

## Effect of strains from different *Saccharomyces* species used in different inoculation schemes on chemical composition and sensory characteristics of Sauvignon blanc wine

Maria Dimopoulou<sup>3</sup>, Vicky Troianou<sup>1</sup>, Chrisavgi Toumpeki<sup>1</sup>, Yves Gosselin<sup>2</sup>, Étienne Dorignac<sup>2</sup>, and Yorgos Kotseridis<sup>4</sup>

<sup>1</sup> Innovino Research & Development, Meg.Alexandrou 21, Pallini 15351, Greece

<sup>2</sup> Fermentis, 137 rue Gabriel Péri, 59703 Marcq-En-Baroeul, France

<sup>3</sup> Department of Wine, Vine and Beverage Sciences, School of Food Science, University of West Attica, Greece.

<sup>4</sup> Department of Food Science & Human Nutrition, Laboratory of Oenology, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece

\*Corresponding author: ykotseridis@aua.gr

### ABSTRACT

Enhancing the sensory profile of wine using different microorganisms has always been a challenge in winemaking. The aim of our work was to evaluate the impact of different fermentation schemes by using mixed and pure cultures of three different *Saccharomyces* species at classic and cold inoculation temperatures on wine chemical composition and sensory profile. All the produced wines were analysed for the main oenological parameters (total acidity, volatile acidity, residual sugars, alcohol, and malic acid), as well as for volatile aromatic compounds, such as higher alcohols, acetate esters, ethyl esters and varietal thiols. In addition, implantation monitoring at the strain level and sensory analyses were performed in all fermentation trials. The wines fermented with *S. pastorianus* strains were characterised by significant lower acetic acid production and greater malic acid degradation compared to the wines fermented with *S. cerevisiae* strains. Interestingly, when the inoculation process for all trials was performed at a cold temperature (13 °C), the typical aromas of Sauvignon blanc were stronger than those in the classic inoculation temperature (18 °C) trials. Furthermore, the co-inoculation of *S. pastorianus* and *S. bayanus* resulted in wines exhibiting a higher intensity of citrus fruit notes than the wines fermented with the commercial *S. cerevisiae* strains. These results show that some strains from different *Saccharomyces* species other than *S. cerevisiae*, such as *S. pastorianus* and *S. bayanus*, could be promising starters for Sauvignon blanc wines and their performance can be modulated by the inoculation conditions.

### KEYWORDS

Non-conventional yeast species, Sauvignon blanc, wine typicity, varietal thiols

## INTRODUCTION

*Saccharomyces cerevisiae* is a commonly used wine yeast species which drives alcoholic fermentation, has a high fermentation rate and contributes to the enrichment of wine with positive and attractive aromas. The use of selected strains of *S. cerevisiae* to drive alcoholic fermentation is a technique which has been applied for years to assure fermentation flow and sugar depletion. More recently, it has been observed that, due to global climate change, grapes are starting to accumulate more sugar at harvest - when their technological maturity has been reached - than in the past. This phenomenon could lead to winemaking problems, such as stuck or delayed fermentation, as the yeasts are not yet well-adapted to the new stressful wine environment (Fleet, 2008; Mira de Orduña, 2010). Furthermore, the wine market's demand for wines with increased sensory properties, such as aromatic complexity and varietal typicity, has turned interest towards non-*Saccharomyces* (nSc) and other non-conventional yeast species (Fleet, 2008; Sadoudi *et al.*, 2012; Albertin *et al.*, 2019). The nSc yeasts are characterised by high genetic diversity, and some species and strains can be detrimental to wine quality, while others can be beneficial (Renault *et al.*, 2009; Tempère *et al.*, 2018). Representative genera of the latter category detected in early fermentations are *Metschnikowia*, *Candida*, *Hanseniaspora*, *Rhodotorula* and *Pichia* (König and Berkelmann-Löhnertz, 2017). In spontaneous fermentation, wild yeasts are metabolically active at the beginning of the fermentation process, and their population starts to decline as the ethanol level increases. In order to mimic the natural evolution of this yeast species during the winemaking process, as well as to ensure the quality of the end-product, the selected strains of nSc yeast are used in co-inoculation or sequential inoculation with the *S. cerevisiae* strain (Bely *et al.*, 2008; Ciani *et al.*, 2010; Comitini *et al.*, 2011; Domizio *et al.*, 2011; Benito *et al.*, 2015). Nevertheless, some interactions, including cell to cell conduct, production of antimicrobial molecules and antagonism for nutrients, have been observed; therefore, the compatible co-existence of nSc species with *S. cerevisiae* in winemaking is not easy (Sadoudi *et al.*, 2012; Branco *et al.*, 2015; Englezos *et al.*, 2018, Englezos *et al.*, 2019; Petitgonnet *et al.*, 2019).

Besides *S. cerevisiae*, other *Saccharomyces* species belonging to the *Saccharomyces* strict sensu group can be isolated from natural environments and be used in the production of alcoholic beverages (Rainieri *et al.*, 2003). The nomenclature of the *Saccharomyces* species has been provided by Dujon and Louis (2017). *Saccharomyces* hybrids, like *S. bayanus*, are used in winemaking, because of their interesting oenological properties (e.g., high osmotolerance and cryotolerance) and their contribution to sensory attributes (Libkind *et al.*, 2011; Salvadó *et al.*, 2011; López-Malo *et al.*, 2013). Moreover, the common lager yeast, *S. pastorianus*, a natural hybrid of *S. cerevisiae* and *S. eubayanus* (Martini and Martini, 1987), has been used in the production of a broad range of active compounds responsible for aroma in beer fermentation (He *et al.*, 2014). Nevertheless, little research has been carried out on these non-conventional yeast species in wine fermentation, whether in pure or mixed cultures.

