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A Novel Approach for Enhancing the Terpenoid Content in Wine Using *Starmerella bacillaris*

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Abstract

In this study, we investigated the impact of two native strains of *Starmerella bacillaris*, used both in pure culture and in a co-inoculation with *Saccharomyces cerevisiae*, on the volatile profile of a chemically defined fermented model must. The focus of this study was the production of monoterpenes and sesquiterpenes and their potential sensory contributions. Geraniol and linalool were detected in all fermentations with *Starmerella bacillaris*, in ranges of 26.7–43.9 µg/L and 34.3–41.3 µg/L, respectively, independent of the inoculation strategy used. Both strains produced concentrations above their respective odour thresholds of 20 µg/L and 25.5 µg/L. Odour activity value (OAV) analysis confirmed that fermentations with *Starmerella bacillaris*, particularly under co-inoculation conditions, generated the highest OAVs for these monoterpenes. Citronellol was only detected in mixed fermentations, while nerolidol and farnesol isomers were produced in variable amounts, depending on the strain and inoculation strategy, at concentrations below the odour threshold. These findings demonstrate the ability of *Starmerella bacillaris* to facilitate de novo biosynthesis of linalool, geraniol, and sesquiterpenes during alcoholic fermentation—in the case of linalool and geraniol, at concentrations exceeding their respective odour thresholds—highlighting the biotechnological potential of these native strains to enhance aroma in wines, particularly those made from neutral grape varieties.

Keywords: non-*Saccharomyces*; Candida; alcoholic fermentation; aromatic compounds; monoterpenoids; sesquiterpenoids; secondary metabolites

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1. Introduction

The terpene family comprises a structurally diverse group of natural compounds, including linear hydrocarbons and carbocyclic skeletons. More than 80,000 compounds have been identified in this category, primarily in plants; however, they have also been isolated from other sources [1,2].

Some of them, such as monoterpenes and sesquiterpenes, play a role in chemical communication between plants and other organisms, acting as attractants, repellents, or signalling compounds [3,4].

In grapes, monoterpenes, sesquiterpenes, and C_{13}-norisoprenoids exist either as free volatile compounds or bound to sugars in a non-volatile form. Although they do not contribute to aroma directly, they have been recognised as essential aroma precursors,

especially for aromatic cultivars [5,6], and can constitute a high proportion of the total monoterpenes, several of which contribute to floral or citrus sensory characteristics [7,8].

The de novo biosynthesis of monoterpenes and sesquiterpenes by wine yeasts has been reported for both *Saccharomyces cerevisiae* (*S. cerevisiae*) [9] and non-*Saccharomyces* (NS) species [10–13]. Among NS yeasts, several species, such as *Metschnikowia pulcherrima*, *Torulaspora delbrueckii*, *Pichia anomala*, and *Hanseniaspora* spp. (*H. uvarum*, *H. guilliermondii*, *H. osmophila*, and *H. vineae*), have demonstrated the ability to release monoterpenes (linalool, geraniol, and nerol) from terpenoid glycosides through β-glucosidase activity during fermentation [14–16]. In this regard, the use of *Starmerella bacillaris* (*S. bacillaris*) in wine fermentation has also been associated with increased terpene concentrations, an effect that has likewise been mainly attributed to its β-glucosidase activity [17–19].

S. bacillaris was first isolated and identified in 2002 by Mills and colleagues from sweet wine fermentations of Botrytis-affected grapes collected in Napa Valley [20]. Since this initial report, in which it was classified under the genus Candida, the species has undergone several taxonomic revisions until consensus was reached about its current nomenclature [21].

In recent years, *S. bacillaris*, a yeast naturally present in vineyards worldwide, has attracted increasing interest due to its potential as a tool for producing wines with enhanced aromatic complexity [22,23] and reduced alcohol content [24].

The main features that distinguish this yeast and support its oenological relevance include its ability to produce high levels of glycerol, tolerance to high osmotic pressure and low temperatures [25], greater resistance to ethanol compared with other NS species [26], persistence until the later stages of fermentation when co-inoculated with *S. cerevisiae*, and lower ethanol yield compared with *S. cerevisiae* [27].

Additionally, *S. bacillaris* exhibits a marked fructophilic behaviour, preferring fructose over glucose as a carbon source. This trait positions it as a valuable complement to *S. cerevisiae*, as it reduces osmotic stress during fermentation and does not compete for the latter's preferred carbon source [28,29].

Despite increasing interest in *S. bacillaris*, to the best of our knowledge, its ability to synthesise monoterpenes and sesquiterpenes de novo in the absence of a glycosidic precursor under winemaking conditions, as well as its potential impact on the sensory characteristics of wine, has not been studied. In this context, the aim of this study was to analyse this capacity in two *S. bacillaris* strains and their possible contribution to the aromatic profile.

2. Materials and Methods

2.1. Yeasts

Two strains of the *S. bacillaris* species (Sb1 and Sb2) were used. These strains were isolated from Cabernet Franc and Merlot grapes and belonged to the collection of native yeasts of the Enology and Fermentation Biotechnology Area of the Faculty of Chemistry. These yeasts were selected for their oenological characteristics. The commercial *S. cerevisiae* yeast Lalvin BM 4 × 4^{TM} (Lallemand) (Sc) was used as a control in all treatments.

2.2. Microfermentation

Each fermentation was carried out in triplicate in 250 mL flasks containing 125 mL of the chemically defined model must (CDMM), semi-anaerobically (the flasks were stopped with cotton plugs), and at a temperature of 20 °C, according to the methods used in [30], with some modifications. The final composition of the medium was as follows: nitrogen components: diammonium phosphate (DAP), 90.4 mg/L (Merck, Darmstadt, Germany); Arg, 135.59 mg/L (J.T. Baker, Phillipsburg, NJ, USA); Glu, 90.4 mg/L; Pro, 90.4 mg/L;

