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Article

Volatile Fingerprints of Synthetic Wines Fermented by Different Oenological Yeast Strains

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Abstract: Background: The role of the *S. cerevisiae* strain in defining the volatile fingerprint is expressed throughout alcoholic fermentation and post-fermentation *sur lie* aging and is crucial for customizing the wine style. Methods: In this study, the alcoholic fermentation was carried out in a synthetic must to exclusively bring out the performance of the yeast in terms of volatile compound production, excluding the effect of the grape. Results: Among the 33 volatile organic compounds identified in the synthetic wines by GC-MS, esters, alcohols, and acids, represented the major groups for the nine different commercial oenological strains tested. All the relevant differences in the volatile fingerprint of the synthetic wines, which were lab-scale fermented, were quantitative rather than qualitative. The clustergram representation of the volatiles revealed an outstanding fingerprint for two strains (VIN13 and VIN7) among those tested, featuring hexanoic acid, octanoic acid, the corresponding esters (ethyl hexanoate, ethyl octanoate), and the acetates (2-phenylethyl acetate, isoamyl acetate), all at the highest levels. No relationship was appreciated between the fermentation rate and the volatile fingerprints. Conclusions: The outcomes of this study address the wine industry's needs, supplying a full characterization of a broad range of commercial yeasts' ability in fermentative volatile production.

Keywords: *S. cerevisiae* strain; commercial oenological yeast; synthetic grape must; alcoholic fermentation; GC-MS analysis; fermentative volatiles

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1. Introduction

The yeast's role in the wine feature setting involves its active metabolism during alcoholic fermentation, which gives rise to the raw young wine, and then it could go on throughout the post-fermentation by the autolysis phenomena during the optional *sur lie* storage when desired. In both stages, the contribution of yeast is crucial to defining the volatile fingerprint of wine [1,2].

Gas chromatography combined with mass spectrometry (GC/MS) has been largely used to determine the wine volatile profile by an untargeted approach [3] and GC techniques combined with olfactometry have been used to identify wine odor-active compounds [4]. Great advances in wine knowledge have been achieved with two-dimensional systems, such as comprehensive two-dimensional GC (GCxGC) [3]. It has been proved that some of the wine flavor components come from the grape, whereas most of them derive from yeast metabolism, which is greatly affected by the composition of grape must (e.g., sugars and nitrogen compounds, micronutrient availability, and pH) as well as by the oenological practices [5,6]. Many research groups worldwide are exploring the ability of different wine yeast strains, inoculated alone or in mixed culture [7,8], to release varietal volatiles (mainly terpenes, thiols, and C 13 -norisoprenoids) from the grape precursors, enhancing the aroma complexity and identity of varietal wines.

The synthesis of fermentative compounds (e.g., esters, acids, and fermentation alcohols), which positively or negatively contribute to the aromatic buffer of the wine and broadly make up the aroma profile of young wines from "neutral" grapes, has been deeply investigated [9,10]; comprehensive reports are available in Romano [11] and Carpena [12] reviews. Moreover, the *de novo* synthesis of terpenes such as linalool and citronellol, even if in the trace, by some *S. cerevisiae* strains has been recently demonstrated [13].

The capability of various commercial yeast strains to produce desirable metabolites during alcoholic fermentation has been explored in real must from various grape varieties [14–18]. However, the application of natural grape must mislead the role of a yeast strain in the level of volatile production as complex interactions between grape variety, must nitrogen content, and other variables may take place [19,20]. Moreover, it is important to consider that most works reported in the literature have been carried out on a single vineyard and a single vintage; therefore, the performance of the yeast strain will not necessarily be reproducible in different grape musts.

Synthetic grapes must have been applied as a valuable option to investigate the role of yeast in volatile production during alcoholic fermentation, since it may be formulated *ad hoc*, mimicking any natural deficiency that could occur in grape must [21]. Moreover, this medium simulates natural grape juice and does not contain grape varietal aromas or odorless precursors that can affect the volatile fingerprint [7,19,22].

A large number of selected yeast strains available from different companies, along with the continuous search for new strains characterized by oenological peculiarities in the production of desired metabolites, has driven and continues to drive some research on their differential ability in the production of fermentative aroma compounds in synthetic grape must [7,19,22–25]. This work was thereby addressed to solely investigate the role of yeast strains on the production of volatile compounds during alcoholic fermentation. For this purpose, a wide selection of different commercial *S. cerevisiae* yeast strains was tested in synthetic grape must, making it possible to exclude the effect of grape variety on the volatile fingerprint of the final wine.

2. Materials and Methods

2.1. Yeast Strains and Fermentation Conditions

The nine different commercial *Saccharomyces cerevisiae* wine strains used in this study are listed in Table 1.

Table 1. *Saccharomyces cerevisiae* wine strains used in this study.

***Saccharomyces cerevisiae* Strain**	**Supplier**	**Abbreviation**
Fermol Sauvignon	AEB, San Polo (BS), Italy	FERM
Lalvin QA23	Lallemand, Montreal, Canada	QA23
NT116	Anchor Yeast, Cape Town, South Africa	NT116
Uvaferm CK	Danstar Ferment GAC, Denmark	CK
VIN13	Anchor Yeast, Cape Town, South Africa	VIN13
VIN7	Anchor Yeast, Cape Town, South Africa	VIN7
Zymaflore VL1	Laffort Oenologie, Bordeaux, France	VL1
Zymaflore VL3	Laffort Oenologie, Bordeaux, France	VL3
Zymaflore X5	Laffort Oenologie, Bordeaux, France	X5

The alcoholic fermentation was performed in a synthetic must (pH 3.3), in order to assure standardized conditions, avoiding variations due to grape juice composition. The synthetic must contain assimilable nitrogen source (300 mg N_2/L): 120 mg as ammoniacal nitrogen (NH_4Cl) and 180 mg as amino acids [26] with 200 g/L of reducing sugars (100 g/L glucose and 100 g/L fructose) [22].

