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Effects of Berry, Cluster Thinning and No-Sulfites Addition on the Sensory Quality of ‘Monastrell’ Organic Wines

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Abstract

This study investigated the impact of berry and cluster thinning on the organoleptic and chemical quality of red wines produced with no-sulfites-added production, using ‘Monastrell’ grapes cultivated under organic viticulture. The experiment was conducted in a commercial vineyard in Murcia (Spain), applying three treatments: control, bunch reduction (BR), and berry thinning (BT). Grapes were vinified under identical conditions, and the resulting wines were analyzed after three months and five years of storage. Physicochemical parameters, volatile organic compounds (VOCs), and sensory profiles were evaluated. Thinning treatments significantly increased alcohol content, reducing sugars, polyphenol index, and the concentration of key aromatic compounds. Sensory analysis revealed that wines from thinned grapes exhibited more intense toasted, vegetal, and fruity notes, and presented greater color stability and fewer defects over time. Notably, only the control wine developed *Brettanomyces*-related off-flavors after five years. Consumer preference tests confirmed higher acceptance of BR and BT wines, based particularly on color, fruity aroma, and aftertaste. These findings suggested that thinning practices, especially bunch thinning, offer a cost-effective strategy to improve wine quality and stability in no-sulfites-added winemaking, reducing the risk of spoilage and enhancing consumer satisfaction.

Keywords: *Vitis vinifera*; sensory profile; volatile organic compounds; *Brettanomyces* sp.; wine quality



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1. Introduction

Wine is a complex beverage resulting from the interaction of volatile and non-volatile components, which can affect its aroma, taste and mouthfeel [1]. The sensory quality of wine depends mainly on its aroma, one of the most appreciated attributes by consumers, that requires the study of volatile compounds [2]. The main volatile compounds responsible for wine aroma are alcohols, aldehydes, esters, ketones, terpenes and lactones, which can be found in the original fruit or formed during the fermentation or wine aging [3]. Some of these compounds play an important role in the aroma and sensory characteristics of wine even at low concentrations; meanwhile, other volatile compounds present at higher concentration have a low sensory impact [4]. The type and concentration of volatile compounds, and therefore the quality of wine produced, can be influenced by several factors such as the environment, grape cultivar, winemaking, aging or agricultural practices [3].

However, the concept of wine quality is very complex and depends on several factors, including the organoleptic characteristics of the product, the production process, price, origin and consumer tastes and preferences [5]. From a technical point of view, many chemical parameters significantly influence wine quality, including alcoholic degree, total acidity, SO₂ levels, reducing sugars and phenolic compounds [6]. Specifically, acidity and phenolic compounds influence aging potential, with wines high in acidity having high aging potential [7].

Food consumption habits are constantly evolving due to various factors, ranging from demographic and cultural changes to technological advances and an increased focus on health and well-being. This explains the recent interest in quality, safe food, with consumers demanding products that are free from chemicals or additives [8]. This trend has also been observed among wine consumers and producers, given that wines can contain high concentrations of sulfites, ranging from 5 to 200 mg/L [9]. Therefore, increased consumer awareness and restrictions by regulatory authorities suggest reducing or replacing sulfites to avoid overexposure [10]. The European Union regulates the use of sulfites as a food preservative and has included them in the list of substances or products causing allergies or intolerances when concentrations exceed 10 mg/kg or 10 mg/L in terms of the total SO₂ and this must be declared on food labelling [11].

In the winemaking process, sulfur dioxide (SO₂) is mainly used for its antioxidant, antiseptic and antimicrobial properties. It can be used at various stages, for instance at grape reception in the cellar to bottling [12]; using SO₂ during the reception of grapes at the winery prevents unwanted fermentation, while different doses can be used during alcoholic fermentation to impact yeast nitrogen metabolism and protect yeast against stressful conditions [13,14]. During bottling, SO₂ prevents undesirable secondary fermentation in the bottle and the growth of various microorganisms, including lactic acid bacteria, acetic acid bacteria, and yeast such as *Brettanomyces* spp., which affects the flavor of the wine and impact consumer acceptability [15,16]. Moreover, the addition of SO₂ not only limits the formation of acetaldehyde, but also fixes acetaldehyde, thereby protecting or enhancing the aroma of wine during aging [17]. However, excessive use of SO₂ will produce unpleasant flavors and aromas, compromising the quality of the wine as well as endangering the health of sensitive consumers [18]. The volatile profile (VOC) of wine changes during storage, which influences the aroma and quality of the wine. Although SO₂ is the most widely used preservative agent in wine, the concentration of SO₂ added influences the organoleptic characteristics of the wine obtained. This is due to the important role of SO₂ in reducing microbiological growth and oxidation, both of which negatively affect the VOC profile [10].

Thus, producing sulfite-free wines poses a significant technological challenge to this sector. Therefore, it is necessary to search for alternative preservatives and innovative technologies that are harmless to health and that can replace the use of SO₂ in the wine-making process, such as natural compounds and physical methods. Specifically, dimethyl dicarbonate (DMDC), bacteriocins, phenolic compounds and lysozymes are natural compounds that have been investigated in recent years [15,19,20]. Physical methods that have been employed to regulate the use of SO₂ in winemaking include pulsed electric fields, ultrasounds, ultraviolet and high pressure [21–24]. While these strategies have some beneficial effects on wine, they also have certain disadvantages. For example, some strategies can cause negative changes in color and aroma [25], be less effective against bacteria than yeast [26–29] and lead to wine haze formation [30].

