






## Article

# Influence of Bunch Compactness and Berry Thinning Methods on Wine Grape Quality and Sensory Attributes of Wine in *Vitis vinifera* L. cv. ‘Monastrell’

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**Abstract:** Presently, there is no information available about the effect of bunch compactness and berry thinning methods on wine grape quality and sensory attributes of wine in the ‘Monastrell’ cultivar. Thus, the main aim of the present study was to determine the influence of bunch compactness and two berry thinning methods, which consisted of the reduction of 25% and 50% of the number of berries in each bunch, on wine grape quality and organoleptic quality of wine in this cultivar. Non-compact bunches and both berry thinning methods showed a significant reduction in total yield, bunch compactness, and bunch fresh mass compared with compact and control ones, respectively. However, these methods, especially the 50% one, significantly increased the content of total soluble solids and total phenolics. Furthermore, both berry thinning methods promoted the increase in total anthocyanins concentration in berries, as well as the hydrophilic total antioxidant activity. Berry thinning methods led to wines with greater sensory descriptors, such as fruity (odor and flavor), sour, sweet, aftertaste, and color, and were preferred by consumers. Finally, 50% berry thinning is the most useful tool to decrease bunch compactness and improve the overall quality of berries and sensory attributes of wines.

**Keywords:** antioxidants; berry architecture; total acidity; total soluble solids; wine quality



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## 1. Introduction

*Vitis vinifera* L. cv. ‘Monastrell’ is the fourth most cultivated red wine grape cultivar in Spain, where Murcia and Alicante are its main production areas [1]. This cultivar is the most representative of Protected Designation of Origin (PDO) Jumilla, growing on over 80% of the cultivated area [2]. In addition, ‘Monastrell’ grapevine cultivar is commonly used in other PDOs in Eastern Spain (Valencia, Alicante, Bullas, Almansa, Yecla, Binissalem, and Pla i Llevant) and in Southern France (Provence), where it is known as ‘Mourvedre’, among other Spanish PDOs [3,4]. Currently, this cultivar is grown mainly in semiarid areas of the southeast of Spain, covering 4.4% of the total vineyard area in the country [5] since it is well adapted to the soil and weather conditions of the area characterized by dry climates of high temperatures and drought cycles [6]. From the point of view of viticulture, this cultivar has an important oenological potential due to new agronomic practices and winemaking methods, leading to high-quality wines with an increase in their exports to other countries and their prices reached at market [6].

The wine is produced with wine grapes whose quality directly affects the composition and quality of the final wine. The main sensory attributes of wine, such as color and taste perceptions such as bitterness, astringency, and mouthfeel are due mainly to the presence

of certain chemical compounds present in wine grapes [7]. Specifically, anthocyanins and other derived pigments are the main chemical compounds responsible for the color of red wine [8]. The synthesis of these anthocyanins starts at the veraison stage and they accumulate in the berry skin tissues during the wine grape ripening [9]. The attributes most valued by consumers in red wines are intense color, with balanced bitterness and astringency, and full-bodied wines. To make wines of high quality, some cultural practices have been established over the years to improve wine grape ripening as one of the most important factors.

The berries of some wine grape cultivars are completely compact and misshapen. These compact bunches are highly susceptible to *Botrytis* incidence due to their high number of internal berries. This problem causes economic losses to farmers due to harvest losses and obtaining berries with a lower content of phenolic compounds (due to the heterogeneity of the bunches), which translates into lower wine production and poorer quality of the wine obtained [10–14]. Several studies have reported that vigorous canopy growth with consequent low bunch light exposure, as well as very high vine yields, can also reduce both grape and wine quality [7,15,16]. Moreover, sun exposure has also a great influence on the flavonol content of berries [17,18]. The greater the sun exposure, the higher the flavonol content [7]. For these reasons, it is necessary to search for methods that reduce the compactness of the bunches to minimize losses and achieve, at the same time, improvements in the quality of the berries obtained.

In this sense, grapevine yield can be controlled by agronomic methods such as bunch thinning by reducing the number of wine grape bunches despite the additional cost of this common practice. Several authors have studied the influence of bunch thinning on wine grape ripening as well as on wine composition and quality [19–25]. Previous studies showed that berry thinning methods improve the concentration of terpenes by ~20% in wine obtained by ‘Chardonnay’, ‘Chardonnay Musqué’, ‘Sauvignon blanc’, and ‘Ribolla Gialla’ cultivars [26–30]. These compounds (in the case of wine, namely: linalool, geraniol, citronellol, nerol, terpineol, among others) are directly related with the fruity sensory descriptor. In addition to improving the phenolic content of wine grapes, bunch thinning tends to reduce total acidity and increase total soluble solids content and pH [24]. Nevertheless, Diago et al. [25] reported that this agronomic method shows a positive effect on wine quality related to an increase in berry size, which decreases the skin-to-flesh ratio. On the other hand, contradictory results have been reported about the effect of bunch thinning on sensory attributes in ‘Grenache’ and ‘Tempranillo’ wines [25].

