

## Article

# Influence of Different Alcohol Reduction Technologies on the Volatile Composition of La Mancha Tempranillo Rosé Wines

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**Abstract:** The objective of the current research was to study the effect of different alcohol reduction technologies on the chemical aromatic composition of La Mancha Tempranillo rosé wines. Volatile compounds were analysed using Gas Chromatography coupled to Mass Spectrometry (GC-MS), with previous isolation by solid phase extraction (SPE). C<sub>6</sub> compounds were the only group of varietal compounds that was modified when the total dealcoholizing process was used. According to their odor descriptor, the volatile compounds were grouped into six odorant series. The total intensity of each aromatic series was calculated by adding the OAVs of the compounds appointed to this series. All wines showed the same sequence, only modified the intensity of the principal aromatic series, mainly in total dealcoholized wines. These studied wines maintain the aromatic typicality independently from ethanol concentration, which highlights the viability of these techniques as an alternative to the traditional winemaking process, which will allow diversifying wine's actual market.

**Keywords:** dealcoholized wine; partially fermented wines; volatile compounds; GC-MS



**Citation:** Osorio Alises, M.; Sánchez-Palomo, E.; González-Viñas, M.A. Influence of Different Alcohol Reduction Technologies on the Volatile Composition of La Mancha Tempranillo Rosé Wines. *Beverages* **2023**, *9*, 63. <https://doi.org/10.3390/beverages9030063>

Academic Editors: Federica Bonello and Maurizio Petrozziello

Received: 19 May 2023

Revised: 28 June 2023

Accepted: 24 July 2023

Published: 31 July 2023



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## 1. Introduction

Wine is considered a unique and distinct product due to its relation to traditions and social and cultural aspects. However, contradictory information about wine healthiness is common in society. Although moderate consumption has always been related to potential health benefits due to its content of grape-derived phenolic compounds, excessive alcohol consumption is harmful and is associated with negative and social consequences [1].

Consumers are increasingly concerned about the impact that lifestyle choices have on their health. In this way, the role of health has become a determinant of consumers' choices for food and beverages, driving a large part of society to change its habits, including a reduction in alcohol consumption, and in consequence, increasing the demand for beverages with a low alcohol content [1–3]. This tendency has motivated manufacturers to create original non-alcoholic products with flowers and fruits [4]. Climatic conditions have been always an important factor to be taken into account in grape growth, and consequently in the production of quality wine, due to the fact that grapevines are one of the most sensitive crops to climate fluctuations [2], but they have become even more important in the last years. Among the climatic variables that have a greater impact on wine quality, we can highlight water status, solar radiation and temperature.

Climate change is being reported to have remarkable impacts on the viticultural sector because of the effects that it is producing in temperatures and precipitations [2,5–7]. Warm wine-producing regions are adversely affected by climate change as the effects of higher temperatures and low precipitations are detrimental for grapevines. One of the principal effects of increasing temperatures due to climate change have in grapevines include an early grape ripening, producing in consequence a higher sugar accumulation and a lower acidity. Moreover, the metabolic routes of synthesis of phenolic and aroma compounds are

also affected by climatic change, so in order to obtain a complete phenolic and aromatic maturity, it would be necessary to delay the harvest day [2,6]. So, in consequence, the oenologists must choose either late harvest, producing highly alcoholic degree and bitter wines; or early harvest, resulting in wines with low color density and poor aroma intensity and complexity being both options rejected by the consumers as the obtained grapes are unbalanced [2]. In this way, the oenological industry has developed different techniques to face climate change. It can be highlighted that the post-fermentative alcohol reduction techniques, such as nanofiltration [8,9], reverse osmosis [10,11], osmotic distillation or evaporative perstraction [12,13], pervaporation [14], vacuum distillation [15] and spinning cone column [16].

As the alcohol reduction process may have a negative impact on sensory properties, and therefore, it could affect the consumers' overall liking, several studies have considered the Spinning Cone Column (SCC) technique as the best and most efficient to reduce ethanol in wine thanks to its operation under vacuum conditions.

On the other hand, according to different studies, reverse osmosis and spinning cone column techniques have been reported as the more efficient in reducing alcohol techniques as an alcohol reduction of around 75% and 85% *v/v*, respectively, has been observed with no significant losses of the main aroma compounds [4].

Thus, the aim of this study was to research the influence of partial fermentation and total dealcoholization in the chemical composition and sensory profile of the aroma of La Mancha rosé Tempranillo wines.

## 2. Materials and Methods

### 2.1. Reagents and Standards

Dichloromethane and methanol were from Merck (Darmstadt, Germany), and anhydrous sodium sulfate was from Panreac (Barcelona, Spain). Ultrapure 18.2 M $\Omega$  cm water was produced from the Milli-Q purification system by Millipore (U.S.). LiChrolut EN resins were supplied by Merck (Darmstadt, Germany). Chromatographically pure standards were pursued by Sigma-Adrich (Madrid, Spain), Merck (Darmstadt, Germany), Fluka (Madrid, Spain), Lancaster (Strasbourg, France) and Firmenich (Geneva, Switzerland). Details are included in Tables 2 and 3. An alkane solution (C7-C24 Supelco, Bellefonte, PA, USA) in dichloromethane was employed in order to determine the linear retention index (RI) of each volatile compound.

### 2.2. Wines

Tempranillo rosé wines were provided by a wine cellar of Castilla-La Mancha region.

#### 2.2.1. Control Wine

At the arrival to the cellar, grapes (50,000 kg) were kept at 12 °C and processed the day after for all grapes to be treated at the same temperature. Grapes were destemmed and crushed with the addition of 100 mg/kg potassium hydrogen sulfite for protection against oxidation and microbial contamination. Musts were then submitted to controlled maceration at 18 °C for 12 h. After maceration, grapes were pressed, and the juice was subdued to the fermentation process. After that, the free run must be transferred to a stainless steel tank and inoculated with *Saccharomyces cerevisiae cerevisiae* (CECT No. 10835). The fermentation was carried out at 18 °C until density acquired a constant value of 995 g/L. The obtained wines were then racked, filtered, bottled and stored at 4 °C. All fermentations were made in duplicate in a steel tank of 25,000 L.

