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Influence of Native *Saccharomyces cerevisiae* Strains from D.O. "Vinos de Madrid" in the Volatile Profile of White Wines

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Abstract: Yeasts during alcoholic fermentation form a vast number of volatile compounds that significantly influence wine character and quality. It is well known that the capacity to form aromatic compounds is dependent on the yeast strain. Thus, the use of native yeast strains, besides promoting biodiversity, encourages the conservation of regional sensory properties. In this work, we studied the volatile profile of Malvar wines fermented with 102 *Saccharomyces cerevisiae* yeast strains, isolated from vineyards and cellars belonging to the D.O. "Vinos de Madrid". The wines elaborated with different *S. cerevisiae* showed a good classification by cellar of origin. Additionally, seven sensory descriptors have helped to classify the wines depending on their predominant aromatic character. Twenty-nine *Saccharomyces* strains, belonging to five of six cellars in the study, were characterized by producing wines with a fruity/sweet character. Floral, solvent, and herbaceous descriptors are more related to wines elaborated with *Saccharomyces* strains from organic cellars A, E, and F. Based on these findings, winemakers may use their best native *S. cerevisiae* strains, which add personality to their wine. Therefore, this study contributes to promoting the use of native *Saccharomyces* yeasts in winemaking.

Keywords: native yeast; *Saccharomyces cerevisiae*; aroma; Malvar (*Vitis vinifera* L. cv.); white wine

1. Introduction

Yeasts contribute to wine aroma by several mechanisms: firstly, by alcoholic fermentation of the grape must; secondly by the de novo biosynthesis of volatile compounds; and lastly, by the transformation of neutral grape compounds into flavor-active components [1,2]. Among fermentation-derived volatiles are esters, higher alcohols, and volatile acids, as well as varietal compounds, i.e., thiols and terpenes; all of these are the most abundant in the total wine aroma composition [3].

Aroma is one of the most influential factors on wine quality and consumers preferences, as well as the prime contributor to overall flavor perception [4,5]. Since the 90s, wine has been described as containing around 600 to 800 volatile aroma compounds arising from the grapes, from alcoholic fermentation, and from the aging process [6]. The particular importance of a specific volatile compound to wine aroma perception is related to its odor threshold value (OTV), which can be considered as the lowest concentration detected by smelling [7]. Another parameter extensively used to estimate the sensory contribution of aromatic compounds to the overall aroma of wine is the odor activity value (OAV). The OAV is obtained from the ratio between the concentration of an individual compound and its perception threshold. A volatile compound contributes to overall aroma when its concentration in wine is above the perception threshold; therefore, odorants with OAV ≥ 1 can be perceived [8,9]. Nevertheless, some authors presented evidence that compounds with low OAV values may act as significant impact odorants [10,11]. Therefore, the characterization of wine aroma compounds and their odorant profiles are currently among the research targets in winemaking [12–14]. In addition, several works have determined the aromatic series as groups of all volatile compounds with similar sensory descriptors [15–17], and a generalized OAV for each aromatic series can be calculated by adding the OAV of each aromatic series component [18].

The monitoring of fermentation is an effective method for modulating the wine aroma [19]. Typically, the use of commercial *Saccharomyces cerevisiae* starter cultures is widespread to obtain control and homogeneity of the fermentation process. In fact, their predominance reduces the risk of wine spoilage, so their dominant growth makes the development of indigenous spoilage species difficult [3,20,21]. However, the continued use of commercial yeasts has resulted in an excessive standardization of wines, regardless of their vinicultural region of origin [22]. For this reason, in recent decades, most studies have focused on using indigenous yeast strains as a way of expressing singular characters and to encourage the aromatic profiles of wines from a given region or appellation [23–25]. This relationship between wine microbiota and *terroir* has gained relevance in the wine industry [26–29]. The concept of *terroir* is linked to the natural environment, the physico-chemical characteristics of the soil, and climatic conditions in a delimited area that affect grape characteristics, so the obtained wine is also affected by this territoriality. Thus, the microbiota from a determined *terroir* is able to confer a unique quality to the wine [30].