Asp, 63.28 mg/L; Thr, 68.28 mg/L; Leu, 54.24 mg/L; Val, 36.16 mg/L; His, 27.12 mg/L; Met, 27.12 mg/L; Phe, 27.12 mg/L; Ala, 18.08 mg/L; Trp, 18.08 mg/L; Gly, 9.04 mg/L; Gln, 36.16 mg/L (Sigma-Aldrich St. Louis, Mo, USA); Lys, 45.2 mg/L; Ser, 72.32 mg/L; Ile, 36.16 mg/L (Applichem, Darmstadt, Germany); Asn, 27.12 mg/L; and Tyr, 3.62 mg/L (Merck, Germany). With this formulation, the concentration of nitrogen was 250 mgN/L, where 200 mgN/L was from amino acids, and the remaining 50 mgN/L was supplied by DAP. Equimolar concentrations of glucose (Carlo Erba Reagents S.A., Val-de-Reuil, France) and fructose (Sigma-Aldrich, St. Louis, MO, USA) were added to reach 200 g/L, and the mixed vitamins and salts used were added in the following concentrations: KH_2PO_4, 1.14 g/L; $MgSO_4.7H_2O$, 1.23 g/L; $CaCl_2.2H_2O$, 0.44 g/L; Myo-inositol, 100 mg/L; pyridoxine.HCl, 2 mg/L; nicotinic acid, 2 mg/L (Sigma-Aldrich, St. Louis, MO, USA); D-pantotenic acid calcium salt, 1 mg/L (Applichem, Darmstadt, Germany); thiamine.HCl, 0.5 mg/L; PABA-K (p-aminobenzoic acid), 0.2 mg/L; riboflavin, 0.2 mg/L; biotin, 0.125 mg/L (Sigma-Aldrich, St. Louis, MO, USA); and folic acid, 0.2 mg/L (Merck, Germany). The acids used were potassium hydrogen tartrate, 2.5 g/L; L-malic acid, 3 g/L; and citric acid, 0.2 g/L. Microelements were added in the following concentrations: $MnCl_2.4H_20$, 200 µg/L; $ZnCl_2$, 135/L; $FeCl_2$, 30 µg/L; $CO(NO_3)_2.6H_2O$, 30 µg/L; KIO_3, 10 µg/L; $Na_2MO_4.2H_2O$, 25 µg/L; $CuCl_2$, 15 µg/L (Sigma-Aldrich, St. Louis, MO, USA); and H_3BO_3, 5 µg/L (Carlo Erba Reagents S.A., Val-de-Reuil, Francia). The pH of the medium was adjusted to 3.5 with NaOH-HCl, and a lipid supplement of 10 mg/L ergosterol was added.

Three treatments were established using pure cultures of each of the *S. bacillaris* strains and the control strain; additionally, mixed fermentations were carried out in a sequential culture and a culture co-inoculated with the control *S. cerevisiae* (Table 1).

Table 1. Fermentation treatments.

Treatments	Type of Inoculum	*S. bacillaris* Inoculum Size (Cells/mL) *	*S. cerevisiae* Inoculum Size (Cells/mL) *
A	Pure strains (Sb1, Sb2, and Sc)	1×10^6	1×10^6
B	Sequential (48 h)	1×10^6	1×10^6
C	Co-inoculated	1×10^6	1×10^5

* The inoculum size was adjusted according to a previous work [31].

2.3. Microvinifications in CDMM Medium with Glycosidic Aroma Precursors (GAPs)

An extract of glycosidic precursors from a young Tannat wine was added to the CDMM. The precursors were obtained by solid-phase extraction using a cartridge filled with 1 g of styrene–divinylbenzene (SDVB) polymer (Biotage, Uppsala, Sweden) [32]. The glycoside precursors eluted from the cartridge with methanol (after elution of the free compounds) were dried using a rotary evaporator and then redissolved in CDMM. The pH was adjusted to 3.5 before adjusting the final volume to 1000 mL.

2.3.1. Microvinifications with Glycosidic Aroma Precursors

Pure culture microvinifications were carried out with the *S. bacillaris* strains and the control using the CDMM medium with glycosidic aroma precursors. Three flasks without yeast inoculation were also included, to which 120 mg/L potassium metabisulfite was added. These flasks were maintained until the end of the NS yeast fermentation process to determine the concentration of terpenes that could be released through acid hydrolysis.

2.3.2. Monitoring of Fermentation

The fermentation progress was indirectly assessed by monitoring weight loss caused by CO_2 release [33]. When fermentation was complete, the yeast population was studied

by surface seeding in a WLN differential culture medium [34] at 28 °C for 48 h. After that time, the growth of the yeasts that were effectively inoculated was controlled, and the colony-forming units were counted to determine the yeast population.

2.4. Analysis and Identification of Terpenoids

The concentrations of the terpenoids linalool, geraniol, nerol, citronellol, nerolidol, and farnesol were determined. For this analysis, calibration curves were developed for each terpenoid using standards in a concentration range between 1 and 250 µg/L in a 12% *v*/*v* hydroalcoholic solution. Analysis of these compounds in the samples and at the points on the calibration curve was performed by headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC-MS). For the samples and calibration curve points, 20 mL vials were used, to which 10 mL of the sample, 2.4 g of NaCl, and 10 µL of the internal standard, 1-heptanol, were added, as described in [35]. A fibre with the following phase characteristics was used for extraction: DVB/Carbon-WR/PDMS; thickness, 50/30 µm; and length, 10 mm (Restek, Bellefonte, PA, USA). For the analysis, we used a Shimadzu GC-20 Plus gas chromatograph coupled with a Shimadzu QP 2020 mass spectrometer equipped with a DW-Wax 30 fused silica capillary column (Agilent Technologies J&W, Santa Clara, CA, USA) coated with polyethylene glycol. The gas chromatographic conditions were as follows: the column temperature was maintained at 40 °C for 8 min, then increased to 180 °C at a rate of 3 °C min^{-1}, and subsequently raised to 230 °C at 20 °C min^{-1}. Both the injector and detector were set to 250 °C. Samples were introduced in splitless mode for 2 min, with hydrogen as the carrier gas at a pressure of 30 kPa. Mass spectral data were acquired at an ionisation energy of 70 eV in full-scan mode over an m/z range of 40–350, with a scan rate of 666 amu s^{-1}.

2.5. β-Glucosidase Activity

To identify β-glucosidase activity in the selected strains, Esculin Agar medium (EGA) was used at pH levels of 4.5 and 6, in accordance with the methods reported in [36]. The yeast strains to be studied were inoculated as radial streaks from fresh cultures. Each plate was incubated at 25 °C and examined at 2, 4, 6, and 10 days. An uninoculated plate served as a negative control, and a *Metschnikowia pulcherrima* strain served as a positive control. Strains with β-glucosidase activity hydrolysed the substrate, forming a dark brown halo on the agar.

2.6. Statistical and Data Analysis

Analysis of variance (ANOVA) was carried out on the obtained data using Statistica 7.1 software. Significant differences were evaluated using Fisher's LSD test or Tukey's test, as appropriate. In all cases, differences with p values ≤ 0.05 were considered statistically significant.

For the analysis of odour activity values (OAVs), a heatmap was generated using the Orange Data Mining software version 3.39.0 (https://orangedatamining.com accessed on 13 June 2025) to visualise the relevance of the terpenoid compounds detected in the different samples.