#### 2.2.2. Partially Fermented Wines

This wine was elaborated in the same way as the control wine, but when the alcoholic degree was about 7% *v/v*, the temperature was decreased to 0 °C in order to stop fermentation. Then, the wine was racked, filtered, bottled and stored at 4 °C. All fermentations were made in duplicate in a steel tank of 25,000 L.

### 2.2.3. Total Dealcoholized Wines by SCC

The dealcoholization process was carried out using the technique of SCC according to the conditions proposed by Sam et al., 2021 [4]. Firstly, a portion of the rosé control wine was passed, at 0.04 atm of vacuum pressure and at 28 °C of temperature, by the SCC with the aim of recovering the volatile compounds which account for approximately 1% of the total volume of wine. The dearomatized wine was then subjected to slightly higher vacuum pressure and a temperature of 38 °C to eliminate the alcohol. Finally, to obtain the dealcoholized wine, the dealcoholized and dearomatized wine is blended with the wine aromas in a proportion of 0.04% *v/v* and concentrated must is added up to a sugar concentration of 60 g/L. Then, the wines were racked, bottled and stored at 4 °C. All fermentations were made in duplicate in a steel tank of 25,000 L.

### 2.3. Conventional Analysis of Wines

In order to determine total and volatile acidity, total and free, sulfur dioxide (SO<sub>2</sub>), pH, alcohol, and residual sugar in studied wines the methods proposed by the International Organization of Vine and Wine (OIV) [17] were used.

### 2.4. Analysis of Major Volatile Compounds

The method proposed by Sánchez–Palomo et al. [18], was used to determine the major volatile compounds of wines by direct injection into HP-5890GC equipped with a FID detector using a CP-Wax-57 column (50 m × 0.25 mm i.d. × 0.25 µm). Operating conditions were: oven temperature program: 40 °C (5 min)—4 °C/min—120 °C (10 min), carrier gas He at 1 mL/min and injector and detector temperatures were 250 °C and 280 °C respectively. One µL of the sample was injected in split mode (1:15). The quantification of major volatile compounds was carried out using calibration curves for each standard at ten different concentration levels. Each standard was prepared in synthetic wine solution (5 g/L of tartaric acid, dissolved in 12% ethanol solution (*v/v*), at pH 3.5, adjusted with NaOH).

### 2.5. Analysis of Minor Volatile Compounds

In order to research minor volatile compounds of La Mancha Tempranillo wines, gas chromatography coupled with mass spectrometry (GC-MS) was used. In the first place, it was carried out an extraction of volatile compounds using the method proposed by Sánchez–Palomo et al. [18], using propylene divinylbenzene cartridges (LiChrolut EN, Merck, 0.5 g of phase). 100 mL of wine added of 40 µL of 4-nonanol (1.20 g/L) as internal standard were eluted through these cartridges with a constant flow of 1 mL/min. In order to eliminate sugars and other low-molecular-weight polar compounds, the cartridge was washed with 50 mL of milli-Q water. To elute minor volatile compounds, 10 mL of dichloromethane was used, and then a nitrogen stream was used to concentrate the organic extract to a final volume of 200 µL.

An Agilent Gas Chromatograph (model 6890 N) coupled to a Mass Selective Detector (model 5973 inert) via a BP-21 column (60 m × 0.25 mm i.d.; 0.25 µm film thickness) was employed to perform gas chromatographic analysis. One µL of the sample was injected in splitless mode and helium at a flow rate of 1 mL/min was used as carried gas. The temperature program involved 5 min at 70 °C, 1 °C/min up to 95 °C (10 min), 2 °C/min to 200 °C and held at this temperature for 40 min. The injector temperature was 250 °C. The scan mode at 70 eV and the mass range was 40–450 *m/z* were set in the mass spectrometer and the ion source temperature of 280 °C.

A series of *n*-alkanes (C7–C24, Supelco, Bellefonte, PA, USA), in dichloromethane was injected in the GC following the same conditions employed for the wine samples and were used for experimental RI calculation according to Van den Dool and Kratz [19]. MS and RI of authentic standards, NBS75K reference database were used for identification of volatile compounds.

For quantification of volatile compounds, when standards were available, calibration curves were used for each standard at eight different concentration levels. Each standard was prepared in synthetic wine solution (5 g/L of tartaric acid, dissolved in 12% ethanol solution (*v/v*), at pH 3.5, adjusted with NaOH). When authentic standards were not available, a comparison of GC-MS signals with the standard internal signal, taking into account the relative response factor equal to one, was executed to calculate the concentration of volatile compounds. In cases where authentic standards were not available.

### 2.6. Odor Activity Values (OAV)

The contribution of each volatile compound to the aroma of studied wines was determined using the odor activity value (OAV). OAV was calculated as the ratio between the concentration and the perception threshold of the individual compound found in the literature [20–22]. A possible contribution to the wine aroma was considered when OAV was higher than one.

### 2.7. Statistical Analysis

The analysis of variance (ANOVA) was carried out to establish significant differences between the results of conventional analysis and concentration of volatile compounds of Tempranillo rosé wines. A Student–Newman–Keuls test was conducted with the level of significance set at  $p < 0.05$  when significant differences were observed between samples. All statistical treatments were performed using SPSS 24.0 for Windows statistical package.

## 3. Results and Discussion

### 3.1. Conventional Analysis

The values of the physicochemical parameters obtained were within the OIV acceptable limits [17] as shown in Table 1. Excluding volatile and total acidity, there was a generally significant difference between control wines and partially fermented and total dealcoholized wines. This can be since a partial fermentation and total dealcoholizing process by SCC affects in a significant way to the physicochemical parameters. Similar results were obtained by [4,23,24] in rosé dealcoholized Pinot Noir wines.

**Table 1.** General composition of rosé Tempranillo control, partially fermented and total dealcoholized wines. Mean values and relative standard deviation ( $n = 2$ ).