The yeast cells were prepared according to the manufacturer's instructions (i. rehydratation: 0.5 g active dry yeast in 5 mL water at 37 °C for 15 min; ii. acclimatation in

400 mL synthetic must) and then inoculated (5×10^6 cells/mL) in a total volume of 2 L synthetic must each.

Microvinification experiments carried out in triplicate were performed in 3 L flasks equipped with a Müller valve at 20 °C, with orbital agitation (20 rpm, Zhicheng ZHWY-200B, Shanghai, China). The alcoholic fermentation progress was monitored by the measurement of the weight loss (Europe 4000 AR balance, Gibertini, Elettronica Srl, Novate, Milan, Italy) throughout the process [24] until there was no weight loss for 3 consecutive days. The complete sugar consumption was also detected by using a Megazyme d-fructose/d-glucose assay kit according to the manufacturer's instructions.

At the end of each fermentation, 60 mg/L of potassium metabisulfite was added to the synthetic wines. After sample centrifugation at 4000 rpm for 5 min, the supernatants were filtered through a 0.45 µm membrane filter (Millipore, Molsheim, France) and stored in bottles at 4 °C for further chemical analysis.

2.2. Estimation of the Alcoholic Fermentation Parameters

The dynamic of weight loss during alcoholic fermentation, suggesting the kinetic of sugar consumption, was analyzed by linear regression analysis (STATA 11.2 software, 95% significance level). The regression coefficients (K), which represent the slope of the regression lines, indicated the fermentation rate of each commercial yeast strain. The quality of the regression was evaluated by the coefficient of determination (R^2).

2.3. Volatile Determination by GC-MS

Volatile compounds were extracted using a solid-phase extraction procedure according to Pati et al. [27]. A Vac Elut 20 station manifold from Varian (Palo Alto, CA, USA) was used. LiChrolut EN resins prepacked in 200-mg cartridges (Merck, Darmstadt, Germany) were rinsed with 4 mL of dichloromethane/hexane (2:3), 4 mL of methanol and, finally, 4 mL of a water–ethanol mixture (12%, *v*/*v*); 50 mL of wine, containing 100 mg/L of 4-methyl-2-pentanol as internal standard (IS), was passed through the cartridge at around 2 mL/min. Afterward, the sorbent was dried by letting air pass through it. The analytes were recovered by elution with 1.3 mL of dichloromethane/hexane (2:3). The sample (1 µL) was injected onto a gas-chromatograph (GC 6890N) coupled to a mass selective detector (MS 5973) and equipped with an HP-INNOWAX capillary column (60 m × 0.25 mm i.d, 0.25 µm film thickness, J&W Scientific Inc., Folsom, CA, USA). The carrier gas was helium at a flow rate of 1 mL/min. The injection was made in splitless mode and the injector temperature was 250 °C. The column oven temperature, initially held at 40 °C, was programmed to 230 °C, at 2.5 °C/min, with a final holding time of 20 min. Spectra were recorded in electron ionization impact mode (EI, 70 eV) in a range of 30–500 *m/z* at 3.2 scans/s. A solvent delay time of 10 min was used to avoid overloading the mass spectrometer with solvent.

Compounds were identified by comparing their retention times and mass spectra with those of pure compounds (Sigma-Aldrich, Milan, Italy), analyzed under the same conditions, except for the compounds ethyl-2-hydroxyhexanoate and ethyl methyl succinate. The compounds ethyl-2-hydroxyhexanoate and ethyl methyl succinate were putatively identified by comparing the mass spectra with the data system library (NIST 02, $p > 80$). The semi-quantitative analysis of individual compounds was performed, normalizing the compound peak area to that of the internal standard and multiplying by the internal standard concentration. The concentration of each volatile compound is expressed as mg equivalents of IS/L synthetic wine.

2.4. Statistical Analysis

All data were statistically analyzed using the STATISTICA 6.0 software package (StatSoft Inc., Tulsa, OK, USA). One-way ANOVA followed by a Duncan post hoc test with a 95% confidence level was applied.

A hierarchical cluster analysis with the ClustVis web tool [28] for visualizing the clustering of multivariate data using heatmap was carried out on the volatile compounds

concentration values. Correlation metrics were used as similarity metrics and unweighted average distance as the linkage criterion.

3. Results and Discussion

3.1. Alcoholic Fermentation Kinetic

The fermentation kinetics depicted in Figure 1 pointed out remarkable differences in the sugar consumption depending on the strain used.

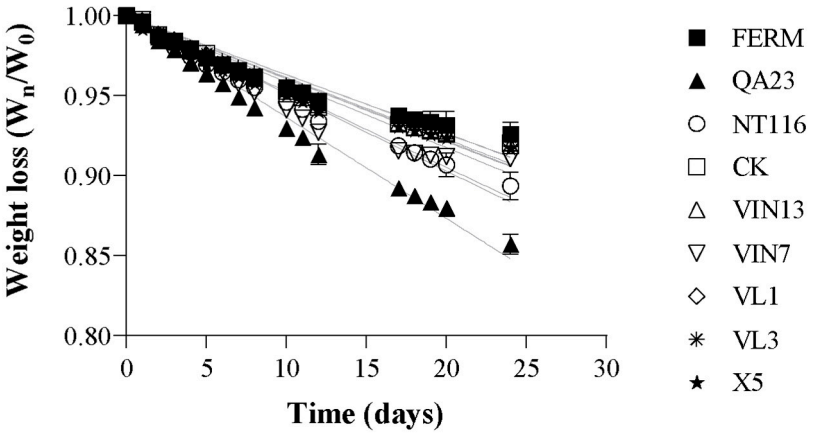

Figure 1. Alcoholic fermentation kinetic: sample weight (W) loss in relation to the strain used.

