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Effect of Two Different Treatments for Reducing Grape Yield in *Vitis vinifera* cv Syrah on Wine Composition and Quality: Berry Thinning versus Cluster Thinning

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ABSTRACT: The influence of two treatments for reducing grape yield, cluster thinning and berry thinning, on red wine composition and quality were studied in a *Vitis vinifera* cv Syrah vineyard in AOC Penedès (Spain). Cluster thinning reduced grape yield per vine by around 40% whereas berry thinning only reduced it by around 20%. Cluster thinning grapes had higher soluble solids content than control grapes, and their resultant wines have greater anthocyanin and polysaccharide concentrations than the control wine. Wine obtained from berry thinning grapes had a higher total phenolic index, greater flavonol, proanthocyanidin, and polysaccharide concentrations, and lower titratable acidity than the control wine. Wines obtained from both treatments were sufficiently different from the control wine to be significantly distinguished by a trained panel in a triangular test. Even though both treatments seem to be effective at improving the quality of wine, berry thinning has the advantage because it has less impact on crop yield reduction.

KEYWORDS: *cluster thinning, berry thinning, wine composition, wine quality, polyphenols, polysaccharides*

INTRODUCTION

Grape is the raw material of wine production, and therefore, grape quality directly affects wine composition and quality. It is well known that some families of chemical compounds from grapes are closely related to such sensory attributes of wine as color and tasting perceptions as bitterness, astringency, and mouthfeel. The color of red wine is mainly due to anthocyanins[1] and other derived pigments.[2] However, wine color is also influenced by other phenolic compounds, such as phenolic acids, flavonols, and flavanols, which exert a copigmentation effect.[3] Flavanols and their polymers (proanthocyanidins) have also been related to the bitterness and astringency of wine,[4] and some relations has been established between these perceptions and the proanthocyanidin structures.[5] As well as polyphenols, the polysaccharide family of grape compounds also has an effect on buccal sensations. Specifically, it has been reported that polysaccharides smooth astringency and increase mouthfeel.[6]

A range of studies have established close relations between grape maturity and wine composition and shown the importance of grape maturity in wine quality. On the one hand, anthocyanin synthesis starts during veraison and remains active throughout grape ripening, which causes gradual accumulation in grape skins.[7] The same thing happens with flavonols.[8] On the other hand, proanthocyanidin content is highest at veraison and subsequently decreases until just before complete ripeness, after which time it remains relatively constant.[8] The structure of proanthocyanidins depends on their origin: while seed proanthocyanidins[9] are smaller polymers made up of (+)-catechin, (−)-epicatechin, and (−)-epicatechin-3-gallate, skin proanthocyanidins[10] also contain (−)-epigallocatechin and a much lower proportion of (−)-epicatechin-3-gallate. Therefore, skins release procyanidins and prodelphinidins with a higher mean degree of polymerization (mDP) whereas seeds only release procyanidins with a higher proportion of galloylation and a lower mDP. Throughout maturity, the progressive enzymatic degradation[11] of skin cell walls has a double effect: it increases the presence of soluble polysaccharides[12] and also improves the phenolic extraction[13] of skins into red wine during maceration. In contrast, seed proanthocyanidin extraction diminishes with ripening probably because of oxidation phenomena and gradual seed lignification[14] that prevent them from dissolving in the wine. It is generally accepted that seed proanthocyanidins are much more astringent (because of their large content of galloylated units) and bitter (probably due to their lower mDP) than skin proanthocyanidins, so it is quite logical that riper grapes lead to better quality wines.

Consumers value deeply colored and full-bodied red wines. To make wines of this nature, grapes need to be just ripe enough from both the technological and the phenolic point of view. When grapes are well ripe, many techniques are available for winemakers to improve color and phenolic compound

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extraction[15] and produce full-bodied and deeply colored red wines with balanced bitterness and astringency. However, if grapes are not well ripe, winemakers have few options: excessive maceration makes the wine too bitter and astringent,[16] while too little maceration leads to bodiless and lightly colored red wines.

One of the most important factors to bear in mind if wines are to be of high quality is grape ripening, and because of this importance, a series of cultural practices have been developed over the years to improve it. Factors such as crop level and the ratio between leaf area and total fruit weight per plant are regarded as being essential to control and ensure the correct maturity and development of berries.[17] Several studies have attempted to relate both parameters with wine composition and sensorial quality. It is well known that a vigorous canopy growth (and consequent low cluster-light exposure) can lead to lower grape quality.[18] This vigorous canopy growth can be controlled by such practices as leaf removal and/or summer pruning. Likewise, it is well accepted that very high crop yields delay ripening and reduce fruit and wine quality.[19] Plant yield can be controlled by such practices as winter pruning and corrected with summer practices such as cluster thinning. All these techniques represent an additional cost because of the workforce required. In the particular case of cluster thinning, this additional cost is especially acute because production is lower.[20]

Despite the economic impact of cluster thinning, it is a common practice, and several studies have been carried out to determine the effect it has on grape maturity and wine composition and quality. In addition to the clearly demonstrated effect of advancing grape maturity,[21] cluster thinning also improve grape composition from a phenolic point of view.[22] It seems to be very effective with cultivars that tend to over crop,[23] because it improves the phenolic content of grapes and therefore the phenolic content of wine. It has been suggested recently that the effectiveness of cluster thinning is related to the vine water regime,[24] and this may explain why the positive effect of cluster thinning often depends on the vintage.[25] Besides improving the phenolic content of grapes, cluster thinning tends to diminish acidity and increase soluble solids and pH.[26] However, it should be pointed out that cluster thinning usually increases the grape size,[27] which decreases the skin-to-pulp ratio and therefore the positive effect on wine quality.

An alternative to traditional cluster thinning practices to improve grape maturity is berry thinning.[28] This practice consists of removing the tips of all the clusters just after flowering to obtain blunted clusters of grapes. This is done because some studies ascribe a better[29] and earlier[30] maturity of grapes from the shoulders and the top of the cluster than from the tips.

Although some scientific articles discuss the influence of berry thinning on grape maturity, to our knowledge they all focus on table grapes,[31] and none of them have studied the influence of this practice on the quality of red wines.

The aim of this paper was to determine the effect of cluster thinning and especially of berry thinning on grape production and wine quality. This study was performed in a vineyard of *Vitis vinifera* cv Syrah that have got problems of over production and insufficient maturity during recent years in order to determine if these treatments may be useful for advancing ripeness and consequently obtaining wines of better quality.

