.
Glories, Y., & Agustin, M. (1993). Maturité phénolique du raisin, conséquences technologiques; application aux millésimes 1991 et 1992. Actes du Colloque ''Journé technique du CIVB" 21 janvier 1993, Bordeaux (pp. 56–61).
Gonzalez-Manzano, S., Rivas-Gonzalo, J. C., & Santos-Buelga, C. (2004). Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration. *Analytica Chimica Acta, 513*, 283–289.
González-San José, M., Diez, C., Santa María, G., & Garrido, J. L. (1988). Análisis por cromatografía líquida de alta eficacia de los pigmentos antociánicos de uvas de *Vitis vinifera* L. *Anales de Química, 84*, 290–293.
Haselgrove, L., Botting, D., van Heeswijck, R., Høj, P. B., Dry, P. R., Ford, C., et al. (2000). Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic composition of *Vitis vinifera* L. cv. Shiraz grape berries. *Australian Journal of Grape and Wine Research, 6*, 141–149.
Herderich, M. J., & Smith, P. A. (2005). Analysis of grape and wine tannins: Methods, applications and challenges. *Australian Journal of Grape and Wine Research, 11*, 205–214.
Jackson, R. S. (2002). Quantitative (technical) wine assessment. In Jackson (Ed.), *Wine tasting. A professional hand book* (pp. 113–186). Birlighton: Academic Press.
Kennedy, J. A., & Jones, G. P. (2001). Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *Journal of Agricultural and Food Chemistry, 49*, 1740–1746.
Kennedy, J. A., Matthewa, M. A., & Waterhouse, A. L. (2002). Effect of maturity and vine water status on grape skin and wine flavonoides. *American Journal of Enology and Viticulture, 53*(4), 268–274.
Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2010). Comparison of methods for estimating phenolic maturity in grapes: Correlation between predicted and obtained parameters. *Analytica Chimica Acta, 660*, 127–133.
Llaudy, M. C., Canals, R., Canals, J. M., Rozes, N., Arola, Ll., & Zamora, F. (2004). New method for evaluating astringency in red wine. *Journal of Agricultural and Food Chemistry, 52*, 742–746.
Llaudy, M. C., Canals, R., Canals, J. M., & Zamora, F. (2008). Influence of ripening stage and maceration length on the contribution of grape skins, seeds and stems to phenolic composition and astringency in wine-simulated macerations. *European Food Research and Technology, 226*, 337–344.
Niketic-Aleksic, G. K., & Hrazdrina, G. (1972). Quantitative analysis of the anthocyanin content in grape juices and wine. *Lebensmittel-Wissenschaft und -Technologie, 5*(5), 163–165.

Noble, A. C. (1990). Bitterness and astringency in wine. In R. L. Rouseff (Ed.). *Bitterness in foods and beverages, development in food science* (Vol. 25, pp. 145–158). New York: Elsevier.

O.I.V. (2005). *Le recueil des méthodes internationales d'analyse des vins et des moûts* (Vols. I, II, Sect. 3). Paris: O.I.V.

Ó-Marques, J., Reguinga, R., Laureano, O., & Ricardo-da-Silva, J. M. (2005). Changes in grape seed, skins and pulp condensed tannins during berry ripening: Effect of fruit pruning. *Ciência Tècnica Vitivinicola, 20*(1), 35–52.

Pérez-Magarin~o, S., & González-San José, M. L. (2006). Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade. *Food Chemistry, 96*(2), 197–208.

Pérez-Magariño, S., & González-Sanjose, M. L. (2003). Application of absorbance values used in wineries for estimating CIELAB parameters in red wines. *Food Chemistry, 81*, 301–306.

Peyrot des Gachons, C., & Kennedy, J. A. (2003). Direct method determining seed and skin proanthocyanidin extraction into red wine. *Journal of Agricultural and Food Chemistry, 51*, 5877–5881.

Prieur, C., Rigaud, J., Cheynier, V., & Moutounet, M. (1994). Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry, 36*, 781–784.

Ribereau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). Phenolic compounds. *Handbook of enology: Vol. 2. The chemistry of wine, stabilization and treatments* (pp. 141–204). Chichester: John Wiley and Sons, Ltd.

Rivas-Gonzalo, J. C. (2003). Analysis of anthocyanins. In C. Santos-Buelga & G. Williamson (Eds.), *Methods in polyphenols analysis* (pp. 338–358). Cambridge: The Royal Society of Chemistry.

Rivas-Gonzalo, J. C., Gutierrez, Y., Hebrero, E., & Santos-Buelga, C. (1992). Comparisons of methods for the determination of anthocyanins in red wines. *American Journal of Enology and Viticulture, 43*(2), 210–214.

Romeyer, F. M., Macheix, J. J., & Sapis, J. C. (1986). Changes and importance of oligomeric procyanidins during maturation of grape seed. *Phytochemistry, 25*(1), 219–221.

Ryan, J. M., & Revilla, E. (2003). Anthocyanin composition of *Cabernet sauvignon* and Tempranillo grapes at different stages of ripening. *Journal of Agricultural and Food Chemistry, 51*, 3372–3378.

Sarneckis, C. J., Dambergs, R. G., Jones, P., Mercurio, M., Herderich, M. J., & Smith, P. A. (2006). Quantification of condensed tannins by precipitation with methyl cellulose: Development and validation of an optimised tool for grape and wine analysis. *Australian Journal of Grape and Wine Research, 12*, 39–49.

Smart, R. E., Robinson, J. B., Due, G. R., & Brien, C. J. (1985). Canopy microclimate modification for the cultivar Shiraz. I. Definition of canopy microclimate. *Vitis, 24*, 17–31.

Souquet, J. M., Cheynier, V., Brossaud, F., & Moutounet, M. (1996). Polymeric proanthocyanidins from grape skins. *Phytochemistry, 43*, 509–512.

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., et al. (2003). The mouth-feel properties of grape and apple proanthocyanidins in a winelike medium. *Journal of the Science of Food and Agriculture, 83*(6), 564–573.

Vivas, N., & Glories, Y. (1996). Role of oak wood ellagitannins in the oxidation process of red wines during aging. *American Journal of Enology and Viticulture, 47*, 103–107.