**Wine Fermentation Performance of Indigenous *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* Strains Isolated in a Piedmont Vineyard**

Antonella Costantini *, Maria Carla Cravero κ, Loretta Panero κ, Federica Bonello κ, Enrico Vaudano, Laura Pulcini and Emilia Garcia-Moruno κ

Citation: Costantini, A.; Cravero, M.C.; Panero, L.; Bonello, F.; Vaudano, E.; Pulcini, L.; Garcia-Moruno, E. Wine Fermentation Performance of Indigenous *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* Strains Isolated in a Piedmont Vineyard. *Beverages* 2021, 7, 30. https://doi.org/10.3390/beverages7020030

1. Introduction

Wine quality depends mainly on the characteristics of the grapes and the diversity of microorganisms (yeasts and bacteria) present during winemaking. During alcoholic fermentation (AF) yeasts of the genus *Saccharomyces* have a predominant role in completing the fermentation of the grape sugars to ethanol. At the end of the fermentation only the species best adapted to the high ethanol content, in many instances *S. cerevisiae* and *S. bayanus*, are found [1].

However, it is known that the grape-wine ecological habitat has a much more complex microbial biodiversity; in fact, wine fermentation is not a ‘single species’ fermentation, but it is the result of a composite community where the indigenous yeasts also play an important role in the final wine complexity [2,3].

In this contest, these yeasts, *Saccharomyces* and non-*Saccharomyces*, are interesting in oenology, because together with the soil or the microclimate they represent the territory of origin of the wine [4]. Actually, their use is growing in response to the ever-increasing need for wine personalization, which is becoming an important aspect of winemaking [5,6]. Nowadays, wineries are dealing with new challenges due to market demands and climate
change and the selection and the use as starter of non-conventional yeasts can be beneficial since they represent an important resource of biodiversity.

*S. cerevisiae* is one of the most extensively studied yeast species because it is the main actor of industrial fermentations, such as wine, beer, and bread production [7]. *S. paradoxus* is phylogenetically closed related to *S. cerevisiae*; it is frequently found in association with oak trees [8–10], but rarely with vineyards. *S. paradoxus*, being the subject of studies on ecology and evolution, was the first *Saccharomyces* yeast to be acknowledged as a non-domesticated species [11,12]. Majdak et al. [13] and Orlic et al. [14] reported the possibility of using *S. paradoxus* strains as starter in fermentation because of their contribution to the aroma of the wines. Alonso del Real et al. [15] tested one *S. paradoxus* strain in a mixed co-culture with one *S. cerevisiae* but in their results they found that during the fermentation *S. cerevisiae* dominated over *S. paradoxus*. Due to these divergent data, further studies must be conducted to increase knowledge about the behavior of *S. paradoxus* in wine.

A very important aspect of quality is determined by the aroma components of the wine: Varietal aromas (originating from the grapes) and fermentative aromas (originating during alcoholic and malolactic fermentations). The contribution of yeast fermentation metabolites to the aromatic profile of wine is well documented [16,17], but yeast can also contribute to wine aroma by other mechanisms, the de novo biosynthesis of volatile compounds and the transformation of neutral grape compounds into flavor-active components [18,19].

Recently, we isolated and characterized the *Saccharomyces* and non-*Saccharomyces* yeasts present in the grapes of a new implantation vineyard of Grignolino in Piedmont (Italy). Grignolino is a red variety cultivated in Piedmont in the northwest of Italy to produce three DOC (Denomination of Controlled Origin) wines. Some of the characterized strains showed good technological basic features such as fermentative vigor and low volatile acidity, as tested in laboratory fermentations [20].

In this work, the specific enological characteristics of two indigenous strains (*S. paradoxus* and *S. cerevisiae*) isolated in this vineyard were determined by comparing their wine fermentation performances in a pilot scale in real cellar conditions. Their dominance was ascertained at the end of alcoholic fermentation (AF) and their impact on the final wines was investigated by quantifying volatile compounds and by performing sensory analysis test.