Sauvignon blanc is a French grape variety which is planted all over the world and which expresses varietal aromas of tropical and citrus fruits, as well as green nuances, due to the production of thiols, esters, higher alcohols and methoxy-pyrazines (Tominaga *et al.*, 1998; Dubourdieu *et al.*, 2006; Šuklje *et al.*, 2016). The impact of microorganisms on the Sauvignon blanc aromatic profile has been well-documented (Dubourdieu *et al.*, 2006; Masneuf-Pomarède *et al.*, 2006; Sadoudi *et al.*, 2012; Šuklje *et al.*, 2016; Hart *et al.*, 2017). Most of the research has focused on the enzymatic action of *Saccharomyces* or non-*Saccharomyces* species in relation to the precursors of volatile thiols, cysteinylated or glutathionylated conjugates, which seems to be strain and culture dependent (Dubourdieu *et al.*, 2006; Zott *et al.*, 2011).

This study evaluated the use of three *Saccharomyces* species in different fermentation schemes as a means of modulating Sauvignon blanc varietal typicity. Under-developed strains of *S. pastorianus* and *S. bayanus* were tested in pure and mixed cultures at two inoculation temperatures for the first time. In parallel, commercial *S. cerevisiae* strains were used for the control conditions. The consequences of all the fermentation schemes on volatile compounds and the sensory profiles of the produced wines were analysed. The use of non-conventional yeast species in winemaking processes can

provide new technological opportunities for the wine industry.

## MATERIALS AND METHODS

### 1. Wine fermentations and vinification protocols

80 kg of white grapes (Sauvignon blanc variety, 2018) was crushed and pressed, and 80 mg/L of potassium metabisulfite was added to the resulting must. The must then underwent clarification by cold/gravity settling for 12 hours, after which it was decanted. Almost 50 L of clarified must was prepared and split into 22 bottle fermentors with almost 2 L per bottle. Twenty-two experimental fermentations (11 duplicated trials) were conducted by adding different strains in pure and mixed cultures and using two different inoculation temperatures. For the cold conditions, yeasts were directly inoculated when the temperature was 13 °C, while for the classic conditions, yeasts were inoculated when temperature reached 18 °C. In all cases, the yeasts were inoculated in the must at 1.10<sup>6</sup> CFU/mL. In the case of mixed cultures, a proportion of 70/30 (*S. pastorianus*/*S. bayanus*) was chosen as *S. pastorianus* cells tend to flocculate. Twenty-four hours after yeast addition, 200 mg/L of SpringFerm™ (Fermentis, France) was added. Upon completion of one third of fermentation, 240 mg/L of diammonium phosphate (DAP) and 200 mg/l SpringFerm™ (Fermentis, France) were added. All the strains used were produced by Fermentis, France. The strains and the corresponding codes are shown in Table 1.

### 2. Microbiological analyses

#### 2.1. Microbial growth

Samples for all the fermentation trials were taken at the time of inoculation, after 48 hours, and when two thirds of the sugars were consumed. 1 ml of each sample was transferred to 1.5 ml tubes and the cells were spun down at 4000 rpm for 3 min. The supernatants were discarded and the cell pellet was washed three times with 1 ml sterile distilled water. Finally, the cells were suspended in 1 ml water and serial dilutions were performed on Wallerstein Laboratory Nutrient agar (WLN) and Lysine medium agar for the numeration of total yeast and wild yeast respectively. The incubation of the plates was carried out at 28 °C and 37 °C, as *S. pastorianus* cannot grow above 30 °C. The plates of all

samples were incubated at both temperatures in order to determine the cultivability of all tested species.

#### 2.2. DNA fingerprinting

In order to analyse the DNA profiles of their Ty1 retrotransposon sequence (Ness *et al.*, 1993), the *Saccharomyces* strains were identified during the vinification process using primers  $\delta 12$  (5'-TCAACAATGGAATCCCAAC-3') and  $\delta 2$  (5'-GTGGATTTTATTCCAACA-3') (Legras and Karst, 2003; Schuller *et al.*, 2004; Xufre *et al.*, 2011). As a template for the PCR, a single colony was picked from the dish of interest in aseptic conditions and the colony was suspended in 20  $\mu$ L of 0.02N NaOH. The cell suspension was incubated in 98 °C for 10 min in order to lyse the cells, and 3  $\mu$ L were used in each PCR reaction. Ten (for pure inoculation cultures) and twenty (for mixed inoculation cultures) colonies from WLN plates which had been incubated at room temperature per sample and per time point were tested through PCR reaction with delta12/delta2 primer set. Each PCR reaction was performed in 25  $\mu$ L, containing 1 U of KapaTaq polymerase, 1xBuffer B, 0.2 mM dNTPs, and 800 nM of each primer. The PCR program was carried out as follows: 4 min at 94 °C, 35 cycles of 30 sec at 94 °C, 30 sec at 49 °C and 60 sec at 72 °C, followed by a final extension step of 10 min at 72 °C. As soon as the PCR programme had been completed, 5  $\mu$ L of 6x loading buffer was added per reaction and the

**TABLE 1.** *Saccharomyces* yeast strains in pure and mixed cultures at two inoculation temperatures (classic and cold).

