OENO One
VINE AND WINE OPEN ACCESS JOURNAL
Volume 50 > Number 4 > 2016

Influence of grape maturity and prefermentative cluster treatment of the Grenache cultivar on wine composition and quality

Olga Pascual[1], Jeanette Ortiz[1], Maruxa Roel[1], Nikolaos Kontoudakis[1], Mariona Gil[1], Sergio Gómez-Alonso[2,4], Esteban García-Romero[3], Joan Miquel Canals[1], Isidro Hermosín-Gutíerrez[2] and Fernando Zamora[1*]

[1]Departament de Bioquímica i Biotecnologia, Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili, C/Marcel.li Domingo s/n, 43007 Tarragona, Spain
[2]Instituto Regional de Investigación Científica Aplicada, Universidad de Castilla-La Mancha, Campus Universitario s/n, 13071 Ciudad Real, Spain
[3]Instituto de la Vid y el Vino de Castilla-La Mancha, Ctra. Toledo-Albacete s/n, 13700 Tomelloso, Ciudad Real, Spain
[4]Fundación Parque Científico y Tecnológico de Albacete, Paseo de la Innovación 1, 02006 Albacete, Spain

Abstract

This work studied how different grape maturity levels and cluster treatments affect the color and phenolic composition of Grenache wines. Specifically, five treatments were undertaken at a microvinification scale for three maturity levels: Control (destemmed and crushed grapes), Whole Berry, Whole Cluster, Crushed Cluster and Submerged Cap. The first three treatments were also reproduced with large-scale wine fermentation in oak barrels but only with well-ripened grapes. The results indicated that the total polyphenol index (TPI), anthocyanin and proanthocyanidin concentrations, as well as the mean degree of polymerization were higher in all the treatments when the grapes were riper. Submerged Cap generated maximum color and polyphenolic extraction at the three maturity levels. Whole Berry wines were the most similar to the controls. The presence of stems (Crushed Cluster and Whole Cluster treatments) produced wines with a significantly higher pH at all maturity levels and with lower color intensity when the grapes were less ripe. The presence of stems also significantly increased the TPI in almost all cases.

Keywords: grape maturity, cluster treatment, stems, color, phenolic compounds

manuscript received 4th December 2015 - accepted 8th March 2016
doi: 10.20870/oeno-one.2016.50.4.1824

*Corresponding author: fernando.zamora@urv.cat

OENO One, 2016, **50**, 4, 169-181

INTRODUCTION

The quality of red wines is highly determined by the composition of phenolic compounds. Some of their sensory attributes, such as color, body and astringency, are mainly associated with the composition of anthocyanins and proanthocyanidins (Gawel, 1998; Vidal *et al.*, 2003). Anthocyanins are only present in grape skins of most grape cultivars, with the exception of teinturier varieties, whereas proanthocyanidins are present in skins, seeds, and stems (Ribereau-Gayon *et al.*, 2000). Seed proanthocyanidins are made up of (+)-catechin, (-)-epicatechin, and (-)-epicatechin-3-gallate (Prieur *et al.*, 1994), whereas skin proanthocyanidins also contain (-)-epigallocatechin and a much lower concentration of (-)-epicatechin-3-gallate (Souquet *et al.*, 1996). Consequently, skin proanthocyanidins include procyanidins and prodelphinidins, whereas seed proanthocyanidins only consist of procyanidins. Little is known about stem proanthocyanidins, but it is thought that they are made up of the four monomers: (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-gallate, and (-)-epigallocatechin (Souquet *et al.*, 2000; del Llaudy *et al.*, 2008). Skin proanthocyanidins have a higher mean degree of polymerization (mDP) than seed proanthocyanidins but the polymerization degree of stem proanthocyanidins is a subject of controversy (Souquet *et al.*, 2000; Vivas *et al.*, 2004; del Llaudy *et al.*, 2008). It has also been reported that molecular sizes, and especially the monomeric composition of proanthocyanidins, have a considerable influence on the perception of astringency. More specifically, a greater degree of polymerization and a higher percentage of galloylation cause a greater perception of astringency (Vidal *et al.*, 2004).

It is well known that the maturity of grapes strongly influences the phenolic composition of red wines (del Llaudy *et al.*, 2008; Gil *et al.*, 2012). Unripe grapes have lower extractability of anthocyanins and skin proanthocyanidins, but higher extractability of seed proanthocyanidins (Peyrot des Gachons and Kennedy, 2003; Canals *et al.*, 2005). For this reason, immature grapes may produce more astringent wines because their seeds can release a greater quantity of highly galloylated proanthocyanidins (del Llaudy *et al.*, 2008). It has also been shown that stems can release highly astringent and bitter proanthocyanidins. Moreover, the presence of stems causes significant color loss and contributes to a 'stemmy flavor' in the wine (Boulton *et al.*, 1995; Hashizume and Samuta, 1997). For this reason, destemming grapes is a common procedure in red winemaking in order to avoid these negative attributes. Other arguments for removing stems are that they reduce the ethanol content and titratable acidity, increase pH and even take up valuable space in the tank (Sun and Spranger, 2005).

On the contrary, some winemakers argue that stems may occasionally have positive effects (Peynaud, 1984; Sun and Spranger, 2005). They claim that retaining stems produces wines with a higher concentration of proanthocyanidins, which helps to stabilize color and improve mouthfeel. Moreover, the presence of stems makes the cap less compact, which favors color extraction. Traditionally, stems have been used in red winemaking in such traditional regions as Châteauneuf-du-Pape (Côtes du Rhône), because their presence increased the polyphenolic content of wines and, therefore, improved their aging ability. Moreover, some winemakers in the Médoc region (Bordeaux) used to include a proportion of stems when grey rot was present, with the aim of inhibiting laccase and protecting wine color. Stems have occasionally been partially or fully used for low-tannin varieties such as Pinot Noir in traditional regions (Peynaud, 1981; Blouin, 2000). Nowadays, winemaking using the whole cluster is especially common in biodynamic/natural wine production, probably because the extra tannin contribution of stems can protect the wine against oxidation, which means that the doses of sulfur dioxide can be decreased.

Operations during winemaking can have a non-negligible effect on color and phenolic compound extraction (Gómez-Plaza *et al.*, 2000). Several studies have been carried out on the influence of temperature, enzymatic addition, maceration length, mechanical treatment of the cap, ethanol content, etc (Sacchi *et al.*, 2005; Gil *et al.*, 2013). However, to our knowledge, very little information exists about the influence of stem presence on winemaking, and wine composition and quality (Goode and Harrop, 2011). For this reason, the aim of this study was to investigate how different grape maturity levels and prefermentative cluster treatments, with or without stems, affected the color and phenolic composition of Grenache wines.

