

Chapter

Distinctive Role of Yeast Strain on Aromatic Profile of Wines Made from Minority Grape Cultivars: Chemical and Sensory Characterization of Aroma Components

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Abstract

This chapter synthesizes the main results that our research group has obtained about the specific influence of a commercial *Saccharomyces cerevisiae* strain on the aromatic profile of fermented musts from four minority grape varieties (*Vitis vinifera* L.) cultivated in Castilla-La Mancha (Spain), that is, Moribel, Tinto Fragoso, Albillo Dorado and Montonera del Casar. In addition, wines made from the grape cultivars Tempranillo and Airén were evaluated. To determine the main yeast-derived odor relevant in these grape varieties, the aromatic profiles of grape cultivars and the resulting wines were studied by gas chromatography coupled to mass spectrometry and wines were subjected to Napping, a rapid sensory evaluation method. The results revealed wine sensory differences which are consequence of different aromatic profiles of wines produced with these grape cultivars. The combination of quantitative chemical analysis of volatile compounds together with sensory analysis of wines point out different patterns of aroma compound formation and release. Thus, the yeast strain used in the fermentation step is one of the main factors that affect the sensory properties of wines.

Keywords: *Saccharomyces cerevisiae*, yeast, grape, wine, volatile compounds, aroma, gas chromatography, sensory analysis

1. Introduction

Wine is a beverage obtained usually from grapes via the alcoholic fermentation process carried out by yeasts. The use of commercial *Saccharomyces cerevisiae* strains has been widespread in wine industry due to its good fermentative properties and the ability to produce quality wines, particularly through modifications of volatile compound

profile [1]. The distinctive aroma of wine is determined by several factors such as grape variety and maturity, viticultural and winemaking practices, and storage conditions [2]. Several volatile compounds are produced in grapes, increasing their concentration during the vegetative growing period although this tendency changes during grape ripening [3]. Among aroma compounds present in grapes, a significant part comes from specific glycosidically linked forms (odorless precursors) which are transformed into free forms (odor compounds) during the winemaking process [4]. In wine, a large number of volatile compounds can be a part of its aromatic profile. However, around 70 odor compounds play major roles in the aromatic characteristics of wines [5]. Most of these volatile compounds belonging to different chemical classes are formed from precursors during fermentation by *Saccharomyces cerevisiae* or grape-derived compounds released and/or modified by the yeast action [6]. Fermentation-derived volatiles including higher alcohols, acids, ethyl esters and acetates are the most abundant in total aroma composition of wine. The modulation of the levels of these compounds by the yeast strain in charge of fermentation leads to sensory differences [7].

Spain is the country with the biggest vineyard surface in the world and has a great varietal biodiversity [8], despite the disappearance of many grape varieties (*Vitis vinifera* L.) in Europe was caused by the attack of the phylloxera in the late nineteenth century [9]. In the last decades, prospecting and recovery works started in the wine-growing areas of the world due to the great interest of wine sector in recovering ancient varieties [10], some of which are minoritarian and in danger of disappearing. In this way, grapevine prospecting work allowed to preserve the old vine heritage of the Castilla-La Mancha Spanish region, which resulted in the identification of more than 40 new grape genotypes not previously registered in any database [11]. Notable among these minority grape varieties are Moribel, Tinto Fragoso, Albillo Dorado and Montonera del Casar (**Figure 1**). These grape genotypes are grown in the Collection

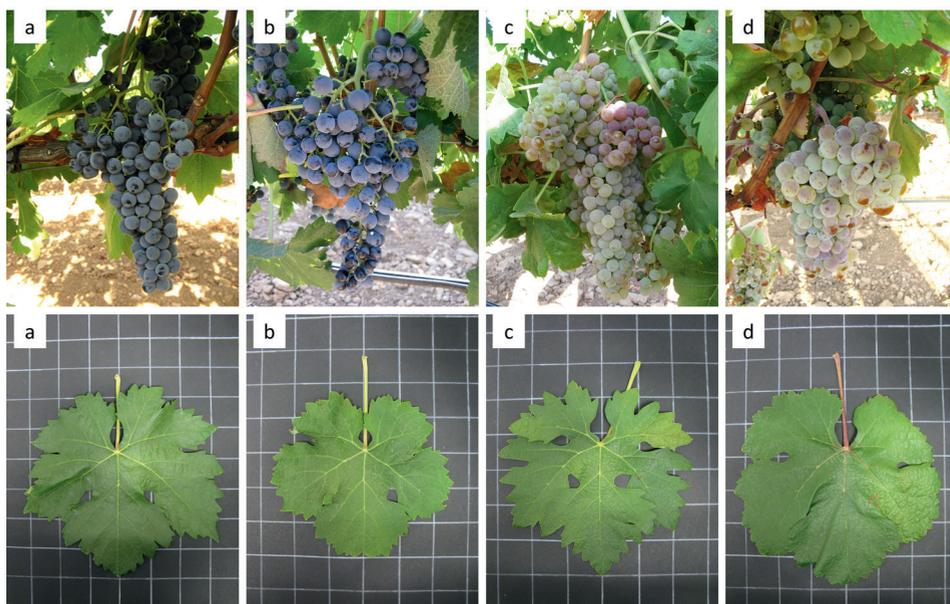


Figure 1. Leaf and bunch morphology in the *Vitis vinifera* L. grape cultivars: Moribel (a), Tinto Fragoso (b), Albillo Dorado (c) and Montonera del Casar (d).

of Grapevine Varieties from Castilla-La Mancha (CGVCLM) created to preserve the local grape diversity of this winemaking region.

The aim of the chapter is to synthesize the main results that our research group has obtained on the impact of a commercial *Saccharomyces cerevisiae* strain on the volatile composition and aroma profile of wines produced by these minority grape cultivars to elucidate the chemical changes in the components potentially responsible for aroma sensory properties. This work was carried out by studying the aromatic profile of these grape varieties and resulting wines by gas chromatography coupled to mass spectrometry (GC-MS) and the sensory properties of wines were evaluated by Napping. These were compared to wines made from Airén and Tempranillo, the most cultivated grape varieties in this winemaking region. To our knowledge, this is the first comprehensive study about the aroma components of these minority grape cultivars and the resulting wines.

2. Materials and methods

2.1 Chemicals

All solvents and reagents were of analytical grade (>99%) and a Milli-Q purification system (Merck-Millipore, Darmstadt, Germany) was used to obtain the pure water. Several commercial standards were employed for volatile compound identification and quantification purposes, which were purchased from Extrasynthese (Genay, France), Fluka (Buchs, Germany), Merck (Darmstadt, Germany), and Sigma-Aldrich Chemie (Steinheim, Germany). 4-nonanol and 4-methyl-2-pentanol were used as internal standards and were supplied by Sigma-Aldrich Chemie (Steinheim, Germany).

2.2 Grape samples

Grape samples of four minority varieties (*Vitis vinifera* L.), two red (Moribel and Tinto Frago), and two white (Albillo Dorado and Montonera del Casar), were evaluated in this chapter. Grapes were harvested at the optimal ripening stage and in good sanitary conditions from an experimental vineyard of the Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal from Castilla-La Mancha (IRIAF) located in Tomelloso (Spain). The minority varieties were compared with two of the most traditionally cultivated grapes in this winemaking region, that is, Tempranillo and Airén. For each variety, a total of three batches of grape clusters were sampled from grapevines in several zones of the vineyard. Sampling was made by selecting randomly berries from the top, central, and bottom of grape bunches in the lab, choosing 500 g of grapes from each batch to evaluate the aromatic potential.

2.3 Winemaking process

Winemaking process was carried out in the winery of IRIAF and all produced wines were dry, i.e. containing less than 5 g/L of residual sugar. White wines were elaborated from 75 kg of grapes. The grapes were destemmed and crushed, with the addition of 80 mg/L of SO₂. Cold pre-fermentation maceration was developed at 5°C for 24 h. Subsequently, the resultant grape must was separated from the solid phase by pressing, and total acidity was adjusted to 4.5 g/L in the case of grape must with lower values. In addition, ascorbic acid (100 mg/L) and lysozyme (100 mg/L) were

added to avoid oxidation and malolactic fermentation respectively. Fermentation was carried out at 17°C in 100 L fermentation vessels using the commercial yeast Uvaferm VN® (Lallemand Inc., Zug, Switzerland) at 20 g/hL. Alcoholic fermentation was controlled by measures of the density. When a relative density of 1.010 was reached, the alcoholic fermentation continued at 20°C. When the glucose + fructose concentration was below 5 g/L, the alcoholic fermentation was terminated. Then, the wines were racked and sulphited (25 mg/L of free SO₂). For 1 month, the fermentation lees were stirred regularly (*Bâtonnage* technique). Wines were subjected to cold treatment at -5°C for 15 days, and the free SO₂ concentration was corrected up to 25 mg/L. Wines were then filtered through 0.2 µm membranes, bottled, and stored at 16–18°C.