### 2. Materials and Methods

#### 2.1. Yeast Strains

*Saccharomyces cerevisiae* and *Saccharomyces paradoxus* strains were previously isolated in a Grignolino Vineyard in Nord Italy [20]. They are included in the Microbial Culture Collection of Oenological and Viticultural Environment (CREA-CMVE) of the Center with the references: *S. cerevisiae* (ISE1567) and *S. paradoxus* (ISE1618). A yeast pre-inoculum, previously grown in YEPG broth, was prepared in commercial grape juice (Bravo, Rauch, Austria) and then inoculated (5 x 10⁶ cells/mL) in Grignolino must. Each strain was tested in triplicate.

#### 2.2. Vinification

The trials were run during the 2018 vintage. Homogeneous samples of Grignolino grapes were harvested in crates. Grapes were divided into six homogeneous lots of 130 kg each, containing equally distributed grapes of each row of the vineyard. After destemming and crushing, 80 mg/kg of potassium metabisulfite was added to all six trials.

AF was carried out at a temperature of 24–25 °C. In the first four days of fermentation, a pump down was carried out in the morning and a pumping over of about 20% of the volume in the evening. From the fifth day onwards, two pump-overs in the air for half the volume were done twice a day. Racking off was carried out after 20 days of maceration. At the end of AF, approximately 70 L of wine was obtained in each fermentation. Wine fermented with *S. paradoxus* is termed SpW, and the one with *S. cerevisiae* ScW.
2.3. Yeast Dominance Analysis

At the end of fermentation, yeasts were isolated by dilution and spreading on WL (Wallerstein Laboratory) agar. After growth, 24 colonies from each sample were randomly collected from plates. Dominance analysis was performed by microsatellite multiplex PCR (MM-PCR) to distinguish *S. cerevisiae* strains [21].

MM-PCR data were managed using Bionumerics software (Applied Maths, Belgium). The band pattern profile obtained on the colonies isolated at the end of fermentation was compared with the profile of the inoculated strain.

Since microsatellite loci amplification is not possible in *S. paradoxus*, the species was assessed by amplifying the D1–D2 domain with primers NL1–NL4 [22] and sequencing. For this, the NS1/ITS2 primer pair was used to amplify the ITS1 region of the 18S rDNA; PCR products were digested with MspI, and separated by electrophoresis [8]. Gels were processed using Bionumerics software as above.

2.4. Chemical Analysis

Density, volatile acidity, titratable acidity, and ethanol content were analysed according to the methods of the OIV (International Organisation of Vine and Wine). Residual sugar and glycerol were quantified using an HPLC with a refractometric detector using the following conditions: Rezex RCM-Monosaccharide column (300 × 7.8 mm, 8 μm, Phenomenex, Torrance, CA, USA), water as eluent with a flow of 0.35 mL/min, column temperature 85 °C, and injection volume 20 μL. Organic acids were quantified by HPLC Agilent 1100 as described [23], and YAN (Yeast Assimilable Nitrogen) was determined by formol titration [24].

2.5. Sensory Analysis

The wine sensory descriptive analysis (sensory profile) was conducted by a trained panel (6 males and 7 females) following a methodology deriving from the ISO norms [25]—similarly to other procedures [26,27]—using ISO (3591-1977) approved glasses in an ISO (8589-2007) tasting room.

In all the sensory sessions, 4 wines were served (50 mL) in a randomized order and identified with a three-digit code. All the wines were tasted in a preliminary tasting session to define the odor descriptors with the help of a predefined odor list [28]. The choice of descriptors was made on the identification frequencies. The second-level descriptors (fresh herbaceous, dry herbaceous, and balsamic/resinous) were chosen when their frequency of identification was higher than 39 (13 assessors × 6 wines/2), and the third level descriptors (e.g., rose, geranium flower, pepper, cloves, raspberry, cherry and jam/marmalade) when their frequency was higher than 19.5 (13 assessors × 6 wines/4). The taste and mouth-feel attributes evaluated were acidity, bitterness, astringency, body (structure) and taste-olfactory persistence. The chosen attributes were confirmed by presenting to the panel appropriate standards, and measured twice in the wines in two different tasting sessions. Qualitative and quantitative sensory analyses were performed by FIZZ (Biosystems, Couternon, France). The intensity of the wine sensory attributes measures was acquired in two repetitions using a non-structured scale (0–100).