MATERIALS AND METHODS

1. Chemicals and equipment

Methanol, acetonitrile, formic acid and acetic acid of high performance liquid chromatography (HPLC) grade (>99%) and absolute ethanol and hydrochloric acid (37 %) were purchased from Panreac (Barcelona, Spain); acetaldehyde, polyvinylpoly-

pyrrolidone, phloroglucinol, ascorbic acid, sodium acetate and ammonium formate were purchase from Sigma-Aldrich (Madrid, Spain) ; the commercial standards *trans*-caftaric acid (≥95%), quercetin 3-glucuronide (≥95%), caffeic acid (≥99%) and *p*-coumaric acids (≥99%) were purchased from Phytolab (Vestenbergsgreuth, Germany) ; the commercial standards malvidin 3-glucoside (≥95%), kaempferol (≥99%), quercetin dihydrate (≥99%), isorhamnetin (≥99%), myricetin (≥99%) and syringetin (≥99%), the 3-glucosides of kaempferol (≥99%), quercetin (≥99%), myricetin (≥99%), isorhamnetin (≥95%) and syringetin (≥99%) were purchased from Extrasynthese (Genay, France). Vitisin A (10-carboxy-pyranomalvidin-3-glucoside) was quantified with a previously obtained standard of ≥95% purity (Blanco-Vega *et al.*, 2011). All spectrophotometric measurements were carried out with a Helios Alpha UV-vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2. Grapes and wines

The experiment was carried out with a Grenache variety (*Vitis vinifera* L) from the AOC Montsant (Spain). About 230 kg of grapes were manually harvested at 3 maturity levels (3, 5, and 7 weeks after veraison). Five different cluster treatments and maceration techniques were performed : Control, Submerged Cap, Whole Berry (destemming without crushing), Crushed Cluster (crushing without destemming), and Whole Cluster. All microvinifications were carried out in triplicate in 25 L tanks. Around 3/5 of the grapes were carefully destemmed (Delta, Bucher-Vaslin, Chalonnes-sur-Loire, France) and the intact berries were randomly distributed in 9 batches of 15 kg. The first three batches were introduced in three tanks without any treatment (Whole Berry), whereas the other six were crushed with a manual crusher. Three of these tanks were considered as "Control" while the other three were employed for "Submerged Cap" winemaking. The remaining 2/5 of the grapes were randomly distributed in batches of 15 kg without destemming. Three of them were crushed with a manual crusher (Crushed Cluster) while the other three were placed in the tanks intact (Whole Cluster). All the tanks were immediately sulfited (100 mg $K_2S_2O_5$/kg), inoculated with 200 mg/kg of selected yeast (EC1118, Lallemand Inc, Montreal, Canada) and maintained at a room temperature of 25 ± 1 °C. All treatments were punched-down once a day until the end of fermentation, excluding the Submerged Cap system, which was carried out according to the winemaking method described by Sampaio *et al.* (2007). After 2 weeks of maceration, the wines were racked into bottles (5 L plastic). All the wines were sulfited (100 mg $K_2S_2O_5$/L) and kept at 4 °C for 1 month for stabilization. Malolactic fermentation was therefore inhibited to prevent any variations caused by it. The wines were subsequently bottled and stored in a dark cellar at 15 °C until analysis.

This experiment was also performed on a larger scale (400 kg each) but only with well-ripe grapes from another vineyard and with 3 treatments only (Control, Whole Berry and Whole Cluster). This experiment was performed without replicates in opened French oak barrels (500 L) placed vertically as fermentation tanks. These wines were aged in 225 L French oak barrels for six months.

3. Standard wine analysis

The analytical methods recommended by the OIV were used to determine the ethanol content, pH and volatile acidity (Organisation Internationale de la Vigne et du Vin, 2014). The total polyphenol index (TPI) was analyzed by measuring the 280 nm absorbance of a 1 : 100 dilution of wine with a spectrophotometer, using a 10-mm quartz cuvette and multiplying the absorbance value by 100 as described by Ribéreau-Gayon *et al.* (2006). Condensed tannin concentration was estimated by precipitation with methyl-cellulose (Sarneckis *et al.*, 2006).

4. Color parameters

Ten microliters of a 10 % (v/v) acetaldehyde solution was added to 1 mL of wine sample 20 min before color measurement to avoid sulfite interferences. The color 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 the method used by Ayala *et al.* (1997) and data processing was performed with MSCV software (Ayala *et al.*, 2001).

5. Analysis of individual low molecular mass (MM) phenolic substances in wine

The individual low MM phenolic substances in wines were prepared with solid phase extraction and analyzed with a reversed-phase HPLC diode array detector-electrospray ionization-tandem mass spectrometry system (RP-HPLC-DAD-ESI-MSn) (Blanco-Vega *et al.*, 2011 ; Lago-Vanzela *et al.*, 2013). The system comprised an Agilent 1100 Series HPLC (Agilent, Waldbronn, Germany), equipped with a DAD (G1315B) and an LC/MSD Trap VL (G2445C VL) ESI-MSn, coupled to an Agilent Chem Station (version B.01.03) data processing station. The

OENO One, 2016, **50**, 4, 169-181

mass spectra data were processed with the Agilent LC/MS Trap software (version 5.3). The samples (0.25 mL of wine diluted with 4.75 mL of water: formic acid, 98.5:1.5) were injected (100 µL) after filtration (0.20 µm, polyester membrane, Chromafil PET 20/25, Macherey-Nagel, Düren, Germany) on a Ascentis Express C18 reversed-phase column (4.6 × 150 mm; 2.7 µm particle size) (Supelco, Sigma-Aldrich, Madrid, Spain), maintained at 16°C. The solvents were A [water/methanol/formic acid (89: 10:1, v/v/v)] and B (methanol), and the flow rate was 0.5 mL/min. The linear gradient for solvent B was: 0 min, 1 %; 2 min, 1%; 60 min, 23 %; 75 min, 70 %; 80 min, 95 %; 90 min, 95 %; 95 min, 1 %; 100 min, 1 %. Two MS scan types were used: enhanced MS for compound identification, and multiple reaction monitoring (MRM) for quantification. The conditions for both MS scan types were ion spray voltage, −4000; ion source temperature, 450 °C; collision gas, high; curtain gas, 15; ion source gas 1, 70; ion source gas 2, 50; declustering potential, −35; entrance potential, −10; collision energy, −30; and collision cell exit potential, −3. Two injections of (+)-catechin standard solution, one at the beginning and the second at the end of every injection series, were performed to update the response factors before quantification. The analyses were carried out in duplicate. The chromatographic system was managed by an Agilent Chem Station (version B.01.03) data processing station. The mass spectral data were processed with the Analyst MDS software (Applied Biosystems, version 1.5).

6. Analysis of wine proanthocyanidins

The proanthocyanidins of the wines were extracted and analyzed by acid depolymerization in the presence of an excess of phloroglucinol (Pastor del Rio and Kennedy, 2006); the products of the reaction were separated by RP-HPLC-DAD (Kennedy and Jones *et al.*, 2001). Proanthocyanidins were analyzed with an Agilent 1200 Series HPLC equipped with a G1362A refractive index detector (RID), a G1315D DAD, a G1311A quaternary pump, a G1316A column oven and a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA). The chromatographic system was managed by an Agilent Chem Station (version B.01.03) data processing station.

7. Sensory analysis

Sensory analyses were only performed with the wines obtained by barrel winemaking because they were considered more representative of what occurs in the wineries than micro-scale wines. Two sensory triangle tests were conducted by eleven expert tasters to compare the control wine versus the wines obtained with the Whole Berry or the Whole Cluster. In all the cases, the main objective was to determine whether the tasters were able to recognize the wine that was different. The secondary objective was to determine which wine was preferred by the panelists who had correctly identified the different wines.