Red wines were elaborated from 75 kg of grapes in vats of 100 L, with skin maceration until the alcoholic fermentation ended. After stemming and crushing, a concentration of 50 mg/L of SO₂ was added before the inoculation with *S. cerevisiae* selected yeast Uvaferm VN® (Lallemand Inc., Zug, Switzerland) at 20 g/hL. The alcoholic fermentation temperature was maintained at 22°C and its development was monitored daily by measuring the density until 0.995. After alcoholic fermentation, the wines were racked and malolactic fermentation was carried out at a temperature of 22°C using *Oenococcus oeni* lactic acid bacteria VP41® (Lallemand Inc., Zug, Switzerland) at 1 g/hL. Malolactic fermentation was monitored by the quantification of L-malic acid in wines. When the malic acid content reached values below 0.2 g/L, the wines were sulphited to 25 mg/L of free SO₂. Subsequently, wines were left for 30 days in vats at 5°C and then underwent cold treatment at -5°C for 15 days. After, the wines were racked and sulphited to obtain 25 mg/L of free SO₂. Finally, wines were filtered through 0.2 µm membranes, bottled, and stored in controlled conditions at 16–18°C. All wines were carried out in duplicate for each grape cultivar.

2.4 Volatile compound extraction

For each variety, an amount of 500 g of grapes was selected and crushed for 3 min with an ULTRA-TURRAX digital T50 crusher and subsequently centrifuged at 3500 rpm for 15 min. The obtained supernatant was then filtered, disregarding the first wort fraction. A volume of 25 mL was collected and 500 µL of 4-nonanol 0.1 g/L (internal standard) was added. The extraction of the free fraction of volatile compounds was performed following the procedure described in the literature [12], using SPE cartridges (LiChrolut EN, Merck, 0.2 g phase for wine, 0.3 g phase for grape must). A volume of 25 mL of grape must or wine was selected and passed through the SPE resin with the internal standard. Subsequently, 25 mL of Milli-Q water was added to remove polar compounds and sugars. The free fraction of volatile compounds was recovered by passing 15 mL of a pentane-dichloromethane solution (2:1, v/v). The eluate obtained was concentrated by distillation on a Vigreux column and then under a nitrogen stream to 150 µL, keeping at -20°C until analysis. The glycosidically bound volatile fraction of grapes was obtained by passing 25 mL of ethyl acetate-methanol (9:1, v/v) after recovering the aroma-free portion from the SPE resin. Subsequently, the obtained fraction was evaporated to dryness in a rotary evaporator and reconstituted with 5 mL of 0.2 M citrate buffer (pH = 5.00). Glycosidic aromatic precursors were released by enzymatic hydrolysis. The reconstituted sample was thawed and 250 mg of enzyme Lallzyme® BETA (Lallemand Inc., Zug, Switzerland) was added, remaining tightly covered in an oven at 40°C for 18 hours. Then it was left to cool and 500 µL of 4-nonanol 0.1 g/L was added. The process of volatile compound extraction described above was repeated once again, using SPE cartridges of 0.2 g of phase and 5 mL capacity.

2.5 GC-MS analysis of volatile compounds

Volatile compound analysis was performed with a Focus GC gas chromatograph system coupled to a mass spectrometer (ISQ, ThermoQuest, Waltham, MA, USA). A BP21, polyethylene glycol treated with nitroterephthalic acid, capillary column (50 m × 0.32 mm i.d.; 0.25 μm thick of Free Fatty Acid Phase (FFAP)) was used. For major volatile compounds, 0.8 μL of a mix solution (100 μL of wine sample, 100 μL of 4-methyl-2-pentanol (50 mg/L) as internal standard, and 1 mL of Milli-Q water) were injected in split mode. The gas chromatographic conditions were as follows: helium was used as carrier gas with a constant flow of 1.2 mL/min, the injector temperature was 195°C and oven temperature program was: 32°C (2 min), 5°C/min to 120°C, 75°C/min to 190°C, and 18 min at 190°C. Minor volatile compounds were analyzed by injecting 1 μL of sample in splitless mode after solid phase extraction (SPE). Operating conditions were as follows: carrier gas was helium (1 mL/min); injector temperature, 220°C; and oven temperature program was: 40°C for 15 min, 2°C/min to 100°C, 1°C/min to 150°C, 4°C/min to 210°C and 55 min at 210°C. The mass spectrometer operated in the electron impact mode with an electron energy of 70 eV, ion source temperature 250°C, and the global run time was recoded in full scan mode (mass scanning range, 40–250 amu). The identification of volatile compounds was performed by chromatographic retention times and mass spectra using commercial standards. These compounds were quantified by analyzing the characteristic m/z fragment for each compound following the internal standard method. When the commercial standards were not available, the volatile compound concentration was expressed as internal standard equivalents obtained by normalizing the compound peak area to that of the internal standard, multiplying by the internal standard concentration.

2.6 Odor activity values

The Odor Activity Values (OAVs) were determined to evaluate the contribution of a chemical compound to the wine aroma, providing the importance of a specific component to the sample odor. OAVs were calculated as the ratio between the individual compound concentration and the perception threshold found in literature [13, 14]. A possible contribution to the aroma of wine was considered when OAV was higher than 0.1.

2.7 Wine sensory analysis

Wines were sensorial evaluated by a trained panel of 11 experienced tasters from the IRIAF. The assessment was carried out under ISO standards related to taster selection and training [15], methodology and vocabulary [16], and tasting room [17], following the Napping technique [18]. Wine-taster members were asked to smell and taste the samples and to place them on white sheet of 40 × 60 cm, according to their similarities and dissimilarities. Samples close together on the sheet had similar sensory properties but if tasters perceived samples very differently, they had to place them far from each other. A second session was carried out providing a list of attributes imposed by the judge-in-chief and previously chosen by an expert panel. Napping sensory maps were obtained by Multiple Factorial Analysis (MFA) which provides useful information on the general perception of evaluated wines.

2.8 Statistical analysis

The statistical treatments were performed using the SPSS software version 23.0 (SPSS Inc., Chicago, Illinois, USA) and XLSTAT 2017 statistical software (Addinsoft, Paris, France). To determine statistically significant differences in the concentration of volatile compounds of wine aroma, a one-way analysis of variance (ANOVA, Student–Newman–Keuls/Tukey test, $p < 0.05$) was made. Sensory data were subjected to MFA to obtain the Napping sensory maps of wines.

3. Results and discussion

3.1 Aroma composition of grapes

The study of the aroma composition of these grapes allowed us to identify and quantify the free and bound volatile fractions usually located in berry skins by GC-MS (Tables 1 and 2).

Different families of volatiles described the varietal composition of these minority cultivars, that is, C6 compounds, terpenes, norisoprenoids, benzenic compounds, and alcohols. C6 compounds are derived from linoleic and lignoceric acids via enzymatic reactions and are responsible for herbaceous aroma in grapes and wines [20]. Among the free C6 compounds, 1-hexanol, *cis*-3-hexenol, *trans*-2-hexenol, hexanal, and *trans*-2-hexenal were present at relatively high concentrations in the six grape varieties, which is in agreement with previous results from *V. vinifera* grapes [21].

The bound fraction of C6 compounds was dominated by 1-hexanol, hexanal, and *trans*-2-hexenal. In all cases, total concentrations of bound C6 compounds were lower than those found in the free forms. Red grapes were characterized by a higher total concentration of free C6 compounds than other grape varieties grown in Spain [4]. Montonera del Casar was the grape genotype with a higher content of the free C6 compounds, reaching 2.72 g/L. However, the most abundant bound fraction of C6 compounds was determined in Tempranillo grapes (0.54 g/L). Terpenes have characteristic fruity and floral notes and are considered a positive quality factor because of their contribution to the varietal aroma although they are present at low levels [22]. Linalool, nerol, and geraniol were the terpenoids identified in these *V. vinifera* grapes. Among the terpenoids, geraniol was predominant in the terpene composition, mainly in Tinto Fragoso genotype. The concentration of these compounds observed in the glycosidically-bound forms was remarkable. Total concentrations of bound terpenes were 7–17 times higher than those observed in the free forms of the six grape cultivars. Similar results were found in a study on Godello and Agudelo grapes [23]. Norisoprenoids are formed by carotenoid degradation and have been identified as potential impact odorants in wine [24]. These compounds were only identified in the bound form and significant differences were found between grape cultivars, showing the highest values in Tempranillo grapes. Total concentrations of norisoprenoids ranged from 0.05 to 0.17 mg/L.