MATERIALS AND METHODS

Chemicals and Equipment. Methanol, acetonitrile, formic acid, and acetic acid were of high-performance liquid chromatography (HPLC) grade (>99%) and purchased from Panreac (Barcelona, Spain). Acetaldehyde, poly(vinylpolypyrrolidone), phloroglucinol, ascorbic acid, sodium acetate, and ammonium formate were purchased from Sigma−Aldrich (Madrid, Spain). The commercial standards were from Phytolab (Vestenbergsgreuth, Germany): malvidin 3-glucoside, *trans*-caftaric acid, caffeic and *p*-coumaric acids, *trans*-resveratrol, and *trans*-piceid. Absolute ethanol and hydrochloric acid were purchased from Panreac (Barcelona, Spain). Commercial standards of kaempferol, quercetin, isorhamnetin, myricetin, and syringetin, and the 3-glucosides of kaempferol, quercetin, isorhamnetin, syringetin, proanthocyanidin dimer B2, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, and (-)-epicatechin-3-*O*-gallate were purchased from Extrasynthese (Genay, France). Other noncommercial flavonol standards (myricetin 3-glucoside and quercetin 3-glucuronide) were kindly supplied by Dr. Ullrich Engelhardt (Institute of Food Chemistry, Technical University of Braunschweig, Germany). The *trans* isomers of resveratrol and piceid (resveratrol 3-glucoside) were transformed into their respective *cis* isomers by UV irradiation (366 nm light for 5 min in quartz vials) of 25% MeOH solutions of the *trans* isomers. Vitisin A (10-carboxy-pyranomalvidin-3-glucoside) and 10-*p*-monohydroxyphenyl-pyranomalvidin-3-glucoside (MHP-pymv-3-glc) were quantified using previously obtained standards.[32] A pullulan calibration kit Shodex P-82 (P-5, M_w = 5.9 kDa; P-10, M_w = 11.8 kDa; P-20, M_w = 22.8 kDa; P-50, M_w = 47.5 kDa; P-100, M_w = 112 kDa; P-200, M_w = 212 kDa; P-400, M_w = 404 kDa; P-800, M_w = 788 kDa) was obtained from Waters (Barcelona, Spain), while a pullulan 1.3 kDa and four dextrans BioChemika (12, 25, 50, and 80 kDa) were obtained from Fluka (St. Louis, Missouri). The polysaccharides used as external standards for quantification were pectins from citrus fruit and dextrans synthesized by *Leuconostoc mesenteroides* purchased from Sigma−Aldrich (St. Louis, MO). The HPLC analysis for proanthocyanidins and polysaccharides was performed using an Agilent 1200 series liquid chromatograph equipped with a G1362A refractive index detector (RID), a G1315D diode array detector (DAD), a G1311A quaternary pump, a G1316A column oven, and a G1329A autosampler (Agilent Technologies, Santa Clara, CA). Low molecular weight wine phenolic compounds (namely, anthocyanin and nonanthocyanin phenolics) were HPLC separated, identified, and quantified on an Agilent 1100 series system (Agilent, Germany), equipped with a DAD (G1315B) and a LC/MSD trap VL (G2445C VL) electrospray ionization mass spectrometer (ESI-MS^n), and coupled to an Agilent Chem Station (version B.01.03) data-processing station. The mass spectra data were processed with the Agilent LC/MS Trap software (version 5.3). All the spectrophotometric measurements were performed using a Helios alpha UV−visible spectrophotometer (Thermo Fisher Scientific Inc., Waltman, MA).

Vineyard. The experiment was carried out in a *Vitis vinifera* cv Syrah (clone 470, ENTAV-INRA, France) vineyard on the Juvé and Camps estate in Mediona (AOC Penedès; 41°31′ 30.1080″ (N) and 1°42′47.4516″ (E)) during the 2011 vintage. The vineyard is located at an altitude of 600 m above sea level. The grapevines, grafted on 110 Richter rootstock, were planted in 2004, trained on a vertical trellis system, pruned using unilateral Royat cordon system, and arranged in north−south rows spaced 2.60 m apart, with 1.0 m between plants in the row. The vineyard was managed according to standard viticultural practices for the cultivar and region. Winter pruning was carried out leaving 10 buds per vine. Canopy management practices were all performed manually. The weather conditions of the vintage were as follows: annual rainfalls, 555 mm; average temperature, 15.6 °C. More specifically, the weather conditions from budburst to harvest were as follows: rainfalls, 206 mm; average temperature, 20.4 °C.

Vine Treatments. Three representative and consecutive rows were selected for this assay. The first one was the control, the second one was used for cluster thinning, and the third row was used for berry thinning. Berry thinning was carried out when grapes were pea size (June 10) by cutting the tip of all the clusters roughly in half (type II)

according to the procedure described by Winkler.[28] Cluster thinning was carried out at mid veraison (August 14) by cutting 50% of the clusters.

Harvest. Grapes were harvested manually on October 18 when it was considered that they were sufficiently ripe (around 13.5% of potential ethanol content). Grapes from each vine were weighed to determine the plant yields for each treatment. All the grapes from each treatment were mixed together after weighing so as to obtain more homogeneous samples for further analysis and winemaking. The grapes were immediately transported to the experimental winery of the Enology Faculty of the Rovira i Virgili University in Constantí (Tarragona).

Clusters Characterization. For each treatment, 10 clusters were randomly selected and weighed. Then, clusters were manually destemmed in order to determine the weight proportion of stems and grapes.

Wines. Nine microvinifications were carried out in an attempt to study how the vine treatment (cluster thinning and berry thinning) influenced wine composition and quality. The berries for each vine treatment were manually destemmed and randomly distributed in three groups of 6 kg each, crushed with a semiautomatic crusher machine (Gual, Villafranca del Penedès, Spain), sulphited (100 mg of $K_2S_2O_5$/kg) and placed in 8 L tanks equipped with a submerged cap system in accordance with the winemaking method described by Sampaio et al.[33] All tanks were immediately inoculated with 200 mg/kg of selected *Saccharomyces cerevisiae* yeast (EC1118, Lallemand Inc., Montreal, Canada) 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 juice using a portable density meter (METTLER TOLEDO, L'Hospitalet de Llobregat, Spain). The cap was mechanically punched down once around 1040 density units in order to improve color extraction. After two weeks of maceration, the wines were racked. Once alcoholic fermentation had completely finished, wines were sulphited (100 mg of $K_2S_2O_5$/L) and maintained at 4 °C for three months to allow tartaric salts to stabilize. Hence, malolactic fermentation was inhibited to avoid any variations introduced by it. Subsequently, wines were bottled and stored in a dark cellar at 15 °C until analysis.

Grapes Characterization. For each vine treatment, a triplicate of 100 manually destemmed grapes was used. The weight of 100 grapes was established by using an analytical scale. The volume of 100 grapes was established by the displacement of a known volume of water into a graduated cylinder. The theoretical surface (S) of skins was calculated from the previously measured berry volume and assuming that the berries were perfect sphere ($S = 4\pi[3v/4\pi]^{2/3}$). The ratio between berry surface and volume was calculated by dividing the theoretical surface by the average berry volume.

To determine the grape density distribution, 12 solutions of sucrose dissolved in distilled water between 1070 and 1125 mg/mL (every 5 mg) were prepared. One hundred berries were placed in the less dense solution (1070 mg/mL). The floating berries were considered to have a lower density than the solution. They were separated from the berries that sank and counted. Then, the berries that sank were removed and placed in the next denser solution (1075 mg/mL). The same process was repeated with all sucrose solutions. All these measurements were carried out in triplicate.

