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Effect of Cluster Thinning and Prohexadione Calcium Applications on Phenolic Composition and Sensory Properties of Red Wines

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ABSTRACT: The overall objective of this study was to investigate the effect of manual cluster thinning (CT) and the application of the growth regulator Prohexadione calcium (ProCa) on the phenolic composition and the sensory profile of Tempranillo and Grenache wines produced from treated vines in La Rioja (Spain). ProCa was applied at preblooming and CT was carried out at veraison in two consecutive years. Different physicochemical parameters and analyses of phenolic compounds were carried out in control, CT and ProCa grapes and wines and wine sensory was performed. Thinning treatments decreased crop yield, besides ProCa application reduced berry size, and berry weight. Color and phenolic composition of Grenache and Tempranillo wines in general were affected by thinning treatments, with an increase in anthocyanin, flavanol and flavonol concentrations. In sensory analysis, wines obtained from thinned vines presented higher values for several aromatic (e.g., white and yellow fruits, fresh flowers) and taste attributes (i.e., astringency, bitterness, persistence). CT and ProCa treatments resulted in an improvement in wine quality. In general, similar results in phenolic composition, sensory properties and quality of wines were obtained by manual and chemical cluster thinning. ProCa as a growth regulator may be an option for a quality vitiviniculture.

KEYWORDS: *manual thinning, prohexadione calcium, wines, phenolic composition, sensory analysis*

INTRODUCTION

Crop load adjustment is widely accepted as an important vineyard management tool for premium-quality wine production. In Europe this assumption is often written into the law, and only relatively low yields are permitted for controlled appellation wines.[1] Crop thinning subsequent to fruit set can help regulate yield and improve fruit composition at harvest.[2] Nowadays, cluster thinning is a very widely used technique to reduce production in vigorous vines. In most cases, cluster thinning induces faster grape ripening and improves quality, depending on the timing of the thinning.[3] Some researchers found that the most effective time for thinning is veraison.[4−6] The influence of thinning on the pH and total acidity is lower than on sugar accumulation. Some authors found a higher content in anthocyanins and phenols in previous studies where total leaf area/fruit ratio was increased through cluster thinning.[5−7] The presence of phenolic compounds in wine is important, on one hand, because moderate consumption is associated with health-promoting properties, such as antioxidant, antibacterial, anti-inflammatory, antiallergic, and antithrombotic activities.[8] On the other hand, some of these compounds, such as phenolic acids, catechins, and some flavonoids, play an important role in wine quality, contributing to flavor and color properties, especially in red wines.[9] Thus, it is important to study the influence of thinning treatments on phenolic composition.

Cluster thinning (CT) has been demonstrated to produce an increase in color intensity (CI), total polyphenol index (TPI), and anthocyanins.[10] Although effective, manual cluster thinning is a very expensive operation because of large labor requirements.[11] An alternative method for controlling production is the use of plant growth regulators. Prohexadione calcium (ProCa, 3-oxido-4-propionyl-5-oxo-3-cyclohexenecarboxylate) is a gibberellin biosynthesis inhibitor with limited persistence.[12] ProCa operates by blocking two oxoglutarate-dependent dioxygenases, which catalyze the later steps in the biosynthetic sequence. The 3-β hydroxylation of GA_{20} (inactive) into GA_1 (biologically active) is especially inhibited, resulting in a reduction of longitudinal shoot growth in plants.[13,14] ProCa is easily applied by spraying and constitutes no apparent risk for consumers or the environment.[15] ProCa has been reported to be absorbed completely within 8 h and to be degraded in plants with a half-life of a few weeks and in soil with a half-life of less than 1 week, without producing toxic metabolites.[16] ProCa has been registered and used on apples as Apogee (27.5% ProCa) in North America and as Regalis (10% ProCa) in Europe by BASF.[17] ProCa has been applied on fruit trees with satisfactory results.[18−21]

The first studies concerning the influence of ProCa on fruit and wine composition were performed by Disegna et al.[22] in cv. Tannat. They observed that the ProCa application produced a higher alcohol content, higher color intensity, and higher volume by mouth in wines produced from treated vines. These wines were also more complex, persistent, and fruity, with higher aromatic

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intensity and higher terpene and norisoprenoid content with respect to the untreated ones.

Lo Giudice et al.[23,24] studied the impact of application of ProCa in *Vitis vinifera* grapevines, especially in Cabernet Franc, Cabernet Sauvignon, Chardonnay, and Seyval. ProCa produced a decrease in berry size. In cv. Cabernet Sauvignon, the reduction of berry weight was correlated with an increase in color intensity, total anthocyanins, and total phenols. The observed effects on grape composition were generally positive, but the effect on the quality and the organoleptic characteristics of the final wine are still unknown.[24]

In previous studies carried out by our research group[25] on cv. Tempranillo, treatment with ProCa produced a reduction in yield, clusters, and berry size. An increase in TPI, tannins, and CI was also observed in wines obtained from treated grapes. The sensory analysis revealed different organoleptic characteristics in these wines. The application of ProCa led to an enhancement of typical sensory attributes and an improvement in sensory characteristics of Tempranillo wines.[25]

The aim of this study was to examine the effect on enological parameters, phenolic composition, and sensory attributes of wines obtained from Tempranillo and Grenache grapevines (major varieties in DOCa Rioja) that were treated with ProCa or cluster-thinned manually.

MATERIALS AND METHODS

Vineyards. The experiments were conducted in two commercial vineyards planted with Tempranillo and Grenache (both *Vitis vinifera* L.), nonirrigated and situated in locations of the Rioja Qualified Denomination of Origin (Aldeanueva de Ebro, Spain) over two consecutive years, 2007 and 2008. Tempranillo vines were planted to goblet training. Vine spacing was 2.6 m (row) × 1.2 m (vine). Vines were cane-pruned, grafted onto 110R rootstock (clone 51), and planted in 2000. Grenache vines were trained and spur-pruned on a wire trellis system for support and the canopy was vertically shoot-positioned. Row and vine spacing was 2.6 m × 1.2 m. Vines were grafted onto 110R rootstock (clone 70) and planted in 1998. Both vineyards were managed according to standard viticultural practices for the cultivar and region. All fertilizer applications and pest and disease management practices were applied as uniformly across the vineyards as possible. Winter pruning was carried out, leaving 12 buds/vine in both vineyards.

Field Treatments. Treatments were carried out along six consecutive rows for each treatment and control. Three rows were left as buffer zone between CT and ProCa treatments. Each row contained approximately 100 vines. ProCa was applied as Regalis, 10% ProCa (BASF, Ludwigshafen, Germany), at a dose of 3 kg/ha at preblooming (BBCH 57). Treatments were applied to both sides of the canopy, wetting the entire shoots. The product was sprayed with a ILEMO-HARDI atomizer (Taastrup, Denmark). Applications were carried out when there was no rain predicted for at least 24 h. Manual cluster thinning was carried out at the beginning of veraison (BBCH 81) in the following ratio: 30% (in Tempranillo) and 50% (in Grenache) of total bunches per vine in both years, except in 2008 when cluster thinning was not carried out in Tempranillo variety because of the lack of grapes in the vines. The distal cluster was removed, leaving only one bunch per shoot at most, as were clusters of weak shoots. Six rows were neither treated nor thinned, and they were used as controls.

Vinification Process. Clusters from 40 vines of the two central rows of each treatment were randomly handpicked. The last five vines from the ends of the row were not considered. Mature grapes were harvested on October 11, 2007 and October 7, 2008. The grapes of each treatment were mixed and fermented in triplicate. Nine lots of 45 kg of grapes were vinified each year per variety. Experimental vinifications were carried out in the experimental winery of the University of La Rioja. Grapes were destemmed, crushed, and fermented into 50 L stainless steel tanks; 100 mg/kg potassium metabisulfite was added. The must was inoculated at a rate of 30 g/hL with commercial yeast strain VRB *Saccharomyces cerevisiae* (Lallemand Edwardstown, Australia). The prefermentation process lasted 24 h. During alcoholic fermentation, the cap was punched down twice a day and pumping-over was carried out once a day. The wines were removed from the skins and seeds and then were drawn off. Commercial lactic bacteria strain Alpha *Oenococcus oeni* was inoculated at a rate of 1 g/hL (Lallemand, Edwardstown, Australia) in 15 L stainless steel tanks. After malolactic fermentation, wines were racked and 90 mg/L potassium metabisulfite was added. Wines were clarified by settling at 4 °C for 4 weeks and then bottled. The wines were stored at 4 °C.

Chemical Analysis. *Reagents.* All chemicals used were of analytical reagent grade. All chromatographic solvents were of HPLC grade. Ultrapure water was obtained from a Milli-Q purification system (Millipore, Molsheim, France). TSK Toyopearl gel HW-50F was purchased from Tosohaas (Montgomeryville, PA). Methanol, formic acid, ethanol, acetonitrile, and sulfuric acid were supplied by Scharlab (Barcelona, Spain). Quinine sulfate dihydrate (98%) was obtained from Alfa Aesar (Karlsruhe, Germany). Potassium and aluminum sulfate and tannic acid were purchased from Panreac (Barcelona, Spain). Ovalbumin (V-grade), catechin, epicatechin, myricetin, kaempferol, gallic acid, caffeic acid, and quercetin were purchased from Sigma−Aldrich (Madrid, Spain). Quercetin 3-glucoside, quercetin 3-galactoside, quercetin 3-glucuronide, kaempferol 3-glucoside, epicatechin gallate, epigallocatechin gallate, epigallocatechin, and *p*-coumaric acid were provided from Extrasynthèse (Genay Cedex, France). Trifluoroacetic acid (TFA) was supplied by Fluka (Buchs, Switzerland).