	% Alcohol ( <i>v/v</i> )	Volatile Acidity (g/L Acetic Acid)	Total Acidity (g/L Tartaric Acid)	pH	Residual Sugars (g/L)	Free SO <sub>2</sub> (mg/L)	Total SO <sub>2</sub> (mg/L)
Control wine	11.44 <sup>a</sup> (0.04)	0.20 <sup>a</sup> (0.01)	4.99 <sup>a</sup> (0.02)	3.30 <sup>a</sup> (0.01)	3.95 <sup>a</sup> (0.21)	23.33 <sup>a</sup> (1.53)	42.67 <sup>a</sup> (1.15)
Partially fermented wine	7.90 <sup>b</sup> (0.17)	0.20 <sup>a</sup> (0.00)	4.93 <sup>a</sup> (0.16)	3.35 <sup>b</sup> (0.01)	56.63 <sup>b</sup> (1.77)	34.33 <sup>b</sup> (1.15)	63.33 <sup>b</sup> (1.53)
Total dealcoholized wine	0.52 <sup>c</sup> (0.03)	0.23 <sup>a</sup> (0.03)	4.91 <sup>a</sup> (0.04)	3.34 <sup>a</sup> (0.01)	66.73 <sup>c</sup> (0.40)	28.67 <sup>c</sup> (1.15)	55.33 <sup>c</sup> (0.58)

<sup>a,b,c</sup>: different letters in the same column indicate significant statistical differences, with a level of 0.05 according to the Student–Newman–Keuls test

Concerning ethanol, the studied wines presented concentrations that are within the values established by Regulation (EU) 2021/2117 [25]. The total dealcoholizing process by SCC and partial fermentation reduced ethanol concentration in 95.40% and 30.94%, respectively, obtaining alcoholic degrees of 0.52° in total dealcoholized wine and 7.90° in partially fermented wine.

According to sugars remaining in the wine, only the rosé control wine can be classified as dry according to OIV acceptable limits [17]. The presence of significant levels of sugars remain are some of the options that enable wines with reduced alcohol levels to protect the wine from microbial contamination.

### 3.2. Volatile Compounds

Independently of their alcoholic content, seventy-four volatile compounds were positively identified and quantified in Tempranillo rosé wines. Tables 2 and 3 showed the varietal volatile compounds and the volatile compounds formed mainly during alcoholic fermentation, respectively. They are grouped according to their chemical structure and include alcohols, esters, acids, terpenes, C<sub>13</sub> norisoprenoids, C<sub>6</sub> and benzenic compounds.

**Table 2.** Mean concentration (µg/L) and relative standard deviation (n = 2) of varietal volatile compounds of Tempranillo rosé control, partially fermented and total dealcoholized wines.

Source *	RI <sup>A</sup>	Compound	Control Wine		Partially Fermented Wine		Total Dealcoholized Wine	
Fluka	1282	1-hexanol	1035.49 <sup>b</sup>	(4.37)	1458.66 <sup>c</sup>	(3.93)	106.46 <sup>a</sup>	(0.96)
Sigma-Aldrich	1286	(E)-3-hexen-1-ol	13.70 <sup>a</sup>	(8.52)	25.03 <sup>b</sup>	(3.98)	24.47 <sup>b</sup>	(1.73)
Sigma-Aldrich	1296	(Z)-3-hexen-1-ol	327.37 <sup>c</sup>	(7.11)	184.16 <sup>b</sup>	(5.69)	5.72 <sup>a</sup>	(2.85)
Sigma-Aldrich	1300	(E)-2-hexen-1-ol	n.d.		n.d.		0.40 <sup>a</sup>	(0.03)
Sigma-Aldrich	1394	2-ethyl-1-hexanol	6.79 <sup>a</sup>	(4.17)	n.d.		n.d.	
Sigma-Aldrich	1197	2-hexanol	37.22 <sup>b</sup>	(5.02)	n.d. <sup>a</sup>		44.27 <sup>c</sup>	(2.96)
		<b>C6 alcohols</b>	<b>1420.56</b>		<b>1667.84</b>		<b>181.31</b>	
Tentatively identified	1455	<i>cis</i> -linalool oxyde furanic	5.50 <sup>b</sup>	(3.99)	0.32 <sup>a</sup>	(0.01)	0.82 <sup>a</sup>	(0.01)
Tentatively identified	1483	<i>trans</i> -linalool oxyde furanic	3.67 <sup>c</sup>	(3.28)	Tr		0.30 <sup>b</sup>	(0.24)
Fluka	1529	Linalool	0.21 <sup>a</sup>	(0.01)	8.65 <sup>c</sup>	(0.57)	3.71 <sup>b</sup>	(3.43)
Fluka	1607	α-terpineol	12.42 <sup>c</sup>	(6.83)	8.97 <sup>b</sup>	(4.97)	4.82 <sup>a</sup>	(4.40)
Fluka	1755	β-citronelol	5.54 <sup>c</sup>	(2.30)	3.15 <sup>b</sup>	(2.47)	Tr	
Fluka	1831	Geraniol	Tr		Tr		Tr	
Tentatively identified	1902	3,7-dimethyl-1-octen-3,7-diol	19.34 <sup>b</sup>	(9.21)	5.92 <sup>a</sup>	(3.58)	21.27 <sup>b</sup>	(4.02)
		<b>Terpenic compounds</b>	<b>46.67</b>		<b>26.99</b>		<b>30.91</b>	
Tentatively identified	1685	Trimethyl dihydronaphthalene	13.22 <sup>b</sup>	(5.03)	0.49 <sup>a</sup>	(0.04)	1.62 <sup>a</sup>	(1.31)
Sigma-Aldrich	1703	4-oxo-isophorone	1.69 <sup>b</sup>	(0.84)	Tr		8.78 <sup>c</sup>	(4.27)
Sigma-Aldrich	1801	β-Damascenone	1.36 <sup>a</sup>	(1.04)	5.03 <sup>b</sup>	(2.81)	6.28 <sup>c</sup>	(5.30)
Tentatively identified	1907	2,3-Dehydro-4-oxo-β-ionol	18.59 <sup>b</sup>	(8.75)	Tr		24.15 <sup>c</sup>	(2.37)
Tentatively identified	2875	7,8-dihydro-3-oxo-α-ionol	15.40 <sup>a</sup>	(3.31)	42.52 <sup>b</sup>	(1.76)	n.d.	
		<b>C13 norisoprenoids</b>	<b>50.26</b>		<b>48.04</b>		<b>40.81</b>	
Sigma-Aldrich	1503	Benzaldehyde	8.85 <sup>c</sup>	(2.10)	1.83 <sup>c</sup>	(1.55)	7.72 <sup>b</sup>	(2.75)
Sigma-Aldrich	1882	Guaicol	0.26 <sup>a</sup>	(0.01)	6.89 <sup>c</sup>	(2.77)	1.25 <sup>b</sup>	(1.13)
Sigma-Aldrich	1895	Benzyl alcohol	85.42 <sup>c</sup>	(1.91)	62.55 <sup>b</sup>	(2.55)	29.90 <sup>a</sup>	(8.07)
Sigma-Aldrich	1971	Phenol	20.42 <sup>c</sup>	(9.18)	9.79 <sup>b</sup>	(6.72)	0.98 <sup>a</sup>	(0.08)
Sigma-Aldrich	2193	Eugenol	Tr		Tr		Tr	
Sigma-Aldrich	2219	4-vinyl-guaiacol	5.06 <sup>a</sup>	(2.10)	33.29 <sup>b</sup>	(3.68)	61.91 <sup>c</sup>	(7.22)
Sigma-Aldrich	2225	2,6-dimethoxy phenol (syringol)	14.59 <sup>b</sup>	(4.41)	31.43 <sup>c</sup>	(3.80)	4.52 <sup>a</sup>	(3.29)
Sigma-Aldrich	2302	Isoeugenol	Tr		Tr		Tr	
Panreac	2511	Vanillin	1.69 <sup>a</sup>	(1.67)	3.61 <sup>b</sup>	(2.94)	4.06 <sup>b</sup>	(1.04)
Sigma-Aldrich	2543	Methyl vanillate	0.88 <sup>a</sup>	(0.06)	1.59 <sup>c</sup>	(1.34)	1.27 <sup>b</sup>	(1.11)
Sigma-Aldrich	2676	Ethyl vanillate	2.02 <sup>a</sup>	(1.75)	4.80 <sup>b</sup>	(0.74)	2.08 <sup>a</sup>	(1.36)
Sigma-Aldrich	2685	Acetovanillone	0.99 <sup>b</sup>	(0.08)	1.20 <sup>b</sup>	(1.18)	Tr	
Sigma-Aldrich	2936	Zingerone	35.21 <sup>b</sup>	(8.03)	6.45 <sup>a</sup>	(4.28)	7.28 <sup>a</sup>	(5.63)
Tentatively identified	3030	Methyl vanillyl eter	5.78 <sup>a</sup>	(2.20)	6.75 <sup>b</sup>	(2.93)	12.97 <sup>c</sup>	(0.93)
		<b>Benzenic compounds</b>	<b>181.14</b>		<b>170.14</b>		<b>133.92</b>	