Standard Grape Juice and Wine Analysis. The titratable acidity was measured by titrimetry using 0.1 N NaOH and bromothymol blue as indicator. pH values were determined by a pH meter Basic-20 (CRISON, Barcelona, Spain). Soluble solids of grape juice were determined by refractometry, and the ethanol content of wine was determined by ebullometry (GAB system, Barcelona Spain). 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.[34]

Color Parameters. A 20 μL amount of a 10% (v/v) solution of acetaldehyde was added to 2 mL of wine sample in order to avoid sulphite interferences. After 20 min of incubation, spectrophotometric measurements were made using a 1 mm quartz cuvette. The color intensity (CI) was estimated using the method described by Glories.[35] The CIELAB coordinates lightness (L*), chroma (C*), hue (h*), red−greenness (a*), and yellow−blueness (b*) were determined according to Ayala et al.,[36] and the data were processed with the MSCV software.[37] All absorbance measurements were taken with a Helios alpha UV−visible spectrophotometer.

Analysis of Low Molecular Weight Phenolic Compounds. *Sample Preparation.* Anthocyanin-free wine fractions were isolated from diluted red wines (3 mL of wines and 3 mL of 0.1N HCl) following the procedure previously described by Castillo-Muñoz et al.[38] using SPE cartridges (Oasis MCX cartridges, Waters Corp., Mildford, MA; 6 mL cartridges filled with 500 mg of adsorbent). The eluate containing nonanthocyanin phenolic compounds was dried in a rotary evaporator (35 °C), redissolved in 3 mL of 25% methanol, and stored at −18 °C until use. These anthocyanin-free fractions were used to analyze nonanthocyanin phenolic compounds, with the exception of flavan-3-ols.

HPLC-DAD-ESI-MS^n Analysis of Wine Phenolic Compounds. All the standards were used for identification and quantification using calibration curves. All the expected concentration ranges were covered (usually 0−100 mg/L, with the exception of malvidin 3-glucoside covering a range 0−1000 mg/L). When a standard was not available, the quantification was made using the calibration curve of the most similar compound (with subsequent molecular mass correction): malvidin 3-glucoside was used for all native grape anthocyanins; vitisin A was used for vitisin B; *p*-coumaric acid was used for coutaric acids; flavonol 3-glycosides with nonavailable standard as their corresponding 3-glucoside derivatives. Anthocyanins and nonanthocyanin phenolic compounds were analyzed separately after the previously described methods had been adapted[38,39] for narrow bore, smaller particle size, chromatography columns. The samples were injected (10 μL for anthocyanin analysis after a 4:1 dilution of wine with 0.1 N HCl; 20 μL of anthocyanin-free wine fraction for the analysis of nonanthocyanin phenolics) after filtration (0.20 μm, polyester membrane, Chromafil PET 20/25, Machery-Nagel, Düren, Germany) on a reversed-phase column Zorbax eclipse XDB-C18 (2.1 mm × 150 mm; 3.5 μm particle; Agilent, Germany), thermostatted at 40 °C. The flow rate was 0.19 mL/min. The solvents were as follows: solvent A (acetonitrile/water/formic acid, 3:88.5:8.5, v/v/v), solvent B (acetonitrile/water/formic acid, 50:41.5:8.5, v/v/v), and solvent C (methanol/water/formic acid, 90:1.5:8.5, v/v/v). The linear solvents gradient for anthocyanin analysis was as follows: 0 min, 94% A and 6% B; 10 min, 70% A and 30% B; 30 min, 50% A and 50% B; 34 min, 100% B; 36 min, 100% B; 42 min, 96% A and 4% B; 50 min, 96% A and 4% B. The linear solvents gradient for nonanthocyanin analysis was as follows: 0 min, 98% A and 2% B; 8 min, 96% A and 4% B; 37 min, 70% A, 17% B, and 13% C; 51 min, 50% A, 30% B, and 20% C; 51.5 min, 30% A, 40% B, and 30% C; 56 min, 50% B and 50% C; 57 min, 50% B and 50% C; 64 min, 96% A and 4% B. For identification, ESI-MS^n was used in both positive (anthocyanins) and negative (flavonols, hydroxycinnamic acid derivatives, and stilbenes) ion modes. The following parameters were set: dry gas, N_2, 8 L/min; drying temperature, 325 °C; nebulizer, 50 psi; ionization and fragmentation parameters were optimized by direct infusion of appropriate standard solutions (malvidin-3-glucoside and quercetin-3-glucoside for positive and negative ionization modes, respectively); scan range, 50−1200 m/z. Identification was mainly based on spectroscopic data (UV−visible and MS/MS) that had been obtained from authentic standards or previously reported.[39,40] For quantification, DAD chromatograms were extracted at 520 nm (anthocyanins), 360 nm (flavonols), and 320 nm (hydroxycinnamic acid derivatives and stilbenes). Analyses were performed in duplicate.

Proanthocyanidin Analysis. Acid-catalyzed depolymerisation of proanthocyanidins in the presence of an excess of phloroglucinol was used to analyze the proanthocyanidin content, their monomeric composition, and their mDP, as described by Kennedy and Jones.[41] A 10 mL amount of wine was evaporated under a low-pressure vacuum (Univapo 100 ECH, Uni Equip, Germany). Then, it was resuspended in 6 mL of distilled water and applied to Set Pak Plus $_t$C18

Table 1. Yield Values and Cluster and Berry Characterization for Each Experimental Condition[a]

	parameter	control	berry thinning	cluster thinning
vine yield	yield[b]	1.88 ± 0.53, b	1.47 ± 0.56, ab	1.08 ± 0.24, a
	yield reduction[c]	–	22%	43%
cluster characterization	% stems[d]	6.0 ± 1.2, a	6.1 ± 0.6, a	6.6 ± 1.3, a
	% berries[e]	94.0 ± 1.2, a	93.9 ± 0.6, a	93.4 ± 1.3, a
	changes in weight per cluster[f]	–	−18.77%	+18.83%
berry characterization	m_{100}[g]	169 ± 10, a	181 ± 9, ab	186 ± 5, b
	V_{100}[h]	152 ± 10, a	165 ± 5, ab	168 ± 8, b
	S/V[i]	2.51 ± 0.22, a	2.27 ± 0.14, a	2.19 ± 0.09, a

[a]Different letters in a row indicate statistical differences ($p < 0.05$) between experimental conditions. [b]Yield expressed as kg/vine. [c]Yield reduction for each treatment respect the control. [d]Percentage (w/w) of stems in grape clusters. [e]Percentage (w/w) of berries in grape clusters. [f]Changes in weight per cluster for each treatment respect the control. [g]Weight of 100 berries expressed as g. [h]Volume of 100 berries expressed as mL. [i]Theoretical ratio skin surface/berry volume expressed as cm^2/mL.