Fractionation of Wine Phenolics by Size-Exclusion Chromatography. TSK Toyopearl gel HW-50F was suspended in the mobile phase and it was packed in a Millipore (Bedford, MA) Vantage L column (120 mm ×12 mm i.d.) at atmospheric pressure. Gel-permeation chromatography data were analyzed by connecting the column to a diode-array detector (DAD; Agilent, G1315B). Two milliliters of wine was directly injected in the column, and flow rate was regulated at 1 mL/min by use of a peristaltic pump. A first fraction (F1) was eluted with 60 mL of ethanol/water/trifluoroacetic acid (54.95:45:0.05 v/v/v). A second fraction (F2) was recovered by elution with 50 mL of acetone/water (60:40 v/v). The two fractions collected were brought to dryness under vacuum. Fractions 1 and 2 were redissolved in 2 mL of formic acid/water (5:95 v/v) and 2 mL of methanol, respectively. Fraction F1 was further analyzed by HPLC-DAD and HPLC-MS (mass spectrometry). All wines were fractionated three times and passed through a 0.45 μm filter before being analyzed.

HPLC-DAD Analysis. Anthocyanins, hydroxycinnamic acids, flavanols, and flavonols were analyzed by direct injection of fraction F1, obtained from size-exclusion chromatography (SEC), into the HPLC system. Analysis of low molecular weight phenolics by HPLC-DAD was performed in an Agilent modular 1100 liquid chromatograph (Waldbronn, Germany), and detection was carried out with a G1315B photodiode array detector. The column was a reversed-phase Kromasil 100-C18 (5 μm packing, 250 mm × 46 mm i.d.), protected with a guard column of the same material (Teknokroma, Barcelona, Spain). Phenolic compounds were eluted under the following conditions: 1 mL/min flow rate; oven 40 °C; solvent A, formic acid/water (5:95 v/v); solvent B, acetonitrile (100%); gradients, isocratic 0% B in 2 min, from 0% to 8% B in 3 min, from 8% to 20% B in 55 min, from 20% to 30% B in 10 min, from 30% to 50% B in 1 min, from 50% to 100% B in 2 min, stay at 100% B for 7 min, from 100% to 0.0% B in 1 min, and then stay at 0% B for 9 min, followed by washing and reconditioning of the column. Fraction F1 (30 μL) obtained from the SEC fractionation (in formic acid/water, 5:95 v/v) was directly injected in the HPLC system and chromatographed. UV−vis spectra were recorded from 220 to 700 nm, with a bandwidth of 2.0 nm.

Quantification was carried out by peak area measurements at 520 nm for anthocyanins, 365 nm for flavonols, 313 nm for hydroxycinnamic acids, and 280 nm for flavanols. Identification of compounds was performed by comparing their retention times and UV−vis spectra to those of authentic standards and was also confirmed by HPLC-MS analysis. Their quantification was performed in triplicate by use of an external standard calibration curve for each compound. Anthocyanin, flavonol, hydroxycinnamic acid, and flavanols contents

were expressed as malvidin 3-*O*-glucoside, quercetin, caffeic acid, and catechin, respectively. Calibration curves were obtained by injecting different concentrations of standards. The range of the linear calibration curves was from 0.01 (limit of quantification) to 1.0 mg/L for the lower concentration of compounds and from 1.0 to 100 mg/L for the higher concentration of compounds. Each measurement was run in triplicate. Quantitative data of the identified compounds were obtained by interpolation of the relative areas in the calibration curves built for pure reference compounds. Good linearity was obtained for all compounds and for the entire range of studied concentrations, with correlation coefficients better than 0.993. The analytical method presented accuracy between 97.1% and 103.6% and precision (repeatability) < 0.9%.

HPLC-ESI-MS Analysis. Mass spectrometric analysis was performed by coupling the Agilent 1200 liquid chromatograph (LC) described above to a Hewlett-Packard 5989 quadrupole mass spectrometer equipped with an electrospray interface (ESI-MS; HP 59987A) and controlled by the MS Agilent 1200 software. Chromatographic separation was performed under the same conditions described above. To ensure a flow of 19 μL/min into the ESI interface during LC-MS, the LC effluent was split by means of a zero dead volume T-piece. This flow was found to be the optimum under these conditions. Nitrogen was used as nebulizing gas at an inlet pressure of 80 psi and a temperature of 225 °C. All mass spectrometry data were acquired from 150 to 700 m/z in the negative-ion mode for hydroxycinnamic acids, flavanols, and flavonols and in the positive-ion mode for anthocyanins. Table 1 shows the identification of all compounds by UV−vis and MS.

Astringency Index: Analysis of Protein-Precipitable Proanthocyanidins. Protein-precipitable proanthocyanidins (PAs) were estimated by use of ovalbumin as the precipitation agent and tannic acid solutions as standards, in accordance with a previously described method.[26]

Determination of Usual Enological Parameters and Color Composition Measurements. Conventional enological parameters (ethanol content, pH, reducing sugars, titratable and volatile acidities, and total and free SO_2) were determined in accordance with official OIV practices.[27] L-Malic and L-lactic acids were determined by enzymatic methods in accordance with official AOAC analysis methods.[28] Color intensity (CI) was calculated as the sum of absorbances at 420, 520, and 620 nm, and the hue of the wine was calculated as the ratio of absorbance at 420 and 520 nm. TPI was estimated as absorbance at 280 nm. Tannins were determined using the method described by Ribéreau-Gayon and Stonestreet.[29] The ethanol index (ETI) reflected the tannin−polysaccharide condensation and it was calculated by the method described by Glories.[30] All determinations were carried out in triplicate.

Sensory Analysis. In February 2008 and 2009, sensory analysis was performed by a panel formed by experts from the Asociación de Enólogos of La Rioja and enology graduates from the University of La Rioja. All wine tasters had participated in previous aroma and mouthfeel sensory descriptive panels and had regularly participated in quality-scoring Tempranillo and Grenache wines sensory panels. The panel had 30 members (13 males and 17 females from 28 to 56 years old) in 2008 and 32 members (17 males and 15 females from 26 to 62 years old) in 2009.

Duo-Trio Test. The first aim of the sensory evaluation was to determine whether the wine obtained from treated vines (CT practice and ProCa application) was significantly different from the control. Since this is a classical application of a discrimination test, a duo-trio test was chosen.[31] Three samples were presented to the experts, one of which was identified as the reference. One of the other two was identical to the reference. The panelists were asked to state which product most closely resembled the reference. Since the question was "Which sample matched the reference sample?" the one-tailed binomial test was used. Panelists considered odor, taste, and mouthfeel perceptions.

Sensory Training. Panelists attended four descriptive sensory training sessions (45 min each). In these sessions, sensory vocabulary describing wine aroma attributes for each variety as well as taste and mouthfeel sensations were described. In a first session, the panelists were asked to describe aroma attributes of Tempranillo and Grenache wines in their own words. Descriptors and their definitions were discussed by panelists and panel leader. Tasters selected eight aroma attributes that were agreed upon as best describing the sensory characteristics of the wines. All the generated terms were usual wine-tasting terms for describing red wines (Table 2). These descriptors were also used in 2009 in order to perform a comparison between

Table 1. Compounds Analyzed by HPLC-DAD and MS in Studied Wines

RT (min)	compd ID	λ_{max} (nm)	$[M - H]^-$ or $[M - H]^+$ (m/z)	MS/MS fragments (m/z)
		Acid[a]		
3.7	gallic acid	216, 272	169	125
		Flavanols[a]		
9.3	catechin 3-gallate	202, 277	441	289
9.6	catechin	203,279	289	203
11.2	epigallocatechin 3-gallate	203, 271	457	305
13.7	epicatechin 3-gallate	203, 279	441	289
17.2	epicatechin	203, 279	289	203
11.9	epigallocatechin	206, 271	305	203
		Hydroxycinnamic Acids[a]		
8.4	(*Z*)-caftaric acid	330	311	179
8.6	(*E*)-caftaric acid	331	311	179
10.6	coutaric acid	313	295	149
11.2	caffeic acid	218, 238, 324	179	135
16.8	coumaric acid	212, 226, 310	163	119
		Flavonols[a]		
20.5	myricetin 3-glucoside	254, 365	479	151
22.7	quercetin 3-galactoside	256, 354	463	301
27.6	quercetin 3-glucoside	256, 355	463	301
35.4	kaempferol 3-glucoside	265, 346	447	285
42.4	myricetin	254, 371	317	151
54.7	quercetin	255, 369	301	151
67.5	kaempferol	253, 362	285	269
		Anthocyanins[b]		
12.8	delphinidin 3-glucoside	277, 342, 524	465	303
15.7	cyanidin 3-glucoside	279, 516	449	287
18.4	petunidin 3-glucoside	277, 347, 525	479	317
22.1	B-type vitisin of petunidin 3-glucoside	492	503	341
25.5	malvidin 3-glucoside	277, 348, 527	493	331
29.2	vitisin A	299, 372, 510	561	399
30.3	delphinidin 3-*O*-6-acetylglucoside	276, 346, 527	507	303
38.8	malvidin 3-glucoside catechin	280, 532	781	619
42.6	petunidin 3-*O*-6-acetylglucoside	270, 529	521	317
46.9	peonidin 3-*O*-6-acetylglucoside	280, 522	505	301
47.9	malvidin 3-*O*-6-acetylglucoside	278, 350, 530	535	331
52.4	delphinidin 3-*O*-6-(*p*-coumaroyl) glucoside	282, 313, 531	611	303
54.3	cyanidin 3-*O*-6-(*p*-coumaroyl) glucoside	284, 314, 524	595	287
55.9	petunidin 3-*O*-6-(*p*-coumaroyl) glucoside	282, 313, 532	625	317
62.6	peonidin 3-*O*-6-(*p*-coumaroyl) glucoside	283, 313, 526	609	301
64.3	malvidin 3-*O*-6-(*p*-coumaroyl) glucoside	282, 313, 532	639	331

[a] $[M - H]^-$ (negative-ion mode). [b] $[M - H]^+$ (positive-ion mode).