Classic inoculation conditions	
Code	Yeast strains in pure and mixed cultures
Sp1Sb1	<i>S. pastorianus</i> 1 70 % and <i>S. bayanus var bayanus</i> 1 30 %
Sp1	<i>S. pastorianus</i> 1
Sb1	<i>S. bayanus var bayanus</i> 1
Sc1	<i>S. cerevisiae</i> 1
Cold inoculation conditions	
Code	Yeast strains in pure and mixed cultures
CldSp1Sb1	<i>S. pastorianus</i> 1 70 % and <i>S. bayanus</i> 1 30 %
CldSp2Sb1	<i>S. pastorianus</i> 2 70 % and <i>S. bayanus</i> 1 30 %
CldSp1	<i>S. pastorianus</i> 1
CldSp2	<i>S. pastorianus</i> 2
CldSb1	<i>S. bayanus var bayanus</i> 1
CldSc1	<i>S. cerevisiae</i> 1
CldSc2	<i>S. cerevisiae</i> 2

whole reaction volume was loaded onto 2 % agarose gel containing 0.01 % Midori Green in 1xSB buffer (5 mM Sodium borate decahydrate) in order to separate the PCR products. The agarose gel was run at 120 Volt for 35 min in 1xSB buffer. DNA profiles obtained from the analysis of Ty1 retrotransposon elements can be used as genetic markers for polymorphism identification, as these DNA elements differ in number and location depending on the *Saccharomyces* strain (Legras and Karst, 2003). As long as there are interdelta retrotransposons in the genome inherited from *S. cerevisiae*, this method can amplify other species like *S. bayanus* and *S. pastorianus* and thus be used to determine if the strains concerned were present or not. In this study, a Ty1 analysis was performed to distinguish the different strains used, rather than to extract data regarding the origin of each strain.

### 3. Chemical analyses

#### 3.1. Analysis of oenological parameters

The must was immediately analysed for the following parameters: glucose/fructose, total acidity, volatile acidity, pH, L-malic acid, Yeast Assimilable Nitrogen (YAN), free and total SO<sub>2</sub> and turbidity using OIV methods (OIV, 2015).

#### 3.2. Quantification of higher alcohols and esters

Fermentation volatile aromas were analysed by GC–MS using the HeadSpace Solid-Phase Microextraction (HS-SPME) methodology. A volume of 25 ml of wine spiked with the internal standard 25 µg of 3-octanol, was placed in a 40 ml vial, to which 3 g of NaCl and a magnetic stir bar were added. The vial was then sealed with a screw-top cap with a silicon septum and placed on a heated stir plate. The solution was equilibrated by magnetic stirring at 750 rpm for 5 min at 30 °C. The SPME needle was then manually inserted into the vial septum, and the fiber (DVB/CAR/PDMS, 75 µm) was exposed to the headspace of the sample for 30 min at 30 °C. The fiber was then retracted, and the SPME device was removed from the vial and inserted into the GC injector for thermal desorption for 10 min. Analysis was performed using an Agilent 7890A GC, equipped with an Agilent 5873C MS detector. An HP-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was used and the gas carrier was helium with a flow rate of 1 mL/min. The injector and MS-transfer line were maintained at 250 °C and

260 °C respectively. Oven temperature was held at 30 °C for 5 min, then raised to 160 °C at 4 °C/min, and finally to 240 °C at 20 °C/min. The selective ion monitoring (SIM) mode was applied and ions were used as quantifiers: ethyl isobutyrate (m/z 88), ethyl butyrate (m/z 71), ethyl 2 methyl butyrate (m/z 102), isoamyl acetate (m/z 87), isobutyl acetate (m/z 116), 3-octanol (m/z 59), ethyl hexanoate (m/z 88), ethyl octanoate (m/z 88), ethyl decanoate (m/z 157), 2 phenyl ethyl acetate (m/z 104), hexyl acetate, (m/z 69), isoamyl alcohol (m/z 87), 2-methyl-1-propanol (m/z 87), heaxanol-1 (m/z 84), 2-phenylethanol (m/z 122), and 3-(methylthio)-1-propanol (m/z 96).

#### 3.3. Quantification of varietal thiols

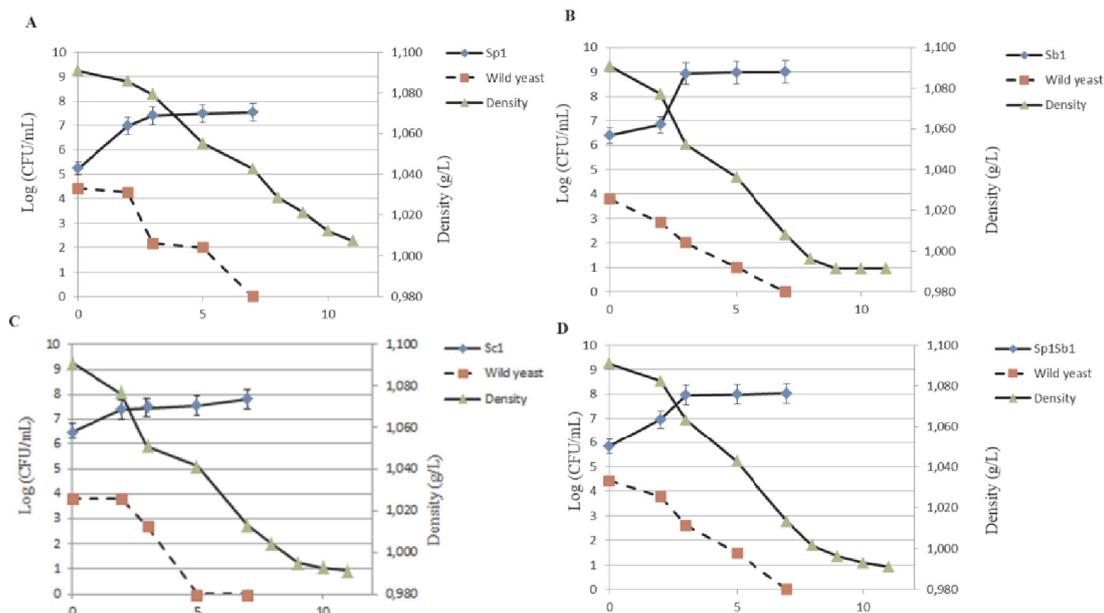
Varietal thiols (4-Methyl-MercaptoPentan-2-one (4MMP), 3-MercaptoHexanol-1 (3MH), and 3-MercaptoHexanol Acetate (3MHA), were quantified using the method described by Tominaga *et al.* (2003).

