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Cluster Thinning Improves Aroma Complexity of White Maraština (*Vitis vinifera* L.) Wines Compared to Defoliation under Mediterranean Climate

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Abstract: Defoliation and cluster thinning are useful canopy management techniques to modulate grapevine carbon distribution and microclimate. Both techniques are directed to achieve the proper balance between fruit and foliage, and to maximize production of well-ripened fruits and quality wines. We performed five canopy treatments on Maraština grapevine grown at a commercial vineyard in the Vrgorac Valley region of Croatia: three different times of basal defoliation, cluster thinning at the veraison, and an untreated control. The effects of the canopy changes on the chemical composition of grapes and wines were studied. The treatments had variable impacts on yield components and basic wine composition. Volatile aroma compounds in produced wines were analyzed using gas chromatography–mass spectrometry coupled with a mass-selective detector. The concentrations of 70 of the 96 individual volatile compounds were significantly influenced by the canopy technique used. The concentrations of 58 of these compounds were different according the timing of defoliation. Cluster thinning at an intensity of 35% produced wines with more terpenes, esters, higher alcohols, other alcohols, volatile phenolic compounds, lactones, and other compounds than other treatments. Among terpenes, cluster thinning increased terpinen-4-ol, linalool, *trans*-β-farnesen, and geraniol. Odor activity value analysis revealed 16 volatile compounds that contributed to the aroma of cluster-thinned wines.

Keywords: grapevine; leaf removal; cluster thinning; varietal aromas; terpenes; fermentation aromas; odor activity value

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1. Introduction

Techniques to manipulate the canopy by restricting vegetative and generative growth are useful to interrupt the natural cycle of grape ripening and sugar accumulation temporarily. The two techniques most commonly used to regulate the grape microclimate are defoliation and cluster thinning. Defoliation triggers a general reprogramming of the transcriptome and metabolome [1]. This leads to changes in enzyme activity and/or kinetics of biochemical reactions in berries during development [2]. Two main factors influencing the effects of defoliation and cluster thinning on vine physiology are the intensity and berry development stage, during which the intervention was carried out [3–6]. Basal defoliation modulates the cluster zone shadow–light ratio, temperature, and airflow [2,5,6]. Basal defoliation significantly disrupted carbon flux between primary and secondary metabolic pathways, delayed sugar accumulation, increased flavonoids, and decreased herbaceous aromas [1,7].

Cluster thinning one week before veraison was shown to increase free and glycosylated terpene concentrations in Sauvignon Blanc berries [4]. Cluster thinning at an intensity of 20% at veraison increased the varietal aroma compounds citronellol, linalool, and vitispirane in sparkling Ribolla Gialla wines, but only in vineyards located in a valley [8]. In Malvasia wines, a limited effect of a low crop level (10 buds per vine) on aroma compounds

was reported. A low crop level affected geranyl acetate, but not C_{13} norisoprenoids, C_6 alcohols, and higher alcohols. On the contrary, the concentration of some esters increased with a higher crop level (20 buds per vine) [9].

Maraština is an old white grape variety (*Vitis vinifera* L.) mostly cultivated in the coastal regions of Croatia (Dalmatia). It produces high-quality wines with fruity flavors and moderate acidity. Maraština is used for dessert-type premium wines called Prošek in Croatia. Bunch rot and powdery mildew can be a problem due to relatively compact clusters and thin berry skin. Maraština is known in Greece and Italy as Pavlos and Malvasia Bianca Lunga (Malvasia del Chianti), respectively [10]. While many studies indicate that canopy-management techniques can result in improved fruit and wine quality, too little fruit or too many leaves can turn the vine toward overcropping and eventually lead to unbalanced wines. There is no information of the impact of canopy techniques on the aroma complexity of Maraština wines. Even more, results on other white varieties grown on dry and shallow karstic soils are scarce, and only a few papers [3,8,9] reported variations in individual wine aroma classes after application of defoliation or cluster-thinning techniques. The effects of three defoliation times (preflowering, after berry set, and at veraison) and cluster thinning at veraison on the aroma profile of white wines have not been compared under the same vineyard conditions previously. The controversial results of the effect of viticulture techniques among other varieties under different climate conditions are not applicable to the improvement of the aroma potential of neutral variety Maraština under Mediterranean conditions.

Here, we fill this information gap and compared the effects of five different canopy techniques: defoliation at preflowering (PF), after berry set (BS), or at veraison (V); cluster thinning at veraison (CT); and an untreated control (C) on the agronomic qualities, cluster architecture, and the physiochemical and aroma composition of cv. Maraština wines. This will provide practical information to optimize cultivation and targeted manipulation of aroma compounds to improve sensory attributes of Maraština wines.

2. Materials and Methods

2.1. Vineyard Site and Climate Conditions

The experiment was conducted in 2019 in a commercial vineyard in the Vrgorac Valley (43°10′9.2″ N, 17°23′44.2″ E, 23asl) in the Dalmatian Hinterland wine region (Croatia). *Vitis vinifera* L. 'Maraština' grafted on 1103 Paulsen rootstock was planted in 2010 in fluvisol soil (undeveloped hydromorphic soil) on karst at a spacing of 0.6 m (within row) × 2.2 m (between row) in an east–west orientation. Vines were Guyot-trained to a single-curtain vertical trellis (VSP) and pruned back in winter to a single cane of 10 buds and one spur of two buds.

The climate in the Vrgorac Valley is Mediterranean, based on climate data from the nearest meteorological station with at least one month of drought in summer. The average annual temperature in 2019 was 15.2 °C, and the annual precipitation was 1389.3 mm. Weather conditions during this experiment in Vrgorac (Figure 1) were very hot and dry. Most of the precipitations during the growing season occurred in May (maximum 235.8 mm). The driest month was August (12.9 mm), which was also the month with the hottest average temperature (27.6 °C). In August, the temperature exceeded 30 °C for three consecutive days. Detailed analysis of meteorological data from June to September revealed 78 days with a maximum daily temperature above 30 °C, and the peak of 39.5 °C was on 12 August. Minimum night temperatures were above 20 °C on 54 days, which may be crucial for the balance of photosynthesis and respiration, as well as for flavor development.

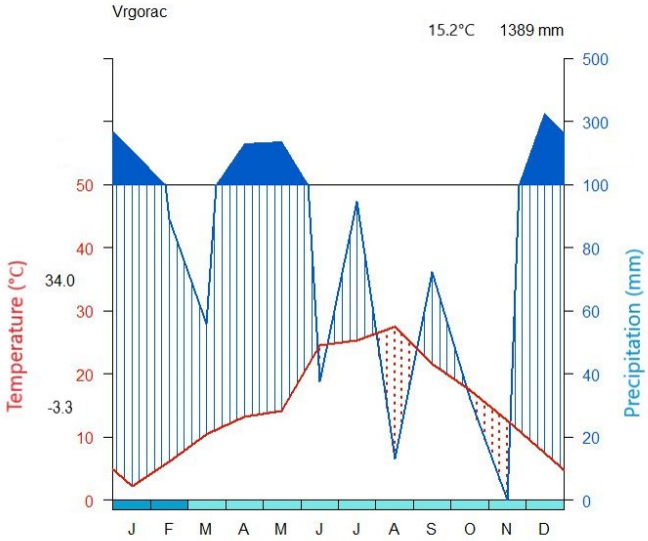


Figure 1. Walter–Lieth climatic diagram for average temperature (red line) and total monthly precipitation (blue line) per month at Vrgorac weather station in 2019. On the left axis, values in black represent the absolute maximum and minimum temperature. At the top right, values in black represent the average values of mean temperature and annual precipitation.

2.2. Experimental Setup

A complete randomized block design was used to allow three repetitions of each treatment. Three rows (experimental blocks), each with 15 intermediate spacing (experimental units composed of 12 vines each), were selected in the central part of the vineyard. Canopy treatments were assigned randomly to each experimental units in three rows. In total, 15 vines per each of five treatments were in one row. Four canopy-manipulation techniques were applied at specific phenological development stages according to the Eichhorn and Lorenz (E-L) standard [11]. The three treatments for basal defoliation consisted of manual removal of six basal leaves from shoots at different grapevine phenological stages of the vine: preflowering (E-L 18), at berry set (bunch at right angle to shoot, berries were 2 to 4 mm diameter) (E-L 27), and at veraison (E-L 35). The cluster thinning was carried out at veraison (E-L 35) by cutting off 35% of the clusters, based on cluster counting. The thinned clusters were positioned on the upper nodules. The interval between the first two treatments was nine days, starting on 11 June, and the third treatment was applied on 6 August 2019. A control treatment without leaf or cluster removal was also carried out. Regardless of treatment, the lateral shoots were removed to the proximal node during berry set, as is common in this region. The grapes were harvested manually at the technological ripeness of the untreated control on 3 October. At harvest, yield components, number of clusters, and total mass were determined for each vine per treatment. The total yield per treatment was mixed after weighing to obtain more homogeneous samples for further analysis and winemaking. Immediately after harvest, grapes were transported to the experimental winery with ampelometry of the Institute for Adriatic Crops and Karst Reclamation.

2.3. Analysis of Physicochemical Components of Grape Juice

Ten representative clusters per each of five treatments were randomly selected and weighed, and the number of berries in each cluster was determined. Three randomly selected 100-berry samples per treatment were weighted (g) and processed into juice for rapid physiochemical analysis of basic maturity indicators [12]. Total soluble solids (°Brix) were measured with a portable density meter (RHW-25/Brix (ATC)); the total acid content was determined using titration to the point of equivalence with 0.1 mol L^{-1} NaOH and the bromthymol blue indicator (expressed as g L^{-1} tartaric acid); and the pH was measured using a pH meter (Methrom 728, Herisau, Switzerland). Single berry weight (g) was calculated by dividing the total mass of 100 berries by the number of berries.

