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Influence of Yeast Strain on Odor-Active Compounds in Fiano Wine

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Featured Application: This research can be useful for the winemaking industry to expand the range of products, offer the customer a typical wine with specific sensory and to improve the wine quality.

Abstract: The type of yeast strain used for wine alcoholic fermentation dramatically affects its final volatile composition and, therefore, its sensory properties. In this study, the influence of four oenological *Saccharomyces* strains (three *S. cerevisiae* and one *S. bayanus*) on wine volatile composition was determined on the Fiano variety, a typical cultivar from the Campania region (Italy), fermented in oak barrique. Fiano wines were analyzed by means of gas chromatography/mass spectrometry (GC/MS) and gas chromatography/olfactometry (GC/O). The results showed that the four selected yeast strains had a significant impact on the majority of volatile compounds as shown by the concentration of volatile compounds and based on the Aroma Extract Dilution Analysis (AEDA) values for many of the odor volatile compounds. This resulted in a dramatic change of the odor impact of the wines, such as the "fruity" attribute, which was higher compared to the control, and caused some changes of other odor attributes, particularly "floral", "phenolic" and "honey". This research demonstrates the potential of using these selected yeast strains and this technological approach of oak fermentation for this typical white wine grape variety.

Keywords: yeasts; volatile compounds; white wine; gas chromatography/olfactometry; Aroma Extract Dilution Analysis (AEDA); alcoholic fermentation; barriques

Citation: Genovese, A.; Caporaso, N.; Moio, L. Influence of Yeast Strain on Odor-Active Compounds in Fiano Wine. *Appl. Sci.* **2021**, *11*, 7767. https://doi.org/10.3390/app11177767

Academic Editor: Maria Kanellaki

Received: 5 July 2021
Accepted: 20 August 2021
Published: 24 August 2021

1. Introduction

Wine aroma is strongly influenced by the yeast strain that conducts the fermentation [1–4]. Each strain has its own characteristics, different from those of the other strains, capable of influencing the gustatory and aromatic balance of the wine [5–15].

Several studies have reported the remarkable influence of the type of yeast strain on the wine aroma. In particular, the yeast strain effect on the biosynthesis of higher alcohols, esters, aldehydes of sulfur compounds and volatile phenols were reviewed by Lambrechts and Pretorius [1]. In some cases, these differences are very slight and detectable only by expert tasters but not the majority of usual consumers; however, in other cases, they are more obvious to all tasters.

A recent research by Cotea, Focea, Luchian, Colibaba, Scutarașu, Marius, Zamfir and Popîrdă [6] investigated the impact of five yeast strains on the quality parameters of sparkling wines from Muscat Ottonel. The authors showed that despite the limited influence of different commercial strains on many physicochemical parameters, the impact on wine volatile compounds was significant. The authors showed that some strains confer more floral odor notes, particularly elderflowers, while others, fruitier notes, for

example green banana. They also correlated the fruity notes with a higher presence of ethyl octanoate, ethyl decanoate or diethyl succinate.

Fraile, Garrido and Ancín [7] reported an investigation on rose wine from the Garnacha variety by comparing selected yeast strains and a control with indigenous yeasts. They reported a higher content of alcohol and acids in the control wine, and particularly for the acid, they noticed a more rapid production in the first phase of fermentation.

Mauriello et al. [16], characterizing wild strains of *S. cerevisiae* isolated from vineyard from Northern and Southern Italy, reported that strains from Southern Italy had a higher production of volatile compounds compared to the Northern Italian strains. Suzzi, Arfelli, Schirone, Corsetti, Perpetuini and Tofalo [13] also tested indigenous *S. cerevisiae* starters for Montepulciano d'Abruzzo wine production. They reported that different strains have different kinetics during the fermentation and thus a different tendency to dominate over other strains. This leads to the production of different volatile profiles, and aroma profiles, as assessed by a sensory panel.

Another more recent review [17] focused on the influence of non-*Saccharomyces* strains on aroma-related compounds. The reader can find extensive information on the formation of volatile compounds and the related metabolic routes in the alcoholic fermentation, in particular, those carried out using a wide range of non-Saccharomyces species. The application of non-*Saccharomyces* species would be for the benefit of consumers that are looking for lower alcohol content in wines, by taking advantage of some strains that naturally produce less ethanol. Varela [18] reported several examples of this application for producing wines with a reduced alcohol content.

Therefore, the choice of the yeast is a primary decision in the wine production process. The chemical composition of the must affects the activity of the yeast strain. In addition to the grape variety, important factors are the grape-ripening level and the pre-fermentation operations, which affect the must composition. This is especially important when wooden barrels are used because of the exchanges of chemical components between the barrel and the wine or must [19]. Generally, the oak barrels influence the sensory quality of wine by releasing volatile compounds with high olfactory activity and non-volatile molecules characterized by gustatory activity [20,21]. Barriques, oak barrels of a well-defined volume, can be used by adding wine after the alcoholic fermentation, or as occurs in the production technology of Chardonnay from Burgundy, immediately after mashing the grapes, allowing the must to ferment into them [22–24]. In the latter case, the yeasts will change their chemical composition, compared to the same must fermented in a traditional inert tank. Therefore, the choice of the yeast strain could be more complex, and the greater complexity of the must has to be taken into account. Whilst wine fermentation in wooden barrels is traditionally carried out for red wines, it has also spread to the production of Italian white wines obtained from native grape varieties [25]. Other recent research evaluated the impact of different fermentation technologies of another typical white wine, namely Palomino Fino, a neutral variety, reporting significant changes of the volatile composition, Odor Activity Values (OAV) and sensory profiles [26].

However, information on the influence of specific yeast strains on the aroma characteristics of wine fermented in barrique is lacking regarding Italian native grape varieties.