#### 3.4. Chemical compounds

Absolute ethanol (analytical grade, 99.97 %) and sodium chloride (99 %) were purchased from Scharlab S.A, Barcelona, Spain. Tartaric acid and sodium hydroxide were acquired from VWR-Prolabo, Fontenay-sous-Bois, France. Reference standard grade purity compounds were obtained from commercial sources as follows: 4-methyl-4-mercaptopentan-2-one, 3-mercaptohexanol-1, 3-mercaptohexanol acetate, ethyl isobutyrate, ethyl butyrate, ethyl-2-methyl butyrate, ethyl caproate, ethyl caprylate, ethyl decanoate, isobutyl acetate, isoamyl acetate, hexyl acetate, 2-phenylethyl acetate, isoamyl alcohol, 2-methyl-1-propanol, heaxanol-1, 2-phenylethanol, and 3-(methylthio)-1-propanol from Sigma Aldrich (Saint Louis, USA); and Divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 75 µm) solid phase microextraction fiber from Supelco.

### 4. Sensory analyses

The sensory assessment was carried out by a group of eight trained and experienced judges. The panelists attended four training sessions over a period of two weeks. The training period included a general initial session in which the panelists were served samples for assessment and asked to identify the sensations and aromas perceived. The selected attributes were grouped into two categories: olfactory descriptors (citrus



**FIGURE 1.** Kinetics (density) and yeast population dynamics of laboratory fermentations of Sauvignon blanc must performed by different *Saccharomyces* species. (A) *S. pastorianus* Sp1, (B) *S. bayanus* Sb1, (C) *S. cerevisiae* Sc1 and (D) co-inoculation of *S. pastorianus* Sp1 (70 %) and *S. bayanus* Sb1 (30 %). The population dynamics of the wild yeast is indicated by a dashed line. Values are means of duplicate fermentations.

fruit, herbaceous, tropical fruit, esters and reduction) and gustative descriptors (acidity, balance, body, after-taste and quality level). During the subsequent sessions, the panelists were trained using appropriate solutions. The tests were conducted in individual booths and each sample was served in random order. The panelists were provided with 30 ml of wine in ISO wine glasses, coded with random three-digit numbers at room temperature (18-20 °C). The intensity of the sensory attributes examined was evaluated using a 10-point scale (1: null; 10: very strong).

## 5. Statistical analyses

Volatile compound concentrations (micrograms per liter) were expressed as mean  $\pm$  standard deviation. For each experiment, a first one-way ANOVA was performed using R (R Development Core Team 2010). ANOVA was followed by a Tukey post hoc test to identify differences between groups, using a 95 % confidence interval.

## RESULTS

At the beginning of the study, one yeast strain of each species in pure and mixed cultures, was inoculated at two inoculation temperatures: the classic inoculation temperature (18 °C) and the

cold inoculation temperature (14 °C). As the cold inoculation gave more promising results for the strain of *S. pastorianus*, a second strain of the species was added to test the effect of cold inoculation. In order to present the findings in a more comprehensible way, the Results Section is divided according to the inoculation temperature.

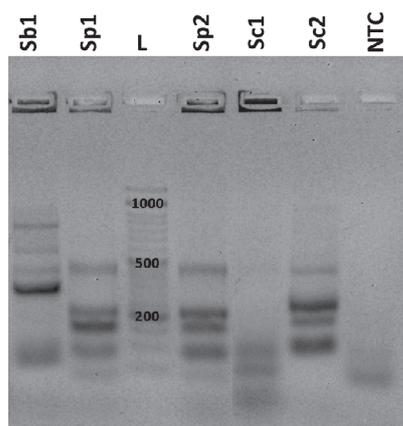
### 1. Yeast population dynamics and fermentative behaviour of pure and mixed cultures of different *Saccharomyces* species in classic inoculation conditions

Fermentations of clarified and slightly sulfited Sauvignon blanc must were conducted at laboratory scale using two different *Saccharomyces* species in pure and mixed cultures. A fermentation trial conducted with a pure culture of *Saccharomyces cerevisiae* was used as a comparative control. The grape must was inoculated with pure cultures of *Saccharomyces pastorianus* strain 1 (Sp1), with pure cultures of *Saccharomyces bayanus* strain 1 (Sb1), with a mixed culture of *S. pastorianus* strain 1 and *S. bayanus* strain 1 in a ratio of 70/30w/w (Sp1Sb1), and with the pure culture of *S. cerevisiae* strain1 (Sc1) (Table 1). All the inoculums of the dry inactivated yeast were first weighed in order to achieve exact ratios of the

strains. They were then activated and the fermentation processes, as well as the population dynamics of the inoculated yeast and the wild yeast, were monitored. The fermentation kinetics was evaluated by monitoring the density of each culture, and the population dynamics was estimated until the first week of fermentation, when at least 70 % of the total alcohol was produced (Figure 1). In addition, the

concentration of glucose and fructose was calculated at the end of the fermentation process in order to confirm the completion of each fermentation trial and to calculate the duration of the process. Oenological parameters were analysed in all final wines (Table 2).