2.4. Microvinifications

A total of 300 kg of grapes, exactly 60 kg per treatment, was mechanically destemmed, crushed, sulfited to 25 mg L^{-1} free sulfur dioxide (SO_2), and immediately pressed. The free-run must from each treatment was divided in three repetitions and clarified in 10 L stainless steel tanks at 10 °C for 24 h. After settling, the must was decanted and inoculated with 250 mg kg^{-1} of the commercial *Saccharomyces cerevisiae* yeast strain (Siha WhiteArome, Eaton, Langenlonsheim, Germany). During rehydration, inactive yeast nutrients (Siha Speedferm, Eaton, Langenlonsheim, Germany) were added to promote formation of active yeast cells and improve fermentation performance. The fermentation temperature was monitored daily and maintained at 18 °C. After six days, 20 g L^{-1} of a yeast nutrient was added (Siha Profermplus, Eaton, Langenlonsheim, Germany). Once alcoholic fermentation was complete, wines were decanted, sulfited to 30 mg L^{-1} SO_2, and stored at 15 °C. After three months, the wines were decanted a second time, the SO_2 was corrected to 20 mg L^{-1} SO_2, and wines were bottled in 750 mL bottles.

2.5. Analysis of Physiochemical Components of Wine

The physiochemical oenological parameters (alcohol, residual sugars, total extract, pH, total acidity, and volatile acidity) of all 15 wines were determined three months after bottling using the official methods of wine and must analysis [12].

2.6. Analysis of Volatile Compounds Using GC-MS

Solid-phase extraction of volatile aroma compounds was performed as previously described [13]. Briefly, cartridges containing 200 mg of LiChrolut EN sorbent (200 mg/3 mL, Merck, Darmstadt, Germany) were preconditioned using successive washing with 3 mL of dichloromethane (UHPLC gradient grade J.T. Baker, Deventer, The Netherlands), methanol (UHPLC gradient grade J.T. Baker, Deventar, The Netherlands), and a 13% aqueous ethanol solution (LiChrosolv, Merck, Darmstadt, Germany). After loading the 50 mL sample onto the column, residual sugars and other polar compounds were washed out with 3 mL of water. The column was dried in a vacuum. The analytes were recovered using elution with 1 mL of dichloromethane. For a quality control, 50 mL of water was injected into the SPE column instead of the sample. Identification and quantification of targeted aromatic compounds in the wines was performed as previously described [14,15]. Gas chromatography–mass spectrometry analysis was performed using an Agilent 6890 system equipped with an Agilent 6890 automatic liquid sampler and a ZB_WAX capillary column (60 m × 0.32 mm i.d., with 0.5 µm film thickness, Phenomenex, Torrance, CA, USA) coupled to a 5973 N mass-selective detector. The injection was performed in splitless mode with volume of 2 µL, while the injector was held at 250 °C. The temperature gradient was as follows: initial oven temperature of 40 °C for 15 min, then increasing to 250 °C in steps of 2 °C/min and held at 250 °C for 15 min. The ion source and MS transfer line were at 250 °C. The carrier gas flow was 1.0 mL/min 5.0 grade helium (Messer, Zaprešić, Croatia) in constant flow mode. The mass detector was operated in electron ionization (EI) mode at an ionization energy of 70 eV with total ion current monitoring. The mass detector ion source and quadrupole were maintained at 250 °C. Compounds were identified by comparing the retention times and mass spectra with those of commercial standards. Six-point calibration was performed for each standard as reported in [14]. Concentration ranges for alcohols, esters, and acids were 5 ug L^{-1} to 5000 ug L^{-1}; and for terpenes, C_{13} norisoprenoids, and lactones: 1 ug L^{-1} to 300 ug L^{-1}. Standard calibration curves were generated using EnhancedChemStation software (Agilent Technologies, Santa Clara, CA, USA) to quantify the compounds. A list of used standards, linear retention indices, and other identification and quantification parameters for the GC-MS are presented in [14]. The results were expressed in µg L^{-1} as the concentration of the compounds calculated from the peak area of each compound compared to that of the internal standard. In the eluate, 10 µL of internal standard (50 mg L^{-1}, 2-octanol) was added.

2.7. Odor Activity Values of Volatile Aromas (OAV)

To evaluate the potential sensory impact to the wine, the odor activity value of 73 volatile aroma compounds was calculated. The OAV was calculated as the quotients of concentration of a specific compound and the corresponding odor detection threshold from the literature [14,15]. The odor detection threshold values and aroma descriptors were taken from the list of 28 references in the Supplementary Materials and are available in Table S1. When possible, the threshold values determined in wine or winelike medium were used to assure accuracy of the OAV. Volatile aroma compounds with an OAV higher than one had a direct impact on aroma and were considered as active odorants. Those with lower values were considered as contributing to wine complexity through synergistic and additive effects of compounds with similar structure or odor (synergistic compounds) [14].

2.8. Statistical Analysis

A one-way analysis of variance (ANOVA) and mean separation using Tukey's test (with different letters representing significant differences at $p \leq 0.05$) was performed to determine differences between the five different treatments. Analyses of variance were performed in SAS (SAS Institute, Inc., Cary, NC, USA). The results of all physiochemical and aroma parameters were expressed as mean ± standard deviation of three repetitions. A multivariate analysis approach was applied to the volatile metabolites using Statistica version 14 software (TIBCO Software Inc., Palo Alto, CA, USA) [16]. A total of 16 volatile compounds with odor-active compounds >1 were selected as the most likely contributors to the aroma of the wines. Principal component analysis (PCA) was performed on the 16 volatile compounds to differentiate the wines from five canopy treatments and to analyze possible relationships among them.

3. Results

3.1. Yield Components and Physicochemical Composition of Maraština Musts and Wines

Yields components and basic physiochemical composition were determined for Maraština must and wine subjected to defoliation at preflowering (PF), after berry set (BS), or at veraison (V); cluster thinning (CT); and an untreated control (C) (Table 1). Significant differences in the number of clusters per vine were determined between PF and CT. Significant differences in yield were determined in untreated C (1.7 ± 0.8) kg and BS (1.2 ± 0.5) kg, with other treatments in-between. The highest cluster weight and number of berries per cluster was in CT, followed by untreated C and V defoliation. BS caused the greatest reduction in yield per vine and cluster architecture parameters. Defoliation significantly reduced the total soluble solids in must, with a maximum reduction in BS (18.7 ± 0.1) °Brix compared to CT (19.6 ± 0.2) °Brix or the untreated C (19.5 ± 0.2) °Brix. BS significantly reduced total acidity (4.3 ± 0.1) g L^{-1}, followed by treatments performed at veraison (CT and V). The highest pH (3.74 ± 0.01) was in treatments conducted at BS and at veraison, which were significantly higher than in PF and C. As expected, defoliation significantly reduced the alcohol content of the wines, regardless of the timing of treatment. The greatest alcohol reduction, determined as alcoholic strength by volume, of 0.8 vol % was in PF wines, while postponing the treatment reduced differences between those and untreated C or CT wines. In wine, the lowest pH was in early defoliation treatments (PF and BS), followed by C and CT.

Table 1. Yield components and basic physiochemical composition of Maraština musts and wines subjected to defoliation and cluster-thinning treatments.

	PF	BS	V	CT	C
Yield components					
Number clusters/vine	8.9 ± 3.2 a	7.5 ± 2.9 ab	7.5 ± 2.4 ab	5.9 ± 1.7 b	7.7 ± 2.5 ab
Yield/vine (kg)	1.3 ± 0.8 ab	1.2 ± 0.5 b	1.4 ± 0.6 ab	1.4 ± 0.7 ab	1.7 ± 0.8 a
Cluster weight (g)	165.6 ± 99.2 b	161.4 ± 54.2 b	183.0 ± 72.0 ab	229.1 ± 84.2 a	222.5 ± 57.3 a
Berries per cluster	225 ± 50 a	192 ± 50 a	224 ± 53 a	253 ± 44 a	227 ± 79 a

Table 1. *Cont.*

	PF	BS	V	CT	C
Must composition					
TSS (°Brix) *	18.9 ± 0.2 b	18.7 ± 0.1 b	18.9 ± 0.1 b	19.6 ± 0.2 a	19.5 ± 0.2 a
Total acidity **	4.7 ± 0.0 a	4.3 ± 0.1 c	4.6 ± 0.1 b	4.5 ± 0.0 b	4.7 ± 0.1 a
pH	3.66 ± 0.01 b	3.74 ± 0.01 a	3.74 ± 0.01 a	3.74 ± 0.01 a	3.52 ± 0.01 c
Wine composition					
AS_v (vol %) ***	10.5 ± 0.1 b	10.6 ± 0.1 b	10.7 ± 0.2 b	11.3 ± 0.1 a	11.3 ± 0.1 a
Residual sugars (g L^{-1})	1.0 ± 0.0 b	1.0 ± 0.1 b	1.1 ± 0.2 b	1.6 ± 0.3 a	1.2 ± 0.2 ab
Total extract (g L^{-1})	16.5 ± 0.6 b	16.9 ± 0.2 b	17.8 ± 0.4 a	18.5 ± 0.6 a	19.0 ± 0.9 a
pH	3.61 ± 0.02 c	3.64 ± 0.04 bc	3.76 ± 0.01 a	3.69 ± 0.01 ab	3.68 ± 0.05 bc
Total acidity (g L^{-1})	4.8 ± 0.1 ab	4.9 ± 0.1 a	4.5 ± 0.1 b	4.6 ± 0.1 b	4.7 ± 0.1 ab
Volatile acidity (g L^{-1}) ****	0.5 ± 0.0 b	0.5 ± 0.0 b	0.7 ± 0.0 a	0.6 ± 0.0 a	0.6 ± 0.0 a

Data were subjected to one-way ANOVA; results are means of biological repetitions ± standard deviation. Means labeled with different letters (a, b, or c) within a row differed significantly according to Tukey's test at $p \leq 0.05$. * TSS: total soluble solids; ** total acidity is expressed as g L^{-1} tartaric acid equivalents; *** AS_v: alcoholic strength by volume; **** volatile acidity is expressed as g L^{-1} acetic acid equivalents; PF: preflowering defoliation; BS: after berry set defoliation; V: veraison defoliation; CT: cluster thinning at veraison; C: untreated control.