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Table 2. Final List of Descriptors Used for Aroma, Taste, and Mouthfeel Descriptive Analysis, with the Corresponding Reference Standards Presented during Panel Training

attribute	reference standard[a]
	Aroma
white and yellow fruits	14 mL of apple juice +14 mL of peach juice + 20 drops of orange extract + 0.5 mL of isoamyl acetate
red and black fruits	15 mL of black-currant-flavored water + 5 g of blackberry jam + 5 g of strawberry jam + 5 g of raspberry jam + 5 g of cherry jam
fresh flowers	50 μL of no. 25 "Le Nez Du Vin" + 50 μL of no. 29 "Le Nez Du Vin" + 5 jasmine petals + 5 rose petals
lactic	30 mL of single cream
spicy	2 g of black pepper + licorice stick + 1 unit of nutmeg
balsamic	1 g of chopped Sintox spearmint candy
alcoholic	15 mL of ethyl alcohol
herbaceous	4 g of chopped fresh grass
	Taste and Mouthfeel
sweetness	0−12 g/L sucrose
sourness	0−1.5 g/L tartaric acid
bitterness	0−10 mg/L quinine sulfate
astringency	0−5 g/L potassium and aluminum sulfate
volume by mouth	0−30 g/L glycerol

[a]Standards were prepared in 30 mL of deionized water.

years, so there was no discussion regarding terms in this panel session. During training, different reference standards representative of aroma, taste, and mouthfeel terms were presented. Standards were either commercially available odorants taken from Sentosphère (Paris, France), "Le Nez du Vin" (Jean Lenoir, Provence, France), or natural products (fruits, juices, spices, vegetables) prepared at the beginning of each session. For taste and astringency, solutions containing different concentrations of table sugar (0−12 g/L) for sweetness, tartaric acid (0−1.5 g/L) for sourness, quinine sulfate (0−10 mg/L) for bitterness, glycerol (0−30 g/L) for volume by mouth, and potassium aluminum sulfate (0−5 g/L) for astringency stimuli were presented to the panel to aid with recognition and discrimination between the different oral sensations. During training phase (four sessions), panelists became familiar with aroma attributes and with intensity rating of sweetness, sourness, bitterness, volume by mouth, astringency, aromatic and retronasal intensity, and overall intensity, as well as persistence. During a typical session, panelists had to evaluate 2−4 different wines, by rating selected aroma attributes, sweetness, acidity, bitterness, volume by mouth, and astringency on a 6-point scale (0 = absence, 1 = very low, and 5 = very high), while retronasal and aromatic intensity, in-mouth overall intensity, and persistence were measured on a 5-point scale (1 = very low and 5 = very high) since, for these last concepts, the 0 has no meaning.

Sample Evaluation. Evaluation for sensory analysis was carried out in duplicate. Wine samples (20 mL) were presented in dark ISO (1977) approved wineglasses labeled with three-digit random codes and covered by plastic Petri dishes according to a random arrangement. Panelists were asked to smell each wine and rate aroma attributes. Then they were asked to rate the sweetness, acidity, bitterness, volume by mouth, astringency, and aromatic and retronasal and overall intensities, as well as the overall persistence of the samples, using the above-mentioned structured scales for each wine. Panelists paused for 5 min intervals between sample evaluations to limit adaptation effects. During that time they were asked to rinse their mouths with water, to have some plain crackers, and finally to rinse their mouths again with water. All wines were served at room temperature and were evaluated in individual booths. Samples were stored at 15 °C.

Statistical Analysis. Statistical analyses were performed with the SPSS 15 package (IBM, Armonk, NY). All chemical data obtained were assessed by one-way analysis of variance (ANOVA) to identify significant differences between control and treated wines. Differences between samples always refer to significant differences with at least $P < 0.05$. Two-way ANOVAS were performed in order to evaluate "treatment × variety" and "treatment × year" interactions for significance ($P < 0.05$).

A mixed-model ANOVA was performed on sensory descriptive analysis data, in which the judges were considered as a random effect. Treatment, replication, and judge factors, as well as the two-way interaction treatment × judge, were evaluated for significance ($P < 0.05$) by the general linear model. The mean differences between treatments were calculated by the least significant difference Fisher's test.

A normalized PCA (principal component analysis) was performed on the mean rating over the panelists for the attributes for wines and color parameters.

RESULTS

Climatic Conditions and Enological Parameters of Grapes. Monthly metereological data from preblooming to harvest are summarized in Table 3. Month temperatures were similar in both years except for August, where temperatures were higher in 2007, and October, with lower temperatures in 2008. Rainfall amounts were different, with lower preciptations in 2008. Total GDD (growing degree days) from June 1 to harvest were higher in 2007, but there were differences between the same months of different years. Table 4 shows the results of several enological parameters at harvest for control grapes and treated grapes. Physical parameters showed that ProCa application produced lower weight and size in grapes. The results showed that the application of ProCa produced a reduction in the crop yield in Grenache of 29% in 2007 and 15% in 2008, and in Tempranillo 24% reduction in 2007, while there was no reduction in 2008. Cluster thinning led to a decrease in Tempranillo production of 25% in 2007, and in Grenache production 41% and 42% decreases in 2007 and 2008, respectively. °Brix data in 2007 showed that grapes from the CT treatment presented the highest sugar content, while grapes from the ProCa treatment showed no significant differences compared to control grapes. In 2008 there were no significant differences in °Brix. In 2007, Tempranillo wines from thinning treatments presented lower pH values than those from control, while Grenache wines from thinning treatments

Table 3. Meteorological Data in Aldeanueva de Ebro (Spain) from Weather Station of La Rioja Government

	max temp[a] (°C)		mean temp[a] (°C)		min temp[a] (°C)		cum precip (L/m²)		GDD[b] (°C)	
month	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
June	27.5	27.3	20.2	20.6	13.4	14.7	11.4	17.0	219.7	230.8
July	29.9	29.6	22.1	21.9	15.0	14.9	0.0	32.8	385.8	331.0
Aug	28.2	30.1	21.2	22.2	15.1	15.3	16.8	1.8	361.9	394.5
Sept	25.3	25.0	18.5	18.2	12.9	12.3	10.2	13.6	272.7	260.0
Oct[c]	22.2	19.5	17.2	12.9	13.7	6.5	51.0	0	87.5	21.2
total							89.4	65.2	1327.6	1237.5

[a]Mean for complete month. [b]Cumulative growing degree days (base 10°C). [c]Data from Oct 1 to harvest day.

Table 4. Several Yield Components and Enological Parameters in Tempranillo and Grenache Grapes[a]

samples	crop yield (t/ha)	avg berry wt (g)	berry size,[b] % 10–12 mm	12–14 mm	14–16 mm	16–18 mm	°Brix	pH	titratable acidity[c] (g/L)	malic acid (g/L)	TPI[d]	tot. anthocyanins (mg/g)	tot. tannins (mg/g)
Tempranillo, 2007													
ctrl	7.9 ± 0.2 a	2.0 ± 0.0 b	8 ± 0 b	54 ± 7 a	32 ± 7 b	8 ± 0 a	22.8 ± 0.1 b	3.5 ± 0.1 a	6.3 ± 1.1 b	2.4 ± 0.1 a	43.0 ± 1.5 b	1.6 ± 0.0 b	2.7 ± 0.2 b
CT	6.0 ± 0.1 c	2.1 ± 0.0 a	0 ± 1 c	44 ± 3 b	50 ± 3 a	6 ± 1 a	24.1 ± 0.4 a	3.4 ± 0.0 b	6.3 ± 1.3 b	2.2 ± 0.0 b	52.0 ± 1.3 a	1.7 ± 0.1 b	3.7 ± 0.2 a
ProCa	6.7 ± 0.1 b	1.7 ± 0.0 c	24 ± 2 a	57 ± 4 a	19 ± 2 c	3 ± 1 b	23.5 ± 0.6 b	3.4 ± 0.0 b	6.8 ± 0.0 a	2.3 ± 0.1 ab	49.8 ± 2.1 a	2.1 ± 0.1 a	2.6 ± 0.1 b
Tempranillo, 2008													
ctrl	7.2 ± 0.1 a	2.3 ± 0.1 a	0 ± 0 a	24 ± 6 a	59 ± 5 a	16 ± 7 a	24.3 ± 0.9 a	3.4 ± 0.1 a	4.1 ± 0.2 a	1.7 ± 0.2 b	49.0 ± 1.5 a	1.2 ± 0.1 a	2.8 ± 0.1 a
ProCa	7.1 ± 0.1 a	2.3 ± 0.2 a	0 ± 0 a	19 ± 4 a	52 ± 8 a	25 ± 4 a	24.4 ± 1.1 a	3.3 ± 0.0 a	4.7 ± 0.4 a	2.0 ± 0.1 a	48.9 ± 5.7 a	1.1 ± 0.1 a	2.4 ± 0.0 b
Grenache, 2007													
ctrl	8.2 ± 0.1 a	2.4 ± 0.0 a	0 ± 0 a	31 ± 6 b	54 ± 5 a	16 ± 4 a	20.6 ± 0.5 b	3.2 ± 0.0 b	6.8 ± 0.1 a	1.7 ± 0.0 a	20.6 ± 5.0 b	0.8 ± 0,2 b	1.3 ± 0.1 b
CT	4.8 ± 0.0 c	2.4 ± 0.1 a	0 ± 0 a	38 ± 7 b	51 ± 4 a	11 ± 3 b	25.3 ± 0.8 a	3.3 ± 0.0 a	6.1 ± 0.2 b	1.2 ± 0.2 b	32.9 ± 2.1 a	1.0 ± 0.1 a	1.8 ± 0.3 a
ProCa	5.8 ± 0.1 b	1.7 ± 0.0 b	3 ± 0 b	64 ± 4 a	27 ± 5 b	6 ± 1 c	20.8 ± 1.9 b	3.3 ± 0.0 a	6.5 ± 0.2 a	1.9 ± 0.2 a	27.6 ± 2.4 ab	0.8 ± 0.2 ab	1.4 ± 0.2 ab
Grenache, 2008													
ctrl	8.0 ± 0.2 a	2.0 ± 0.1 a	0 ± 0 a	57 ± 2 c	38 ± 4 a	6 ± 2 a	25.5 ± 0.7 a	3.3 ± 0.0 a	5.6 ± 0.2 a	1.3 ± 0.2 a	16.7 ± 0.6 b	0.4 ± 0.0 b	0.5 ± 0.1 b
CT	4.5 ± 0.1 c	1.9 ± 0.1 b	0 ± 0 a	64 ± 2 b	32 ± 3 a	4 ± 2 ab	25.8 ± 0.4 a	3.3 ± 0.0 a	5.4 ± 0.1 a	1.0 ± 0.1 b	28.0 ± 2.6 a	0.8 ± 0.1 a	1.0 ± 0.0 a
ProCa	6.8 ± 0.1 b	1.7 ± 0.1 c	0 ± 0 a	74 ± 3 a	24 ± 4 b	2 ± 1 b	25.5 ± 0.8 a	3.3 ± 0.0 a	5.7 ± 0.4 a	0.9 ± 0.1 b	24.1 ± 2.5 a	0.7 ± 0.1 a	0.8 ± 0.3 ab