The wild yeast population level was high at the beginning of all fermentation trials ( $4 \pm 0.5$  Log CFU/mL) but started to decrease during the fermentation process (Figure 1). The wild yeast could not be detected after five days in the control culture (Sc1), and after seven days in the other cases, showing that the inoculated strain(s) dominated the fermentation process. The highest population level ( $9 \pm 0.35$  Log CFU/mL) was reached by Sb1 after 72 h. The control entered the stationary phase within the first 38 h, in contrast to the other schemes which reached the stationary phase after 72 h. The Ty1 sequence analysis (Figure 2) for the *S. bayanus* strain in the Sb1Sp1 mode showed that its population was three times higher than the *S. pastorianus* population after 48 h and was slightly elevated at the end of the fermentation, as resulted by the colonies tested. Given that there are more than twice the living cells per g in the dry *S. bayanus* product than in the *S. pastorianus* (data not shown), the initial ratio of inoculation in terms of living cells was about 50 % *S. bayanus* and 50 % *S. pastorianus*; this means that Sb1 took the lead during the fermentation, whereas Sp1 remained until the end of the fermentation.



**FIGURE 2.** Ty1 sequence analysis. DNA profiles of the five *Saccharomyces* strains used in this study using delta12/delta2 primer pairs. Sb1: *S. bayanus*, Sp1: *S. pastorianus* 1, Sp2: *S. pastorianus* 2, Sc1: *S. cerevisiae* 1, Sc2: *S. cerevisiae* 2, NTC: PCR no template control, L: DNA ladder 50 bp (NipponGenetics),

**TABLE 2.** Mean concentration with standard deviation of oenological parameters of Sauvignon blanc wines with different inoculation schemes.

Oenological parameters	Mixed cultures		Pure cultures	
	Sp1Sb1	Sp1	Sb1	Sc1
Fermentation duration (days)	11.0±0ab	16.00±0c	9.0±0a	13.00±0b
pH	3,55±0,2a	3,58±0,1a	3,57±01a	3,5±0a
Total acidity (tartaric acid g/L)	4,7±0,8b	4,81±0,5b	4,79±0,5b	5,47±0,4a
Volatile acidity(acetic acid g/L)	0.13±0.01a	0.09±0.01a	0.1±0a	0.33±0.01b
Glycerol (g/L)	6.15±0.49a	6.25±0.07a	5.8±0.14a	6.15±0.21a
SO2 production (mg/L)	7.0±12.83a	25.0±0b	10.0±2.83a	14.50±0.71a
L-Malic acid degradation (%)	32±0.01b	33±0b	34±0b	22±0.01a
Color intensity (A420nm)	0.09±0b	0.09±0b	0.07±0a	0.07±0a
Alcohol (vol%)	12,85±0a	12,65±0,01a	12,85±0,05a	12,7±0,05a
Fermentation rate (sugars/%vol)	16.88±0a	16.83±0.02a	17.02±0.19ab	17.16±0.01b

Values with different superscript roman letters (ac) in the same row are significantly different according to Tukey’s post hoc test ( $p < 0.05$ ).

Fermentative behaviour was different for all schemes (Table 2). After eight days of fermentation, the remaining sugars were  $16 \pm 1$  g/L for Sp1Sb1,  $72 \pm 0.4$  g/L for Sp1,  $5 \pm 0.3$  g/L for Sb1 and  $19.5 \pm 0.5$  g/L for Sc1. In all cases, the remaining sugars after the first week of fermentation comprised fructose in a proportion of more than 80 % compared to glucose. The duration of the fermentation process was 9 days for Sb1 and 11 days for Sp1Sb1. Both cases were significantly ( $p < 0.05$ ) shorter than the fermentation of Sp1 (16 days). In the control conditions (Sc1), the fermented sugar was consumed within 13 days, showing an intermediate fermentation capacity compared to the tested conditions. All the trials achieved dryness, but differed in fermentation rates. The presence of *S. pastorianus* in either mixed or pure culture significantly decreased the fermentation rate compared to *S. cerevisiae* (control).

In both pure or mixed cultures, the two *Saccharomyces* species, *S. pastorianus* and *S. bayanus*, produced less ( $p < 0.05$ ) acetic acid than the pure culture of *S. cerevisiae*. Even if the level of volatile acidity was not high ( $0.33 \pm 0.01$ ) for the control condition, the tested fermentation schemes with the other *Saccharomyces* species expressed lower levels of production, almost close to zero. Furthermore, these three inoculation modes (Sp1Sb1, Sp1 and Sb1) attained higher levels ( $p < 0.05$ ) of malic

acid degradation than in the control conditions (Sc1).