<sup>A</sup> Linear retention index on a BP21 capillary column. <sup>a,b,c</sup> Different letters in the same row indicate statistical differences with a level of 0.05 according to Student–Newman Keuls test. \* Only the compound with Tentatively identified are quantified using the relative response factor equal one. n.d., not detected; Tr, Traces [ $<0.05$  µg/L].

**Table 3.** Mean concentration ( $\mu\text{g/L}$ ) and relative standard deviation ( $n = 2$ ) of volatile compounds formed mainly during alcoholic fermentation of Tempranillo rosé control, partially fermented and total dealcoholized wines.

Source *	RI <sup>A</sup>	Compound	Control Wine		Partially Fermented Wine		Total Dealcoholized Wine	
Sigma-Aldrich	800	Acetaldehyde	84,200.00 <sup>b</sup>	(1.18)	74,150.00 <sup>a</sup>	(1.58)	89,000.00 <sup>c</sup>	(1.75)
		<b>Aldehydes</b>	<b>84,200.00</b>		<b>74,150.00</b>		<b>89,000.00</b>	
Sigma-Aldrich	879	Methanol	85,200.00 <sup>a</sup>	(2.49)	90,200.00 <sup>a</sup>	(10.19)	90,200.00 <sup>a</sup>	(5.49)
Sigma-Aldrich	1060	1-Propanol	32,700.00 <sup>c</sup>	(6.49)	2415.00 <sup>b</sup>	(2.05)	15,930.00 <sup>a</sup>	(5.15)
Sigma-Aldrich	1190	2-methyl-1-propanol	460.57 <sup>c</sup>	(3.15)	398.65 <sup>b</sup>	(3.36)	290.35 <sup>a</sup>	(7.67)
Merck	1214	Isobutanol	34,690.00 <sup>b</sup>	(10.19)	23,605.00 <sup>a</sup>	(2.73)	20,965.00 <sup>a</sup>	(4.42)
Sigma-Aldrich	1221	2-methyl-1-butanol	50,000.00 <sup>a</sup>	(14.14)	3820.00 <sup>a</sup>	(1.48)	34,000.00 <sup>a</sup>	(2.91)
Sigma-Aldrich	1221	3-methyl-1-butanol	198,800.00 <sup>a</sup>	(3.20)	192,695.0 <sup>a</sup>	(0.25)	188,280.00 <sup>a</sup>	(0.72)
Sigma-Aldrich	1301	2-Methyl-2-butanol	4.01 <sup>a</sup>	(1.41)	8.04 <sup>b</sup>	(6.68)	7.46 <sup>b</sup>	(4.74)
Fluka	1545	2,3-Butanediol (levo)	65.96 <sup>b</sup>	(5.18)	43.40 <sup>a</sup>	(7.23)	46.37 <sup>a</sup>	(4.78)
Fluka	1585	2,3-Butanediol (meso)	3.29 <sup>a</sup>	(2.11)	4.82 <sup>b</sup>	(2.81)	7.68 <sup>c</sup>	(3.02)
Sigma-Aldrich	1725	3-(methylthio)-1-propanol	129.62 <sup>a</sup>	(1.06)	160.68 <sup>b</sup>	(5.93)	199.25 <sup>c</sup>	(3.64)
Fluka	1892	2-Phenylethanol	15,901.89 <sup>c</sup>	(4.93)	12,891.41 <sup>b</sup>	(6.44)	10,081.29 <sup>a</sup>	(6.41)
		<b>Alcohols</b>	<b>417,955.34</b>		<b>382,357.00</b>		<b>360,007.40</b>	
Sigma-Aldrich	834	Ethyl acetate	29,080.00 <sup>c</sup>	(3.02)	21,165.00 <sup>b</sup>	(2.24)	12,385.00 <sup>a</sup>	(1.77)
Fluka	1080	Ethyl butyrate	780.20 <sup>c</sup>	(3.41)	253.55 <sup>b</sup>	(6.82)	173.05 <sup>a</sup>	(8.05)
Sigma-Aldrich	1145	Isoamyl acetate	2369.15 <sup>c</sup>	(8.01)	1424.89 <sup>b</sup>	(6.01)	300.75 <sup>a</sup>	(4.47)
Fluka	1185	Ethyl hexanoate	266.35 <sup>b</sup>	(2.23)	394.38 <sup>c</sup>	(6.64)	48.14 <sup>a</sup>	(6.18)
Sigma-Aldrich	1294	Hexyl acetate	72.09 <sup>b</sup>	(2.82)	249.67 <sup>c</sup>	(1.12)	17.29 <sup>a</sup>	(1.09)
Sigma-Aldrich	1326	Ethyl lactate	1870.36 <sup>b</sup>	(6.65)	652.73 <sup>a</sup>	(1.74)	794.73 <sup>a</sup>	(1.95)