3.2. Aroma Composition of Maraština Wines

A total of 96 volatile aroma compounds were identified and quantified in the 15 wines made using the five treatments. These compounds were grouped into 10 chemical classes. The terpenes and C_{13} norisoprenoids are carriers of varietal aromas (Table 2). Fermentation aromas include esters, higher alcohols, other alcohols, aldehydes, fatty acids, volatile phenols, lactones, and other compounds. Although some aroma compounds were present only in trace amounts, their suppressor or enhancer contributions to the overall wine aroma were very important. The odor activity values with corresponding odor detection thresholds and aroma descriptors of each compound analyzed are listed in the Supplementary Materials (Tables S1 and S2).

Table 2. The concentrations of varietal aroma compounds of Maraština wines subjected to defoliation and cluster-thinning treatments.

Compound (μg L^{-1})	PF	BS	V	CT	C
α-Pinene	0.89 ± 0.07 b	0.50 ± 0.06 c	1.53 ± 0.07 a	1.45 ± 0.15 a	0.64 ± 0.04 c
Tetrahydrolinalool	7.86 ± 0.45 ab	5.53 ± 0.46 c	5.75 ± 0.32 bc	7.97 ± 1.23 a	8.52 ± 1.08 a
Ethyl linalyl acetal	0.05 ± 0.03 b	0.08 ± 0.01 ab	0.17 ± 0.20 ab	0.09 ± 0.03 ab	0.30 ± 0.01 a
cis-Linalool oxide, furan	0.18 ± 0.03 b	0.26 ± 0.04 b	0.14 ± 0.02 b	0.17 ± 0.05 b	0.46 ± 0.07 a
Linalool	14.98 ± 2.35 b	12.27 ± 0.95 b	12.35 ± 2.14 b	20.40 ± 0.82 a	15.29 ± 0.81 b
Terpinen-4-ol	0.78 ± 0.24 c	1.60 ± 0.41 b	0.66 ± 0.30 c	3.33 ± 0.28 a	0.77 ± 0.06 c
Hotrienol	2.74 ± 0.29 a	0.80 ± 0.06 b	2.39 ± 0.09 a	2.67 ± 0.28 a	0.78 ± 0.20 b
trans-β-Farnesene	0.72 ± 0.17 c	0.55 ± 0.05 c	2.11 ± 0.31 b	2.62 ± 0.22 a	0.53 ± 0.01 c
cis-β-Farnesene	0.95 ± 0.04 a	1.00 ± 0.08 a	1.00 ± 0.09 a	0.92 ± 0.03 a	1.07 ± 0.09 a
α-Farnesene	0.89 ± 0.02 a	0.94 ± 0.06 a	0.93 ± 0.09 a	0.91 ± 0.12 a	1.08 ± 0.06 a
2,6-dimethyl-7-octene-2,6-diol	4.32 ± 0.56 a	4.04 ± 0.41 a	2.50 ± 0.12 b	2.36 ± 0.12 b	5.23 ± 0.83 a
α-Terpineol	1.16 ± 0.24 a	1.50 ± 0.36 a	1.39 ± 0.29 a	1.31 ± 0.16 a	1.22 ± 0.10 a
Citronellol	12.02 ± 0.62 b	12.61 ± 1.74 b	12.31 ± 2.12 b	19.26 ± 3.05 a	15.87 ± 2.17 ab
Nerol	0.57 ± 0.07 a	0.50 ± 0.13 a	0.44 ± 0.06 a	0.42 ± 0.03 a	0.49 ± 0.08 a
ε-Fenchene	0.92 ± 0.11 a	0.90 ± 0.04 a	1.10 ± 0.11 a	1.03 ± 0.09 a	1.03 ± 0.11 a
Geraniol	3.56 ± 0.41 b	3.25 ± 0.11 b	3.59 ± 0.40 b	4.53 ± 0.38 a	3.61 ± 0.17 b
6,7-dihydro-7-hydroxylinalool	4.18 ± 0.01 b	4.24 ± 0.14 b	4.36 ± 0.25 b	4.51 ± 0.33 b	5.23 ± 0.28 a
Geranyl acetate	2.64 ± 0.09 a	2.55 ± 0.04 a	2.68 ± 0.14 a	2.42 ± 0.17 a	2.78 ± 0.29 a
8-Hidroxylinalool	5.93 ± 0.88 a	9.83 ± 0.45 ab	13.89 ± 1.04 a	12.60 ± 3.88 a	7.21 ± 0.43 b
Farnesol	3.61 ± 0.45 c	6.52 ± 0.46 a	5.07 ± 0.09 b	4.53 ± 0.19 bc	5.21 ± 0.65 b
Neric acid	2.57 ± 0.25 a	2.40 ± 0.09 a	2.50 ± 0.04 a	3.78 ± 1.66 a	2.62 ± 0.26 a
1,8-Terpin	0.35 ± 0.04 d	0.70 ± 0.08 bc	0.90 ± 0.08 ab	0.43 ± 0.11 cd	1.13 ± 0.18 a
Neralidol	0.66 ± 0.02 a	0.70 ± 0.06 a	0.82 ± 0.05 a	0.73 ± 0.05 a	0.70 ± 0.13 a
∑Terpenes	72.52 ± 3.41 b	73.34 ± 2.73 b	78.58 ± 4.63 b	98.44 ± 8.52 a	81.76 ± 2.59 b
TDN *	0.72 ± 0.16 a	0.63 ± 0.03 a	0.61 ± 0.10 a	0.74 ± 0.17 a	0.65 ± 0.16 a

Table 2. *Cont.*

Compound (μg L^{-1})	PF	BS	V	CT	C
β-Damascenone	3.61 ± 0.44 a	2.38 ± 0.40 a	2.43 ± 0.25 a	3.10 ± 1.23 a	3.46 ± 0.66 a
Dihydroactinidiolide	0.83 ± 0.06 a	1.16 ± 0.22 a	0.82 ± 0.20 a	0.93 ± 0.18 a	1.02 ± 0.09 a
$\sum C_{13}$-norisoprenoids	5.15 ± 0.33 a	4.17 ± 0.60 a	3.86 ± 0.12 a	4.77 ± 1.55 a	5.13 ± 0.68 a

Data were subjected to one-way ANOVA; results are means of three biological repetitions ± standard deviation. Means labeled with different letters (a, b, c, or d) within a row differed significantly according to Tukey's test at $p \leq 0.05$. PF: preflowering defoliation; BS: after berry set defoliation; V: veraison defoliation; CT: cluster thinning at veraison; C: untreated control; * TDN: 1,1,6-trimethyl-1,2-dihydronaphthalene.

3.2.1. Varietal Volatile Composition of Maraština Wine

The CT wines had the most linalool and citronellol: (20.40 ± 0.82) μg L^{-1} and (19.26 ± 3.05) μg L^{-1}, respectively. Those were the most abundant terpene compounds in Maraština wines. In addition, CT significantly increased the concentrations of terpinen-4-ol, *trans*-*β*-farnesene, and geraniol. Consequently, the greatest total sum of terpenes was in CT at 98.44 ± 8.52 μg L^{-1}. There were no significant differences among defoliation treatments (PF, BS, and V) and C in linalool, citronellol, or geraniol concentration or in total sum of terpenes. Defoliation reduced terpene compounds; the lowest total sum of terpene compounds (72.52 ± 3.41 μg L^{-1}) was in PF wines.

Among defoliation treatments, PF wines had a significantly lower concentration of linear sesquiterpene farnesol and 1,8-terpin, although no difference was seen between PF and CT wines in those compounds. Defoliation at berry set led to wines with a significantly higher level of farnesol (6.52 ± 0.46 μg L^{-1}) among all treatments, while a significant increase in terpinen-4-ol (1.60 ± 0.41 μg L^{-1}) in BS wines was seen when defoliation treatments were compared. BS wines had significantly lower concentrations of hotrienol and *α*-pinene, but had no difference from untreated C. Treatments at veraison (V and CT) had significantly more terpenic hydrocarbon *α*-pinene. Significantly more *cis*-linalool oxide and 6,7-dihydro-7-hydroxylinalool were in wines of the untreated C. The treatments did not differ in concentrations of individual and total C_{13} norisoprenoid compounds.