Table 1 - Effect of grape maturity and prefermentative cluster treatment on the general parameters of micro-scale wines

Parameter	Maturity Level	Control	Submerged Cap	Whole Berry	Crushed Cluster	Whole Cluster
Ethanol (%v/v)	1	14,2 ± 0,1 A β	14,3 ± 0,1 A β	14,1 ± 0,1 A αβ	14,3 ± 0,1 A β	13,9 ± 0,1 A α
	2	16,0 ± 0,2 B αβ	16,2 ± 0,1 B β	15,8 ± 0,1 B α	15,9 ± 0,2 B αβ	15,7 ± 0,1 B α
	3	16,5 ± 0,1 C αβ	16,7 ± 0,1 C β	16,6 ± 0,0 C β	16,7 ± 0,1 C β	16,3 ± 0,1 C α
pH	1	3,17 ± 0,03 A α	3,19 ± 0,03 A α	3,20 ± 0,03 A α	3,38 ± 0,07 A β	3,28 ± 0,03 A β
	2	3,55 ± 0,06 B α	3,59 ± 0,01 B α	3,61 ± 0,03 B α	3,73 ± 0,03 B β	3,73 ± 0,01 B β
	3	3,79 ± 0,02 C α	3,79 ± 0,02 C α	3,77 ± 0,02 C α	3,89 ± 0,04 C β	3,94 ± 0,01 C β
AV (g/L)	1	0,27 ± 0,02 A α	0,28 ± 0,04 A α	0,26 ± 0,00 A α	0,29 ± 0,03 A α	0,22 ± 0,04 A α
	2	0,48 ± 0,04 B δ	0,39 ± 0,02 B γ	0,27 ± 0,02 A α	0,41 ± 0,02 B γ	0,32 ± 0,02 B β
	3	0,51 ± 0,04 B α	0,48 ± 0,02 C α	0,55 ± 0,07 B α	0,54 ± 0,04 C α	0,48 ± 0,02 C α
TPI	1	42,0 ± 2,2 A α	53,0 ± 1,9 A β	46,0 ± 0,7 A α	43,9 ± 1,6 A α	50,5 ± 3,0 A β
	2	51,7 ± 0,8 B α	55,3 ± 2,6 A α	52,6 ± 2,8 B α	54,5 ± 1,3 B α	54,9 ± 2,3 A α
	3	55,0 ± 3,1 B α	66,4 ± 2,6 B β	61,9 ± 2,1 C β	63,1 ± 0,9 C β	64,6 ± 1,1 B β
Tannins (mg/L)	1	509 ± 45 A α	637 ± 28 A γ	584 ± 10 A β	529 ± 58 A αβ	612 ± 66 A βγ
	2	566 ± 63 AB α	590 ± 52 A α	588 ± 25 A α	646 ± 55 B α	619 ± 30 A α
	3	655 ± 74 B α	839 ± 39 B β	688 ± 36 B α	811 ± 87 C β	779 ± 41 B β

Different letters indicate significant differences ($p<0.05$). Capital letters are used to compare the different maturity levels and Greek letters are used to compare the different treatments with the control (by using one-way ANOVA, and employing the Student-Newman-Keuls method for multiple comparisons). AV: Volatile acidity; TPI: Total Polyphenol Index of wines.

8. Statistics

All the data for micro-scale wines are expressed as the arithmetic average ± standard deviation of three replicates. One-factor ANOVA tests were carried out with XLSTAT software, and multiple comparisons were performed using the Student–Newman–Keuls post-hoc test. The level of significance of sensory triangle tests was determined following Jackson's method (Jackson, 2002).

RESULTS AND DISCUSSION

Table 1 shows the general parameters of the micro-scale wines. In overall terms, these results indicate clearly that the maturity level exerts a major influence on wine composition regardless of the cluster treatment and maceration procedure. As expected, the greater the maturity the higher the ethanol content, pH, TPI and tannin concentration in all the treatments. All these data confirm that grapes underwent the correct maturation process. Volatile acidity also increased with grape maturity, probably due to the higher ethanol content.

The different prefermentative cluster treatment of the grapes showed some interesting differences in some of the general parameters. The ethanol content was very similar in all the treatments at each maturity level. However, the ethanol content of Whole Cluster wines was slightly lower than in the other treatments, and these differences were significant in some cases. This somewhat lower ethanol content may be related to the presence of stems which can absorb ethanol and release water (Hashizume *et al.*, 1998). It has been reported that the moisture content of stems is around 65 % (González-Centeno *et al.*, 2010). Considering this value and that stems represent a percentage of about 4-5 % of the cluster weight, the observed decrease in ethanol content can be considered as quite logic once the osmotic equilibrium is reached. However, the Crushed Cluster wines showed similar values to the other experimental treatments although stems were also present. The influence of the presence of stems was clearer on the pH since both treatments containing stems, Crushed Cluster and Whole Cluster, had significantly higher values in this parameter. This is probably because stems can release potassium which neutralizes the acids (Hashizume *et al.*, 1998).

Table 2 - Effect of prefermentative cluster treatment on the general parameters of barrel-scale wines

Parameter	Control	Whole Berry	Whole Cluster
Ethanol (%v/v)	16,6	16,5	16,1
pH	3,86	3,76	4,03
AV (g/L)	0,51	0,49	0,49
TPI	38,3	45,7	51,1
Tannins (mg/L)	300	371	474

AV : Volatile acidity ; TPI : Total Polyphenol Index of wines.

Overall Submerged Cap wines have higher TPI and tannin concentrations than Control wines, although in some of the maturity levels these differences were not significant. These results confirm that this winemaking procedure improves polyphenol extraction, as has been previously reported (Bosso *et al.*, 2011 ; Ichikawa *et al.*, 2012). Whole Berry wines also presented generally higher TPI and tannin concentration than the Control wines, although these differences were only significant in some maturity levels. These differences were in any case smaller than those observed in Submerged Cap wines. As expected, Crushed Cluster and Whole Cluster wines also had higher TPI and tannin concentrations than the Control wines, although these differences were only significant in some cases. This data confirms that stems are a source of tannins (Suriano *et al.*, 2015).

Table 2 shows the general parameters of the barrel-scale wines. Since no replicates were performed in that experiment, it is impossible to draw statistical differences. However, some tendencies can be confirmed in comparison with the micro-scale experiments. For example, the ethanol content of Whole Cluster wine was lower and the pH higher than in Control and Whole Berry wines in a similar way to observations in the micro-scale trials. Whole Berry and especially Whole Cluster wines also have higher TPI and tannin concentrations than the Control wine. This data suggests that winemaking with Whole Berry favors phenolic compound extraction. This behavior was also observed in micro-scale assays, although the differences were smaller and not always significant. These results also confirm that the stems enrich wine in tannins, and probably also favor the extraction of phenolic compounds from skins and seed because their presence makes the cap less compact (del Llaudy *et al.*, 2008).