[a]Mean ± standard deviation, $n = 3$. Different letters in same column and year mean significant differences at 5% confidence level. [b]Percentage of berries for each diameter category is listed. [c]Expressed as grams of tartaric acid per liter. [d]Total polyphenol index.

showed higher pH values. Titratable acidity in 2007 was higher for Tempranillo and Grenache wines from ProCa treatment. In 2008 there were no significant differences in pH and titratable acidity. CT wines presented the lowest values of malic acid content for both varieties.

With respect to TPI, in general treatments performed to reduce production provided higher TPI in grapes. However, differences in the effect of treatments on varieties were observed. In Tempranillo grapes, ProCa treatment seemed to increase anthocyanin content while grapes from manual cluster thinning increased total tannins. However, these results were not confirmed in the second year of study for Tempranillo cv. In the case of Grenache, higher content of anthocyanins and tannins were obtained in CT grapes for both years.

Enological Parameters of Wines. Results of usual enological analysis are shown in Table 5. There was a significant increase in alcoholic content in wines produced from CT vines compared to control wines for the two varieties in 2007 and in Grenache in 2008, while treatment with ProCa produced an ethanol percentage similar to control wines. pH did not show significant differences between treatments and control for both varieties and years. Titratable acidity was significantly higher for ProCa treatment in both varieties and both years. All wines performed malolactic fermentation as shown by the values of lactic acid. CT practice and ProCa application led to wines with greater TPI in two varieties, which is consistent with an increase in total tannins. With regard to the ethanol index, there were significant differences in the two years and varieties, producing a decrease in wines from thinned vines. For astringency index, significant differences for the two varieties were found in 2007, with higher values in wines from CT and ProCa-treated vines. This index was not significantly higher in wines of both varieties in year 2008.

Color Parameters and Anthocyanic Composition. Color parameters and anthocyanin composition of wines from both varieties and treatments are summarized in Table 6. ProCa treatment caused an increase in CI compared to control wines for both varieties, except in the case of Tempranillo in 2008. CT also produced higher CI in Grenache control wines; however, Tempranillo wines from CT vines presented values for CI between control and ProCa wines, with no differences between these wines.

Wines from both thinning treatments presented similar anthocyanin concentrations, which were higher when compared to the control wines. This difference is mainly due to the concentration of nonacylated anthocyanins, representing approximately 80% of total anthocyanins in both varieties. However, it is worthy of comment that Tempranillo wines in both years and Grenache in 2007, with treatments aimed at reducing production, led to an increase in the concentration of pyranoanthocyanins, which are more stable to oxidation and discoloration by sulfur dioxide. These anthocyanins represented between 0.5% and 2% of total anthocyanins in Tempranillo and between 6% and 8.5% in the case of the Grenache variety. Tempranillo wines presented no significant differences in acylated (6′-*p*-coumaroyl) and total condensed anthocyanins concentrations for both years, while higher amounts of acylated (6′-acetyl) anthocyanins were found in wines from thinning treatments in 2007. As for Grenache wines, higher levels of acylated (6′-*p*-coumaroyl) anthocyanins were determined in wines from treated vines for both years, and these wines presented higher total condensed anthocyanins concentration in 2008. CT wines presented higher acylated (6′-acetyl) anthocyanins contents in 2008.

Table 5. Conventional Enological Analysis of Wines[a]

samples	ethanol, %	titratable acidity[b] (g/L)	pH	volatile acidity[c] (g/L)	lactic acid (g/L)	malic acid (g/L)	TPI[d]	tot. tannins	ethanol index	astringency index[e]
					Tempranillo, 2007					
ctrl	13.7 ± 0.3 b	4.4 ± 0.4 b	3.8 ± 0.1 a	0.2 ± 0.0 b	1.5 ± 0.1 a	0.1 ± 0.0 a	43.7 ± 1.2 b	1.5 ± 0.1 b	91.0 ± 0.0 a	2.1 ± 0.4 b
CT	14.2 ± 0.4 a	4.8 ± 0.4 b	3.8 ± 0.0 a	0.2 ± 0.0 a	1.4 ± 0.0 b	0.1 ± 0.1 a	49.1 ± 0.8 a	2.1 ± 0.1 a	87.4 ± 0.0 b	3.4 ± 0.6 a
ProCa	13.5 ± 0.4 b	5.7 ± 0.3 a	3.8 ± 0.0 a	0.2 ± 0.0 a	1.4 ± 0.0 ab	0.1 ± 0.0 a	45.9 ± 1.6 ab	1.6 ± 0.1 ab	86.6 ± 0.0 b	3.1 ± 0.3 a
					Tempranillo, 2008					
ctrl	14.1 ± 0.2 a	3.9 ± 0.3 b	3.8 ± 0.1 a	0.2 ± 0.0 a	1.6 ± 0.1 a	0.3 ± 0.0 a	42.9 ± 2.6 b	0.6 ± 0.1 b	88.3 ± 0.3 a	5.8 ± 0.4 a
ProCa	14.5 ± 0.2 a	4.5 ± 0.1 a	3.9 ± 0.1 a	0.1 ± 0.0 a	1.6 ± 0.1 a	0.2 ± 0.1 a	49.8 ± 2.0 a	1.6 ± 0.0 a	84.3 ± 0.0 b	6.1 ± 0.1 a
					Grenache, 2007					
ctrl	12.8 ± 0.9 b	5.4 ± 0.3 b	3.4 ± 0.1 a	0.2 ± 0.1 b	1.1 ± 0.0 a	0.1 ± 0.1 b	19.6 ± 0.6 b	0.5 ± 0.0 a	94.9 ± 0.1 a	1.2 ± 0.2 b
CT	14.5 ± 0.2 a	5.7 ± 0.2 b	3.5 ± 0.0 a	0.3 ± 0.0 a	0.7 ± 0.1 a	0.7 ± 0.0 a	24.7 ± 0.8 a	0.5 ± 0.2 a	91.0 ± 0.0 b	2.5 ± 0.2 a
ProCa	13.0 ± 0.9 b	6.8 ± 0.2 a	3.4 ± 0.1 a	0.2 ± 0.0 b	1.2 ± 0.2 a	0.1 ± 0.1 b	22.5 ± 0.6 ab	0.5 ± 0.1 a	90.8 ± 0.0 b	2.6 ± 0.4 a
					Grenache, 2008					
ctrl	14.0 ± 0.2 b	5.8 ± 0.1 b	3.5 ± 0.0 a	0.2 ± 0.0 a	0.6 ± 0.0 b	0.3 ± 0.0 b	26.9 ± 1.9 b	0.8 ± 0.1 b	94.0 ± 0.6 a	4.6 ± 0.7 a
CT	14.9 ± 0.3 a	6.0 ± 0.1 b	3.5 ± 0.0 a	0.1 ± 0.0 b	0.7 ± 0.1 a	0.2 ± 0.1 b	39.8 ± 2.0 a	1.3 ± 0.1 a	90.6 ± 0.2 b	5.1 ± 0.3 a
ProCa	14.1 ± 0.2 b	6.3 ± 0.2 a	3.4 ± 0.0 b	0.1 ± 0.0 b	0.6 ± 0.0 b	0.7 ± 0.0 a	38.7 ± 1.4 a	1.1 ± 0.1 a	90.8 ± 0.1 b	4.8 ± 0.4 a

[a]Mean ± standard deviation, $n = 3$. Different letters in same column and year mean significant differences at 5% confidence level. [b]Expressed as grams of tartaric acid per liter. [c]Expressed as grams of acetic acid per liter. [d]Total polyphenol index. [e]Expressed as grams of tannic acid per liter.