The production of high-quality wine depends on the use of premium grapes, which in turn depends on the agronomic management of the vineyard. Among the cultural practices, bunch compactness is a trait specific to grapevines that affects disease susceptibility, berry ripening, and other characteristics of wine and table grapes [31]. A previous study showed

that reducing the number of berries in each bunch by 25% or 50% increased the total soluble solids, total anthocyanin concentrations and total phenolic content of the ‘Monastrell’ cultivar [32]. Other authors demonstrated the effectiveness of both berry and cluster thinning methods in reducing grape yield in *Vitis vinifera* cv. Syrah [33], thereby improving wine quality in both cases compared to the control group. Notably, berry thinning was the treatment that had less impact on reducing crop yield. Berry or bunch thinning has been shown to affect the composition of the fruit, increasing color, flavor and sugar concentration [33,34]. However, despite producing an optimal wine composition and quality, berry thinning can have a great economic impact on agronomic practices.

‘Monastrell’ (*Vitis vinifera* L.) is a grapevine variety extensively cultivated in viticultural regions with semi-arid Mediterranean climates. Its presence is particularly prominent in southeastern Spain, where it represents the dominant cultivar in Denominations of Origin such as Jumilla, Bullas, Yecla, Alicante, and Valencia, accounting for approximately 80% of the vineyard surface in these areas [35]. Internationally, ‘Monastrell’ is also grown in France (where it is referred to as Mourvèdre), California (under the name Mataró), as well as in Chile and Australia, demonstrating adaptability to similar climatic conditions.

The present study therefore aimed to evaluate the impact of thinning practices (bunches or berries) on the physicochemical, sensory and aromatic qualities of red wine (after 3 months and 5 years of aging) produced from ‘Monastrell’ grapes, without adding sulfites during the elaboration, and storage, process.

2. Materials and Methods

2.1. Experimental Design

The experiment was carried out during the 2019 growing season in a commercial plot located in Cehegín (Murcia, Spain, 38°00′08.5″ N, 1°45′10.4″ W). The vineyard is located along the margins of a south-facing ephemeral watercourse, where alternating lithological deposits of gravel and clay have formed a clay-loam soil profile with embedded coarse fragments (gravels and stones) present both at the surface and throughout the subsurface horizons. The experiment was conducted in a 43-year-old vineyard comprising *Vitis vinifera* cv. ‘Monastrell’ vines grafted onto 110 R rootstock, with a within-row spacing of 2.30 × 2.30 m. The vineyard was managed according to organic viticultural practices for the cultivar and the region (four sulfur treatments (cumulus 4%) and two copper treatments (Scoltyflow 4%)) and was cultivated in a Mediterranean semi-arid climate with hot, dry summers and scarce annual rainfall. The weather conditions of the vintage for 2019 season were as follows: annual rainfall of 426.60 mm and average temperature of 15.08 °C. These data were obtained from a weather station located close to the experimental site (38°6′39.35″ N, 1°40′59.06″ W).

Three different blocks, each comprising 250 vines per method with 3 replicates, were randomly selected: control, cluster thinning or bunch reduction (BR) and berry thinning (BT). For the berry thinning method, approximately 50% of the berries were manually (freehand) removed from each cluster, on the same day (third week of June), when the grapes were pea-sized, corresponding to stage E–L 31 in the modified E–L system [36]. Cluster thinning was carried out during veraison (the first week of August) by removing 50% of the clusters from each vine, manually (scissors). The control vineyard was grown without any intervention regarding the fruit quantity.

The grapes were manually harvested on 8 October 2019, when they had reached their optimum ripening stage (with a potential ethanol content of around 14–14.5%). The grapes were weighed to determine the total yield of each evaluated method, and the results were expressed as total production and yield (kg/vine). The grapes were then

transported by truck to the Jorge Piernas Bodegas y Viñedos, located in Mula (Murcia, Spain) for vinification.

2.2. Winemaking

Grapes for the control, bunch reduction and berry thinning were selected according to their optimal health status before entering the tanks. The grapes were destemmed and transferred to temperature-controlled 500-litre tanks, where fermentation began naturally on the second day. The wines were produced under identical conditions and, following the same fermentation protocol and temperature regime (12 d at 26 ± 2 °C). The nine tanks (three each treatment) were pumped over and pressed on the same day. Afterwards, the wine was transferred to 225 L barrels (Tonnellerie Quintessence Bordeaux, Beychac et Caillau, France) with the same light toasting light which were purchased in 2016. The nine barrels rested in the same place at the same height, and equal aging conditions were used with a maximum aging temperature of 18 °C and 70% relative humidity. Once alcoholic and malolactic fermentation had finished, the wine remained in the barrels from 30 October 2019 to 23 April 2020. The wines were then bottled directly from the barrel without the addition of sulfur. The bottles were stored at room temperature and used for physicochemical, volatile organic compound and sensory analyses after three months and five years.