3. Results

3.1. Analysis of Terpenoid Compounds

Fermentation was carried out in the CDMM medium following different inoculation strategies: pure cultures and mixed cultures with *S. cerevisiae*. The de novo synthesis of monoterpenes and sesquiterpenes by the two *S. bacillaris* strains (Sb1 and Sb2) was initially evaluated in the CDMM medium. Table 2 shows the concentrations of the terpenoid compounds detected in each fermentation.

Table 2. Concentration of terpenoid compounds detected in fermentations carried out in the CDMM medium, according to the yeast strain and inoculation strategy. The odour descriptors and thresholds of perception reported in the literature are also included.

		Strains and Inoculation Strategy								
		SC	Sb1			Sb2				
KI	Compounds (mg/L)		Pure	Co-Inoculation	Sequential	Pure	Co-Inoculation	Sequential	Odour Threshold (µg/L)	Odour Descriptor
1860	Geraniol	nd	26.7 ± 1.3 a	37.0 ± 3.5 c	33.3 ± 2.0 b	37.9 ± 4.8 c	43.9 ± 1.6 c	43.9 ± 14.6 c	20 [37]	Rose and geranium
1558	Linalool	nd	35.9 ± 3.8 a,b	36.3 ± 7.7 a,b	34.6 ± 4.3 a,b	37.7 ± 1.9 a,b	41.3 ± 2.8 b	34.3 ± 5.2 a	25.2 [38]	Citrus, floral, lavender
1765	Citronellol	nd	nd	4.1 ± 1.5 b	2.6 ± 0.9 a,b	nd	3.1 ± 0.5 a,b	2.4 ± 0.1 a	100 [39]	Citronella, rose, green
2025	*cis*-Nerolidol	nd	6.4 ± 0.1 a	7.6 ± 2.3 a	nd	nd	6.6 ± 0.1 a	nd	700 [39]	Floral, green, citrus, woody
2042	*trans*-Nerolidol	12.4 ± 0.7 a	20.6 ± 4.2 b	22.2 ± 3.6 b	13.8 ± 2.8 a	20.5 ± 1.7 b	32.8 ± 1.8 c	12.6 ± 1.5 a	700 [39]	Floral and woody
2331	*trans*,*cis*-Farnesol	nd	1.7 ± 0.7 a	nd	nd	5.9 ± 0.1 b	nd	nd	1000 [40]	Sweet floral
2350	*trans*,*trans*-Farnesol	3.4 ± 1.8 a	7.9 ± 2.6 a	8.6 ± 0.9 a	nd	13.3 ± 4.4 b	26.3 ± 1.6 c	nd	1000 [40]	Sweet floral

The data are presented as means (from three biological replicates) ± standard deviation. Values followed by different letters within the same row indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test. nd: not detected; KI: Kovats index.

Geraniol and linalool formation was detected in all fermentations performed with *S. bacillaris*. In the case of geraniol, no statistically significant differences were observed between the different inoculation strategies for strain Sb2, with concentrations close to 40 μg/L. On the other hand, Sb1 showed significant variations according to the fermentation conditions, with the lowest concentration of this compound (26.7 μg/L) found in the monoculture. As for linalool, fermentations with strain Sb1 showed no significant differences between the different cultivation conditions, and Sb2 showed small statistically significant variations, with the lowest concentration found for the sequential mixed culture strategy (34.3 μg/L). In all evaluated conditions, both strains produced concentrations of these monoterpenes above their respective thresholds of perception.

Citronellol was only detected in the mixed fermentations at low concentrations compared with the concentrations of linalool and geraniol. The highest concentration was observed in the co-inoculation with Sb1 of about 4.1 μg/L, while the lowest concentration was 2.4 μg/L in the sequential mixed culture with Sb2.

It should be noted that no monoterpene production was detected following the fermentation carried out with the control strain (*S. cerevisiae*).

Regarding sesquiterpenes, significant differences were observed in the production of nerolidol and farnesol isomers, depending on the strain used and the inoculation strategy.

For the *trans*-nerolidol isomer, *S. cerevisiae* produced 12.4 μg/L in the monoculture. Strain Sb1 showed significantly higher concentrations under all conditions, with a maximum of 22.2 μg/L in the co-inoculation. Sb2 also achieved the highest production of the *trans*-nerolidol isomer in the co-inoculation (32.8 μg/L), while the levels decreased to 12.6 μg/L in the sequential fermentation, similar to those achieved with *S. cerevisiae*. In all cases, the obtained concentration was below the sensory threshold reported for this compound.

The *cis*-nerolidol isomer was only detected in fermentations with Sb1 (in the monoculture and co-inoculation) and in the sequential condition with Sb2, at concentrations between 6.4 and 7.6 μg/L (which are also below the sensory threshold).

As for the farnesol isomers, *trans*,*cis*-farnesol was detected at low concentrations only in the Sb1 monoculture and the Sb2 co-inoculation. *Trans*,*trans*-farnesol was produced by *S. cerevisiae* (3.4 μg/L) and Sb1 under all conditions, reaching a maximum of 8.6 μg/L in sequential inoculation. In Sb2, co-inoculation resulted in the highest concentration of this isomer (26.4 μg/L). However, in no case did the concentration exceed the reported threshold of perception.

3.2. Sensory Relevance of the Detected Terpenoid Compounds

In order to evaluate the sensory significance of the detected terpenoids, the corresponding OAVs were calculated for each inoculation strategy. The results are represented in a heatmap (Figure 1), which allows for a comparative visualisation of the compounds and the different fermentation conditions. The areas of greater chromatic intensity (according to the proposed scale) indicate compounds whose aroma units exceed a value of 1, suggesting a higher probability of contributing to the aromatic profile of the wine. In this regard, all fermentations involving *S. bacillaris* presented higher aroma units for geraniol and linalool, indicating that, through their metabolism, these strains could generate components that contributed to the aroma of the wine, independent of the inoculation strategy employed. Although some strain-specific variations were observed, both strains of *S. bacillaris* produced lower levels of these terpenes in isolated fermentations compared with mixed cultures. In particular, the co-inoculation strategy showed the highest aroma unit values for both strains of *S. bacillaris*. On the other hand, values significantly below the threshold of perception were observed for citronellol and the isomers of nerolidol and farnesol, indicating that

these components make no relevant individual sensory contributions. Finally, the control (*S. cerevisiae*) presented low values or values below the threshold of perception for all the studied terpenes, indicating no sensory contribution of terpene aroma.