The commercial strain QA23 had the highest fermentation rate, followed by NT116, VIN7, and X5 (Table 2). Similarly to the results observed in synthetic must, the alcoholic fermentation of real wines from Godello and Albariño grapes QA23 also had the fastest rates in comparison to autochthonous yeasts (*S. cerevisiae* XG1 and XG3) [5]. Contrariwise, the slowest fermentation kinetic was revealed for FERM and VL3 yeast strains. No significant differences were found comparing the other tested strains.

Table 2. The fermentation rate indicates the alcoholic fermentation progress. K values represent the regression coefficients of the ratio W_n/W_0 (where W_n and W_0 represent the current and initial weight, respectively) over time. All data are expressed as mean value ± standard deviation (n = 3). Different letters indicate significant differences according to Duncan's post hoc test at a significant value of 95% ($p < 0.05$).

Saccharomyces cerevisiae Strain	K	R^2
FERM	0.0037 ± 0.0001 d	0.967
QA23	0.0063 ± 0.0001 a	0.980
NT116	0.0048 ± 0.0001 b	0.988
CK	0.0039 ± 0.0001 cd	0.971
VIN13	0.0039 ± 0.0001 cd	0.965
VIN7	0.0047 ± 0.0002 b	0.975
VL1	0.0039 ± 0.0001 cd	0.965
VL3	0.0037 ± 0.0001 d	0.967
X5	0.0041 ± 0.0002 c	0.970

3.2. Volatile Profile as Affected by Yeast Strains

The volatile organic compound (VOC) profile of synthetic wines produced with nine *S. cerevisiae* yeast strains, namely FERM, QA23, NT116, CK, VIN13, VIN7, VL1, VL3, and X5, has been assessed. Table 3 summarizes information on the 33 VOCs identified, including the chemical family, their retention time, mg equivalents IS/L of synthetic wine, ANOVA results, and odor description. The volatile metabolites included alcohols, esters, acids, aldehydes, and ketones, and S-compounds, and all of them have been previously reported as *S. cerevisiae* yeast metabolites [23]. VOC significant differences were observed among

all nine types of yeast strains; all the differences resulted to be quantitative rather than qualitative. Concerning the number of compounds identified, esters (13 VOCs), alcohols (9 VOCs), and acids (7 VOCs) represent the major groups for all the *S. cerevisiae* strains, in accordance with Lambrechts and Pretorius [29].

Higher alcohols were the most abundant volatile group in the tested synthetic wines. They are reported to arise from sugar catabolism, as well as from decarboxylation and deamination of amino acids; and small amounts are produced by yeast through the reduction in the corresponding aldehydes. Isoamyl alcohol and 2-phenylethanol were the main alcohols present in the VOC metabolic profile from all yeast strains; the first is mainly produced through degradation of the branched-chain amino acid leucine; meanwhile, 2-phenylethanol is produced by phenylalanine. In particular, the 2-phenylethanol, which is an important yeast fermentation metabolic by-product responsible for rose flavor [30], was found at the highest levels in wines fermented with the X5 yeast strain. A high production of higher alcohols is not desired as it could have negative impacts on the aroma of wine (concentrations exceeding 400 mg/L); however, higher alcohols are precursors for esters, which are associated with positive aroma notes, such as the isoamyl acetate [31].

Great differences among synthetic wines were detected in the produced esters including acetates of higher alcohols and ethyl esters of fatty acids. These compounds are produced during carbohydrate, fatty acid, and/or amino acid metabolism. Apolar esters, including isoamyl acetate, ethyl octanoate, ethyl hexanoate, and 2-phenylethyl acetate, have been demonstrated to be responsible in large part for the pleasant, fresh, and fruity aroma of young wine. They were produced in the highest concentration in VIN7 and VIN13 fermented synthetic wines. Meanwhile, polar esters, although produced in larger amounts than apolar ones, have been shown to have a low impact on wine aroma, contributing more to wine body [32]. Within apolar esters, the monoethyl succinate represented the major ester for all the strains and was the highest in X5; other abundant apolar esters for all strains were diethyl succinate, ethyl lactate, and diethyl malate.

C6 to C10 fatty acids are generally correlated to soapy, cheesy, fatty, and rancid notes and constitute an important group of aroma compounds because they are precursors of the corresponding ethyl esters. The main fatty acids were the hexanoic and octanoic acids and the highest amount was found in synthetic wines fermented with VIN13 and VIN7.

The synthetic wines showed the presence of two sulfur compounds, i.e., 3-(methylthio)-1-propanol and dihydro-2-methyl-3(2H)-thiophenone, known to be degradation products of sulfur-containing amino acids (methionine and cysteine) by the Ehrlich pathway and to confer negative odors of 'garlic' and 'chlorine' in the wine [30]. The lowest concentration of dihydro-2-methyl-3(2H)-thiophenone was found in NT116, while the lowest level of 3-(methylthio)-1-propanol was detected in VIN13 and VIN7.

A detailed report on yeast metabolites and their importance to wine aroma has been published by Lambrechts and Pretorius [29] and by Swiegers et al. [31].

A Hierarchical Clustering Analysis (HCA), applied to concentration values of volatiles in synthetic wine samples, was conducted to further investigate the relationships with the yeast strain (Figure 2). The cluster heatmap allowed better graphic visualization of the link between molecules and yeast strain, confirming the variability of the identified VOCs as a function of the yeast strain. It is worth noting that VIN13 and VIN7 stand out from the other strains. The differential production of volatile compounds by hybrid strains VIN13 and VIN7 could result, at least in part, from the genetic contribution of the other parental strain to their genetic background [19,32].

Table 3. Volatile compounds (mg/L) identified by HS-SPME-GC/MS in synthetic wines.