Therefore, the aim of this study was to determine the influence of four yeast strains on the volatile composition of white wine, whose grape-must was fermented in oak barriques. The experiment was conducted on the "Fiano" cultivar, the most representative white wine variety in the Campania region of Italy.

2. Materials and Methods

2.1. Wines and Yeast Strains

Fiano grapes were harvested at 22° Brix in vineyards located in the town of Taurasi (AV-coordinates 41.0125° N, 14.9681° E), a DOCG area in the Campania region, Italy.

A total of 1200 kg of grapes were crushed (0.9 atm), using a pneumatic press of 80 q. SO_2 (50 mg/L), and pectic enzyme (1 g/hL) were added to the must. Then, it was

immediately cooled to 10 °C and submitted to static decantation to 80–100 NTU (Number Torbidity Unity) in stainless-steel tanks (15 hL).

Four tanks were inoculated each with a selected yeast strain, while the fifth tank was not inoculated (control). Inoculations were carried out at 30 g/hL, after the yeasts were rehydrated in warm water for 30 min, as described by the manufacturers. Then, a homogenization was carried out for 10 min, and the must from each stainless-steel tank was transferred to a new barrique (Troncay, MTL+). Fermentation took place in five barriques (Troncay) at 12 °C. Upon completion of alcoholic fermentation (30 days), wines were cold stabilized for a 3-month period at 10 °C, filtered on 5 µm membranes and bottled, previously added with 30 mg/L of SO_2.

Three strains of *S. cerevisiae* (Lalvin D47; Lalvin CY3079; Zymaflore VL1) and one strain of *S. bayanus* (Lalvin QA23) were used. The D47, CY3079 and QA23 strains were supplied by LALLEMAND S.A. company (Montreal, QC, Canada) as dry active yeasts, while Zymaflore VL1 by Laffort Oenologie (Bordeaux, France). The *S. cerevisiae* strains were previously selected for white winemaking.

The QA23 strain is defined as "aromatic", due to its higher production of esters and alcohols. It has an alcohol resistance of up to 15% and works an optimal fermentation temperature of 15–35 °C. The CY3079 strain is defined as "varietal", due to its ability to release the primary aroma of the grapes. It ferments over a wide temperature range above 13 °C and has an alcohol resistance of up to 16%. The D47 strain has a good aptitude for fermenting in wooden barrels. It has an alcohol resistance of up to 15% and an optimal fermentation temperature of 12–30 °C. The VL1 strain is defined as highly varietal. It has an alcohol resistance of up to 14.5% and an optimal fermentation temperature of 16–20 °C.

The chemical standards were supplied by Sigma-Aldrich (St. Louis, MO, USA).

2.2. Extraction and Analysis of Volatile Compounds

The volatile compounds of the wine were determined, using an extraction procedure previously reported by Moio et al. [27]. A total of 200 mL of wine, obtained after mixing three equal bottles, was submitted to continuous liquid–liquid extraction for 3 h with 20 mL of di-chloromethane. As the internal standard, 2-methyl-1-pentanol at a final concentration of 1 mg L^{-1} was added. The organic layer was recovered in a separating funnel. Residual water was removed by means of the addition of Na_2SO_4, and the solvent was concentrated first in a Kuderna–Danish concentrator to 1 mL and finally under a low stream of nitrogen (1.5 L min^{-1}) to 500 µL. Extraction of each sample was performed in triplicate.

GC/MS was performed using an Agilent 6890 chromatograph equipped with a split/splitless injector (Agilent Technologies, Folsom, CA, USA), a J&W DB-Wax column (30 m length × 0.25 i.d. × 0.25 film thickness; J&W Scientific, Folsom, CA), and 5973 Network series quadrupole mass spectrometric detector (Agilent Technologies, Folsom, CA, U.S.A.). The temperature program used was 40 °C for 3 min, raised at 4 °C min^{-1} to 220 °C, and held for 20 min at maximum temperature. The carrier gas (He) velocity was 37 cm/s. The injector port and the ion source were maintained at 250 and 230 °C, respectively. Electron impact mass spectra were recorded with an ion source energy of 70 eV. A 1 µL aliquot of each concentrated extract was injected in splitless mode.

Volatile compound identification was performed by comparing retention times and mass spectra obtained by analyzing pure reference compounds under the same conditions. The identification was further confirmed by comparing mass spectra with those of the NIST database. Compounds for which pure reference standards were not available were tentatively identified only based on mass spectra comparison.

GC/O analysis was performed on extracted volatile compounds, using a 5890 Hewlett-Packard gas-chromatograph equipped with a same column of GC/MS analysis and connected with a Hewlett-Packard "Y splitter deactivated", allowing the effluent to be split between the sniffing port and flame ionization detector (FID). Dilutions of Aroma Extract Dilution Analysis (AEDA) were done sequentially by volume (1:5) [28]. A 1.5 µL splitless injection of extract was made. The gas chromatographic conditions were the same as

those described for GC/MS analysis. Two experienced judges operated independently for the assessment.

2.3. Data Analysis

Partial least squares (PLS) regression analysis was chosen as an exploratory technique to investigate the correlation between GC/O data and volatile organic compounds of wines in relation to the yeast strain used. Tukey's test was used to assess the significance of the differences among the mean values of the variables. Partial least squares and Tukey's test were carried out using XLStat (Version 2014.5.03), an add-in software package for Microsoft Excel (Addinsoft Corp., Paris, France). When not otherwise indicated, differences were considered statistically significant when $p < 0.05$, and strongly statistically significant at $p < 0.001$.

