applied sciences

MDPI

Article

Influence of *Saccharomyces pastorianus* and *Saccharomyces bayanus* Inoculation Ratio to Oenological Characteristics of Sauvignon Blanc Wine

Maria Dimopoulou [1,*], Vicky Troianou [2], Chrisavgi Toumpeki [2], Despina Lola [3], Elli Goulioti [3], Aikaterini Tzamourani [1], Etienne Dorignac [4], Spiros Paramithiotis [5,*] and Yorgos Kotseridis [3]

1 Department of Wine, Vine and Beverage Sciences, School of Food Science, University of West Attica, 28 Ag. Spyridonos St., 12243 Athens, Greece
2 Innovino Research & Development, 21 Meg. Alexandrou St., 15351 Pallini, Greece
3 Laboratory of Oenology, Department of Food Science and Human Nutrition, Agricultural University of Athens, 75 Iera Odos St., 11855 Athens, Greece
4 Fermentis, 137 Rue Gabriel Péri, 59703 Lille, France
5 Laboratory of Food Process Engineering, Department of Food Science and Human Nutrition, Agricultural University of Athens, 75 Iera Odos St., 11855 Athens, Greece
* Correspondence: mdimopoulou@uniwa.gr (M.D.); sdp@aua.gr (S.P.)

Abstract: The aim of the present study was to assess the effect of the inoculation ratio between *Saccharomyces pastorianus* strain SP2 and *S. bayanus* strain BCS103 on the enological properties and aroma profile of Sauvignon Blanc wines. For that purpose, a total of eight different fermentation trials on Sauvignon Blanc must took place. These included spontaneous fermentation as well as inoculation with pure and mixed cultures of the *S. pastorianus* and *S. bayanus* strains. The mixed cultures contained different proportions of the two strains (*S. pastorianus* SP2 to *S. bayanus* BCS103: 99–1%, 97–3%, 95–5%, 90–10% and 70–30% *w*/*w*). Classical oenological analyses were employed to assess the course of fermentation and classical microbiological enumeration combined with inter-delta sequence profile analysis was used for yeast population dynamics estimation. The volatile compounds of each wine were analyzed with GC/MS. The fermentation was completed between 11 and 13 days, while the inoculation ratio significantly affected the chemical composition and the sensorial evaluation of the resulting wines. Based on the sensory evaluation, the least-appreciated Sauvignon Blanc wine was the one resulting from spontaneous fermentation, and the higher the ratio of the *S. bayanus* strain in the inoculum, the higher the level of appreciation of the wine.

Keywords: species interaction; wine yeast; Sauvignon Blanc; wine typicity; *Saccharomyces* sensu stricto group

Citation: Dimopoulou, M.; Troianou, V.; Toumpeki, C.; Lola, D.; Goulioti, E.; Tzamourani, A.; Dorignac, E.; Paramithiotis, S.; Kotseridis, Y. Influence of *Saccharomyces pastorianus* and *Saccharomyces bayanus* Inoculation Ratio to Oenological Characteristics of Sauvignon Blanc Wine. *Appl. Sci.* **2023**, *13*, 3393. https://doi.org/10.3390/app13063393

Academic Editor: Niel Van Wyk

Received: 9 February 2023
Revised: 28 February 2023
Accepted: 6 March 2023
Published: 7 March 2023

1. Introduction

Wine quality depends on the inherent characteristics of the grape varieties, the pedoclimatic environment, the agricultural and winemaking practices as well as their interaction [1–8]. The outcome of the fine balance between these factors is the production of wines with varietal typicity expressing the unique character of each region. This is also the case of Sauvignon Blanc, a variety that is among the most preferred for the production of aromatic white wines. Sauvignon Blanc wines are characterized by a very wide palette of aromas and flavors. Among the most distinctive ones are grapefruit, passion fruit and box tree notes, the occurrence of which has been attributed mainly to the presence of aromatic volatile thiols, namely 3-mercaptohexan-1-ol, 3-mercaptohexyl acetate and 4-mercapto-4-methylpentan-2-one, respectively [9–11]. Especially regarding the first two, their presence and concomitantly, the intensity of the respective aromas seems to be largely dependent upon the yeast strain carrying out alcoholic fermentation [12–14].

In recent years, two additional factors have emerged that demanded specific attention and proper adjustment of the winemaking practices, namely the modification of the climatic conditions and consumer preferences [15–17]. The first resulted in a series of effects on vine phenology and grape ripening, which significantly affected the microbial ecology of the must and ultimately yeast metabolism and alcoholic fermentation [15]. At the same time, a consumer trend towards increased aromatic complexity and modified varietal typicity has also been noted [16,17]. Research has initially addressed this demand through the use of non-*Saccharomyces* yeasts in combination with *S. cerevisiae*. However, such coexistence is not always feasible due to the antagonistic relationship that may develop [12,18–26]. Interestingly, such an issue has not yet been reported when strains within the *Saccharomyces* sensu stricto group are combined.

Saccharomyces pastorianus and *S. bayanus* are interesting alternatives that have been successfully employed to modulate Sauvignon Blanc typicity [27]. More specifically, the wines fermented with *S. pastorianus* monoculture contained less acetic and malic acids, while those fermented with the coculture of *S. pastorianus and S. bayanus*, at a ratio of 70/30 (*w*/*w*), were characterized by higher citrus fruit notes, compared to the wines fermented with *S. cerevisiae*. In addition, the significance of the inoculation ratio between *S. pastorianus* and *S. cerevisiae* was also recently highlighted [28]. A series of inoculation ratios were assessed and each of them significantly affected the enological properties and the aroma profile of the produced Sauvignon Blanc wines. Therefore, the aim of the present study was to further assess the effect of the inoculation ratio between *S. pastorianus* and *S. bayanus* on the enological properties and aroma profile of Sauvignon Blanc wines. For that purpose, five different inoculation ratios were employed for alcoholic fermentation and the resulting wines were thoroughly assessed from a chemical and organoleptic perspective.

2. Materials and Methods

2.1. Microorganisms and Culture Conditions

Saccharomyces bayanus strain BCS103 (Fermentis, Marcq-En-Baroeul, France) and *S. pastorianus* strain SP2 [28] were used throughout this study. The first was stored in its commercially available dry form, while the latter was stored in Nutrient Broth supplemented with 20% glycerol at −20 °C. Both strains were activated by cultivating twice in YM broth (1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract) at 25 °C for 48 h.

2.2. Winemaking Conditions

Grapes of the Sauvignon Blanc variety, grown in Asprokampos (Nemea, Greece), were harvested, manually destemmed and crushed, and 50 mg/L sodium metabisulfite (Scharlab S.A, Barcelona, Spain) was added. The initial must density and total acidity were determined at 11.87 °Be and 6 g tartaric acid/L, respectively. After cold clarification, the must was decanted into 16 glass fermenters (30 L each) and inoculated with the yeast strains at final populations of 10^6 CFU/mL. The cases that were assessed are presented in Table 1.

The different inoculation ratios were achieved by mixing the appropriate serial dilutions of the two yeast strains. Two fermentation vessels were inoculated for each case. The addition of 200 mg/L SpringFerm™ (Fermentis, France) took place 24 h after inoculation. Fermentation took place at 18 °C.

Table 1. Inoculation strategies employed in the present study.