COMPOUNDS	t_R	FERM	QA23	NT116	CK	VIN13	VIN7	VL1	VL3	X5	Odor Description
ALCOHOLS											
1-Propanol + ethylbutanoate *	11.71	0.47 ± 0.08 c	0.56 ± 0.16 c	0.47 ± 0.13 c	0.71 ± 0.18 b,c	0.9 ± 0.2 a,b	0.99 ± 0.05 a	0.63 ± 0.07 c	0.52 ± 0.18 c	0.67 ± 0.03 c	-
2-Methyl-1-propanol	13.57	5.0 ± 0.3 b,c	5.07 ± 0.16 b,c	4.89 ± 0.18 c	4.9 ± 0.2 c	5.395 ± 0.016 a,b,c	5.7 ± 0.5 a	5.3 ± 0.3 a,b,c	5.5 ± 0.2 a,b	5.34 ± 0.09 a,b,c	alcoholic
1-Butanol	15.85	0.089 ± 0.002 b	0.106 ± 0.006 a	0.092 ± 0.004 b	0.092 ± 0.004 b	0.063 ± 0.007 c	0.064 ± 0.005 c	0.093 ± 0.005 b	0.087 ± 0.002 b	0.088 ± 0.004 b	fusel odour
Isoamyl alcohol	19.48	75 ± 4 b	77.8 ± 1.4 a,b	78.0 ± 1.1 a,b	78 ± 2 a,b	80.5 ± 0.9 a,b	80 ± 7 a,b	79 ± 1.0 a,b	79 ± 6 a,b	83 ± 3 a	bitter, harsh
1-Hexanol	23.32	2.2 ± 0.2 a	2.091 ± 0.013 a,b	2.14 ± 0.04 a,b	2.13 ± 0.03 a,b	0.64 ± 0.04 c	0.68 ± 0.03 c	2.13 ± 0.15 a,b	1.95 ± 0.14 b	2.23 ± 0.09 a	toasted, green, dry
3-Ethoxy-1-propanol	27.55	0.037 ± 0.002 e	0.049 ± 0.002 b,c	0.042 ± 0.003 d,e	0.054 ± 0.007 a,b	0.054 ± 0.003 b	0.059 ± 0.003 a	0.0555 ± 0.0007 a	0.041 ± 0.003 d,e	0.046 ± 0.002 c,d	-
(Z)-3-Hexen-1-ol	27.89	0.106 ± 0.004 a	0.0984 ± 0.0014 a,b	0.101 ± 0.004 a,b	0.101 ± 0.002 a,b	0.0584 ± 0.0015 d	0.059 ± 0.002 d	0.097 ± 0.006 b,c	0.091 ± 0.007 c	0.105 ± 0.006 a	fresh, cut grass
1-Heptanol	31.43	0.022 ± 0.003 c	0.02186 ±0.00011 c	0.0213 ± 0.0009 c	0.0218 ± 0.0005 c	0.134 ± 0.003 b	0.142 ± 0.007 a	0.0198 ± 0.0007 c	0.0194 ± 0.0011 c	0.023 ± 0.003 c	herbal, musty, weak alcohol
2-Phenylethanol	52.43	51 ± 3 a,b	48.3 ± 0.4 a,b	48 ± 3 a,b	48.8 ± 1.9 a,b	39.5 ± 1.4 c,d	37 ± 2 d	42 ± 11 b,c,d	45 ± 2 a,b,c	51.7 ± 1.7 a	floral, rose, pollen
ESTERS											
Ethyl 3-methylbutanoate	12.87	0.061 ± 0.006 a	0.0526 ± 0.0003 b	0.048 ± 0.002 b,c	0.049 ± 0.002 b,c	0.0118 ± 0.0007 d	0.0123 ± 0.0006 d	0.050 ± 0.004 b,c	0.045 ± 0.003 c	0.049 ± 0.006 b,c	fruity, apple
Isoamyl acetate	15.02	0.147 ± 0.014 c	0.133 ± 0.006 c	0.1382 ± 0.0004 c	0.125 ± 0.002 c	2.1 ± 0.07 b	2.30 ± 0.09 a	0.160 ± 0.016 c	0.117 ± 0.011 c	0.135 ± 0.017 c	banana, pear
Ethyl hexanoate	20.24	0.53 ± 0.03 b	0.458 ±0.002 b,c,d	0.43 ± 0.04 c,d	0.46 ± 0.03 b,c,d	0.83 ± 0.03 a	0.77 ± 0.06 a	0.51 ± 0.03 b,c	0.41 ± 0.04 d	0.40 ± 0.09 d	apple, banana, strawberry, fruity
Ethyl lactate	25.84	9.0 ± 0.3 e	9.34 ± 0.06 d,e	9.30 ± 0.10 d,e	9.5 ± 0.2 c,d,e	10.31 ± 0.04 a	10.1 ±0.2 a,b	9.71 ± 0.19 b,c,d	9.6 ± 0.6 b,c,d	9.9 ± 0.4 a,b,c	sweet, fruity, creamy
Ethyl octanoate	30.29	0.076 ± 0.006 c	0.068 ± 0.003 c	0.08 ± 0.03 c	0.08 ± 0.02 c	0.638 ± 0.013 a	0.42 ± 0.03 b	0.071 ± 0.010 c	0.055 ± 0.005 c	0.072 ± 0.006 c	pineapple, pear, sweet, fruity
Isobutyl lactate	31.57	0.036 ± 0.002 b	0.0325 ± 0.0013 b	0.032 ± 0.003 b	0.032 ± 0.003 b	0.043 ± 0.002 a	0.045 ± 0.002 a	0.032 ± 0.005 b	0.033 ± 0.002 b	0.0350 ± 0.0009 b	buttery
Ethyl 3-hydroxybutanoate	34.55	0.091 ± 0.002 c	0.101 ± 0.004 b	0.091 ± 0.003 c	0.099 ± 0.005 b,c	0.142 ± 0.007 a	0.150 ± 0.004 a	0.0957 ± 0.0007 b,c	0.098 ± 0.008 b,c	0.102 ± 0.005 b	fruity
Ethyl 2-hydroxyhexanoate	35.69	0.35 ± 0.04 a	0.300 ± 0.019 b,c	0.311 ± 0.019 a,b,c	0.312 ± 0.009 a,b,c	0.140 ± 0.010 d	0.176 ± 0.006 d	0.31 ± 0.03 a,b,c	0.29 ± 0.04 c	0.336 ± 0.015 a,b	fruity, green
Ethyl methyl succinate	40.01	0.050 ± 0.005 a	0.0479 ± 0.0014 a	0.051 ± 0.007 a	0.050 ± 0.003 a	0.022 ± 0.002 b	0.0218 ± 0.0008 b	0.044 ± 0.004 a	0.049 ± 0.003 a	0.050 ± 0.005 a	-
Diethyl succinate	41.99	22 ± 2 a,b	20.54 ± 0.05 b,c	21.4 ± 1.1 a,b,c	21.0 ± 0.8 a,b,c	9.0 ± 1.0 d	9.0 ± 0.4 d	21.0 ± 1.3 a,b,c	19.4 ±1.0 c	23.3 ±1.6 a	cheese, earthy, spicy
2-Phenylethyl acetate	48.20	0.019 ± 0.002 c	0.016 ± 0.002 c	0.0183 ± 0.0003 c	0.0161 ± 0.0012 c	0.338 ± 0.032 a	0.312 ± 0.025 b	0.025 ± 0.002 c	0.0151 ± 0.0013 c	0.022 ± 0.002 c	rose, honey, fruity
Diethyl malate	57.44	20.7 ± 0.5 b	21.7 ± 0.2 b	22 ± 2 b	22 ± 2 b	3.83 ± 0.10 c	3.54 ± 0.09 c	40 ± 16 a	22.6 ± 1.0 b	23.9 ±1.9 b	fruity
Monoethyl succinate	69.66	45 ± 4 b,c	51.8 ± 0.2 a,b	50 ± 3 a,b,c	52 ± 2 a,b	30.4 ±1.6 d	27.6 ± 0.7 d	42 ± 12 c	50 ± 5 a,b,c	56 ± 3 a	-