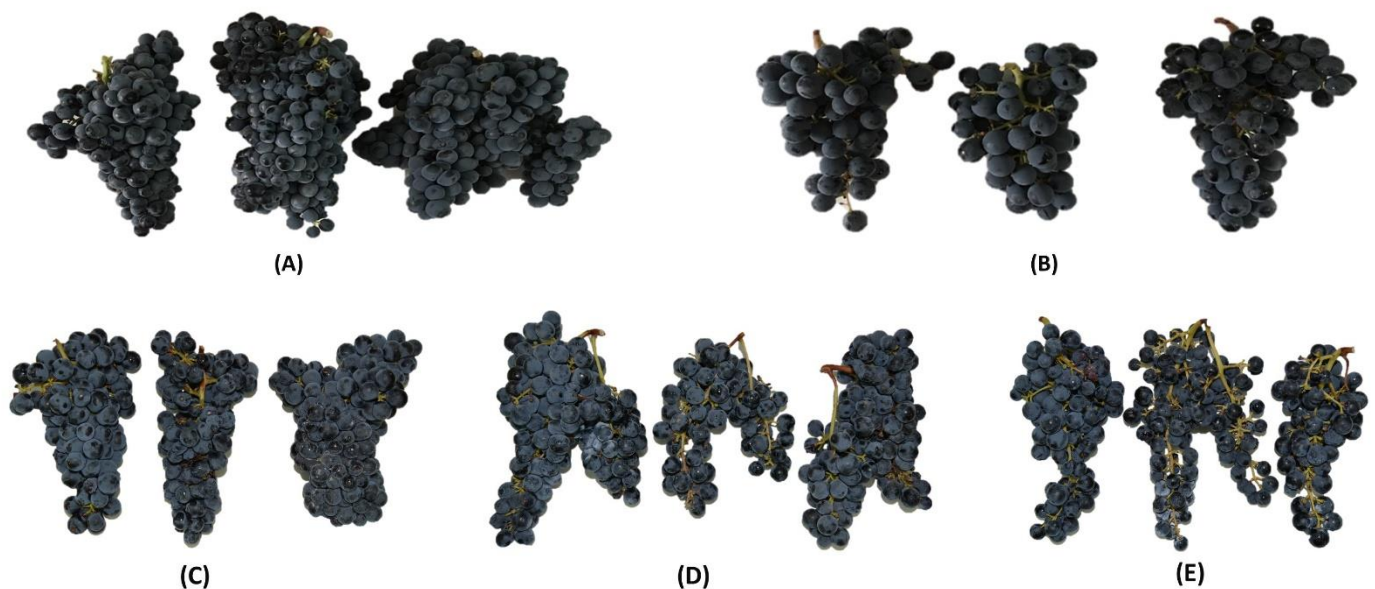
Berry thinning is an alternative method to the traditional one of bunch thinning in order to improve wine grape ripening [7]. This agronomic practice consists of the removal of the tips of all the bunches once the flowering has started to obtain blunted bunches of grapes. This can be linked with some previous studies where a higher wine grape quality was observed and an earlier ripening can be reached in berries from the shoulders and top of the bunch rather than from the tips [31,32]. Liu et al. [33] reported that the use of berry thinning for the reduction in bunch compactness resulted in a greater increase in sugar and anthocyanin content than bunch thinning of the ‘Cabernet Sauvignon’ cultivar, according to a previous study [34]. Recently, Han et al. [35] elucidated that berry thinning significantly increases the accumulation of berry sugars in this cultivar through the upregulation of cell wall invertase (CWI) activity—a key regulator of the carbon partitioning during berry ripening—or the cooperation with soluble acid invertase (SAI) to control sink strength of the remaining berries in response to berry removing. These mechanisms suggested that reducing bunch compactness might change the chemical composition of the wine obtained. Although some scientific articles discuss the influence of bunch compactness and berry thinning on grape ripening, to the best of our knowledge, they all focus on table grapes [36,37] or ‘Syrah’ and ‘Cabernet Sauvignon’ wine grape cultivars [7,35]. None of them have studied the influence of these practices on the wine grape quality in the ‘Monastrell’ cultivar. Furthermore, as far as we know, the effect of berry thinning on the sensory attributes of ‘Monastrell’ red wine has not been previously studied. Thus, the aim

of this study was to determine the influence of bunch compactness and especially of two berry thinning methods, which consisted of a reduction of 25% and 50% of the number of berries in each bunch, on wine grape quality and organoleptic quality of wine of the ‘Monastrell’ cultivar.

## 2. Materials and Methods

### 2.1. Plant Material and Experimental Design

Two experiments were carried out during two growing seasons (2018 and 2019) in a 42-year-old vineyard of the *Vitis vinifera* cv. ‘Monastrell’ in Cehegín, Murcia, Spain (38°00′08.5″ N, 1°45′10.4″ W). The vineyard was located at an altitude of 759 m above sea level and was managed according to standard viticulture practices for the cultivar and region. Grapevines were grafted onto 110-Richter rootstock spaced at 2.30 × 2.30 m. Canopy management practices were all performed manually. Winter pruning was carried out leaving around 10 buds per vine. Meteorological data were obtained from a weather station located close to the experimental site (38°6′39.35″ N, 1°40′59.06″ W), (CR32, Cehegín, Murcia, Spain). The weather conditions of the vintage for 2018 and 2019 seasons were as follows: annual rainfall, 392.60 mm and 426.60 mm, respectively; average temperature, 14.98 °C and 15.08 °C, respectively. Two and three representative and consecutive rows were selected for the 2018 and 2019 vintages, respectively. The experimental design used for the first season was randomized blocks with three biological replicates of five grapevines for each replication and two methods studied: compact bunch (Figure 1A) and non-compact bunch (Figure 1B). In the second season, three methods randomized with three biological replicates of five grapevines per replicate were studied. The first one was the control (Figure 1C; the same as the compact bunch of the previous season) and the second and third methods consisted of a removal of 25% and 50% of berries in each bunch, respectively (Figure 1D,E, respectively). Berry thinning was carried out when grapes were pea size, corresponding to stage E–L 31 on the modified E–L system [38], and they were removed manually producing different bunch compactness.



**Figure 1.** Visual appearance of bunches for each experiment: (A) compact bunch, (B) non-compact bunch, (C) control, (D) 25% Berry thinning method, and (E) 50% berry thinning method.

Grapes were harvested manually according to their ripening stage (*ca.* 24 °Brix and 14–14.5% of potential ethanol content). Grapes from each grapevine were weighed to determine the total yield for each method studied. The grapes were immediately transported to the laboratory and the experimental winery of the Food Technology Department at Miguel Hernández University (Alicante, Spain). The grapes of the three replicates from

each method were mixed after being weighed and bunched based on the physicochemical and functional characterizations in order to obtain more homogeneous samples for winemaking.