2.3. Determination of Physicochemical Parameters

Alcohol content (acquired alcoholic degree and total alcoholic strength (% vol)), pH, total acidity (g/L tartaric acid), volatile acidity (g/L acetic acid), acetic acid (g/L), reducing sugars (g/L glucose), and total polyphenol index (UA) were determined according to the methods of the International Organisation of Vine and Wine [37]. Analyses were conducted in triplicate.

2.4. Volatile Organic Compounds (VOCs)

The volatile compounds in the samples were extracted by adding 10 mL of wine to a hermetic vial with polypropylene cap and PTFE (polytetrafluoroethylene)/silicone septa, together with 1 g of NaCl, using headspace solid-phase microextraction (HS-SPME). A50/30 mm DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA, USA) measuring 1 cm in length was used for the extraction. The samples were exposed for 40 min at 40 °C, with constant agitation at (250 rpm using a Shimadzu AOC-6000 Plus autosampler (Shimadzu Corporation, Kyoto, Japan), and then, were analyzed in a Shimadzu GC2030 chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA), coupled with a Shimadzu TQ8040 NX mass spectrometer detector. The column and chromatographic conditions were performed according to Pérez-López et al. [38].

Volatile compounds were identified by comparing: (i) experimentally obtained mass spectra and those available in the NIST 17 Mass Spectral database, and (ii) the linear retention indices, which were calculated using the C6-C20 n-alkane mix (Sigma-Aldrich, Steinheim, Germany). Only compounds with a similarity of >90% in their spectra were considered as correct hits; the linear retention index threshold was also set at ± 10 units. Calibration curves were prepared for each analyzed compound using pure standards (Sigma-Aldrich, Madrid, Spain). Analyses were conducted in triplicate and results were expressed as mg/L.

2.5. Sensory Analysis

2.5.1. Descriptive Sensory Analysis

A trained panel consisting of ten panelists (four males and six females) from the Miguel Hernández University (CIAGRO-UMH) performed the descriptive sensory analysis of wines. The lexicon used was based on Piernas et al. [32] with some modifications. The panel

analyzed the following descriptors: (i) odor: alcohol, fruity, floral, vegetable, spicy, animal, toasted and defects; (ii) flavor: alcohol, fruity, floral, vegetable, spicy, animal, toasted, sweet, sour, bitter, astringent and defects; (iii) global: aftertaste and imbalances; (iv) visual: cleanliness, color and layer. The panelists used a 0–10-point scale for evaluation, where 0 represented extremely low intensity or absence and 10 represented extremely high intensity, with increments of 0.5. The analyses were conducted in a standardized tasting room with natural and white light, at a temperature of 22 ± 1 °C. To prevent color influencing sensory perception, the olfactory-gustatory and overall phases were first evaluated using a black cup. The visual phase was then evaluated using a transparent cup. Panelists were provided with water and breadsticks to cleanse their palates between samples.

2.5.2. Affective Sensory Analysis

An affective sensory analysis was conducted with a consumer panel three months (in 2020) and five years (in 2025) after bottling, using a sample group of 95 and 102 consumers, respectively, according to Issa-Issa et al. [39]. The consumers' panel was recruited from Miguel Hernandez University of Elche (UMH), Spain, and consisted of 50% men and 50% women aged between 22 and 67 years. The wines were served at 15 ± 2 °C, coded with 3-digit random numbers and presented one at a time with a 5-min gap between samples. Water and unsalted crackers were provided to panelists between samples for palate cleansing. In each questionnaire, consumers were asked to rate their level of satisfaction with different descriptors of each wine sample using a hedonic scale from 0 to 10 points (0 = dislike extremely, 5 = neither like or dislike, 10 = like extremely). The descriptors evaluated were: (i) visual: color; (ii) odor: alcohol, fruity and toasted; (iii) flavor: alcohol, fruity, toasted, sweetness, sourness, bitterness, astringency and aftertaste; (iv) global; (v) preference, evaluated using the ranking test.

2.6. Statistical Analysis

All the data included in this study are the mean of three replicates for the physicochemical and volatile analyses, ten for the descriptive sensory data, and 95 and 102 consumers for the affective sensory data in 2020 and in 2025, respectively. All the data were subjected to ANOVA and Tukey's tests. Furthermore, a ranking test (Friedman) was used to determine the preferred samples in the affective sensory study. XLSTAT Premium 2016 (Addinsoft, New York, NY, USA) was used to perform the statistical analyses. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Effect of Thinning Method on Total Production (kg), Yield (kg/vine) and Berry Size (mm) of Wine Grape

As expected, the total production of the control vines was significantly higher than that of the vines subjected to thinning methods. Specifically, the total production of the control vines was 2168 kg, followed by the vines subjected to BR (1560 kg) and BT (1388 kg). These differences represented a reduction in total production of 36% and 28% in the BT and BR vines, respectively, compared to the control vines. Consequently, vine yield was significantly higher in the control vines (2.89 kg/vine) than in those subjected to bunch reduction (2.08 kg/vine) or berry thinning (1.85 kg/vine), with no significant differences observed between the two thinning methods.