The sensory profile of each wine obtained from the average of the two-tasting session of each of the 3 wines produced with the same yeast is represented with radar diagrams. The quantitative sensory results (sensory profiles) were processed with ANOVA and Tukey’s test (p = 95%).

2.6. Free Volatile Compounds Analysis

The volatile compounds were extracted by solid-phase extraction (SPE) as follows: 30 mL wine was diluted threefold with water and 300 μL and added with 1-heptanol (51.43 mg/L) as internal standard; samples were loaded onto a 1 g C18-EC cartridge (Biotage AB, Uppsala, Sweden), extracted with 5 mL dichloromethane and concentrated to 100 μL under a weak nitrogen flow. After the addition of 1-pentanol and stirring, the
aqueous phase was extracted with dichloromethane and concentrated to 100 μL as before. The GC-MS analysis was performed with an Agilent 7890 Series gas chromatograph with an Agilent 5975 N Mass Selective Detector. The chromatographic conditions were: Helium carrier gas with a flow of 1 mL/min; the sample (1 μL) was injected in splitless mode on a Zebron ZB-WAX column (60 m × 0.25 mm, 0.25 μm, Phenomenex, Torrance, CA, USA); the source and the transfer line were kept at 230 °C and the injector at 250 °C [29]. Data were acquired in TIC mode (Total Ion Current) and processed with the ChemStation software (Agilent Technologies, Santa Clara, CA, USA). The identifications were achieved by comparing the retention times with those of pure reference compounds (when available), or comparing the LRI (linear retention index) to those reported in the literature. All high purity standards were purchased from Sigma–Aldrich (Milan, Italy).

2.7. Statistical Analysis

ANOVA, Tukey’s test \((p = 95\%)\) and PCA were performed using XLSTAT (Addinsoft, France).

3. Results and Discussion

3.1. Enological Characteristics of S. paradoxus and S. cerevisiae Strains

To overcome any inhomogeneity during ripening of the grapes that could result in differences in acidity, alcohol, color or polyphenol content of wines, the initial grape mass was collected and randomized distributed in boxes. The musts had the following mean chemical parameters: Brix 23.44°; titrable acidity (expressed as tartaric acid) 6.4 g/L; pH 3.49; YAN 287.7 mg/L.

Microbiological and biomolecular analyses were performed to evaluate the dominance of the yeasts at the end of fermentation. In S. cerevisiae wines, microsatellite analysis showed that the inoculated S. cerevisiae strain dominated the fermentations (Figure 1).

Figure 1. Electrophoretic profile obtained by MM-PCR of 16 colonies of S. cerevisiae strains showing the same profile of the inoculated strain (Lane 17). Lanes M: Molecular marker 100 bp.

As expected, no amplification was achieved in S. paradoxus wine isolated colonies by MM-PCR because this method is S. cerevisiae species-specific; thus, ARDRA analysis was conducted, confirming that they belonged to the S. paradoxus species (Figure 2).
The progress of alcoholic fermentation (13 days) in the different trials was similar. At the end of the AF, significant differences ($p \leq 0.05$) in glycerol (6.70 vs 8.62 g/L) and malic acid (3.27 vs 2.50 g/L) content between \textit{S. cerevisiae} and \textit{S. paradoxus} were recorded (Table 1).