environmental cartridges (Waters, Milford, MA) that had previously been activated with 10 mL of methanol and 15 mL of water. The samples were washed with 15 mL of distilled water, and then, the proanthocyanidins were eluted with 12 mL of methanol, immediately evaporated under a vacuum, and redissolved in 2 mL of methanol. Finally, 100 μL of this sample was reacted with a 100 μL of 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. Reversed-phase HPLC analysis (Agilent series 1200 HPLC-DAD) was carried out with an Agilent Zorbax eclipse XDB C18, 4.6 mm × 250 mm, 5 μm column (Agilent Technologies, Santa Clara, CA) as described below, and the injection volume was 30 μL. The solvents used were 1% aqueous acetic acid (solvent A) and methanol (solvent B) at a flow rate of 1 mL/min. The elution conditions were 1.0 mL/min. Elution was performed with a gradient starting at 5% B for 10 min, a linear gradient from 5−20% B in 20 min, and a linear gradient from 20−40% B in 25 min. The column was then washed with 90% B for 10 min and re-equilibrated with 5% B for 5 min before the next injection. The monomers (+)-catechin, (-)-epicatechin, and (-)-epicatechin-3-*O*-gallate were identified by comparing their retention times with those of the pure compounds. The phoroglucinol adducts of (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, and (-)-epicatechin-3-*O*-gallate were identified by their retention time (described in the literature) and confirmed through a HPLC-MS analysis. Analyses were performed with the Agilent 1200 series HPLC using an Agilent 6210 time-of-flight (TOF) mass spectrometer equipped with an electrospray ionization system (ESI). Elution was carried out under the same HPLC analysis conditions as described below. The capillary voltage was 3.5 kV. Nitrogen was used both as a dry gas at a flow rate of 12 L/min at 350 °C and as a 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 the addition of phloroglucinol to measure the flavan-3-ol monomers that are naturally present in wine. The number of terminal subunits was considered to be the difference between the total monomers measured in normal conditions (with phoroglucinol) and thus obtained when the analysis was performed without phloroglucinol addition. The number of extension subunits was considered as the addition of all the phloroglucinol adducts. The mDP was calculated by adding terminal and extension subunits (in moles) and dividing by the terminal subunits. Because 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 from wine.

Polysaccharide Analysis. Wine samples were processed using the methodology described by Ayestarán et al.[42] Briefly, 10 mL of wine was centrifuged (8500 rpm, 20 min) in a Biofuge Primo (Heraeus, Hanau, Germany), and the supernatant was concentrated to a final volume of 2 mL using a vacuum evaporator (Univapo 100ECH, Uniequip, Martinsried, Germany). Total soluble polysaccharides were precipitated by addition of 10 mL of cold acidified ethanol (0.3 M HCl in absolute ethanol) and kept for 24 h at 4 °C. Then, the samples were centrifuged (8500 rpm, 20 min, 4 °C), the supernatants were discarded, and the pellets were washed four times with cold ethanol to remove the interfering materials. Finally, the precipitates were dissolved in 1 mL of ultra pure water, frozen to −80 °C, and freeze-dried using a Christ Alpha 1-4 (Martin Christ, Osterode am Harz, Germany). In order to determine the molecular distribution and quantify the polysaccharides obtained from wines, the soluble fractions were analyzed by high-resolution size-exclusion chromatography (HRSEC) using a refraction index detector (RID). The lyophilized samples were resuspended in 1 mL of 30 mM ammonium formate and filtered through a 0.45 μm pore size nylon membrane; then, 100 μL was injected onto the column. Separation was carried out at 20 °C using two Shodex OHpak SB-803 HQ and SB-804 HQ columns connected in series (300 mm × 8 mm i.d.; Showa Denko, Japan). The mobile phase consisted of an aqueous solution of 30 mM ammonium formate applied with a constant flow of 0.6 mL/min for 60 min, and the temperature of the cell RID was 35 °C. The molecular weight distribution of the wine fractions was followed by calibration with pullulan and dextran standards of different molecular weight (see above). The polysaccharides were quantified according to the peak area for each fraction, using the external standard method with pectin and dextran commercial standards. The calibration curve was obtained by injection of standard solutions, under the same conditions as for the samples analyzed, in the range between 0 and 2 g/L.

Sensory Analysis. All the wines were tasted by a group of 24 expert oenologists from the Rovira i Virgili University 12 months after bottling. Three triangular tests were performed comparing the three wines in pairs.

Statistics. All the physical and chemical data are expressed as the arithmetic average ± the standard deviation. In the case of yield production, the statistics were drawn up from data from 20 vines. In the case of cluster characterization, the statistics were drawn up with data from 10 clusters. All the remaining statistics, grapes, grape juice, and wines, were done in triplicate. One-factor analysis of variance (ANOVA) was carried out with SPSS software (SPSS Inc., Chicago, IL), and comparison between samples was performed by using the least-squares differences (LSD) posthoc test. The level of significance of the sensory triangle tests was determined using Jackson's method.[43]

RESULTS AND DISCUSSION

Table 1 shows the yield production per vine of three experimental conditions. As expected, both of the treatments for reducing grape yield decreased the grape production per vine although this was only statistically significant in the case of cluster thinning. Specifically, berry thinning reduced yield by only 22% whereas cluster thinning reduced it by 43% with respect to the control. Several articles have been published on the effect of cluster thinning on vine yield production, and our data is generally very similar to those described by other

authors.[23,44] Very little information exists about the influence of berry thinning on yield production, all of which was obtained using table grapes. Moreover, all this information was published a long time ago. Specifically, Winkler[28,45] reported a decrease in yield production of around 20−30% that is in agreement with our results. Our data indicate that berry thinning decreases production less than cluster thinning, which is an advantage from the economic standpoint.

Data about cluster characterization is shown in Table 1. No significant differences were found in the proportion of grapes versus stems in any of the experimental conditions. However, grape bunches were around 19% heavier after cluster thinning. This increase in bunch weight in cluster thinning vines is in agreement with previous data.[21,46] In contrast, the average weight of bunches in berry thinning vines was approximately 19% lighter than those in control vines. In general, this reduction is very similar to the little information available in the literature on the subject.[28,45,47] These bunch weight variations were in agreement with the observed yield production per vine. In the case of cluster thinning, it must be taken into account that half of clusters were removed that should theoretically reduce production by half. However, the real decrease in production was somewhat lower because the bunches treated in this way were heavier on average than the controls. Similar results have been described by other authors[46,48] who have attributed this phenomenon to the fact that the vines distribute the resources available among a smaller number of grapes.[31]

The application of these treatments in the vineyard modified the appearance of the clusters, at least in the case of berry thinning (see Figure 1). As expected, after berry thinning, clusters were more rounded, since the tip had been cut. No evident visual differences between control and cluster thinning were detected. However, some differences were found when the berries were characterized. Specifically, the weight and the volume of the berries from the cluster-thinned vines were significantly higher (10%) than those from control vines. These data are in agreement with the increase in the weight of bunches mentioned above. A similar tendency was observed in grapes from berry thinning vines (7%) although in this case differences were not significant. Although the theoretical surface/volume ratio of the berries seemed to be lower in berries from both treatments than in controls, no significant differences were detected in any of the experimental conditions.

Figure 1. Appearance of clusters for each experimental condition. C: control; D: berry thinning; A: cluster thinning.