3. Results and Discussion

3.1. Impact of In-Barrique Fermentation Using Selected Yeast Strains on Wine Volatile Compound Composition

White wines obtained from a Southern Italian variety called "Fiano", typical of the Campania region, were analyzed for their volatile composition by focusing on key aroma compounds. Table 1 reports the content of volatile compounds with concentration measured in the wines fermented, using four different yeast strains, and shows the control fermented, using a spontaneous fermentation.

Table 1. Content (mg L^{-1}) of volatile compounds in "Fiano" white wines obtained by using four selected yeast strains and fermenting the must in oak barrels. The compounds are grouped according to their chemical composition.

Compound	Control	s.d.	QA23	s.d.	D47	s.d.	CY3079	s.d.	VL1	s.d.	*p*-Value
Alcohols											
1-Propanol	0.352	0.027	0.396	0.002	0.388	0.041	0.519	0.053	0.374	0.010	***
2-Methyl-1-propanol	2.645	0.100	0.920	0.091	1.598	0.247	2.654	0.787	3.082	0.419	***
1-Butanol	0.048	0.019	0.046	0.012	0.061	0.018	0.065	0.001	0.044	0.007	NS
3 + 2-Methyl-1-butanol	97.870	5.237	96.216	5.426	89.024	4.292	84.125	5.701	108.606	6.566	*
1-Hexanol	2.918	0.287	3.050	0.068	2.718	0.602	2.769	0.171	2.993	0.212	NS
cis-3-Hexen-1-ol	0.030	0.001	0.033	0.002	0.023	0.003	0.026	0.003	0.035	0.008	*
Linalool	0.030	0.001	0.032	0.002	0.031	0.001	0.031	0.003	0.023	0.006	*
α-Terpineol	0.081	0.005	0.083	0.004	0.078	0.015	0.084	0.005	0.078	0.008	NS
Benzyl alcohol	0.191	0.005	0.198	0.011	0.412	0.117	0.365	0.098	0.190	0.003	**
2-Phenylethanol	50.854	1.034	69.411	4.210	47.705	7.100	50.375	4.137	57.107	4.286	**
Esters											
Ethyl butanoate	0.352	0.027	0.396	0.002	0.388	0.041	0.519	0.053	0.374	0.010	***
3-Methylbutyl acetate	0.390	0.017	0.345	0.008	0.348	0.074	0.330	0.022	0.246	0.012	**
Ethyl hexanoate	0.979	0.025	1.340	0.059	0.784	0.130	0.847	0.014	0.904	0.038	***
Hexyl acetate	0.132	0.015	0.022	0.002	0.084	0.010	0.076	0.002			***
Ethyl lactate	37.655	0.635	15.459	6.822	32.481	3.086	46.027	2.075	43.613	2.232	***
Ethyl octanoate	2.054	0.090	2.405	0.094	1.408	0.299	1.730	0.084	1.764	0.113	***
3-Hydroxyethyl butanoate	0.043	0.012	0.060	0.013	0.095	0.014	0.075	0.001	0.085	0.006	***
Ethyl decanoate	0.679	0.036	0.906	0.012	0.550	0.122	0.544	0.052	0.518	0.031	***
Diethyl ester of butanedioic acid	5.292	0.111	6.386	0.470	7.589	1.302	7.162	0.088	7.131	0.570	*
2-Phenylethyl acetate	0.103	0.004	0.088	0.001	0.092	0.017	0.088	0.007	0.069	0.012	***
Aldehydes and ketones											
Acetoin	0.791	0.070	0.386	0.054	0.616	0.053	0.661	0.099	0.066	0.013	***
Furfural	0.176	0.005	2.866	0.118	2.648	0.418	0.299	0.010	0.225	0.012	***
Ethylphenyl acetaldehyde	0.021	0.007	0.066	0.005	0.028	0.007	0.033	0.005	0.030	0.009	***
β-Damascenone	0.012	0.001	0.006	0.002	0.011	0.000			0.012	0.000	NS
5-Hydroxymethyl furfural	0.358	0.043	0.129	0.015	0.293	0.083	0.351	0.083	0.077	0.028	***

Table 1. *Cont.*

Compound	Control	s.d.	QA23	s.d.	D47	s.d.	CY3079	s.d.	VL1	s.d.	*p*-Value
Lactones											
Butyrolactone	5.085	0.131	4.220	0.659	4.262	1.068	8.200	0.428	5.428	0.127	***
trans-3-Methyl-γ-octalactone	0.053	0.002	0.011	0.001	0.067	0.020	0.068	0.004	0.044	0.004	***
cis-3-Methyl-γ-octalactone	0.151	0.007	0.114	0.012	0.091	0.022	0.136	0.009	0.143	0.015	**
3-Hydroxy-2-pyranone	0.089	0.009	0.618	0.017	0.225	0.077	0.156	0.041	0.107	0.016	***
Pantolactone	0.012	0.001	0.021	0.008	0.013	0.001			0.011	0.001	**
Volatile phenols											
4-Vinylguaiacol	0.248	0.038	0.466	0.200	0.278	0.074	0.190	0.018	0.336	0.038	NS
Syringol	0.013	0.002	0.015	0.002			0.037	0.004	0.009	0.001	***
Vanillin	0.018	0.001	0.047	0.001					0.016	0.004	NS
Acids											
Acetic acid	0.062	0.006	0.260	0.060	0.220	0.007	0.069	0.004	0.081	0.027	*
Propanoic acid			0.017	0.004					0.015	0.001	***
Hexanoic acid	11.369	0.354	7.591	2.685	8.261	0.427	8.405	0.523	10.172	0.743	*
2-Hexenoic acid	0.150	0.012	0.111	0.012	0.198	0.071	0.133	0.012	0.148	0.024	NS
Octanoic acid	38.185	1.990	32.460	3.183	20.110	7.852	29.693	1.417	37.226	2.702	**
Decanoic acid	9.464	0.570	6.535	0.705	4.811	0.723	6.805	0.505	9.300	0.732	***
Furans											
2-Propyl furan	1.247	0.228	0.425	0.005	1.562	0.364	2.222	0.572	0.656	0.055	***
2-Furanmethanol	0.392	0.032			0.422	0.033	0.604	0.094	0.695	0.084	***
Others											
3-Methylthiopropanol	0.180	0.003	0.192	0.032	0.140	0.010	0.227	0.025	0.229	0.032	**
N-2-Phenyl-acetamide	0.128	0.012	0.075	0.012	0.061	0.014	0.236	0.010	0.221	0.031	***
N-butyl-acetamide	0.191	0.005	0.198	0.011	0.412	0.117	0.365	0.098	0.190	0.003	**