**Table 1.** Chemical parameters of the wines at the end of the alcoholic fermentation.

| Parameter                | SpW     | ScW     |
|--------------------------|---------|---------|
| Density 20/20            | 0.99456 | 0.99368 |
| Ethanol % v/v            | 12.73   | 13.29   |
| Residual sugars g/L      | $\leq 1$| $\leq 1$|
| Titratable acidity g/L   | 6.32    | 6.35    |
| Volatile acidity g/L     | 0.19    | 0.25    |
| Malic acid g/L           | $2.50^a$| $3.27^b$|
| pH                       | 3.54    | 3.52    |
| Glycerol g/L             | $8.62^a$| $6.70^b$|

Results are the average of three independent fermentations; titratable acidity was expressed as tartaric acid. Volatile acidity was expressed as acetic acid. SpW: \textit{S. paradoxus} wines; ScW: \textit{S. cerevisiae} wines. Different letters “a” and “b” mean statistical differences at ANOVA and Tukey’s test ($p = 95\%$).

Remarkably, the \textit{S. paradoxus} strain production of glycerol, a very important compound for wine quality that provides sweetness and fullness [30], was high, thereby confirming previous data indicating that \textit{S. paradoxus} produces a higher amount of glycerol than \textit{S. cerevisiae} [31]. The observed ability of \textit{S. paradoxus} to degrade malic acid is an interesting property in fermentation of musts with a high acidity; several studies demonstrated that fermentations with this species lead to a degradation of malic acid [10,32,33]; therefore, our results are in agreement with these previous works. In particular, Bovo et al. [32] affirmed that \textit{S. paradoxus} strains were able to degrade high amounts of malic acid in ripe grape must, i.e., high glucose and low malic acid concentration.

No significant differences ($p \leq 0.05$) were found for the other parameters analyzed, even though the quantity of ethanol produced was slightly lower in the fermentations of \textit{S. paradoxus} ($\bar{x} = 12.73$ g/L) than in those of \textit{S. cerevisiae} ($\bar{x} = 13.29$ g/L). This result agrees with Orlic et al. [31] who reported that \textit{S. paradoxus} always produced lower ethanol concentrations than \textit{S. cerevisiae}.

It is important to note that this lower ethanol production was not accompanied by a higher volatile acidity. At the same time, as mentioned before, glycerol content shows significantly higher values in \textit{S. paradoxus} than in \textit{S. cerevisiae} fermentations. These data lead to hypothesize that in the \textit{S. paradoxus} strain a carbon flow balance shifted towards glycerol
to a greater extent than *S. cerevisiae*, thus showing interesting technological prospects for natural reduction of alcohol content in wines [32,33].

3.2. Free Volatile Compounds and Sensory Profile of the Wines

Grignolino variety is cultivated in Piedmont, northwest Italy, to produce DOC wines that are generally described as dry and slightly tannic, with a moderately bitter taste and a persistent aftertaste. In a previous study [34] carried out on a suitable number (*n* = 36) of commercial Grignolino wines, the olfactory descriptors were violet-rose, geranium, pepper, raspberry, straw-hay. These attributes were also present in this study, even though the odor complexity was higher, which could be due to various reasons (grape quality, evolution of grape growing and winemaking techniques, different yeasts).

Regarding the organoleptic characteristics of the under-study wines, the sensory profiles obtained with the two yeasts (Figure 3) were different only for the odor attribute rose, which was significantly higher in the ScW wines (ANOVA and Tukey’s test, *p* = 95%). Raspberry odor, cherry and dry herbaceous were also slightly higher in these wines, but all wines were very similar for taste and mouth-feel attributes.

![Figure 3](image)

*Figure 3*. Sensory profile of Grignolino wines obtained by the two autochthonous strains. Different letters indicate significant statistical differences with ANOVA and Tukey’s test (*p* = 95%). (ScWs: Average profile of wines produced with *S. cerevisiae*; SpWs: Average profile of wines produced with *S. paradoxus*).