Sigma-Aldrich	1436	Ethyl octanoate	612.75 <sup>b</sup>	(4.10)	594.03 <sup>b</sup>	(2.12)	20.36 <sup>a</sup>	(8.32)
Tentatively identified	1499	3-hidroxy-ethyl butyrate	141.29 <sup>b</sup>	(3.87)	22.12 <sup>a</sup>	(6.90)	210.31 <sup>c</sup>	(7.38)
Sigma-Aldrich	1605	Diethyl malonate	4.22 <sup>a</sup>	(0.84)	n.d.		n.d.	
Fluka	1655	Ethyl decanoate	207.26 <sup>b</sup>	(2.81)	137.42 <sup>a</sup>	(5.56)	n.d.	
Fluka	1702	Diethyl succinate	2986.58 <sup>b</sup>	(5.02)	130.56 <sup>a</sup>	(2.68)	42.50 <sup>a</sup>	(2.82)
Tentatively identified	1827	4-hidroxy-ethyl butyrate	n.d.		n.d.		8.02 <sup>a</sup>	(0.76)
Fluka	1936	2-phenylethyl acetate	446.72 <sup>c</sup>	(7.47)	185.84 <sup>b</sup>	(6.98)	21.26 <sup>a</sup>	(6.23)
Sigma-Aldrich	2070	Diethyl malate	1.37 <sup>a</sup>	(0.62)	176.20 <sup>c</sup>	(1.65)	63.17 <sup>b</sup>	(5.64)
Tentatively identified	2331	Ethyl monosuccinate	114.04 <sup>a</sup>	(9.14)	n.d.		n.d.	
		<b>Esters</b>	<b>38,952.38</b>		<b>25,386.39</b>		<b>14,084.57</b>	
Sigma-Aldrich	1426	Acetic acid	24.72 <sup>c</sup>	(5.63)	16.16 <sup>b</sup>	(5.63)	9.36 <sup>a</sup>	(3.84)
Sigma-Aldrich	1546	Propanoic acid	6.14 <sup>b</sup>	(3.96)	10.06 <sup>c</sup>	(2.55)	1.92 <sup>a</sup>	(1.72)
Fluka	1583	Isobutyric acid	115.66 <sup>b</sup>	(5.83)	53.84 <sup>a</sup>	(4.21)	273.40 <sup>c</sup>	(5.26)
Fluka	1600	Butyric acid	122.78 <sup>a</sup>	(3.53)	223.30 <sup>b</sup>	(5.00)	546.70 <sup>c</sup>	(3.55)
Sigma-Aldrich	1642	Isovaleric acid	394.94 <sup>c</sup>	(1.25)	288.85 <sup>b</sup>	(0.68)	265.96 <sup>a</sup>	(2.03)
Fluka	1703	Pentanoic acid	12.33 <sup>a</sup>	(6.27)	13.78 <sup>a</sup>	(2.82)	16.55 <sup>b</sup>	(2.01)
Fluka	1816	Hexanoic acid	1300.20 <sup>c</sup>	(3.91)	1195.53 <sup>b</sup>	(2.08)	1007.67 <sup>a</sup>	(2.13)
Sigma-Aldrich	1929	(E)-2-hexenoic acid	24.59 <sup>b</sup>	(3.32)	12.20 <sup>a</sup>	(8.70)	23.61 <sup>b</sup>	(3.83)
Sigma-Aldrich	1957	(E)-3-hexenoic acid	25.05 <sup>a</sup>	(8.49)	41.45 <sup>c</sup>	(2.96)	37.09 <sup>b</sup>	(3.30)
Fluka	2024	Octanoic acid	4241.85 <sup>b</sup>	(6.43)	4320.21 <sup>a</sup>	(2.40)	1690.47 <sup>b</sup>	(1.05)
Sigma-Aldrich	2108	Nonanoic acid	n.d.		n.d.		40.33 <sup>a</sup>	(1.26)
Sigma-Aldrich	2289	Decanoic acid	1340.91	(5.23)	1287.92	(5.46)	163.46	(6.12)
Sigma-Aldrich	2439	Dodecanoic acid	n.d.		25.32 <sup>a</sup>	(5.26)	n.d.	
		<b>Acids</b>	<b>7609.17</b>		<b>7488.62</b>		<b>4076.51</b>	
Sigma-Aldrich	1650	$\gamma$ -butyrolactone	15.31 <sup>a</sup>	(5.04)	2.84 <sup>a</sup>	(0.99)	11.82 <sup>a</sup>	(9.69)
Sigma-Aldrich	1902	Pantoic lactone	29.00 <sup>a</sup>	(8.06)	31.40 <sup>a</sup>	(3.69)	25.96 <sup>b</sup>	(6.49)
		<b>Lactones</b>	<b>44.30</b>		<b>34.24</b>		<b>37.78</b>	

<sup>A</sup> Linear retention index on a BP21 capillary column, <sup>a,b,c</sup> Different letters in the same row indicate statistical differences with a level of 0.05 according to Student-Newman Keuls test. \* Only the compound with Tentatively identified are quantified using the relative response factor equal one. n.d., not detected; Tr, Traces [ $<0.05 \mu\text{g/L}$ ].