Numbers indicate the concentration of volatile compounds, as the average from three replicates, and the standard deviation. NI = control; QA23 = *S. bayanus* Lalvin; D47 = *S. cerevisae* Lalvin; CY3079 = *S. cerevisae* Lalvin; VL1 = *S. cerevisae* Zymaflore. NS = not significant ($p > 0.05$). Asterisks indicate significant differences between treatment-related samples (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Alcohols, ethyl esters, acetates and fatty acids are the major fermentation compounds, exclusively due to the metabolic activity of the yeasts. In fact, yeasts are able to synthesize all the needed amino acids from ramified amino acids by the Ehrlich pathway. In this case, some yeast by-products are represented by main fusel alcohols, i.e., 2-methyl-1-propanol, 3 and 2-methyl-1-butanol. During yeast fermentation, many medium- and long-chain fatty acids are also formed via the fatty acid synthesis pathway from acetyl-CoA, while acetates and esters are resulted from the equilibrium reaction between an alcohol and an acid [3]. The major fermentation compounds, such as alcohols, ethyl esters and fatty acids constitute a main and common part of the wine flavor and are considered to play a positive role in wine fruity notes [29].

Alcohols. The most abundant fusel alcohols in all wines analyzed were 3 and 2-methyl-1-butanol, whose content reached a maximum for LV1, while CY3079 had the lowest concentration. 2-Phenylethanol had a significantly higher content in QA23 compared to the control, while D47 showed the lowest content. A strong statistically significant difference ($p < 0.001$) was observed for 2-methyl-1-propanol, whose content for QA23 was the lowest, while VL1 had the highest content, with an almost 4 times higher content. On the contrary, VL1 showed the lowest level for linalool, while QA23 had the highest one. Non-significant differences were observed for 1-butanol, 1-hexnaol and α-terpineol. Linalool and α-terpineol are terpene alcohols obtained by the metabolism of mevalonic acid and are responsible for the typical floral aromas of Muscat and Gewurztraminer wines [3].

Esters. The major compound belonging to this class was ethyl lactate, whose content statistically significant changed according to the yeast strains used. The lowest content was observed for QA23, while CY3079 and VL1 had more than double the concentration of QA23. All volatile compounds were significantly affected by the yeast strain used. Generally, with a few exceptions, esters had higher content for QA23 and lower content for D47. Diethyl ester of butanedioic acid had a different trend, with the highest concentration

being recorded for D47, while the lowest one was obtained for the control sample. Ester biosynthesis was widely studied in *S. cerevisiae* during wine fermentation.

It is known that acetate esters are formed by the activity of alcohol acetyltransferases (Atf1p and Atf2p), isoamyl alcohol acetyltransferase and ethanol acetyltransferase, while ethyl esters follow a different pathway, as they are synthetized by the activity of two acyl-CoA: ethanol O-acyltransferase enzymes, Eeb1p and Eht1p [3].

Acids. Octanoic acid was statistically significantly lower in D47, with a concentration almost half the concentration of the control. Stronger statistically differences ($p < 0.001$) were observed for propanoic acid and decanoic acid. Regarding the use of the selected yeast strain in contrast with using mixtures of yeasts that naturally occur in the vineyard, Fraile, Garrido and Ancín [7] reported an investigation on rose wine from the Garnacha variety by comparing selected yeast strains and control indigenous yeasts. They reported a higher content of alcohol and acids in the control wine; particularly for the acid, they noticed a more rapid production in the first phase of fermentation.

Aldehydes and ketones. All compounds from these classes were strongly and significantly affected ($p < 0.001$) by the treatment. The only exception was for β-damascenone, a norisoprenoid compound that might originate from the direct degradation of grapes carotenoids, such as β-carotene, lutein, neoxanthin and violaxanthin, for which no significant changes were observed. Furfural had particularly high concentrations. Yeasts QA23 and D47 had about 10 times higher content than the other samples, including the control.

Lactones. Butyrolactone was the most abundant lactone in the analyzed wines, with a significantly higher content for wines produced using CY3079. Those produced using QA23 were significantly higher in 3-hydroxy-2-pyranone, whose content in any treatment was higher than the control.

Volatile phenols. Two of the 3 volatile phenols analyzed did not have statistically significant differences depending on the treatment, with syringol being more abundant in CY3079. Whist volatile phenols are always present in wines, even at very low concentrations, their contribution to the wine aroma is not always positive [4]. Their formation occurs through a decarboxylation on the p-coumaric acid and ferulic acid in a non-oxidative process by *Saccharomyces cerevisiae*.

Furans. 2-Propyl furan had a strong statistically lower concentration in QA23, followed by VL1. On the other hand, D47 and CY3079 had higher concentrations, with more than 3 times the abundance recorded for D47, but similar to the control.

Other compounds. 3-Methylthiopropanol and N-2-phenyl-acetamide had a similar trend, with D47 showing the lowest content, while CY3079 and VL1 showed statistically significant higher concentrations. N-butyl-acetamide had a very similar content to the control for QA23 and VL1, while significantly ($p < 0.01$) higher contents were observed for D47 and CY3079.