The Denomination of Origin (D.O.) "Vinos de Madrid", created in 1990, is located in the center of Spain and covers an area of 8390 ha. This D.O. comprises 46 wineries in three regions: Arganda (27 wineries), Navalcarnero (5 wineries), and San Martín de Valdeiglesias (14 wineries). Recently, the new region of El Molar has become part of this D.O. The climate of this region is Mediterranean continental, with temperatures ranging from −8 °C minimum in winter to a maximum of 41 °C in summer [31]. The annual rainfall ranges between 460 and 660 mm. Winemakers in this region base their production on the cultivation of the vine varieties Airen and Malvar (white), and Garnacha and Tempranillo (red) (*Vitis vinifera* L. cv.). Malvar is an autochthonous cultivar for this D.O., while Airen, Garnacha, and Tempranillo have major extensions all over the Iberian Peninsula.

In the last few years, our research activity has been directed to the exploitation of native microbiota potential to enhance the quality of regional Malvar wines. In the present investigation, small volume fermentations were carried out with 101 autochthonous *S. cerevisiae* strains isolated from vineyards and cellars of D.O. "Vinos de Madrid" and compared with a control of *S. cerevisiae* CLI889 previously isolated and exhibiting good oenological aptitudes [32,33]. This work seeks to study the impact of *S. cerevisiae* strains isolated from their oenological region on the volatile composition of Malvar wines, providing an opportunity for wineries to elaborate products with their own typicity.

2. Materials and Methods

2.1. Yeast Strains, Origin, and Vinification Procedure

A total of 101 native *S. cerevisiae* yeast strains have been used for wine elaboration in this study. These strains were isolated from six vineyards and commercial cellars (A-F) belonging to the D.O. "Vinos de Madrid" as stated by Tello et al. [34]. The location of cellars is shown in Figure 1. As published by Tello et al. [34] and García et al. [35], four of the wineries (A, D, E, and F) use an organic system of wine production, in contrast to cellars B and C, that utilize a conventional production system. In wineries A, E, and F, the fermentation was spontaneous, and different commercial *S. cerevisiae* strains induced the fermentation in cellars B, C, and D. One autochthonous strain, *S. cerevisiae* CLI 889 from the IMIDRA collection, selected by our group for Airen white wine elaboration, was used as a control [32,33]. This strain has been deposited in the Spanish Type Culture Collection (CECT 13145).

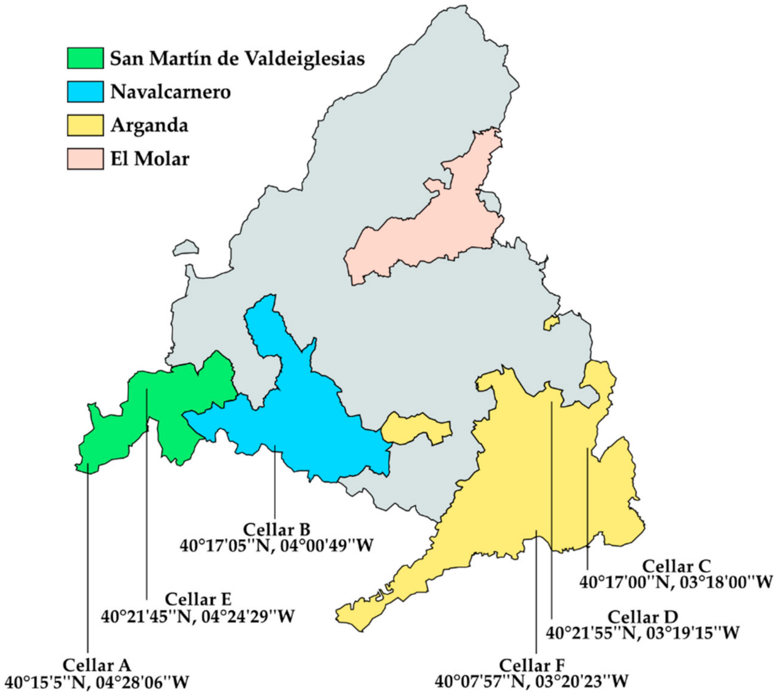

Figure 1. Map of D.O. "Vinos de Madrid". Different regions and location of cellars (A-F) included in this study are given in this figure.