3.2.2. Fermentation Volatile Composition of Maraština Wine

Wines treated at veraison, CT and V had significantly higher levels of the most abundant ester, isoamyl acetate, and total sum of esters than the other treatments (Table 3). CT wines had significantly higher levels of six individual esters (isobutyl acetate, ethyl lactate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl furoate, and diethyl succinate) than V treatments. Ten individual esters and the total sum of esters reached a minimum in BS wines, with isobutyl acetate, isoamyl acetate, 2-phenylethyl acetate, ethyl butanoate, ethyl-2-methylbutanoate, ethyl-3-methylbutanoate, ethyl-2-hydroxy-3-methylbutanoate, ethyl hexadecanoate, ethyl-3-hydroxyhexanoate, and diethyl succinate being significantly less abundant than in C wines.

Table 3. The concentration of fermentation aromas in Maraština wines subjected to defoliation and cluster-thinning treatments.

Compound (μg L^{-1})	PF	BS	V	CT	C
Isobutyl acetate	22.18 ± 1.60 c	16.62 ± 0.47 d	24.30 ± 1.06 c	27.89 ± 0.95 b	31.37 ± 1.89 a
Ethyl butanoate	165.94 ± 9.33 c	145.79 ± 2.41 d	210.23 ± 3.08 a	221.89 ± 2.52 a	187.91 ± 5.45 b
Ethyl 2-methylbutanoate	4.48 ± 0.39 a	3.39 ± 0.22 b	2.09 ± 0.08 c	4.51 ± 0.26 a	4.43 ± 0.26 a
Ethyl 3-methylbutanoate	9.55 ± 0.44 b	7.78 ± 0.81 bc	6.12 ± 0.64 c	12.06 ± 0.89 a	12.16 ± 0.57 a
Isoamyl acetate	955.88 ± 37.78 b	650.86 ± 20.22 c	1373.66 ± 110.61 a	1403.59 ± 4.97 a	1031.45 ± 22.90 b
Ethyl hexanoate	258.46 ± 24.48 c	322.49 ± 8.91 bc	440.27 ± 36.02 a	364.94 ± 28.57 b	351.05 ± 11.05 b
Hexyl acetate	15.30 ± 1.12 c	17.28 ± 0.26 c	27.15 ± 1.91 a	21.95 ± 1.33 b	14.42 ± 1.09 c
Ethyl lactate	586.12 ± 15.73 a	463.50 ± 29.66 b	358.71 ± 13.27 c	561.73 ± 36.89 a	439.51 ± 33.59 b
Ethyl 2-hydroxy-3-methylbutanoate	95.68 ± 3.49 ab	61.26 ± 1.12 d	101.22 ± 1.31 a	90.06 ± 1.97 b	81.68 ± 2.55 c
Ethyl octanoate	516.94 ± 20.99 ab	362.08 ± 32.57 c	547.21 ± 47.58 a	444.28 ± 25.05 b	438.25 ± 11.46 bc
Methyl 2-furoate	149.72 ± 0.92 a	150.84 ± 0.67 a	149.91 ± 0.10 a	149.74 ± 1.11 a	150.38 ± 0.99 a
Ethyl furoate	1.52 ± 0.19 a	1.10 ± 0.08 abc	0.80 ± 0.15 c	1.35 ± 0.25 ab	1.05 ± 0.05 bc
Ethyl decanoate	110.80 ± 7.23 b	82.87 ± 5.16 c	150.04 ± 6.29 a	145.90 ± 8.78 a	97.01 ± 3.79 bc
Diethyl succinate	267.11 ± 35.33 bc	205.91 ± 4.96 d	252.80 ± 21.43 cd	412.96 ± 19.04 a	319.80 ± 6.12 b