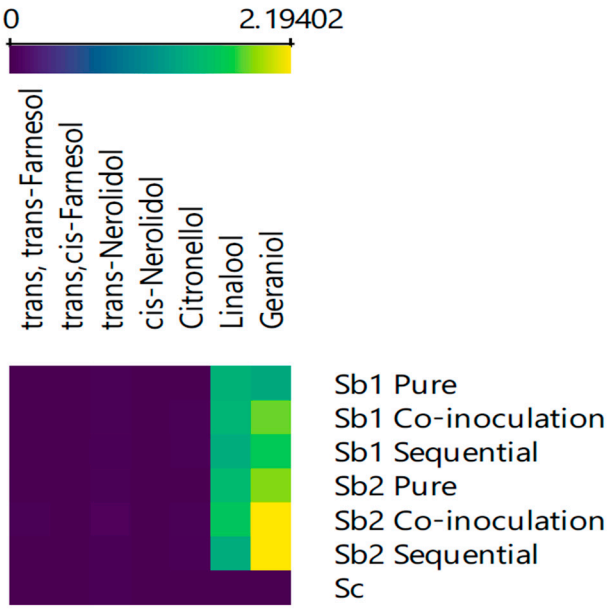

Figure 1. Heatmap of OAVs of terpenoid compounds detected in fermentations carried out in CDMM medium with *S. bacillaris* (Sb1 and Sb2) and *S. cerevisiae* (Sc) using different inoculation strategies.

3.3. β-Glucosidase Activity and Fermentations in the Presence of GAPs

Considering previous reports on the β-glucosidase activity of *S. bacillaris* species [25], tests were performed on esculin agar plates at pH levels of 4 and 6 with strains Sb1 and Sb2 [36], but no detectable activity was observed under these conditions for the studied strains. In this sense, the authors of [41], who isolated 63 strains of *S. bacillaris* from grapes in Italy, only found 5% with this enzymatic activity.

Complementing this plate assay, fermentations were carried out in CDMM medium supplemented with GAP to analyse whether these strains were able to release monoterpenes and norisoprenoids from glycosylated precursors under fermentative conditions.

Table 3 shows the results obtained from fermentation in the CDMM medium supplemented with GAP for the *S. bacillaris* strains Sb1 and Sb2, the control strain *S. cerevisiae*, and the non-yeast controls. As reported in [42], neither norisoprenoids nor other terpenes typically associated with the Tannat variety—such as α-terpineol, which is derived from the hydrolysis of GAP—were detected.

Table 3. Concentrations of terpenoid compounds detected in pure fermentations of Sb1, Sb2, and Sc, and in the control without inoculation, carried out in the CDMM medium with GAP.

	Compounds (μg/L)						
Strains	**Linalool**	**Geraniol**	**Citronellol**	***cis*-Nerolidol**	***trans*-Nerolidol**	***trans,cis*-Farnesol**	***trans,trans*-Farnesol**
Sc	nd	nd	nd	nd	26.0 ± 4.8	nd	23.8 ± 13.0 c
Sb1	37.1 ± 3.8 a	44.4 ± 4.9 b	nd	nd	23.9 ± 5.5	1.3 ± 0.6 a	6.3 ± 0.7 b
Sb2	40.1 ± 5.8 a	48.2 ± 13.1 b	nd	nd	33.4 ± 8.0	nd	11.8 ± 5.2 b,c
Control	nd	nd	nd	nd	nd	nd	nd

The data are presented as means (from three biological replicates) ± standard deviation. Values followed by different letters within the same row indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test. nd: not detected.

In this fermentative context, linalool and geraniol were again detected in the fermentations with Sb1 and Sb2, where they reached concentrations comparable with or slightly higher than those observed in CDMM. In particular, linalool was quantified at 37.1 μg/L for Sb1 and 40.1 μg/L for Sb2, while geraniol reached 44.4 μg/L and 48.2 μg/L, respectively. These differences could be associated with variations between assays performed at different times. No linalool or geraniol was detected in the fermentations carried out with *S. cerevisiae* or in the controls without inoculation.

In contrast to the results obtained in the CDMM medium (Table 2), the cis-nerolidol isomer was not detected in any of the fermentations carried out in the CDMM medium supplemented with GAP. On the contrary, concentrations of *trans*-nerolidol were similar to those previously observed, reaching a maximum value of 33.4 μg/L in strain Sb2.

Regarding the farnesol isomers, the highest concentration of *trans,trans*-farnesol was recorded in Sb2 (11.8 μg/L). Furthermore, the *trans,cis*-farnesol isomer was only detected in fermentations with Sb1 (1.4 μg/L) and in the control without yeast (0.5 μg/L).

4. Discussion

4.1. Terpenoid Compounds Generated by S. bacillaris

The results demonstrate the ability of *S. bacillaris* strains to produce monoterpenes and sesquiterpenes de novo. To the best of our knowledge, this is the first report documenting the de novo production of monoterpenes by this species, highlighting the generation of linalool and geraniol. This finding reinforces the findings from previous reports suggesting that this strain, when used in winemaking, contributes fruity and floral aromas to wine [23].

Although specific assays are needed, these results suggest that there are differences in the mevalonate pathway (MVA) between *S. bacillaris* and *S. cerevisiae*. This pathway, the main function of which is the synthesis of ergosterol, also provides intermediate clues for the formation of other cellular compounds, such as ubiquinone, quinones, dolichols, etc. This pathway starts from acetyl-CoA and leads to the production of isopentenyl pyrophosphate and its isomer dimethylallyl pyrophosphate, which are essential precursors in terpenoid biosynthesis [43,44].

In *S. cerevisiae*, monoterpene generation is restricted to only a few wine strains [9]. This restriction is attributed, on one hand, to the absence of monoterpene synthases and, on the other hand, to the low availability of geranyl pyrophosphate (GPP), the key intermediate in the synthesis of these compounds. Although the enzyme farnesyl pyrophosphate synthase (encoded by the *ERG20* gene) has the capacity to synthesise GPP, this intermediate is rapidly converted to farnesyl pyrophosphate (FPP) by the same enzyme, restricting its intracellular accumulation [45–47].

In contrast, the *S. bacillaris* strains tested appear to possess active monoterpene synthases, specifically a linalool synthase and a geraniol synthase. In this context, Shen et al. [48] identified a group of genes related to terpene biosynthesis, as well as increased availability of GPP, in a strain of *S. bacillaris*. This difference with respect to *S. cerevisiae* could be due to the presence of a functional variant of farnesyl pyrophosphate synthase with lower affinity for GPP, which would favour its intracellular accumulation and, consequently, the synthesis of monoterpenes.