Table 6. Color Parameters and Anthocyanin Concentrations of Wines[a,b]

			anthocyanin concn (mg/L)					
samples	CI	hue	total	nonacylated	acylated (6′-acetyl)	pyranoanthocyanins	acylated (6′-*p*-coumaroyl)	total condensed (A−F)
					Tempranillo, 2007			
ctrl	10.4 ± 0.7 b	0.6 ± 0.0 a	92.8 ± 13.9 b	74.6 ± 12.3 b	7.3 ± 0.1 b	1.7 ± 0.0 b	8.4 ± 0.7 a	0.8 ± 0.1 a
CT	10.8 ± 1.0 ab	0.6 ± 0.0 a	122.0 ± 9.9 a	99.3 ± 9.1 ab	10.6 ± 1.1 a	2.8 ± 0.6 a	8.4 ± 1.7 a	0.8 ± 0.1 a
ProCa	11.5 ± 0.5 a	0.6 ± 0.0 a	145.5 ± 17.4 a	118.8 ± 18.7 a	11.2 ± 1.4 a	3.0 ± 0.5 a	11.3 ± 2.9 a	1.2 ± 0.6 a
					Tempranillo, 2008			
ctrl	12.6 ± 0.6 a	0.5 ± 0.0 a	68.1 ± 12.4 b	57.5 ± 11.2 b	3.6 ± 0.5 a	0.3 ± 0.1 a	6.3 ± 0.9 a	0.4 ± 0.1 a
ProCa	13.6 ± 0.5 a	0.5 ± 0.0 a	88.7 ± 8.4 a	76.8 ± 7.3 a	3.7 ± 0.5 a	0.4 ± 0.1 a	7.3 ± 1.1 a	0.5 ± 0.1 a
					Grenache, 2007			
ctrl	5.2 ± 0.3 b	0.6 ± 0.1 a	15.4 ± 0.0 b	12.1 ± 0.0 b	1.0 ± 0.0 a	1.0 ± 0.0 b	0.5 ± 0.0 b	nd[c]
CT	7.5 ± 0.6 a	0.6 ± 0.1 a	17.3 ± 0.0 a	12.6 ± 0.0 a	1.1 ± 0.0 a	1.5 ± 0.1 a	0.7 ± 0.1 a	nd
ProCa	7.3 ± 0.1 a	0.6 ± 0.1 a	17.4 ± 0.0 a	12.6 ± 0.0 a	1.1 ± 0.0 a	1.3 ± 0.1 a	0.7 ± 0.1 a	nd
					Grenache, 2008			
ctrl	5.9 ± 0.3 b	0.5 ± 0.0 a	27.5 ± 3.7 b	23.4 ± 3.1 b	1.3 ± 0.2 b	nd	2.6 ± 0.5 b	0.2 ± 0.1 c
CT	7.4 ± 0.8 a	0.5 ± 0.0 a	51.9 ± 4.5 a	44.5 ± 3.8 a	2.7 ± 0.3 a	nd	4.4 ± 0.3 a	0.3 ± 0.0 b
ProCa	8.0 ± 0.0 a	0.5 ± 0.0 a	48.4 ± 7.7 a	42.0 ± 4.1 a	1.6 ± 0.1 b	nd	4.3 ± 0.2 a	0.5 ± 0.0 a

[a]Mean ± standard deviation, $n = 3$. Different letters in same column and year mean significant differences at 5% confidence level. [b]Treatment × year interaction was not significant for all columns except for pyranoanthocyanins, 0.013. Treatment × variety interaction was not significant for all columns. [c]Not detected.

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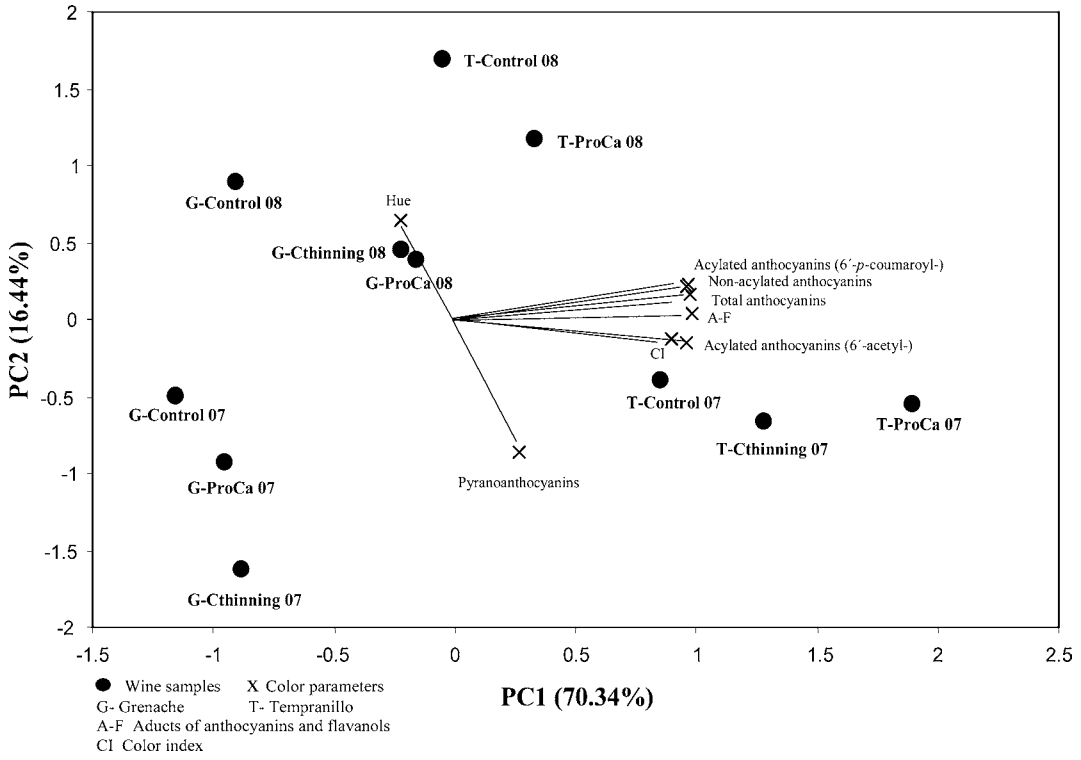

Figure 1. Projection of 11 wines and color-related parameters on the first two principal components of the PCA.

The results showed that, in general, there were no variety and year effects on treatments for anthocyanin composition (Table 6) and therefore the treatments had a similar effect in both varieties and years studied.

Figure 1 shows the projection of the two principal components obtained with samples and color-related variables. The first principal component explained 70.34% of the original variance and was characterized positively by CI and all types of anthocyanins except pyranoanthocyanins. Component 2 explained 16.44% of the original variance and was characterized by hue on the positive side and pyranoanthocyanins with negative loading. The PCA plane showed that Tempranillo wines are situated to the right side of PC1, which showed that these wines had higher CI and higher anthocyanin content than the Grenache wines.

As for Tempranillo cv., wines from vines treated with ProCa were always situated to the right side of the corresponding control samples, indicating wines with more color and greater anthocyanin concentration than control wines. Wines obtained from CT were located between ProCa and control, so that these wines presented intermediate characteristics. In relation to PC2, wines from vines that had undergone thinning treatments were wines with higher pyranoanthocyanin concentration and lower hue compared to control wines. Similarly, in the case of cv. Grenache, wines obtained from vines subjected to thinning treatments were situated in the PCA plane on the right-hand side and further down than the control wines, showing higher CI, higher concentration of all types of anthocyanins, pyranoanthocyanins, and lower hue. In 2007, Grenache wines obtained from vines subjected to ProCa treatment were located between control wines and wines from CT vines. However, in 2008 it was the opposite situation although the position of the wines was very close, suggesting that these wines were very similar.

Phenolic Composition. Table 7 shows the results for the analysis of polyphenolic compounds in wines. Tempranillo wines presented higher amounts of phenolic compounds than Grenache wines in both studied years, except for the concentration of (*Z*)-caftaric acid and quercetin 3-galactoside.

In both Tempranillo and Grenache varieties, the higher concentrations of phenolic acids were generally obtained in wines from CT vines. In general, wines from vines treated with ProCa presented lower concentrations of several acids [(*Z*)-caftaric, (*E*)-caftaric, and coumaric acids] than those from CT vines. With regard to flavanols, Tempranillo wines produced in 2007 had a higher concentration of flavanols than wines obtained in 2008. Treatments carried out to reduce yield produced a significant increase in the concentration of all compounds analyzed for Tempranillo wines in 2007. Treatment with ProCa caused a higher increase in several compounds such as catechins (catechin and catechin 3-gallate). In 2008, no significant differences were observed between control wines and those produced from vines treated with ProCa except for epigallocatechin and epicatechin 3-gallate, which were found at a higher concentration in wines from ProCa treated vines. Similarly, in Grenache cv., wines obtained from treated vines presented higher content of several flavanols than control wines for both years.

Concerning flavonol compounds for Tempranillo cv., wines with higher concentrations were generally obtained from vines which had been treated with ProCa. In 2008, Tempranillo wines did not differ in concentrations of studied compounds. Wines produced in 2008 generally presented higher flavonol concentrations than those obtained in 2007. For Grenache wines, most determined flavonols were below the limit of quantification (LOQ), except for quercetin 3-glucopyranoside and quercetin 3-glucuronide. Kaempferols were not detected in 2008 in either of the varieties. However, other compounds such as myricetin 3-glucoside and quercetin 3-galactoside showed higher concentrations in 2008. Thinning treatments produced wines with higher concentrations of quantified flavonols than control wines.

Table 7. Phenolic Compound Concentrations in Wines[a]