Table 3. *Cont.*

Compound (μg L^{-1})	PF	BS	V	CT	C
Methyl geranoate	2.67 ± 0.25 ab	2.37 ± 0.35 b	3.40 ± 0.28 a	2.52 ± 0.37 b	2.56 ± 0.05 b
2-Phenylethyl acetate	6.55 ± 0.39 bc	5.22 ± 0.30 c	8.43 ± 1.14 a	7.60 ± 0.37 ab	7.04 ± 0.43 ab
Ethyl 3-hydroxyhexanoate	1.68 ± 0.34 a	0.83 ± 0.14 b	1.62 ± 0.14 ab	2.34 ± 0.42 a	2.02 ± 0.35 a
Ethyl hexadecanoate	0.26 ± 0.04 ab	0.14 ± 0.04 c	0.08 ± 0.02 c	0.18 ± 0.01 bc	0.29 ± 0.06 a
∑Esters	3,170.85 ± 79.85 c	2,500.35 ± 80.45 d	3,658.03 ± 48.58 b	3,875.49 ± 28.09 a	3,172.37 ± 42.68 c
Isobutanol	2,555.41 ± 126.36 bc	2,225.97 ± 18.98 c	2,545.03 ± 121.52 bc	3,235.33 ± 255.55 a	2,752.73 ± 103.98 b
1-Butanol	36.84 ± 1.83 b	39.20 ± 3.78 b	40.86 ± 1.13 b	58.69 ± 1.35 a	41.10 ± 3.14 b
2-Methyl-1-butanol	9,752.26 ± 126.73 c	7,997.82 ± 85.59 d	10,115.13 ± 112.48 bc	12,452.80 ± 466.25 a	10,787.12 ± 381.73 b
Isoamyl alcohol	2,397.85 ± 232.52 c	1,609.71 ± 100.10 c	9,411.80 ± 503.45 a	7,487.32 ± 408.50 b	8,482.01 ± 420.15 a
1-Pentanol	5.36 ± 0.46 c	5.34 ± 0.03 c	8.21 ± 0.26 b	11.35 ± 2.12 a	6.32 ± 0.50 bc
3-Methyl-1-pentanol	132.52 ± 3.26 ab	124.94 ± 5.19 b	103.70 ± 4.68 c	146.56 ± 4.89 a	100.66 ± 9.17 c
1-Hexanol	0.42 ± 0.03 b	0.49 ± 0.09 b	0.60 ± 0.08 b	1.19 ± 0.23 a	0.30 ± 0.03 b
trans-3-Hexen-1-ol	22.64 ± 2.66 c	24.79 ± 0.94 c	32.00 ± 1.06 b	44.17 ± 2.94 a	28.59 ± 3.10 bc
2-Ethyl-1-hexanol	1.16 ± 0.16 a	0.98 ± 0.07 a	1.23 ± 0.14 a	1.35 ± 0.21 a	1.00 ± 0.12 a
1-Octen-3-ol	1.56 ± 0.27 b	1.37 ± 0.24 b	2.56 ± 0.18 a	2.78 ± 0.25 a	2.25 ± 0.19 a
1-Heptanol	11.62 ± 0.63 a	8.96 ± 0.19 b	4.86 ± 0.84 c	6.34 ± 0.62 c	11.69 ± 0.98 a
1-Octanol	7.88 ± 1.29 a	7.97 ± 1.11 a	10.57 ± 1.34 a	10.44 ± 1.07 a	11.08 ± 1.45 a
1-Nonanol	0.44 ± 0.14 bc	0.14 ± 0.02 c	0.75 ± 0.15 ab	0.90 ± 0.09 a	0.91 ± 0.15 a
1-Decanol	4.76 ± 0.17 a	1.86 ± 0.55 c	3.41 ± 0.44 b	4.10 ± 0.62 ab	3.76 ± 0.18 ab
Phenylethyl alcohol	5,126.42 ± 154.61 a	4,917.57 ± 69.47 a	5,055.14 ± 225.33 a	4,861.04 ± 94.37 a	5,272.90 ± 184.53 a
∑Higher alcohols	20,057.14 ± 201.50 b	16,967.12 ± 130.06 c	27,335.85 ± 709.84 a	28,324.36 ± 215.46 a	27,502.42 ± 367.05 a
1,2-Propanediol	1.65 ± 0.21 b	3.34 ± 0.38 a	1.50 ± 0.21 b	3.75 ± 0.44 a	1.93 ± 0.22 b
2,3-Butanediol	31.07 ± 1.51 ab	23.52 ± 2.63 c	32.46 ± 2.61 ab	33.72 ± 1.82 a	27.01 ± 1.17 bc
Furfuryl alcohol	2.40 ± 0.15 a	2.59 ± 0.35 a	2.68 ± 0.28 a	2.66 ± 0.32 a	2.46 ± 0.31 a
Benzyl alcohol	8.03 ± 1.35 b	7.81 ± 1.73 b	10.65 ± 0.72 ab	11.79 ± 1.61 a	7.75 ± 0.57 b
1,4-Butanediol	1.31 ± 0.48 a	1.25 ± 0.16 a	0.60 ± 0.06 b	1.01 ± 0.03 ab	1.22 ± 0.13 ab
∑Other alcohols	44.45 ± 3.04 bc	38.51 ± 4.06 c	47.89 ± 3.13 ab	52.93 ± 0.48 a	40.37 ± 1.81 bc
2-Octenal	274.53 ± 19.69 b	219.22 ± 8.76 c	315.23 ± 6.75 a	280.30 ± 8.58 b	263.40 ± 12.70 b
2,4-Heptadienal	0.38 ± 0.07 ab	0.32 ± 0.04 b	0.45 ± 0.04 ab	0.62 ± 0.20 a	0.40 ± 0.03 ab
Furfural	1.11 ± 0.11 c	0.72 ± 0.04 d	1.41 ± 0.03 c	2.25 ± 0.21 a	1.7 ± 0.16 b
Decanal	1.45 ± 0.38 a	1.30 ± 0.06 a	1.37 ± 0.11 a	1.54 ± 0.40 a	1.61 ± 0.32 a
Benzaldehyde	8.06 ± 1.79 b	6.26 ± 0.38 b	11.91 ± 0.33 a	12.47 ± 0.70 a	6.91 ± 0.27 b
Benzeneacetaldehyde	7.42 ± 0.37 b	8.55 ± 0.29 a	6.21 ± 0.60 c	5.77 ± 0.22 c	8.48 ± 0.13 a
Neral	8.84 ± 0.08 a	7.13 ± 1.14 ab	4.11 ± 0.36 c	3.33 ± 0.83 c	6.2 ± 0.86 b
2,4-Decadienal	0.20 ± 0.03 a	0.15 ± 0.02 ab	0.08 ± 0.03 bc	0.05 ± 0.02 c	0.19 ± 0.06 a
∑Aldehydes	301.99 ± 19.32 b	243.65 ± 8.11 c	340.78 ± 5.61 a	306.33 ± 7.40 b	289.02 ± 13.77 b
Propanoic acid	3.01 ± 0.18 c	4.88 ± 0.19 a	3.84 ± 0.36 bc	4.29 ± 0.42 ab	4.03 ± 0.35 b
2-Methylpropionic acid	3.52 ± 0.40 b	2.63 ± 0.23 c	4.60 ± 0.45 a	2.79 ± 0.17 bc	3.19 ± 0.19 bc
Butanoic acid	2.71 ± 0.36 c	3.80 ± 0.11 a	3.02 ± 0.09 bc	3.17 ± 0.24 bc	3.30 ± 0.18 ab
Isovaleric acid	7.67 ± 1.37 a	3.02 ± 0.41 b	5.94 ± 1.17 ab	5.29 ± 0.52 ab	8.09 ± 1.58 a
Hexanoic acid	1,112.94 ± 103.53 b	1,315.46 ± 85.31 ab	1,493.72 ± 176.20 a	1,455.99 ± 127.64 a	1,307.05 ± 95.62 ab
Heptanoic acid	3.57 ± 0.33 b	8.95 ± 0.85 a	3.69 ± 0.20 b	8.75 ± 0.33 a	9.07 ± 0.83 a
Nonanoic acid	8.24 ± 0.16 a	7.50 ± 0.77 a	8.21 ± 0.26 a	8.45 ± 0.46 a	8.24 ± 0.43 a
Decanoic acid	864.94 ± 51.74 ab	737.23 ± 83.28 bc	863.64 ± 33.27 ab	876.59 ± 22.24 a	696.02 ± 43.03 c
∑Fatty acids	2,006.59 ± 75.06 b	2,083.47 ± 151.65 ab	2,386.66 ± 155.65 a	2,365.30 ± 126.77 a	2,038.99 ± 137.26 ab
Eugenol	0.35 ± 0.03 ab	0.14 ± 0.05 c	0.22 ± 0.07 bc	0.39 ± 0.04 a	0.49 ± 0.09 a
4-Ethylphenol	0.04 ± 0.02 a	0.06 ± 0.04 a	0.06 ± 0.03 a	0.07 ± 0.02 a	0.05 ± 0.04 a
4-Vinylguaiacol	30.54 ± 6.03 b	32.38 ± 3.01 b	35.02 ± 1.81 b	50.09 ± 0.80 a	50.66 ± 4.77 a
Vanillin	0.92 ± 0.09 bc	1.26 ± 0.14 a	0.80 ± 0.07 bc	0.75 ± 0.15 c	1.12 ± 0.16 ab
Homovanillyl alcohol	30.90 ± 6.13 a	30.91 ± 5.22 a	37.64 ± 4.46 a	37.15 ± 7.80 a	33.29 ± 6.79 a
∑Volatile phenols	62.76 ± 12.16 b	64.76 ± 4.53 b	73.74 ± 3.41 ab	88.45 ± 6.84 a	85.61 ± 3.20 a
γ-Butyrolactone	311.44 ± 30.80 c	380.71 ± 34.38 bc	392.35 ± 15.52 b	562.58 ± 27.68 a	358.31 ± 25.18 bc
γ–Hexalactone	1.73 ± 0.15 b	1.63 ± 0.43 b	2.64 ± 0.32 a	2.50 ± 0.29 ab	2.34 ± 0.41 ab
γ-Octalactone	0.33 ± 0.04 a	0.36 ± 0.01 a	0.40 ± 0.04 a	0.41 ± 0.02 a	0.37 ± 0.06 a
γ-Nonalactone	5.52 ± 0.39 ab	5.07 ± 0.33 b	6.34 ± 0.65 ab	6.57 ± 0.22 ab	6.94 ± 1.02 a
γ–Decalactone	1.54 ± 0.16 b	1.59 ± 0.13 b	1.79 ± 0.19 b	2.24 ± 0.15 a	1.89 ± 0.15 ab
δ-Decalactone	2.11 ± 0.22 a	2.03 ± 0.02 a	1.96 ± 0.12 a	2.17 ± 0.13 a	2.17 ± 0.29 a
γ-Undecalactone	0.58 ± 0.10 a	0.62 ± 0.04 a	0.56 ± 0.09 a	0.66 ± 0.07 a	0.58 ± 0.06 a
∑Lactones	323.24 ± 30.85 c	392.00 ± 33.76 bc	406.04 ± 16.59 b	577.13 ± 28.07 a	372.59 ± 26.69 bc
2-Pentylfuran	228.35 ± 11.34 a	224.63 ± 2.79 a	201.88 ± 14.39 a	213.08 ± 17.82 a	207.61 ± 18.96 a
6-Methyl-5-hepten-2-one	114.17 ± 4.70 a	89.90 ± 5.40 b	62.49 ± 4.16 c	113.50 ± 14.41 a	117.64 ± 4.10 a
Acetoin	4.07 ± 0.22 c	13.20 ± 1.64 a	6.16 ± 0.75 bc	7.04 ± 0.50 b	5.03 ± 0.99 bc
Acetylfurane	1.13 ± 0.20 a	1.03 ± 0.14 a	1.10 ± 0.14 a	1.20 ± 0.21 a	1.12 ± 0.06 a
∑Other compounds	110.92 ± 15.03 b	107.11 ± 7.81 b	124.90 ± 6.52 ab	145.05 ± 6.58 a	129.67 ± 4.89 ab

Data were subjected to one-way ANOVA; results are means of three biological repetitions ± standard deviation. Means labeled with different letters (a, b, c, or d) within a row differed significantly according to Tukey's test at $p \leq 0.05$. PF: preflowering defoliation; BS: after berry set defoliation; V: veraison defoliation; CT: cluster thinning at veraison; C: untreated control.

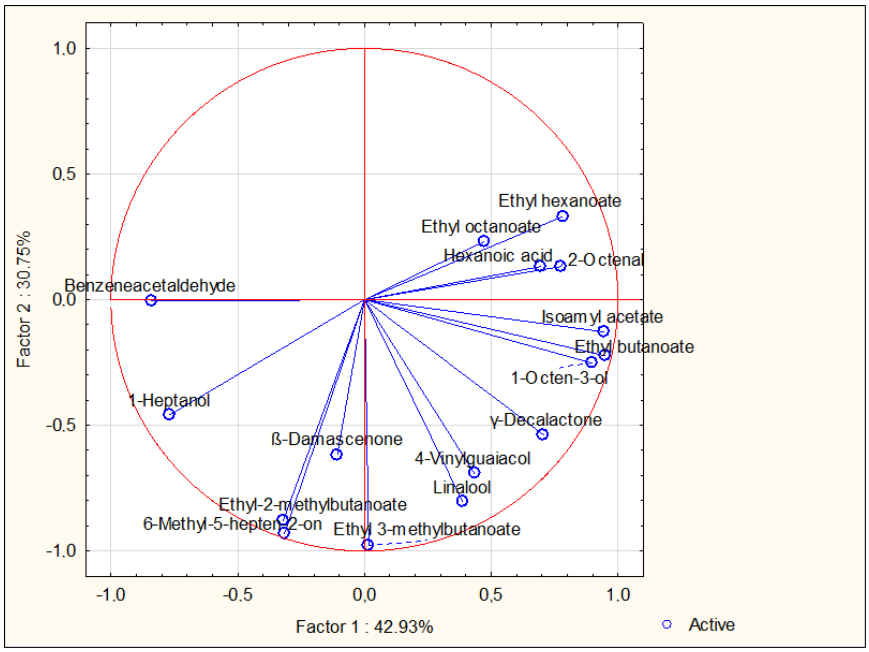
The most abundant higher alcohol in Maraština wines was 2-methyl-1-butanol regardless of treatment. Concentrations of this compound ranged from 7,997.82 ± 85.59 μg L^{-1} in BS wines to 12,452.80 ± 466.25 μg L^{-1} in CT wines. Wines of early defoliation treatments (PF and BS) had significantly lower concentrations of 3-methyl-1-butanol, isoamyl alcohol, 1-octen-3-ol, and total sum of higher alcohols. CT wines had a significantly higher concentration of 1-hexanol, *trans*-3-hexen-1-ol, isobutanol, 1-butanol, and 1-pentanol.