## 2. Overall production of volatile compounds from pure and mixed cultures of *Saccharomyces* species at classic inoculation temperature

All the tested schemes were significantly different regarding their capacity to release volatile thiols. The best producer was Sp1 which released almost three times more than Sb1 (Table 3). Out of all the tested thiols, 3-mercaptohexanol-1 was found in the highest concentrations, with Sp1 containing the highest concentration ( $877.77 \pm 8.14$  ng/L). Sp1 also produced the highest amounts of 4-methyl-4-mercaptopentan-2-one ( $9.32 \pm 0.5$  ng/L). The control (Sc1) was the second best producer for the three tested volatile thiols. Regarding total higher alcohols, Sp1 contained the most ( $26.07 \pm 0.5$  ng/L) and was significantly different from the control wine. 2-phenylethanol was the alcohol that discriminated the four tested schemes, in which Sp1 produced 4 times more than Sc1. The Sp1Sb1 and Sb1 schemes showed an intermediate production capacity of higher alcohols. The last group of aromatic compounds analysed was the esters. The wine fermented with the mixed culture of *S. pastorianus* and *S. bayanus* contained the greatest amounts of tested esters ( $9.30 \pm 0.75$  mg/L in total). This predominance was mainly due to the high level of isoamyl acetate ( $7.14 \pm 0.57$  mg/L), the

**TABLE 3.** Mean concentrations with standard deviation of volatile compounds at the end of alcoholic fermentation performed with different inoculation schemes.

Oenological parameters	Mixed cultures		Pure cultures	
	Sp1Sb1	Sp1	Sb1	Sc1
Fermentation duration (days)	11.0±0ab	16.00±0c	9.0±0a	13.00±0b
pH	3,55±0,2a	3,58±0,1a	3,57±01a	3,5±0a
Total acidity (tartaric acid g/L)	4,7±0,8b	4,81±0,5b	4,79±0,5b	5,47±0,4a
Volatile acidity(acetic acid g/L)	0.13±0.01a	0.09±0.01a	0.1±0a	0.33±0.01b
Glycerol (g/L)	6.15±0.49a	6.25±0.07a	5.8±0.14a	6.15±0.21a
SO <sub>2</sub> production (mg/L)	7.0±12.83a	25.0±0b	10.0±2.83a	14.50±0.71a
L-Malic acid degradation (%)	32±0.01b	33±0b	34±0b	22±0.01a
Color intensity (A420nm)	0.09±0b	0.09±0b	0.07±0a	0.07±0a
Alcohol (vol%)	12,85±0a	12,65±0,01a	12,85±0,05a	12,7±0,05a
Fermentation rate (sugars/%vol)	16.88±0a	16.83±0.02a	17.02±0.19ab	17.16±0.01b

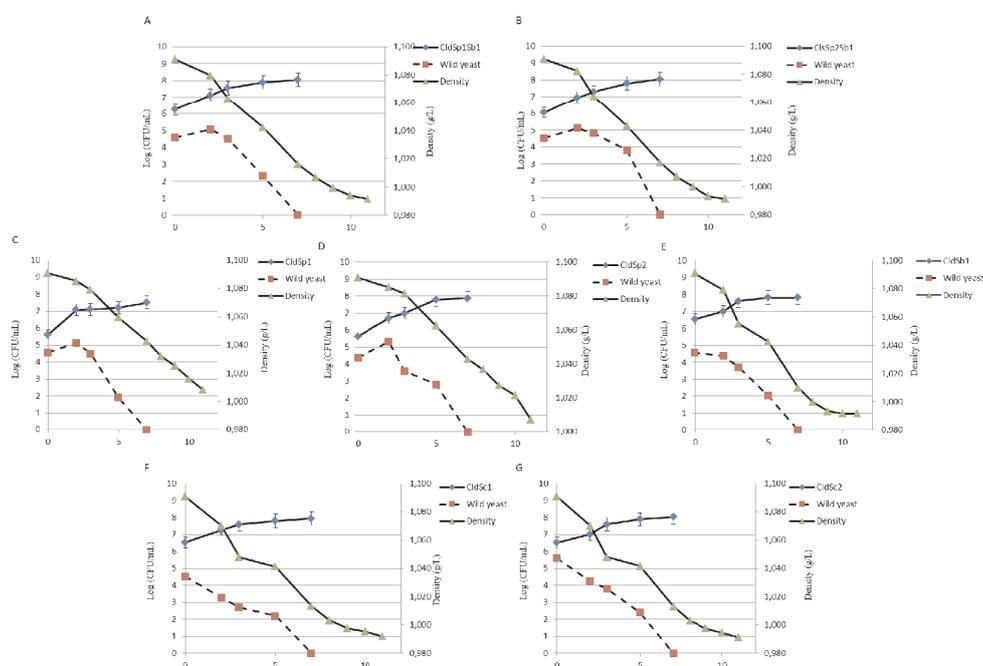
Values with different superscript roman letter (a-d) in the same row are significantly different according to Tukey's post hoc test ( $p < 0.05$ ).

compound representing around 75 % of the total examined esters. *S. pastorianus* performed the least well regarding the production of volatile esters, although it produced two to three times more 2-phenylethyl acetate in our fermentation trials of Sauvignon blanc, which is most certainly linked to its very high production of 2-phenylethanol.