### 3.3. Varietal Compounds

#### 3.3.1. C<sub>6</sub> Compounds

Tempranillo rosé wines showed high quantities of C<sub>6</sub> compounds, 1-Hexanol being the major compound, followed by (Z)-3-hexen-1-ol in the three studied wines, regardless of the treatment. 1-Hexanol is related to green notes in wines, but if it is found in a higher concentration than its olfactory odor threshold (8000  $\mu\text{g/L}$ ) [21], it can contribute negatively to the total wine aroma. However, neither of these wines exceeded its olfactory

odor threshold. The total dealcoholizing process significantly affects the total concentration of C<sub>6</sub> compounds, reaching a reduction of 87% in comparison with the control wine. These results were similar to the obtained by [23] in dealcoholized rosé Pinot Noir wines.

In partially fermented wines there have not been found significant differences in the total concentration of the principal C<sub>6</sub> compounds with respect to control wine. This can be probably explained because partially fermented wines were produced by an alcoholic fermentation stop, and these compounds are formed during the first stages of the process [26].

### 3.3.2. Terpene and C<sub>13</sub>-Noirsoprenoids Compounds

Two other important groups of varietal aroma compounds are terpenes and C<sub>13</sub> norisoprenoids, even though they are present at relatively low levels because of their lower perception thresholds. Seven terpene compounds have been identified and quantified in these wines, all of them in higher concentrations than their olfactory odor thresholds, so it can be said that they do not contribute individually to the global wine aroma, but they could contribute to it due to the synergetic effect with other components that are present in the wine matrix [27].

Concerning C<sub>13</sub>norisoprenoids, five compounds have been identified, being  $\beta$ -damascenone and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) the only ones that were present in every wine.  $\beta$ -damascenone is considered a potent odorant due to its low olfactory odor threshold (0.05  $\mu\text{g/L}$ ), which was exceeded in every case. The alcoholic concentration reduction in partially fermented and total dealcoholized wines caused a decrease in these two compound groups, coinciding with the results found in [4,24,28]. The absence of certain compounds in partially fermented and total dealcoholized wines could be attributed to both the dealcoholizing processes of the original wine [28].

### 3.3.3. Benzene Compounds

Among benzene compounds, volatile phenols are considered important compounds of wine aroma, and some of these are shikimic acid and its derivatives, which are associated with varietal aroma [21] and have been related to pleasant notes such as vanilla, almond, clove and smoky aromas [29]. Vanillin and its derivatives are the shikimic acid derivatives that have been identified in the highest concentrations in this research, giving sweet notes to wine aroma.

In general, it can be observed a reduction in benzenic compounds in total and partially fermented dealcoholized wines, with the exception of vanillin and its derivatives, which were found in higher concentrations in these wines than in control wine. Similar results have been found in other research using different reducing alcohol techniques [28].

## 3.4. Volatile Compounds Formed Principally during Alcoholic Fermentation

Although varietal compounds are responsible for the aromatic typicality of wines, the compounds that are formed during alcoholic fermentation may have a positive or negative effect on wine aroma [22].

### 3.4.1. Aldehydes

Acetaldehyde provides a fruity aroma with nut notes when it is found in low concentrations, but when its concentration is higher, it can contribute negatively to wine aroma because it provides rotten apple notes [20]. Results show that total dealcoholized wines presented the highest acetaldehyde concentration of the studied wines. This can be due to the possible oxidation of wines because of their handling during the dealcoholizing process. On the other hand, partially fermented wines showed a lower acetaldehyde concentration probably due to the duration of alcoholic fermentation.

#### 3.4.2. Alcohols

Higher alcohols are the main group of volatile compounds synthesized during the alcoholic fermentation process by yeast [30]. Higher alcohols can contribute to wine aroma in a positive or negative way, depending on the concentration in which they are present; it is generally accepted that concentrations above 300 mg/L contribute negatively to the aroma of the wine by endowing it with unpleasant notes [31].

For the wines studied, the concentrations of alcohols were lower than the mentioned threshold values in all cases, the reason for which the higher alcohols contribute positively to the aromatic complexity of the analyzed wines. The most abundant aliphatic alcohols were amylic alcohols (3-methyl-1-butanol and 2-methyl-1-butanol), 1-propanol, methanol and isobutanol. 2-phenylethanol is present in concentrations above its threshold of olfactory perception (10,000 µg/L) and has been associated with the floral notes of wines [20].

In general, controlled wine showed the highest alcohol concentration, and partially fermented and total dealcoholized wines showed lower concentrations due to the elimination of these components during the dealcoholizing process. These results agree with those of Ma et al. [23].

#### 3.4.3. Esters

Principally, esters are synthesized enzymatically by yeasts throughout alcoholic fermentation and their concentrations may vary during malolactic fermentation due to the action of lactic acid bacteria [32]. In this study, there have been identified and quantified a total of 15 esters or acetates, although not all of them have been identified in the three studied wines. The highest concentrations of these compounds were found in control wines, followed by partially fermented wines. The total dealcoholized wines presented the lowest concentration of esters due to the highly hydrophobic character of esters and to the formation of groups with alcohols, so, during the dealcoholizing process, they are lost with alcohols [33]. Isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl monosuccinate and diethyl succinate were the esters more affected by total dealcoholizing and partial fermentation techniques [34]. The reduction in the total ester concentration could affect negatively wine aroma because of the loss of fruity notes [20].

#### 3.4.4. Acids

Acids are an important volatile group formed during alcoholic fermentation, and their total concentration depends on the initial composition of the must and on fermentation conditions [35]. Short and medium-chain organic acids are related to fruity, cheesy and oily notes [36], but they can contribute positively to the fruity character of red wines.