3.2. Effect of Thinning on Physicochemical Composition of Wine

After analyzing the physicochemical parameters of the wines in the year of their production, statistically significant differences were found in six of the nine determinations (Table 1). Samples that underwent thinning (BR and BT) had a higher acetic acid content

(~75% and 100% more than the control samples, in the BR and BT samples, respectively). Furthermore, thinning also increased the content of reducing sugars and increased the alcohol content by 1%. Both parameters are directly related, as it is logical to assume that an initial sample with a higher number of reducing sugars has greater potential to transform these into alcohol, resulting in a higher final alcohol content. Similarly, the total polyphenol index was ~10% higher in the BR and BT samples than in the control sample.

Table 1. Physicochemical determinations in wine samples made using control grapes (C), obtained by bunch reduction (BR) and by berry thinning (BT).

Determinations	ANOVA [†]		2020			2025		
	2020	2025	C	BR	BT	C	BR	BT
Total acidity (g/L tartaric acid)	NS	NS	6.57	6.38	6.7	6.61	6.35	6.72
Volatile acidity (g/L acetic acid)	*	NS	0.59 b [‡]	0.76 ab	0.82 a	0.88	0.88	0.91
Acetic acid (g/L)	*	NS	0.30 c	0.53 b	0.61 a	0.86	0.76	0.84
Reducing sugars (g/L glucose)	*	NS	<1.5 b	<1.5 b	2.1 a	2.8	2.4	2.9
Acquired alcoholic degree (% vol)	*	**	13.57 b	14.50 a	14.75 a	13.92 c	14.28 b	14.57 a
Total alcoholic strength (% vol)	*	**	13.61 b	14.52 a	14.78 a	13.95 c	14.30 b	14.62 a
Total polyphenol index (UA)	**	NS	43 b	50 a	52 a	49	50	51
pH	NS	*	3.35	3.43	3.39	3.39 b	3.46 a	3.45 a
Total sulfur (mg/L)	NS	NS	28	31	27	10	<10	<10

[†] NS: not significant; * and **, significant differences $p \leq 0.05$ and 0.01 , respectively. [‡] Different letters, within the same determination and year, correspond to statistically significant differences $p \leq 0.05$ (Tukey test).

Statistically significant differences were only found in the acetic acid and reducing sugars content when considering only the samples in which thinning was carried out. For both parameters, the sample in which berry thinning was performed obtained higher values than the sample in which bunch reduction was performed (0.53 and 0.61 g/L for volatile acidity in BR and BT, respectively; and <1.5 and 2.1 g/L for reducing sugars in BR and BT, respectively) (Table 1). Implementing thinning methods, whether bunch- or berry-thinning, had no impact on total acidity content (expressed as tartaric acid), pH, and total sulfur (Table 1).

After five years of storage, statistically significant differences were found in both the acquired and total alcohol content, as well as in the pH (Table 1). Samples that had undergone thinning showed higher values for both alcohol content and pH. These results are consistent with those obtained in the first sampling. However, comparing the results across the different years of the study reveals that storage time reduces the differences between treatments by equalizing the acidity and reducing sugars and polyphenol content of the samples, parameters that were determinant in the first sampling.

3.3. Volatile Compounds

When analyzing the aromatic profile of the wines under study, 32 volatile compounds were identified and quantified (Table 2). The largest family of compounds was esters (21 compounds), followed by alcohols (7 compounds), aldehydes (3 compounds), and carboxylic acids (1 compound). However, despite being the largest group, esters did not contribute the highest concentration to the wine under study. In this regard, alcohols (~9.5 mg/L) had the highest concentration, followed by esters (~5.0 g/L), carboxylic acids (~0.022 g/L), and aldehydes (~0.018 g/L) (Table 3). As expected, ethanol was the compound found in the highest concentration in the samples under study (~7.2 mg/L), followed by diethyl succinate (~1.5 mg/L), 3-methyl-1-butanol (~1.5 mg/L), ethyl acetate (~1.3 mg/L), phenyl ethyl alcohol (~1.1 mg/L), and ethyl octanoate (~1.0 mg/L). The presence of compounds such as ethyl decanoate, ethyl hexanoate, ethyl hexadecanoate,

ethyl lactate, and 2-methyl-1-butanol was also significant, with average concentrations ranging from ~0.1 to 0.3 mg/L. The rest of the compounds presented concentrations below 0.1 mg/L.

Table 2. Identification of volatile compounds of wine samples.