The different genotypes of *S. cerevisiae* were identified by microsatellite multiplex PCR analysis using the highly polymorphic loci SC8132X, YOR267C, and SCPTSY7 [36]. The size of the fragments was determined by automatic electrophoresis with an ABI 3130 Genetic Analyzer (Applied Biosystems, Tres Cantos, Madrid, Spain), whose results were published by Tello et al. [34].

Grapes from the Malvar cultivar (*Vitis vinifera* L. cv.) were hand-collected from IMIDRA's experimental vineyard located in the Madrid winegrowing region, Spain (40°31′ N, 3°17′ W and 610 m altitude) during the 2010 vintage at commercial maturity. The must was clarified at 4 °C by pectolityc enzymes (Enozym Altair, Agrovin, Spain) (0.01 g/L) and stored at −20 °C until needed. In order to carry out the study under the same conditions, the grape must was adjusted to 200 g/L of reducing sugars; then, the pH value was 3.2, total acidity (as g/L of tartaric acid) was 5.0, and there was 165 mg/L of yeast assimilable nitrogen (YAN). The fermentations were performed in sterile flasks with 100 mL of pasteurized Malvar must with constant agitation (150 rpm) under anaerobic conditions. Each *S. cerevisiae* strain was inoculated in grape must at a concentration of 10^6 cells/mL, from a culture grown for 48 h in YPD liquid medium at 28 °C. The fermentation was performed at 20 °C in a JP Selecta™ incubator (Abrera, Barcelona, Spain), and the alcoholic fermentation kinetics was controlled daily by weight loss. When its value was constant for two consecutive days, the fermentation process was considered complete, and clarified wine samples were frozen in order to carry out volatile composition analyses. All experiments were performed in duplicate.

2.2. Volatile Fraction Analysis

Quantification of major volatile compounds was undertaken by gas chromatography coupled to flame ionization detector (GC-FID) (Agilent Technologies, Santa Clara, CA, USA). The column was DB-Wax column (60 m × 0.32 mm × 0.5 µm film thickness) from J&W Scientific (Folsom, CA, USA). The oven temperature program was: 40 °C for 5 min, then increased at 3 °C/min up to 200 °C. Helium was used as carrier gas at 2 mL/min. Two µL of aroma extract were injected at 250 °C in splitless mode. Total run time was 75 min per sample. The extraction and analysis methodologies of volatile compounds were done following the procedures proposed by Ortega et al. [37]. Analyses were carried out in duplicate.

2.3. Statistical Analysis

The odor activity values of all volatile compounds have been statistically analyzed to study how the use of different *S. cerevisiae* strains affects the aromatic profile of wines. Thus, a discriminant analysis was carried out to determine the impact of wine aroma profiles on their classification by cellar. In addition, a principal component analysis (PCA) was elaborated to link wines produced by *Saccharomyces* strains with the seven aromatic descriptors to describe the volatile profiles of Malvar young wines. Both statistical analyses were performed using SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

Most of the native strains were capable of completing the vinification (residual sugars below 5 g/L), although there are differences in the time required, ranging between 8 to 18 days. Only 13% of *Saccharomyces* strains did not complete the alcoholic fermentation, which stopped at 5−59 g/L residual sugar.

Volatile acidity expressed as acetic acid (g/L) content affects the wine quality. In general, most of the elaborated wines contained moderate levels of acetic acid, i.e., between 0.23–0.70 g/L, except for the wines elaborated with the strains G8 (1.14) and G16 (0.98) from Cellar A, G462 (1.08) and G493 (0.90) from Cellar E, and G529 (1.37) from Cellar F. The legal limit is 1.2 g/L of acetic acid under European legislation [38]. However, acetic acid may provide an unpleasant vinegar aroma and an undesirable acidic taste to wine at concentrations above 0.8 g/L [39].