	phenolic compd concn (mg/L)												
	Tempranillo 2007			Tempranillo 2008		Grenache 2007			Grenache 2008				
	ctrl	CT	ProCa	ctrl	ProCa	ctrl	CT	ProCa	ctrl	CT	ProCa	× year[b]	× variety[c]
Acid													
gallic acid	47.9 ± 0.5 b	54.3 ± 0.2 a	49.8 ± 0.4 b	41.7 ± 3.8 a	42.1 ± 5.1 a	11.3 ± 0.3 b	18.20 ± 0.15 a	7.61 ± 0.13 c	31.0 ± 2.0 a	37.7 ± 7.4 a	37.8 ± 3.1 a	ns[d]	ns
Hydroxycinnamic Acids													
(*Z*)-caftaric acid	nd[e]	nd	nd	2.3 ± 0.2 a	2.0 ± 0.4 b	nd	nd	nd	4.5 ± 0.6 a	4.1 ± 1.0 a	1.8 ± 0.3 b	0.012	0.014
(*E*)-caftaric acid	27.7 ± 0.6 b	32.1 ± 1.0 a	17.4 ± 0.3 c	15.9 ± 1.5 a	15.8 ± 4.5 a	30.3 ± 0.6 b	32.0 ± 0.9 a	30.5 ± 0.9 b	52.5 ± 1.4 a	53.6 ± 3.5 a	50.9 ± 5.0 b	ns	0.029
caffeic acid	2.4 ± 0.3 a	2.1 ± 0.4 b	1.1 ± 0.2 c	1.11 ± 0.18 a	1.1 ± 0.1 a	0.5 ± 0.2 c	1.6 ± 0.4 a	1.1 ± 0.3 b	1.1 ± 0.1 a	1.0 ± 0.2 a	0.9 ± 0.0 a	ns	ns
coutaric acid	25.0 ± 1.3 b	28.0 ± 0.4 a	14.1 ± 0.2 c	15.11 ± 3.6 b	14.7 ± 3.0 a	12.4 ± 0.3 b	14.2 ± 0.7 a	13.7 ± 0.7 b	16.0 ± 1.7 a	15.5 ± 1.1 a	15.8 ± 1.2 a	ns	0.029
coumaric acid	3.2 ± 0.6 b	4.3 ± 0.3 a	2.5 ± 0.1 c	1.45 ± 0.10 a	0.7 ± 0.1 b	0.64 ± 0.26 c	2.15 ± 0.44 a	1.3 ± 0.7 b	2.1 ± 0.11 a	2.1 ± 0.10 a	0.9 ± 0.2 b	ns	ns
Flavanols													
catechin	33.0 ± 1.5 c	41.9 ± 0.4 b	44.6 ± 051 a	8.9 ± 1.2 a	8.3 ± 0.9 a	10.3 ± 0.0 b	19.0 ± 0.1 a	19.0 ± 0.0 a	3.1 ± 1.6 b	12.6 ± 1.8 a	10.3 ± 2.2 a	ns	0.005
epicatechin	27.9 ± 0.3 b	33.7 ± 1.3 a	31.8 ± 0.9 a	3.0 ± 0.4 a	2.7 ± 0.3 a	2.5 ± 0.0 b	4.0 ± 0.0 a	2.2 ± 0.0 b	5.2 ± 0.6 b	5.3 ± 0.2 b	6.4 ± 0.3 a	ns	ns
epigallocatechin	37.3 ± 0.9 b	40.3 ± 1.4 a	40.3 ± 0.5 a	8.3 ± 1.4 b	10.6 ± 0.3 a	7.8 ± 0.0 b	11.9 ± 0.0 a	8.6 ± 0.0 b	9.9 ± 1.8 a	12.6 ± 2.5 a	12.3 ± 0.0 a	ns	ns
catechin 3-gallate	22.3 ± 0.5 c	26.9 ± 1.6 b	28.5 ± 0.4 a	10.3 ± 1.8 a	12.6 ± 2.5 a	11.5 ± 0.1 b	17.1 ± 0.3 a	17.6 ± 0.3 a	2.2 ± 0.4 b	12.1 ± 1.9 a	13.3 ± 0.3 a	ns	ns
epicatechin 3-gallate	48.5 ± 0.6 b	55.8 ± 1.1 a	56.6 ± 1.1 a	11.4 ± 1.1 b	14.8 ± 1.9 a	26.7 ± 1.2 c	57.9 ± 1.7 a	35.2 ± 1.5 b	7.2 ± 1.8 b	10.2 ± 0.6 a	11.5 ± 1.6 a	ns	ns
epigallocatechin 3-gallate	26.4 ± 0.10 b	31.8 ± 2.05 a	30.0 ± 0.5 a	5.8 ± 1.1 a	6.4 ± 0.7 a	7.1 ± 0.2 b	10.5 ± 0.6 a	7.7 ± 0.1 b	2.5 ± 1.0 a	3.3 ± 0.3 a	2.6 ± 0.3 a	ns	ns
Flavonols													
myricetin 3-glucoside	4.2 ± 0.0 b	6.3 ± 0.3 a	6.0 ± 0.3 a	8.4 ± 1.8 a	9.5 ± 0.9 a	<LOQ[f] a	<LOQ a	<LOQ a	1.1 ± 0.2 b	3.1 ± 0.5 a	3.1 ± 0.2 a	ns	ns
quercetin 3-glucopyranoside	1.3 ± 0.0 b	1.4 ± 0.1 b	2.5 ± 0.1 a	1.4 ± 0.3 a	1.6 ± 0.4 a	0.1 ± 0.0 b	0.2 ± 0.0 a	0.1 ± 0.0 b	nd	2.7 ± 0.3 a	2.8 ± 0.2 b	ns	ns
quercetin 3-galactoside	0.2 ± 0.1 b	0.5 ± 0.0 a	0.5 ± 0.0 a	2.4 ± 0.4 a	2.6 ± 0.3 a	nd	nd	nd	1.9 ± 0.1 b	3.4 ± 0.6 a	3.3 ± 0.1 a	0.008	ns
quercetin 3-glucuronide	0.5 ± 0.1 b	0.6 ± 0.0 b	0.8 ± 0.1 a	3.5 ± 0.5 a	3.4 ± 0.9 a	<LOQ b	0.1 ± 0.0 a	0.1 ± 0.0 a	nd	nd	nd	ns	ns
kaempferol 3-glucopyranoside	0.5 ± 0.0 b	1.7 ± 0.0 a	1.8 ± 0.0 a	nd	nd	nd	nd	nd	nd	nd	nd	ns	0.011
myricetin	0.7 ± 0.1 b	0.9 ± 0.0 b	1.1 ± 0.0 a	1.5 ± 0.1 a	1.6 ± 0.1 a	<LOQ a	<LOQ a	<LOQ a	2.0 ± 0.2 b	2.9 ± 0.0 a	nd	0.000	0.000
quercetin	0.3 ± 0.0 b	0.5 ± 0.0 b	1.0 ± 0.0 a	nd	nd	nd	<LOQ a	<LOQ a	nd	nd	nd	ns	ns
kaempferol	1.6 ± 0.0 a	1.3 ± 0.0 b	1.1 ± 0.0 b	nd	nd	<LOQ a	<LOQ a	<LOQ a	nd	nd	nd	ns	ns

[a]Mean ± standard deviation, $n = 3$. Different letters in same row and year mean significant differences at 5% confidence level [b]Treatment × year interaction. [c]Treatment × variety interaction. [d]Not significant. [e]Not detected. [f]Below limit of quantification (0.01 mg/L).

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Table 8. Duo-Trio Test Results

	Tempranillo				Grenache			
	2007		2008		2007		2008	
test	corr/tot.[a]	*P*	corr/tot.[a]	*P*	corr/tot.[a]	*P*	corr/tot.[a]	*P*
ctrl vs CT	26/30	<0.001			27/30	<0.001	28/32	<0.001
ctrl vs ProCa	22/30	0.01	18/32	0.30	26/30	<0.001	37/32	<0.001
CT vs ProCa	20/30	0.1			20/30	0.1	21/32	0.1

[a]Correct/total answers.

In the case of phenolic compounds in general, no significant treatment × year and treatment × variety interactions were found, except in the case of hydroxycinnamic acids, which showed a significant treatment × variety interaction (Table 7).

Sensory Analysis. The duo-trio test (Table 8) showed that control wines differed from wines obtained from CT practice and ProCa application at a significance level of 5%. The duo-trio test performed between wines obtained from CT vines and ProCa-treated vines proved that there were not any significant differences ($P > 0.05$). These results were the same for both years and varieties except for Tempranillo in 2008, when wines showed no difference.

Two-way ANOVA calculated for each attribute in order to assess panel consistency confirmed that the judge effect was significant for all attributes. This effect is common in sensory analysis and is explained by the physiological differences between individuals. As there was no wine−replicate interaction (W−R), a consistent evaluation of the attributes and panel reproducibility was obtained. Moreover, according to one-way ANOVA with repeated measurements (judges considered as replicate), the wine effect was significant at a significance level of 5% for all attributes ($P < 0.05$) except for sweetness ($F = 6.324$; $P = 0.092$) and balsamic attribute ($F = 3.248$, $P = 0.142$). This fact indicated that sweetness and balsamic attributes did not supply differences between wine samples, and for this reason, these terms were not considered in the study.

The mean scores for the 14 evaluated attributes are shown in Table 9, and one-way ANOVA for each attribute showed that Tempranillo wines obtained from thinning treatments (CT and ProCa) presented higher values ($P < 0.05$) in 2007 compared to the control wine for aromatic attributes white and yellow fruits, fresh flowers, lactic, and spicy and lower values for herbaceous attribute. Moreover, wines from CT presented significant differences with respect to wines from ProCa application in white and yellow fruits and lactic attributes. No significant differences were found in 2008 between ProCa treatment and control wines. As for mouth attributes, Tempranillo wines from vines subjected to thinning treatments in 2007 received higher scores in bitterness, astringency, and persistence compared to the control. In 2008, these differences disappeared and retronasal intensity and persistence were the only differences, and wines from vines with ProCa application showed the most retronasal intensity and persistence.

Grenache wines produced from vines with thinning treatments in 2007 presented significant differences compared to control wines in terms of red and black fruits, white and yellow fruits, and alcohol. In 2008, control wines and those from ProCa-treated vines showed higher values for fresh flower and white and yellow fruit descriptors than wines made from CT practice. However, these wines had higher values of red and black fruits than the other wines. Regarding taste and mouthfeel attributes in 2007, wines from CT were more persistent and presented higher volume by mouth and sourness than control wines and those from vines treated with ProCa. However, in 2008 wines did not differ in any of the attributes evaluated by mouth.

Figure 2 shows the two-dimensional plane with the projection of all evaluated sensory descriptors and wine samples. The first two principal components (PC) accounted for over 70.16% of the original variance. PC1 explained 47.62% of the original variance and it was mainly characterized by persistence, volume by mouth, astringency, and retronasal intensity on the positive side and herbaceous attribute with negative loading. The PC2 explained 22.54% of the original variance and presented sourness and white and yellow fruits having positive loading and alcoholic attribute placing on the negative side. Correlation matrix showed that volume by mouth was positively correlated with astringency (93%), persistence (92%), retronasal intensity (89%), and bitterness (81%), and was also positively correlated with the aromatic attributes red and black fruits (79%), lactic (75%), and spicy (67%). Persistence was positively correlated with astringency (83%), retronasal intensity (74%), bitterness (67%), and aromatic intensity, as well as red and black fruits (79%) and lactic attributes (72%).