Overall, our results based on the quantification of volatile compounds showed that the yeast strain used has a statistically significant impact on the majority of target volatile compounds. This result is in line with previous research showing that the yeast strain has to be selected carefully also depending on the type of wine to be produced.

A recent paper by Cotea, Focea, Luchian, Colibaba, Scutarașu, Marius, Zamfir and Popîrdă [6] showed that, despite the limited influence of five different commercial yeast strains on many physico-chemical parameters of sparkling wines, the impact on wine volatile compounds was statistically significant. By quantifying 20 volatile compounds and carrying out sensory analysis of the wines, the authors showed that some strains confer more floral odor notes, particularly elderflowers, while others, fruitier notes, e.g., green banana. They also reported a positive correlation of the fruity notes with a higher presence of ethyl octanoate, ethyl decanoate or di-ethyl succinate.

Moreover, it was also noticed that not in all wines the inoculated yeast strain is predominant, compared to the indigenous *S. cerevisiae* strains [7]. Suzzi, Arfelli, Schirone, Corsetti, Perpetuini and Tofalo [13] also tested indigenous *S. cerevisiae* starters for Montepulciano d'Abruzzo wine production. They reported that different strains have a different kinetics during the fermentation and thus a different tendency to dominate over other strains. This leads to the production of different volatile profiles and aroma profiles, as assessed by a sensory panel. This conclusion is in line with the results reported in the present paper.

Modulation of the wine flavor can also be approached from a biotechnology point of view, by working toward the development of a recombinant *S. cerevisae* strains that can enhance wine flavor. For example, grapes contain glycoconjugates that have a potential odor impact but are they are "bound" and need to be released to exert their potential activity. The main glycosylated compounds are monoterpene alcohols that can play an important role in the development of varietal aroma of wine. A wide range of yeasts could be potentially used for their glycosidase activity. Whilst previous research has shown that all yeasts have some glycosidic hydrolyzing activity, their activity might be different, according to the different chemical structure of the sugar and the aglycon moieties [30]. A study on Fiano wine specifically investigated the release of free and bound volatile compounds as affected by the enzymatic or acid hydrolysis. The authors showed that linalool, geraniol, teprinen-4-ol,1,1,6-trimethyl-1,2-dihydronaphtalene (TDN), β-damascenone, (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB), ethyl cinnamate, and 4-vinylguaiacol were the most abundant odorous compounds in Fiano wine originated from the hydrolysis of odorless precursors. It was also shown that the formation of linalool and geraniol is mostly attributed to the enzymatic activity [31]. In the present case, a statistically significant effect of the yeast strain used was verified for VL1 compared to all other treatments and the control, which is likely due to the lower hydrolytic activity of this strain toward the odorless precursor of Fiano wine to release linalool.

In general, the results show that "signature" volatile compounds exist, as for the majority of compounds analyzed, we verified that the change in concentration is significant but not yeast-specific. However, a few compounds were particularly more abundant or were absent in some selected strains, compared to the others and to the control, e.g., 3-hydroxy-2-pyranone was particularly abundant in QA23, while 2-furanmethanol was not detected in this same strain, or, for example, hexyl acetate was found in all yeast strains except VL1.

Based on the state-of-the-art of the literature, the combined use of biotechnical and chemical methods can help in improving the final aroma of wines, for example taking advantage of yeast strains enhanced for their β-glycosidases activity. However, often an empirical approach based on testing a range of yeast strains for specific wine varieties has shown that their suitability depends on the wine style, or the target that the winemaker has set.

3.2. Gas Chromatography–Olfactometry Analysis

To better understand the interaction among those volatiles and the potential resulting impact to the consumer, Aroma Extract Dilution Analysis (AEDA) was used to screen for those volatiles with an odor impact. The results of the olfactometric analysis carried out by AEDA are reported in Table 2.

Table 2. Results of olfactometric analysis of white wines cv. "Fiano" fermented in oat barrels using four different yeast strains, three *S. cerevisiae* and one *S. bayanus*, compared to the control without yeast inoculation.