The V wines contained significantly more total aldehydes, particularly 2-octenal. In contrast, BS wines had the lowest concentrations of these compounds. Wines from the two veraison treatments did not differ in their benzaldehyde or benzeneacetaldehyde concentration. Both had a significantly higher concentration of benzaldehyde and a lower concentration of benzeneacetaldehyde in comparison to other treatments. The PF wines differed significantly in the total sum of fatty acids compared to the V and CT treatments. In general, the greatest proportion of fatty acids was found in V wines. Delaying the time of defoliation increased the total amount of fatty acids in the wines, reflecting the trend of the most abundant hexanoic acid among them. These wines also had the greatest proportion of 2-methylpropionic acid. Defoliation generally led to significantly lower total volatile phenols and the most abundant among them, 4-vinylguaiacol, which increased with the later defoliation, while the highest concentrations were in CT and C wines. The concentration of γ-butyrolactone reached a maximum of 562.58 $\pm$ 27.68 μg L^{-1} in CT wines. A total sum of lactones followed this trend. Among other compounds, significantly higher concentrations of acetoin were seen in BS wines, contrary to other treatments that did not differ from untreated C. The lowest concentration of C_{13} norisoprenoid ketone 6-methyl-5-hepten-2-one was in V wines.

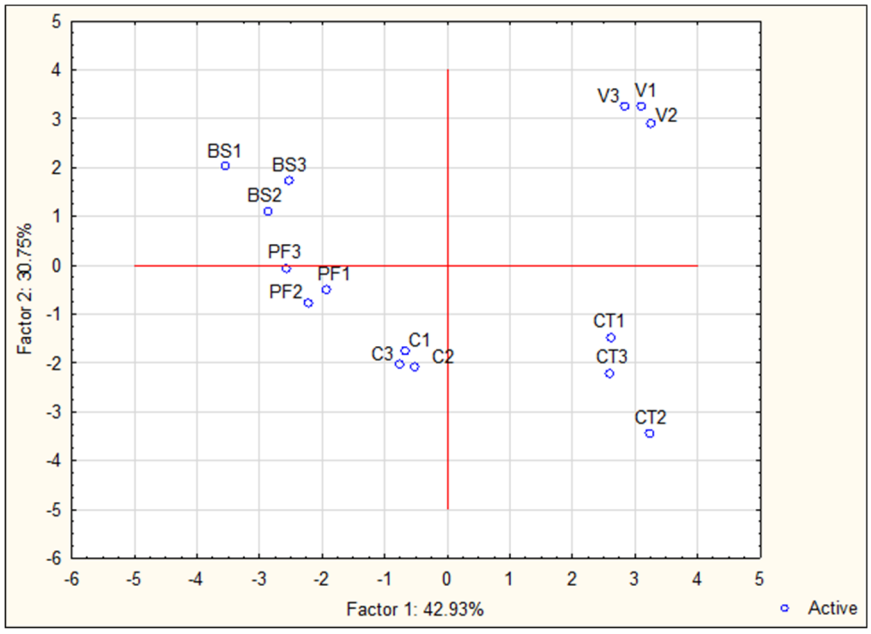
3.3. Odor Activity Values of Volatile Compounds in Maraština Wine

A total of 16 volatile compounds out of 96 identified in the Maraština wines were present at concentrations above their thresholds with an OAV > 1, and contributed to the aroma of the wines. Fourteen compounds had OAV > 1 in all wines, regardless of treatment: β-damascenone, isoamyl acetate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl octanoate, 1-octen-3-ol, 1-heptanol, 2-octenal, benzeneacetaldehyde, hexanoic acid, γ-decalactone, and 6-methyl-5-hepten-2-one. Compounds with the greatest OAV across all treatments were ethyl octanoate (273.61), carrier of pineapple, pear, and floral notes; and 2-octenal (105.08), carrier of herbal and nutty notes. PF wines differed from the others in having higher OAV values for β-damascenone than ethyl hexanoate. Linalool had an OAV > 1 in wines from PF, CT, and untreated C, while 4-vinylguaiacol had an OAV > 1 in CT and C.

The data for 16 volatile variables with OAV > 1 were processed using principal component analysis (PCA) to differentiate among wines from the five different canopy-management treatments according to their volatile compounds. In a projection of 16 volatile variables that defined the principal components F1 and F2, the first two principal components with eigenvalues > 1 explained 73.7% of the variance. The contribution of F1 was 42.93%, and of F2, 30.75% (Figure 2A). According the PCA, wines of different treatments were clearly separated (Figure 2B). Wine sample replicates grouped together for all five canopy-management treatments. V and CT wines were located on the positive side of PC1 and were characterized by 11 OAV values: linalool, isoamyl acetate, four ethyl esters (ethyl hexanoate, ethyl octanoate, ethyl butanoate, and ethyl-3-methylbutanoate), 2-octenal, hexanoic acid, 4-vinylguaiacol, and γ-decalactone. Nine compounds belonged to floral, tropical fruity, sweet, spicy, or nutty aroma odor classes [17], with only two from the green and mineral class, 1-octen-3-ol and hexanoic acid (Flavornet, http://www.flavornet.org/flavornet.html, accessed on 28 February 2022). Veraison samples were located in different quadrants: V1, V2, and V3 in the first; and CT1, CT2, CT3 in the second. Early defoliation treatments (BS and PF) had little or no influence on wine OAV values compared to control wines, all of which were located on the negative side of PC1. Those wines correlated with five OAVs: 6-methyl-5-hepten-2-one, ethyl-2-methylbutanoate, β-damascenone, 1-heptanol, and benzeneacetaldehyde, compounds associated with floral, vegetable, pome, and citrus fruit characteristics. Control and preflowering wines (C1, C2, C3, PF1, PF2, PF3) were located in the third quadrant of the plot, while berry set wines (BS1, BS2, BS3) were in the fourth quadrant. PCA confirmed that the canopy-management treatment affected the volatile profiles of the wine samples.



(**A**)



(**B**)

Figure 2. PCA performed using the concentrations of 16 volatile compounds with OAV > 1 discriminated among wine samples produced from five canopy-management treatments. (**A**) Projections of 16 volatile characteristics based on latent PC factors 1 and 2, which together explained 73.68% of total variability. (**B**) Principal component analysis (PCA) of 15 wines (C1, C2, C3: control; V1, V2, V3: defoliation at veraison; CT1, CT2, CT3: cluster thinning; PF1, PF2, PF3: defoliation at preflowering; and BS1, BS2, BS3: defoliation at berry set) on the plane defined by the first two principal components.

4. Discussion

The lowest yield per vine and cluster weight was found in BS vines, and not those cluster-thinned at an intensity of 35%, as expected. It is probable that early source removal of six basal leaves immediately after berry set reduced assimilate supply and pressured the vine to select a certain number of berries that would ripen properly [5]. The lack of a significant decrease in yield, despite the low number of clusters left on the vine after

cluster thinning, was due to self-compensation increasing the grape cluster and berry weight [18,19]. Those larger berries could decrease the skin-to-pulp ratio and aroma compound concentrations [18].

Regulation of yield with cluster-thinning or defoliation practices depends on vintage [5,19,20], agroecological conditions of location, and variety [19,21,22]. Despite studies describing the impact of defoliation or cluster thinning on aromatic compounds in berries, there are little data concerning their effect on the physiochemical and aroma composition of wine.

Defoliation reduced berry TSS, final alcohol content, and pH, and increased the total acidity of Maraština wines, with the greatest effect with early defoliation treatments (BS and PF). Terpene and C_{13} norisoprenoid compounds are important carriers of varietal aroma. In this study, defoliation at intensity of six basal leaves, regardless of the timing, reduced the total concentration of terpenes in Maraština wines compared to the untreated C. Despite use of the same training system (Guyot) in this and in an experiment conducted on Malvazija Istarska [23], the reported beneficial impact of early defoliation on linalool, citronellol, nerol, and geraniol was missing. Among the three defoliation treatments, the most individual and total sum of terpene compounds was in V wines, as reported previously for Sauvignon Blanc with 50% or 100% (six leaves) intensity of basal defoliation [6]. Defoliation early in the season had a strong impact on temperature and increase in evaporative and respiration rate of berries [24]. It could be that heat stress during herbaceous stage of berries led to a delay in the onset of accumulation of those compounds and an increase in the degradation and volatilization of already-synthetized compounds [25,26]. Previous research placed the peak of terpene synthesis at two weeks after veraison, and the hypothesis that defoliation at veraison would increase certain terpenes was partly confirmed [27].

The CT wines had the highest total sum of terpenes, with the most abundant being linalool, terpinen-4-ol, *trans*-β-fernesene, and geraniol. Significantly, the increases in linalool and geraniol in CT wines while others remained constant may have been an indicator of a fully mature grape [28]. One of the most important norisoprenoids is β-damascenone, a carrier of sweet, fruity, floral, and honey aromatic notes. In white wines, the perception threshold of β-damascenone was 0.05 µg L^{-1}, as in hydroalcoholic solution (10–12%, v/v, water/ethanol mixture) [29]. In our study, wines of all treatments had high concentrations of this compound. The esters that carry floral and fruity aromas in wine are yeast-derived compounds. CT wines had the highest concentrations of isoamyl acetate, isobutyl acetate, ethyl lactate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl furoate, diethyl succinate, and total sum of esters. The positive effect of cluster thinning on the overall concentration of esters was reported previously in Ribolla Gialla wines [8]. Similar to terpenes, an increase in esters could not be attributed only to a lower crop load, as we saw the minimum of esters in the BS wines that also had a lowest yield. It was probably a consequence of the enhancement of ripening after the cluster thinning.

The Maraština wines from early defoliation treatments had fewer higher alcohols, contrary to previous reports for Istrian Malvasia [9], which could be explained by a special viticultural condition in certain vintages. V wines did not differ from C in the total sum of higher alcohols, nor in isobutanol or isoamyl alcohol, as found previously for Sauvignon Blanc and Riesling from Continental Croatia [3]. Higher alcohols are the result of sugar or deamination and decarboxylation of certain amino acids during fermentation by the Ehrlich pathway.