It is noticeable that aromatic intensity was correlated with aromatic attributes such as fresh flowers (78%), white and yellow fruits (72%), and red and black fruits (65%) and mouth attributes such as persistence (60%) and sourness (60%). Moreover, retronasal intensity, apart from correlations with aromatic terms such as, for example, spicy (83%) and lactic (72%), was also correlated with taste and mouthfeel attributes such as astringency (93%), bitterness (82%), and volume by mouth (89%).

Similarly in both years and varieties, wines from CT and ProCa vines were located to the right of control wine, with higher scores of PC1. This suggest that wines from both thinning treatments present better mouth and aromatic characteristics and lower herbaceous notes than their respective control wines. Concerning of grape varieties, a different effect of studied treatments could be observed, so in the case of cv. Tempranillo, wines from vines treated with ProCa had higher scores of PC1 than wines from CT vines, and vice versa in the case of cv. Grenache.

Finally, it is noteworthy that the decrease in the percentage of tannins bound to polysaccharides (ethanol index) in wines from thinned vines (CT and ProCa) of both varieties and both years is correlated ($F = 30.14$ $P = 0.005$; $F = 28.47$ $P = 0.013$) with the increase in astringency.

DISCUSSION

The low production in Tempranillo in 2008 meant that CT practice could not be performed and therefore the production of wines only from control vines and chemically thinned (by ProCa treatment) vines was possible for this variety in this year. The absence of significant differences in the chemical composition and in the organoleptic characteristics of wines could be due to a poor fruit set, with control vines showing

Table 9. Mean Scores for Aroma and Taste Attributes Evaluated by Sensory Panel[a]

	Aroma Attributes							
	white and yellow fruits	red and black fruits	fresh flowers	spicy	alcoholic	herbaceous	lactic	aromatic intensity
				Tempranillo, 2007				
ctrl	1.79 ± 0.06 c	2.52 ± 0.06 a	1.00 ± 0.05 b	1.30 ± 0.05 b	1.89 ± 0.06 a	1.87 ± 0.06 a	1.12 ± 0.06 c	2.30 ± 0.12 b
CT	2.06 ± 0.04 b	2.48 ± 0.07 a	1.48 ± 0.05 a	1.74 ± 0.05 a	2.04 ± 0.05 a	1.14 ± 0.05 b	1.42 ± 0.05 b	2.86 ± 0.05 a
Pro-Ca	2.46 ± 0.05 a	2.67 ± 0.05 a	1.59 ± 00.6 a	1.68 ± 0.06 a	1.89 ± 0.06 a	1.13 ± 0.04 b	1.69 ± 0.06 a	2.81 ± 0.06 a
treatment	0.003	ns[b]	0.010	0.030	ns	0.030	0.010	0.050
				Tempranillo, 2008				
ctrl	2.03 ± 0.08 a	2.43 ± 0.06 a	0.97 ± 0.05 a	1.71 ± 0.05 a	1.31 ± 0.05 a	1.26 ± 0.06 a	1.49 ± 0.06 a	2.63 ± 0.06 a
Pro-Ca	1.91 ± 0.07 a	2.49 ± 0.05 a	1.06 ± 0.04 a	1.74 ± 0.05 a	1.48 ± 0.05 a	1.27 ± 0.05 a	1.61 ± 0.07 a	2.71 ± 0.07 a
treatment	ns	ns	ns	ns	ns	ns	ns	ns
				Grenache, 2007				
ctrl	1.85 ± 0.05 b	1.85 ± 0.05 b	1.31 ± 0.05 a	0.64 ± 0.04 a	1.92 ± 0.07 a	1.00 ± 0.06 a	0.88 ± 0.04 a	2.20 ± 0.04 a
CT	2.19 ± 0.06 a	2.19 ± 0.04 a	1.23 ± 0.05 a	0.96 ± 0.05 a	1.27 ± 0.05 b	1.08 ± 0.05 a	0.92 ± 0.05 a	2.37 ± 0.05 a
Pro-Ca	2.31 ± 0.07 a	2.31 ± 0.07 a	0.96 ± 0.05 a	0.52 ± 0.04 a	1.28 ± 0.06 b	1.46 ± 0.07 a	0.92 ± 0.06 a	2.17 ± 0.06 a
treatment	0.020	0.001	ns	ns	0.004	ns	ns	ns
				Grenache, 2008				
ctrl	3.26 ± 0.08 a	2.29 ± 0.10 b	1.86 ± 0.07 a	1.57 ± 0.06 a	1.43 ± 0.08 a	0.83 ± 0.12 a	1.66 ± 0.06 a	3.06 ± 0.12 a
CT	2.37 ± 0.09 b	2.95 ± 0.11 a	1.03 ± 0.05 b	1.20 ± 0.06 a	1.51 ± 0.09 a	1.13 ± 0.10 a	1.63 ± 0.06 a	2.54 ± 0.10 a
Pro-Ca	2.94 ± 0.08 a	2.20 ± 0.10 b	1.80 ± 0.07 a	1.26 ± 0.07 a	1.40 ± 0.07 a	1.06 ± 0.09 a	1.46 ± 0.06 a	3.00 ± 0.12 a
treatment	0.030	0.002	0.005	ns	ns	ns	ns	ns

	Taste Attributes					
	sourness	bitterness	astringency	volume by mouth	retronasal intensity	persistence
			Tempranillo, 2007			
ctrl	1.70 ± 0.05 a	1.93 ± 0.05 b	2.00 ± 0.07 b	2.37 ± 0.04 a	2.12 ± 0.06 a	2.52 ± 0.06 b
CT	1.74 ± 0.05 a	2.44 ± 0.06 a	2.46 ± 0.05 a	2.67 ± 0.04 a	2.23 ± 0.06 a	2.85 ± 0.06 a
Pro-Ca	1.59 ± 0.06 a	2.32 ± 0.06 a	2.64 ± 0.08 a	2.70 ± 0.05 a	2.35 ± 0.08 a	2.85 ± 0.08 a
treatment	ns	0.001	0.002	ns	ns	0.020
			Tempranillo, 2008			
ctrl	1.54 ± 0.06 a	2.46 ± 0.06 a	2.29 ± 0.05 a	2.26 ± 0.06 a	1.77 ± 0.07 b	2.49 ± 0.07 b
Pro-Ca	1.64 ± 0.06 a	2.57 ± 0.05 a	2.40 ± 0.05 a	2.37 ± 0.05 a	2.49 ± 0.05 a	3.00 ± 0.06 a
treatment	ns	ns	ns	ns	0.002	0.005
			Grenache, 2007			
ctrl	1.73 ± 0.05 b	1.44 ± 0.06 a	1.36 ± 0.05 a	1.15 ± 0.04 b	1.04 ± 0.04 a	1.52 ± 0.06 b
CT	2.14 ± 0.04 a	1.64 ± 0.06 a	1.48 ± 0.05 a	1.78 ± 0.04 a	1.31 ± 0.04 a	2.48 ± 0.05 a
Pro-Ca	2.19 ± 0.05 a	1.52 ± 0.06 a	1.56 ± 0.06 a	1.37 ± 0.05 b	1.27 ± 0.05 a	1.62 ± 0.05 b
treatment	0.040	ns	ns	0.010	ns	0.005
			Grenache, 2008			
ctrl	2.51 ± 0.10 a	2.20 ± 0.08 a	2.03 ± 0.08 a	2.29 ± 0.11 a	2.06 ± 0.09 a	2.80 ± 0.08 a
CT	2.26 ± 0.09 a	2.11 ± 0.08 a	1.82 ± 0.07 a	2.17 ± 0.10 a	1.79 ± 0.08 a	2.65 ± 0.07 a
Pro-Ca	2.46 ± 0.10 a	1.97 ± 0.08 a	2.03 ± 0.08 a	2.03 ± 0.10 a	1.90 ± 0.08 a	2.60 ± 0.07 a
treatment	ns	ns	ns	ns	ns	ns

[a]Analysis of variance *P*-values for treatment (n = 30 judges × 1 wine/treatment × 2 reps/wine). Mean values within columns were separated by Fisher's test (P = 0.05), where a, b, and c indicate statistical outcomes. [b]Not significant.

similar behavior to thinned vines. This fact was possibly due to climatic conditions during flower induction, which came about in early June of the previous year. This process was promoted by high temperatures and low rainfalls in 2006 (data not shown), while in 2007 climatic conditions were not favorable (more wet and rainy days).

There were differences in grape ripening between years. In 2008, grapes from both varieties presented higher °Brix and lower titratable acidity and malic acid amount ($P < 0.05$). This could be due to differences in weather conditions during the ripening period. High temperatures and low precipitation in August 2008 led to accelarated sugar ripening and acid degradation. In 2007, high precipitation in the late ripening stage (September and October) produced lower sugar content. However, no relationship between weather conditions and phenolic content in grapes was found.

The effectiveness in yield reduction by the application of ProCa in both Grenache and Tempranillo varieties in this study confirms previous studies carried out by Lo Giudice et al.[23,24] and Vaquero-Fernández et al.[25] It is noteworthy that the differences in the alcoholic content of wines is related to the differences observed in the content of °Brix measured in grapes. On the contrary, this relationship does not exist in the case of titratable acidity or pH parameters. Wines from ProCa treatment presented a higher significant titratable acidity; however, neither the value of grapes' titratable acidity (Table 4) nor the content of other acids such as malic and lactic (Table 5) in the wines can explain the differences found. Nevertheless, the higher values of titratable acidity of these wines can be of particular interest in red wines due to the improvement in sensory characteristics perceived in mouth and in the stability of wine during aging.