Code	Inoculum
SP2	*S. pastorianus* strain SP2
BCS103	*S. bayanus* strain BCS103
70/30	*S. pastorianus* SP2—*S. bayanus* strain BCS103 70%-30% (*w*/*w*)
90/10	*S. pastorianus* SP2—*S. bayanus* strain BCS103 90%-10% (*w*/*w*)
95/5	*S. pastorianus* SP2—*S. bayanus* strain BCS103 95%-5% (*w*/*w*)
97/3	*S. pastorianus* SP2—*S. bayanus* strain BCS103 97%-3% (*w*/*w*)
99/1	*S. pastorianus* SP2—*S. bayanus* strain BCS103 99%-1% (*w*/*w*)
spontaneous	no inoculum

2.3. Chemical Analyses

2.3.1. Standard Enological Parameters

Alcoholic fermentation was monitored in daily intervals through the determination of glucose and fructose; their depletion (less than 2 g/L) signified the end of fermentation. Analysis of glucose/fructose, along with malic acid, acetic acid and glycerol as well as initial and final pH value and total acidity were determined using enzymatic kits adapted for Biosystems Auto-analyser (Barcelona, Spain). Determination of free and total SO_2 was performed by titrimetric methods. NIR spectrometry was employed for ethanol quantification [29].

2.3.2. Volatile Compounds Quantification

Head-Space Solid Phase Micro Extraction (HS-SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS) was employed to analyze wine volatile compounds. The analysis was performed according to Dimopoulou et al. [27]. In brief, DVB/CAR/PDMS, 75 µm fiber was used for volatile compounds absorption. Analysis was performed with an Agilent 7890A GC (Santa Clara, CA, USA) equipped with an Agilent 5873C MS detector. The DBWAX capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was employed for the separation of the volatile compounds using helium as a gas carrier at a flow rate of 1.2 mL/min. The temperature of the injector and the MS-transfer line was 250 °C and 260 °C, respectively. The oven temperature was maintained at 30 °C for 5 min, then raised to 220 °C at 4 °C/min and held at this temperature for 20 min.

2.3.3. Quantification of Varietal Thiols

The method described by Tominaga et al. [10] was employed for the quantification of varietal thiols, namely 3-mercaptohexan-1-ol (3MH), 3-mercaptohexyl acetate (3MHA) and 4-methyl-4-methylpentan-2-one (4MMP).

2.4. Microbiological Analyses

Microbiological analyses were performed 48 h after inoculation, after completion of approximately 2/3 of fermentation and at the end of fermentation. Since the speed of fermentation differed between samples, the latter two corresponded to different days. The enumeration of *Saccharomyces* and non-*Saccharomyces* yeasts population was performed by plating serial dilutions on Wallerstein Laboratory Nutrient (WLN) agar and Lysine medium agar, respectively. The latter was incubated at 28 °C for 48–72 h. Phenotypic differentiation between *S. bayanus* and *S. pastorianus* was achieved by incubating the WLN plates at elevated temperatures. More specifically, incubation at 37 °C for 24 h resulted in the enumeration of the *S. bayanus* colonies due to the inability of *S. pastorianus* to provide adequate growth at temperatures above 30 °C. Conversely, incubation of the WLN plates at 28 °C for 48 h will result in the enumeration of colonies of both yeast strains. Verification

of the yeast identity was performed by the inter-delta sequence profile analysis according to Dimopoulou et al. [28].

2.5. Sensory Analysis

Sensory analysis was performed by a panel of eight trained and experienced judges, according to Dimopoulou et al. [27]. The trained judges belonged to the age range from 25 to 55 years old, all working in the academic departments of the present study (affiliations 1 and 3). The descriptors assessed were grouped into four categories: 1. Visual; 2. Olfactory (aroma intensity, freshness feeling, floral, citrus, amylic, tropical fruits, vegetal/herbaceous, reduction, complexity); 3. Gustatory (acidity, body/roundness, sweetness, balance, aftertaste/persistence); 4. Overall quality.

2.6. Statistical Analysis

Statistical analysis was performed using JMP version 3.1.5 software (SAS Institute Inc., Cary, NC, USA). The differences between the chemical parameters and sensorial descriptors were assessed with a one-way analysis of variance (ANOVA) with Tukey's post-hoc test ($p < 0.05$).

3. Results

Table 2 shows the concentration of glucose and fructose (in g/L) during the alcoholic fermentation of Sauvignon Blanc must under the different inoculation cases assessed. Alcoholic fermentation was considered as completed (glucose + fructose < 2 g/L) after 10 days, when fermentation was carried out by *S. bayanus* BCS103 as a monoculture and in coculture with *S. pastorianus* SP2, with the population of the latter up to 95% of the total inoculum. The fermentation was completed after 12 days when it was carried out spontaneously by *S. pastorianus* SP2 as a monoculture and in coculture with *S. bayanus* BCS103 at a ratio of 97/3. Finally, the fermentation was completed after 13 days when it was carried out by the coculture of *S. bayanus* BCS103 with *S. pastorianus* SP2 at a ratio of 1/99.

Table 2. Concentration of glucose and fructose (in g/L) during alcoholic fermentation of Sauvignon Blanc must.

Time (Days)	Spontaneous	Inoculum						
		BCS103	SP2	70/30	90/10	95/5	97/3	99/1
0	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}
1	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}	210.0 (0.0) ^{a}
2	204.0 (0.8) ^{g}	182.0 (1.5) ^{c}	202.0 (0.9) ^{g}	169.0 (1.3) ^{a}	173.0 (1.5) ^{b}	194.0 (1.4) ^{e}	188.0 (1.3) ^{d}	199.0 (1.0) ^{f}
3	189.0 (1.2) ^{g}	150.0 (2.9) ^{c}	186.0 (1.3) ^{g}	125.0 (2.7) ^{a}	134.0 (2.0) ^{b}	173.5 (2.1) ^{e}	164.0 (1.7) ^{d}	179.0 (1.2) ^{f}
4	135.0 (2.7) ^{c}	120.0 (5.4) ^{b}	145.0 (6.6) ^{d}	112.0 (6.3) ^{a}	116.0 (2.6) ^{ab}	137.0 (2.8) ^{c}	138.0 (2.7) ^{c}	152.0 (2.9) ^{e}
5	75.0 (7.4) ^{a}	78.0 (2.3) ^{a}	110.0 (1.0) ^{c}	79.0 (3.8) ^{a}	83.0 (6.9) ^{a}	97.0 (7.1) ^{b}	108.0 (5.3) ^{c}	116.0 (6.3) ^{c}
6	45.0 (2.2) ^{c}	37.6 (1.4) ^{a}	69.0 (1.2) ^{f}	40.4 (2.1) ^{b}	48.0 (1.1) ^{d}	61.8 (0.3) ^{e}	73.0 (1.6) ^{g}	78.0 (1.3) ^{h}
7	27.3 (1.4) ^{c}	18.5 (0.8) ^{a}	38.4 (0.2) ^{e}	19.3 (0.2) ^{a}	24.1 (1.2) ^{b}	35.1 (0.9) ^{d}	45.1 (0.8) ^{f}	47.3 (1.4) ^{g}
8	15.5 (0.3) ^{d}	7.6 (0.1) ^{b}	29.4 (0.1) ^{f}	6.7 (0.1) ^{a}	10.5 (0.1) ^{c}	21.1 (0.1) ^{e}	31.8 (0.1) ^{g}	37.1 (0.2) ^{h}
10	2.2 (0.3) ^{c}	<0.1	8.4 (0.0) ^{e}	<0.1	0.1 (0.0) ^{a}	1.5 (0.2) ^{b}	6.9 (0.1) ^{d}	12.9 (0.1) ^{f}
12	0.2 (0.0) ^{a}	<0.1	1.6 (0.0) ^{d}	<0.1	<0.1	0.4 (0.1) ^{b}	1.3 (0.0) ^{c}	2.1 (0.1) ^{e}
13	0.1 (0.0) ^{a}	<0.1	0.3	<0.1	<0.1	<0.1	<0.1	0.4 (0.0) ^{b}

The average values are presented. Standard deviation is given in parenthesis. Different letters in each row designate statistically significant differences ($p < 0.05$).

3.1. Microbiological Analyses

A total of 250 colonies were randomly selected from the agar plates used for the enumeration of the *S. bayanus* BCS103 and *S. pastorianus* SP2 strains and subjected to inter-delta fingerprinting in order to verify their identity. The inability of the *S. pastorianus* strain to grow adequately at 37 °C was verified in all cases; therefore, the temperature-based differentiation that was employed in the present study was deemed accurate.