Figure 2 shows the density distribution curve of berries in the three experimental conditions. In all the cases, a classical Gaussian bell curve is observed that confirms the expected heterogeneity in the maturity of the berry grapes. These data have been previously described[49] and indicate that, in any grape crop, there is always a certain proportion of unripe and overripe grapes. Analyzing the geometry of these curves, it can be observed that the height of the control curve is maximum at a lower density than both treatments. This confirms that both cluster thinning and berry thinning advance the maturity of the grapes. Moreover, berry thinning presents a more symmetrical, narrower, and higher curve, which indicates lower heterogeneity. In principle, this should be positive because the proportion of unripe grapes, which can affect negatively the quality of the wine, is lower.[49]

Table 2 shows the grape juice characterization of the three experimental conditions. In general, the parameters of both treatments indicate that the maturity of the grapes is better than in the control. The total soluble solids and the pH of the juice from cluster thinning grapes was significantly higher than in controls. The grape juice density was also somewhat higher although the differences were not significant. In contrast, titratable acidity was significant lower. In the case of berry thinning, only titratable acidity was significant lower. In general, these results agree with those of Weaver and Winkler[47] who previously described that berry thinning leads to grapes with lower titratable acidity.

Table 3 shows the general parameters of wines made from grapes from the three experimental conditions. No significant differences were found in ethanol content, pH, titratable acidity, and TPI between cluster-thinning and control wines that indicated that similar maturity levels were reached. Berry-thinning wine also have similar values in ethanol content and pH, but its titratable acidity was significant lower, and its TPI was significant higher than in control wines. These results suggest that the phenolic maturity of grapes from berry-thinning was greater than that of control grapes.

Table 4 shows the color parameters of wines from the different experimental conditions. All the wines present similar color intensities. However, some slight but statistically significant differences were found in chroma (C*), hue (H*), green−red (a*), and blue−yellow (b*) components. To verify whether these small differences were large enough to be distinguished by the human eye, the total color differences (ΔEab*) between the wines were calculated. The average of ΔEab* between control and berry thinning was 3.51 ± 1.16, between control and cluster thinning was 3.60 ± 0.95, and finally, between both treatments was 2.84 ± 0.43. The human eye can generally distinguish two colors when ΔEab* ≥ 1.[50] However, it is also generally accepted that tasters can only distinguish the color of two wines through the glass when ΔEab* ≥ 5.[50] Since this parameter was not higher than five units in any case, the effect of both treatments on wine color was not enough to be distinguished by the human eye.

Table 5 shows the monomeric anthocyanin concentration of wines made with grapes from each experimental condition. The total anthocyanin concentration in cluster-thinning wine was significantly higher than in control (24%) and berry-thinning wines (18%). No significant differences were found in the percentages of the five nonacylated anthocyanins among the three experimental conditions. However, some slight but

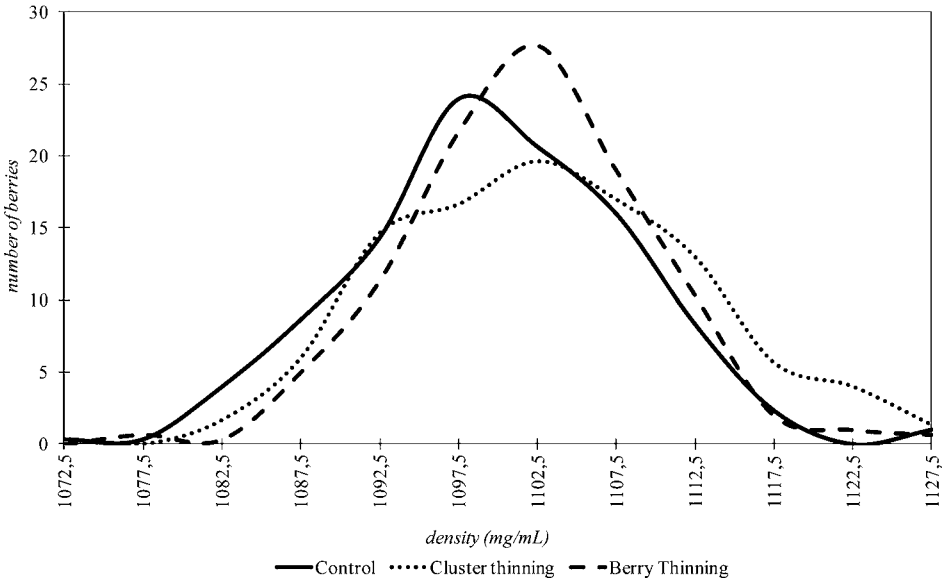

Figure 2. Density distribution of berries for each experimental condition.

Table 2. Grape Juice Characterization for the Three Experimental Conditions[a]

	control	berry thinning	cluster thinning
pH	3.33 ± 0.04, a	3.41 ± 0.07, ab	3.46 ± 0.01, b
titratable acidity[b]	4.4 ± 0.2, b	3.9 ± 0.2, a	3.6 ± 0.1, a
soluble solids[c]	21.73 ± 0.46, a	22.42 ± 0.49, ab	22.57 ± 0.20, b
initial density of grape juice[d]	1095.2 ± 1.2, a	1098.1 ± 2.2, a	1098.2 ± 0.8, a

[a]Different letters in a row indicate statistical differences ($p < 0.05$) between treatments. [b]Titratable acidity expressed as g/L of tartaric acid. [c]Soluble solids expressed as °Brix. [d]Initial density of grape juice expressed as mg/mL.

Table 3. General Parameters of Wines Made from Grapes for the Three Experimental Conditions[a]

	control	berry thinning	cluster thinning
% vol[b]	14.0 ± 0.6, a	14.3 ± 0.4, a	14.6 ± 0.1, a
pH	3.69 ± 0.07, a	3.78 ± 0.06, a	3.74 ± 0.03, a
TA[c]	5.5 ± 0.1, b	5.2 ± 0.1, a	5.4 ± 0.1, ab
TPI[d]	65.4 ± 2.1, a	70.2 ± 3.5, b	67.0 ± 0.9, ab

[a]Different letters in a row indicate statistical differences ($p < 0.05$) between treatments. [b]Ethanol content of wines, expressed as a percentage (v/v). [c]Titratable acidity, expressed as g/L of tartaric acid. [d]Total polyphenolic index.

significant differences were found in some of the acetylated or *p*-coumarylated anthocyanins. These slight differences were mainly in malvidin-3-*O*-(6″-acetyl)glucoside, which was found in lower proportions in the cluster-thinning wine than in the control and berry-thinning wines and in malvidin-3-*O*-(6″-*trans*-*p*-coumaroyl)glucoside, which was found in lower percentages in control wines than in cluster- or berry-thinning wines. Similarly, the wines of the three experimental conditions showed similar concentrations of vitisins A and B although a small but significant difference was detected in 10-*p*-monohydroxyphenyl-pyranomalvidin-3-glucoside (MHP-pymv) content, which was slightly lower in control wines.