Table 3 shows the influence of grape maturity and prefermentative cluster treatment on the anthocyanin concentration and color parameters of micro-scale wines. In overall terms, total anthocyanins tended to increase with maturity in all treatments, although the differences were not always significant. This tendency was also observed in non-acylated

OENO One, 2016, **50**, 4, 169-181

Table 3 - Effect of grape maturity and prefermentative cluster treatment on anthocyanins and color parameters of micro-scale wines

Parameter	Maturity Level	Control			Submerged Cap			Whole Berry			Crushed Cluster			Whole Cluster		
Total Anthocyanins	1	280 ± 69	A	a	388 ± 29	A	bg	291 ± 56	A	α	342 ± 34	A	αβ	416 ± 8	A	g
	2	322 ± 34	A	a	401 ± 24	A	β	388 ± 39	A	αβ	434 ± 23	B	β	506 ± 15	B	g
	3	365 ± 42	A	a	426 ± 15	A	β	361 ± 13	A	α	439 ± 7	B	β	497 ± 7	B	g
Non-Acylated	1	259 ± 61	A	a	352 ± 26	A	bg	259 ± 50	A	α	308 ± 31	A	αβ	373 ± 4	A	g
	2	287 ± 31	A	a	360 ± 22	A	β	346 ± 35	B	αβ	401 ± 20	B	β	464 ± 13	B	g
	3	338 ± 39	A	a	394 ± 13	A	β	333 ± 10	B	α	411 ± 6	B	β	465 ± 6	B	g
Acetylated	1	8 ± 2	A	a	8 ± 1	A	α	7 ± 1	A	α	9 ± 1	A	α	11 ± 2	A	a
	2	10 ± 1	A	a	10 ± 1	A	α	10 ± 2	A	α	9 ± 1	A	α	11 ± 2	A	a
	3	8 ± 1	A	a	8 ± 1	A	α	7 ± 1	A	α	6 ± 2	A	α	8 ± 2	A	a
p-Coumaroylated	1	22 ± 6	A	a	29 ± 3	AB	ab	25 ± 5	AB	αβ	25 ± 2	A	α	32 ± 2	B	b
	2	25 ± 3	A	a	31 ± 2	B	β	32 ± 3	B	β	30 ± 2	B	αβ	32 ± 2	B	b
	3	20 ± 2	A	a	24 ± 1	A	β	21 ± 2	A	α	22 ± 2	A	α	25 ± 3	A	a
Pyranoanthocyanins	1	21 ± 4	A	b	13 ± 2	A	α	9 ± 2	A	α	28 ± 7	B	β	38 ± 16	B	b
	2	32 ± 2	B	g	24 ± 2	B	β	19 ± 1	B	α	34 ± 7	B	g	50 ± 4	B	d
	3	27 ± 3	AB	b	29 ± 3	B	β	24 ± 9	B	β	9 ± 1	A	a	10 ± 1	A	a
CI	1	6,4 ± 0,1	A	β	7,6 ± 0,2	A	γ	6,1 ± 0,2	A	β	5,4 ± 0,3	A	α	5,5 ± 0,3	A	α
	2	7,8 ± 0,1	B	β	9,7 ± 1,0	B	γ	7,9 ± 0,3	B	β	7,7 ± 0,6	B	β	6,7 ± 0,3	B	α
	3	10,5 ± 1,0	C	αβ	13,5 ± 0,6	C	γ	11,6 ± 0,4	C	β	11,0 ± 1,0	C	β	9,6 ± 0,4	C	α
L*	1	64,4 ± 0,4	C	β	59,7 ± 0,8	C	α	65,6 ± 0,8	C	β	68,6 ± 1,5	C	γ	68,1 ± 1,5	C	γ
	2	59,5 ± 0,2	B	β	53,1 ± 3,6	B	α	58,8 ± 1,2	B	β	59,4 ± 2,1	B	β	63,2 ± 1,2	B	γ
	3	48,0 ± 3,0	A	βγ	39,8 ± 1,4	A	α	44,6 ± 1,0	A	β	45,8 ± 2,7	A	βγ	49,8 ± 1,4	A	γ
C*	1	41,7 ± 0,8	A	β	50,8 ± 1,1	A	γ	41,0 ± 0,8	A	β	33,7 ± 1,9	A	α	38,3 ± 3,2	A	β
	2	47,6 ± 0,8	B	β	53,0 ± 2,4	A	γ	49,5 ± 1,1	B	β	42,9 ± 1,1	B	α	41,1 ± 1,1	A	α
	3	44,9 ± 2,6	AB	αβ	50,7 ± 0,4	A	γ	47,5 ± 0,9	B	β	43,3 ± 2,3	B	α	40,7 ± 2,3	A	α
h*	1	357,3 ± 0,2	A	γ	355,5 ± 0,8	A	αβ	356,4 ± 0,5	A	βγ	356,8 ± 0,1	A	βγ	354,5 ± 0,8	A	α
	2	357,7 ± 0,6	A	β	358,2 ± 1,3	B	β	356,2 ± 0,3	A	α	358,5 ± 0,6	B	β	355,7 ± 0,9	A	α
	3	362,3 ± 0,7	B	α	362,2 ± 0,6	C	α	361,8 ± 0,4	B	α	361,5 ± 0,8	C	α	362,4 ± 2,1	B	α

Different letters indicate significant differences ($p<0.05$). Capital letters are used to compare the different maturity levels and Greek letters are used to compare the different treatments with the control (by using one-way ANOVA, and employing the Student-Newman-Keuls method for multiple comparisons). All wine pigments (determined by RP-HPLC-ESI-MSn) are expressed as mg/L of malvidin-3-*O*-glucoside; Non-acylated: Summation of malvidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside; Acetylated: Summation of malvidin-3-*O*-(6-acetyl)-glucoside, petunidin-3-*O*-(6-acetyl)-glucoside and delphinidin-3-*O*-(6-acetyl)-glucoside; Coumaroylated: Summation of malvidin-3-*O*-(6-p-coumaroyl)-glucoside, petunidin-3-*O*-(6-p-coumaroyl)-glucoside and peonidin-3-*O*-(6-p-coumaroyl)-glucoside; Pyranoanthocyanins: Vitisin A; CI: Color intensity of wines; L*: Lightness values (CIELab coordinates); C*: Chroma values (CIELab coordinates); h*: Hue values (CIELab coordinates).

anthocyanins but was not clear in acylated anthocyanins (acetylated and coumaroylated), maybe because the latter are minor anthocyanins in Grenache wines (Noriega and Casp, 2007). The influence of ripeness on wine color was also very clear. In specific terms, the color intensity (CI) and hue (h*) increased whereas the luminosity (L*) decreased when the grapes were riper. This data confirms that riper grapes produced wines richer in anthocyanins and with a deeper color.

The total anthocyanin concentration of Submerged Cap wine was significantly higher than in the Control wine at all maturation levels. This trend was also observed in non-acylated anthocyanins but not in acylated anthocyanins. Submerged Cap wine also had higher CI, C* and lower L* than the Control wines, although these differences were not significant in some of the maturity levels. This data confirms that this winemaking procedure improves the anthocyanin extraction.