Table 3. *Cont.*

COMPOUNDS	t_R	FERM	QA23	NT116	CK	VIN13	VIN7	VL1	VL3	X5	Odor Description
ACIDS											
Acetic acid	30.85	2.05 ± 0.11 c	2.23 ± 0.09 c	2.1 ± 0.2 c	2.1 ±0.2 c	2.49 ± 0.19 b	2.77 ± 0.11 a	1.993 ± 0.002 c	2.01 ± 0.14 c	2.02 ± 0.13 c	vinegar, pungent
Propanoic acid	35.29	0.040 ± 0.002 a,b	0.042 ± 0.002 a,b	0.0391 ± 0.0012 b,c	0.041 ± 0.004 a,b	0.036 ± 0.002 c	0.04071 ± 0.00002 a,b	0.038 ± 0.002 b,c	0.038 ± 0.002 b,c	0.0434 ± 0.0007 a	rancid, slightly pungent
2-Methyl-Propanoic acid	36.74	0.209 ± 0.005 b,c	0.20303 ± 0.00013 b,c	0.1936 ± 0.0010 c	0.207 ± 0.004 b,c	0.41 ± 0.03 a	0.43 ± 0.02 a	0.202 ± 0.006 b,c	0.226 ± 0.009 b	0.218 ± 0.011 b	pungent, phenolic, fatty
Butanoic acid	39.57	0.271 ± 0.014 d	0.28 ± 0.02 c,d	0.286 ± 0.007 c,d	0.2971 ± 0.0014 c,d	0.39 ± 0.05 b	0.429 ± 0.003 a	0.280 ± 0.009 c,d	0.297 ± 0.009 c,d	0.315 ± 0.011 c	pungent, cheese
3-Methyl- + 2-methyl-butanoic acid *	41.50	0.50 ± 0.03 b	0.48 ± 0.04 b	0.47 ± 0.03 b	0.52 ± 0.03 b	0.619 ± 0.007 a	0.58 ± 0.03 a	0.49 ± 0.04 b	0.52 ± 0.03 b	0.51 ± 0.04 b	-
Hexanoic acid	49.31	4.9 ± 0.3 b	4.57 ± 0.03 b	4.58 ± 0.13 b	4.60 ± 0.10 b	5.6 ± 0.3 a	5.79 ± 0.09 a	4.1 ± 1.1 b	4.3 ± 0.3 b	4.65 ± 0.15 b	sour, cheese, sweaty, rancid, fatty
Octanoic Acid	57.95	5.4 ± 0.2 a,b,c	4.99 ± 0.10 b,c,d	4.7 ± 0.3 c,d	4.8 ± 0.2 b,c,d	5.7 ± 0.3 a,b	5.94 ± 0.09 a	4.3 ± 1.2 d	4.6 ± 0.3 c,d	4.8 ± 0.3 b,c,d	soapy, cheese, oily, rancid, fatty
ALDEHYDES AND KETONES											
Acetoin	22.77	0.100 ± 0.002 c	0.1428 ± 0.0012 b	0.070 ± 0.002 e	0.153 ± 0.014 a	0.053 ± 0.004 f	0.077 ± 0.005 e	0.042 ± 0.004 g	0.086 ± 0.003 d	0.0368 ± 0.0008 g	fatty, wet
Benzaldehyde	34.74	0.0044 ± 0.00015 b	0.00314 ± 0.00011 b	0.0046 ± 0.0004 b	0.0039 ± 0.0008 b	0.0127 ± 0.0005 a	0.0126 ± 0.0013 a	0.0036 ± 0.0009 b	0.0029 ± 0.0002 b	0.010 ± 0.004 a	almond
SULFUR COMPOUNDS											
Dihydro-2-methyl-3(2H)-thiophenone	35.07	0.0546 ± 0.0012 a	0.047 ± 0.002 b,c,d	0.038 ± 0.003 e	0.041 ± 0.004 d,e	0.0520 ± 0.0014 a,b	0.043 ± 0.003 c,d,e	0.043 ± 0.006 d,e	0.047 ± 0.006 b,c,d	0.050 ± 0.002 a,b,c	chlorine, wet
3-(Methylthio)-1-propanol	43.94	0.25 ± 0.03 a	0.26 ± 0.02 a	0.27 ± 0.02 a	0.27 ± 0.03 a	0.148 ± 0.003 b	0.121 ± 0.002 b	0.224 ± 0.008 a	0.24 ± 0.02 a	0.24 ± 0.06 a	raw potato, garlic

The data, the means of three replicates, are expressed as the mg internal standard equivalent/L of wine ± standard deviation. In each row, different letters indicate significant differences according to Duncan test at a significant value of 95% ($p < 0.05$); tR indicates Retention time. * 1-propanol and ethylbutanoate, and 3-methyl- and 2 methyl-butanoic acid were grouped together as they were not separated chromatographically. Odor descriptors using published data [29,30,33] and the website https://www.thegoodscentscompany.com/, accessed on 12 July 2024.