Benzaldehyde, benzyl alcohol, 2-phenylethanol, phenylacetaldehyde, vanillin, and acetovanillone were the benzenic compounds found in the six grape varieties. Eugenol was only present in bound form. The concentration of each bound benzenic compound was, in general, higher than those observed in the free one, mainly benzyl alcohol and 2-phenylethanol which have been described as a floral aroma component [25]. Eugenol contributes to the spicy and smoky notes and has a low perception

Compounds	Tempranillo		Moribel		Tinto Fragoso	
	Free	Bound	Free	Bound	Free	Bound
1-Hexanol ¹	0.31 ± 0.02 b	0.15 ± 0.03 a	0.34 ± 0.03 b	0.08 ± 0.01 a	0.61 ± 0.09 c	0.14 ± 0.02 a
<i>trans</i> -3-Hexenol ²	5.72 ± 0.27 a	ND	5.95 ± 0.45 a	ND	11.51 ± 2.70 b	ND
<i>cis</i> -3-Hexenol ¹	0.21 ± 0.02 d	0.08 ± 0.00 b	0.36 ± 0.03 e	0.05 ± 0.01 a	0.14 ± 0.02 c	0.03 ± 0.00 a
<i>trans</i> -2-Hexenol ¹	0.29 ± 0.03 d	0.03 ± 0.00 a	0.24 ± 0.02 c	0.01 ± 0.00 a	0.52 ± 0.03 e	0.07 ± 0.01 b
<i>cis</i> -2-Hexenol ²	7.45 ± 0.50 d	2.55 ± 0.19 b	4.67 ± 0.38 c	1.45 ± 0.45 a	8.48 ± 0.84 e	2.26 ± 0.37 ab
Hexanal ¹	1.50 ± 0.07 d	0.12 ± 0.01 a	1.34 ± 0.11 c	0.08 ± 0.00 a	0.96 ± 0.11 b	0.06 ± 0.01 a
<i>trans</i> -3-Hexenal ²	9.96 ± 1.00 a	ND	8.88 ± 0.96 a	ND	10.28 ± 1.26 a	ND
<i>trans</i> -2-Hexenal ¹	0.12 ± 0.01 ab	0.16 ± 0.02 c	0.17 ± 0.01 c	0.09 ± 0.01 a	0.13 ± 0.01 b	0.11 ± 0.02 ab
<i>cis</i> -2-Hexenal ²	82.61 ± 4.95 d	0.95 ± 0.21 a	66.07 ± 12.07 c	0.68 ± 0.07 a	34.77 ± 7.63 b	0.68 ± 0.13 a
<i>C6 compounds</i> ¹	2.54 ± 0.15 b	0.54 ± 0.07 a	2.52 ± 0.13 b	0.30 ± 0.01 a	2.42 ± 0.27 b	0.41 ± 0.06 a
Linalool ²	0.60 ± 0.15 ab	0.81 ± 0.24 b	0.25 ± 0.05 a	0.67 ± 0.35 ab	0.57 ± 0.01 ab	0.49 ± 0.14 ab
Nerol ²	0.43 ± 0.07 a	6.88 ± 0.56 c	0.33 ± 0.05 a	3.02 ± 0.36 b	0.81 ± 0.10 a	7.40 ± 0.96 c
Geraniol ²	1.35 ± 0.28 a	28.23 ± 2.48 b	1.57 ± 0.31 a	12.66 ± 1.89 a	6.85 ± 0.61 a	51.20 ± 11.15 c
<i>Terpenes</i> ²	2.37 ± 0.34 a	35.91 ± 3.26 c	2.15 ± 0.37 a	16.35 ± 2.20 b	8.22 ± 0.71 ab	59.09 ± 11.87 d
3-Oxo- α -ionol ¹	ND	0.15 ± 0.02 b	ND	0.08 ± 0.01 a	ND	0.08 ± 0.01 a
β -ionol ²	ND	16.51 ± 1.29 c	ND	2.96 ± 0.20 a	ND	6.80 ± 0.83 b
<i>Norisoprenoids</i> ¹	ND	0.17 ± 0.02 b	ND	0.09 ± 0.01 a	ND	0.08 ± 0.02 a
Benzaldehyde ²	5.41 ± 0.77 a	31.71 ± 6.41 bc	6.77 ± 0.39 a	36.18 ± 3.46 c	5.51 ± 1.06 a	27.87 ± 2.21 b
Benzyl alcohol ¹	0.01 ± 0.00 a	1.12 ± 0.22 c	0.08 ± 0.00 a	1.50 ± 0.20 d	0.02 ± 0.00 a	0.72 ± 0.04 b
Phenylacetaldehyde ²	3.63 ± 1.08 ab	4.32 ± 0.51 ab	6.99 ± 0.53 c	3.17 ± 0.54 a	5.72 ± 1.96 bc	2.74 ± 0.31 a
2-Phenylethanol ¹	0.02 ± 0.00 a	0.57 ± 0.07 c	0.19 ± 0.03 b	0.58 ± 0.03 c	0.01 ± 0.00 a	0.19 ± 0.01 b

Compounds	Tempranillo		Moribel		Tinto Fragoso	
	Free	Bound	Free	Bound	Free	Bound
Eugeno ²	ND	14.80 ± 1.88 b	ND	2.84 ± 0.62 a	ND	26.30 ± 1.83 c
Vanillin ¹	0.01 ± 0.00 a	0.05 ± 0.00 b	0.11 ± 0.04 c	0.04 ± 0.00 ab	0.01 ± 0.00 a	0.02 ± 0.00 ab
Acetovanillone ¹	0.01 ± 0.00 a	0.15 ± 0.03 c	0.01 ± 0.00 a	0.08 ± 0.00 b	0.00 ± 0.00 a	0.15 ± 0.01 c
Benzeric compounds ¹	0.04 ± 0.00 a	1.94 ± 0.31 d	0.40 ± 0.06 b	2.24 ± 0.24 e	0.05 ± 0.01 a	1.13 ± 0.04 c
1-Butanol ²	12.23 ± 1.25 a	98.88 ± 4.09 e	20.17 ± 1.73 b	32.81 ± 1.74 c	30.76 ± 2.62 c	38.28 ± 4.27 d
3-Methyl-2-butanol ¹	ND	0.08 ± 0.01 b	ND	0.03 ± 0.00 a	ND	0.03 ± 0.00 a
3-Methyl-3-butanol ¹	1.79 ± 0.12 b	0.25 ± 0.02 a	4.53 ± 0.39 c	0.11 ± 0.01 a	2.48 ± 0.73 b	0.05 ± 0.01 a
1-Pentanol ²	6.63 ± 0.86 a	40.70 ± 1.82 c	9.78 ± 0.93 a	25.48 ± 0.84 b	7.98 ± 0.54 a	26.45 ± 3.73 b
2-Ethyl-1-hexanol ¹	0.01 ± 0.00 bc	0.04 ± 0.00 e	0.02 ± 0.00 cd	0.02 ± 0.00 d	0.01 ± 0.00 e	0.01 ± 0.00 a
1-Octanol ²	1.07 ± 0.11 a	11.97 ± 1.31 d	1.40 ± 0.26 a	5.68 ± 0.42 c	1.32 ± 0.19 a	3.37 ± 0.44 b
3-Octanol ²	2.58 ± 0.09 b	2.51 ± 0.11 b	2.66 ± 0.05 b	2.49 ± 0.16 b	2.59 ± 0.02 b	2.12 ± 0.17 a
Alcohols ¹	0.04 ± 0.00 a	0.52 ± 0.03 d	0.05 ± 0.00 a	0.23 ± 0.01 c	0.06 ± 0.01 a	0.15 ± 0.02 b
Total ¹	2.62 ± 0.16 bc	3.20 ± 0.43 c	2.98 ± 0.08 bc	2.88 ± 0.26 bc	2.53 ± 0.27 b	1.83 ± 0.11 a

¹mg/L.²µg/L.

Abbreviation: ND = not detected.

Different letters in the same row indicate significant differences (ANOVA, Student–Neuman–Keuls test, $p < 0.05$).Mean value ± standard deviation, $n = 3$.

Adapted from Pérez-Navarro et al. [19].

Table 1. Volatile composition of Tempranillo, Moribel, and Tinto Fragoso grape musts.