In general terms, Crushed Cluster and Whole Cluster wines also had significant higher anthocyanin concentration than control wines. This higher anthocyanin concentration may seem surprising because stems have been reported as being able to absorb anthocyanins (Suriano *et al.*, 2015) and their presence should consequently reduce anthocyanin concentration. By contrast, stems release tannins and other phenolic compounds that can protect anthocyanins against oxidation (Bautista-Ortín *et al.*, 2005). Moreover, the presence of stems makes the cap less compact, which favors anthocyanin

Table 4 - Effect of prefermentative cluster treatment on anthocyanins and color parameters of barrel-scale wines

Parameter	Control	Whole Berry	Whole Cluster
Total Anthocyanins	236	331	297
Non-acylated	225	313	280
Acetylated	3	5	4
p-Coumaroylated	7	13	12
Pyranoanthoxyanins	16	19	14
CI	6,8	7,8	7,1
L*	65,2	59,3	63,1
C*	32,6	40,3	32,5
h*	12,4	2,4	7,8

All wine pigments (determined by RP-HPLC-ESI-MSn) are expressed as mg/L of malvidin-3-*O*-glucoside; Non-acylated: Summation of malvidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside; Acetylated: Summation of malvidin-3-*O*-(6-acetyl)-glucoside, petunidin-3-*O*-(6-acetyl)-glucoside and delphinidin-3-*O*-(6-acetyl)-glucoside; Coumaroylated: Summation of malvidin-3-*O*-(6-p-coumaroyl)-glucoside, petunidin-3-*O*-(6-p-coumaroyl)-glucoside and peonidin-3-*O*-(6-p-coumaroyl)-glucoside; Pyranoanthocyanins: Vitisin A; CI: Color intensity of wines; L*: Lightness values (CIELab coordinates); C*: Chroma values (CIELab coordinates); h*: Hue values (CIELab coordinates).

extraction (del Llaudy *et al.*, 2008). In our particular case, the presence of stems enhanced total anthocyanin concentration at almost all maturity levels. This behavior was similar for non-acylated anthocyanins but not in acylated anthocyanins. However, C* of Crushed Cluster and Whole Cluster wines tended to be lower than in Control wines, although these differences were not always significant. In the case of Whole Cluster wines, CI also tended to be lower and L* to be higher than in Control wines. However, this tendency was not observed in Crushed Cluster wines. As a whole, these results indicate that the presence of stems has a negative effect on wine color, in contrast to the higher anthocyanin concentration detected in these wines. A possible cause for this higher anthocyanin concentration and poorer color is probably related to the higher pH observed in the wines made in the presence of stems.

In general terms, Whole Berry wine showed similar anthocyanin concentrations to Control wine. The color parameters of Whole Berry wines were also very similar to Control wines in the first harvest. However, CI and C* tended to be somewhat higher and L* to be lower in Whole Berry wines of the third harvest, when the grapes were riper.

The pigments derived from anthocyanins, pyranoanthocyanins, did not show a clear tendency according to the maturity level of the grapes or of the prefermentative cluster treatment. This result could be expected, as the main pyranoanthocyanins found in the Grenache wines, vitisin A, at this stage, namely young red wines, were produced by alcoholic fermentation yeast by-products (Blanco-Vega *et al.*, 2011) and we used the same yeast strain for all vinifications.

Table 4 shows the anthocyanin concentration and color parameters of barrel-scale wines. Although it is not possible to draw statistical conclusions because no replicates were performed, some tendencies can be highlighted. Whole Berry wine has a higher anthocyanin concentration than Control wine. However, these data do not match with those obtained at micro-scale level, in which only small differences were found. A possible reason for this may be related to the fact that on a micro-scale level, the solubilization of anthocyanins from skins during maceration process is easier than on a barrel-scale because the punch down are more effective in small volume. This fact has probably reduced the differences. Whole Berry wine also has higher CI and C*, and lower L* and H* than the Control wine.

Whole Cluster wine also has higher anthocyanin concentrations than the Control wine, but in this case the color parameters CI, C* and L* were very similar. This higher anthocyanin concentration of Whole Cluster wine is consistent with those obtained in the micro-scale trials, which would confirm that the presence of stems favors anthocyanin extraction and/or provides protection against oxidation. The

OENO One, 2016, **50**, 4, 169-181

Table 5 - Effect of grape maturity and prefermentative cluster treatment on the composition of the phenolic compounds of micro-scale wines

Parameter		Maturity Level	Control	Submerged Cap	Whole Berry	Crushed Cluster	Whole Cluster
Non-flavonoids	Total hydroxycinnamic acids and derivatives	1	102 ± 5 A a	167 ± 12 A b	94 ± 55 A a	82 ± 28 A a	89 ± 31 A a
		2	133 ± 1 B b	163 ± 14 A g	136 ± 24 A bg	85 ± 13 A a	107 ± 12 A a
		3	93 ± 32 A a	164 ± 9 A b	133 ± 19 A ab	148 ± 10 B b	165 ± 7 B b
Flavonols	Total flavonols	1	27 ± 5 A b	47 ± 9 A g	23 ± 8 A ab	16 ± 4 A a	26 ± 12 A ab
		2	47 ± 2 B b	55 ± 10 AB b	46 ± 5 B bg	30 ± 11 AB a	31 ± 5 AB a
		3	36 ± 13 AB a	64 ± 3 B b	49 ± 15 B ab	49 ± 10 B a	43 ± 3 B a
	Aglycones	1	6 ± 2 A a	13 ± 3 A b	9 ± 1 A ab	6 ± 2 A a	12 ± 4 A b
		2	11 ± 2 B a	14 ± 6 A a	15 ± 2 B a	13 ± 3 B a	13 ± 3 AB a
		3	14 ± 7 B a	26 ± 1 B b	20 ± 3 B a	17 ± 4 B a	17 ± 1 B a
Flavan-3-ols	Proanthocyanidins (mg/L)	1	876 ± 59 A α	919 ± 315 A α	846 ± 59 B α	835 ± 106 A α	1118 ± 137 B α
		2	987 ± 114 A α	882 ± 79 A α	972 ± 53 C α	955 ± 55 A α	913 ± 101 AB α
		3	776 ± 197 A α	921 ± 260 A α	731 ± 12 A α	711 ± 151 A α	794 ± 86 A α
	mDP	1	5,24 ± 0,26 A α	5,70 ± 0,39 A α	5,36 ± 0,05 A α	5,17 ± 0,28 A α	5,94 ± 0,47 A α
		2	6,96 ± 0,32 B α	6,93 ± 0,26 B α	6,56 ± 0,09 B α	6,33 ± 0,60 B α	6,13 ± 0,21 A α
		3	8,19 ± 0,60 C β	8,92 ± 0,45 C αβ	7,94 ± 0,23 C αβ	7,80 ± 0,30 C αβ	6,74 ± 0,99 A α
	Prodelphindins (%)	1	19,2 ± 0,3 A αγ	22,4 ± 0,4 A β	19,7 ± 0,2 A αγ	18,2 ± 0,5 A α	21,9 ± 1,1 A β
		2	22,2 ± 0,5 B βγ	23,1 ± 0,8 A β	21,5 ± 1,0 B βδ	19,9 ± 0,6 AB α	20,4 ± 0,0 A αδ
		3	20,1 ± 0,3 A α	22,3 ± 0,3 A αβ	20,9 ± 0,5 AB α	22,4 ± 2,4 B αβ	24,5 ± 1,4 B β
	Galloylation (%)	1	6,2 ± 0,6 B β	4,8 ± 0,3 A α	5,9 ± 0,5 B β	6,2 ± 0,2 B β	5,8 ± 0,2 A β
		2	4,6 ± 0,1 A α	4,4 ± 0,1 A α	4,2 ± 0,1 A α	4,3 ± 0,3 A α	3,8 ± 1,0 A α
		3	6,9 ± 0,2 B β	7,0 ± 0,1 B β	6,8 ± 0,3 C β	6,8 ± 0,3 C β	4,4 ± 1,3 A α

Different letters indicate significant differences ($p<0.05$). Capital letters are used to compare the different maturity levels and Greek letters are used to compare the different treatments with the control (by using one-way ANOVA, and employing the Student-Newman-Keuls method for multiple comparisons). Total amount of hydroxycinnamic acids and derivatives (determined by RP-HPLC-ESI-MSn) is expressed as mg/L of caffeic acid; Total amount of flavonols (determined by RP-HPLC-ESI-MSn) is expressed as mg/L of quercetin-3-*O*-glucoside; Total proanthocyanidin concentration (mg/L) was calculated by the addition of the total monomeric unit released during the phloroglucinolysis reaction; mDP, Mean degree of polymerization of wine proanthocyanidins; Prodelphinidin ratio of proanthocyanidins is expressed as a percentage; Galloylation degree of proanthocyanidins is expressed as a percentage.