### 3. Yeast population dynamics and fermentative behaviour of pure and mixed cultures of different *Saccharomyces* species under cold inoculation conditions

The laboratory fermentations of Sauvignon blanc must were carried out and analysed as previously described. For this experiment, the inoculation temperature was 13 °C (cold condition), and extra *Saccharomyces* strains were tested in order to examine possible intra-species differentiation. More precisely, a second *S. pastorianus* strain was tested in pure culture (Sp2) and in mixed culture with 30 % *S. bayanus* (Sp2Sb1). A second strain of *S. cerevisiae* in pure culture (Sc2) (Table 1) was used as well. The wild yeast population kinetics was estimated in all the trials. The population dynamics and the fermentative behaviour of the examined schemes are shown in Figure 3 and Table 4.

The results of the Ty1 sequence analysis (Figure 2) show that in both the CldSp1Sb1 and CldSp2Sb1 schemes there was 30-40 % of *S. pastorianus* at the beginning and after 48 h of inoculation, and around 30 % at the end of fermentation; i.e., it was slightly above, but similar to classic inoculation populations, as resulted by microbiological analysis. The tested inoculation schemes showed different fermentation behaviour. Fermentation lasted 11 days in all schemes, except for the two cases of inoculation with pure culture of *S. pastorianus* (CldSp1 and CldSp2) for which the duration was significantly ( $p < 0.05$ ) longer (16 and  $17 \pm 1$  days). In both the CldSp1 and CldSp2 schemes, after 13 days of fermentation the sugar of the medium was only fructose at a concentration of  $10 \pm 3$ g/L, while glucose had been depleted. The first plateau of the *Saccharomyces* yeast was reached by CldSp1 within 48 hours of inoculation, while the highest yeast population was recorded by CldSp1Sb1 ( $8 \pm 1.12$  Log CFU/mL). The *Saccharomyces* yeast dynamics were not really different in the tested schemes. On the other hand, the wild yeast of the initial Sauvignon blanc must followed a different population kinetics depending on the inoculation mode. When the two *S. pastorianus*



**FIGURE 3.** Kinetics (density) and yeast population dynamics of laboratory fermentations of Sauvignon blanc must performed by different *Saccharomyces* species. (A) *S. pastorianus* Sp1, (B) *S. bayanus* Sb1, (C) *S. cerevisiae* Sc1 and (D) co-inoculation of *S. pastorianus* Sp1 (70 %) and *S. bayanus* Sb1 (30 %). The population dynamics of the wild yeast is indicated with a dashed line. Values are means of duplicate fermentations.

**TABLE 4.** Mean concentrations with standard deviation of oenological parameters of Sauvignon blanc wines using different inoculation schemes.

Oenological parameters	Mixed cultures						Pure cultures					
	CldSp1Sb1	CldSp2Sb1	CldSp1	CldSp2	CldSb1	CldSc1	CldSp1Sb1	CldSp2	CldSb1	CldSc2	CldSc1	
Fermentation duration (days)	11.0±0a	11.00±0a	16.00±0b	17.00±1b	11.00±0a	11.00±0a	11.00±0a	17.00±1b	11.00±0a	11.00±0a	11.00±0a	
pH	3.57±0a	3.55±0,2a	3.6±0a	3.66±0,3a	3.55±0a	3.5±0,1a	3.55±0a	3.66±0,3a	3.55±0a	3.5±0,1a	3.5±0a	
Total acidity (tartaric acid g/L)	4.74±0,2b	4.7±0,4b	4.86±0,5b	4.85±0,2b	4.71±0,3b	5.34±0,2a	4.71±0,3b	4.85±0,2b	4.71±0,3b	5.34±0,2a	5.2±0,2a	
Volatile acidity (acetic acid g/L)	0.1±0,01b	0.11±0b	0.01±0,01a	0.00±0a	0.09±0b	0.20±0c	0.09±0b	0.00±0a	0.09±0b	0.20±0c	0.32±0,02d	
Glycerol (g/L)	5.3±0a	5.30±0,14a	5.95±0,07bc	6.15±0,21c	4.95±0,21a	5.15±0,07a	4.95±0,21a	6.15±0,21c	4.95±0,21a	5.15±0,07a	5.45±0,21ab	
SO <sub>2</sub> production (mg/L)	11.0±1,41ab	8.00±0a	21.50±6,36bc	23.50±2,12c	8.00±2,83a	36.00±1,41d	8.00±2,83a	23.50±2,12c	8.00±2,83a	36.00±1,41d	18.00±1,41ac	
L-malic acid degradation (%)	33±0,01c	34±0,01c	36±0,0c	39±0d	35±0c	20±0,01a	35±0c	39±0d	35±0c	20±0,01a	28±0,01b	
Colour intensity (A420 nm)	0.1±0b	0.08±0b	0.09±0b	0.09±0b	0.07±0a	0.07±0b	0.07±0a	0.09±0b	0.07±0a	0.07±0b	0.07±0a	
Alcohol (vol%)	12.75±0a	12.73±0,03a	12.83±0,03a	12.85±0a	12.7±0,05a	12.9±0,05a	12.7±0,05a	12.85±0a	12.7±0,05a	12.9±0,05a	12.6±0a	
Fermentation rate (sugars/% vol)	17.0±0ab	17.05±0,05ab	16.83±0,02a	16.81±0,03a	17.02±0,19ab	16.76±0,07a	17.02±0,19ab	16.81±0,03a	17.02±0,19ab	16.76±0,07a	17.16±0,01b	

Values with different superscript roman letter (a-d) in the same row are significantly different according to Tukey's post hoc test ( $p < 0.05$ ).

strains were present, either in pure or mixed cultures, the wild yeast was able to maintain its high population (average  $5 \pm 1.15$  Log CFU/mL) during the first 48 h of inoculation of the *Saccharomyces* strains, while in the other cases their population lost more than 1 Log CFU/mL. In all cases, the wild yeast completely depleted after one week into the fermentation process.