Along these lines, Lemos et al. [49] analysed the genome of two *S. bacillaris* strains and detected five major translocations. Among the genes located near the breakpoints, one was found to encode for a farnesyl diphosphate synthase, suggesting possible functional alterations in this protein. In addition, although no specific studies on ergosterol biosynthesis in *S. bacillaris* have been reported, the same authors observed differences between the two species in the high osmolarity glycerol response. This response is directly related to the

regulation of ergosterol metabolism in *S. cerevisiae* [50], which reinforces the hypothesis of differences in the MVA with *S. bacillaris*.

Moreover, although statistically significant differences in the final geraniol and linalool concentrations between the different inoculation strategies were observed in some cases, these variations do not seem to be clearly correlated with the *S. bacillaris* cell population or the duration of fermentation. The evaluated conditions differed in both aspects: in the co-inoculation, the *S. bacillaris* population remained around 5×10^7 CFU/mL during most of the fermentation process, which lasted 16 days, whereas in the sequential inoculation and monocultures, the population was 1×10^8 CFU/mL throughout the fermentation process, which lasted 22 and 16 days, respectively (Table S1 and Figure S1).

In fungi, secondary metabolism plays a fundamental role in interactions with the environment, especially in defence against other organisms and communication mechanisms [51]. In this context, although linalool and geraniol have been described for their antifungal activity, the concentrations obtained in this work were too low to consider them effectively responsible for defensive functions [52,53]. In relation to cell communication, certain terpenes, such as farnesol, have been shown to act as quorum-sensing signals in some yeast species [54]. However, no clear correlation between cell density and monoterpene production was observed in the tested strains [51]. Taken together, these results suggest that although *S. bacillaris* could have the potential to synthesise these compounds, their specific ecological function remains unclear; as such, future studies are required.

Citronellol was found only in mixed cultures and at low concentrations compared with the other monoterpenes. One explanation for the presence of citronellol under these conditions only is the previously reported ability of *S. cerevisiae* to reduce geraniol to citronellol under fermentative conditions [55,56].

The other terpenoids detected in this work were two isomers of farnesol and two isomers of nerolidol. The detection of these compounds varied depending on the inoculation strategy. Nerolidol and farnesol are derived from farnesyl diphosphate, an intermediary in the biosynthesis of ergosterol. At an acidic pH, instability of the diphosphate group leads to the release of farnesol and its isomer, nerolidol. Although the exact function of these sesquiterpenes in *S. cerevisiae* is not known, farnesol is recognised to have antimicrobial properties at concentrations approaching 50 µg/L [57,58]. Increased production of these sesquiterpenes was observed in yeast co-inoculation, accompanied by increased production of linalool and geraniol in this culture strategy.

4.2. Sensory Relevance of the Terpenoid Compounds Generated by S. bacillaris

Fermented beverages constitute complex chemical matrices containing a wide array of volatile compounds that can significantly influence the beverage's final aroma profile. Despite the presence of synergistic and antagonistic interactions among these volatiles—whereby one compound may enhance or suppress the perception of another—a widely accepted method for estimating the individual impact of specific compounds is the calculation of odour activity values (OAVs) [59]. This metric reflects the frequency at which the concentration of a given compound exceeds its odour threshold, facilitating the identification of key aroma-active molecules [60].

Terpenes are among the most relevant classes of volatile compounds in wine, primarily due to their low odour thresholds and their association with pleasant floral and fruity attributes. These compounds are strongly linked to a varietal character, as they are naturally present in grape skins in both free and glycosidically bound forms and can be released into the must during maceration or enzymatically liberated during fermentation [61]. In addition, certain terpenes may be produced de novo or modified through yeast metabolism, particularly when non-*Saccharomyces* species are employed.

From a sensory perspective, monoterpenes contribute notes described as citrus, lavender, litchi, citronella, tropical fruits, and geranium, with some also exhibiting resinous aromas [61]. These compounds have been reported to interact synergistically, enhancing the overall aroma intensity or generating novel aromatic impressions not attributable to individual components alone [62].

Linalool is a key monoterpene in wine and is typically associated with floral and fruity notes. Depending on the matrix and the presence of other volatiles, it has been linked to descriptors such as grapefruit, ginger [62], and violet [63,64]. Reconstitution experiments have demonstrated its synergistic effect with other terpenes, particularly in the expression of dark fruit aromas [65].

Geraniol—another important terpene—plays a critical role in the aromatic profile of Muscat and other white grape varieties. While it is generally linked to geranium and jasmine when perceived in isolation, its combination with linalool, citronellol, and α-terpineol at elevated concentrations may evoke apricot and peach notes in white wines [61,63].

The capacity of *S. bacillaris* to biosynthesise linalool and geraniol during alcoholic fermentation, particularly at concentrations surpassing their respective odour thresholds, offers promising prospects for its biotechnological application in winemaking.

5. Conclusions

The use of *Starmerella bacillaris* in pure and mixed fermentations with *Saccharomyces cerevisiae* not only produced terpenoid compounds but also ensured the completion of fermentation, addressing one of the limitations commonly associated with non-*Saccharomyces* species.

These findings suggest that the impacts of these strains of *Starmerella bacillaris* on wine aroma are linked to the de novo synthesis of terpenoid compounds, given the absence of detectable β-glycosidase activity.

This approach could be particularly valuable for enhancing aromatic complexity in wines produced from neutral grape varieties with an inherently low terpene content, whether in free or glycosylated form.

The metabolic pathways involved in the biosynthesis of these components, as well as their biological roles in *Starmerella bacillaris* species, should be explored in future studies. Moreover, scaling up these assays under real winemaking conditions, with a subsequent sensory evaluation, will be essential to confirm the results obtained.

These results provide evidence, once again, that the use of non-*Saccharomyces* yeasts is a crucial tool for innovation in the wine industry.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation11090496/s1, Figure S1: Fermentation kinetics for each inoculation strategy. Data are present as means of three biological replicates; Table S1: Colony-forming units for each inoculation strategy at the end of alcoholic fermentation.