### 2.2. Grapevine Yield, Bunch Characterization, and Physicochemical Determinations of Wine Grape

Grapevine production for each method was expressed as total yield ( $\text{kg vine}^{-1}$ ) and data were the mean  $\pm$  SE of three biological replicates of five grapevines (15 grapevines in total). In relation to the bunch compactness assessment, fifteen bunches from the three replicates (five bunches per replicate) were randomly selected for each method studied and weighed in order to determine bunch fresh mass (g) by using an analytical balance 'Radwag WLC 2/A2' (Radwag Wagi Elektroniczne, Radom, Poland) with a precision of two decimal places. After that, bunch length (cm), as the distance from the uppermost to the lowest berry of the bunch, was determined by measuring with a ruler. Bunch compactness index was calculated according to Tello and Ibáñez [39], using bunch weight (g)/[bunch length (cm)]<sup>2</sup> and was expressed as  $\text{g cm}^{-2}$ . All data of parameters related to bunch architecture were the mean  $\pm$  SE of fifteen bunches ( $n = 3$ ) for all methods studied. Then, 25 manually destemmed wine berries from each replicate (5 wine berries from each bunch) were ground to obtain a homogeneous juice sample in which total soluble solids (TSS) and total acidity (TA) content were determined according to the protocol described previously by García-Pastor et al. [40]. TSS were determined in duplicate using an Atago PR-101 digital refractometer (Atago Co. Ltd., Tokyo, Japan) at 20 °C, and expressed as  $\text{g } 100 \text{ g}^{-1}$ . TA was also measured in duplicate in the same juice by automatic titration (785 DMP Titrino, Metrohm) with 0.1 N NaOH up to pH 8.10, and results were expressed as g tartaric acid equivalent  $100 \text{ g}^{-1}$ , which is the major organic acid in wine grape berries. Finally, the ripening index (RI) was calculated as the ratio of TSS/TA. Data of TSS, TA, and RI were also the mean  $\pm$  SE ( $n = 3$ ) for each method evaluated. All of these parameters were analyzed in both the 2018 and 2019 seasons.

### 2.3. Determination of Functional Parameters in Wine Grape

The juice of the other 25 wine berries from each replicate ( $n = 3$ ) was also obtained and used to measure the functional parameters of wine grape, namely, total phenolic compounds, total anthocyanins, and total antioxidant activity of hydrophilic fraction. Total phenolic content was quantified in the two experiments (2018 and 2019 seasons), although total anthocyanins content and total antioxidant activity were determined only in the second trial during the 2019 season. Total phenolic compounds were extracted by homogenizing 5 mL of juice sample with 10 mL of water: methanol (2:8, *v/v*) containing 2 mM NaF, in order to inactivate polyphenol oxidase activity and prevent phenolic degradation, for 30 s by using a homogenizer (Ultraturrax, T18 basic, IKA, Berlin, Germany). The homogenate was centrifuged at  $10,000 \times g$  for 10 min at 4 °C and total phenolics content was quantified in duplicate in the supernatant using the Folin–Ciocalteu reagent, as previously described by García-Pastor et al. [41]. Results were expressed as mg gallic acid equivalent (GAE)  $100 \text{ g}^{-1}$  of fresh weight (FW) and are the mean  $\pm$  SE of three replicates. To extract total anthocyanins, 5 mL of juice sample was homogenized in the same way with 15 mL of methanol: formic acid: water (25:1:24, *v/v/v*) and then centrifuged at  $10,000 \times g$  for 10 min at 4 °C. Total anthocyanin content in the supernatant was quantified spectrophotometrically at 520 nm in duplicate by using a spectrophotometer (UNICAM Helios- $\alpha$ , Artisan Technology Group, Champaign, IL, USA). The results were expressed as mg of malvidin 3-glucoside equivalent (molar absorption coefficient of  $27,000 \text{ L cm}^{-1} \text{ mol}^{-1}$  and molecular weight of  $493.4 \text{ g mol}^{-1}$ )  $100 \text{ g}^{-1}$  FW (mean  $\pm$  SE,  $n = 3$ ), as previously reported [36]. Total antioxidant activity (TAA) was quantified according to the procedure described by Sayyari et al. [42], which leads to determining TAA related to both hydrophilic (HTAA) and lipophilic (LTAA) compounds in the same extraction. In this study, only the results of HTAA in wine grape for each method studied have been reported. Briefly, 5 mL of each sample was homogenized in 10 mL of 50 mM phosphate buffer pH 7.80 and 5 mL of ethyl

acetate and then centrifuged at  $10,000 \times g$  for 15 min at 4 °C. The upper fraction was used to quantify the HTAA of extracts. This functional parameter was determined using the enzymatic system composed of the chromophore 2,20-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the horseradish peroxidase enzyme and its oxidant substrate (hydrogen peroxide), in which ABTS<sup>+</sup> radicals are generated and monitored at 730 nm. The decrease in absorbance after 60 s of adding the wine grape extract was calculated and used to quantify HTAA by comparison with a calibration curve performed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid) (0–20 nmol) from Sigma (Madrid, Spain), and results are expressed as mg 100 g<sup>-1</sup> Trolox equivalent (TE) (mean  $\pm$  SE of three replicates).