Analysis of the aromatic compounds of wines at the end of the alcoholic fermentation (Table 2) showed no statistical differences in alcohols and fermentation esters between the two yeasts, but statistical differences in the concentration of terpene compounds were recorded, which can explain the sensory difference for the descriptor “rose”. Terpenes are characterized by floral, muscatel or fruity aromas, and their concentrations in grapes and wines depend on various factors, including cultivar, region, wine-making techniques and yeasts.

|  |  |  |
|---|---|---|
| **Alcohols** |  |  |
| Isoamylalcohol | 12,261 ± 1447 | 13,140 ± 2888 |
| Cis-3-hexenol | 73 ± 6 | 57 ± 21 |
| 1-hexanol | 1359 ± 66 | 1402 ± 298 |

Table 2. Volatile compounds in the wines at the end of the alcoholic fermentation (*μ*g/L).
Table 2. Volatile compounds in the wines at the end of the alcoholic fermentation (µg/L).

|                  | SpW         | ScW         |
|------------------|-------------|-------------|
| **Alcohols**     |             |             |
| Isoamylalcohol   | 12,261 ± 1447 | 13,140 ± 2888 |
| Cis-3-hexenol    | 73 ± 6      | 57 ± 21     |
| 1-hexanol        | 1359 ± 66   | 1402 ± 298  |
| Benzylalcohol    | 7 ± 2       | 7 ± 1       |
| 2-phenylethanol  | 14,337 ± 1000 | 17,465 ± 5000 |
| **Esters**       |             |             |
| Ethylhexanoate   | 279 ± 60    | 185 ± 31    |
| Isoamylacetate   | 406 ± 151   | 418 ± 27    |
| Ethyllactate     | 1284 ± 118  | 585 ± 42    |
| Ethyloctanoate   | 267 ± 62    | 177 ± 13    |
| Ethylhexanoate   | 271 ± 46    | 185 ± 31    |
| Ethyldecanoate   | 55 ± 20     | 37 ± 4      |
| Diethylsuccinate | 1187 ± 360  | 1351 ± 293  |
| Ethylpalmitate   | 12 ± 1      | 16 ± 4      |
| **Acids**        |             |             |
| Isovaleric acid  | 150 ± 11    | 99 ± 24     |
| Octanoic acid    | 1869 ± 242  | 1559 ± 342  |
| Decanoic acid    | 444 ± 172   | 377 ± 57    |
| Lauric acid      | 37 ± 13     | 29 ± 4      |
| **Terpenic compounds** |         |             |
| Linalool         | 0 a         | 16 ± 2 b    |
| Citronellol      | 14 ± 0      | 24 ± 2      |
| Citronellol oxide| 4 ± 1       | 5 ± 1       |
| HO trienol       | 0           | 7 ± 5       |
| Alpha terpineol  | 10 ± 1      | 11 ± 2      |
| Geranic acid     | 40 ± 13     | 43 ± 4      |
| **Aldehydes ketones** |       |             |
| Benzaldehyde     | 6 ± 2       | 8 ± 2       |
| Butyrolactone    | 36 ± 20     | 40 ± 27     |
| Methoxyacetophenone | 0     | 248 ± 95    |
| Vanillin         | 0           | 14 ± 1      |
| β-damascenone    | 11 ± 5      | 8 ± 1       |
| **Total terpenes** | 53 a       | 318 b       |
| **Total acids**  | 2500        | 2064        |
| **Total esters** | 3761        | 2954        |

Different letters “a” and “b” indicate significant statistical differences with ANOVA and Tukey’s test (p = 95%).

In this study, particularly significant was the difference between the linalool content in both strains, as linalool (16 µg/L) was only present in the S. cerevisiae fermentation (Table 2). The ability of S. cerevisiae yeast to synthesize terpenes has already been reported [35,36]. The threshold level for linalool in wine is 50 µg/L, but it can be detected in lower concentrations (10–20 µg/L) when similar aroma-based chemicals are also present [37]. The individual terpenes quantified in this study are not present at levels close to their sensory limits, but they should nevertheless contribute collectively to the floral aspect of the wine aroma descriptor. Rose aroma contribution should collectively include linalool, citronellol and HO-trienol, which are aromatic compounds characterized by floral notes, and the sum of these terpenes supports the difference perceived by the panel in the sensory analysis.