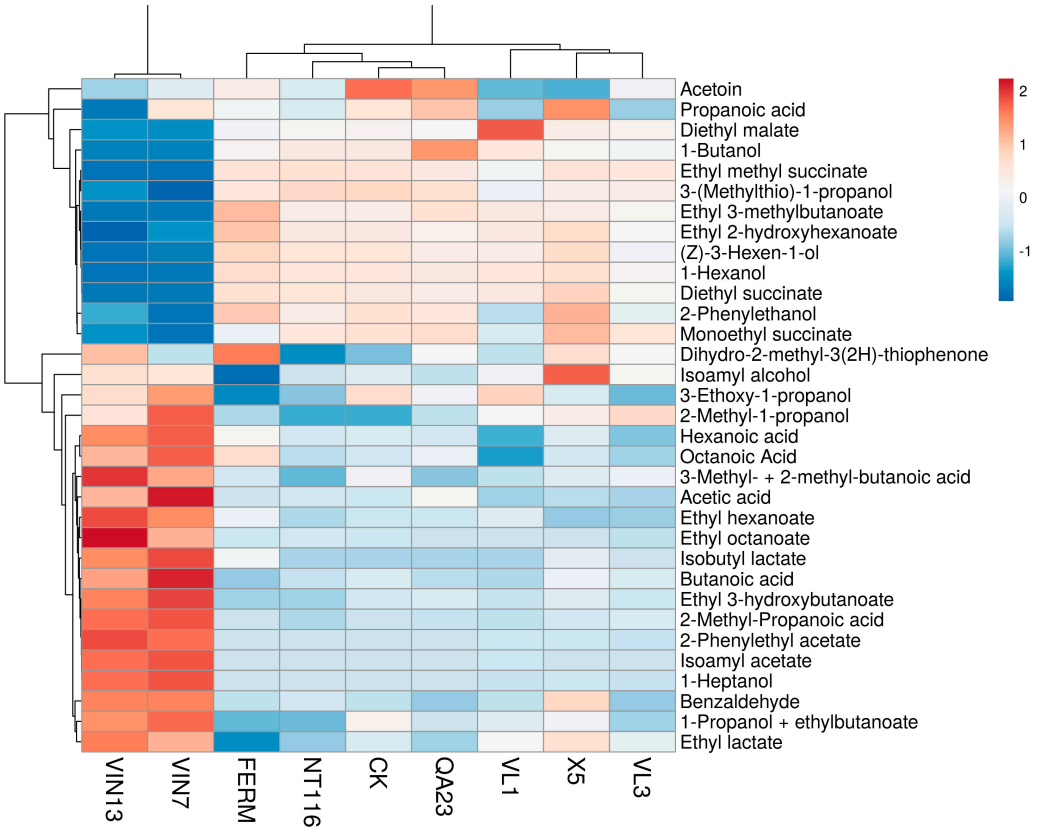

Figure 2. Clustergram (heatmap and hierarchical clustering) performed on VOC concentration values.

The compounds mainly produced by these strains and grouped in the same first large cluster (I group), hexanoic acid, octanoic acid, the corresponding esters (ethyl hexanoate, ethyl octanoate), acetic acid, and the acetates (2-phenylethyl acetate, isoamyl acetate) were observed to strongly discriminate VIN13 and VIN7 from the others. The aforementioned esters are known to be responsible for the fruit odors of wine, such as banana and pear. These results were in accordance with what was reported by Molina et al. [19] for VIN13. The authors found that musts fermented with VIN13 exhibited the highest concentration of short and branched-chain ethyl esters and were characterized by sweet and fruity aroma characters. Both features constitute positive traits for VIN13 in the production of fresh and young white wines. This 'aroma producer' capacity is complemented by the good ability of VIN13 to release thiol precursors from grapes, thus enhancing the tropical flavor in Sauvignon Blanc wines [17].

The other abundant compounds detected in VIN13 and VIN7 were 3-methyl- + 2-methyl-butanoic acid (coeluting peaks), ethyl 3-hydroxybutanoate, 2-methyl-propanoic acid, 1-heptanol, isobutyl lactate, benzaldehyde, and ethyl lactate. High levels of ethyl lactate have been shown to contribute to negative odor scores for wines [34]. On the other hand, VIN13 and VIN7 were characterized by the lowest amount of several compounds (II group), including 2-phenylethanol, known to confer rose notes, hexen-1-ol, and 1-hexanol, which are responsible for the vegetal character of wines, ethyl 3-methylbutanoate, ethyl 2-hydroxyhexanoate, and 3-(methylthio)-1-propanol. Finally, VIN7 and VIN13 showed the lowest amount of diethyl malate, 1-butanol, monoethyl succinate, ethyl methyl succinate, and diethyl succinate.

With regard to the other strains, minor differences were observed. Generally, the opposite behavior with respect to VIN13 and VIN7 was reported. FERM, QA23, NT116, CK, VL1, VL3, and X5 produced a greater amount of II group metabolites and a minor amount of I group metabolites, compared to VIN13 and VIN7. Furthermore, the heatmap highlighted that X5 was the greatest producer of isoamyl alcohol and propanoic acid, CK and QA23

were the largest producers of acetoin, FERM produced the highest amount of dihydro-2-methyl-3(2H)-thiophenone, and VL1 produced the highest amount of diethyl malate. Finally, VL3 was the strain with the lowest production of metabolites. Therefore, this could suggest its use when the minimization of fermentative aroma is desired. Ultimately, no relationship was appreciated between the fermentation rate and the volatile fingerprints.