#### 2.4. Winemaking

Nine microvinifications in total (three per method,  $n = 3$ ) were performed in the 2019 experimental season in order to study how the berry thinning methods performed; a 0% (control), 25%, and 50% decrease in berry compactness influenced sensory attributes of wine. Wine grapes from each method, which were mixed together after physicochemical and functional analyses to obtain a homogeneous sample, were manually destemmed and randomly distributed in three groups of 2 kg each. Thus, all vinifications were made in triplicate ( $n = 3$ ) and placed in 2.5 L Speidel plastic fermenters equipped with a submerged cap system, using 6 kg of grapes in total for each method studied. Then, berries were manually crushed and sulfited with potassium metabisulfite (K<sub>2</sub>S<sub>2</sub>O<sub>5</sub>) at 80 mg kg<sup>-1</sup>. Total acidity was corrected to 6 g L<sup>-1</sup> with tartaric acid. All wine fermenters were immediately inoculated with the selected *Saccharomyces cerevisiae* yeast (EC1118, Lallemand Inc., Montreal, Que, Canada) at 150 mg kg<sup>-1</sup> and maintained at 25  $\pm$  1 °C. All microvinifications were controlled daily by measuring the temperature and density of juice samples to control the delays or stoppages in the fermentation process. Density was determined by using a portable density meter (METTLER TOLEDO, L'Hospitalet de Llobregat, Spain). To improve color extraction, the cap was punched down once around 1035 density units. After the 10 days of maceration–fermentation time, the wines were racked to other clean wine fermenters and the lees were discarded. Once alcoholic fermentation had completely finished, wines were sulfited with 100 mg of K<sub>2</sub>S<sub>2</sub>O<sub>5</sub> L<sup>-1</sup> and cold stabilized at 4 °C for three months. Subsequently, red wines were bottled and stored at room temperature until descriptive sensory analysis.

#### 2.5. Descriptive Sensory Analysis and Consumer Acceptance of Wine

The analysis of the sensory properties of the wine samples was carried out by a trained panel composed of 12 panelists (7:5, women:men, aged 30–62). The panelists have extensive experience in the use of sensory techniques and their application to wine and alcoholic products because all of them belong to accredited panels of sensory analysis of wine in different Protected Designations of Origin (PDO) in Spain. Two sessions of homogenization of scales, to guarantee that the panelists evaluated the samples in a homogeneous way, were carried out two weeks before the assay with the samples under study. During these sessions, a pooling of sensory descriptors was performed at different intensities and a tasting of 15 wine samples was analyzed, including replicates to ensure the reproducibility of the panel scores. For the descriptive sensory analysis, the lexicon and methodology previously described by Issa-Issa et al. was used [43]. In this case, the following descriptors were used: (i) odor: alcohol, animal, floral, fruity, spicy, toasted, and vegetable; (ii) flavor: alcohol, animal, floral, fruity, spicy, toasted, and vegetable; (iii) basic tastes: bitter, sour, and sweet; (iv) chemical feelings: astringent; (v) global: aftertaste; (vi) appearance: limpidity, color (hue), and color intensity; and (vii) defects: cauliflower, cork, earthy, glue, horse, onion, rotten apple, rotten egg, soap, sulfur, vegetal, and vinegar. Panelists used an 11-point scale for the evaluation, in which 0 was an extremely low or nonperceptible intensity and 10 was an extremely high intensity, except in the case of defects in which an absence/presence scale was used. Each sample was served in two stages: firstly, in a black tasting cup, in

order to avoid any influence of visual characteristics of the sample on the objective analysis of panelists to determine the descriptors of odor, flavor, basic flavors, chemical feelings, global, and defects; and, secondly, a transparent tasting cup was used for the evaluation of the visual phase. Samples were randomly coded with three digits, monadically presented according to a Williams Latin square design balanced for order and carryover effects, and in both cases (stage 1 and 2), 35 mL of the sample was served at a temperature of  $15 \pm 1$  °C. Between each of the samples, 5 min breaks were established in which the panelists had osmotic water and unsalted crackers at their disposal to clean their palate. Reference materials for each attribute were prepared and made available for all panelists.

All analyses were carried out in an official tasting room at the Food Technology Department at Miguel Hernández University (Alicante, Spain), which had natural and white light and a temperature of  $22 \pm 1$  °C. Evaluations were carried out in three sessions of 1 h to have three replicates ( $n = 3$ ) and, in each session, replicate samples were used. In addition to descriptive sensory analysis, a study of the degree of acceptance of the samples under study was performed with 60 habitual wine consumers, who declared consuming wine at least three times per week. Consumers (55:45, women:men, aged 25–60) tested the samples (control, 25%, and 50% berry thinning methods) and ordered them from the most preferred sample to the least preferred one (overall ranking). Samples were monadically presented according to a Williams Latin square design balanced for order and carryover effects. In addition, samples were also randomly coded by three digits. The affective study was developed in the same conditions of the sample and tasting room as the descriptive analysis.

#### 2.6. Statistical Analysis and Principal Components Analysis (PCA)

A Student's *t*-test ( $p \leq 0.05$ ) was performed to detect significant differences between compact bunch and non-compact bunch samples during the first experiment (2018 season). Data for the analytical determinations and sensory analysis of the 2019 experiment were subjected to analysis of variance (ANOVA) using the method studied as the factor. Thus, one-way ANOVA was used to determine the significance of mean differences among the three berry thinning methods; 0% (control), 25%, and 50% reduction in bunch compactness. Mean comparisons of analytical determinations were performed using a multiple range test (Tukey's test) to examine if differences among the methods were significant at  $p \leq 0.05$ . To determine significant differences among methods studied for sensory analysis, mean comparisons were carried out using a Tukey's multiple range test, which shows significant differences at  $p \leq 0.05$ ,  $p \leq 0.01$ , and  $p \leq 0.001$ . All analyses were performed with SPSS software package v. 17.0 for Windows (Microsoft: Redmond, WA, USA). Additionally, data of the parameters analyzed with significant differences were subjected to principal component analysis (PCA). The software used for PCA was XLSTAT (Addinsoft, 2016.02.27444 version, Paris, France).