The evolution of *Saccharomyces* and non-*Saccharomyces* yeast populations during the alcoholic fermentation of Sauvignon Blanc must under the different inoculation cases assessed is presented in Figure 1. In all cases, alcoholic fermentation was driven by *Saccharomyces* yeasts while the non-*Saccharomyces* kept a low population along the fermentation process. Worth to mention the exception of the spontaneous one, in which the non-*Saccharomyces* population prevailed at the 48th hour of fermentation (Figure 1A) while the indigenous *Saccharomyces* dominated the rest of the process but in a lower population compared to the inoculated conditions. (Figure 1B,C).

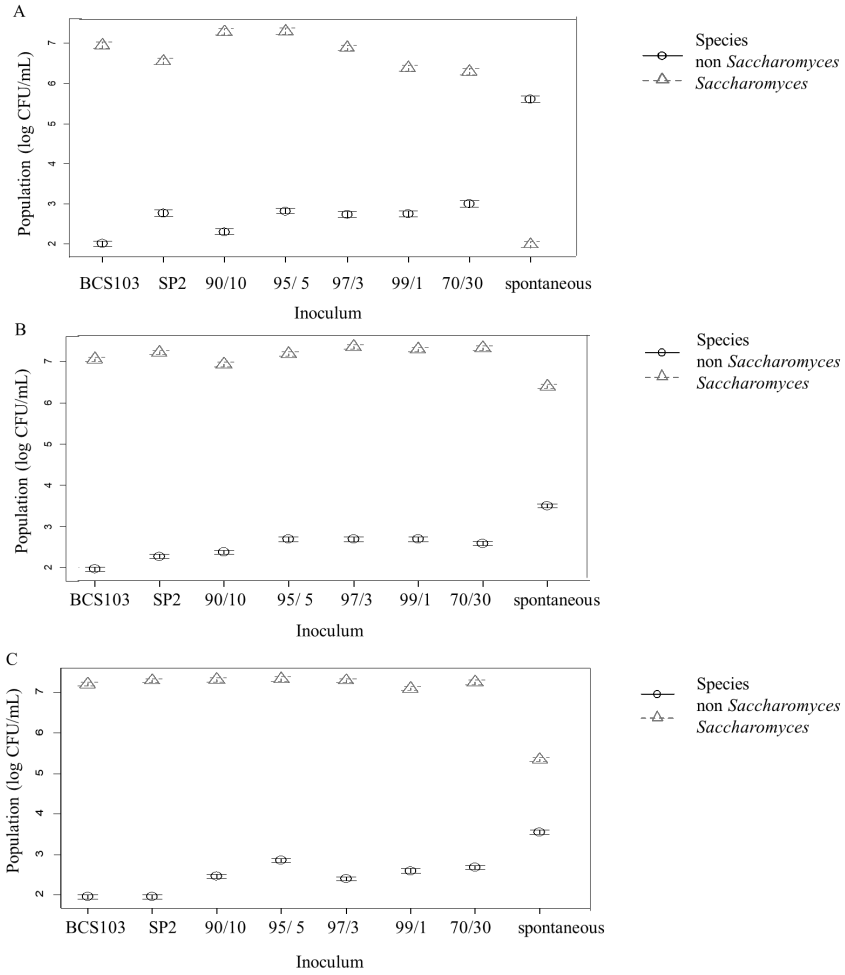

Figure 1. Evolution of *Saccharomyces* and non-*Saccharomyces* populations (in log CFU/mL) during alcoholic fermentation of Sauvignon Blanc must after 48 h (**A**), 2/3 of the process (**B**) and at the end of fermentation (**C**). The must was fermented under different inoculation schemes using *S. bayanus* BCS103 (BCS103) and *S. pastorianus* SP2 (SP2) as monoculture and co-inoculation of *S. pastorianus* SP2 and *S. bayanus* BCS103 at a ratio of 90/10, 95/5, 97/3, 99/1 and 70/30. The condition with no inoculation scheme was also tested (spontaneous).

In Figure 2, the evolution of *S. bayanus* BCS103 and *S. pastorianus* SP2 populations during the alcoholic fermentation of Sauvignon Blanc must is shown. Despite the different initial populations that were used when co-inoculation of *S. bayanus* BCS103 and *S. pas-*

torianus SP2 was employed, at the 48th hour of fermentation, no statistically significant differences between their populations were observed, and the fermentation seemed to be driven by both strains (Figure 2A). This was also the case at the 2/3 of fermentation and at the end of fermentation with the exception of the 90/10 and the 99/1 co-inoculation experiments, in which the population of the *S. bayanus* strain was higher than the respective of the *S. pastorianus* strain ($p < 0.05$) (Figure 2B,C).

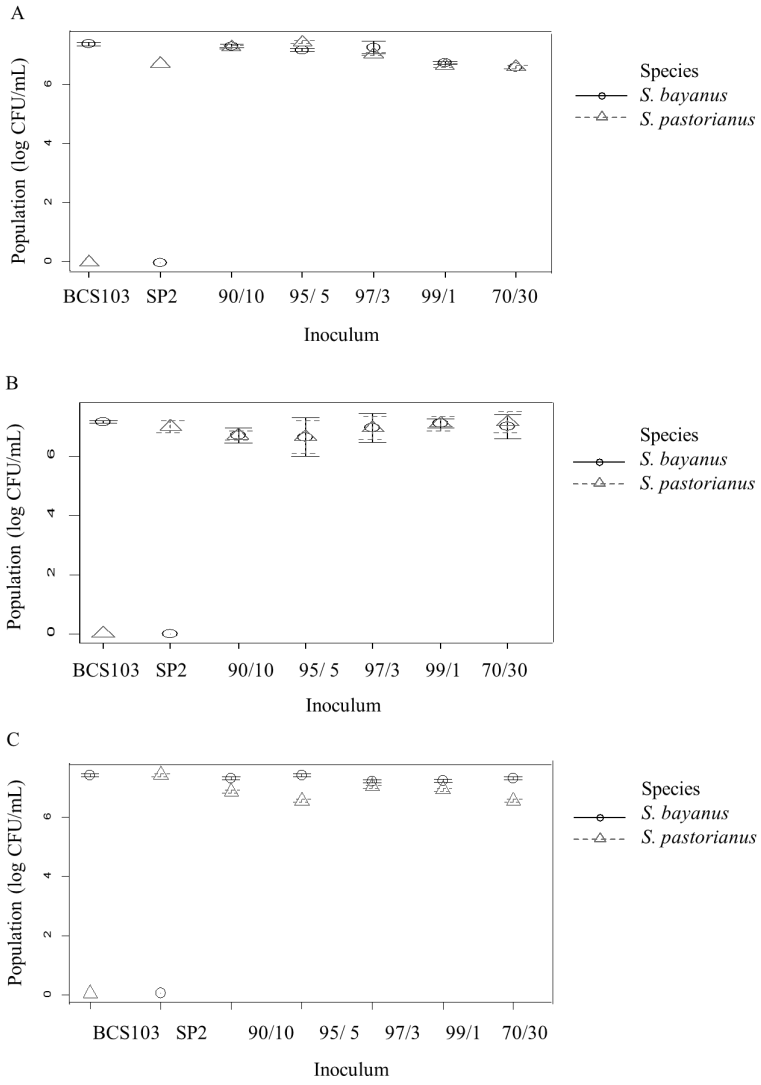

Figure 2. Evolution of *S. bayanus* BCS103 and *S. pastorianus* SP2 populations (in log CFU/mL) during alcoholic fermentation of Sauvignon Blanc must after 48 h (**A**), 2/3 of the process (**B**) and at the end of fermentation (**C**). The must was fermented under different inoculation schemes using *S. bayanus* BCS103 (BCS103) and *S. pastorianus* SP2 (SP2) as monocultureç as well as co-inoculation of *S. pastorianus* SP2 and *S. bayanus* BCS103 at a ratio of 90/10, 95/5, 97/3, 99/1 and 70/30.