4. Conclusions

The findings of this study contribute to deeply characterizing the volatile fingerprint as largely dependent on the wine yeast strain used during a lab-scale alcoholic fermentation. The 33 volatile organic compounds, identified in the synthetic wines, well discriminated the differential ability in the fermentative profile of nine different commercial oenological strains. Discriminating features were in the esters including acetates of higher alcohols and ethyl esters of fatty acids, as pointed out by the Hierarchical Clustering Analysis.

Particularly noteworthy were the VIN13 and VIN7 strain volatile fingerprints, with the highest amount of apolar esters (isoamyl acetate, ethyl octanoate, ethyl hexanoate, and 2-phenylethyl acetate), some fatty acids (hexanoic and octanoic acids), along with the lowest amount of 2-phenylethanol, hexen-1-ol, 1-hexanol, ethyl 3-methylbutanoate, ethyl 2-hydroxyhexanoate, and 3-(methylthio)-1-propanol. No relationship was appreciated between the fermentation rate and the volatile fingerprints.

Overall, the results of this research offer a new perspective to the oenological sector for addressing the wine volatile fingerprint and the aromatic wine style, as affected by the *S. cerevisiae* strain used in guided alcoholic fermentation.

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References

1. Gonzalez, R.; Morales, P. Truth in wine yeast. *Microb. Biotechnol.* **2022**, *15*, 1339–1356. [CrossRef] [PubMed]
2. Comitini, F.; Agarbati, A.; Canonico, L.; Ciani, M. Yeast interactions and molecular mechanisms in wine fermentation: A comprehensive review. *Int. J. Mol. Sci.* **2021**, *22*, 7754. [CrossRef]
3. Welke, J.E.; Hernandes, K.C.; Lago, L.O.; Silveira, R.D.; Marques, A.T.B.; Zini, C.A. Flavoromic analysis of wines using gas chromatography, mass spectrometry and sensory techniques. *J. Chromatogr. A* **2024**, *1734*, 465264. [CrossRef] [PubMed]
4. Welke, J.E.; Hernandes, K.C.; Nicolli, K.P.; Barbará, J.A.; Biasoto, A.C.T.; Zini, C.A. Role of gas chromatography and olfactometry to understand the wine aroma: Achievements denoted by multidimensional analysis. *J. Sep. Sci.* **2021**, *44*, 135–168. [CrossRef] [PubMed]
5. Blanco, P.; Mirás-Avalos, J.M.; Pereira, E.; Orriols, I. Fermentative aroma compounds and sensory profiles of Godello and Albariño wines as influenced by *Saccharomyces cerevisiae* yeast strains. *J. Sci. Food Agric.* **2013**, *93*, 2849–2857. [CrossRef]
6. Pereira, C.; Mendes, D.; Dias, T.; Garcia, R.; da Silva, M.G.; Cabrita, M. Revealing the yeast modulation potential on amino acid composition and volatile profile of Arinto white wines by a combined chromatographic-based approach. *J. Chromatogr. A* **2021**, *1641*, 461991. [CrossRef]
7. Rollero, S.; Bloem, A.; Ortiz-Julien, A.; Camarasa, C.; Divol, B. Altered fermentation performances, growth, and metabolic footprints reveal competition for nutrients between yeast species inoculated in synthetic grape juice-like medium. *Front. Microbiol.* **2018**, *9*, 196. [CrossRef]