3.1. Aromatic Profile of Wines Elaborated with Different S. cerevisiae Strains

Table 1 shows the major volatile compounds quantified in Malvar white wines. This table also contains the odor threshold values and descriptors for each aromatic compound. Moreover, each compound was attributed to one or more aromatic series depending on its principal sensory description: solvent, sweet, herbaceous, floral, fruity, microbiological, and fatty. These seven classes of sensory descriptors were employed to link odorous compounds with similar sensory descriptors into classes (aromatic series) [16,18,40,41] and give an organoleptic profile of wines elaborated with the different *S. cerevisiae* native strains. Moreover, the contribution of each volatile compound to each series can be determined. This procedure, which is based on more objective criteria than other existing alternatives, allows for the connection of quantitative information obtained from chemical analysis to sensory perceptions in order to achieve an aroma profile for the wine [16].

In order to analyze the aroma composition of wines, the OAVs were calculated for each of the 31 volatile compounds quantified in the wines (Table S1, Supplementary Materials). As can be seen, only isoamyl alcohol, several esters such as ethyl butyrate, ethyl isovalerate, isoamyl acetate, ethyl hexanoate, acids, i.e., isobutyric acid, isovaleric acid, hexanoic acid, and octanoic acid, and one ketone compound, i.e., diacetyl, has OAVs above the unity in all wines. The OAV for β-phenylethyl alcohol was greater than 1 in all wines elaborated with *S. cerevisiae* from D.O. "Vinos de Madrid" cellars, whereas that for the *S. cerevisiae* CLI 889 (control), this OAV value was lower than unity. In the case of 2-phenylethyl acetate, only two strains (G12, Cellar A and G507, Cellar F) did not exceed the unity. In contrast, it should be pointed out that the OAVs of 1-butanol, benzyl alcohol, ethyl-3-hydroxybutyrate, diethyl succinate, furfural, benzaldehyde, and acetoin, were below 0.1 in all cases.

Fusel alcohols (isoamyl alcohol, isobutanol, and β-phenylethyl alcohol) contribute to the wine odor of the analyzed Malvar wines. These alcohols are usually present in wines, formed as the fermentation products by yeasts. High concentrations of these volatiles (above 300 mg/L) can have a detrimental effect on wine, whereas concentrations below this value add a desirable level of complexity to the wine [2,42]. Esters are one of the most important classes of volatiles, and are responsible for the fruity and floral character in wines; their synthesis is mainly dependent on yeasts [6]. However, these compounds in excess can mask varietal aromas; for example, ethyl acetate over 90 mg/L, or 200 mg/L of total esters, can have a negative effect [42]. In our work, the total esters of samples ranged between 6.42 mg/L and 88.04 mg/L, in no case exceeding 200 mg/L (data not shown).

Table 1. Major aroma compounds quantified in wines. Odor description (ODE), Odor threshold value (OTV) (in mg/L), and assignation of compounds to different aromatic series.

Compound	ODE	OTV [1]	Aromatic Serie [2]
1-Propanol	Alcohol, ripe fruit	9 [a]	1, 5
1-Butanol	Soap, fatty, diesel	150 [a]	1
Isobutanol	Bitter, fusel, alcohol	40 [b]	1
Isoamyl alcohol	Harsh, bitter	30 [b]	1
(Z)-3-Hexen-1-ol	Lemon, fresh	0.4 [b]	3, 5
1-Hexanol	Green grass, fresh	8 [b]	3
Metionol	Garlic	1 [b]	3
Benzyl alcohol	Pleasant, soft	200 [c]	2, 4
β-Phenylethyl alcohol	Flowery, roses	14 [b]	2, 4
Ethyl butyrate	Fruity, sweet, apple	0.02 [b]	2, 5
Ethyl isovalerate	Fruity, sweet, banana	0.003 [b]	2, 5
Isoamyl acetate	Banana, sweet, fruity	0.03 [b]	2, 5
Ethyl hexanoate	Pineapple, apple	0.014 [b]	5
Ethyl-3-hydroxybutyrate	Fruity	20 [c]	5
Hexyl acetate	Fruity, green, pear	1 [d]	5
2-Phenylethyl acetate	Flowery, lilac	0.25 [b]	4
Diethyl succinate	Camphor	100 [d]	5, 6
Ethyl octanoate	Fresh, flowery, pineapple	0.58 [a]	2, 4, 5
Ethyl lactate	Lactic	154 [a]	6
Isobutyric acid	Rancid, butter, cheese	0.05 [e]	7
Butyric acid	Butter, cheese, stinky	0.173 [b]	7
Isovaleric acid	Cheese	0.033 [b]	7
Hexanoic acid	Cheese	0.42 [b]	7
Octanoic acid	Sweet, cheesy	0.5 [b]	7
Decanoic acid	Rancid, fatty	1 [b]	7
Diacetyl	Butter	0.1 [b]	7
Furfural	Bread, toasty, candy	15 [d]	6
Benzaldehyde	Sweet, candy, wood	5 [b]	2, 4
Phenylacetaldehyde	Roses	1 [f]	4
Acetoin	Butter	150 [a]	7
γ-Butyrolactone	Coconut	35 [c]	2