3.2. Chemical Analyses

In Table 3, the standard enological parameters after the completion of alcoholic fermentation are presented. The pH value ranged between 3.28 and 3.39, with the lowest value observed in the wine made with the monoculture of *S. bayanus* BCS103 and the highest observed in the wine made with the monoculture of *S. pastorianus* SP2. The total acidity of the wines ranged between 5.78 and 6.04 g tartaric acid/L, without statistically significant differences between them. The total SO_2 ranged between 18 and 40 mg/L. The lowest concentration was observed in the wine made by spontaneous fermentation as well as the one made with the monoculture of *S. bayanus* BCS103. The highest amount of total SO_2 was

observed in the wine made with the co-culture of the two strains at a ratio of 99/1. No free SO_2 was detected in any wine. In all cases, the ethanol produced ranged between 12.5 and 12.8 % vol.

Table 3. Standard enological parameters at the beginning and the end of alcoholic fermentation of Sauvignon Blanc must.

Inoculum	pH	Total SO_2 (mg/L)	Total Acidity (g Tartaric acid/L)	Ethanol (% vol)
uninoculated must	3.23 (0.02) ^{a}	24 (1.8) ^{bc}	6.18 (0.26) ^{a}	-
BCS103	3.28 (0.05) ^{ab}	18 (2.5) ^{a}	5.89 (0.32) ^{a}	12.7 (0.01) ^{bc}
SP2	3.39 (0.02) ^{d}	39 (2.3) ^{f}	5.96 (0.33) ^{a}	12.6 (0.00) ^{ab}
70/30	3.30 (0.06) ^{bc}	21 (1.7) ^{ab}	5.78 (0.41) ^{a}	12.8 (0.02) ^{c}
90/10	3.30 (0.05) ^{bc}	26 (2.4) ^{cd}	5.89 (0.29) ^{a}	12.6 (0.02) ^{ab}
95/5	3.33 (0.03) ^{bcd}	28 (1.9) ^{de}	5.96 (0.35) ^{a}	12.6 (0.03) ^{ab}
97/3	3.34 (0.04) ^{bcd}	31 (2.1) ^{e}	6.00 (0.51) ^{a}	12.6 (0.00) ^{ab}
99/1	3.36 (0.02) ^{cd}	40 (2.6) ^{f}	6.04 (0.21) ^{a}	12.6 (0.01) ^{ab}
spontaneous	3.35 (0.04) ^{cd}	18 (3.2) ^{a}	6.04 (0.57) ^{a}	12.5 (0.03) ^{a}

The average values are presented. Standard deviation is given in parentheses. Different letters in each column designate statistically significant differences ($p < 0.05$).

The volatile and non-volatile compounds quantified at the end of alcoholic fermentation of Sauvignon Blanc must, under the different inoculation cases studied, are exhibited in Table 4. The wine produced by spontaneous fermentation presented significant differences from those produced by inoculation; it contained the highest amounts of malic acid, acetic acid and hexyl acetate and the lowest amounts of glycerol, ethyl 2-methyl butyrate, ethyl isobutyrate, 2-phenyl ethanol, propanol and isoamyl alcohol. Similarly, the inoculation strategy affected the concentration of the volatile and non-volatile compounds. More specifically, the wine made with the monoculture of *S. pastorianus* SP2 had more ethyl octanoate, ethyl hexanoate, ethyl butyrate, ethyl hydroxy propanoate, ethyl 3-hydroxy butanoate, isobutanol, butanol, isoamyl alcohol, 3-sulfanylhexan-1-ol and 3-sulfanylhexan-1-ol acetate and less ethyl 2-hydroxy isobutyrate, hexyl acetate, isoamyl acetate, 2-phenylethyl acetate and 4-methyl-4-sulfanylpentan-2-one than the wine made with the monoculture of *S. bayanus* BCS103. Co-inoculation of the two strains affected significantly the volatile and non-volatile content of the produced wines, with the exception of glycerol and propanol, the amount of which presented no statistically significant differences between the wines produced with the monoculture of *S. pastorianus* SP2 and the ones produced with any co-culture employed. The wines produced with any co-culture had less ethyl hexanoate, ethyl butyrate, ethyl hydroxy propanoate and 3-sulfanylhexan-1-ol acetate than those produced with the monoculture of *S. pastorianus* SP2. Such reduction was also observed in the case of malic acid, ethyl octanoate, ethyl 3-hydroxy butanoate, hexyl acetate, isobutanol, butanol and 3-sulfanylhexan-1-ol, but under different co-culture ratios. Conversely, an increase in the amount of acetic acid, ethyl decanoate, ethyl 2-methyl butyrate, ethyl isobutyrate, ethyl 2-hydroxy isobutyrate, isoamyl acetate, 2-phenyl ethanol and isoamyl alcohol was observed, but not in all co-culture ratios. Interestingly, the amount of 2-phenylethyl acetate and 4-methyl-4-sulfanylpentan-2-one increased at co-culture ratios of 70/30 and 90/10 but decreased at ratios 97/3 and 99/1.

Table 4. Volatile and non-volatile compounds at the end of alcoholic fermentation of Sauvignon Blanc must.