8. King, E.S.; Swiegers, J.H.; Travis, B.; Francis, I.L.; Bastian, S.E.; Pretorius, I.S. Coinoculated fermentations using *Saccharomyces* yeasts affect the volatile composition and sensory properties of *Vitis vinifera* L. cv. Sauvignon blanc wines. *J. Agric. Food Chem.* **2008**, *56*, 10829–10837. [CrossRef]
9. Escudero, A.; Gogorza, B.; Melús, M.A.; Ortín, N.; Cacho, J.; Ferreira, V. Characterization of the aroma of a wine from maccabeo. Key role played by compounds with low odor activity values. *J. Agric. Food Chem.* **2004**, *52*, 3516–3524. [CrossRef]
10. Arias-Pérez, I.; Ferrero-Del-Teso, S.; Sáenz-Navajas, M.P.; Fernández-Zurbano, P.; Lacau, B.; Astraín, J.; Barón, C.; Ferreira, V.; Escudero, A. Some clues about the changes in wine aroma composition associated to the maturation of "neutral" grapes. *Food Chem.* **2020**, *320*, 126610. [CrossRef]
11. Romano, P.; Braschi, G.; Siesto, G.; Patrignani, F.; Lanciotti, R. Role of Yeasts on the Sensory Component of Wines. *Foods* **2022**, *11*, 1921. [CrossRef] [PubMed]
12. Carpena, M.; Fraga-Corral, M.; Otero, P.; Nogueira, R.A.; Garcia-Oliveira, P.; Prieto, M.A.; Simal-Gandara, J. Secondary Aroma: Influence of Wine Microorganisms in Their Aroma Profile. *Foods* **2021**, *10*, 51. [CrossRef] [PubMed]
13. Carrau, F.M.; Medina, K.; Boido, E.; Farina, L.; Gaggero, C.; Dellacassa, E.; Versini, G.; Henschke, P.A. De novo synthesis of monoterpenes by *Saccharomyces cerevisiae* wine yeasts. *FEMS Microbiol. Lett.* **2005**, *243*, 107–115. [CrossRef]
14. Massera, A.; Assof, M.; Sari, S.; Ciklic, I.; Mercado, L.; Jofré, V.; Combina, M. Effect of low temperature fermentation on the yeast-derived volatile aroma composition and sensory profile in Merlot wines. *LWT Food Sci. Technol.* **2021**, *142*, 111069. [CrossRef]
15. Genovese, A.; Caporaso, N.; Moio, L. Influence of yeast strain on odor-active compounds in Fiano wine. *Appl. Sci.* **2021**, *11*, 7767. [CrossRef]
16. King, E.S.; Francis, I.L.; Swiegers, J.H.; Curtin, C. Yeast strain-derived sensory differences retained in Sauvignon blanc wines after extended bottle storage. *Am. J. Enol. Vitic.* **2011**, *62*, 366–370. [CrossRef]
17. Swiegers, J.H.; Kievit, R.L.; Siebert, T.; Lattey, K.A.; Bramley, B.R.; Francis, I.L.; King, E.S.; Pretorius, I.S. The influence of yeast on the aroma of Sauvignon Blanc wine. *Food Microbiol.* **2009**, *26*, 204–211. [CrossRef] [PubMed]
18. Patrignani, F.; Chinnici, F.; Serrazanetti, D.I.; Vernocchi, P.; Ndagijimana, M.; Riponi, C.; Lanciotti, R. Production of Volatile and Sulfur Compounds by 10 *Saccharomyces cerevisiae* Strains Inoculated in Trebbiano Must. *Front. Microbiol.* **2016**, *7*, 243. [CrossRef]
19. Molina, A.M.; Guadalupe, V.; Varela, C.; Swiegers, J.H.; Pretorius, I.S.; Agosin, E. Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. *Food Chem.* **2009**, *117*, 189–195. [CrossRef]
20. Ugliano, M.; Bartowsky, E.J.; McCarthy, J.; Moio, L.; Henschke, P.A. Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three *Saccharomyces* yeast strains. *J. Agric. Food Chem.* **2006**, *54*, 6322–6331. [CrossRef]
21. Liu, P.; Wang, Y.; Ye, D.; Duan, L.; Duan, C.; Yan, G. Effect of the addition of branched-chain amino acids to non-limited nitrogen synthetic grape must on volatile compounds and global gene expression during alcoholic fermentation. *Aust. J. Grape Wine Res.* **2018**, *24*, 197–205. [CrossRef]
22. Beltran, G.; Esteve-Zarzoso, B.; Rozès, N.; Mas, A.; Guillamón, J.M. Influence of the timing of nitrogen additions during synthetic grape must fermentations on fermentation kinetics and nitrogen consumption. *J. Agric. Food Chem.* **2005**, *53*, 996–1002. [CrossRef] [PubMed]
23. Alves, Z.; Melo, A.; Figueiredo, A.R.; Coimbra, M.A.; Gomes, A.C.; Rocha, S.M. Exploring the *Saccharomyces cerevisiae* Volatile Metabolome: Indigenous versus Commercial Strains. *PLoS ONE* **2015**, *10*, e0143641. [CrossRef] [PubMed]
24. Romano, P.; Siesto, G.; Capece, A.; Pietrafesa, R.; Lanciotti, R.; Patrignani, F.; Granchi, L.; Galli, V.; Bevilacqua, A.; Campaniello, D.; et al. Validation of a Standard Protocol to Assess the Fermentative and Chemical Properties of *Saccharomyces cerevisiae* Wine Strains. *Front. Microbiol.* **2022**, *13*, 830277. [CrossRef]
25. Pérez, D.; Jaehde, I.; Guillamón, J.M.; Heras, J.M.; Querol, A. Screening of *Saccharomyces* strains for the capacity to produce desirable fermentative compounds under the influence of different nitrogen sources in synthetic wine fermentations. *Food Microbiol.* **2021**, *97*, 103763. [CrossRef]
26. Riou, C.; Nicaud, J.M.; Barre, P.; Gaillardin, C. Stationary-phase gene expression in *Saccharomyces cerevisiae* during wine fermentation. *Yeast* **1997**, *13*, 903–915. [CrossRef]
27. Pati, S.; Esti, M.; Leoni, A.; Liberatore, M.T.; La Notte, E. Polysaccharide and volatile composition of Cabernet wine affected by different over-lees ageing. *Eur. Food Res. Technol.* **2012**, *235*, 537–543. [CrossRef]
28. Tauno, M.; Vilo, J. Clustvis: A web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. *Nucleic Acids Res.* **2015**, *43*, W566–W570.
29. Larnbrechts, M.G.; Pretorius, I.S. Yeast and its Importance to Wine Aroma—A Review. *S. Afr. J. Enol. Vitic.* **2000**, *21*, 97–129.
30. Aznar, M.; Lopez, R.; Cacho, J.F.; Ferreira, V. Identification of impact odorants of aged red wines from Rioja. GC-Olfactometry, quantitative GCeMS, and odor evaluation of HPLC fractions. *J. Agric. Food Chem.* **2001**, *49*, 2924–2929. [CrossRef]
31. Swiegers, J.H.; Bartowksy, E.J.; Henschke, P.A.; Pretorius, I.S. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005**, *11*, 127–138. [CrossRef]
32. Gamero, A.; Belloch, C.; Querol, A. Genomic and transcriptomic analysis of aroma synthesis in two hybrids between *Saccharomyces cerevisiae* and *S. kudriavzevii* in winemaking conditions. *Microb. Cell Fact.* **2015**, *14*, 128. [CrossRef] [PubMed]

33. Rocha, M.S.; Rodrigues, F.; Coutinho, P.; Delgadillo, I.; Coimbra, M.A. Volatile composition of Baga red wine: Assessment of the identification of the would-be impact odorants. *Anal. Chim. Acta* **2004**, *513*, 257–262. [CrossRef]
34. Nikolaou, E.; Soufleros, E.H.; Bouloumpasi, E.; Tzanetakis, N. Selection of indigenous *Saccharomyces cerevisiae* strains according to their oenological characteristics and vinification results. *Food Microbiol.* **2006**, *23*, 205–211. [CrossRef]