Only V wines contained significantly more total aldehydes, particularly 2-octenal, a compound that is considered as one of are markers for oxidative deterioration of wine [30]. Wines from the two veraison treatments had a significantly higher concentration of benzaldehyde (almond) and a lower concentration of benzeneacetaldehyde (2-phenylacetaldehyde; rose) in comparison to other treatments.

CT wines had peak values for 7 among 10 aroma compound classes, with a significant increase in the total sum of terpenes and esters. Cluster thinning improved the aromatic maturity of Maraština wines by enhancing grape ripeness. The odor activity values (OAVs)

of volatile compounds were calculated to gain insight into perceptible changes in the concentrations of volatile compounds in wine caused by five different vineyard treatments. According the odor thresholds and description of each aroma compound reported in the literature (Table S1), wines of Maraština have a fruity character. Ethyl octanoate and 2-octenal were two most odor-potent compounds regardless of treatment. A predominance of β-damascenone in PF wines, compared to ethyl hexanoate in other wines, could be important due to its role as an enhancer of other compounds in the wine matrix. CT wines had a more complex aromatic profile, as all 16 compounds in those had an OAV > 1, while in wines from the defoliation treatments, linalool and 4-vinylguaiacol were <1. Previously, high OAV values for linalool and β-damascenone were reported in the aroma of greenhouse-dried Maraština grapes [31].

5. Conclusions

A total of 96 individual volatile compounds were identified and quantified in 15 wines from five different canopy manipulation treatments. Among varietal aromas, terpenes (23) and norisoprenoids (3) were measured. Among fermentation aromas, esters (18), higher alcohols (15), other alcohols (5), aldehydes (8), fatty acids (8), volatile phenols (5), lactones (7), and other compounds (4) were measured. The canopy-manipulation treatment significantly affected the aroma profile of Maraština wines. Early defoliation treatments had a dampening effect on the total concentrations of terpene, esters, higher alcohols, and aldehydes in wines compared to treatments at veraison. Cluster thinning of Maraština at an intensity of 35% at veraison led to wines higher in alcohol, terpenes, esters, higher alcohols, other alcohols, volatile phenolic compounds, lactones, and other compounds. CT treatment stimulated production of varietal aroma compounds: four individual terpenes (terpinen-4-ol, linalool, *trans*-β-farnesen, and geraniol). An odor activity value analysis showed that cluster thinning improved the quality of Maraština wines by increasing the concentrations of 16 compounds: linalool, β-damascenone, isoamyl acetate, ethyl butanoate, ethyl-2-methylbutanoate, ethyl-3-methylbutanoate, ethyl hexanoate, ethyl octanoate, 1-octen-3-ol, 1-heptanol, 2-octenal, benzeneacetaldehyde, hexanoic acid, 4-vinylguaiacol, γ-decalactone, and 6-methyl-5-hepten-2-one, the carriers of floral and fruity aromas. Cluster thinning led to an improved quality of Maraština wines without a negative impact on yield, while defoliation in our specific region under Mediterranean climate conditions was the least risky canopy technique. In this context, further research on different intensities and timings of cluster thinning is required to understand the effect of this management technique on improvement of the wine quality in a Mediterranean climate.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app12147327/s1, Table S1. Odor description and odor threshold (ODT) of 73 aroma compounds detected in 'Maraština' wines; Table S2. Odor activity values (OAV) for volatile compounds in the wine of 'Maraština' from five different canopy-management treatments: preflowering (PF), after berry set (BS), and veraison (V) defoliation; cluster thinning without defoliation; 35% clusters thinned in veraison (CT); and untreated control (C). Refs. [32–58] are cited in Supplementary Materials.

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References

1. Pastore, C.; Santo, S.D.; Zenoni, S.; Movahed, N.; Allegro, G.; Valentini, G.; Filippetti, I.; Tornielli, G.B. Whole plant temperature manipulation affects flavonoid metabolism and the transcriptome of grapevine berries. *Front. Plant Sci.* **2017**, *8*, 929. [CrossRef] [PubMed]
2. Young, P.R.; Eyeghe-Bickong, H.A.; du Plessis, K.; Alexandersson, E.; Jacobson, D.A.; Coetzee, Z.; Deloire, A.; Vivier, M.A. Grapevine plasticity in response to an altered microclimate: Sauvignon Blanc modulates specific metabolites in response to increased berry exposure. *Plant Physiol.* **2016**, *170*, 1235–1254. [CrossRef] [PubMed]
3. Kozina, B.; Karoglan, M.; Herjavec, S.; Jeromel, A.; Orlic, S. Influence of basal leaf removal on the chemical composition of Sauvignon Blanc and Riesling wines. *J. Food Agric. Environ.* **2008**, *6*, 28.
4. Kok, D. Influences of pre- and post-veraison cluster thinning treatments on grape composition variables and monoterpene levels of *Vitis vinifera* L. cv. Sauvignon Blanc. *J. Food Agric. Environ.* **2011**, *9*, 22–26.
5. Sivilotti, P.; Falchi, R.; Herrera, J.C.; Škvarč, B.; Butinar, L.; Lemut, M.S.; Bubola, M.; Sabbatini, P.; Lisjak, K.; Vanzo, A. Combined effects of early season leaf removal and climatic conditions on aroma precursors in Sauvignon Blanc grapes. *J. Agric. Food Chem.* **2017**, *65*, 8426–8434. [CrossRef]
6. Yue, X.; Ma, X.; Tang, Y.; Wang, Y.; Wu, B.; Jiao, X.; Zhang, Z.; Ju, Y. Effect of cluster zone leaf removal on monoterpene profiles of Sauvignon Blanc grapes and wines. *Food Res. Int.* **2020**, *131*, 109028. [CrossRef]
7. Šuklje, K.; Antalick, G.; Coetzee, Z.; Schmidtke, L.M.; Česnik, H.B.; Brandt, J.; du Toit, W.J.; Lisjak, K.; Deloire, A. Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of *Vitis vinifera* L. cv. Sauvignon Blanc wine. *Aust. J. Grape Wine Res.* **2014**, *20*, 223–233. [CrossRef]
8. Škrab, D.; Sivilotti, P.; Comuzzo, P.; Voce, S.; Degano, F.; Carlin, S.; Arapitsas, P.; Masuero, D.; Vrhovšek, U. Cluster thinning and vineyard site modulate the metabolomic profile of Ribolla Gialla base and sparkling wines. *Metabolites* **2021**, *11*, 331. [CrossRef]
9. Bubola, M.; Rusjan, D.; Lukić, I. Crop level vs. leaf removal: Effects on Istrian Malvasia wine aroma and phenolic acids composition. *Food Chem.* **2020**, *312*, 126046. [CrossRef]
10. Maletić, E.; Pejić, I.; Kontić, J.K.; Zdunić, G.; Preiner, D.; Šimon, S.; Andabaka, Ž.; Mihaljević, M.Ž.; Bubola, M.; Marković, Z.; et al. Ampelographic and genetic characterization of Croatian grapevine varieties. *Vitis. J. Grapevine Res.* **2015**, *54*, 93–98.
11. Coombe, B.G. Growth Stages of the Grapevine: Adoption of a System for Identifying Grapevine Growth Stages. *Aust. J. Grape Wine Res.* **1995**, *1*, 104–110. [CrossRef]
12. Office International de la Vigne et du Vin. *Compendium of International Methods of Wine and Must Analysis—Volume I*; Organisation Internationale de la Vigne et du Vin (OIV): Paris, France, 2015.
13. López, R.; Aznar, M.; Cacho, J.; Ferreira, V. Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *J. Chromatogr. A* **2002**, *966*, 167–177. [CrossRef]
14. Korenika, A.M.J.; Preiner, D.; Tomaz, I.; Jeromel, A. Volatile profile characterization of Croatian commercial sparkling wines. *Molecules* **2020**, *25*, 4349. [CrossRef]
15. Maslov, L.; Tomaz, I.; Žulj, M.M.; Jeromel, A. Aroma characterization of predicate wines from Croatia. *Eur. Food Res. Technol.* **2017**, *243*, 263–274. [CrossRef]
16. TIBCO Software Inc. Data Science Workbench, Version 14. 2020. Available online: http://tibco.com (accessed on 2 March 2022).
17. Flavornet. Available online: http://www.flavornet.org/flavornet.html (accessed on 28 February 2022).
18. Condurso, C.; Cincotta, F.; Tripodi, G.; Sparacio, A.; Giglio, D.M.L.; Sparla, S.; Verzera, A. Effects of cluster thinning on wine quality of Syrah cultivar (*Vitis vinifera* L.). *Eur. Food Res. Technol.* **2016**, *242*, 1719–1726. [CrossRef]
19. Palliotti, A.; Cartechini, A. Cluster thinning effects on yield and grape composition in different grapevine cultivars. *Acta Hortic.* **2000**, *512*, 111–120. [CrossRef]
20. Keller, M.; Mills, L.J.; Wample, R.L.; Spayd, S.E. Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. *Am. J. Enol. Vitic.* **2005**, *56*, 91–103.
21. Poni, S.; Casalini, L.; Bernizzoni, F.; Civardi, S.; Intrieri, C. Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. *Am. J. Enol. Vitic.* **2006**, *57*, 397–407.
22. Hernandez-Orte, P.; Concejero, B.; Astrain, J.; Lacau, B.; Cacho, J.; Ferreira, V. Influence of viticulture practices on grape aroma precursors and their relation with wine aroma. *J. Sci. Food Agric.* **2015**, *95*, 688–701. [CrossRef]
23. Bubola, M.; Lukić, I.; Radeka, S.; Sivilotti, P.; Grozić, K.; Vanzo, A.; Bavčar, D.; Lisjak, K. Enhancement of Istrian Malvasia wine aroma and hydroxycinnamate composition by hand and mechanical leaf removal. *J. Sci. Food Agric.* **2019**, *99*, 904–914. [CrossRef]