	Monocultures		Co-Cultures of SP2 with BCS103 at Ratio					Spontaneous Fermentation
	BCS103	**SP2**	**70/30**	**90/10**	**95/5**	**97/3**	**99/1**	
Polyols (g/L)								
glycerol	7.03 (0.34) ^{b}	7.25 (0.32) ^{bc}	7.36 (0.27) ^{bc}	7.42 (0.23) ^{bc}	7.85 (0.42) ^{c}	7.71 (0.33) ^{c}	7.67 (0.18) ^{c}	5.82 (0.58) ^{a}
Organic acids (g/L)								
Malic acid 1	1.21 (0.02) ^{c}	1.20 (0.02) ^{c}	1.21 (0.04) ^{c}	1.10 (0.04) ^{a}	1.12 (0.03) ^{ab}	1.16 (0.03) ^{bc}	1.19 (0.02) ^{c}	1.30 (0.05) ^{d}
Acetic acid	0.01 (0.00) ^{a}	0.02 (0.00) ^{a}	0.03 (0.01) ^{b}	0.07 (0.01) ^{c}	0.1 (0.01) ^{d}	0.18 (0.00) ^{e}	0.03 (0.00) ^{b}	0.35 (0.02) ^{f}
Ethyl esters (mg/L)								
Ethyl decanoate	557 (90.6) ^{ab}	401 (100.3) ^{a}	540 (104.7) ^{ab}	543 (95.8) ^{ab}	715 (106.3) ^{bc}	802 (110.6) ^{c}	799 (107.3) ^{c}	639 (102.8) ^{bc}
Ethyl octanoate	1533 (67.3) ^{a}	2077 (89.4) ^{e}	1598 (47.1) ^{a}	1717 (26.9) ^{b}	1827 (54.9) ^{bc}	1934 (85.2) ^{cd}	1978 (56.9) ^{de}	1867 (63.1) ^{cd}
Ethyl hexanoate	1147 (27.6) ^{a}	1617 (41.5) ^{e}	1201 (32.5) ^{ab}	1239 (37.1) ^{bc}	1304 (36.8) ^{c}	1419 (44.2) ^{d}	1457 (39.3) ^{d}	1266 (54.2) ^{bc}
Ethyl butyrate	529 (6.3) ^{d}	585 (7.2) ^{f}	501 (9.6) ^{c}	445 (8.1) ^{a}	461 (8.4) ^{b}	501 (9.7) ^{c}	547 (6.3) ^{e}	504 (8.2) ^{c}
Ethyl hydroxy propanoate	9561 (215.3) ^{a}	15024 (309.4) ^{e}	10034 (388.2) ^{a}	12162 (406.2) ^{b}	13159 (370.9) ^{c}	13875 (502.4) ^{d}	14345 (429.7) ^{d}	12358 (417.3) ^{b}
ethyl 3-hydroxy butanoate	323 (13.5) ^{a}	397 (21.6) ^{c}	345 (33.2) ^{ab}	368 (30.5) ^{bc}	334 (26.9) ^{ab}	357 (24.7) ^{abc}	354 (23.1) ^{abc}	360 (25.3) ^{abc}
ethyl 2- methyl butyrate	11.3 (0.62) ^{b}	13.2 (1.23) ^{bc}	13.4 (1.87) ^{c}	16.1 (0.66) ^{de}	17.2 (1.75) ^{e}	17.0 (1.24) ^{e}	14.8 (1.06) ^{cd}	5.2 (0.44) ^{a}
ethyl isobutyrate	105 (3.1) ^{b}	109 (2.8) ^{b}	109 (3.6) ^{b}	139 (4.5) ^{d}	129 (4.9) ^{c}	141 (5.2) ^{d}	123 (4.4) ^{c}	72 (3.2) ^{a}
ethyl 2-hydroxyisobutyrate	38 (2.2) ^{d}	24 (1.5) ^{a}	35 (3.5) ^{cd}	34 (2.4) ^{cd}	28 (2.8) ^{ab}	25 (1.7) ^{a}	24 (3.2) ^{a}	31 (2.4) ^{bc}
Acetate esters (mg/L)								
hexyl acetate	302 (11.7) ^{e}	198 (12.3) ^{cd}	220 (21.5) ^{d}	179 (20.6) c	150 (13.6) ^{b}	120 (14.2) ^{a}	110 (11.5) ^{a}	338 (23.3) ^{f}
Isoamyl acetate	6026 (956.2) ^{d}	2959 (885.3) ^{ab}	5123 (1023.1) ^{cd}	4257 (982.3) ^{bc}	3036 (907.6) ^{ab}	2154 (709.4) ^{a}	1988 (752.2) ^{a}	3481 (937.3) ^{ab}
2-phenylethyl acetate	385 (25.3) ^{e}	264 (24.2) ^{c}	358 (31.6) ^{de}	331 (30.8) ^{d}	248 (28.1) ^{bc}	209 (20.5) ^{ab}	199 (24.3) ^{a}	273 (21.8) ^{c}
Higher alcohols (mg/L)								
2- phenyl-ethanol	18.61 (0.36) ^{bc}	18.23 (0.24) b	18.98 (0.51) ^{c}	19.81 (0.36) ^{d}	20.5 (0.49) ^{e}	19.89 (0.44) ^{de}	18.78 (0.37) ^{bc}	15.72 (0.28) ^{a}
propanol	27.9 (1.84) ^{b}	28.2 (1.62) ^{b}	27.0 (2.67) ^{b}	28.1 (2.35) ^{b}	27.8 (2.53) ^{b}	31.0 (3.17) ^{b}	28.0 (2.31) ^{b}	22.0 (1.69) ^{a}
isobutanol	14.1 (4.36) ^{a}	30.0 (5.21) ^{c}	16.7 (3.78) ^{ab}	22.5 (6.07) ^{bc}	27.1 (5.05) ^{c}	28.0 (5.12) ^{c}	26.0 (4.68) ^{c}	21.7 (3.69) ^{abc}
butanol	0.73 (0.036) ^{a}	1.24 (0.027) ^{e}	0.81 (0.085) ^{bc}	1.08 (0.124) ^{d}	1.14 (0.058) ^{de}	0.91 (0.069) ^{c}	1.18 (0.074) ^{de}	1.08 (0.044) ^{d}
isoamyl alcohol	166.4 (0.69) ^{b}	170.2 (0.75) ^{cd}	170.0 (0.87) ^{cd}	171.5 (0.90) ^{d}	168.6 (0.96) ^{c}	173.3 (1.08) ^{e}	168.6 (1.41) ^{c}	141.4 (0.92) ^{a}
Thiols (ng/L)								
3-sulfanylhexan-1-ol	206 (7.3) ^{ab}	295 (6.5) ^{d}	295 (9.2) ^{d}	281 (8.2) ^{d}	224 (8.5) ^{c}	211 (8.7) ^{bc}	195 (9.2) ^{a}	192 (7.2) ^{a}
3-sulfanylhexan-1-ol acetate	ND	5 (0.6)	ND	ND	ND	ND	ND	ND
4-methyl-4-sulfanylpentan-2-one	24.2 (1.14) ^{e}	6.7 (0.87) ^{b}	17.1 (1.21) ^{d}	12.2 (1.69) ^{c}	5.5 (0.71) ^{ab}	4.8 (0.63) ^{a}	4.6 (0.47) ^{a}	4.7 (0.39) ^{a}

1 Initial malic acid concentration was 1.89 g/L. The average values are presented. Standard deviation is given in parentheses. Different letters in each column designate statistically significant differences ($p < 0.05$).

The diversity of the volatile compounds produced under the different inoculation schemes is illustrated by the Principal Component Analysis in Figure 3. The PCA analysis of the volatile compounds clearly distinguished in different quadrants the monoculture fermentation schemes and the spontaneous fermentation while the co-inoculation schemes were distributed mainly on the upper part of the biplot. The co-inoculation modality of SP2 90%-BCS103 10% was the closest to the monoculture condition BCS103, both characterized by the production of ethyl 2-hydroxyisobutyrate, isoamyl acetate, 2-phenylethyl acetate and 4-methyl-4-sulfanylpentan-2-one. Diametrically opposed, the fermentation schemes of *S. pastorianus* as monoculture, as well as the coculture with *S. bayanus* at a ratio of 70/30 and 99/1, were positioned to the right part of the biplot. At this side of the plot, the majority of the ethyl esters as well as 3-sulfanylhexan-1-ol, butanol and isobutanol were grouped. Finally, the spontaneous fermentation is clearly distinguished by the rest fermentation conditions and positioned on the lower left quadrant, characterized by high hexyl acetate production.

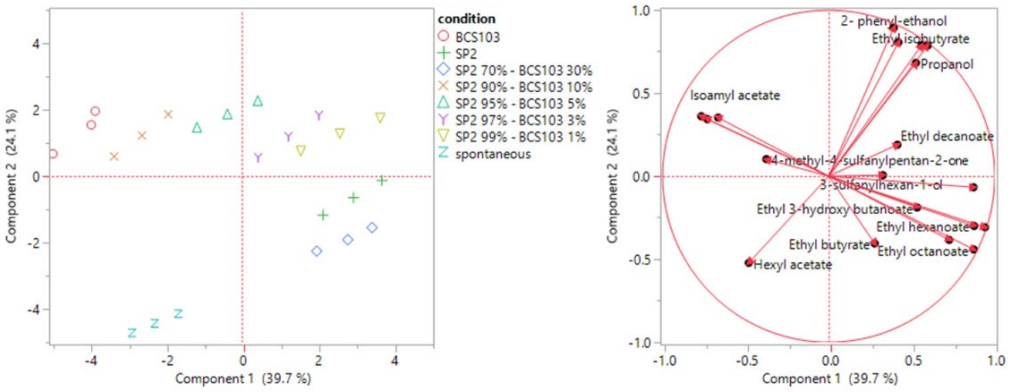

Figure 3. Principal component analysis of 20 volatile compounds of Sauvignon blanc wines fermented with monocultures of *S. bayanus* (BCS103) and *S. pastorianus* (SP2), mixed cultures of both species in different inoculation ratio as well as the spontaneous fermentation condition.