Compounds	Airen		Albillo Dorado		Montonera del Casar	
	Free	Bound	Free	Bound	Free	Bound
1-Hexanol ¹	0.17 ± 0.01 c	0.03 ± 0.00 a	0.27 ± 0.06 d	0.10 ± 0.02 b	0.46 ± 0.04 e	0.04 ± 0.00 a
<i>trans</i> -3-Hexenol ²	5.28 ± 0.26 a	ND	4.49 ± 0.84 a	ND	31.17 ± 2.79 b	ND
<i>cis</i> -3-Hexenol ¹	0.45 ± 0.02 e	0.03 ± 0.00 a	0.37 ± 0.03 d	0.08 ± 0.01 b	0.16 ± 0.02 c	0.01 ± 0.00 a
<i>trans</i> -2-Hexenol ¹	0.25 ± 0.03 b	0.01 ± 0.00 a	0.27 ± 0.01 b	0.02 ± 0.00 a	0.60 ± 0.04 c	0.02 ± 0.00 a
<i>cis</i> -2-Hexenol ²	3.80 ± 0.22 b	0.93 ± 0.35 a	6.17 ± 1.56 c	1.86 ± 0.93 a	12.08 ± 1.15 d	0.94 ± 0.32 a
Hexanal ¹	0.84 ± 0.04 b	0.06 ± 0.00 a	1.10 ± 0.14 c	0.06 ± 0.00 a	1.24 ± 0.12 d	0.06 ± 0.00 a
<i>trans</i> -3-Hexenal ²	7.29 ± 0.15 a	ND	10.89 ± 0.27 c	ND	10.26 ± 0.18 b	ND
<i>trans</i> -2-Hexenal ¹	0.19 ± 0.01 b	0.08 ± 0.01 a	0.15 ± 0.00 c	0.19 ± 0.02 d	0.11 ± 0.02 b	0.17 ± 0.02 c
<i>cis</i> -2-Hexenal ²	56.35 ± 1.83 b	0.51 ± 0.11 a	79.79 ± 8.79 c	0.44 ± 0.12 a	84.79 ± 8.34 c	0.46 ± 0.06 a
<i>C6 compounds</i> ¹	1.90 ± 0.11 c	0.21 ± 0.02 a	2.27 ± 0.13 d	0.45 ± 0.03 b	2.72 ± 0.18 e	0.30 ± 0.03 ab
Linalool ²	0.26 ± 0.02 a	0.62 ± 0.11 a	0.24 ± 0.03 a	0.55 ± 0.35 a	0.25 ± 0.02 a	0.62 ± 0.18 a
Nerol ²	0.39 ± 0.04 a	6.27 ± 0.55 d	0.36 ± 0.06 a	2.81 ± 0.23 b	0.65 ± 0.08 a	4.51 ± 0.44 c
Geraniol ²	1.39 ± 0.26 a	19.14 ± 2.16 b	3.16 ± 0.38 a	21.27 ± 7.77 b	2.09 ± 0.63 a	38.65 ± 4.35 c
<i>Terpenes</i> ²	2.04 ± 0.26 a	26.02 ± 1.91 b	3.76 ± 0.41 a	24.63 ± 7.93 b	3.83 ± 0.98 a	43.79 ± 4.83 c
3-Oxo- α -ionol ¹	ND	0.04 ± 0.01 a	ND	0.13 ± 0.01 c	ND	0.07 ± 0.01 b
β -Ionol ²	ND	5.20 ± 0.78 b	ND	14.91 ± 0.87 c	ND	1.71 ± 0.20 a
<i>Norisoprenoids</i> ¹	ND	0.05 ± 0.02 a	ND	0.14 ± 0.01 c	ND	0.07 ± 0.01 b
Benzaldehyde ²	4.40 ± 0.50 a	16.39 ± 1.78 a	7.76 ± 0.69 a	25.49 ± 1.32 a	17.76 ± 0.87 a	498.96 ± 32.79 b
Benzyl alcohol ¹	0.01 ± 0.00 a	0.49 ± 0.03 b	0.01 ± 0.00 a	0.61 ± 0.03 c	0.02 ± 0.00 a	0.84 ± 0.14 d
Phenylacetaldehyde ²	4.76 ± 0.34 b	2.65 ± 0.40 a	5.11 ± 0.08 b	2.81 ± 0.26 a	12.27 ± 1.99 c	3.79 ± 0.27 ab

Compounds	Airén		Albillo Dorado		Montonera del Casar	
	Free	Bound	Free	Bound	Free	Bound
2-Phenylethanol ¹	0.01 ± 0.00 a	0.25 ± 0.03 b	0.02 ± 0.00 a	0.46 ± 0.04 d	0.03 ± 0.00 a	0.34 ± 0.03 c
Eugenol ²	ND	5.50 ± 1.56 b	ND	2.09 ± 0.36 a	ND	1.00 ± 0.22 a
Vanillin ²	6.75 ± 2.39 a	24.71 ± 6.55 b	27.68 ± 8.64 b	9.93 ± 1.05 a	66.42 ± 11.29 c	9.37 ± 0.65 a
Acetovanillone ²	0.89 ± 0.58 a	79.25 ± 10.71 c	4.16 ± 0.69 a	62.71 ± 3.57 b	5.27 ± 1.45 a	55.52 ± 5.86 b
Benzenic compounds ¹	0.03 ± 0.00 a	0.87 ± 0.06 b	0.08 ± 0.01 a	1.17 ± 0.07 c	0.15 ± 0.01 a	1.74 ± 0.17 d
1-Butanol ²	2.72 ± 0.27 a	6.92 ± 1.08 b	23.10 ± 2.22 d	45.67 ± 1.36 e	6.35 ± 0.29 b	10.65 ± 1.23 c
3-Methyl-2-butanol ²	ND	48.19 ± 3.81 a	ND	44.88 ± 4.71 a	ND	47.28 ± 1.87 a
3-Methyl-3-butanol ²	1.74 ± 0.24 a	94.26 ± 9.15 b	2.05 ± 0.12 a	104.86 ± 14.51 b	2.41 ± 0.17 a	92.79 ± 10.81 b
1-Pentanol ²	5.96 ± 0.88 a	15.88 ± 2.65 c	9.80 ± 1.12 b	28.06 ± 1.94 e	9.12 ± 0.25 b	19.28 ± 2.00 d
2-Ethyl-1-hexanol ²	9.94 ± 0.39 a	22.60 ± 1.69 c	11.58 ± 0.73 a	27.08 ± 3.29 d	14.82 ± 0.63 b	22.60 ± 0.59 c
1-Octanol ²	1.15 ± 0.24 a	3.42 ± 0.77 b	1.37 ± 0.20 a	3.43 ± 1.07 b	1.15 ± 0.14 a	2.52 ± 0.37 ab
3-Octanol ²	2.14 ± 0.05 a	2.38 ± 0.10 b	2.61 ± 0.12 b	2.40 ± 0.16 b	2.61 ± 0.07 b	2.02 ± 0.13 a
Alcohols ¹	0.02 ± 0.00 a	0.19 ± 0.01 c	0.05 ± 0.00 b	0.25 ± 0.02 d	0.04 ± 0.00 ab	0.20 ± 0.02 c
Total ¹	1.96 ± 0.11 b	1.34 ± 0.07 a	2.40 ± 0.91 c	2.03 ± 0.09 b	2.91 ± 0.17 d	2.35 ± 0.21 c

¹mg/L.

²µg/L.

Abbreviation: ND = not detected.

Different letters in the same row indicate significant differences (ANOVA, Student–Neuman–Keuls test, $p < 0.05$).

Mean value ± standard deviation, $n = 3$.

Adapted from Pérez-Navarro et al. [19].

Table 2.

Volatile composition of Airén, Albillo Dorado, and Montonera del Casar grape musts.

threshold (6 µg/L) [26], thus may have a significant impact on wine aroma. In all grapes evaluated, the concentrations of this compound exceeded its odor threshold with the highest concentration in Tinto Fragoso grapes (26.30 µg/L). In this study, red grape cultivars were characterized by a higher eugenol concentration than white ones. Several alcohols in both free and bound forms were identified in the studied grapes. However, 3-methyl-2-butanol was only detected in the free form. The six grape cultivars had greater levels of bound alcohols than the free fraction. The higher amounts of alcohol in the glycosidic form were 5 to 13 times higher than those in the free forms. These compounds derived from grapes do have not a significant impact on wine aroma due to the number of alcohols formed during alcoholic fermentation as secondary yeast metabolites [6]. The total concentration of free volatiles varied according to grape cultivar, with values between 1.96 and 2.98 mg/L. In general, total concentration of the bound fraction of volatile compounds was lower than those observed in the free forms. However, the proportion of glycosylated fraction present in all grapes may have a relevant role in the aromatic potential of these grapes, with a concentration ranging from 1.34 to 3.20 mg/L. The bound volatile compounds can be converted into free form by hydrolysis, modifying the aromatic profile of wines and enhancing the varietal character [27].

3.2 Aroma composition of wines

Wines fermented by the commercial *Saccharomyces cerevisiae* strain used were analyzed by gas chromatography and mass spectrometry to evaluate the influence of the yeast strain on aromatic profile of wines made from Airén, Tempranillo, and the minority grape varieties. The results of the GC-MS analysis of wines are given in **Table 3**. Differences in the aroma components of wines were noticeable even the typical varietal aroma was preserved.

Volatile compounds affecting the secondary aroma of wine are produced *via* yeast metabolism during alcoholic fermentation and have a significant impact on sensory properties of wine [30]. Some of these compounds are alcohols and esters which are present at high concentrations in the evaluated wines. Alcohols are secondary metabolites of yeast and optimal levels of them provide fruity odors. However, an excessive amount of these compounds can have negative impact on aroma and flavor of wine, resulting in pungent smell and taste [31].