### 3. Results

#### 3.1. Effect of Bunch Compactness on Grapevine Yield, Bunch Architecture, Physicochemical, and Functional Parameters of Wine Grape

As expected, the total yield per grapevine of non-compact bunch group was significantly reduced. Specifically, grapevines with non-compact bunches showed a 1.18-fold decrease compared with those with compact bunches. Bunch fresh mass (g) was also significantly decreased by 55% in non-compact bunches, resulting in a lowering of 1.80-fold of bunch compactness compared with the compact bunch, although the bunch length did not significantly decrease accordingly for those harvested in 2018 (Table 1). Regarding the physicochemical parameters, wine grapes from non-compact bunches increased significantly in content of TSS than compact ones (Table 1). However, both TA and RI did not show significant differences among compact and non-compact bunches. As a functional parameter, total phenolic content was quantified. The content of total phenolics showed a

1.14-fold increase in wine grapes from non-compact bunches compared with compact ones, leading to berries with approximately 39 mg 100 g<sup>-1</sup> FW (Table 1).

**Table 1.** Effects of bunch compactness on parameters related to bunch architecture and berry chemical quality at harvest in the 2018 growing season.

	Student's <i>t</i> -Test †	Compact Bunch	Non-Compact Bunch
Bunch fresh mass (g)	*	359.90 ± 20.60	232.00 ± 12.20
Bunch length (cm)	NS	16.45 ± 0.51	17.74 ± 0.35
Bunch compactness (g cm <sup>-2</sup> )	*	1.33 ± 0.05	0.74 ± 0.05
Total soluble solids (g 100 g <sup>-1</sup> )	*	23.58 ± 0.16	24.41 ± 0.27
Total acidity (g 100 g <sup>-1</sup> )	NS	0.72 ± 0.01	0.73 ± 0.02
Ripening index	NS	32.30 ± 0.89	33.44 ± 1.30
Total phenolics (mg 100 g <sup>-1</sup> FW)	*	34.20 ± 0.38	39.03 ± 0.54

† Values are the mean ± SE of three biological replicates; significant differences ( $p \leq 0.05$  according to Student's *t*-test) were expressed as \* symbol placed in each row showing differences between both methods for each parameter at harvest; NS = not significant.

### 3.2. Effect of Berry Thinning Methods on Grapevine Yield, Bunch Architecture, Physicochemical and Functional Parameters of Wine Grape

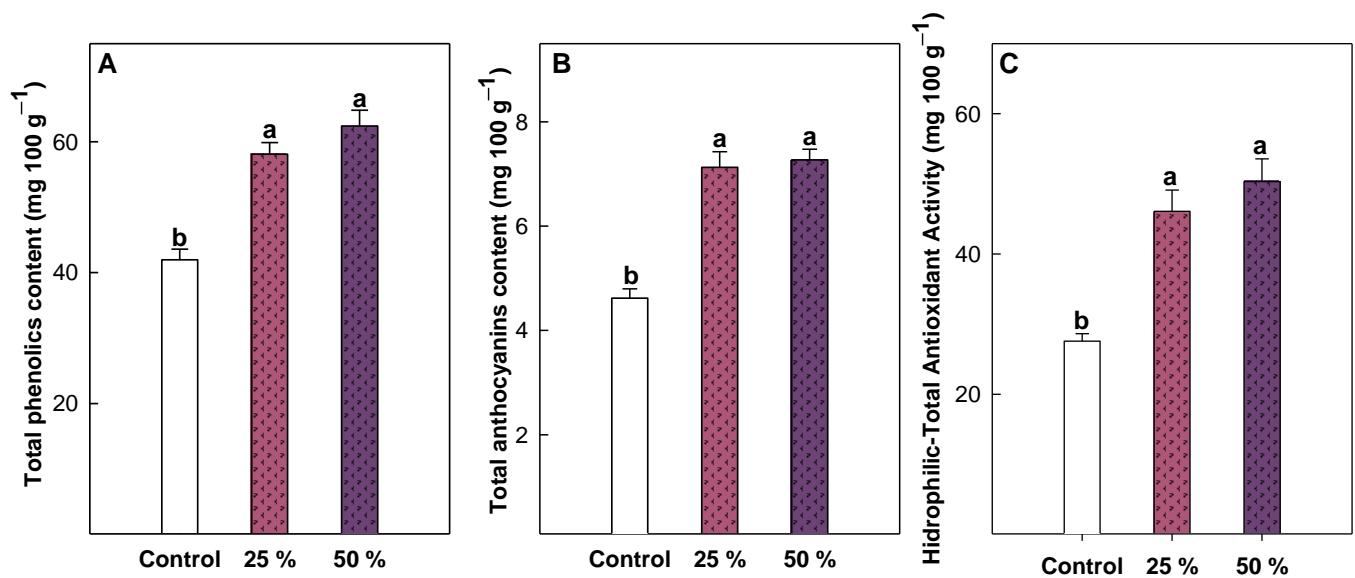
Total yield of control grapevines ( $3.11 \pm 0.21$  kg vine<sup>-1</sup>) was significantly reduced with the 25% and 50% berry thinning ( $2.72 \pm 0.15$  and  $2.49 \pm 0.19$  kg vine<sup>-1</sup>, respectively). Both methods decreased the bunch fresh mass by 19% and 31%, respectively, resulting in a greater lowering of bunch compactness than the control. Specifically, the compactness of bunches showed a 1.28- and 1.58-fold decrease for 25% and 50% berry thinning methods, respectively, compared with the control bunches. Nevertheless, both methods did not influence the bunch length in the 2019 growing season (Table 2). On the other hand, the content of TSS was significantly increased by the two berry thinning methods tested (Table 2). The 50% berry thinning leads to wine berries with a higher content of TSS, followed by those bunches thinned by 25% and the control ones. However, both TA and RI parameters did not show significant differences among the methods studied (Table 2).