3.3. Sensory Analysis

In Figure 4, the sensory analysis of the wines produced from the Sauvignon Blanc variety is presented. In general, no statistically significant differences were observed in the grades received in the visual descriptor 'color', the olfactory descriptors 'floral' and 'citrus' as well as the gustatory descriptor 'sweetness' between the wines produced. The wines produced by spontaneous fermentation received lower grades in the olfactory descriptor 'aroma intensity', the gustatory descriptor 'balance' as well as the overall quality, compared to the wines produced by inoculation. The different inoculation strategies also affected the sensory analysis of the wines to some extent. The wines produced with the monocultures of the strains under study received different grades ($p < 0.05$) in the overall quality and the olfactory descriptor 'aroma intensity'. More specifically, the grades received by the wine made with the monoculture of the *S. bayanus* BCS103 were higher than the respective received by the wine made with the monoculture of the *S. pastorianus* SP2. Co-inoculation with both strains had also a significant effect on the sensory analysis of the wines. More specifically, the olfactory descriptor 'aroma intensity' of the wine made with the inoculum ratio 90/10 and the overall acceptance of the wines made with the inoculum ratios 70/30 and 90/10 were improved compared to the wine made by the monoculture of the *S. pastorianus* SP2. Conversely, the olfactory descriptors 'aroma intensity', amylic' and 'complexity' as well as the gustatory descriptors 'body/roundness' and 'aftertaste/persistence' and the overall quality of the wine made with the inoculum ratio 99/1 received lower grades than the respective made with the monoculture of the *S. bayanus* BCS103. Similarly, the wine made with the inoculum ratio 99/1 received a higher grade in the olfactory descriptor 'reduction' compared to the one made with the monoculture of the *S. bayanus* BCS103.

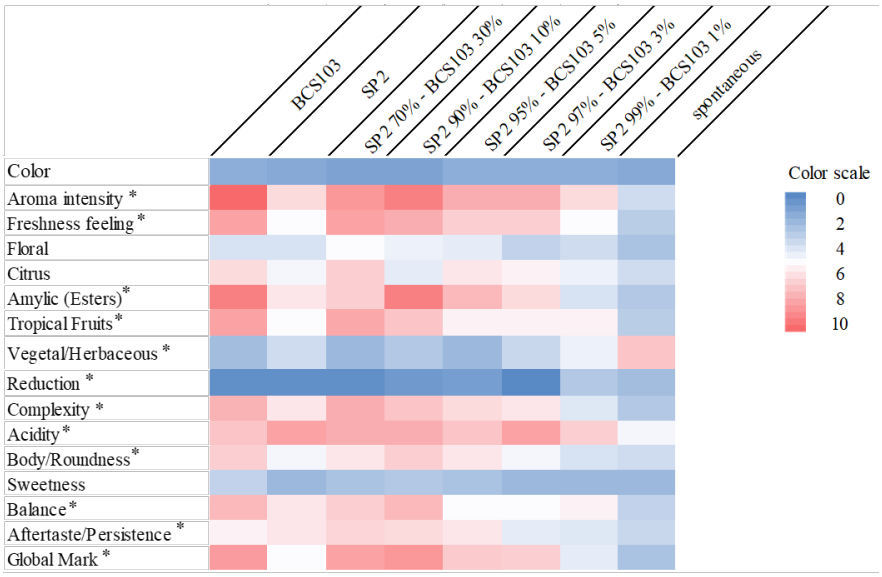

Figure 4. Heatmap analysis representation corresponding to Sauvignon Blanc wine sensory evaluation fermented under different inoculation schemes. The relative content of each sensory attribute is illustrated through a color scale (from dark blue, minimum, to dark red, maximum). The asterisk denotes a statistically significant difference ($p < 0.05$) between each attribute and the fermentation schemes.

4. Discussion

Enhancing aromatic complexity without altering varietal typicity is the key to effectively addressing the recent consumer trends toward new sensorial challenges. The strategy that has been employed for that purpose includes the utilization of yeast strains that unfold the typical varietal aromas and at the same time supplement them through the production of fermentation aromas. Thus, novel combinations are created, at least from the varietal typicity perspective. *S. pastorianus* is not that common in winemaking while *S. bayanus* is used more frequently, however, usually for difficult fermentations, i.e., for high levels of sugar musts or stuck fermentations; however, they have provided very interesting results regarding the alcoholic fermentation of Sauvignon Blanc must, especially under co-inoculation mode [27]. The aim of the present study was to expand our knowledge of the interactions between two strains of the aforementioned species and their effect on the sensorial quality of Sauvignon Blanc wine. Thus, the chemical composition and the sensorial properties of wines made by the co-culture of these strains at different ratios were evaluated and compared with the ones made by each strain separately and through spontaneous fermentation.

Controlled fermentation through the utilization of suitable strains is the strategy employed in order to obtain reproducible results. In the case of alcoholic fermentation, strains belonging to the *Saccharomyces* genus are employed for ethanol production, and strains belonging to other genera, such as *Hanseniaspora*, *Metschnikowia*, *Issatchenkia*, *Pichia*, *Schizosaccharomyces*, *Torulaspora* and *Lachancea*, for the production of aroma compounds [23,30–36]. Spontaneously fermented wines are usually characterized by higher sensorial complexity and richer body, which has been attributed to the larger variety of metabolites produced by the larger yeast consortium that contributes to alcoholic fermentation [16,37,38]. However, this consortium is uncontrolled, and, therefore, the outcome of the fermentation is unpredictable. Thus, spontaneously fermented wines may be characterized as highly acceptable [39,40], or, as in the present study, sensorially unsatisfactory [41]. Additionally, according to our results, the volatile and sensory profiles of the spontaneously fermented wines were well distinguished compared to the inoculated ones. It seems that the dominance of non-*Saccharomyces* yeast after 48h of alcoholic fermentation clearly has an impact on the final produced wines [42].

Even if the impact of non-*Saccharomyces* yeast in wine has been well documented in the literature [43,44], the prediction of their intra- and inter-species interaction along the winemaking process is not yet established. The main obstacle of both spontaneous and co-inoculation strategies in wine production is the compatibility of the microbial strains, in terms of population growth and metabolite production. The antagonistic interactions that possibly develop may have a negative impact on wine quality [45–47]. Regarding the relationship between the *S. bayanus* and the *S. pastorianus* strains used in the present study, good compatibility was observed between the strains since no negative oenological attributes were detected. In terms of coexistence during fermentation, *S. bayanus* seems to grow more efficiently in the must environment, since it managed to grow at populations equal to or higher than the respective population of *S. pastorianus*, even though it was inoculated at a lower initial population. Therefore, it can be concluded that there are no evident indirect interactions of mutualism or commensalism between the two strains as their co-existence didn't affect their growth compared to the pure culture conditions [47,48].

Interestingly, the inoculation of Sauvignon Blanc must with different ratios of the two strains resulted in the production of wines with different chemical compositions and concomitantly sensory evaluation. These differences could not be correlated with the population of the two strains during fermentation, since in the majority of the cases no statistically significant differences were observed. Our findings suggest that the interaction of the two strains could be based mainly on the presence of metabolites through physical contact between these microorganisms. Wine yeasts have been reported to secrete small quorum-sensing molecules to assess their population density and adapt their behavior under stress conditions [49,50]. For instance, the aromatic alcohol 2-phenylethanol has been reported to play the role of a signaling molecule in *S. cerevisiae* [51]. In our case, the production of 2-phenylethanol was significantly higher when the inoculation rate of *S. pastorianus* was higher than 90% compared to the monoculture conditions. It would be interesting to study in the future the kinetics of 2-phenylethanol under a co-inoculation mode of the two species and also study more metabolites through non-targeted metabolomics analysis.

5. Conclusions

The present study aimed to assess the effect of different *S. pastorianus* and *S. bayanus* inoculation ratios on the sensory profile of Sauvignon Blanc wines, towards the modification of the varietal typicity. The good compatibility of the strains employed was exhibited by the absence of oenological attributes. The *S. bayanus* strain seemed to grow more efficiently in the must environment, and the higher the ratio of the *S. bayanus* strain in the inoculum, the higher the level of appreciation of the Sauvignon Blanc wine.