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References

1. Christianson, D.W. Structural and Chemical Biology of Terpenoid Cyclases. *Chem. Rev.* **2017**, *117*, 11570–11648. [CrossRef]
2. Déclaire Mabou, F.; Belinda, I.; Yossa, N. Terpenes: Structural Classification and Biological Activities. *IOSR J. Pharm. Biol. Sci.* **2021**, *16*, 25–40. [CrossRef]
3. Dunlevy, J.D.; Kalua, C.M.; Keyzers, R.A.; Boss, P.K. The Production of Flavour & Aroma Compounds in Grape Berries. In *Grapevine Molecular Physiology and Biotechnology*, 2nd ed.; Springer: Dordrecht, The Netherlands, 2009; pp. 293–340. [CrossRef]
4. Chizzola, R. Regular Monoterpenes and Sesquiterpenes (Essential Oils). In *Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes*; Springer: Berlin/Heidelberg, Germany, 2013; pp. 2973–3008. [CrossRef]
5. Maicas, S.; Mateo, J.J. Hydrolysis of Terpenyl Glycosides in Grape Juice and Other Fruit Juices: A Review. *Appl. Microbiol. Biotechnol.* **2005**, *67*, 322–335. [CrossRef] [PubMed]
6. Hjelmeland, A.K.; Ebeler, S.E. Glycosidically Bound Volatile Aroma Compounds in Grapes and Wine: A Review. *Am. J. Enol. Vitic.* **2015**, *66*, 1–11. [CrossRef]
7. Mele, M.A.; Kang, H.M.; Lee, Y.T.; Islam, M.Z. Grape Terpenoids: Flavor Importance, Genetic Regulation, and Future Potential. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 1429–1447. [CrossRef]
8. Black, C.A.; Parker, M.; Siebert, T.E.; Capone, D.L.; Francis, I.L. Terpenoids and Their Role in Wine Flavour: Recent Advances. *Aust. J. Grape Wine Res.* **2015**, *21*, 582–600. [CrossRef]
9. 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]
10. Carrau, F.M.; Boido, E.; Dellacassa, E. Terpenoids in Grapes and Wines: Origin and Micrometabolism during the Vinification Process. *Nat. Prod. Commun.* **2008**, *3*, 577–592. [CrossRef]
11. Drawert, F.; Barton, H. Biosynthesis of Flavor Compounds by Microorganisms. 3. Production of Monoterpenes by the Yeast Kluyveromyces Lactis. *J. Agric. Food Chem.* **1978**, *26*, 765–766. [CrossRef]
12. Hock, R.; Benda, I.; Schreier, P. Formation of Terpenes by Yeasts during Alcoholic Fermentation. *Z. Lebensm.-Unters.-Forsch.* **1984**, *179*, 450–452. [CrossRef]
13. Klingenberg, A.; Sprecher, E. Production of Monoterpenes in Liquid Cultures by the Yeast *Ambrosiozyma monospora*. *Planta Med.* **1985**, *51*, 264–265. [CrossRef]
14. Morata, A.; Escott, C.; Bañuelos, M.A.; Loira, I.; Del Fresno, J.M.; González, C.; Suárez-lepe, J.A. Contribution of Non-*Saccharomyces* Yeasts to Wine Freshness. A Review. *Biomolecules* **2020**, *10*, 34. [CrossRef]
15. Ferreira, A.M.; Clímaco, M.C.; Faia, A.M. The Role of Non-*Saccharomyces* Species in Releasing Glycosidic Bound Fraction of Grape Aroma Components—A Preliminary Study. *J. Appl. Microbiol.* **2001**, *91*, 67–71. [CrossRef]
16. López, S.; Mateo, J.; Maicas, S. Characterisation of Hanseniaspora Isolates with Potential Aroma-Enhancing Properties in Muscat Wines. *S. Afr. J. Enol. Vitic.* **2014**, *35*, 292–303. [CrossRef]
17. Beckner Whitener, M.E.; Stanstrup, J.; Panzeri, V.; Carlin, S.; Divol, B.; Du Toit, M.; Vrhovsek, U. Untangling the Wine Metabolome by Combining Untargeted SPME–GCxGC-TOF-MS and Sensory Analysis to Profile Sauvignon Blanc Co-Fermented with Seven Different Yeasts. *Metabolomics* **2016**, *12*, 53. [CrossRef]
18. Englezos, V.; Rantsiou, K.; Cravero, F.; Torchio, F.; Giacosa, S.; Ortiz-Julien, A.; Gerbi, V.; Rolle, L.; Cocolin, L. Volatile Profiles and Chromatic Characteristics of Red Wines Produced with *Starmerella bacillaris* and *Saccharomyces cerevisiae*. *Food Res. Int.* **2018**, *109*, 298–309. [CrossRef]
19. Sadoudi, M.; Tourdot-Maréchal, R.; Rousseaux, S.; Steyer, D.; Gallardo-Chacón, J.; Ballester, J.; Vichi, S.; Guérin-Schneider, R.; Caixach, J.; Alexandre, H. Yeast-Yeast Interactions Revealed by Aromatic Profile Analysis of Sauvignon Blanc Wine Fermented by Single or Co-Culture of Non-*Saccharomyces* and Saccharomyces Yeasts. *Food Microbiol.* **2012**, *32*, 243–253. [CrossRef]
20. Mills, D.A.; Johannsen, E.A.; Cocolin, L. Yeast Diversity and Persistence in Botrytis-Affected Wine Fermentations. *Appl. Environ. Microbiol.* **2002**, *68*, 4884–4893. [CrossRef] [PubMed]
21. Duarte, F.L.; Pimentel, N.H.; Teixeira, A.; Fonseca, A. *Saccharomyces bacillaris* Is Not a Synonym of *Candida stellata*: Reinstatement as *Starmerella bacillaris* Comb. Nov. *Antonie Van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **2012**, *102*, 653–658. [CrossRef] [PubMed]
22. Englezos, V.; Torchio, F.; Cravero, F.; Marengo, F.; Giacosa, S.; Gerbi, V.; Rantsiou, K.; Rolle, L.; Cocolin, L. Aroma Profile and Composition of Barbera Wines Obtained by Mixed Fermentations of *Starmerella bacillaris* (Synonym *Candida zemplinina*) and *Saccharomyces cerevisiae*. *LWT* **2016**, *73*, 567–575. [CrossRef]