In wine, variations in the alcohol concentration can occur using different yeast strains during alcoholic fermentation [32]. Total alcohol concentrations ranged from 284 to 519 mg/L in the evaluated samples, showing greater values in red wines. Isoamyl alcohol is synthesized in the yeast cell through the Ehrlich pathway [6]. In all wines, this compound was the most abundant alcohol produced (173–305 mg/L). Most of ethyl esters of carboxylic acids are biosynthesized by the yeast metabolism during fermentation. All samples contained ethyl lactate which was produced at high levels in red wines due to the malolactic fermentation. Ethyl acetate is a compound related to fruity aromas and was the most abundant ester determined in wines, with concentration values between 50 and 85 mg/L. Other significant esters are isoamyl acetate (banana odor) and 2-phenylethyl acetate (honey, fruity and flowery aromas).

Acetaldehyde is also produced by the metabolism of yeasts and its production is affected by SO₂ content of the media [33]. Significant differences in acetaldehyde content were observed among wines, showing lower levels in red wines due to the least amount of SO₂ used in red winemaking. An important group of volatile compounds in wines are carboxylic acids and their lactones which are products of the lipid

Compounds	Tempranillo	Morribel	Tinto Fragoso	Airén	Albillo Dorado	Montonera del Casar
1-Hexanol ¹	2.50 ± 0.02 d	1.82 ± 0.04 c	3.16 ± 0.09 e	0.80 ± 0.18 b	0.66 ± 0.03 b	0.39 ± 0.01 a
<i>trans</i> -3-Hexenol ¹	0.07 ± 0.01 a	0.15 ± 0.01 b	0.09 ± 0.01 a	0.17 ± 0.04 b	0.16 ± 0.00 b	0.08 ± 0.00 a
<i>cis</i> -3-Hexenol ¹	0.99 ± 0.07 b	1.25 ± 0.08 b	1.11 ± 0.01 b	1.86 ± 0.37 c	1.01 ± 0.01 b	0.05 ± 0.00 a
<i>C6 compounds</i> ¹	3.56 ± 0.05 c	3.21 ± 0.13 c	4.36 ± 0.11 d	2.83 ± 0.59 c	1.82 ± 0.09 b	0.52 ± 0.01 a
Linalool ²	2.16 ± 0.04 ab	1.40 ± 0.17 a	4.76 ± 0.54 c	2.98 ± 0.58 b	2.78 ± 0.20 b	3.17 ± 0.19 b
Citronellol ²	2.88 ± 0.38 b	1.60 ± 0.09 a	4.25 ± 0.08 c	2.11 ± 0.29 ab	2.21 ± 0.35 ab	2.49 ± 0.04 b
Geraniol ²	12.18 ± 0.84 c	14.64 ± 0.77 d	18.12 ± 0.43 e	4.94 ± 0.91 a	4.12 ± 0.60 a	6.75 ± 0.43 b
<i>Terpenes</i> ²	17.22 ± 0.42 c	17.64 ± 0.69 c	27.13 ± 0.89 d	10.02 ± 1.78 ab	9.11 ± 1.14 a	12.41 ± 0.66 b
β -Damasenone ²	4.73 ± 0.90 ab	2.30 ± 0.65 a	6.41 ± 0.22 ab	3.50 ± 0.52 a	10.57 ± 2.98 b	7.76 ± 2.67 ab
β -Ionone ²	0.52 ± 0.01 b	0.47 ± 0.05 b	0.58 ± 0.06 b	0.10 ± 0.02 a	0.08 ± 0.01 a	0.02 ± 0.00 a
<i>Norisoprenoids</i> ²	5.24 ± 0.91 ab	2.78 ± 0.70 a	6.99 ± 0.16 ab	3.60 ± 0.54 ab	10.66 ± 3.00 b	7.78 ± 2.67 ab
Benzaldehyde ²	ND	ND	ND	2.32 ± 0.79 b	0.98 ± 0.15 a	44.13 ± 0.31 c
Eugenol ²	4.17 ± 0.45 a	13.39 ± 1.41 b	59.24 ± 5.82 c	2.86 ± 0.15 a	0.93 ± 0.17 a	0.82 ± 0.18 a
Guaiacol ²	10.89 ± 0.28 b	7.38 ± 0.13 ab	9.93 ± 4.07 b	2.14 ± 0.47 a	2.30 ± 0.66 a	11.82 ± 1.43 b
2-Phenyletanol ¹	28.62 ± 3.09 b	20.93 ± 1.97 a	39.69 ± 3.15 c	17.70 ± 5.85 a	17.86 ± 1.19 a	11.74 ± 0.29 a
Vanillin ²	22.82 ± 1.51 b	28.61 ± 5.97 b	31.97 ± 3.82 b	7.33 ± 1.26 a	9.44 ± 2.51 a	5.75 ± 0.40 a
Acetovanillone ¹	0.09 ± 0.00 a	0.09 ± 0.00 a	0.44 ± 0.03 b	ND	ND	ND
<i>Benzenic compounds</i> ¹	28.74 ± 3.09 b	21.08 ± 1.97 ab	40.24 ± 3.19 c	17.71 ± 5.85 a	17.87 ± 1.19 a	11.80 ± 0.29 a
Methanol ¹	80.97 ± 29.53 a	84.51 ± 5.42 a	118.16 ± 40.50 a	48.95 ± 1.90 a	40.53 ± 1.24 a	120.37 ± 3.28 a
1-Propanol ¹	32.35 ± 5.66 a	38.36 ± 1.99 a	46.68 ± 11.50 a	ND	ND	ND
Isoamyl alcohol ¹	262.11 ± 24.31 c	242.84 ± 8.16 bc	305.28 ± 7.17 d	200.17 ± 0.75 ab	231.18 ± 21.49 bc	173.87 ± 0.71 a
3-Ethoxy-propanol ²	27.36 ± 10.92 a	35.23 ± 4.76 a	115.55 ± 70.69 a	3.55 ± 1.63 a	2.22 ± 1.59 a	14.58 ± 1.90 a
3-Methylthio-propanol ¹	0.26 ± 0.06 a	0.23 ± 0.05 a	0.85 ± 0.06 a	0.25 ± 0.08 a	0.22 ± 0.10 a	0.25 ± 0.04 a

Compounds	Tempranillo	Moribel	Tinto Fragoso	Airén	Albillo Dorado	Montonera del Casar
Isobutanol ¹	41.13 ± 1.25 a	36.92 ± 3.12 a	48.34 ± 4.24 a	34.88 ± 3.54 a	52.19 ± 11.76 a	50.95 ± 1.34 a
Alcohols ¹	416.84 ± 49.48 a	402.90 ± 2.43 a	519.41 ± 55.06 b	284.26 ± 2.73 a	324.12 ± 34.58 a	345.45 ± 1.27 a
Isobutyric acid ¹	0.23 ± 0.01 a	0.23 ± 0.05 a	0.29 ± 0.16 b	ND	ND	ND
Butyric acid ¹	0.48 ± 0.07 a	0.50 ± 0.07 a	0.28 ± 0.17 a	0.49 ± 0.07 a	0.32 ± 0.09 a	0.58 ± 0.04 a
Isovaleric acid ¹	1.06 ± 0.06 a	1.20 ± 0.23 a	1.27 ± 0.83 a	1.08 ± 0.30 a	0.81 ± 0.22 a	0.91 ± 0.11 a
Hexanoic acid ¹	12.15 ± 1.37 b	14.01 ± 0.47 b	5.75 ± 3.42 a	12.29 ± 1.83 b	10.87 ± 0.52 ab	10.16 ± 0.39 ab
Octanoic acid ¹	19.57 ± 2.67 ab	26.39 ± 2.06 b	10.42 ± 6.06 a	48.48 ± 7.08 c	38.65 ± 1.28 c	43.93 ± 4.87 c
Decanoic acid ¹	4.18 ± 0.98 a	6.46 ± 1.15 a	2.90 ± 1.68 a	12.26 ± 0.35 b	12.91 ± 0.38 b	19.99 ± 3.71 c
Acids ¹	37.68 ± 5.16 ab	48.79 ± 4.03 bc	20.91 ± 12.32 a	74.61 ± 8.93 c	63.56 ± 1.47 c	75.58 ± 9.13 c
Acetaldehyde ¹	10.69 ± 1.30 a	8.72 ± 0.43 a	11.16 ± 1.36 a	76.06 ± 6.43 d	41.43 ± 3.46 c	32.17 ± 3.58 b
Aldehydes ¹	10.69 ± 1.30 a	8.72 ± 0.43 a	11.16 ± 1.36 a	76.06 ± 6.43 d	41.43 ± 3.46 c	32.17 ± 3.58 b
Ethyl acetate ¹	63.42 ± 12.04 a	85.49 ± 1.91 b	52.09 ± 3.42 a	43.35 ± 5.13 a	50.21 ± 5.49 a	60.28 ± 2.18 a
Isoamyl acetate ¹	717 ± 2.38 b	10.34 ± 1.34 b	2.13 ± 0.27 a	708 ± 1.13 b	6.97 ± 0.60 b	6.32 ± 0.41 b
2-Phenylethyl acetate ¹	0.28 ± 0.04 b	0.22 ± 0.06 b	0.13 ± 0.01 a	0.26 ± 0.03 b	0.26 ± 0.02 b	0.26 ± 0.02 b
Ethyl lactate ¹	36.35 ± 0.43 c	24.34 ± 1.08 b	38.87 ± 4.22 c	5.06 ± 0.52 a	7.41 ± 0.72 a	6.47 ± 0.44 a
Ethyl butyrate ¹	0.33 ± 0.03 a	0.47 ± 0.03 b	0.22 ± 0.01 a	0.25 ± 0.06 a	0.30 ± 0.04 a	0.26 ± 0.01 a
Ethyl hexanoate ¹	0.57 ± 0.15 a	0.61 ± 0.01 a	0.59 ± 0.04 a	1.03 ± 0.12 b	0.89 ± 0.06 ab	0.85 ± 0.00 ab
Ethyl octanoate ¹	0.68 ± 0.10 a	0.66 ± 0.01 a	0.55 ± 0.06 a	1.47 ± 0.19 b	1.34 ± 0.17 b	1.39 ± 0.04 b
Ethyl decanoate ¹	0.12 ± 0.02 a	0.12 ± 0.00 a	0.11 ± 0.02 a	0.27 ± 0.01 b	0.36 ± 0.07 c	0.41 ± 0.01 c
Esters ¹	108.94 ± 15.17 bc	122.24 ± 1.53 c	94.68 ± 5.04 b	58.75 ± 5.45 a	67.73 ± 5.70 a	76.24 ± 3.01 a
Furaneol ²	12.30 ± 0.78 a	23.54 ± 0.05 a	14.18 ± 4.07 a	27.34 ± 4.71 a	15.24 ± 8.36 a	17.40 ± 3.93 a
Furanic compounds ²	12.30 ± 0.78 a	23.54 ± 0.05 a	14.18 ± 4.07 a	27.34 ± 4.71 a	15.24 ± 8.36 a	17.40 ± 3.93 a