Author Contributions: Conceptualization, Y.K., V.T. and E.D.; methodology, V.T., C.T., D.L., E.G., A.T., M.D. and Y.K.; formal analysis, M.D., S.P. and Y.K.; investigation, V.T.; data curation, V.T., C.T., D.L., E.G. and A.T.; writing—original draft preparation, M.D., S.P. and Y.K.; writing—review and editing, M.D. and S.P.; visualization, M.D. and S.P. 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: The data presented in this study are available in the manuscript.

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

References

1. Makra, L.; Vitányi, B.; Gál, A.; Mika, J.; Matyasovszky, I.; Hirsch, T. Wine quantity and quality variations in relation to climatic factors in the Tokaj (Hungary) winegrowing region. *Am. J. Enol. Vitic.* **2009**, *60*, 312–321. [CrossRef]
2. Ubalde, J.M.; Sort, X.; Zayas, A.; Poch, R.M. Effects of soil and climatic conditions on grape ripening and wine quality of Cabernet Sauvignon. *J. Wine Res.* **2010**, *21*, 1–17. [CrossRef]

3. Lorenzo, M.N.; Taboada, J.J.; Lorenzo, J.F.; Ramos, A.M. Influence of climate on grape production and wine quality in the Rias Baixas, north-western Spain. *Reg. Environ. Chang.* **2013**, *13*, 887–896. [CrossRef]
4. Smith, P.A.; McRae, J.M.; Bindon, K.A. Impact of winemaking practices on the concentration and composition of tannins in red wine. *Aust. J. Grape Wine Res.* **2015**, *21*, 601–614. [CrossRef]
5. Alatzas, A.; Theocharis, S.; Miliordos, D.-E.; Leontaridou, K.; Kanellis, A.K.; Kotseridis, Y.; Hatzopoulos, P.; Koundouras, S. The effect of water deficit on two Greek *Vitis vinifera* L. cultivars: Physiology, grape composition and gene expression during berry development. *Plants* **2021**, *10*, 1947. [CrossRef]
6. Blackford, M.; Comby, M.; Zeng, L.; Dienes-Nagy, Á.; Bourdin, G.; Lorenzini, F.; Bach, B. A review on stems composition and their impact on wine quality. *Molecules* **2021**, *26*, 1240. [CrossRef]
7. Miliordos, D.E.; Kanapitsas, A.; Lola, D.; Goulioti, E.; Kontoudakis, N.; Leventis, G.; Tsiknia, M.; Kotseridis, Y. Effect of nitrogen fertilization on Savvatiano (*Vitis vinifera* L.) grape and wine composition. *Beverages* **2022**, *8*, 29. [CrossRef]
8. Yan, H.K.; Ma, S.Y.; Lu, X.; Zhang, C.C.; Ma, L.; Li, K.; Wei, Y.C.; Gong, M.S.; Li, S. Response of wine grape quality to rainfall temperature and soil properties in Hexi corridor. *HortScience* **2022**, *57*, 1593–1599. [CrossRef]
9. Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J.-N.; Dubourdieu, D. Identification of a powerful aromatic component of *Vitis vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour Fragr. J.* **1995**, *10*, 385–392. [CrossRef]
10. Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour Fragr. J.* **1998**, *13*, 159–162. [CrossRef]
11. Hart, R.S.; Jolly, N.P.; Mohamed, G.; Booyse, M.; Ndimba, B.K. Characterization of *Saccharomyces cerevisiae* hybrid yeasts selected for low volatile acidity formation and the production of aromatic Sauvignon blanc wine. *Afr. J. Biotechnol.* **2016**, *15*, 2068–2081. [CrossRef]
12. Englezos, V.; Rantsiou, K.; Cravero, F.; Torchio, F.; Pollon, M.; Fracassetti, D.; Ortiz-Julien, A.; Gerbi, V.; Rolle, L.; Cocolin, L. Volatile profile of white wines fermented with sequential inoculation of *Starmerella bacillaris* and *Saccharomyces cerevisiae*. *Food Chem.* **2018**, *257*, 350–360. [CrossRef] [PubMed]
13. Masneuf-Pomarède, I.; Mansour, C.; Murat, M.-L.; Tominaga, T.; Dubourdieu, D. Influence of fermentation temperature on volatile thiols concentrations in Sauvignon blanc wines. *Int. J. Food Microbiol.* **2006**, *108*, 385–390. [PubMed]
14. Hart, R.S.; Ndimba, B.K.; Jolly, N.P. Characterization and evaluation of thiol-releasing and lower volatile acidity forming intra-genus and inter-genus hybrid yeast strains for Sauvignon blanc wine. *Afr. J. Microbiol. Res.* **2017**, *11*, 740–755.
15. Mira de Orduña, R. Climate change associated effects on grape and wine quality and production. *Food Res. Int.* **2010**, *43*, 1844–1855. [CrossRef]
16. Varela, C.; Siebert, T.; Cozzolino, D.; Rose, L.; McLean, H.; Henschke, P.A. Discovering a chemical basis for differentiating wines made by fermentation with 'wild' indigenous and inoculated yeasts: Role of yeast volatile compounds. *Aust. J. Grape Wine Res.* **2009**, *15*, 238–248. [CrossRef]
17. Capozzi, V.; Fragasso, M.; Romaniello, R.; Berbegal, C.; Russo, P.; Spano, G. Spontaneous food fermentations and potential risks for human health. *Fermentation* **2017**, *3*, 49. [CrossRef]
18. Sadoudi, M.; Tourdot-Maréchal, R.; Rousseaux, S.; Steyer, D.; Gallardo-Chacón, J.-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]
19. Branco, P.; Viana, T.; Albergaria, H.; Arneborg, N. Antimicrobial peptides (AMPs) produced by *Saccharomyces cerevisiae* induce alterations in the intracellular pH, membrane permeability and culturability of *Hanseniaspora guilliermondii* cells. *Int. J. Food Microbiol.* **2015**, *205*, 112–118. [CrossRef]
20. Englezos, V.; Rantsiou, K.; Giacosa, S.; Río Segade, S.; Rolle, L.; Cocolin, L. Cell-to cell contact mechanism modulates *Starmerella bacillaris* death in mixed culture fermentations with *Saccharomyces cerevisiae*. *Int. J. Food Microbiol.* **2019**, *289*, 106–114. [CrossRef]
21. Bely, M.; Stoeckle, P.; Masneuf-Pomarède, I.; Dubourdieu, D. Impact of mixed *Torulaspora delbrueckii-Saccharomyces cerevisiae* culture on high-sugar fermentation. *Int. J. Food Microbiol.* **2008**, *122*, 312–320. [CrossRef] [PubMed]
22. Ciani, M.; Comitini, F.; Mannazzu, I.; Domizio, P. Controlled mixed culture fermentation: A new perspective on the use of non-*Saccharomyces* yeasts in winemaking. *FEMS Yeast Res.* **2010**, *10*, 123–133. [CrossRef] [PubMed]
23. Comitini, F.; Gobbi, M.; Domizio, P.; Romani, C.; Lencioni, L.; Mannazzu, I.; Ciani, M. Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. *Food Microbiol.* **2011**, *28*, 873–882. [CrossRef] [PubMed]
24. Domizio, P.; Romani, C.; Lencioni, L.; Comitini, F.; Gobbi, M.; Mannazzu, I.; Ciani, M. Outlining a future for non-*Saccharomyces* yeasts: Selection of putative spoilage wine strains to be used in association with *Saccharomyces cerevisiae* for grape juice fermentation. *Int. J. Food Microbiol.* **2011**, *147*, 170–180. [CrossRef] [PubMed]
25. Benito, S.; Hofmann, T.; Laier, M.; Lochbühler, B.; Schüttler, A.; Ebert, K.; Fritsch, S.; Röcker, J.; Rauhut, D. Effect on quality and composition of Riesling wines fermented by sequential inoculation with non-*Saccharomyces* and *Saccharomyces cerevisiae*. *Eur. Food Res. Technol.* **2015**, *241*, 707–717. [CrossRef]
26. Petitgonnet, C.; Klein, G.L.; Roullier-Gall, C.; Schmitt-Kopplin, P.; Quintanilla-Casas, B.; Vichi, S.; Julien-David, D.; Alexandre, H. Influence of cell-cell contact between *L. thermotolerans* and *S. cerevisiae* on yeast interactions and the exo-metabolome. *Food Microbiol.* **2019**, *83*, 122–133. [CrossRef]