[1] References: a, thresholds from Etievant et al. [43]; b, thresholds from Ferreira et al. [15]; c, thresholds from Aznar et al. [44]; d, thresholds from Chaves et al. [45]; e, thresholds from Van Gemert and Nettenbreijer [46]; f, thresholds from Culleré et al. [47]. [2] 1, solvent; 2, sweet; 3, herbaceous; 4, floral; 5, fruity; 6, microbiological; 7, fatty.

The family of fatty acids has been reported to derive not only from yeasts, but from grapes as well [48], providing fruity, cheese, fatty, and rancid notes to wines [7]. Among these fatty acids, we mention the importance of isobutyric, isovaleric, hexanoic, and octanoic acids as active odorants, whose OAVs were higher than 1 in all the studied wines. A greater fatty acid proportion than other aromatic descriptors was found in samples G462 and G475 (Cellar E). Finally, we denoted the relevant content of the ketone diacetyl in the Malvar samples, showing the highest amounts in G7 (1.50 mg/L) and G502 (1.51 mg/L) from cellars A and E, respectively. Diacetyl concentrations exceeding 5–7 mg/L are considered undesirable, although depending on the style and type of wine, this compound is recognized to contribute a desirable buttery and butterscotch-like flavor at amounts around 1–4 mg/L [49].

To determine whether the volatile composition of wines is related to the cellar to which the yeast strains belong, the OAV data from the 31 compounds were submitted to discriminant statistical analysis to find the canonical parameters that explain the maximum variability between the studied wines (Figure 2). The results of this analysis showed six discriminant functions, where the first two accounted for 41.3% and 28.5% of the total variance, respectively, so the total variance explained by these two functions reached 69.8%. The wines elaborated with different *S. cerevisiae* genotypes presented a good correlation by cellar of origin. These results are in agreement with those obtained by Knight et al. [50], who revealed that there is a significant correlation between the region of isolation of *S. cerevisiae* and the aroma profile in New Zealand wines. In relation with discriminant function 1, 1-propanol, (Z)-3-hexen-1-ol, 1-hexanol, and isovaleric acid were the most significant compounds in the differentiation between wines. In the case of function 2, 1-butanol, 2-phenylethyl acetate and benzaldehyde contributed most to the discriminant model. The same analysis exhibited correct classification of 86.7% of the wines elaborated with *S. cerevisiae* native strains, according to their cellar of origin (data not shown). Figure 2 shows that the most aromatically-different wines were those elaborated with strains CLI 889 (control), G113 (Cellar B) and G114 (Cellar C). As previously indicated, the fermentation process was induced by commercial *S. cerevisiae* strains in these two cellars, and these strains were the only ones which were isolated throughout the fermentation from each winery [34]. The use of these starter yeast cultures for winemaking guarantees that the must ferments in the expected way [51]. In contrast, some authors have found that native yeasts produced wines with high concentrations of pleasant aromas and special bouquets not which are available with commercial yeast strains [23,24,52,53]. In our case, these wines showed a fruity character, highlighting ethyl isovalerate and ethyl hexanoate concentrations, while acetate ester contents were lower than those in wines elaborated with the native strains.