23. Tufariello, M.; Fragasso, M.; Pico, J.; Panighel, A.; Castellarin, S.D.; Flamini, R.; Grieco, F. Influence of Non-Saccharomyces on Wine Chemistry: A Focus on Aroma-Related Compounds. *Molecules* **2021**, *26*, 644. [CrossRef]
24. Lemos Junior, W.J.F.; Treu, L.; Nadai, C.; da Silva Duarte, V.; Campanaro, S.; Fabrega-Prats, M.; Giacomini, A.; Corich, V. Genomic Insights into the Glutathione Metabolism of the Wine Yeast *Starmerella bacillaris*. *Oeno One* **2021**, *55*, 105–117. [CrossRef]
25. García, M.; Esteve-Zarzoso, B.; Cabellos, J.M.; Arroyo, T. Advances in the Study of *Candida stellata*. *Fermentation* **2018**, *4*, 74. [CrossRef]
26. Englezos, V.; Giacosa, S.; Rantsiou, K.; Rolle, L.; Cocolin, L. *Starmerella bacillaris* in Winemaking: Opportunities and Risks. *Curr. Opin. Food Sci.* **2017**, *17*, 30–35. [CrossRef]
27. Rosa, A.L.; Miot-Sertier, C.; Laizet, Y.; Salin, F.; Sipiczki, M.; Bely, M.; Masneuf-Pomarede, I.; Albertin, W. Draft Genome Sequence of the *Starmerella bacillaris* (Syn., *Candida zemplinina*) Type Strain CBS 9494. *Microbiol. Resour. Announc.* **2018**, *7*, e00872-18. [CrossRef]
28. Raymond Eder, M.L.; Rosa, A.L. Genetic, Physiological, and Industrial Aspects of the Fructophilic Non-*Saccharomyces* Yeast Species, *Starmerella bacillaris*. *Fermentation* **2021**, *7*, 87. [CrossRef]
29. Tufariello, M.; Capozzi, V.; Spano, G.; Cantele, G.; Venerito, P.; Mita, G.; Grieco, F. Effect of Co-Inoculation of *Candida zemplinina*, *Saccharomyces cerevisiae* and *Lactobacillus plantarum* for the Industrial Production of Negroamaro Wine in Apulia (Southern Italy). *Microorganisms* **2020**, *8*, 726. [CrossRef]
30. Martin, V.; Giorello, F.; Fariña, L.; Minteguiaga, M.; Salzman, V.; Boido, E.; Aguilar, P.S.; Gaggero, C.; Dellacassa, E.; Mas, A.; et al. De Novo Synthesis of Benzenoid Compounds by the Yeast *Hanseniaspora vineae* Increases the Flavor Diversity of Wines. *J. Agric. Food Chem.* **2016**, *64*, 4574–4583. [CrossRef] [PubMed]
31. Massera, A.; Soria, A.; Catania, C.; Krieger, S.; Combina, M. Simultaneous Inoculation of Malbec (*Vitis vinifera*) Musts with Yeast and Bacteria: Effects on Fermentation Performance, Sensory and Sanitary Attributes of Wines. *Food Technol. Biotechnol.* **2009**, *47*, 192–201.
32. Boido, E.; Lloret, L.; Medina, K.; Fariña, L.; Carrau, F.; Versini, G.; Dellacassa, E. Aroma Composition of *Vitis vinifera* Cv. Tannat: The Typical Red Wine from Uruguay. *J. Agric. Food Chem.* **2003**, *51*, 5408–5413. [CrossRef] [PubMed]
33. Carrau, F.M.; Medina, K.; Farina, L.; Boido, E.; Henschke, P.A.; Dellacassa, E. Production of Fermentation Aroma Compounds by *Saccharomyces cerevisiae* Wine Yeasts: Effects of Yeast Assimilable Nitrogen on Two Model Strains. *FEMS Yeast Res.* **2008**, *8*, 1196–1207. [CrossRef]
34. Pallmann, C.L.; Brown, J.A.; Olineka, T.L.; Cocolin, L.; Mills, D.A.; Bisson, L.F. Use of WL Medium to Profile Native Flora Fermentations. *Am. J. Enol. Vitic.* **2001**, *52*, 198–203. [CrossRef]
35. Fariña, L.; Boido, E.; Ares, G.; Gonzalez, N.; Lado, J.; Curbelo, R.; Almeida, L.; Medina, K.; Carrau, F.; Dellacassa, E. Solid Phase Microextraction for the Characterization of Food Aroma and Particular Sensory Defects. In *Flavors and Fragrances in Food Processing: Preparation and Characterization Methods*; ACS Symposium Series; American Chemical Society: Washington, DC, USA, 2022; Volume 1433, pp. 299–325. ISBN 9780841297319.
36. Pérez, G.; Fariña, L.; Barquet, M.; Boido, E.; Gaggero, C.; Dellacassa, E.; Carrau, F. A Quick Screening Method to Identify β-Glucosidase Activity in Native Wine Yeast Strains: Application of Esculin Glycerol Agar (EGA) Medium. *World J. Microbiol. Biotechnol.* **2011**, *27*, 47–55. [CrossRef]
37. Aznar, M.; Lopez, R.; Cacho, J.; Ferreira, V. Prediction of aged red wine aroma properties from aroma chemical composition. *Partial least squares regression models. J. Agric. Food Chem.* **2003**, *51*, 2700–2707. [CrossRef]
38. Ferreira, V.; López, R.; Cacho, J.F. Quantitative Determination of the Odorants of Young Red Wines from Different Grape Varieties. *J. Sci. Food Agric.* **2002**, *80*, 1659–1667. [CrossRef]
39. Condurso, C.; Cincotta, F.; Tripodi, G.; Sparacio, A.; Giglio, D.M.L.; Sparla, S.; Verzera, A. Effects of Cluster Thinning on Wine Quality of Syrah Cultivar (*Vitis vinifera* L.). *Eur. Food Res. Technol.* **2016**, *242*, 1719–1726. [CrossRef]
40. Zea, L.; Moyano, L.; Moreno, J.; Cortes, B.; Medina, M. Discrimination of the Aroma Fraction of Sherry Wines Obtained by Oxidative and Biological Ageing. *Food Chem.* **2001**, *75*, 79–84. [CrossRef]
41. Englezos, V.; Rantsiou, K.; Torchio, F.; Rolle, L.; Gerbi, V.; Cocolin, L. Exploitation of the Non-Saccharomyces Yeast *Starmerella bacillaris* (Synonym *Candida zemplinina*) in Wine Fermentation: Physiological and Molecular Characterizations. *Int. J. Food Microbiol.* **2015**, *199*, 33–40. [CrossRef] [PubMed]
42. Fariña, L.; Villar, V.; Ares, G.; Carrau, F.; Dellacassa, E.; Boido, E. Volatile Composition and Aroma Profile of Uruguayan Tannat Wines. *Food Res. Int.* **2015**, *69*, 244–255. [CrossRef]
43. Ninkuu, V.; Zhang, L.; Yan, J.; Fu, Z.; Yang, T.; Zeng, H. Biochemistry of Terpenes and Recent Advances in Plant Protection. *Int. J. Mol. Sci.* **2021**, *22*, 5710. [CrossRef]
44. Maury, J.; Asadollahi, M.A.; Møller, K.; Clark, A.; Nielsen, J. Microbial Isoprenoid Production: An Example of Green Chemistry through Metabolic Engineering. *Adv. Biochem. Eng. Biotechnol.* **2005**, *100*, 19–51.