Table 6 - Effect of prefermentative cluster treatment on the composition of the phenolic compounds of barrel-scale wines

Parameter		Control	Whole Berry	Whole Cluster
Non-flavonids	Total hydroxycinnamic acids and derivatives	79	119	72
Flavonols	Total flavonols	15	24	22
	Aglycones	5	9	9
Flavan-3-ols	Proanthocyanidins (mg/l)	447	555	647
	mDP	5,7	6,6	6,4
	Prodelphinidins (%)	12,3	15,6	16,0
	Galloylation (%)	5,0	5,4	5,8

Total amount of hydroxycinnamic acids and derivatives (determined by RP-HPLC-ESI-MSn) is expressed as mg/L of caffeic acid; Total amount of flavonols (determined by RP-HPLC-ESI-MSn) is expressed as mg/L of quercetin-3-O-glucoside; Total proanthocyanidin concentration (mg/L) was calculated by the addition of the total monomeric unit released during the phloroglucinolysis reaction; mDP, Mean degree of polymerization of wine proanthocyanidins; Prodelphinidin ratio of proanthocyanidins is expressed as a percentage; Galloylation degree of proanthocyanidins is expressed as a percentage.

Table 7 - Sensory analysis of the wines elaborated in oak barrels using different cluster treatments

Triangular test	Positive identifications	*P*	Preference		
			Control	Whole berry	Whole cluster
Control vs Whole Berry	9/11	< 0.05	2	7	-
Control vs Whole Cluster	8/11	< 0.05	5	-	3

lack of differences in CI, C* and L* between Whole Cluster wine and Control wine despite the differences in anthocyanin concentration can be attributed to the higher pH of Whole Cluster wine, as mentioned in the comments on the micro-scale trials.

The hue (h*) of the control wine at barrel-scale was somewhat higher than in Whole Berry and Whole Cluster wines. This high value indicates that the color of the Control wine was more yellowish and consequently indicates a greater oxidation. Grenache is a cultivar with a great tendency to color oxidation (De Andres-De Prado *et al.*, 2007). Winemaking in open barrel can favor a greater oxygen intake that may be the cause of the higher h*. The other winemaking conditions did not have this disadvantage, probably due to two different reasons. In the case of Whole Berry, the extraction of anthocyanins took place inside the berry at the beginning of the alcoholic fermentation, protecting the anthocyanins against oxygen. In the case of Whole Cluster, the presence of stems releases tannins and other phenolic compounds that can act as antioxidants, protecting anthocyanins from oxidation.

Table 5 shows the influence of grape maturity and prefermentative cluster treatment on the hydroxycinnamic acid and derivative, flavonol and flavan-3-ol concentration of micro-scale wines. In general, the total hydroxycinnamic acid and derivative concentration showed an erratic behavior throughout the maturity process. In the case of the Control wines, the total hydroxycinnamic acids and derivatives increased significantly between the first and second harvest but decreased in the third. In the case of Submerged Cap, the concentration did not change throughout ripening. Finally, in the other three prefermentative cluster treatments, the total hydroxycinnamic acids and derivatives tended to increase, although the differences were not always significant. It is therefore very difficult to draw conclusions.

In overall terms, the total flavonols and their aglycones tended to increase when the grapes were riper in all the experimental conditions, with the Submerged Cap wines being the richest in these substances. The Control and Whole Berry wines had similar levels of flavonols. By contrast, when stems were present, Whole Cluster and Crushed Cluster wines, the total flavonol concentration was significantly lower in some of the maturity levels than in Control wines.

The total proanthocyanidin concentration obtained by phloroglucinolysis is also shown in Table 5. These data are higher than those obtained by the methyl cellulose method and do not show a similar tendency than that observed for TPI or tannin concentration obtained by the methyl cellulose method. In fact, the total proanthocyanidin concentration measured by

OENO One, 2016, **50**, 4, 169-181

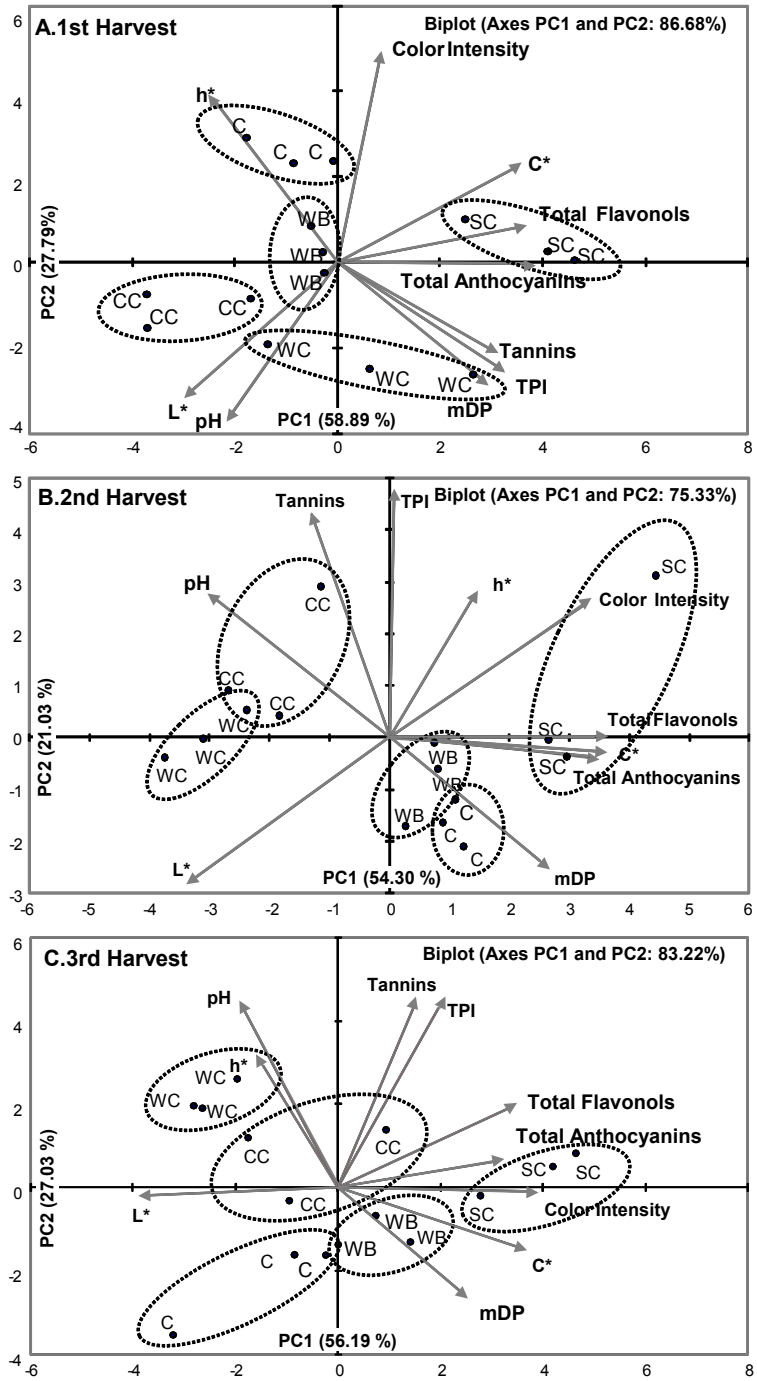

Figure 1 - Principal Component Analysis

C: Control wines; SC: Submerged Cap wines; WB: Whole Berry wines; CC: Crushed Cluster wines; WC: Whole Cluster wines