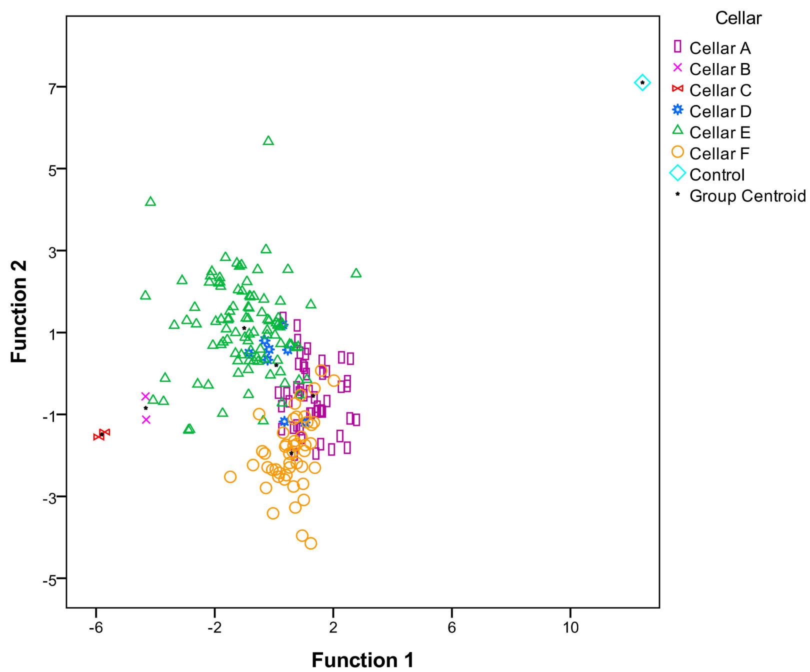

Figure 2. Application of discriminant analyses of the OAV data of volatile compounds studied in wines, classifying the samples by cellar of origin.

3.2. Principal Component Analysis

A PCA analysis was done to cluster yeast fermentations according to the aromatic descriptors (Figure 3). In reference to the seven defined classes of aromatic descriptors (Figure 3a), a generalized OAV for each class of sensory descriptor was calculated by adding up the OAVs of all compounds belonging to that class. Then, this generalized OAV calculated by wine sample was used to calculate the proportion (% OAV; Table S2, Supplementary Materials) that each aromatic descriptor represents into wines elaborated with the *Saccharomyces* strains (Figure 3b). Calculation of the aroma series by the accumulation of OAVs cannot be considered as an arithmetical addition of the odorant sensations, and the assignment of some compounds in a particular series or in several series may be questionable [40,54]. However, several authors have employed the proposed method, which groups the compounds into odorant series, since it reduces the number of variables to be interpreted and, consequently, is a valid and simple way to compare a wine's aroma character [18,41]. It can be particularly useful in many contexts where a sensorial study is not available or affordable, and a first analysis of wine aroma peculiarity is outlined [18].