The qualitative composition of the acids is the same independent of the dealcoholizing technique, being octanoic, hexanoic and decanoic acids the compounds that were found in higher concentrations in the three studied wines. The total dealcoholizing process reduced significantly the total acid concentration in wines, while control and partially fermented wines presented very similar concentrations. These results are in agreement with the obtained in [37].

#### 3.4.5. Lactones

Lactones were a minor qualitative and quantitative group in wine aroma, independent of the alcohol content. They identified and quantified three lactones,  $\gamma$ -butyrolactone and pantoic lactone, and their concentration were almost not modified by the dealcoholizing process.

### 3.5. Odor Activity Values (OAV)

Among all the aroma compounds that exist in wine, there are only a few of them which are considered as important in wine aroma.

The OAV of each volatile compound was used with the aim of determining the impact on the final aroma of wine

It is generally accepted that all compounds having an OAV  $\geq 1$  contribute individually and significantly to wine aroma. However, other authors propose that, when OAV is lower than 1, it can contribute to wine aroma due to the synergetic effect with other compounds present in the matrix.

Table 4 [20–22] shows that 26 out of 74 quantified volatile compounds (Tables 2 and 3) were found in the studied wines with OAVs  $> 0.1$ , but only acetaldehyde, ethyl octanoate, isoamyl acetate, ethyl butyrate,  $\beta$ -dama-scenone, ethyl hexanoate, isovaleric acid, octanoic acid, 3-methyl-1-butanol, ethyl acetate, hexanoic acid, 2-phenylethyl acetate, 2-phenyl alcohol and ethyl decanoate, were found in higher concentrations than their corresponding olfactory odor threshold, so therefore, they are considered as potential contributors to the global bouquet of wine.

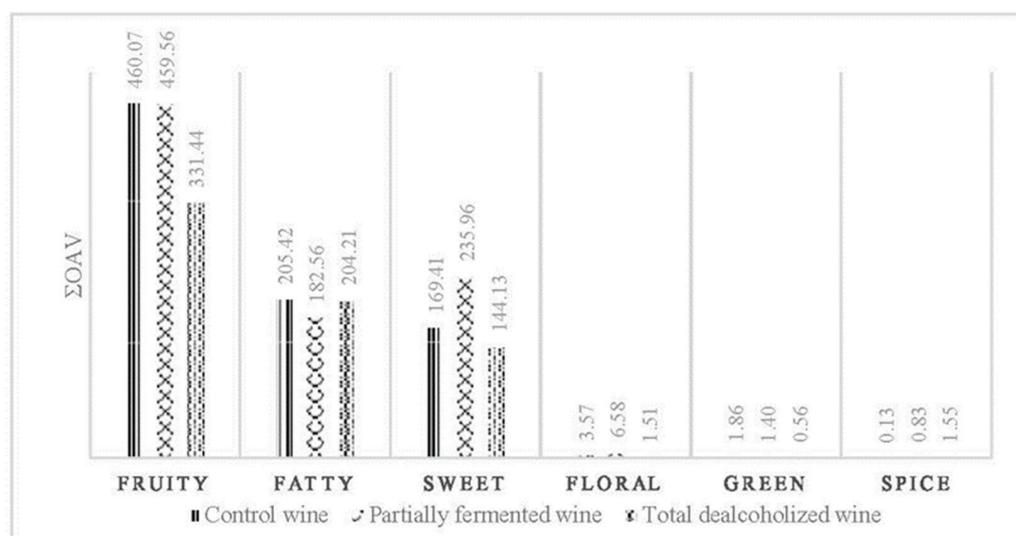
**Table 4.** Odor descriptor, odor threshold ( $\mu\text{g/L}$ ), aromatic series and odor activity values of rosé Tempranillo control, partially fermented and total dealcoholized wines.

Compound	Odor Descriptor	Odor Threshold ( $\mu\text{g/L}$ )	Aromatic Series	OAV Control Wine	OAV Partially Fermented Wine	OAV Total Dealcoholized Wine
Acetaldehyde	Rough, ripe apple	500	1, 6	168.40	148.30	178.00
Ethyl octanoate	Caramel, fruity	5	1, 4	122.55	118.81	4.07
Isoamyl acetate	Banana	30	1	78.97	47.50	10.03
Ethyl butyrate	Fruity	20	1	39.01	12.68	8.65
$\beta$ -Damascenone	Sweet, fruity	0.05	1, 4	27.20	100.60	125.60
Ethyl hexanoate	Green apple	14	1	19.02	28.17	3.44
Isovaleric acid	Acid, rancid	33	4, 6	11.97	8.75	8.06
Octanoic acid	Sweet, cheese	500	6	8.48	8.64	3.36
3-methyl-1-butanol	Burnt, alcohol	30,000	4, 6	6.63	6.42	6.28
Ethyl acetate	Fruity, solvent	7500	1, 6	3.88	2.82	1.65
Hexanoic acid	Sweet	420	6	2.86	3.44	2.44
2-phenylethyl acetate	Floral	250	2	1.79	0.74	0.09
2-phenylethyl alcohol	Floral, rose	10,000	2	1.59	4.91	1.15
Decanoic acid	Rancid fat	1000	6	1.34	1.29	0.17
Ethyl decanoate	Caramel, fruity	200	1, 4	1.04	0.69	0.00
Isobutanol	Bitter, green	40,000	3, 6	0.87	0.59	0.52
(Z)-3-hexen-1-ol	Green, cut grass	400	3	0.82	0.46	0.01
Butyric acid	Rancid, cheese, sweet	173	6	0.71	1.29	3.16
3-(methylthio)-1-propanol	Cooked vegetable	1000	6	0.13	0.16	0.20
1-hexanol	Resinous, floral, green	8000	2, 3	0.13	0.18	0.01
Methanol	Chemical, medicine	668,000	6	0.13	0.14	0.14
4-vinyl-guaiacol	Spicy, curry	40	5	0.13	0.83	1.55
Hexyl acetate	Green, floral	1500	2, 3	0.05	0.17	0.01
Guaicol	Medicine, caramel, smoke	10	4, 6	0.03	0.69	0.13
Linalool	Floral	15	2	0.01	0.58	0.25
Isobutyric acid	Rancid, butter, cheese	2300	6	0.01	0.02	0.12

1 = fruity; 2 = floral; 3 = green, fresh; 4 = sweet; 5 = spicy; 6 = fatty.