**Table 2.** Effects of berry thinning methods on parameters related to bunch architecture and berry chemical quality at harvest in the 2019 growing season<sup>1</sup>.

	Control	Berry Thinning Methods	
		25%	50%
Bunch fresh mass (g)	381.80 ± 10.40 a	308.30 ± 8.90 b	264.10 ± 7.50 c
Bunch length (cm)	17.32 ± 0.64 a	17.57 ± 0.35 a	18.14 ± 0.52 a
Bunch compactness (g cm <sup>-2</sup> )	1.27 ± 0.06 a	0.99 ± 0.04 b	0.80 ± 0.05 c
Total soluble solids (g 100 g <sup>-1</sup> )	25.14 ± 0.28 c	26.12 ± 0.22 b	26.95 ± 0.16 a
Total acidity (g 100 g <sup>-1</sup> )	1.01 ± 0.02 a	1.01 ± 0.01 a	1.02 ± 0.02 a
Ripening index	24.84 ± 0.60 a	25.89 ± 0.67 a	26.37 ± 0.94 a

<sup>1</sup> Values are the mean ± SE of three biological replicates. Data in the same row with different letters indicate significant differences among methods at  $p \leq 0.05$  according to the Tukey's multiple range test.

Furthermore, wine grapes from berry thinning methods showed significantly increased bioactive compounds content, namely, total phenolics and total anthocyanins, and HTAA than control ones (Figure 2). In this sense, bunches thinned by both methods showed 1.44-, 1.56-, and 1.75-fold increases in phenolics content, total anthocyanins content, and HTAA compared with control bunches. However, no significant differences between both berry thinning methods were observed for these functional parameters, with the increases being statistically equal for both of them.



**Figure 2.** Effects of berry thinning methods on total phenolics (A) and total anthocyanins (B) content, as well as hydrophilic total antioxidant activity (C) of the whole berry (flesh and skin tissues) at harvest in the 2019 growing season. Data are the mean  $\pm$  SE of three biological replicates. Different letters show significant differences among methods for each parameter at  $p \leq 0.05$  according to the Tukey's multiple range test.

### 3.3. Effect of Berry Thinning Methods on Descriptive Sensory Analysis and Consumer Acceptance of Wine

After the descriptive sensory analysis of the wine samples by a trained panel, statistically significant differences were found in seven of the 22 sensory descriptors evaluated (Table 3): fruity (odor), fruity (flavor), sour, sweet, aftertaste, color and color intensity. In the case of the odor and flavor descriptors, the wine sample in which 25% of the berry thinning was performed during its crop cycle had a higher intensity of fruity (7.63 odor and 7.81 flavor) than the control sample (6.38 odor and 6.63 flavor). The wine sample of the 50% berry thinning method did not show significant differences in fruity (odor) compared with the control and the 25% berry thinning, however, it had a significantly higher intensity of fruity flavor (7.56) than the control.

Regarding the basic tastes, both berry thinning methods led to a wine with a higher intensity of sweetness and sourness, as well as a higher intensity of aftertaste, than the control wine (Table 3). In the case of color, as the percentage of thinning increased, the wine lost less color during the winemaking process. Thus, the wine maintained the characteristic color of a young wine (red-purplish) for a longer time, as well as its intensity. Furthermore, the wine made from bunches thinned by 50% was the favorite among consumers, while the control wine was the least favorite one. The wine sample of the 25% berry thinning did not show significant differences in the overall ranking compared with the other methods (Table 3).



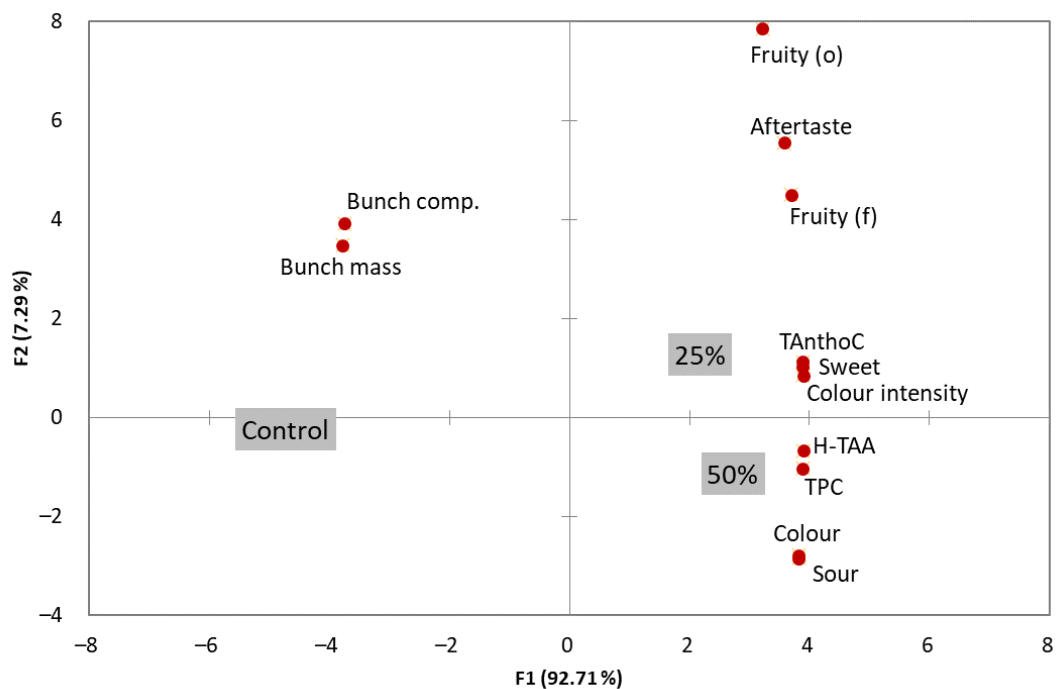
**Table 3.** Descriptive sensory analysis and consumer acceptance of wines made from grapes for control, 25%, and 50% of berry thinning methods in the 2019 growing season <sup>1</sup>.