Significant differences were also found in aldehyde and ketone compounds, the total amount of these compounds was higher in ScW than in SpW, in particular for vanillin and for methoxyacetophenone. These compounds increase wine complexity, conferring vanilla, nutty and floral notes.
In general, wines obtained in this study have good enological properties and distinctive characteristics; this is also shown by PCA analysis (Figure 4): Wines obtained by \textit{S. cerevisiae} have higher malic acid, total aldehydes and ketones and ethanol; \textit{S. paradoxus} wines are mainly characterized by a higher content of glycerol.

![Figure 4. Biplot obtained by PCA analysis on data of the three repetitions.](image)

**4. Conclusions**

In conclusion, both indigenous \textit{S. cerevisiae} and \textit{S. paradoxus} strains isolated from vineyard possess oenological properties of interest for the wine industry. \textit{S. paradoxus} is characterized by a high production of glycerol and the ability to degrade malic acid. This, together with a lower production of ethanol and a low volatile acidity, makes this \textit{S. paradoxus} strain a very interesting starter from an oenological point of view, in particular for the production of low alcohol content wines. On the other hand, the strain of \textit{S. cerevisiae} gives the wine a pleasant smell of rose, as highlighted in the sessions of sensory analysis. Grignolino is a neutral variety with a very low content of terpene compounds, and thus, in these kinds of varieties, the role of yeast in the production of aromatic compounds is even more important.

Further studies should be conducted to better investigate the use of \textit{S. paradoxus}, in particular its use in mixed cultures with \textit{S. cerevisiae} or other yeast species, or in sequential inoculation, in order to obtain specific results in terms of ethanol content and glycerol production.

**Author Contributions:** Conceptualization, E.G.-M.; methodology A.C., L.P. (Loretta Panero), M.C.C. and F.B.; writing E.G.-M., A.C., L.P. (Loretta Panero), M.C.C. and F.B.; review and editing, E.G.-M., A.C., L.P. (Loretta Panero), M.C.C., F.B., E.V. and L.P. (Laura Pulcini); supervision, E.G.-M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** All of the data is contained within the article.
Acknowledgments: We thank Valter Pierini of the Portacomaro (Asti, Italy) municipality, Riccardo Durando and Carlo Cerrato responsible for the project “Vigna del Papa” for the permission to sample their vineyard.