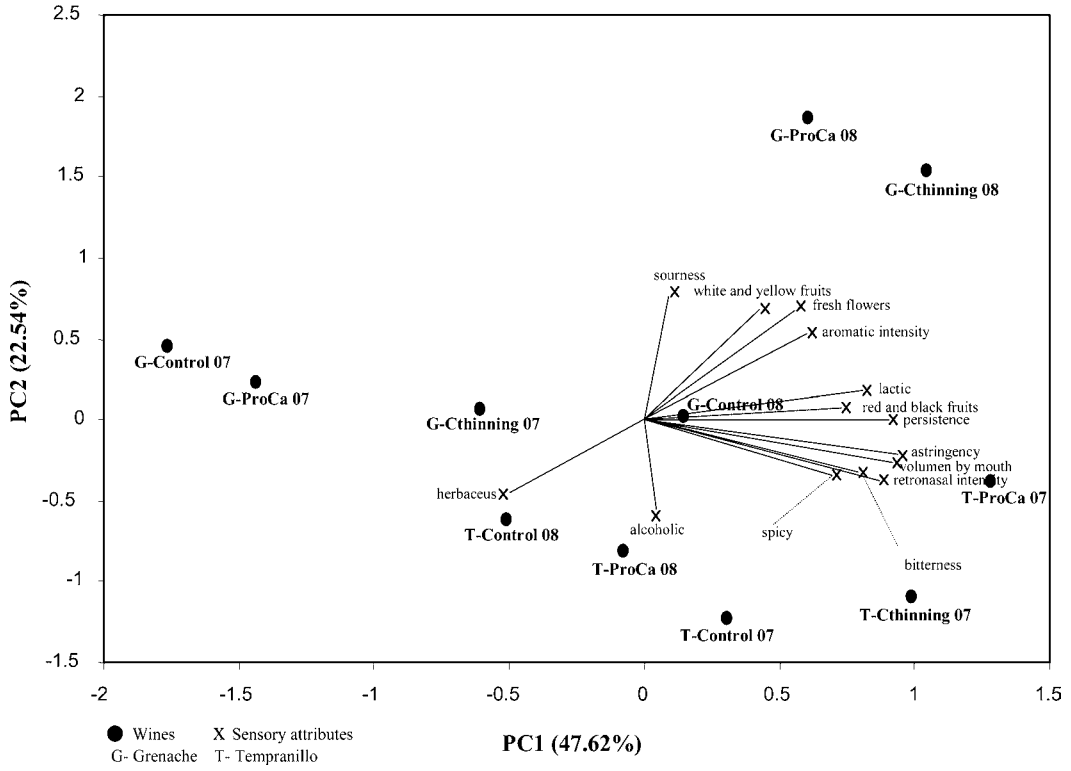

Figure 2. Projection of 11 wines and sensory attributes on the first two principal component of the PCA.

In this study, there was an important increase in CI and TPI in grapes and wines obtained from CT and ProCa treatments with respect to those obtained from control vines. In the ProCa treatment, one of the reasons for this increase is the reduced yield and berry size of vines treated with this growth regulator. These results confirm a previous study performed by our research group,[25] where the vines treated with ProCa presented a lower yield with smaller berry size and the corresponding wines showed a higher TPI. Concerning CT treatment, Gil-Muñoz et al.[32] found that cluster thinning at veraison improved the grape quality, and in particular, Syrah and Tempranillo wines produced with cluster thinning practices had significantly better chromatic characteristics than control wines. Similarly, García-Escudero et al.[10] and Puertas et al.[33] observed that CT produced an increase in TPI and CI. The increase in CI in both years is consistent with an increase in the concentration of total anthocyanins in both grapes (Table 4) and wines (Table 6). The individual analysis of anthocyanins in wines for both varieties reveals that nonacylated anthocyanin contents in wines obtained from treated grapes were significantly higher than concentrations found in control wines. Besides, in Grenache wines an increase in acylated and total condensed anthocyanins was observed in wines from thinning treatments. Some authors note that the actions which improve total leaf area/production ratio through CT presented a higher content in anthocyanins.[6,34] In this present study, PCA results (Figure 1) confirm the differences in color and in the concentration of anthocyanins between control wines and wines from thinning treatments. These ones produced a similar effect on Grenache variety in terms of anthocyanin composition and color, whereas in Tempranillo, the studied treatments produced more differentiated wines.

Apart from a decrease in yield and berry size, the increase in CI in wines produced with thinning treatments in both Grenache and Tempranillo varieties and in the two studied years can be explained by the increase in copigments such as flavanols and in pyranoanthocyanins (Tables 6 and 7). The higher content in copigments found in wines from both thinning treatments could contribute to an increase in the color of these wines by the copigmentation effect.[35] This finding is consistent with that of Peña-Neira et al.,[36] who studied the phenolic composition of Syrah grapes in vines that had been thinned manually at veraison. They found an increase in some phenolic derivatives related to wine color stability through copigmentation and polymerization reactions. Also noteworthy is the increase in the amount of pyranoanthocyanins (Table 6) in wines produced from CT and ProCa treatments. These compounds play an important role in the color stabilization of red wines, because the formation of these compounds reduces the increase of yellow color of wines, as stated in a recent publication by Sáenz-Navajas et al.[37]

Manual cluster thinning reportedly produces an increase in total anthocyanins and total phenolic compounds.[38,39] Fanzone et al.[40] observed that Malbec grapes from thinned vines presented a higher concentration of most phenolic compounds, indicating a greater potential for more complex wines. They found that CT encouraged the biosynthesis of individual anthocyanins in skins (nonacylated, acylated, and total anthocyanins), affecting the content of flavanols (catechin, epicatechin 3-gallate) and flavonols (quercetins) in skins and seeds. In this study (Table 5), an increase in the total phenolic content of wines obtained from thinned vines was observed. This fact is consistent with the results of Valdés et al.,[41] who observed noticeably higher contents of phenolic compounds in Tempranillo wines made from grapes that had been thinned. However, a different content was observed in several phenolic compounds obtained from ProCa vines (Table 7). These wines presented higher concentrations of several flavanols and

flavonols (flavonoid compounds) and a lower accumulation of several hydroxycinnamic acids (nonflavonoid compounds) than wines from CT practice in both studied varieties. Puhl et al.[42] reported alterations in flavonoid composition and a reduction of hydroxycinnamic acids content in grapevines by the application of grape growth regulator ProCa. The reduction of hydroxycinnamic acids is remarkable, since a reduction in these compounds may reduce the risk of oxidation of the wines[43] and may have an influence on the synthesis of ethylphenols (olfactory default) by *Brettanomyces* yeasts.[44]

As for season and grape variety effects on phenolic composition, evaluated interactions showed that the treatments affected phenolic composition similarly regardless of year and variety, except for hydroxycinnamic acids.

Besides the fact that a decrease in crop yield and berry size results in an increase in anthocyanins and flavonoids,[45] there is a second hypothesis. It has been shown that ProCa directly affects the biosynthetic pathway of anthocyanins and other flavonoids. This fact is possibly due to the role of 2-oxoglutarate-dependent dioxygenases.[46−49] As ProCa is able to alter flavonoid metabolism, therefore, novel flavonoids are formed that were previously identified as 3-deoxycatechins in young apple leaves,[49,50] pear,[51] and grapevine leaves and berries.[20,42]

In several works related to cluster thinning, an improvement in the organoleptic characteristics and sensory attributes of wines from treated vines has been observed.[22,25,52−54] In this study, duo-trio tests proved that control wines and wines produced from thinned vines were different in both years and varieties. In the descriptive sensory analysis performed with the aroma profile, wines obtained from thinned vines (CT and ProCa) presented in general higher values for white and yellow fruits and fresh flowers in both varieties. There were also some differences between wines from both thinning treatments and control wines for spicy and red and black fruit attributes in Tempranillo and Grenache varieties, respectively, as is shown in Table 9. This is consistent with the results of Di Profio et al.,[55] who showed that viticultural practices such as cluster thinning enhanced intensities of several aroma and retronasal descriptors (e.g., black fruit, black pepper). Similarly, Naor et al.[56] and Roberts et al.[57] observed an increase of fruit and floral flavors in wines from Sauvignon blanc and Chardonnay musqué vines that had undergone CT practice. It is noteworthy that Tempranillo control wines in 2007 showed a vegetal/herbaceous character. Diago et al.[11] observed that a lower intensity rating had been given for the herbaceous attribute for Tempranillo wines proceeding from thinning treatments. This decrease in herbaceous character can lead to increased product quality, since these aromas resulted in lower perceived quality, as noted by Sáenz-Navajas et al.[58]

Concerning taste and mouthfeel perceptions, results were very noticeable (Table 9). Wines from thinning treatments were generally evaluated with higher values of persistence, astringency, bitterness, and retronasal intensity than control wines for Tempranillo cv.; in general, Grenache wines from treated vines showed higher values of sourness, volume by mouth, and persistence. Similarly, Diago et al.[11] have observed a higher astringency in wines obtained from vines with cluster removal. Recently, Sáenz-Navajas et al.[58] have reported that the in-mouth sensory perception of red wine is primarily driven by the perception of astringency and by the chemicals compounds causing it. The higher concentration of phenolic compounds found in wines obtained from both thinning treatments is therefore consistent with the higher score in mouth attributes evaluated in these wines. This fact could result in an increase in wine quality since Sáenz-Navajas et al.[59] noted that both astringency and persistence are very important attributes in perceived quality of wines.

With regard to the thinning treatments studied, different influences on varieties can be observed. Thus, Tempranillo wines with higher values for mouth attributes were those from ProCa treatment, while Grenache wines from CT obtained higher scores for mouth attributes.

The preblooming application of ProCa and CT modified the sensory attributes of wines obtained. ProCa treatment can be considered as an alternative to CT. The application of ProCa can be used as a new tool for controlling production and therefore improving wine quality for both studied varieties. Further research is necessary to confirm that this growth regulator performs similarly to crop thinning under climatic conditions not assessed in this two-season study and to assess what the implication is for this growth regulator in the biosynthesis of phenolic compounds.

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Notes

The authors declare no competing financial interest.

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