Attribute	ANOVA	Control	Berry Thinning Methods	
			25%	50%
<i>Odor</i>				
Alcohol	NS	6.31	6.94	6.94
Animal	NS	1.19	1.25	1.00
Floral	NS	4.75	5.44	5.19
Fruity	**	6.38 b	7.63 a	7.06 ab
Spicy	NS	3.31	3.81	3.81
Toasted	NS	1.25	1.81	1.88
Vegetable	NS	2.88 b	3.75 a	4.19 a
<i>Flavor</i>				
Alcohol	NS	6.56	6.19	7.00
Animal	NS	1.38	1.31	1.13
Floral	NS	5.06	5.63	5.69
Fruity	*	6.63 b	7.81 a	7.56 a
Spicy	NS	4.38	4.44	4.81
Toasted	NS	1.88	2.50	3.00
Vegetable	NS	2.81	3.50	3.69
<i>Basic tastes</i>				
Bitter	NS	2.13	2.13	3.19
Sour	*	4.00 b	4.63 ab	4.94 a
Sweet	*	2.56 b	3.50 a	3.56 a
<i>Chemical feeling</i>				
Astringent	NS	3.56	3.50	4.50
<i>Global</i>				
Aftertaste	**	6.31 b	7.11 a	6.88 a
<i>Appearance</i>				
Color	*	8.88 c	9.50 b	9.81 a
Color intensity	*	8.19 b	9.06 ab	9.13 a
Limpidity	NS	8.31	8.44	8.38
Overall ranking	**	b	ab	a

<sup>1</sup> NS = not significant; \* and \*\* significant at  $p < 0.05$  and  $0.01$ , respectively; data in the same row with different letters indicate significant differences among methods according to the Tukey's multiple range test. Mean satisfaction degree of 60 consumers is denoted by overall ranking obtained by the statistical Friedman's test.

### 3.4. Principal Components Analysis (PCA)

For better understanding of the relationships among the twelve statistically significant variables studied for the wine grapes and wines, a PCA (linear dimensionality reduction method for processing of multivariate data) was conducted to project the samples depending on the instrumental and sensory parameters applied to the experimental results (Figure 3). This statistical test was run for all samples including the only significantly different variables: bunch compactness, bunch mass, total anthocyanins content, hydrophilic total antioxidant activity, total phenolics content, color, color intensity, fruity (odor and flavor), sweet, sour, and aftertaste (Figure 3). The PCA explained analytical variables in two axes, F1 (92.71%) and F2 (7.29%). The results explained, easily, that functional properties of wine grape, as well as the main sensory attributes of wine, can be improved with berry thinning methods (25% and 50%) as can be seen in the differences between the control and both methods along the F1-axis. All the sensory attributes in which significant differences were found (color, color intensity, fruity, sweet, sour, and aftertaste) appear grouped with the wine samples in which a berry reduction (25% and 50%) was applied. Similarly, it can be seen in the functional determinations (total anthocyanins content, hydrophilic total antioxidant activity, and total phenolics content).



**Figure 3.** Principal component analysis (PCA) scores plot showing the relationship among parameters related to bunch architecture, physicochemical and functional properties of wine grape, as well as sensory parameters of wine. (o): odor; (f): flavor; TAnthoC: total anthocyanins content; H-TAA: hydrophilic total antioxidant activity; TPC: total phenolics content; bunch comp.: bunch compactness.

#### 4. Discussion

Some authors have reported that a high crop yield of grapevines delays ripening and reduces the quality of wine grapes and the subsequent wine [7,16]. In this sense, berry thinning is a usual method used by farmers to control total yield [19–21]. Reduction in bunch compactness is a method of berry thinning which is becoming more common to control berry quality [11,35,44]. To the best of our knowledge, there is a research gap regarding the effect of bunch compactness and berry thinning methods on the quality traits of the ‘Monastrell’ wine grape and, above all, pertaining to its influence on the sensory qualities of wine. This study focuses on filling this knowledge gap. In two consecutive growing seasons, 2018 and 2019, the total yield per grapevine of non-compact bunches, as well as those grapevines in which 25% and 50% berry thinning were performed, was significantly reduced. Very few reports exist about the influence of berry thinning on total yield of the ‘Monastrell’ wine grape because most of them were performed on table grape cultivars. According to our results, some authors have observed a decrease of 20–30% on total yield using berry thinning methods [7,45,46].

The bunch compactness showed a higher decrease in both growing seasons in the non-compact bunches group and those where the berry thinning method was applied compared with the compact or control ones, respectively (Figure 1). In the 2018 season, the bunch fresh mass significantly decreased by 55% in non-compact bunches compared with the compact ones, while this parameter related to bunch architecture showed a lower decline of 19% and 31% with 25% and 50% berry thinning, respectively, in the 2019 season. Nevertheless, none of the studied methods significantly influenced the bunch length (Tables 1 and 2). Accordingly, Gil et al. [7] reported that the average weight of bunches in grapevines where berry thinning is performed was around 19% lighter than the control grapevines. This reduction in bunch fresh mass is also in accordance with the little information available on this research subject [45–47]. The small decline observed on the bunch fresh mass from the two berry thinning methods tested could be related to higher berry mass and size, according to Han et al. [35]. Other previous studies on bunch compactness report that

berries have more growing space after a decrease in bunch compactness [48], and the berry thinning method in early berry development could have a positive effect on berry size mediated by an increase in carbon and remobilized nutrient supply available for remaining berry growth [49]. The application of these methods in the vineyard could modify the appearance of the bunches. In a previous study, clusters were more rounded after berry thinning, since the tip had been cut [7]. However, the bunches of the present study thinned by removing 25% and 50% of berries did not show a rounded appearance since berries were removed manually producing a different bunch compactness, although evident visual differences were observed among them (Figure 1).