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

References

1. Pretorius, I.S. Tailoring wine yeast for the new millennium: Novel approaches to the ancient art of winemaking. *Yeast* **2000**, *16*, 675–729. [CrossRef]
2. Jolly, N.P.; Varela, C.; Pretorius, I.S. Not your ordinary yeast: Non-Saccharomyces yeasts in wine production uncovered. *FEMS Yeast Res.* **2014**, *14*, 215–237. [CrossRef]
3. Liu, Y.; Rousseaux, S.; Tourdot-Maréchal, R.; Sadoudi, M.; Gougeon, R.; Schmitt-Kopplin, P.; Alexandre, H. Wine microbiome: A dynamic world of microbial interactions. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 856–873. [CrossRef] [PubMed]
4. Tristezza, M.; Fantastico, L.; Vetrano, C.; Corallo, D.; Grieco, F.; Mita, G. Molecular and Technological Characterization of Saccharomyces cerevisiae Strains Isolated from Natural Fermentation of Susumaniello Grape Must in Apulia, Southern Italy. *Int. J. Microbiol.* **2014**, *2014*, 1–11. [CrossRef] [PubMed]
5. Ilieva, F.; Velčikovska, S.K.; Dimovska, V.; Mirhosseini, H.; Spasov, H. Selection of 80 newly isolated autochthonous yeast strains from the Tîkveš region of Macedonia and their impact on the quality of red wines produced from Vanec and Cabernet Sauvignon grape varieties. *Food Chem.* **2017**, *216*, 309–315. [CrossRef] [PubMed]
6. Vigentini, L.; Cardenas, S.B.; Valdetara, F.; Piccioni, C.; Foschino, R. Use of Native Yeast Strains for In-Bottle Fermentation to Face the Uniformity in Sparkling Wine Production. *Front. Microbiol.* **2017**, *8*, 1225. [CrossRef] [PubMed]
7. Nielsen, J. Yeast systems biology: Model organism and cell factory. *Biotechnol. J.* **2019**, *14*, e1800421. [CrossRef] [PubMed]
8. Redžepović, S.; Orlić, S.; Sikora, S.; Majdak, A.; Pretorius, I.S. Identification and characterization of Saccharomyces cerevisiae and Saccharomyces paradoxus paradoxus strains isolated from Croatian vineyards. *Lett. Appl. Microbiol.* **2002**, *33*, 305–310. [CrossRef] [PubMed]
9. Sniegowski, P.D.; Dombrowski, F.G.; Fingerman, E. Saccharomyces cerevisiae and Saccharomyces paradoxus coexist in a natural woodland site in North America and display different levels of reproductive isolation from European conspecifics. *FEMS Yeast Res.* **2002**, *2*, 299–306. [CrossRef] [PubMed]
10. Yurkov, A.M. First Isolation of the Yeast Saccharomyces paradoxus in Western Siberia. *Microbiology* **2005**, *74*, 459–462. [CrossRef]
11. Boynton, P.; Greig, D. The ecology and evolution of non-domesticated Saccharomyces species. *Yeast* **2014**, *31*, 449–462. [CrossRef]
12. Bleuven, C.; Dubé, A.K.; Nguyen, G.Q.; Gagnon-Arsenault, I.; Martin, H.; Landry, C.R. A collection of barcoded natural isolates of Saccharomyces paradoxus to study microbial evolutionary ecology. *Microbiologiyopen* **2019**, *8*, e773. [CrossRef] [PubMed]
13. Majdak, A.; Herjavec, S.; Orlić, S.; Redžepović, S.; Mirošević, N. Comparison of Wine Aroma Compounds Produced by Saccharomyces paradoxus and Saccharomyces cerevisiae Strains. *Food Technol. Biotechnol.* **2002**, *40*, 103–109. [CrossRef] [PubMed]
14. Orlić, S.; Redžepović, S.; Jeromel, A.; Herjavec, S.; Iacumin, L. Influence of indigenous Saccharomyces paradoxus strains on Chardonnay wine fermentation aroma. *Int. J. Food Sci. Technol.* **2007**, *42*, 95–101. [CrossRef]
15. Alonso-Del-Real, J.; Lairón-Peris, M.; Barrio, E.; Querol, A. Effect of Temperature on the Prevalence of Saccharomyces Non cerevisiae Species against a S. cerevisiae Wine Strain in Wine Fermentation: Competition, Physiological Fitness, and Influence in Final Wine Composition. *Front. Microbiol.* **2017**, *8*, 150. [CrossRef]
16. González-Barreiro, C.; Rial-Otero, R.; Cancho-Grande, B.; Simal-Gándara, J. Wine Aroma Compounds in Grapes: A Critical Review. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 202–218.
17. García, M.; Esteve-Zarzoso, B.; Crespo, J.; Cabellos, J.M.; Arroyo, T. Influence of Native Saccharomyces cerevisiae Strains from D.O. “Vinos de Madrid” in the Volatile Profile of White Wines. *Fermentation* **2019**, *5*, 94. [CrossRef]