Compounds	Tempranillo	Moribel	Tinto Fragoso	Airén	Albillo Dorado	Montonera del Casar
γ -Butyrolactone ¹	0.07 ± 0.01 a	0.05 ± 0.00 a	0.16 ± 0.04 b	0.04 ± 0.01 a	0.07 ± 0.02 a	0.11 ± 0.00 a
γ -Nonalactone ²	10.13 ± 0.02 c	12.85 ± 1.16 d	23.38 ± 2.09 e	3.29 ± 0.68 a	7.19 ± 0.50 b	6.29 ± 0.13 b
δ -Dodecalactone ²	46.11 ± 5.28 a	45.73 ± 3.79 a	51.46 ± 0.70 a	108.12 ± 28.01 b	70.54 ± 17.90 a	63.00 ± 0.07 a
Lactones ¹	0.13 ± 0.00 a	0.12 ± 0.01 a	0.24 ± 0.04 b	149.15 ± 37.65 ab	144.67 ± 39.34 ab	175.37 ± 3.37 ab

¹mg/L.

²µg/L.

Abbreviation: ND = not detected.

Different letters in the same row indicate significant differences (ANOVA, Student–Newman–Keuls test, $p < 0.05$).

Mean value ± standard deviation, $n = 2$.

Adapted from Pérez-Navarro et al. [19, 28, 29].

Table 3.

Volatile composition of red and white wines.

metabolism of yeast and provide fatty and wax-like smell [34]. The concentrations of these compounds were under the odor threshold and did not influence final aroma of wines.

Green and grassy odors are important for the final aroma of wines and are represented by C6 compounds. In all wine samples, 1-hexanol and *trans*-3-hexenol were quantified in a concentration below the odor threshold value [35], except for *cis*-3-hexenol which is the predominant isomer of 3-hexenol in wines [36].

The production of monoterpenes from different precursors depends on *S. cerevisiae* strain, although its hydrolysis action on bonded terpene fraction in the first fermentation stages is one of the most relevant contributions [37]. Linalool, citronellol, and geraniol were the terpenes identified and quantified in the evaluated wines, which are associated with floral odors [38]. In general, geraniol was the most abundant terpene determined in all samples (4.12–18.12 µg/L). These values were close to those reported previously in literature [39]. The concentrations of these compounds were under the odor thresholds so the contribution of terpenes to resulting wine aroma was minimal.

The concentrations of β -damascenone were higher than those of β -ionone in all wines. The odor threshold of β -damascenone was exceeded, contributing to wine aroma profile [40]. Among benzenic compounds, benzaldehyde was only determined in white wines with a concentration that did not exceed the odor threshold (2000 µg/L) [38] although it could have a synergic effect on wine aroma, providing fruity notes. Tinto Fragoso wine was characterized by a volatile composition rich in eugenol. This was also observed in grapes so this compound may be considered as a varietal marker for this grape cultivar. The 2-phenylethanol was determined at significantly higher concentration than the odor threshold in red wines, supporting rose odors of terpenes like geraniol. Red wines showed 2-phenylethanol concentrations similar to those reported for wines made from Bobal grapes [41].

A large number of volatile compounds were determined in the evaluated wines. However, the impact of these compounds on the overall aroma character is different. To evaluate the influence of each volatile compound on wine aroma, Odor Activity Values (OAVs) were calculated as the ratio between the concentration of the aroma compound and its odor threshold concentration (**Table 4**).

The OAVs higher than 0.1 were grouped into several aromatic series to estimate the overall wine aroma and each compound was assigned to one or several aromatic series (fruity, floral, green/fresh, sweet, spicy, fatty, and other odors) based on similar odor descriptors.

A plot of the values obtained for the aromatic series in the wines made from the six grape cultivars is shown in **Figure 2**, indicating the mean values of each series. The main aroma categories were series I (fruity), III (green, fresh), and VII (other odors) which contributed most to the aroma profile of wines. A great impact of odor compounds on the aromatic profile was observed in white wines, mainly in series I and VII. Tinto Fragoso was characterized by higher OAVs in the spicy aromatic series, which may be explained by its eugenol concentration.

3.3 Sensory profiles

The Napping technique was employed to evaluate the sensory attributes identified in the produced wines from the grape cultivars studied. **Figure 3A** shows Napping data obtained from the sensory analysis of red wines.

A clear separation between red wines was observed in the plot, which indicates that wines made from Tempranillo, Moribel, and Tinto Fragoso showed a distinctive sensory

Compounds	Odor descriptors	Odor threshold (µg/L)	Aromatic series
1-Hexanol	Flower, green, cut grass	8000 [35]	II, III
<i>trans</i> -3-Hexenol	Green	400 [38]	III
<i>cis</i> -3-Hexenol	Green, cut grass	400 [35]	III
Linalool	Floral	15 [35]	II
Citronellol	Floral	100 [35]	II
Geraniol	Roses, geranium	30 [35]	II
β-Damascenone	Sweet, fruit	0.05 [35]	I, IV
β-Ionone	Floral, violet	0.09 [26]	II
Benzaldehyde	Sweet, cherry, almond	2000 [38]	I, IV
Eugenol	Spices, clove, honey	6 [26]	IV, V, VII
Guaiacol	Medicine, caramel, smoke	10 [26]	IV, VI
2-Phenyletanol	Floral, roses	10,000 [35]	II
Vanillin	Vanilla	60 [42]	V, VII
Acetovainillone	Vanilla, clove	1000 [42]	VI
Methanol	Chemical, medicinal	668,000 [38]	VI
1-Propanol	Ripe fruit, alcohol	830,000 [38]	I, VI
Isoamyl alcohol	Solvent, fusel	30,000 [35]	VII
3-Ethoxy-propanol	Fruity	100 [43]	I
3-Methylthio-propanol	Cooked, vegetable	500 [35]	VI
Isobutanol	Bitter, green	40,000 [35]	III, VI
Isobutyric acid	Rancid, butter, cheese	2300 [26]	VI
Butyric acid	Rancid, cheese, sweat	173 [26]	VI
Isovaleric acid	Acid, rancid	33 [26]	IV, VI
Hexanoic acid	Sweat	420 [26]	VI
Octanoic acid	Sweat, cheese	500 [26]	VI
Decanoic acid	Rancid fat	1000 [26]	VI
Acetaldehyde	Pungent, ripe apple	500 [35]	I, VI
Ethyl acetate	Fruity, solvent	7500 [35]	I, VI
Isoamyl acetate	Banana	30 [26]	I
2-Phenylethyl acetate	Floral, roses	250 [35]	II
Ethyl lactate	Acid, medicine	154,636 [42]	VI
Ethyl butyrate	Fruity	20 [35]	I
Ethyl hexanoate	Green apple	14 [26]	I
Ethyl octanoate	Sweet, fruity	5 [26]	I, II, IV
Ethyl decanoate	Sweet, fruity	200 [26]	I, IV
Furaneol	Burnt sugar, caramel, maple	5 [44]	IV
γ-Butyrolactone	Sweet, toast, caramel	35,000 [40]	IV

Compounds	Odor descriptors	Odor threshold ($\mu\text{g/L}$)	Aromatic series
γ -Nonalactone	Coconut	30 [26]	IV
δ -Dodecalactone	Coconut	88 [38]	IV

Aromatic series: I (fruity), II (floral), III (green, fresh), IV (sweet), V (spicy), VI (fatty), and VII (other odors).