It is complicated to determine the complete aroma impact of these wines from the volume of the data and for this aim the aromatic series were obtained by grouping the volatile compounds identified and quantified in the wine aroma according to odor descriptors used. The series used in this study, have been fruity, floral, green/fresh, sweet, spicy and fatty [38–40]. Due to the high complexity of aromatic assessments, one volatile compound can be assigned to one or more aromatic series based on the studies of some research [41,42]. The total intensity of each aromatic series was calculated by adding the OAVs of the compounds appointed to this series and are graphed in Figure 1. This method

makes it possible to correlate the quantitative data obtained by GC-MS, to sensory profile and has been used by some authors [37,42].



**Figure 1.** Aromatic series of La Mancha Tempranillo rosé control, partially fermented and total dealcoholized wines.

The principal aromatic series, independent of the dealcoholizing technique, are the same (fruity, fatty and sweet), showing only differences in their total intensity. Total dealcoholized wine showed the lowest intensities in the series sweet and fruity, probably due to the lower ester concentration (Table 3). On the other hand, the aromatic series floral, green and spice were minor series although some of these attributes are characteristic in the sensory profile of the studied wines.

As odor thresholds are affected by high imprecision due to synergic, additive and antagonist influences these values should be taken as an approximation to the volatile compounds that influence the aroma of these wines. The principal odorants of the studied wines are basically identical and only differ in relative importance from one wine to another [40,43].

As can be observed, there is a great similarity between the studied wines in the compounds that are considered as the potential contributors to the global bouquet of wine, existing only differences in the relative order between them. Table 5 shows the group of volatile compounds responsible for the aroma differences in the three studied wines. The ratio between OAV max and OAV min was estimated in order to understand which volatile compounds are responsible for the differences in the final aroma of the three types of wine studied. From these results, some important conclusions can be obtained. The compounds that have a greater capacity of differentiation (ratio between the maximum and the minimum OAVs was >10) in the aromatic profile of the studied wines include varietal aromas such as (*Z*)-3-hexen-1-ol, linalool, 1-hexanol, guaiacol and 4-vinylguaiacol, and aromas generated by the yeast's metabolism, such as ethyl octanoate, 2-phenylethyl acetate, hexyl acetate and isobutyric acid. A second group is made up of components with a maximum/minimum OAV ratio between 2 and 10. This group includes ethyl hexanoate, decanoic acid,  $\beta$ -damascenone, ethyl butyrate, butyric acid, 2-phenylethanol, octanoic acid and ethyl acetate. The last group is mainly formed by the compounds formed during yeast metabolism, and they are organic acids, alcohols and aldehydes. Some of these compounds have high OAVs, but the maximum/minimum OAV ratio is well below 2.0, which confirm secondary importance.

**Table 5.** Determination of OAV max/OAV min in the aroma compounds of the three studied wines.

Compound	OAV Max.	OAV Min.	OAV Max./OAV Min.
(Z)-3-hexen-1-ol	0.82	0.01	82.00
Linalool	0.58	0.01	58.00
Ethyl octanoate	122.55	4.07	30.09
Guaicol	0.69	0.03	23.00
2-phenylethyl acetate	1.79	0.09	19.89
1-hexanol	0.18	0.01	18.00
Hexyl acetate	0.17	0.01	17.00
Isobutyric acid	0.12	0.01	12.00
4-vinyl-guaiacol	1.55	0.13	11.92
Ethyl hexanoate	28.17	3.44	8.19
Decanoic acid	1.34	0.17	7.88
Isoamyl acetate	78.97	10.03	7.88
$\beta$ -Damascenone	125.60	27.20	4.62
Ethyl butyrate	39.01	8.65	4.51
Butyric acid	3.16	0.71	4.45
2-phenylethyl alcohol	4.91	1.15	4.27
Octanoic acid	8.64	3.36	2.57
Ethyl acetate	3.88	1.65	2.35
Isobutanol	0.87	0.52	1.67
3-(methylthio)-1-propanol	0.20	0.13	1.54
Isovaleric acid	11.97	8.06	1.49
Hexanoic acid	3.44	2.44	1.41
Acetaldehyde	178.00	148.30	1.20
Methanol	0.14	0.13	1.08
3-methyl-1-butanol	6.63	6.28	1.06

#### 4. Conclusions

The current study brings to light the effects that partial fermentation and total dealcoholization may have on the volatile composition of La Mancha Tempranillo rosé wines. The total dealcoholizing process decreased the concentration of higher alcohols, esters, acids and C<sub>6</sub> compounds in the wines, while not modifying the concentration of terpenes, benzenic compounds and C<sub>13</sub>-nor-isoprenoids. Fruity, fatty and sweet were the principal aromatic series and only their total intensity was modified by the dealcoholizing process. The compounds that have greater capacity of differentiation in the aromatic profile of the studied wines (Z)-3-hexen-1-ol, linalool, ethyl octanoate, guaiacol, 2-phenylethyl acetate, 1-hexanol, hexyl acetate, isobutyric acid and 4-vinylguaiacol.

According to the volatile composition and aromatic series of partially fermented and total dealcoholized wines, it may be concluded that these alcohol-reducing techniques provide a viable alternative to traditional rosé winemaking methods to increase the offer of La Mancha Tempranillo rosé wines to the consumer.

**Author Contributions:** Conceptualization, M.A.G.-V.; methodology, E.S.-P. and M.O.A.; formal analysis, M.O.A.; investigation M.A.G.-V.; resources, E.S.-P.; data curation, M.O.A.; writing—original draft preparation, M.O.A.; writing—review and editing, M.A.G.-V. and E.S.-P.; visualization, M.A.G.-V. and E.S.-P.; supervision, M.A.G.-V. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by University of Castilla-La Mancha grant number project 2022-GRIN-34333.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** We choose to exclude this statement because the study did not report any data.

**Acknowledgments:** María Osorio Alises would like to thank to the UCLM for the award of a grant. The authors are grateful for financial support to the UCLM under the project 2022-GRIN-34333.

**Conflicts of Interest:** The authors declare no conflict of interest.

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