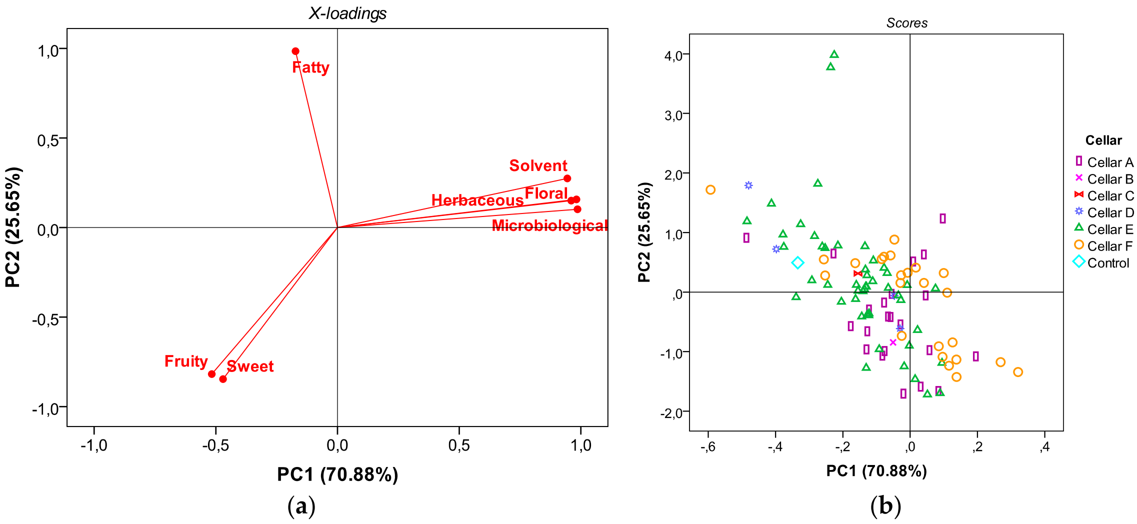

Figure 3. Results of the principal component analysis carried out on the aromatic series matrix: (**a**) loadings of the variables on the first and second principal components; (**b**) scores of the % OAV on each sensory descriptor adding up the different strains by cellar of origin in the plane formed by the first and second principal components. Values are the mean of two % OAV ratios.

The PCA explained the 96.53% of the total variance. Wine samples closely related with sweet and fruity descriptors appear in the left bottom corner of the PC plane. These two sensory descriptors are mainly determined by ester content in wines; specifically, the fruity descriptor represents the highest proportion of aroma composition in most wines (Table S2, Supplementary Materials). Five of the six cellars studied are included in this group (Figure 3b); therefore, we have not found a direct correlation between fruity/sweet descriptors and a determined area or cellar. Wine samples classified at the top right plane are more associated with compounds related to floral, solvent, microbiological, and herbaceous descriptors. However, it is worth noting that the volatile compounds comprising microbiological character (diethyl succinate, ethyl lactate, and furfural) have an OAV lower than unity (OAV < 1) in all Malvar wines (Table S1, Supplementary Materials). The floral parameter was mainly constituted by β-phenylethyl alcohol and 2-phenylethyl acetate; solvent is mostly related to isoamyl alcohol; and herbaceous is determined by metionol. In this quarter, we can find the wines elaborated with the native strains G3, G9, and G19 from Cellar A, G465 from Cellar F, and G513, G514, G515, and G518 from Cellar E. In this case, a connection point between the strains named above is that their cellars of origin utilize spontaneous fermentation and an organic system of wine production. In accordance with these results, Lorenzo et al. [55] observed that the volatile composition of wines from organic or non-organic grapes was considerably different. In particular, they concluded that the OAVs of wines from ecologically-grown grapes had more chemical and floral aromas, while the wines from conventional practices presented a fruitier character. Finally, the fatty character was nearly correlated with two samples from Cellar E (G475 and G462), due to the high proportion of fatty acids within these two samples.

Although compounds with OAV ≥ 1 are called critical compounds essential to total aroma [8], the statistical treatments of this work also considered the compounds with OAV < 1, in agreement with the theory that sub-threshold volatile compounds may contribute to wine aroma through the additive effects of compounds with a similar odor or structure [56]. In contrast, some compounds can mask the perception of others, so they remain undetected at supra-threshold concentrations [57,58]. Atanasova et al. [59] concluded that the fruity character of wine might be masked by woody components when presented at supra-threshold concentrations.

In a previous work by our group, some of these *S. cerevisiae* native strains that showed a pleasant aromatic profile were also recognized for their good fermentation abilities and for resistance to the stresses inherent to wine fermentation in warm areas [35].

4. Conclusions

The knowledge of the volatile profile of wines elaborated with different *S. cerevisiae* strains, together with their fermentation aptitudes and stress resistance, provide important information which contributes to promoting the use of these autochthonous strains in winemaking. Thus, we suggest that each winery uses their best native *S. cerevisiae* strains, which may add personality to their wines. However, more studies are necessary to know the fermentative behavior of these *Saccharomyces* strains at industrial scales. Furthermore, it could be considered an opportunity for some of these *S. cerevisiae* strains to become commercially available.

Supplementary Materials: The following are available online at http://www.mdpi.com/2311-5637/5/4/94/s1, Table S1: Odor activity value (OAV) for the aroma compounds studied in Malvar wines, Table S2: Odor activity value proportion (% OAV) of each aromatic descriptor in wines elaborated with the *Saccharomyces cerevisiae* yeast strains.

Author Contributions: M.G., T.A. and B.E.-Z. designed the experiments, analyzed the results, discussion of the results and wrote the manuscript. M.G., J.C., and J.M.C. performed experiments and analyzed results.

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