Nr.	Compounds	Odor Descriptor	AEDA Value					Aromatic Series
			Control	QA23	D47	CY3079	VL1	
1	N.I.	Fruity, sweety	1	1	0	1	25	Fruity
2	Ethyl acetate	Fruity, apple	5	25	125	625	625	Fruity
3	Diacetyl	Butter	125	5	5	5	5	Butter/cheese
4	1-Propanol	Fruity, sweety	5	125	25	25	25	Fruity
5	Ethyl butanoate	Fruity, apple	25	5	125	0	125	Fruity
6	3-Methylbutyl acetate	Banana	25	25	5	5	5	Fruity
7	1-Butanol	Winey, grass	0	25	5	0	0	Winey
8	2+3-Methyl-1-butanol	Winey, grass	125	625	125	125	625	Winey
9	Ethyl hexanoate	Apple	25	125	5	25	25	Fruity
10	2-Propyl furan	Winey, pungent	125	25	25	625	625	Winey
11	N.I.	Toasted nutty	625	125	125	25	125	Nutty/toasty
12	Ethyl octanoate	Ananas	125	625	625	625	625	Fruity
13	Acetic acid	Vinegar	5	5	5	5	5	Vinegar
14	Linalool	Orange flowers	5	5	25	5	5	Floral
15	2-Methylpropanoic acid	Cheese	1	1	1	5	5	Butter/cheese
16	Butanoic acid	Cheese	25	25	25	5	25	Butter/cheese
17	Acetophenone	Acacia honey	125	0	0	125	125	Honey
18	3-Methylbutanoic acid	Cheese	125	125	625	625	125	Butter/cheese
19	3-Methylthio-1-propanol	Potato, garlic	25	1	5	5	5	-
20	N.I.	Toasty	1	0	0	1	5	Nutty/toasty
21	2-Phenylethyl acetate	Floral	5	5	0	0	0	Floral
22	β-Damascenone	Honey, tea	625	625	625	625	625	Honey
23	Hexanoic acid	Cheese	25	5	25	5	25	Butter/cheese
24	N.I.	Smoked	625	625	125	125	125	Smoked/phenolic
25	trans-3-Methyl-γ-octalactone	Coconut	5	5	25	25	25	Fruity
26	2-Phenylethanol	Floral, rosa	625	625	625	625	625	Floral
27	N.I.	Floral, rosa	0	5	1	5	5	Floral
28	Pantolactone	Floral	5	5	5	5	5	Floral
29	Hydroxy diethyl butanoate	Caramel	25	25	125	25	125	Caramel
30	Octanoic acid	Cheese	1	5	5	5	0	Butter/cheese
31	N.I.	Medicinal/phenolic	25	0	0	1	1	Smoked/phenolic
32	N.I.	Smoked	0	5	0	0	5	Smoked/phenolic
33	N.I.	Caramel	125	125	125	125	125	Caramel
34	N.I.	Apricot	125	125	125	5	125	Fruity
35	N.I.	Medicinal/phenolic	25	25	25	25	25	Smoked/phenolic
36	N.I.	Smoked	5	1	1	5	1	Smoked/phenolic
37	4-Vinylguaiacol	Smoked	625	625	625	125	625	Smoked/phenolic
38	Syringol	Smoked	25	125	25	25	5	Smoked/phenolic
39	N.I.	Smoked	25	5	1	25	25	Smoked/phenolic
40	N.I.	Medicinal/phenolic	125	125	125	25	5	Smoked/phenolic
41	N.I.	Floral	625	125	125	125	25	Floral
42	Phenylacetic acid	Honey	125	625	125	25	25	Honey
43	N.I.	Floral	625	625	625	25	25	Floral

The data in the above table originate from a triplicate extraction followed by a homogenous mixture of the extracts and a GC/O analysis carried out by two expert assessors. Odor descriptors are based on empirical data obtained by assessors through the GC/O analysis. For each compound, the corresponding aromatic group is indicated, and it was used to build the aromatic series. N. I. = not identified volatile compound. The abbreviations for the different yeasts used are the ones corresponding to the names provided by the yeast manufacturer: QA23 = S. bayanus Lalvin; D47 = S. cerevisae Lalvin; CY3079 = S. cerevisae Lalvin; VL1 = S. cerevisae Zymaflore.

This approach offers a closer understanding of the potential sensory impact of each volatile compound, as it is widely known that the concentration of volatile compounds does not directly link to their odor impact due to multiple factors, such as their odor activity value as well as competition with other volatiles and the matrix. GC/O analysis revealed 43 odorous compounds in Fiano wines but only 28 of them were identified based on the comparison of their chromatographic profile with pure standards and GC/MS analysis.

All four selected yeasts had a much lower AEDA value for diacetyl, which is described as being associated with "butter" notes. Similarly, several compounds—some of which were not identified—were lower in the selected yeast trails compared to the control, namely a compound described as "roasted/nutty", 3-methylthio-1-propanol, a non-identified compound described as "medicinal/phenolic" and a compound described with "smoked" notes. On the other hand, the control sample generally had lower values for 1-propanol (fruity, sweety), the N.I. compound n. 27 ("floral/rose" description) and a very few others. For those compounds described as "smoked/phenolics", it was difficult to find a general trend, as several compounds like the N.I. volatiles n. 24 and 40 had high value in CY3079. This yeast also resulted in a wine with lower value for 4-vinylguaiacol and 1-propanol.

In the case of QA23, higher AEDA values compared to the other yeast strains and the control were obtained for 1-propanol, while lower values were recorded for 2-propyl furan and 3-methylthio-1-propanol. Wines fermented using yeast D47 had lower values for the N.I. compound n.1, ethyl hexanoate and acetophenone, while linalool and hydroxy diethyl butanoate had higher values. Yeast VL1 resulted in wines with much higher values of the N.I. compound n. 1 described as "fruity", while compounds described as "floral" or "honey" had low values, namely the N.I. compounds n. 41 and 43, and phenylacetic acid. Additionally, some compounds described with a "smoked/phenolic" note had lower AEDA value, i.e., syringol and N.I. volatile n. 40.

In order to understand how the odor active compounds could affect the global sensory characteristics of a wine, aromatic series were constructed on the available data. An aromatic series is defined as a group of volatile compounds sharing the same, or similar, aroma descriptor [32]. Generally, the value of an aromatic series is obtained by the sum of the OAVs of the selected volatile compounds.

The OAV is obtained by dividing the concentration of each volatile compound by its perception threshold [33]. The series used in this experiment were built by grouping the odor compounds as previously mentioned and reporting the sum of the AEDA values (expressed as $\log_{10}$ value) detected by GC/O analysis (Table 2). Because of the high complexity of the olfactory perception, some aroma compounds were included in two or more odorant series such that their AEDA values could be better linked to the sensory perceptions, based on literature data [34–36].

Accordingly, fruity, floral, winey, vinegar, butter/cheese, caramel, honey, smoked, phenolic and nutty/toasty odor series were built (Table 2). These odor descriptors can be useful to better show the potential aromatic impact of the different yeasts. The results of this approach are shown in Figure 1.

For yeast QA23, the major difference compared to the control wine was obtained for the "fruity" attribute, with QA23 leading to a statistically significant ($p < 0.05$) higher value, while lower values were obtained for "phenolic" and "honey", and only a minor difference for "butter/cheese". These results were very similar to those obtained from the yeast strain D47, which led to a slight stronger decrease in the phenolic, honey and smoked notes.