RT (min)	Compound	Chemical Family	Sensory Descriptor	RI (Exp)	RI (Lit)
2.152	Ethanol	Alcohols	Alcohol	491	489
2.781	Ethyl acetate	Esters	Aniseed, pineapple	604	600
2.899	2-Methyl-1-propanol	Alcohols	Fruity, wine	648	650
4.273	3-Methyl-1-butanol	Alcohols	Oil, whiskey	735	737
4.346	2-Methyl-1-butanol	Alcohols	Toasted, fruity, whiskey	741	740
4.738	Ethyl isobutyrate	Esters	Citrus, strawberry	749	750
5.085	Butyl acetate	Esters	Banana, fruity, green	805	807
5.301	2,3-Butanediol	Alcohols	Fruity, creamy, fatty	781	780
5.791	Ethyl butyrate	Esters	Banana, pineapple, sweet	802	800
6.120	Ethyl lactate	Esters	Buttery, fruity	815	815
7.413	Ethyl 2-methylbutyrate	Esters	Fruity, green, sweet	852	850
7.579	Ethyl isovalerate	Esters	Apple	858	858
8.191	1-Hexanol	Alcohols	Green, wood, sweet	871	869
8.495	Isoamyl acetate	Esters	Banana, pear, sweet	878	876
9.945	γ -Butyrolactone	Esters	Candy	920	918
14.915	Ethyl hexanoate	Esters	Apple, banana, pineapple	996	998
21.840	Nonanal	Aldehydes	Fruity, fat, floral	1103	1101
22.166	Phenyl ethyl alcohol	Alcohols	Honey, floral, pink	1108	1112
25.663	Ethyl succinate	Esters	Fruity, apple, floral	1175	1176
26.495	Octanoic acid	Carbox. Acid	Oily, cheese	1182	1180
26.933	Diethyl succinate	Esters	Fruity, chocolate, earthy	1189	1188
28.122	Ethyl octanoate	Esters	Fruity, floral	1195	1193
31.454	2-Phenyl ethyl acetate	Esters	Honey, fruity, floral	1224	1224
33.931	Ethyl nonanoate	Esters	Oily, fruity, nutty	1290	1294
38.979	Ethyl decanoate	Esters	Grapes, oily, pear	1382	1380
39.640	Dodecanal	Aldehydes	Herbal, wax, floral	1422	1420
40.464	Ethyl isopentyl succinate	Esters	Not found	1438	1436
41.367	Isoamyl octanoate	Esters	Coconut, green, fruity	1442	1446
47.670	Ethyl dodecanoate	Esters	Green, fruity, floral	1561	1563
48.439	1-Tetradecanal	Aldehydes	Oily, incense, musk	1620	1618
54.328	Ethyl tetradecanoate	Esters	Wax, soap	1795	1790
57.353	Ethyl hexadecanoate	Esters	Wax	1970	1975

RT: retention time. RI (exp.): experimental retention index. RI (lit): literature retention index [40].

Statistically significant differences were found in 6 of the 11 major compounds identified (Table 3). Wines obtained through thinning had higher concentrations of volatile ethanol than control wines. However, this result contrasts with the total alcohol content, which was also statistically higher in the samples where thinning was performed. The same result was observed for ethyl acetate concentrations (wines made with thinning had higher concentrations). Ethyl acetate is a highly valued compound in wines, when is in lower concentration, due to its fruity aroma, which is easily identifiable as a tropical fruit aroma, mainly pineapple. When this compound is found in values above 18 mg/L, it is sensorially perceived as a defect [41]. However, the opposite occurred with ethyl octanoate and ethyl decanoate compounds, for which lower concentration values were observed in the BR and BT samples in both years of the study. These compounds are sensorially characterized by fruity descriptors (Table 2).

Table 3. Analysis of volatile compounds (mg/L) of wine samples made using control grapes (C), obtained by bunch reduction (BR) and by berry thinning (BT).