The TSS content of the juice from berries of the non-compact bunches group was significantly higher than those juices from compact bunches (Table 1). However, the bunch compactness did not influence the content of the TA and RI of each sample in the 2018 growing season. In the same way, berries from bunches thinned by removing 25% and 50% of berries from each bunch showed a significantly higher increase in content of TSS compared with the control bunches (Table 2), although no significant differences in TA content and RI parameters were observed among the methods studied. Accordingly, Han et al. [35] also reported that berries of the 'Cabernet Sauvignon' cultivar, from 25% and 50% berry thinning methods, showed more soluble solids content, mainly sugars, and had less acid on the same growth season. Other authors observed that only TA content was significantly lower in those berries harvested from grapevines in which berry thinning methods were applied compared with the control ones [7,47]. These results on the increase in TSS content by these methods, together with those discussed above, could be related with a possible effect of increasing the carbon and remobilized nutrient supply available [49]. Recently, Han et al. [35] concluded that treated berries with thinning methods showed a higher content of glucose and fructose, reporting that bunch compactness significantly affects sucrose partitioning and metabolism in grapes. This is further supported by the upregulation of activities of two isoforms of acid invertases; SAIs and CWIs, which was mediated by a reduction in bunch compactness. Both isoforms are involved in the control of sink activity through the hydrolytic cleavage of sucrose into hexose monomers, thus regulating the supply of photoassimilates, such as carbon partitioning from the source, throughout the berry ripening cycle. This fact was correlated with the accumulation of sugars in those treated berries [35].

On the other hand, the content of bioactive compounds, namely, total phenolics and total anthocyanins, increased after reducing the bunch compactness of 'Monastrell' wine grape (Table 1 and Figure 2). Similarly, other reports have concluded that wine grapes or wines made with berries from bunches that have been treated with berry thinning methods increase the content of total phenolics compounds [35], as well as total flavonols concentration [7]. Regarding the effect of sun exposure on the increase in flavonol content [7,17,18], berries from clusters thinned by 25% and 50% could have a higher content of total phenolics due to those berries receiving more sunlight, since the bunch compactness and the shadowing effect are lower compared with the control bunches. In this sense, a higher phenolic content on wine grapes could increase the possibility of copigmentation effects on its subsequent wine, which could enhance the redness color and the purplish nuances of the red [50]. Furthermore, color heterogeneity increases in compact bunches because they bear more inner berries that receive little sun exposure and more heat [13], which is unbeneficial to improve the biosynthesis and accumulation of secondary metabolites in the skin and flesh of berries [14], especially the accumulation of total anthocyanins [51,52]. Our results show that total anthocyanins content was significantly higher in berries from 25% and 50% berry thinning methods than the control (Figure 2), although no significant differences were observed among the two percentages assayed. Therefore, color accumulation of the berry skin was deeper in those bunches where the two methods were performed, since those bunches would present more solar radiation and efficient aeration. Recently, one previous study indicated that reducing bunch compactness significantly increased the content of individual anthocyanins, mainly petunidin derivatives, at harvest [35]. On

the other hand, Gil et al. [7] also observed a significantly higher proanthocyanidin concentration in berry-thinning wine than control wines. In addition, the responses to bunch compactness might result from F3'H and F3'5'H transcript abundance, which can regulate the key enzymes involved in the production of the shared precursors for biosynthesis of individual anthocyanins [53,54]. Nevertheless, further studies are still necessary to elucidate the impact of bunch compactness and berry thinning methods on anthocyanin metabolism. In general, the improvement of the functional content observed by both methods resulted in an increase in the HTAA, since phenolic compounds, mainly anthocyanins, are hydrophilic antioxidant metabolites (Figure 2). This fact has been reported for the first time in the present study.

Berry thinning during grape growing increases the fruitiness, sourness, sweetness, and color of the wine (Table 3). Torres et al. [55] observed similar results performing shoot thinning, leaf removal, and a combination of both techniques in wine obtained from a 'Cabernet Sauvignon' cultivar. These techniques increased the acidity, sweetness, and color of wines. Other authors have also determined that when berry thinning is applied on grape as an agronomic technique, the content of sugars and organic acids increases, as well as antioxidant compounds that are responsible for color, which is mainly due to the fact that the plant allocates a greater quantity of nutrients to the remaining grains [56,57]. Finally, Figure 3 allows us to conclude that berry thinning methods, mainly the 50% one, during the wine-grape crop cycle provide wine grapes with higher quality and produce a wine with greater sensory attributes, which is more valued by consumers.

## 5. Conclusions

In conclusion, non-compact bunches (2018 season) and the 15% and 50% berry thinning methods (2019 season) show a reduction in total yield, bunch compactness, and bunch fresh mass compared with the compact and control ones, respectively. However, this reduction in bunch compactness effectively improves the quality of the 'Monastrell' wine grape, mainly in terms of total soluble solids and total phenolics content. Specifically, the 50% berry thinning was the most effective, increasing the content of total soluble solids compared with 25% berry thinning and control. Furthermore, both the 25% and 50% methods promote the accumulation of total anthocyanins in the whole berry, as well as the hydrophilic total antioxidant activity; this last effect is being reported for the first time in the present study. The berry thinning methods led to wines with greater sensory descriptors, such as fruity (odor), fruity (flavor), sour, sweet, aftertaste, color, and color intensity. On the other hand, the wine made from bunches thinned by 50% was the favorite among consumers. Finally, the 50% berry thinning method is the most useful agronomic tool to decrease bunch compactness and improve the physicochemical and functional quality of berries and sensory attributes of wines in the 'Monastrell' cultivar. It is necessary to mention that, in order to obtain results that allow standardizing of the methods described in the present study, it is advisable to expand the studies with different wine grape cultivars and in areas with different edaphoclimatic properties.

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