Table 4. Color Parameters of Wines Made from Grapes for the Three Experimental Conditions[a]

	control	berry thinning	cluster thinning
CI[b]	22.1 ± 0.1, a	21.2 ± 1.7, a	21.3 ± 0.3, a
L*[c]	30.4 ± 0.6, a	30.9 ± 1.8, a	31.9 ± 1.8, a
C*[d]	61.3 ± 1.0, ab	60.0 ± 1.0, a	61.7 ± 0.2, b
H*[e]	18.0 ± 0.5, b	15.3 ± 0.3, a	15.2 ± 1.3, a
a*[f]	58.4 ± 1.0, ab	57.9 ± 0.9, a	59.5 ± 0.2, b
b*[g]	18.9 ± 0.4, b	15.9 ± 0.5, a	16.2 ± 1.4, a
SAT[h]	2.02 ± 0.01, a	1.95 ± 0.14, a	1.94 ± 0.02, a

[a]Different letters in a row indicate statistical differences ($p < 0.05$) between treatments. [b]Color intensity of wine. [c]Lightness values. [d]Chroma values. [e]Hue values. [f]Green−red component. [g]Blue−yellow component. [h]Saturation value (expressed as the chroma-to-lightness ratio).

This similarity among the three wines may be related to the youth of the wines at the moment of analysis and was expected because of the origin of the anthocyanin derivatives:[32] vitisins A and B come from the reaction of malvidin-3-glucoside with some yeast metabolites (pyruvic acid and acetaldehyde, respectively), and the same yeast strain was used for all wines; at this stage of winemaking, MHP-pymv was formed from *p*-coumaric acid only by the enzymic way induced by yeast.

Table 6 shows the flavonol concentration of wines made from grapes for each experimental condition. The total flavonol concentration of berry thinning wines is considerably higher than that of control (83%) and cluster-thinning (43%) wines. The percentages of the different flavonols were quite similar in all the experimental conditions although some slight but significant differences were found in myricetin and syringetin glycoside groups, which were somewhat lower in berry-thinning wine. It has been reported that such cultivars as Brancellao have higher amounts of total flavonols in the skins of the grapes from the shoulders than in grapes from the tips.[30] However, these differences were not found in other Galician cultivars[51] such as Mouraton. It seems therefore that there is a cultivar-dependent effect that supports previous suggestions that the flavonol profile is a cultivar characteristic for both grapes and their

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Table 5. Anthocyanin and Derived Pigments Analysis by HPLC-DAD-ESI/MS^n of Wines Made from Grapes for Each Experimental Condition[a]

		control	berry thinning	cluster thinning
anthocyanins	delphinidin-3-glucoside	4.76 ± 0.09, a	4.72 ± 0.62, a	5.09 ± 0.11, a
	cyanidin-3-glucoside	0.41 ± 0.01, a	0.44 ± 0.07, a	0.47 ± 0.02, a
	petunidin-3-glucoside	8.03 ± 0.15, a	8.00 ± 0.78, a	8.79 ± 0.18, a
	peonidin-3-glucoside	6.81 ± 0.44, a	6.47 ± 0.29, a	6.89 ± 0.26, a
	malvidin-3-glucoside	50.68 ± 0.60, a	50.86 ± 1.52, a	49.96 ± 1.28, a
	delphinidin-3-(6″-acetyl)glucoside	2.10 ± 0.10, a	1.95 ± 0.35, a	1.89 ± 0.41, a
	petunidin-3-(6″-acetyl)glucoside	2.59 ± 0.09, b	2.13 ± 0.26, a	2.26 ± 0.10, ab
	peonidin-3-(6″-acetyl)glucoside	2.75 ± 0.10, a	2.39 ± 0.21, a	2.39 ± 0.18, a
	malvidin-3-(6″-acetyl)glucoside	11.33 ± 0.03, b	11.82 ± 0.58, b	10.57 ± 0.04, a
	delphinidin-3-(*trans*-*p*-coumaroyl)glucoside	1.33 ± 0.02, a	0.97 ± 0.35, a	1.00 ± 0.19, a
	cyanidin-3-(*trans*-*p*-coumaroyl)glucoside	0.79 ± 0.25, a	0.58 ± 0.19, a	0.55 ± 0.15, a
	petunidin-3-(*trans*-*p*-coumaroyl)glucoside	1.25 ± 0.07, a	1.26 ± 0.09, a	1.27 ± 0.07, a
	malvidin-3-(*cis*-*p*-coumaroyl)glucoside	0.19 ± 0.00, a	0.24 ± 0.02, b	0.20 ± 0.01, a
	peonidin-3-(*trans*-*p*-coumaroyl)glucoside	2.23 ± 0.01, a	2.48 ± 0.19, ab	2.74 ± 0.18, b
	malvidin-3-(*trans*-*p*-coumaroyl)glucoside	4.73 ± 0.02, a	5.67 ± 0.19, b	5.93 ± 0.72, b
	total anthocyanins	321.5 ± 11.3, a	337.0 ± 44.3, a	397.9 ± 24.0, b
pyranoanthocyanins	vitisin A	4.54 ± 0.29, a	4.34 ± 0.28, a	4.93 ± 1.79, a
	vitisin B	4.97 ± 1.08, a	4.44 ± 1.29, a	3.68 ± 0.54, a
	MHPpymv[b]	0.31 ± 0.01, a	0.33 ± 0.02, b	0.35 ± 0.01, b

[a]Different letters in a row indicate statistical differences ($p < 0.05$) between treatments. Each monomeric anthocyanin is quantified as malvidin-3-glucoside and expressed as a molar ratio (%). Total anthocyanins are expressed as mg/L of malvidin-3-glucoside. The pyranoanthocyanin data is expressed as mg/L. [b]10-*p*-Monohydroxyphenyl-pyranomalvidin-3-glucoside.

Table 6. Flavonol Analysis by HPLC-DAD-ESI/MS^n of Wines Made From Grapes for Each Experimental Condition[a]

	control	berry thinning	cluster thinning
K-type[b]	1.77 ± 0.33, a	2.52 ± 0.20, a	1.79 ± 0.35, a
Q-type[c]	42.49 ± 3.58, a	49.66 ± 3.36, a	44.62 ± 1.52, a
I-type[d]	14.12 ± 1.12, a	13.66 ± 1.06, a	14.67 ± 0.52, a
M-type[e]	26.62 ± 0.57, b	22.30 ± 1.13, a	25.57 ± 1.44, b
L-type[f]	7.88 ± 0.75, a	7.11 ± 0.36, a	7.45 ± 0.28, a
S-type[g]	7.12 ± 0.85, b	4.74 ± 1.17, a	5.90 ± 0.44, ab
total	108.7 ± 1.1, a	198.8 ± 47.3, b	139.5 ± 10.4, a

[a]Different letters in a row indicate statistical differences ($p < 0.05$) between treatments. Total amounts are expressed as mg/L of quercetin-3-*O*-glucoside. Flavonols are classified by their aglycone backbone and the data of each aglycone-type are expressed as molar ratios (%). [b]Kaempferol glycosides group. [c]Quercetin glycosides group. [d]Isorhamnetin glycosides group. [e]Myricetin glycosides group. [f]Laricitrin glycosides group. [g]Syringetin glycosides group.