24. Ferrari, V.; Disegna, E.; Dellacassa, E.; Coniberti, A. Influence of timing and intensity of fruit zone leaf removal and kaolin applications on bunch rot control and quality improvement of Sauvignon blanc grapes, and wines, in a temperate humid climate. *Sci. Hortic.* **2017**, *223*, 62–71. [CrossRef]
25. Scafidi, P.; Pisciotta, A.; Patti, D.; Tamborra, P.; Di Lorenzo, R.; Barbagallo, M.G. Effect of artificial shading on the tannin accumulation and aromatic composition of the Grillo cultivar (*Vitis vinifera* L.). *BMC Plant Biol.* **2013**, *13*, 175. [CrossRef] [PubMed]
26. Rienth, M.; Torregrosa, L.; Luchaire, N.; Chatbanyong, R.; Lecourieux, D.; Kelly, M.T.; Romieu, C. Day and night heat stress trigger different transcriptomic responses in green and ripening grapevine (*Vitis vinifera*) fruit. *BMC Plant Biol.* **2014**, *14*, 108. [CrossRef] [PubMed]
27. Friedel, M.; Frotscher, J.; Nitsch, M.; Hofmann, M.; Bogs, J.; Stoll, M.; Dietrich, H. Light promotes expression of monoterpene and flavonol metabolic genes and enhances flavour of winegrape berries (*Vitis vinifera* L. cv. Riesling). *Aust. J. Grape Wine Res.* **2016**, *22*, 409–421. [CrossRef]
28. Baron, M.; Prusova, B.; Tomaskova, L.; Kumsta, M.; Sochor, J. Terpene content of wine from the aromatic grape variety "Irsai Oliver" (*Vitis vinifera* L.) depends on maceration time. *Open Life Sci.* **2017**, *12*, 42–50. [CrossRef]
29. Pineau, B.; Barbe, J.C.; van Leeuwen, C.; Dubourdieu, D. Which impact for β-damascenone on red wines aroma? *J. Agric. Food Chem.* **2007**, *55*, 4103–4108. [CrossRef]
30. Escudero, A.; Asensio, E.; Cacho, J.; Ferreira, V. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. *Food Chem.* **2002**, *77*, 325–331. [CrossRef]
31. Budić-Leto, I.; Humar, I.; Gajdoš Kljusurić, J.; Zdunić, G.; Zlatić, E. Free and bound volatile aroma compounds of 'Maraština' grapes as influenced by dehydration techniques. *Appl. Sci.* **2020**, *10*, 8928. [CrossRef]
32. Plotto, A.; Margaría, C.A.; Goodner, K.L.; Goodrich, R.; Baldwin, E.A. Odour and flavour thresholds for key aroma components in an orange juice matrix: Terpenes and aldehydes. *Flavour Fragr. J.* **2004**, *19*, 491–498. [CrossRef]
33. Ribereau-Gayon, P.; Boidron, J.N.; Terrier, A. Aroma of Muscat grape varieties. *J. Agric. Food Chem.* **1975**, *23*, 1042–1047. [CrossRef]
34. Fariña, L.; Villar, V.; Ares, G.; Carrau, F.; Dellacassa, E.; Boido, E. Volatile composition and aroma profile of Uruguayan Tannat wines. *Food Res. Int.* **2015**, *69*, 244–255. [CrossRef]
35. Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032. [CrossRef]
36. Sánchez-Palomo, E.; Trujillo, M.; García Ruiz, A.; González Viñas, M.A. Aroma profile of Malbec red wines from La Mancha region: Chemical and sensory characterization. *Food Res. Int.* **2017**, *100*, 201–208. [CrossRef] [PubMed]
37. Slaghenaufi, D.; Luzzini, G.; Samaniego Solis, J.; Forte, F.; Ugliano, M. Two sides to one story—Aroma chemical and sensory signature of Lugana and Verdicchio wines. *Molecules* **2021**, *26*, 2127. [CrossRef]
38. Marais, J. Terpenes in the aroma of grapes: A review. *S. Afr. J. Enol. Vitic.* **1983**, *4*, 49–60.
39. Ferreira, V.; López, R.; Cacho, J.F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80*, 1659–1667. [CrossRef]
40. Francis, L.; Newton, J. Determining wine aroma from compositional data. *Aust. J. Grape Wine Res.* **2005**, *11*, 114–126. [CrossRef]
41. Pardo, E.; Rico, J.; Gil, J.V.; Orejas, M. De novo production of six key grape aroma monoterpenes by a geraniol synthase-engineered *S. cerevisiae* wine strain. *Microb. Cell Fact.* **2015**, *14*, 136. [CrossRef]
42. Sacks, G.L.; Gates, M.J.; Ferry, F.X.; Lavin, E.H.; Kurtz, A.J.; Acree, T.E. Sensory threshold of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and concentrations in young Riesling and non-Riesling wines. *J. Agric. Food Chem.* **2012**, *60*, 2998–3004. [CrossRef]
43. Ferreira, V.; Ortín, N.; Escudero, A.; López, R.; Cacho, J. Chemical characterization of the aroma of Grenache rosé wines: Aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *J. Agric. Food Chem.* **2002**, *50*, 4048–4054. [CrossRef]
44. Gómez-Míguez, M.J.; Cacho, J.F.; Ferreira, V.; Vicario, I.M.; Heredia, F.J. Volatile components of Zalema white wines. *Food Chem.* **2007**, *100*, 1464–1473. [CrossRef]
45. Vilanova, M.; Genisheva, Z.; Masa, A.; Oliveira, J.M. Correlation between volatile composition and sensory properties in Spanish Albariño wines. *Microchem. J.* **2010**, *95*, 240–246. [CrossRef]
46. Culleré, L.; Escudero, A.; Cacho, J.; Ferreira, V. Gas chromatography− olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *J. Agric. Food Chem.* **2004**, *52*, 1653–1660. [CrossRef] [PubMed]
47. Peinado, R.A.; Moreno, J.; Bueno, J.E.; Moreno, J.A.; Mauricio, J.C. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chem.* **2004**, *84*, 585–590. [CrossRef]
48. Gammacurta, M.; Tempere, S.; Marchand, S.; Moine, V.; de Revel, G. Ethyl 2-hydroxy-3-methylbutanoate enantiomers: Quantitation and sensory evaluation in wine. *OENO One* **2018**, *52*, 57–65. [CrossRef]
49. Piombino, P.; Moio, L.; Genovese, A. Orthonasal vs. retronasal: Studying how volatiles' hydrophobicity and matrix composition modulate the release of wine odorants in simulated conditions. *Food Res. Int.* **2019**, *116*, 548–558. [CrossRef]
50. Azcarate, S.M.; Cantarelli, M.A.; Marchevsky, E.J.; Camiña, J.M. Evaluation of Geographic Origin of Torrontés Wines by Chemometrics. *J. Food Res.* **2013**, *2*, 48. [CrossRef]
51. Moyano, L.; Zea, L.; Moreno, J.; Medina, M. Analytical study of aromatic series in sherry wines subjected to biological aging. *J. Agric. Food Chem.* **2002**, *50*, 7356–7361. [CrossRef]
52. Guadagni, D.G.; Buttery, R.G.; Okano, S. Odour thresholds of some organic compounds associated with food flavours. *J. Sci. Food Agric.* **1963**, *14*, 761–765. [CrossRef]

53. Sonni, F.; Moore, E.G.; Chinnici, F.; Riponi, C.; Smyth, H.E. Characterisation of Australian Verdelho wines from the Queensland Granite Belt region. *Food Chem.* **2016**, *196*, 1163–1171. [CrossRef]
54. Buttery, R.G.; Turnbaugh, J.G.; Ling, L.C. Contribution of volatiles to rice aroma. *J. Agric. Food Chem.* **1988**, *36*, 1006–1009. [CrossRef]
55. Cai, J.; Zhu, B.Q.; Wang, Y.H.; Lu, L.; Lan, Y.B.; Reeves, M.J.; Duan, C.Q. Influence of pre-fermentation cold maceration treatment on aroma compounds of Cabernet Sauvignon wines fermented in different industrial scale fermenters. *Food Chem.* **2014**, *154*, 217–229. [CrossRef] [PubMed]
56. Escudero, A.; Gogorza, B.; Melús, M.A.; Ortín, N.; Cacho, J.; Ferreira, V. Characterization of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *J. Agric. Food Chem.* **2004**, *52*, 3516–3524. [CrossRef] [PubMed]
57. Ferreira, V.; Jarauta, I.; Ortega, L.; Cacho, J. Simple strategy for the optimization of solid-phase extraction procedures through the use of solid–liquid distribution coefficients: Application to the determination of aliphatic lactones in wine. *J. Chromatogr. A* **2004**, *1025*, 147–156. [CrossRef] [PubMed]
58. Liu, H.; Bao, M.L.; Chen, H.L.; Li, Q. Impact of sucrose addition on the physiochemical properties and volatile compounds of "Shuangyou" red wines. *J. Food Qual.* **2017**, *2017*, 2926041. [CrossRef]