this method showed an undefined behavior throughout maturity and among the different cluster treatments. However, phloroglucinolysis provides some interesting information about the structural characteristics of proanthocyanidins, such as the mDP, the percentage of prodelphinidins and the percentage of galloylation (Kennedy and Taylor, 2003). The mDP of the proanthocyanidins clearly tended to increase with maturity in all the prefermentative cluster treatments, although in the case of Whole Cluster wine this increase was not significant. The mDP of the proanthocyanidins of all treatments was similar in wines from the first harvest and tended to be lower in wines fermented in the presence of the stems in the other two harvests, although these differences were only significant in

the case of Whole Cluster wines. Finally, the percentage of prodelphinidins and galloylation did not show any clear tendency in terms of either the maturity level or the prefermentative cluster treatments.

Table 6 shows the influence of grape maturity and prefermentative cluster treatment on hydroxycinnamic acid and derivative, flavonol and flavan-3-ol concentration in barrel-scale wines. Although no replicates were performed, some conclusions can be drawn. Whole Berry wine has a higher concentration of hydroxycinnamic acids and derivatives, flavonols and proanthocyanidins than Control wine. The mDP and the percentage of prodelphinidins were also higher in Whole Berry wine than in Control wine, whereas the percentage of galloylation was similar. By contrast, Whole Cluster wine has similar levels of hydroxycinnamic acids and derivatives than the Control wine, but the total flavonol and proanthocyanidin concentrations were higher than in the Control wine. The mDP and the percentage of prodelphinidins were also higher than in the controls.

Table 7 shows the results of the sensory analysis of the various wines produced in oak barrels. The Whole Berry and Whole Cluster wines were compared with the Control wine by means of triangular tests. The results were very clear. The tasters were able to distinguish significantly between the Whole Berry wine and the Control wine (9/11). Of all the tasters who distinguished them correctly, seven preferred the Whole Berry wine, whereas the other two tasters preferred the Control wine. The tasters were also able to distinguish significantly between the Whole Cluster wine and the Control wine (8/11) and 5 of the tasters that selected the wines correctly preferred the Control wine, while the other three tasters preferred the Whole Cluster wine.

A principal component analysis was performed in order to obtain a better understanding of the influence of prefermentative cluster treatment on wine composition. Figure 1 shows the plots of varimax-rotated principal component analyses of wines from the three harvests. In the first harvest (Fig 1A), the first component explains 58.89 % of the variance, and the second accounts for 27.79 % (meaning that the aggregate variance explained by the first two components was 86.68 %). The loadings are presented as vectors, and their length and direction indicate the contribution made by both components. A clear trend can be observed in this plot, and it is possible to separate the different experimental groups. The two experimental wines produced with the presence of stems, the Crushed Cluster and Whole Cluster wines, were located on the lower side of the graph, where the vectors for pH, tannins, TPI and L* were directed. By contrast, the Control, Submerged Cap and Whole Berry wines were located in higher positions, and nearly all the points were at the top of the graph, where the vectors for CI and C* were directed.

This behavior was generally consistent in the other two levels of maturity. In the second harvest (Fig 1B), the first component accounts for 54.30 % of the variance, and the second component accounts for 21.03 % (making the aggregate variance explained by the first two components 75.33 %). This time, the Crushed Cluster and Whole Cluster wines were located on the left side of the graph, in the direction of the pH, tannins and L* vectors, whereas the other experimental wines were located on the right side, where the CI and C* vectors were again directed. Finally, in the case of the third harvest (Fig 1C), the first component accounts for 56.19 % of the variance, and the second accounts for 27.03 % (making the aggregate variance explained by the first two components 83.22 %). Once again, the experimental wines produced in the presence of stems were separated from those produced without them. In this case, Crushed Cluster and Whole Cluster wines were located towards the top of the graph, in the direction of the pH and tannins vectors. By contrast, the Control wines were located in the lower left quadrant and the other two experimental wines, Whole Berry and Submerged Cap, were located on the right side of the graph, where once again the CI and C* vectors were directed.

In general, the PCA of the three levels of maturity confirms that the presence of stems generates wines with higher tannin extraction, less color and higher pH than in wines produced without stems.

It can be concluded that grape ripening and cluster treatments have a clear effect on color and polyphenol extraction. Specifically, TPI, anthocyanin, proanthocyanidin concentrations and mDP were higher when the grapes were riper. Regarding the different treatments, there is strong evidence to suggest that Submerged Cap presents a higher polyphenol extraction than conventional winemaking. The presence of stems under Crushed Cluster and Whole Cluster conditions increases proanthocyanidin extraction. However, stems also decrease color and anthocyanin concentration, increase pH, and produce wines with poor sensory attributes. The analytical micro-scale results of Whole berry wines were very similar to the Control

OENO One, 2016, **50**, 4, 169-181

wine, but these presented a clearly better composition in oak winemaking, which was also preferred by the tasters.

Further studies are required on a more realistic scale, for a better understanding of how cluster treatment affects the composition and quality of red wine.

Acknowledgments : We would like to thank CICYT (Projects AGL2011-29708-C02-01, AGL2011-29708-C02-02, AGL2014-56594-C2-1-R and AGL2014-56594-C2-2-R) for its financial support.

REFERENCES

Ayala F., Echavarri J.F. and Negueruela A.I., 1997. A new simplified method for measuring the color of wines. I. Red and rose wines. *Am. J. Enol. Vitic.*, **48**, 357-363.

Ayala F., Echávarri J.F. and Negueruela A.I., 2001. MSCVes.zip http://www.unizar.es/negueruela/MSCV.es.

Bautista-Ortín A.B., Martínez-Cutillas A., Ros-García J.M., López-Roca J.M. and Gómez-Plaza E., 2005. Improving colour extraction and stability in red wines : the use of maceration enzymes and enological tannins. *Int. J. Food Sci. Technol.*, **40**, 867-878.

Blanco-Vega D., López-Bellido F.J., Alía-Robledo J.M. and Hermosín-Gutiérrez I., 2011. HPLC-DAD-ESI-MS/MS characterization of pyranoanthocyanins pigments formed in model wine. *J. Agric. Food Chem.*, **59**, 9523-9531.

Blouin J., 2000. La vinificación bordelesa de las uvas tintas. In : Madrid AMV (ed) *Enología : fundamentos científicos tecnológicos*. Cord, Flanzys, pp. 465-467.

Bosso A., Panero L., Petrozziello M., Follis R., Motta S. and Guaita M., 2011. Influence of submerged-cap vinification on polyphenolic composition and volatile compounds of barbera wines. *Am. J. Enol. Vitic.*, **62**, 503-511.

Boulton R.B., Singleton V.L., Bisson L.F. and Kunkee R.E., 1995. *Principles and practices of winemaking*. Chap man and Hall, New York.

Canals R., Llaudy M.C., Valls J., Canals J.M. and Zamora F., 2005. Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *J. Agric. Food Chem.*, **53**, 4019-4025.