Table 4.
 Aromatic series, odor descriptors, and thresholds of some volatile compounds in wines.

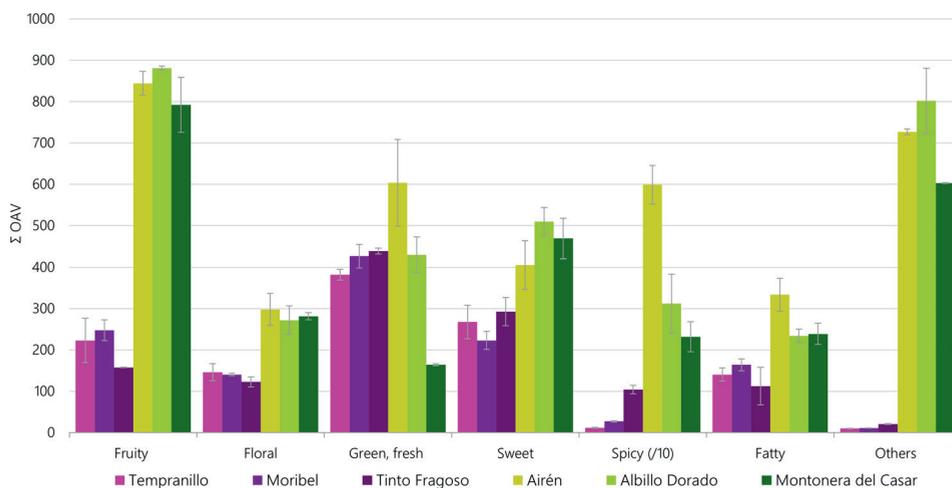


Figure 2.
 Aromatic series in red and white wines (mean value and standard deviation, $n = 2$). Adapted from Pérez-Navarro et al. [19, 28, 29].

profile. Tinto Fragoso wine was characterized by its color with more intensity and purplish nuances. This wine had the most aromatic profile of evaluated red wines, providing spicy notes and persistence in mouth. Wines made from Tempranillo and Moribel grapes showed different odor descriptors. Tempranillo was characterized by red fruity odors, while forest berry and ripe fruit notes defined the aromatic profile of Moribel wine.

The sensory evaluation of white wines by a panel of experienced wine tasters is shown in **Figure 3B**. Airén wine was characterized by green apple odors that are well-known for this grape cultivar [39], and it was located close to Albillo Dorado wine in the plot, which had floral and fruity aromas. More herbaceous odors were determined in Montonera del Casar wine.

4. Conclusions

This chapter gathers the first comprehensive characterization of the chemical aroma composition and sensory properties of wines made from several minority grape cultivars from Castilla-La Mancha (Spain), showing the importance of a proper yeast stain for the development of the desired aroma components in wine. The strong impact exerted by the strain of *Saccharomyces cerevisiae* on wine aroma composition becomes in many cases only from the presence of aromatic fractions from grapes,

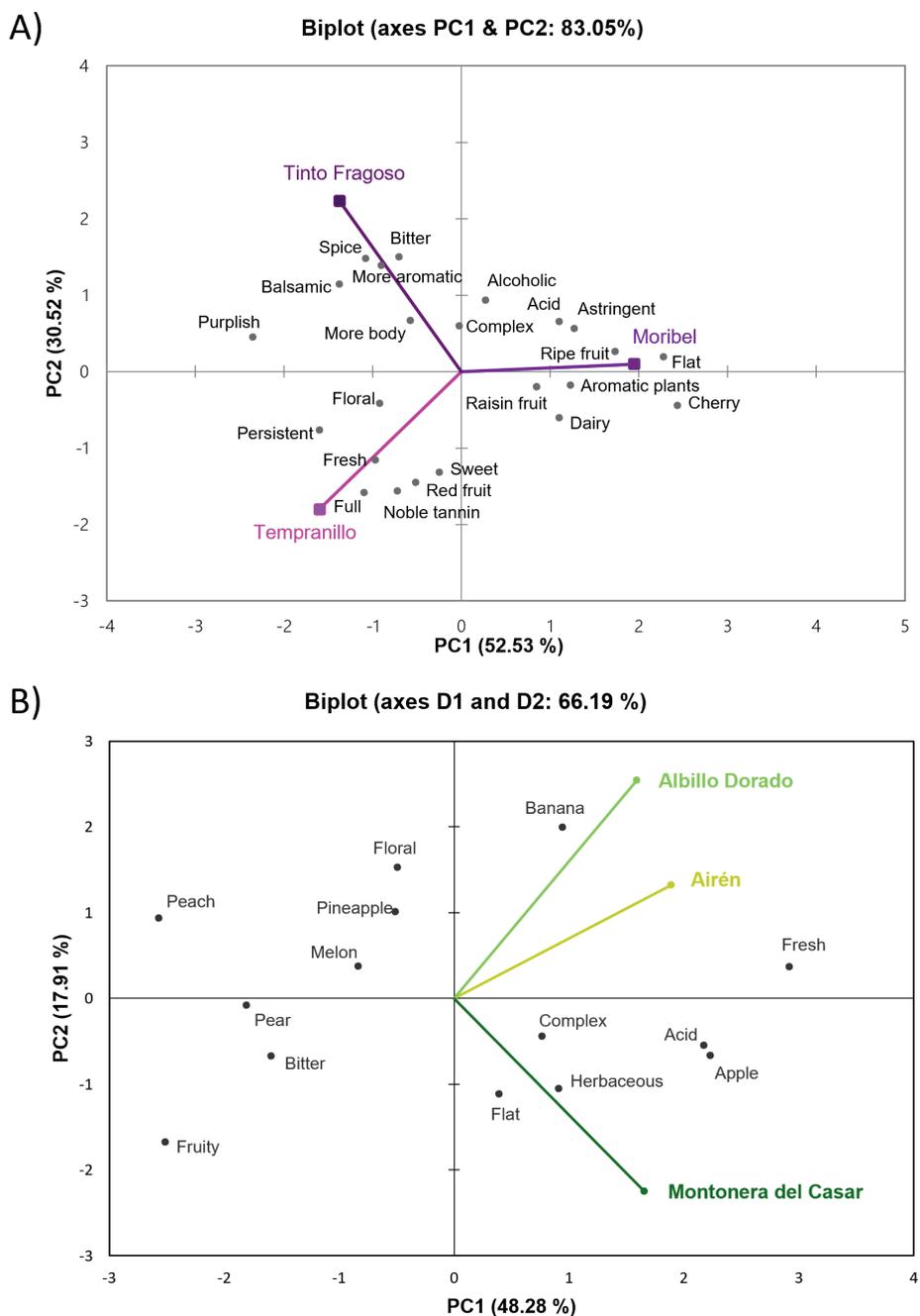


Figure 3. Sensory characterization of red (A) and white (B) wines. Adapted from Pérez-Navarro et al. [19, 28, 29].

having a notable influence on yeast metabolism. Based on the results obtained, two patterns of aroma formation and release from the yeast employed in the vinification of the six grape cultivars are the production of ethyl esters and acetates that provide fruit and fresh attributes and the release of aroma components from grape precursors which enhance the aromatic complexity of wines.

Therefore, the yeast strain selection is pivotal in grape must fermentation due to its significant impact on wine sensory properties, affecting consumer preferences. In addition, this work displayed that these minority grape varieties present an aroma profile characteristic and may be considered a viable alternative to well-known grape varieties used in wine production in this region.

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Conflict of interest

The authors declare no conflict of interest.