On the other hand, both CY3079 and VL1 had an opposite trend. The former had almost the same level of the "fruity" value and a lower "floral" value, while the latter led to the highest "fruity" value and the lowest value of "floral" of all the five samples.

As a general trend, all selected yeast strains resulted in wines with a higher value for the "fruity" attribute, but two of them had lower "floral" notes compared to the control. The "phenolic" note was in any case lower, with VL1 having the lowest value of this attribute. Other odor attributes were similar in all cases, namely "butter/cheese" and "caramel", while "honey" only showed some minor changes compared to the control, except D47 for which a lower value of this sensory note was obtained.

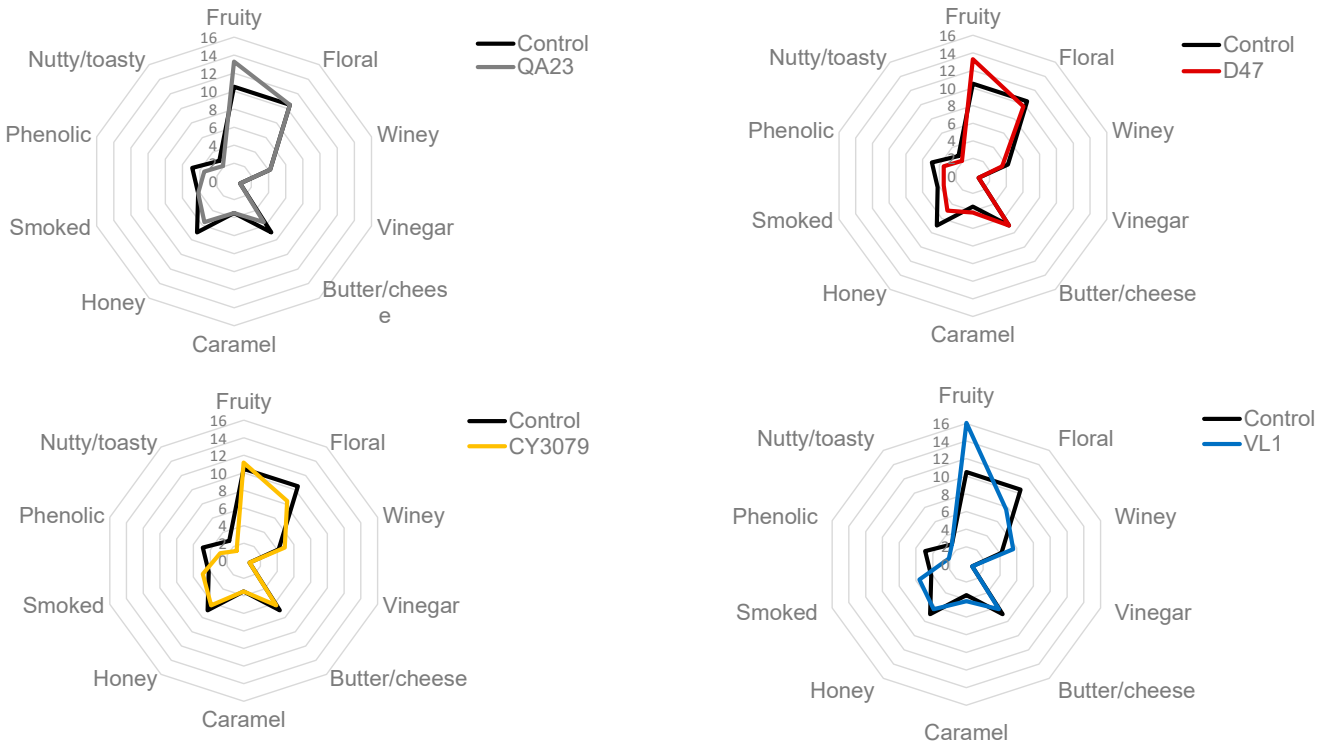

Figure 1. Effect of different yeast strains on the aromatic series of white Fiano wine fermented in barriques. The aromatic series for each of the yeast strain is shown as compared to the control fermentation. The series used in this work groups the odor compounds with similar odor descriptors and reporting as value the sum of the AEDA values, expressed as $\log_{10}$, detected by GC/O analysis (Table 2).

3.3. Statistical Analysis

PLS regression analysis was chosen as an exploratory technique to investigate the correlation of quantitative level of volatile compounds and aromatic series resulting from GC/O analysis from wines fermented using different yeast strains.

Figure 2 displays the result of the PLS by excluding seven volatile compounds that were not statistically affected by the different yeasts used for the fermentation. The PLS loading plot did not show a strong separation or clustering, with some exceptions. Diethyl ester of butanedioic acid and 3-hydroxyethyl butanoate were clustered very closely, and they were fairly well separated from all other volatile compounds. These two volatiles were more associated to "caramel" and "fruity", as shown in the plot. On the other hand, compounds such as hexyl acetate, phenylethyl acetate, acetoin and 5-hydroymethyl furfural were close to each other and correlated to the sensory attribute "phenolic". Regarding the yeast strains used, the control was associated more to this latter group, where attributes such as "butter/cheese" and "floral" are located.

On the other hand, yeast strains D47 and QA23 were clustered closely, and more correlated with compounds such as furfural, acetic acid, 3-hydrody-2-pyranone and others. Wines resulting from fermentation using VL1 or CY3079 were more correlated with "nutty/roasted", "smoked" and "winey" notes. Interestingly, the latter two attributes were clustered very closely. Several acids, alcohols and lactones were associated with these attributes and these yeast strains.