De Andres-De Prado R., Yuste-Rojas M., Sort X., Andres-Lacueva C., Torres M. and Lamuela-Raventos R.M., 2007. Effect of soil type on wines produced from *Vitis vinifera* L. cv. Grenache in commercial vineyards. *J. Agric. Food Chem.*, **55**, 779-786.

Del Llaudy M.C., Canals R., Canals J.M. and 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. *Eur. Food Res. Technol.*, **226**, 337-344.

Gawel R., 1998. Red wine astringency : a review. *Aust. J. Grape Wine Res.*, **4**, 74-95.

Gil M., Kontoudakis N., González E., Esteruelas M., Fort F., Canals J.M. and Zamora F., 2012. Influence of grape maturity and maceration length on color, polyphenolic composition, and polysaccharide content of Cabernet Sauvignon and Tempranillo wines. *J. Agric. Food Chem.*, **60**, 7988-8001.

Gil M., Esteruelas M., González E., Kontoudakis N., Jimenez J., Fort F., Canals J.M., Hermosín-Gutiérrez I. and Zamora F., 2013. Effect of two different treatments for reducing grape yield in *Vitis vinifera* cv Syrah on wine composition and quality : berry thinning versus cluster thinning. *J. Agric. Food Chem.*, **61**, 4968-4978.

Glories Y., 1984. La couleur des vins rouges. 2ème partie : mesure, origine et interpretation. *Conn. Vigne Vin*, **18**, 253-271.

Gómez-Plaza E., Gil-Muñoz R., López-Roca J.M. and Martínez A., 2000. Color and phenolic compounds of a young red wine. Influence of wine-making techniques, storage temperature, and length of storage time. *J. Agric. Food Chem.*, **48**, 736-741.

González-Centeno M.R., Rosselló C., Simal S., Garau M.C., López F. and Femenia A., 2010. Physico-chemical properties of cell wall materials obtained from ten grape varieties and their byproducts : grape pomaces and stems. *LWT - Food Sci. Technol.*, **43**, 1580-1586.

Goode J. and Harrop S., 2011. *Authentic wine : toward natural and sustainable winemaking*. Berkeley, University of California Press.

Hashizume K. and Samuta T., 1997. Green odorants of grape cluster stem and their ability to cause a wine stemmy flavor. *J. Agric. Food Chem.*, **45**, 1333-1337.

Hashizume K., Kida S. and Samuta T., 1998. Effect of steam treatment of grape cluster stems on the methoxypyrazine, phenolic, acid and mineral content of red wines fermented with stems. *J. Agric. Food Chem.*, **46**, 4382-4386.

Ichikawa M., Ono K., Hisamoto M., Matsudo T. and OkudaT., 2012. Effect of cap management technique on the concentration of proanthocyanidins in Muscat bailey A wine. *Food Sci. Technol. Res.*, **18**, 201-207.

Jackson R.S., 2002. Quantitative (technical) wine assessment. In : *Wine Tasting. A professional handbook*. Taylor S.L. (Ed.), Academic Press, Hong Kong, pp. 113-185.

Kennedy J.A. and Jones G.P., 2001. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.*, **49**, 1740-1746.

Kennedy J.A. and Taylor A.W., 2003. Analysis of proanthocyanidins by high-performance gel permeation chromatography. *J. Chromatogr. A*, **995**, 99-107.

Lago-Vanzela E.S., Rebello L.P.G., Ramos A.M., Stringheta P.C., Da-Silva R., García-Romero E., Gómez-Alonso S. and Hermosín-Gutiérrez I., 2013. Chromatic characteristics and color-related phenolic composition of Brazilian young red wines made from the hybrid grape cultivar BRS Violeta ('BRS Rúbea' × 'IAC 1398-21'). *Food Res. Int.*, **54**, 33-43.

Noriega M.J. and Casp A., 2007. Anthocyanin characterization of young red wines from Appellation of Origin Navarra (Spain). *J. Int. Sci. Vigne Vin*, **41**, 111-119.

Organisation Internationale de la Vigne et du Vin, 2014. Methods of analysis of wines and must. http://www.oiv.int/oiv/info/enmethodesinternationalesvin.

Pastor del Rio J.L. and Kennedy J.A., 2006. Development of proanthocyanidins in *Vitis vinifera* L. cv. Pinot noir grapes and extraction into wine. *Am. J. Enol. Vitic.*, **57**, 125-132.

Peynaud E., 1981. *Connaissance et travail du vin*. Bordas, Paris.

Peynaud E., 1984. *Connaissance et travail du vin*. Dunod, Bordas, Paris, pp. 133-135.

Peyrot des Gachons C. and Kennedy J.A., 2003. Direct method for determining seed and skin proanthocyanidin extraction into red wine. *J. Agric. Food Chem.*, **51**, 5877-5881.

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

Ribereau-Gayon P., Glories Y., Maujean A. and Dubourdieu D., 2000. *Handbook of enology. The chemistry of wine stabilisation and treatments*, vol 2, Wiley, Chichester, pp. 232-234.

Ribéreau-Gayon P., Glories Y., Maujean A. and Dubourdieu D., 2006. Chapter 6 : Phenolic compounds. In : *Hanbook of enology. The chemistry of wine stabilisation and treatments*, vol. 2, Chichester : John Wiley and Sons, pp. 141-203.

Sacchi K.L., Bisson L.F. and Adams D.O., 2005. A review of the effect of winemaking techniques on phenolic extraction in red wines. *Am. J. Enol. Vitic.*, **56**, 197-206.

Sampaio T.L., Kennedy J.A. and Vasconcelos M.C., 2007. Use of microscale fermentations in grape and wine research. *Am. J. Enol. Vitic.*, **58**, 534-539.

Sarneckis C.J., Dambergs R.G., Jones P., Mercurio M., Herderich M.J. and 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. *Aust. J. Grape Wine Res.*, **12**, 39-49.

Souquet J.M., Cheynier V., Brossaud F. and Moutounet M., 1996. Polymeric proanthocyanidins from grape skins. *Phytochemistry*, **43**, 509-512.

Souquet J.M., Cheynier V. and Moutounet M., 2000. Les proanthocyanidines du raisin (pépin, pellicule, rafle). *Bull. O.I.V.*, **73**, 601-609.

Sun B. and Spranger M.I., 2005. Changes in phenolic composition of Tinta Miúda red wines after 2 years of ageing in bottle : effect of winemaking technologies. *Eur. Food Res. Technol.*, **221**, 305-312.

Suriano S., Alba V., Tarricone L. and Di Gennaro D., 2015. Maceration with stems contact fermentation : effect on proanthocyanidins compounds and color in Primitivo red wines. *Food Chem.*, **177**, 382-389.

Vidal S., Francis L., Guyot S., Marnet N., Kwiatkowski M., Gawel R., Cheynier V. and Waters E.J., 2003. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *J. Sci. Food Agric.*, **83**, 564-573.

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

Vivas N., Nonier M.F., Vivas de Gaulejac N., Absalon C., Bertrand A. and Mirabel M., 2004. Differentiation of proanthocyanidin tannins from seeds, skins and stems of grapes (*Vitis vinifera*) and heartwood of Quebracho (*Schinopsis balansae*) by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and thioacidolysis/liquid chromatography/electrospray ionization mass spectrometry. *Anal. Chim. Acta*, **513**, 247-256.AV : Volatile acidity ; TPI : Total Polyphenol Index of wines.