wines.[52] Since wines from berry thinning have a considerably higher concentration of flavonols, it is quite probable that Syrah berries from the shoulder have higher amounts of flavonols than berries from the tips. It has also been reported that exposure to the sun has a considerable effect on the flavonol content of berries.[53,54] The greater the exposure, the higher the flavonol content. In this regard, it is clear that grapes from the shoulders receive more sunlight than grapes from the tips because of the shadowing effect exerted by the shoulders. These data also explain why berry thinning wines have significantly higher flavonol concentrations. A higher flavonol content increases the possibility of copigmentation effects, which enhance the redness of the wine and the purplish nuances of the red.[3] In fact, berry-thinning wines had a lower anthocyanin content than cluster-thinning wines (Table 5), but the color parameters did not differ so much between the two wines (Table 4). A likely explanation for this apparent discrepancy is that, in berry-thinning wines, the lower content of anthocyanins was balanced by the higher content of flavonols, which probably promoted the formation of copigmentation complexes to a greater extent.[3]

Table 7 shows the hydroxycinnamic acids and their derivatives in wines from the three experimental conditions. No significant differences were found in total hydroxycinnamic acids and derivatives among the three experimental wines although some slight but significant differences were found in some percentages. It seems therefore that none of the treatments affects the wine composition as far as these compounds are concerned. However, cluster-thinning wine has a significantly higher concentration of *cis*-resveratrol than control and berry-thinning wines. In contrast, no significant differences were found in any of the other stilbenes. In general, these data agree with Prajitna et al.[55] who found that cluster thinning increased the stilbene concentration in total wine. It is well known that cluster thinning usually increases berry size, and in our experimentation, cluster-thinning berries were significantly heavier (10%) than control berries. Larger berries may lead to clusters being more compact in some cultivars especially those that have naturally compacted bunches. Greater compactness may also favor the development of fungal diseases,[56] which in turn can favor synthesis of the stilbene by the plant.[57] Berry-thinning grapes were also somewhat heavier than control grapes (7%), but in this case, the differences were not significant. It has also been reported that berry-thinning decreases bunch compactness, which explains why no differences were found between *cis*-resveratrol and the control. In fact, table-grape growers usually apply this treatment in an attempt to obtain less compact clusters.[31]

Table 8 shows the proanthocyanidin HPLC-DAD analysis of adducts formed by acid depolymerization in the presence of an excess of phloroglucinol. Berry-thinning wine has a considerably higher proanthocyanidin concentration than control (46%) and cluster-thinning (38%) wines. Moreover, the mDP

Table 7. Non-flavonoid Phenolics of Wine Analyzed by HPLC-DAD-ESI/MS^{n}[a]

		control	berry thinning	cluster thinning
hydroxycinnamic acids and derivatives	caftaric acid	50.49 ± 0.77, a	49.47 ± 1.01, a	51.02 ± 0.70, a
	caffeic acid	5.58 ± 0.73, a	5.91 ± 0.49, a	6.79 ± 0.92, a
	ethyl caffeate	1.01 ± 0.43, a	0.96 ± 0.55, a	1.47 ± 0.13, a
	coutaric acid	34.37 ± 0.83, b	35.38 ± 1.44, b	31.68 ± 0.29, a
	p-coumaric acid	2.22 ± 0.22, ab	1.90 ± 0.08, a	2.60 ± 0.40, b
	ethyl *p*-coumarate	2.41 ± 0.83, a	2.45 ± 0.44, a	2.80 ± 0.21, a
	fertaric acid	3.91 ± 0.33, a	3.93 ± 0.12, a	3.64 ± 0.26, a
	total	123.2 ± 9.4, a	139.8 ± 11.9, a	121.4 ± 1.5, a
stilbenes	*t*-piceid	10.17 ± 1.35, a	9.93 ± 0.65, a	10.70 ± 1.26, a
	t-resveratrol	12.64 ± 2.64, a	13.72 ± 0.75, a	13.36 ± 2.25, a
	c-piceid	9.90 ± 2.42, a	12.83 ± 0.93, a	12.28 ± 1.57, a
	c-resveratrol	7.06 ± 2.95, a	9.79 ± 1.14, a	20.33 ± 8.16, b
	total	31.44 ± 6.64, a	36.81 ± 2.59, a	47.13 ± 4.76, b

[a]Hydroxycinnamic acids and derivatives data for each moiety are expressed as molar ratios (%) and the total amounts are expressed as mg/L of caftaric acid. Stilbene data are expressed as mg/L, and total amounts are expressed as *t*-resveratrol equivalents. Different letters in a row indicate statistical differences ($p < 0.05$) between treatments.

Table 8. Proanthocyanidin Analysis of Wines Made From Grapes for Each Experimental Condition[a]

	control	berry thinning	cluster thinning
total PA[b]	513.5 ± 54.0, a	748.9 ± 102.4, b	544.6 ± 9.4, a
mDP[c]	4.66 ± 0.15, a	5.46 ± 0.60, b	4.30 ± 0.04, a
%PC[d]	66.7 ± 1.4, a	68.2 ± 1.0, a	66.7 ± 0.5, a
%PD[e]	33.3 ± 1.4, a	31.8 ± 1.0, a	33.3 ± 0.5, a
%Gal[f]	3.8 ± 0.0, b	3.4 ± 0.1, a	3.4 ± 0.3, a

[a]Analysis was performed by HPLC-DAD of adducts formed by acid depolymerization in the presence of an excess of phloroglucinol. Different letters in a row indicate statistical differences ($p < 0.05$) between treatments. [b]Total proanthocyanidin amount expressed as mg/L. [c]Mean degree of polymerization of analyzed proanthocyanidins. [d]Percentage of procyanidins. [e]Percentage of prodelphinidins. [f]Percentage of galloylation.

of proanthocyanidins from berry-thinning wine was also significant higher than in the other experimental conditions. The proportion of procyanidins and prodelphinidins was similar in all experimental conditions. However, the percentage of galloylation of the proanthocyanidins from the control wine was significantly higher than in wines from both treatments. Some authors have reported that well-ripe grapes release higher amounts of proanthocyanidins from skins than unripe grapes.[13] In contrast, seed proanthocyanidin extraction decreases with ripening.[14] Berry-thinning wine has greater proanthocyanidin concentrations, higher mDP, and lower percentages of galloylation than control wine. Since skin proanthocyanidins have higher mDPs and lower percentages of galloylation than seed proanthocyanidins, it seems that berry-thinning wine has a higher proportion of skin proanthocyanidins and/or a lower proportion of seed proanthocyanidins. Consequently, our data confirm that berry-thinning grapes have reached a higher level of phenolic maturity than control grapes. In contrast, the values for cluster-thinning wine are similar to those for control wine with the only exception of the percentage of galloylation.