45. Zhao, J.; Li, C.; Zhang, Y.; Shen, Y.; Hou, J.; Bao, X. Dynamic Control of ERG20 Expression Combined with Minimized Endogenous Downstream Metabolism Contributes to the Improvement of Geraniol Production in *Saccharomyces cerevisiae*. *Microb. Cell Fact.* **2017**, *16*, 17. [CrossRef] [PubMed]
46. Jiang, G.Z.; Yao, M.D.; Wang, Y.; Zhou, L.; Song, T.Q.; Liu, H.; Xiao, W.H.; Yuan, Y.J. Manipulation of GES and ERG20 for Geraniol Overproduction in *Saccharomyces cerevisiae*. *Metab. Eng.* **2017**, *41*, 57–66. [CrossRef] [PubMed]
47. Zhao, J.; Bao, X.; Li, C.; Shen, Y.; Hou, J. Improving Monoterpene Geraniol Production through Geranyl Diphosphate Synthesis Regulation in *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 4561–4571. [CrossRef] [PubMed]
48. Shen, Y.; Bai, X.; Zhou, X.; Wang, J.; Guo, N.; Deng, Y. Whole-Genome Analysis of *Starmerella bacillaris* CC-PT4 against MRSA, a Non-Saccharomyces Yeast Isolated from Grape. *J. Fungi* **2022**, *8*, 1255. [CrossRef]
49. Lemos Junior, W.J.F.; Duarte, V.d.S.; Treu, L.; Campanaro, S.; Nadai, C.; Giacomini, A.; Corich, V. Whole Genome Comparison of Two *Starmerella bacillaris* Strains with Other Wine Yeasts Uncovers Genes Involved in Modulating Important Winemaking Traits. *FEMS Yeast Res.* **2018**, *18*, foy069. [CrossRef]
50. Jordá, T.; Puig, S. Regulation of Ergosterol Biosynthesis in *Saccharomyces cerevisiae*. *Genes* **2020**, *11*, 795. [CrossRef]
51. Keller, N.P. Fungal Secondary Metabolism: Regulation, Function and Drug Discovery. *Nat. Rev. Microbiol.* **2019**, *17*, 167–180. [CrossRef]
52. Scariot, F.J.; Pansera, M.S.; Delamare, A.P.L.; Echeverrigaray, S. Citral and Geraniol Induce Necrotic and Apoptotic Cell Death on *Saccharomyces cerevisiae*. *World J. Microbiol. Biotechnol.* **2021**, *37*, 42. [CrossRef]
53. Scariot, F.J.; Pansera, M.S.; Longaray Delamare, A.P.; Echeverrigaray, S. Antifungal Activity of Monoterpenes against the Model Yeast *Saccharomyces cerevisiae*. *J. Food Process Preserv.* **2021**, *45*, e15433. [CrossRef]
54. Nickerson, K.W.; Atkin, A.L.; Hornby, J.M. Quorum Sensing in Dimorphic Fungi: Farnesol and Beyond. *Appl. Environ. Microbiol.* **2006**, *72*, 3805–3813. [CrossRef]
55. Steyer, D.; Erny, C.; Claudel, P.; Riveill, G.; Karst, F.; Legras, J.L. Genetic Analysis of Geraniol Metabolism during Fermentation. *Food Microbiol.* **2013**, *33*, 228–234. [CrossRef]
56. King, A.; Dickinson, J.R. Biotransformation of Monoterpene Alcohols by *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* and *Kluyveromyces lactis*. *Yeast* **2000**, *16*, 499–506. [CrossRef]
57. Steyer, D.; Ambroset, C.; Brion, C.; Claudel, P.; Delobel, P.; Sanchez, I.; Erny, C.; Blondin, B.; Karst, F.; Legras, J.-L. QTL Mapping of the Production of Wine Aroma Compounds by Yeast. *BMC Genom.* **2012**, *13*, 573. [CrossRef]
58. Chauhan, N.M.; Mohan Karuppayil, S. Dual Identities for Various Alcohols in Two Different Yeasts. *Mycology* **2021**, *12*, 25–38. [CrossRef]
59. Giungato, P.; Di Gilio, A.; Palmisani, J.; Marzocca, A.; Mazzone, A.; Brattoli, M.; Giua, R.; de Gennaro, G. Synergistic Approaches for Odor Active Compounds Monitoring and Identification: State of the Art, Integration, Limits and Potentialities of Analytical and Sensorial Techniques. *TrAC—Trends Anal. Chem.* **2018**, *107*, 116–129. [CrossRef]
60. Ren, L.; Hu, Y.; Liang, W. A Comprehensive Review and Variability Analysis of Odor Detection Thresholds and Their Influence on Building Material Odor Assessments. *Build. Environ.* **2025**, *280*, 113122. [CrossRef]
61. Santana, G.R.O.; Crepalde, L.T.; Hernandes, K.C.; Silveira, R.D.; Padilha, G.; Reitenbach, A.F.; de Souza, A.L.K.; Welke, J.E.; Caliari, V.; Burin, V.M. Impact of Skin Contact on the Volatile Composition and Sensory Properties of White Wines from Resistant Varieties. *Food Chem.* **2025**, *489*, 144967. [CrossRef]
62. Chigo-Hernandez, M.M.; Dubois, A.; Tomasino, E. Aroma Perception of Rose Oxide, Linalool and α-Terpineol Combinations in Gewürztraminer Wine. *Fermentation* **2022**, *8*, 30. [CrossRef]
63. Darnal, A.; Ceccon, A.; Magni, M.; Robatscher, P.; Poggesi, S.; Boselli, E.; Longo, E. Enantioselective-GCxGC Determination of α-Terpinyl Ethyl Ether in Wine: Quantitative Analysis and Identification of Main Terpene Precursors. *Appl. Food Res.* **2024**, *4*, 100538. [CrossRef]
64. Chen, H.; Bai, S.; Yang, B.; Ren, R.; Tang, Z.; Zhang, Z.; Zeng, Q. Inter- and Intra-Varietal Clonal Differences Influence the Aroma Compound Profiles of Wines Analyzed by GC–MS and GC-IMS. *Food Chem. X* **2025**, *25*, 102136. [CrossRef]
65. Garbay, J.; Tempere, S.; Ballet, L.; Barbe, J.-C.; Lytra, G. Investigating the Olfactory Impact of Monoterpenic Compounds on Fruity Aroma Perception in Model Red Wine Solutions. *J. Agric. Food Chem.* **2025**, *73*, 4208–4218. [CrossRef]

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