Compound	ANOVA [†]		2020			2025		
	2020	2025	C	BR	BT	C	BR	BT
Ethanol	**	**	6.531 b [‡]	7.779 a	7.234 ab	6.204 b	7.025 a	7.220 a
Ethyl acetate	***	**	1.235 b	1.403 a	1.484 a	1.180 b	1.352 a	1.405 a
2-Methyl-1-propanol	**	NS	0.096 a	0.095 a	0.082 b	0.080	0.075	0.094
3-Methyl-1-butanol	NS	NS	1.538	1.639	1.843	1.488	1.520	1.505
2-Methyl-1-butanol	NS	NS	0.155	0.208	0.085	0.135	0.130	0.120
Ethyl isobutyrate	***	NS	0.022 a	0.020 ab	0.015 b	0.015	0.016	0.018
Butyl acetate	NS	NS	0.005	0.006	0.005	0.010	0.012	0.008
2,3-Butanediol	NS	NS	0.065	0.174	0.277	0.095	0.120	0.111
Ethyl butyrate	NS	NS	0.014	0.014	0.052	0.034	0.020	0.057
Ethyl lactate	**	*	0.145 b	0.166 b	0.281 a	0.125 c	0.156 b	0.170 a
Ethyl 2-methylbutyrate	***	NS	0.014 a	0.011 ab	0.009 b	0.022	0.034	0.026
Ethyl isovalerate	NS	NS	0.014	0.013	0.014	0.010	0.008	0.014
1-Hexanol	**	NS	0.018 b	0.035 a	0.039 a	0.008	0.015	0.022
Isoamyl acetate	NS	NS	0.070	0.123	0.152	0.054	0.074	0.068
γ-Butyrolactone	NS	NS	0.018	0.024	0.031	0.025	0.023	0.019
Ethyl hexanoate	NS	NS	0.235	0.195	0.180	0.284	0.222	0.241
Nonanal	NS	NS	0.010	0.011	0.011	0.009	0.007	0.010
Phenyl ethyl alcohol	NS	NS	1.003	1.169	1.169	0.095	0.080	0.088
Ethyl succinate	NS	NS	0.051	0.103	0.181	0.069	0.094	0.105
Octanoic acid	NS	NS	0.023	0.019	0.013	0.031	0.026	0.023
Diethyl succinate	NS	NS	1.631	1.539	1.749	1.520	1.581	1.632
Ethyl octanoate	**	*	1.259 a	0.762 b	0.605 b	1.204 a	0.990 b	0.925 b
2-Phenyl ethyl acetate	NS	NS	0.011	0.015	0.015	0.010	0.008	0.011
Ethyl nonanoate	**	NS	0.004 a	0.002 b	0.001 b	0.002	0.001	0.004
Ethyl decanoate	***	*	0.418 a	0.212 b	0.175 b	0.480 a	0.251 b	0.226 b
Dodecanal	***	NS	0.007 a	0.000 b	0.000 b	0.004	0.002	0.004
Ethyl isopentyl succinate	NS	NS	0.025	0.021	0.026	0.023	0.031	0.018
Isoamyl octanoate	***	NS	0.007 a	0.005 b	0.004 b	0.004	0.007	0.006
Ethyl dodecanoate	**	NS	0.010 a	0.004 b	0.004 b	0.004	0.005	0.005
1-Tetradecanal	***	NS	0.003 a	0.000 b	0.000 b	0.004	0.002	0.007
Ethyl tetradecanoate	NS	NS	0.012	0.009	0.013	0.018	0.010	0.012
Ethyl hexadecanoate	**	NS	0.144 b	0.205 a	0.188 ab	0.120	0.182	0.167
Family								
Alcohols	**	**	9.405 b	11.099 a	10.729 a	8.105 b	8.965 a	9.160 a
Esters	NS	NS	5.344	4.850	5.182	5.213	5.077	5.134
Aldehydes	**	NS	0.020 a	0.011 b	0.011 b	0.017	0.011	0.021
Carboxylic acids	NS	NS	0.023	0.019	0.013	0.031	0.026	0.023
Total	**	*	14.792 b	15.979 a	15.935 a	13.366 b	14.079 a	14.338 a

[†] NS: not significant; *, ** and ***, significant differences $p \leq 0.05$, 0.01 and 0.001, respectively. [‡] Different letters, for the same compound and year, correspond to statistically significant differences $p \leq 0.05$, according to the Tukey test.

3.4. Sensory Analysis

A descriptive sensory analysis of the freshly bottled samples (2020) revealed that the wines made from grapes that underwent thinning (BR and BT) had a more toasted flavor, vegetal, alcohol, and spicy notes, as well as higher sourness and bitterness. Additionally, the BT sample stood out for its more intense vegetal aroma and a longer aftertaste (Table 4). Five years after bottling (in 2025), the samples prepared using thinning grapes (BR and BT) maintained these differences, showing a stronger alcoholic odor and flavor, as well as greater color intensity. In contrast, the control sample presented a defect after 5 years

that the sensory judges identified as a Brett aroma and flavor. This defect was not present in either the BR or in the BT wines. In summary, it can be concluded that the thinning treatment carried out on the vines, whether in the form of grains or clusters, resulting in wines with enhanced aromatic intensity (alcoholic, vegetal, toasted, and fruity notes), increased sourness, and improved resistance to color degradation and sensory defects. That is, thinning improves the preservation of wine in terms of its organoleptic properties, regardless of whether it is done on the vine or the grains.

Table 4. Descriptive sensory analysis of wine samples made using control grapes (C), obtained by bunch reduction (BR) and by berry thinning (BT).