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References

- [1] Karabegović I, Malićanin M, Danilović B, Stanojević J, Stojanović SS, Nikolić N, et al. Potential of non-Saccharomyces yeast for improving the aroma and sensory profile of Prokupac red wine. *Oeno One*. 2021;**55**(2):181-195
- [2] Gambetta JM, Bastian SE, Cozzolino D, Jeffery DW. Factors influencing the aroma composition of chardonnay wines. *Journal of Agricultural and Food Chemistry*. 2014;**62**(28):6512-6534
- [3] Hashizume K, Samuta T. Grape maturity and light exposure affect berry methoxypyrazine concentration. *American Journal of Enology and Viticulture*. 1999;**50**(2):194-198
- [4] Canosa P, Oliveira JM, Masa A, Vilanova M. Study of the volatile and glycosidically bound compounds of minority *Vitis vinifera* red cultivars from NW Spain. *Journal of the Institute of Brewing*. 2011;**117**(3):462-471
- [5] Ferreira V, De-la-Fuente-Blanco A, Sáenz-Navajas M. Wine aroma vectors and sensory attributed. In: Reynolds AG, editor. *Managing Wine Quality*. 2nd ed. Cambridge, UK: Woodhead Publishing Limited; 2022. pp. 3-39
- [6] Swiegers J, Bartowsky E, Henschke P, Pretorius I. Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research*. 2005;**11**(2):139-173
- [7] Denat M, Ferreira V, Pérez D, Sáenz-Navajas MP, Heras J. Impact of two yeast strains on Tempranillo red wine aroma profiles throughout accelerated ageing. *OENO One*. 2021;**55**(4):181-195
- [8] Roca P. State of the World Vine and Wine Sector. OIV Press Conference; 2022. DOI: 10.1787/9789264244047-44-en
- [9] Wilmink J, Breuer M, Forneck A. Effects of grape phylloxera leaf infestation on grapevine growth and yield parameters in commercial vineyards: A pilot study. *OENO One*. 2022;**56**(1):197-208
- [10] Robinson J, Harding J, Vouillamoz J. *Wine Grapes: A Complete Guide to 1,368 Vine Varieties, Including their Origins and Flavours*. Harmondsworth, UK: Allen Lane/Penguin; 2012
- [11] Mena A, Martínez J, Fernández-González M. Recovery, identification and relationships by microsatellite analysis of ancient grapevine cultivars from Castilla-La Mancha: The largest wine growing region in the world. *Genetic Resources and Crop Evolution*. 2014;**61**:625-637
- [12] Ibarz MJ, Ferreira V, Hernández-Orte P, Loscos N, Cacho J. Optimization and evaluation of a procedure for the gas chromatographic-mass spectrometric analysis of the aromas generated by fast acid hydrolysis of flavor precursors extracted from grapes. *Journal of Chromatography A*. 2006;**1116**(1-2):217-229
- [13] Francis I, Newton J. Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research*. 2005;**11**(2):114-126
- [14] Vilanova M, Masa A, Tardaguila J. Evaluation of the aroma descriptors variability in Spanish grape cultivars by a quantitative descriptive analysis. *Euphytica*. 2009;**165**:383-389
- [15] International Organization for Standardization. ISO 8586-1:1993 - Sensory Analysis. General Guidance for the Selection, Training and Monitoring of Assessors. Part 1: Selected Assessors; 1993

- [16] International Organization for Standardization. ISO 11035:1994 - Sensory Analysis. Identification and Selection of Descriptors for Establishing a Sensory Profile by a Multidimensional Approach; 1994
- [17] International Organization for Standardization. ISO 8589:2007 - Sensory Analysis. General Guidance for the Design of Test Rooms; 2007
- [18] Pagès J. Recueil direct de distances sensorielles: Application à l'évaluation de dix vins blancs du Val-de-Loire. *Sciences Des Aliments*. 2003;23(5-6):679-688
- [19] Pérez-Navarro J. Calidad enológica de nuevos genotipos de uva identificados en el Banco de Germoplasma de Vid de Castilla-La Mancha [thesis]. Ciudad Real: University of Castilla-La Mancha; 2019
- [20] Kalua CM, Boss PK. Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). *Journal of Agricultural and Food Chemistry*. 2009;57(9):3818-3830
- [21] Qian X, Sun L, Xu X-Q, Zhu B-Q, Xu H-Y. Differential expression of VvLOXA diversifies C6 volatile profiles in some *Vitis vinifera* table grape cultivars. *International Journal of Molecular Sciences*. 2017;18(12):2705
- [22] Mateo J, Jiménez M. Monoterpenes in grape juice and wines. *Journal of Chromatography A*. 2000;881(1-2):557-567
- [23] Vilanova M, Genisheva Z, Bescansa L, Masa A, Oliveira JM. Changes in free and bound fractions of aroma compounds of four *Vitis vinifera* cultivars at the last ripening stages. *Phytochemistry*. 2012;74:196-205
- [24] Vilanova M, Martínez C. First study of determination of aromatic compounds of red wine from *Vitis vinifera* cv. Castañal grown in Galicia (NW Spain). *European Food Research and Technology*. 2007;224:431-436
- [25] Lamikanra O, Grimm CC, Inyang ID. Formation and occurrence of flavor components in Noble muscadine wine. *Food Chemistry*. 1996;56(4):373-376
- [26] Ferreira V, López R, Cacho JF. Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture*. 2000;80(11):1659-1667
- [27] Mendes Ferreira A, Clímaco M, Mendes FA. The role of non-Saccharomyces species in releasing glycosidic bound fraction of grape aroma components—A preliminary study. *Journal of Applied Microbiology*. 2001;91(1):67-71
- [28] Pérez-Navarro J, Izquierdo-Canas PM, Mena-Morales A, Martínez-Gascuena J, Chacon-Vozmediano JL, Garcia-Romero E, et al. First chemical and sensory characterization of Moribel and Tinto Fragoso wines using HPLC-DAD-ESI-MS/MS, GC-MS, and napping techniques: Comparison with Tempranillo. *Journal of the Science of Food and Agriculture*. 2019;99(5):2108-2123
- [29] Pérez-Navarro J, Izquierdo-Cañas PM, Mena-Morales A, Chacón-Vozmediano JL, Martínez-Gascuña J, García-Romero E, et al. Comprehensive chemical and sensory assessment of wines made from white grapes of *Vitis vinifera* cultivars Albillo Dorado and Montonera del Casar: A comparative study with Airén. *Food*. 2020;9(9):1282
- [30] Ferreira V, Fernández P, Peña C, Escudero A, Cacho JF. Investigation on the role played by fermentation esters in the aroma of young Spanish wines

- by multivariate analysis. *Journal of the Science of Food and Agriculture*. 1995;**67**(3):381-392
- [31] Swiegers JH, Pretorius IS. Yeast modulation of wine flavor. *Advances in Applied Microbiology*. 2005;**57**:131-175
- [32] Giudici P, Romano P, Zambonelli C. A biometric study of higher alcohol production in *Saccharomyces cerevisiae*. *Canadian Journal of Microbiology*. 1990;**36**(1):61-64
- [33] Herraiz T, Martín-Alvarez PJ, Reglero G, Herraiz M, Cabezudo MD. Differences between wines fermented with and without Sulphur dioxide using various selected yeasts. *Journal of the Science of Food and Agriculture*. 1989;**49**(2):249-258
- [34] Vilanova M, Oliveira JM. Application of gas chromatography on the evaluation of grape and wine aroma in Atlantic viticulture (NW Iberian Peninsula). In: Salih B, Celikbicak O, editors. *Gas Chromatography in Plant Science, Wine Technology, Toxicology and Some Specific Applications*. London, UK: IntechOpen; 2012. pp. 109-146
- [35] Guth H. Identification of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry*. 1997;**45**(8):3022-3026
- [36] Boido E. Modificaciones producidas por la fermentación maloláctica en la fracción aromática de los vinos Tannat. *Sección Enología*. 2002
- [37] Ugliano M, Bartowsky EJ, McCarthy J, Moio L, Henschke PA. Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three *Saccharomyces* yeast strains. *Journal of Agricultural and Food Chemistry*. 2006;**54**(17):6322-6331
- [38] Etievant P. *Volatile Compounds in Food and Beverages: Wine*. New York: Marcel Dekker; 1991
- [39] Palomo ES, Hidalgo MD, Gonzalez-Vinas M, Perez-Coello M. Aroma enhancement in wines from different grape varieties using exogenous glycosidases. *Food Chemistry*. 2005;**92**(4):627-635
- [40] Escudero A, Campo E, Fariña L, Cacho J, Ferreira V. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry*. 2007;**55**(11):4501-4510
- [41] García-Carpintero EG, Sánchez-Palomo E, González-Viñas M. Aroma characterization of red wines from cv. Bobal grape variety grown in La Mancha region. *Food Research International*. 2011;**44**(1):61-70
- [42] Gómez-Míguez MJ, Cacho JF, Ferreira V, Vicario IM, Heredia FJ. Volatile components of Zalema white wines. *Food Chemistry*. 2007;**100**(4):1464-1473
- [43] Peinado R, Moreno J, Medina M, Mauricio J. Changes in volatile compounds and aromatic series in sherry wine with high gluconic acid levels subjected to aging by submerged flor yeast cultures. *Biotechnology Letters*. 2004;**26**:757-762
- [44] Escudero A, Gogorza B, Melus M, Ortin N, Cacho J, Ferreira V. Characterization of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *Journal of Agricultural and Food Chemistry*. 2004;**52**(11):3516-3524