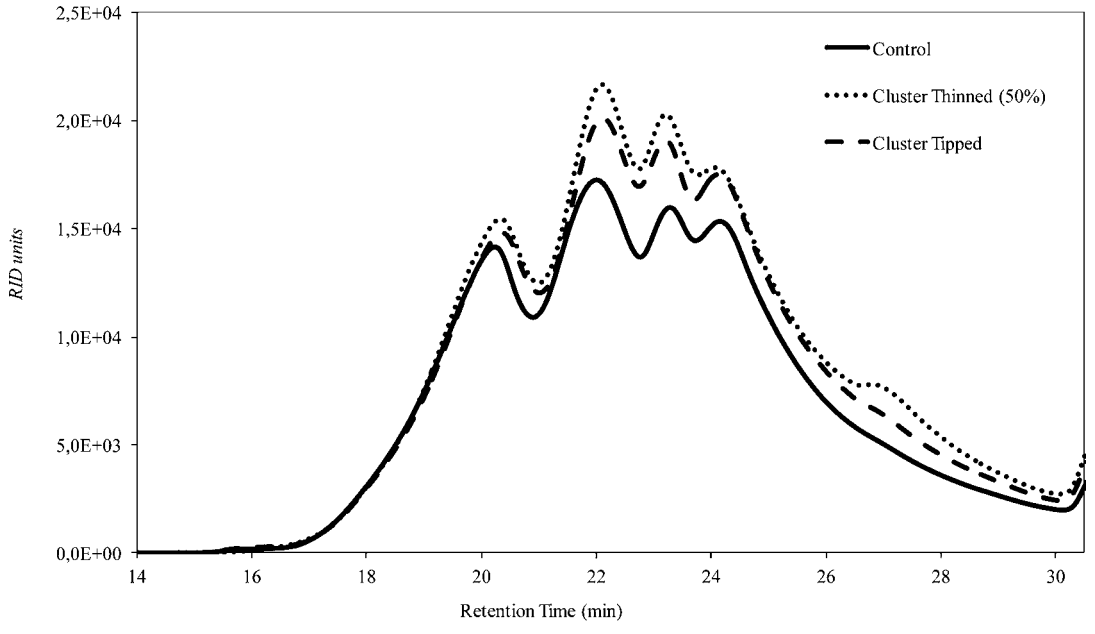

Figure 3. HRSEC-RID profiles for wine polysaccharides for each experimental condition.

Therefore, it seems that cluster thinning affects grape phenolic maturity less than berry thinning.

Figure 3 shows the HRSEC-RID analysis of polysaccharides from wines for the three experimental conditions. Each curve is the average of three replicates. Four molecular weight fractions were obtained in all the wines with the following average molecular weights: F1 (around 177 kDa); F2 (around 71 kDa); F3 (around 39 kDa); and F4 (around 25 kDa). A visual comparison of the chromatograms of the different wines reveals that wines from both treatments have higher areas, especially of the lower molecular weight fractions. Table 9 shows the

Table 9. Analysis of Polysaccharides by HRSEC-RID[a]

polysaccharide fraction		control	berry thinning	cluster thinning
F1	range[b]	2878−131	2873−124	2288−124
	M_n[c]	184.0 ± 2.6, b	172.6 ± 6.7, a	174.9 ± 2.5, a
	conc[d]	133 ± 6, a	142 ± 5, ab	146 ± 4, b
F2	range[b]	131−50	124−50	124−50
	M_n[c]	73.3 ± 0.8, b	69.9 ± 0.7, a	70.3 ± 0.7, a
	conc[d]	125 ± 6, a	134 ± 4, ab	142 ± 3, b
F3	range[b]	50−30	50−31	50−30
	M_n[c]	38.0 ± 0.8, a	39.5 ± 0.5, b	39.5 ± 0.3, b
	conc[d]	67 ± 9, a	78 ± 3, ab	89 ± 7, b
F4	range[b]	30−1	31−1	30−1
	M_n[c]	24.6 ± 1.2, a	24.6 ± 0.6, a	25.4 ± 0.6, a
	conc[d]	191 ± 7, a	229 ± 12, b	239 ± 4, b
total	conc[e]	516 ± 26, a	583 ± 20, b	617 ± 14, b

[a]Different letters in a row indicate statistical differences ($p < 0.05$) between experimental conditions. [b]Range of molecular weights considered in each fraction. The end points are expressed as kDa. [c]Number average molecular weight (M_n) of each fraction expressed as kDa. [d]Quantification of polysaccharides of each fraction expressed as mg/L. [e]Total quantification of polysaccharides expressed as mg/L.

polysaccharide concentration, the number average molecular weight (M_n), and the molecular weight range (MWr) of the different fractions. These data confirm that berry-thinning and especially cluster-thinning wines have significantly higher total polysaccharide concentrations than the control wine. In the case of cluster-thinning wine, all the fractions were significantly higher than in the control wine whereas only F4 was significantly higher in the case of berry-thinning wine. The M_n and MWr results show some interesting differences. In general, the M_n of the higher molecular weight fractions (F1 and F2) from berry-thinning and particularly cluster-thinning wines are significantly lower than controls, and their MWr has intervals with somewhat smaller molecular weight end points. In contrast, a smaller molecular weight fraction (F3) from both treatments has a significantly higher M_n than controls. During ripening, the grape berries undergo a softening process that has been attributed to the enzymatic hydrolysis of cell wall pectins.[12] Our results indicate that wines from both treatments have a higher polysaccharide concentration, a lower M_n, and a molecular weight distribution that tends toward lower values than control wine. Therefore, these data suggest that skins from berry-thinning and cluster-thinning grapes were riper than those of control grapes.

Table 10 shows the results of the triangular sensory analysis of the three pairs of wines. The tasters distinguished significantly ($p < 0.05$) between the wine from both treatments and the control wine. However, they were unable to distinguish between cluster-thinning and berry-thinning wines ($p > 0.05$).

Table 10. Triangular Test Results for Sensorial Analysis of Wines Made of Grapes for Each Experimental Condition

			preference		
triangular test	positive identification	P	control	cluster thinning	berry thinning
control vs cluster thinning	14/24	<0.05	7	7	−
control vs berry thinning	13/24	<0.05	4	−	9
cluster thinning vs berry thinning	9/24	ns	−	5	4

The tasters who distinguished correctly between cluster-thinning and control wines were divided in their preferences: more specifically, seven preferred control wine whereas seven preferred cluster-thinning wine. However, in the comparison between berry-thinning and control wines, they clearly opted for the berry-thinning wine. More specifically, nine preferred berry-thinning wine whereas only four preferred the control wine.

In summary, cluster thinning led to wines with a significantly higher ethanol content, increased anthocyanin and polysaccharide concentrations, and generally higher concentrations of most of the other phenolic compounds although these differences were not significant. Berry thinning led to wines with significantly higher TPI, flavonol, proanthocyanidin and polysaccharide concentrations, and lower titratable acidity. Moreover, the proanthocyanidin mDP of berry-thinning wine was significant higher than that of control wine. Wines obtained from both treatments were sufficiently different from the control wine to be significantly distinguished by a trained panel in a triangular test. It can be concluded therefore that both treatments for reducing yield effectively enhanced grape maturity and improved wine quality. However, cluster thinning involves a considerable reduction in yield, which is a real economic disadvantage. In contrast, berry thinning seems to be more effective than cluster thinning, and the yield reduction is lower.

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