Descriptors	ANOVA [†]		2020			2025		
	2020	2025	C	BR	BT	C	BR	BT
Odor								
Alcohol	***	**	3.3 b [‡]	3.7 ab	4.2 a	4.3 b	5.5 a	5.3 a
Fruity	NS	NS	5.9	5.1	5.3	4.1	4.2	3.9
Floral	NS	NS	1.8	1.5	2.0	2.1	1.8	1.9
Vegetable	**	**	1.8 b	1.6 b	2.6 a	2.1 b	2.2 b	3.0 a
Spicy	NS	NS	1.5	1.8	1.8	1.4	1.3	1.5
Animal	NS	**	0.9	1.0	1.2	0.9 b	1.5 a	1.0 b
Toasted	***	***	1.2 c	3.7 a	2.2 b	1.5 b	3.5 a	3.6 a
Defects	NS	***	0.1	0.1	0.1	2.0 a	0.6 b	0.5 b
Flavor								
Alcohol	**	**	2.7 b	3.6 a	3.8 a	3.2 b	4.3 a	4.5 a
Fruity	NS	*	4.9	5.1	4.3	4.5 b	5.3 a	5.2 a
Floral	NS	NS	2.1	2.0	2.1	2.0	1.8	1.7
Vegetable	**	**	1.6 b	2.3 a	2.3 a	2.2 b	2.4 a	2.6 a
Spicy	***	NS	0.8 b	1.7 a	1.6 a	0.4	0.3	0.4
Animal	NS	*	0.3	0.7	0.7	0.4 b	1.1 a	0.9 a
Toasted	***	**	1.7 b	2.6 a	2.3 ab	1.0 b	2.2 a	2.1 a
Sweetness	**	**	2.4 b	3.3 a	2.7 ab	2.3 b	2.9 a	3.0 a
Sourness	***	***	2.3 b	4.3 a	3.8 a	1.5 c	3.2 a	2.5 b
Bitterness	**	NS	1.9 b	2.3 a	2.2 a	2.0	2.2	2.1
Astringency	NS	NS	1.9	2.5	2.0	2.0	2.1	2.2
Defects	NS	**	0.1	0.2	0.1	1.8 a	0.5 c	1.2 b
Global								
Aftertaste	***	*	3.7 b	3.2 b	4.8 a	4.2 b	5.1 a	5.3 a
Imbalances	NS	NS	0.3	0.4	0.2	0.1	0.2	0.1
Visual								
Cleanliness	NS	NS	9.0	9.6	9.5	9.2	9.3	9.1
Color	NS	*	8.8	9.1	9.3	6.8 b	7.3 a	7.5 a
Layer	***	NS	6.5 b	7.5 a	6.8 b	5.8	6.0	6.1

[†] NS: not significant; *, ** and ***, significant differences $p \leq 0.05$, 0.01 and 0.001, respectively. [‡] Different letters, within the same determination, correspond to statistically significant differences $p \leq 0.05$, according to the Tukey test.

Consumers reported differences in 4 of the 12 sensory descriptors used (Table 5). Wines made from thinned grapes were preferred for their color (~7.15 in BR and BT, compared to ~6.30 for the control sample). Similarly, this was the case for fruity aroma, aftertaste, and overall satisfaction. The BR and BT samples presented greater satisfaction among consumers than the control samples. Additionally, when consumers were asked to rank samples based on their preference (Ranking test), in 2020 the most preferred samples were those from thinning; and, in 2025, the bunch reduction sample stood out above the rest. Consumers mentioned leaning towards the fruity flavor (55 and 60% in 2020 and 2025, respectively), aftertaste (~50 and 45% in 2020 and 2025, respectively), and color (~22 and 26% in 2020 and 2025, respectively) of the samples.

Table 5. Affective sensory analysis ($n = 95$ in 2020 and, $n = 102$ in 2025) of wine samples made using control grapes (C), obtained by bunch reduction (BR) and berry thinning (BT).

Descriptors	ANOVA [†]		2020			2025		
	2020	2025	C	BR	BT	C	BR	BT
Visual								
Color	*	**	6.5 b [‡]	7.3 a	7.2 a	6.2 b	7.2 a	7.3 a
Odor								
Alcohol	NS	NS	5.6	5.9	5.6	4.8	5.2	5.1
Fruity	**	*	5.7 b	6.0 a	6.0 a	6.0 b	7.1 a	7.0 a
Toasted	NS	NS	5.3	5.4	5.2	5.1	5.3	5.2
Flavor								
Alcohol	NS	NS	5.0	5.1	4.8	5.4	5.2	5.4
Fruity	NS	NS	5.6	6.1	5.6	5.3	5.2	5.0
Toasted	NS	NS	5.2	5.5	5.0	5.2	5.3	5.1
Sweetness	NS	NS	5.6	6.1	5.3	5.8	5.6	5.5
Sourness	NS	NS	5.4	5.7	5.0	5.4	5.5	5.6
Bitterness	NS	NS	5.6	5.4	4.8	5.0	5.1	5.3
Astringency	NS	NS	5.5	5.2	4.8	5.1	5.2	5.0
Aftertaste	**	*	5.2 b	6.2 a	6.1 a	5.0 b	6.5 a	6.2 a
Global	*	**	5.2 b	6.1 a	6.2 a	5.5 b	7.0 a	6.8 a
Ranking test 2020	***	***	a	b	b	a	c	b

[†] NS: not significant; *, ** and ***, significant differences $p \leq 0.05$, 0.01 and 0.001, respectively. [‡] Different letters, within the same determination, correspond to statistically significant differences $p \leq 0.05$, according to the Tukey test.

4. Discussion

This is the first study to evaluate the combined influence of berry and cluster thinning on wines produced with non-added sulfites and their changes over a long-term aging. Thinning practices, such as cluster thinning and berry thinning, are designed to reduce yield with the aim of improving grape and wine quality [32,33]. Previous studies showed that cluster thinning reduced grape yield per vine by approximately 40% compared to the control [33]. Although reducing clusters by half should reduce production by half, the decrease was less because the remaining clusters tend to be heavier, which partially offset the reduction. This phenomenon is attributed to the fact that vines distribute available resources among fewer grapes [33]. Previous results showed that both the 25% and 50% berry thinning methods significantly reduced the total yield per vine in the ‘Monastrell’ variety compared to control vines [32]. In the present study berry reduction produced the lowest total production of the two thinning methods assessed; however, no significant differences were observed for yield per vine between both methods. These results contrast with those obtained by Gil et al. [33] when comparing both thinning methods in ‘Syrah’ grape variety, who observed that berry thinning had a lower impact on reducing crop yield compared to cluster thinning.