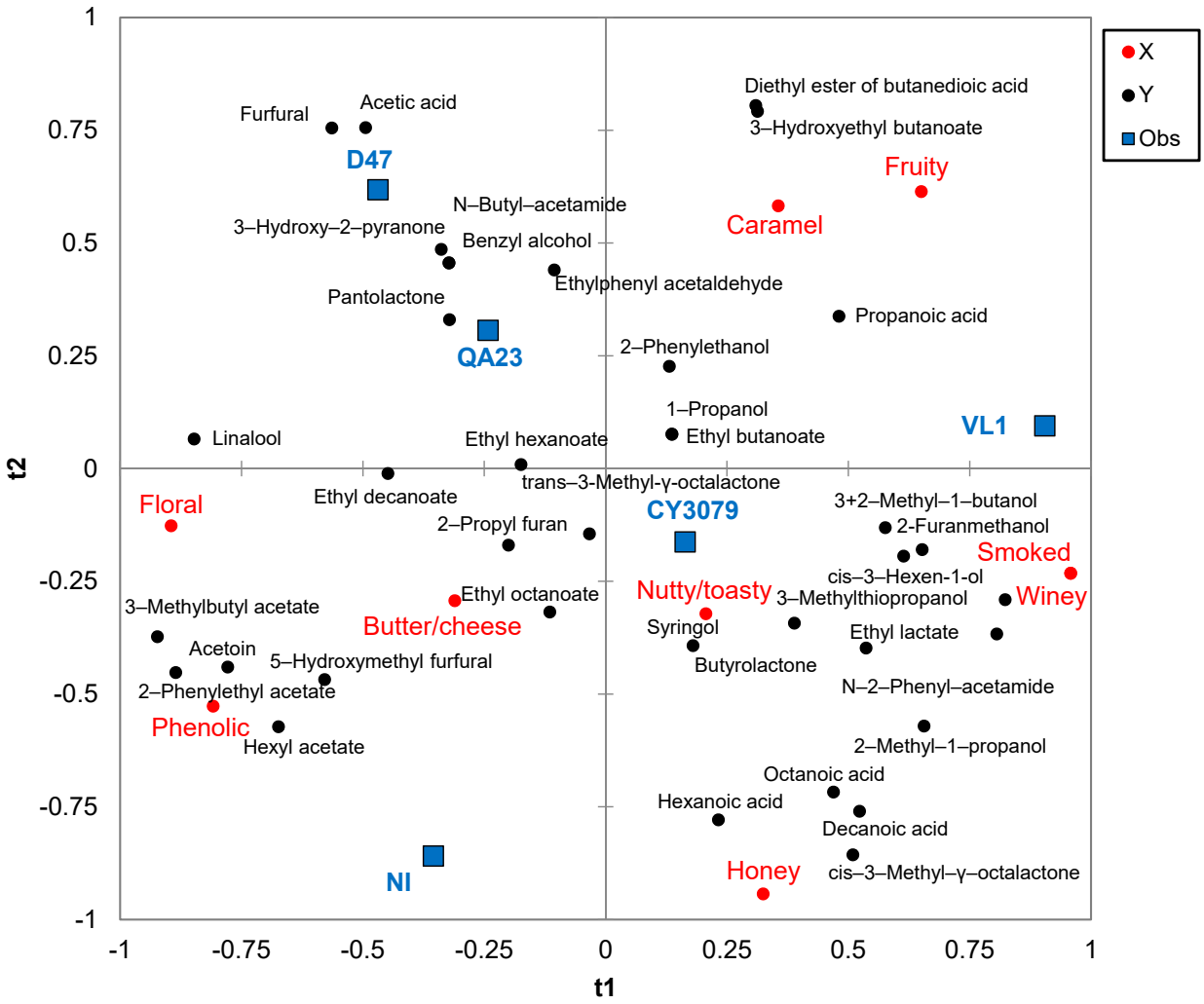

Figure 2. Plot of PLS regression analysis of volatile compounds and aromatic series in Fiano white wine fermented in barriques using four yeast strains. Only those volatile compounds having a statistically significant difference from ANOVA test were used for PLS. The attributes indicated in red are those resulting from the aromatic series obtained from GC/O analysis, while the observations are the different yeast strains used.

4. Conclusions

The current research reported on the impact of using different selected yeast strains for the fermentation of a typical Italian white grape variety from Campania region, named Fiano, which is very appreciated locally, and its market value is increasing over the years. The must was fermented directly in oak barrels, and this technological process was used for all treatments tested. The resulting volatile composition was analyzed by GC/MS, and further analyses were carried out by GC/O to describe the odor impact of the wines. As a means of synthetically describing the whole odor impact of the wine instead of a single volatile compound, the aromatic series approach was used. The results showed that the majority of volatile compounds were strongly and significantly affected by the yeast strain used, and this resulted in an important change in the odor impact of the wines, as shown by the differences in AEDA values for many of the volatile compounds. In addition, all four selected yeast strains had a significant impact on the "fruity" attribute, which was higher compared to the control, and caused some changes of other attributes, particularly "floral", "phenolic" and "honey", showing the potential of using these selected yeast strains and this technological approach of oak fermentation for this typical white wine grape variety.

The results of this research can be useful for winemakers to produce a wider range of sensory characteristics and better differentiate themselves from other competitors on

the market by providing new distinct characteristics of this wine. Our results also prompt further studies to measure (e.g., by consumer testing) the sensorial properties of chemically different wines from the volatile composition point of view here described that can be obtained using different yeast strains.

Author Contributions: Conceptualization, L.M.; methodology, L.M. and A.G.; formal analysis, A.G.; resources, L.M.; writing—original draft preparation, A.G. and N.C.; writing—review and editing, A.G. and N.C.; visualization, A.G. and N.C.; supervision, L.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Additional material is available upon request.

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

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