18. Fleet, G.H. Yeast interactions and wine flavour. *Int. J. Food Microbiol.* **2003**, *86*, 11–22. [CrossRef]
19. Styger, G.; Prior, B.; Bauer, F.F. Wine flavor and aroma. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 1145–1159. [CrossRef] [PubMed]
20. Vaudano, E.; Quintero, G.; Costantini, A.; Pulcini, L.; Pessione, E.; Garcia-Moruno, E. Yeast distribution in Grignolino grapes growing in a new vineyard in Piedmont and the technological characterization of indigenous Saccharomyces spp. strains. *Int. J. Food Microbiol.* **2019**, *289*, 154–161. [CrossRef] [PubMed]
21. Vaudano, E.; Garcia-Moruno, E. Discrimination of Saccharomyces cerevisiae wine strains using microsatellite multiplex PCR and band pattern analysis. *Food Microbiol.* **2008**, *25*, 56–64. [CrossRef] [PubMed]
22. Kurtzman, C.P.; Robnett, C.J. Identification and phylogeny of ascomycete yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* **1998**, *73*, 331–371. [CrossRef]
23. Cane, P. Il controllo di qualità dei vini mediante HPLC: Determinazione di acidi organici. *L’Enotecnico* **1990**, *26*, 69–72.
24. Zeecklein, B.; Fugelsang, K.C.; Gump, B.; Nury, F.S. *Wine Analysis and Production*; Springer: New York, NY, USA, 1999.
25. Cravero, M.C.; Bonello, F.; Tsolakis, C.; Piano, F.; Borsa, D. Comparison between Nero d’Avola wines produced with grapes grown in Sicily and Tuscany. *Ital. J. Food Sci.* **2012**, *24*, 384–387.
26. Campo, E.; Do, B.V.; Ferreira, V.; Valentin, D. Aroma properties of young Spanish monovarietal white wines: A study using sorting task, list of terms and frequency of citation. *Aust. J. Grape Wine Res.* **2008**, *14*, 104–115. [CrossRef] [PubMed]
27. Wirth, J.; Morel-Salmi, C.; Souquet, J.M.; Dieval, J.B.; Aagaard, O.; Vidal, S.; Fulcrand, H.; Cheynier, V. The impact of oxygen exposure before and after bottling on the polyphenolic composition of red wines. *Food Chem.* **2010**, *123*, 107–116. [CrossRef]
28. Guinard, J.X.; Noble, A.C. Proposition d’une terminologie pour une description analytique de l’arome des vins. *Sc. Alim.* 1986, 6, 657–662.
29. Petrozziello, M.; Guaita, M.; Motta, S.; Panero, L.; Bosso, A. Analytical and Sensory Characterization of the Aroma of “Langhe D.O.C. Nebbiolo” Wines: Influence of the Prefermentative Cold Maceration with Dry Ice. *J. Food Sci.* 2011, 76, C525–C534. [CrossRef] [PubMed]
30. Scanes, K.T.; Hohrmann, S.; Prior, B.A. Glycerol Production by the Yeast Saccharomyces cerevisiae and its Relevance to Wine: A Review. *S. Afr. J. Enol. Vitic.* 2017, 19, 17–24. [CrossRef]
31. Orlic, S.; Arroyo-Lopez, F.N.; Huic-Babic, K.; Lucilla, I.; Querol, A.; Barrio, E. A comparative study of the wine fermentation performance of Saccharomyces paradoxus under different nitrogen concentrations and glucose/fructose ratios. *J. Appl. Microbiol.* 2010, 108, 73–80. [CrossRef] [PubMed]
32. Ozturk, B.; Anli, E. Different techniques for reducing alcohol levels in wine: A review. In Proceedings of the BIO Web of Conferences, Mendoza, Argentina, 9–14 November 2014; Aurand, J.-M., Ed.; EDP Sciences: Les Ulis Cedex, France, 2014; Volume 3, p. 02012. [CrossRef]
33. Caballero, A.; Segura, A. The quest for lower alcoholic wines. *Microb. Biotechnol.* 2017, 10, 238–241. [CrossRef] [PubMed]
34. Cravero, M.C.; Ubigli, M. L’analisi sensoriale e la tipicità del Grignolino. *OICCE TIMES* 2003, 4, 13–18.
35. 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] [PubMed]
36. Hernandez-Orte, P.; Cersosimo, M.; Loscos, N.; Cacho, J.; Garcia-Moruno, E.; Ferreira, V. Aroma development from non-floral grape precursors by wine lactic acid bacteria. *Food Res. Int.* 2009, 42, 773–781. [CrossRef]
37. Ferreira, V. A base química do aroma do vinho: Moléculas e sensações olfactogustativas. Parte 1: Álcool e efeito do tampão aromático. *Rev. Internet Vitiv. Enol.* 2009, 9, 1–7.