One of the results observed when analyzing the physicochemical factors was an increase in acidity in all the wine samples. In the form of H_2SO_3 , SO_2 helps to revert phenolic compounds to a stable form and contributes to modulating the available reactive quinones. However, SO_2 has some drawbacks, such as toxicity, the potential for unpleasant flavors, legal maximum limits based on its potential allergenic properties and degradation leading to the formation of sulfuric acid and increased total acidity in wine [42]. In modern winemaking, achieving the same acidity (or even higher acidity in berry thinning) alongside a higher alcohol content (and consequently, higher IPT) is advantageous, as these wines were harvested on the same day. The treatments enabled us to produce wines with a higher

alcohol content and the same or higher acidity, resulting in fresher wines with greater aging potential.

Thinning treatments had a higher IPT index, indicating higher quality wines with greater aging potential. However, after five years, the total polyphenol content also decreases slightly because tannins can also be repolymerized by H_2O_2 due to oxidation. If their amount increases excessively, they will settle at the bottom of the bottle. Indeed, if wine contains large amounts of tannins that do not condense sufficiently through oxidation, both processes can occur simultaneously at the beginning of storage period. Over longer storage periods, tannins tend to degrade, significantly reducing repolymerization and leading to reduced levels over time [43].

One of the most notable quality parameters is volatile acidity, which increased significantly in the control group (a negative aspect in wines), compared to the thinning treatments, where volatiles remained more stable. Acetic acid can be produced during alcoholic fermentation due to the metabolism of acetic acid bacteria, as well as through the additional oxidation of acetaldehyde in the bottle. In addition to being involved in many chemical reactions (e.g., the formation of pyranoanthocyanins), acetaldehyde is constantly formed by ethanol oxidation and greatly contributes to the oxidative change of wine, having the potential to become a dominant aroma over time [44]. Wines with a higher tannin content mask the aroma of undesirable volatile acidity very well compared to lighter wines with lower tannin content. In fact, numerous Protected Designation of Origin (PDO) regulations permit volatile acidity from 0.6 g/L in young wines to 1.0 g/L in aged wines, given their higher tannin content. In addition to this aroma, the sensory panel determined that, after 5 years in the bottle, the control wine had developed aromas such as Brett in the organoleptic analysis resulting in a wine that could be considered defective aromatically. The presence of Brett in wine depends on various factors, including the alcohol content. In this study, only the control wine had aromas produced by this yeast, as its alcohol content was lower, and this yeast thrives at alcohol levels below 14.0–14.5% [45]. Using grapes with high acidity of adding acids during the winemaking process can reduce *Brettanomyces* growth [46]. In the current case, the higher acidity of one of the treatments, along with a higher alcohol content, could have prevented the appearance of Brett. These yeasts are facultative anaerobes that can produce high levels of acetic acid and ethanol in an aerobic condition. They contribute to both the volatile acidity of the wine [47] and the content of volatile phenols [48]. Thus, the increase in volatile acidity in the only wine that presented Brett is understandable.

Both bunch and berry thinning increased the total content of volatile compounds in the wine samples studied. Similar results have previously been reported in other grape varieties and different thinning strategies [49,50]. This increase is closely related to sensory perception. In the descriptive sensory analysis thinning samples had more intensity of fruity descriptors. Moreover, overall acceptance was higher in these samples.

The possibility of producing wine without the use of sulfur dioxide (SO_2) is an increasingly relevant topic in modern oenology. Thinning practices have proven to be effective to produce wines without the addition of sulfites, improving their organoleptic properties and the perception by consumers. However, it must be acknowledged that the absence of this compound entails greater microbiological and oxidative risks in the winery, which many winemakers are not willing to assume. For this reason, the production of wines without added sulfites is currently a complementary approach with significant growth potential in markets that value authenticity, quality, and sustainability, but it is not yet a viable alternative to meet global wine demand.

5. Conclusions

Thinning treatments increased the concentration of volatile compounds and enhanced the sensory acceptance of the wine, with the bunch reduction treatment achieving the highest consumer ratings. Furthermore, a clear correlation between grape thinning (either at the cluster or berry stage) and the production of ‘Monastrell’ wines that are significantly less prone to develop *Brettanomyces* sensory defects. This positive outcome appears to be driven by the physiological changes induced by thinning; specifically, thinned grapes resulted in wines with higher volatile acidity and increased alcohol content compared to control samples. This suggested that the altered ‘Monastrell’ grape composition, influenced by the thinning process, created an environment that was less favorable for *Brettanomyces* development, allowing for a successful production of wines with no-sulfites-added production. Bunch thinning offered a simpler and more cost-effective approach to enhancing wine quality, given its similarly positive effects than berry thinning. To strengthen these results, it would be highly advisable to repeat this experiment using other grape varieties to evaluate whether the results are exclusive to this variety, given its high compactness, or can be extended to other varieties.

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