27. Dimopoulou, M.; Troianou, V.; Toumpeki, C.; Gosselin, Y.; Dorignac, É.; Kotseridis, Y. Effect of strains from different *Saccharomyces* species used in different inoculation schemes on chemical composition and sensory characteristics of Sauvignon blanc wine. *OENO One* **2020**, *54*, 745–759. [CrossRef]
28. Dimopoulou, M.; Goulioti, E.; Troianou, V.; Toumpeki, C.; Paramithiotis, S.; Gosselin, Y.; Dorignac, E.; Papadopoulos, G.; Kotseridis, Y. Effect of *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* co-inoculation on alcoholic fermentation behavior and aromatic profile of Sauvignon Blanc wine. *Fermentation* **2022**, *8*, 539. [CrossRef]
29. International Organisation of Vine and Wine. *Compendium of International Methods of Wine and Must Analysis*; OIV: Paris, France, 2020.
30. Moreira, N.; Mendes, F.; Guedes de Pinho, P.; Hogg, T.; Vasconcelos, I. Heavy sulphur compounds, higher alcohols and esters production profile of *Hanseniaspora uvarum* and *Hanseniaspora guilliermondii* grown as pure and mixed cultures in grape must. *Int. J. Food Microbiol.* **2008**, *124*, 231–238. [CrossRef]
31. Andorrà, I.; Berradre, M.; Mas, A.; Esteve-Zarzoso, B.; Guillamón, J.M. Effect of mixed culture fermentations on yeast populations and aroma profile. *LWT Food Sci. Technol.* **2012**, *49*, 8–13. [CrossRef]
32. Gobbi, M.; Comitini, F.; Domizio, P.; Romani, C.; Lencioni, L.; Mannazzu, I.; Ciani, M. *Lachancea thermotolerans* and *Saccharomyces cerevisiae* in simultaneous and sequential co-fermentation: A strategy to enhance acidity and improve the overall quality of wine. *Food Microbiol.* **2013**, *33*, 271–281. [CrossRef]
33. Anfang, N.; Brajkovich, M.; Goddard, M.R. Co-fermentation with *Pichia kluyveri* increases varietal thiol concentrations in Sauvignon Blanc. *Aust. J. Grape Wine Res.* **2009**, *15*, 1–8. [CrossRef]
34. Azzolini, M.; Tosi, E.; Lorenzini, M.; Finato, F.; Zapparoli, G. Contribution to the aroma of white wines by controlled *Torulaspora delbrueckii* cultures in association with *Saccharomyces cerevisiae*. *World J. Microbiol. Biotechnol.* **2015**, *31*, 277–293. [CrossRef] [PubMed]
35. Loira, I.; Morata, A.; Comuzzo, P.; Callejo, M.J.; González, C.; Calderón, F.; Suarez-Lepe, J.A. Use of *Schizosaccharomyces pombe* and *Torulaspora delbrueckii* strains in mixed and sequential fermentations to improve red wine sensory quality. *Food Res. Int.* **2015**, *76*, 325–333. [CrossRef] [PubMed]
36. Kim, D.H.; Hong, Y.A.; Park, H.D. Co-fermentation of grape must by *Issatchenkia orientalis* and *Saccharomyces cerevisiae* reduces the malic acid content in wine. *Biotechnol. Lett.* **2008**, *30*, 1633–1638. [CrossRef]
37. Varela, C.; Bartel, C.; Espinase Nandorfy, D.; Bilogrevic, E.; Tran, T.; Heinrich, A.; Balzan, T.; Bindon, K.; Borneman, A. Volatile aroma composition and sensory profile of Shiraz and Cabernet Sauvignon wines produced with novel *Metschnikowia pulcherrima* yeast starter cultures. *Aust. J. Grape Wine Res.* **2021**, *27*, 406–418. [CrossRef]
38. Mendes, S.D.C.; Arcari, S.G.; Werner, S.S.; Valente, P.; Ramirez-Castrillon, M. Wild *Saccharomyces* produced differential aromas of fermented Sauvignon Blanc must. *Fermentation* **2022**, *8*, 177. [CrossRef]
39. Uzkuç, N.M.Ç.; Şişli, B.; Ay, M.; Togay, S.Ö.; Yüceer, Y.K.; Bayhan, A.; Toklucu, A.K. Effects of spontaneous fermentation on Karalahna and Cabernet Sauvignon young red wines: Volatile compounds, sensory profiles and identification of autochthonous yeasts. *Eur. Food Res. Technol.* **2020**, *246*, 81–92. [CrossRef]
40. Medina, K.; Boido, E.; Fariña, L.; Gioia, O.; Gomez, M.E.; Barquet, M.; Gaggero, C.; Dellacassa, E.; Carrau, F. Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with *Hanseniaspora vineae*. *Food Chem.* **2013**, *141*, 2513–2521. [CrossRef]
41. Canonico, L.; Agarbati, A.; Comitini, F.; Ciani, M. Assessment of spontaneous fermentation and non-*Saccharomyces* sequential fermentation in Verdicchio wine at winery scale. *Beverages* **2022**, *8*, 49. [CrossRef]
42. Jolly, N.; Augustyn, O.; Pretorius, I. The role and use of non-*Saccharomyces* yeasts in wine production. *S. Afr. J. Enol. Vitic.* **2006**, *27*, 15–39. [CrossRef]
43. Belda, I.; Ruiz, J.; Esteban-Fernández, A.; Navascués, E.; Marquina, D.; Santos, A.; Moreno-Arribas, M.V. Microbial contribution to wine aroma and its intended use for wine quality improvement. *Molecules* **2017**, *22*, 189. [CrossRef]
44. Roudil, L.; Russo, P.; Berbegal, C.; Albertin, W.; Spano, G.; Capozzi, V. Non-*Saccharomyces* commercial starter cultures: Scientific trends, recent patents and innovation in the wine sector. *Recent Pat. Food Nutr. Agric.* **2019**, *10*, 27–39. [CrossRef]
45. Fleet, G.H. Yeast interactions and wine flavour. *Int. J. Food Microbiol.* **2003**, *86*, 11–22. [CrossRef] [PubMed]
46. Ivey, M.; Massel, M.; Phister, T.G. Microbial interactions in food fermentations. *Annu. Rev. Food Sci. Technol.* **2013**, *4*, 141–162. [CrossRef] [PubMed]
47. 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]
48. Nissen, P.; Nielsen, D.; Arneborg, N. Viable *Saccharomyces cerevisiae* cells at high concentrations cause early growth arrest of non-*Saccharomyces* yeasts in mixed cultures by a cell-cell contact-mediated mechanism. *Yeast* **2003**, *20*, 331–341. [CrossRef]
49. Avbelj, M.; Zupan, J.; Raspor, P. Quorum-sensing in yeast and its potential in wine making. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 7841–7852. [CrossRef]

50. González, B.; Vázquez, J.; Morcillo-Parra, M.Á.; Mas, A.; Torija, M.J.; Beltran, G. The production of aromatic alcohols in non-*Saccharomyces* wine yeast is modulated by nutrient availability. *Food Microbiol.* **2018**, *74*, 64–74. [CrossRef]
51. Zupan, J.; Avbelj, M.; Butinar, B.; Kosel, J.; Šergan, M.; Raspor, P. Monitoring of quorum-sensing molecules during mini fermentation studies in wine yeast. *J. Agric. Food Chem.* **2013**, *61*, 2496–2505. [CrossRef]