A "blank" fermentation (not inoculated) was also included in this study. The yeast cells responsible for the fermentation of the inoculated sample were mostly either non-*Saccharomyces* species that did not reveal any DNA profile, or *Saccharomyces* species different to the inoculated species, which gave completely different DNA profiles after analysis of their Tyl1 sequences (data not shown).

The analysis of the oenological parameters in the final wines revealed important differences between the tested inoculation schemes (Table 4). The wines fermented with the two pure cultures of *S. pastorianus* (CldSp1 and CldSp2) had low volatile acidity, with concentrations going as low as zero (acetic acid g/L) and significantly lower ( $p < 0.05$ ) values than the other schemes, especially the two control conditions (CldSc1 and CldSc2). In addition, the levels of glycerol in the final wines increased to as much as  $6.1 \pm 0.14$  g/L in the CldSp1 and CldSp2 trials. The degradation of malic acid was highest (39 %) in CldSp2 and lowest ( $p < 0.05$ ) in CldSc1 and CldSc2 (20 % and 28 % respectively). The fermentation with CldSc2 produced the most SO<sub>2</sub> ( $36 \pm 1.41$  mg/L), four times that in the CldSb1 and CldSp2Sb1 trials.

#### 4. Overall production of volatile compounds of pure and mixed cultures of *Saccharomyces* species under cold inoculation conditions

The seven fermentation schemes under the cold inoculation conditions were also examined for the three categories of aromatic compounds that have an impact on wine sensory characteristics (Table 5). The produced amounts of varietal thiols significantly discriminated all the tested inoculation schemes. The best and the worst producer were the two schemes with *S. cerevisiae*, Sc2 and Sc1, which produced  $1744.95 \pm 25.22$  ng/L and  $748.95 \pm 18.12$  ng/L respectively. The mixed culture with the *S. pastorianus* 2 strain produced 256 ng/L more of total thiol compounds in the final wine than

**TABLE 5.** Mean concentrations with standard deviation of volatile compounds at the end of alcoholic fermentation performed with different inoculation schemes under cold inoculation.

Aromatic compounds	Mixed cultures				Pure cultures		
	CldSp1Sb1	CldSp2Sb1	CldSp1	CldSp2	CldSb1	CldSc2	CldSc1
<b>Thiols (ng/L)</b>							
4-methyl-4-mercaptopentan-2-one	3.97±0.18a	15.18±0.38e	9.25±0.50c	6.58±0.38b	11.10±0.45d	16.50±0.30b	7.00±0.30f
3-mercaptohexanol-1	1020.08±4.88c	1258.56±16.45d	800.87±20.14b	821.29±12.04b	1035.17±12.27c	1677.90±23.91a	693.25±15.92e
3-mercaptohexanol acetate	32.72±1.24ab	36.24±1.55ab	33.69±0.80ab	37.31±1.08ab	41.19±2.09ab	50.55±1.01a	48.70±1.90b
Totalthiols	1056.77±6.3c	1309.98±18.38e	843.81±21.44b	865.18±13.5b	1087.46±14.81d	1744.95±25.22f	748.95±18.12a
<b>Higher alcohols (mg/L)</b>							
Isoamyl alcohol	16.49±0.10bc	16.22±0.48bc	15.05±1.00ab	14.13±0.11a	15.86±0.04ab	17.76±0.30ab	15.43±0.10c
2-methyl-1-butanol	6.33±0.69a	6.63±1.05a	5.63±0.13a	5.49±0.01a	6.54±0.01a	6.83±1.15a	7.15±0.11a
hexanol-1	0.08±0a	0.08±0a	0.08±0a	0.08±0a	0.07±0a	0.08±0a	0.08±0a
2-phenyl ethanol	2.84±0.33bc	2.77±0.09bc	7.87±0.38d	7.11±0.08d	2.25±0ab	3.21±0.09c	1.63±0.06a
3-(methylthio)-1-propanol	0.55±0b	0.57±0.02b	0.44±0.04a	0.42±0.01a	0.61±0.01b	0.45±0.03a	0.46±0a
Totalalcohols	26.28±1.13b	26.26±0.49b	29.08±0.71c	27.23±0.18ac	25.34±0.05a	28.33±0.97bc	24.75±0.16a
<b>Esters (mg/L)</b>							
Isobutyl acetate	0.11±0.01cd	0.12±0d	0.06±0.01a	0.06±0.01a	0.07±0ab	0.09±0.01bc	0.11±0cd
Isoamyl acetate	7.07±0.09b	7.28±0.28b	4.69±0.14a	4.21±0.04a	6.76±0.49b	7.51±0.61b	4.95±0.01a
Hexyl acetate	0.15±0.02a	0.16±0.02a	0.11±0.03a	0.12±0a	0.15±0.02a	0.13±0.01a	0.12±0.01a
2-phenylethyl acetate	0.27±0.05b	0.28±0b	0.64±0.06c	0.61±0.01c	0.22±0.01ab	0.24±0ab	0.15±0.01a
Ethyliso butyrate	0.04±0.03a	0.07±0.01a	0.03±0.02a	0.04±0.03a	0.08±0a	0.08±0.02a	0.12±0.02a
Ethyl butyrate	0.42±0.03bc	0.44±0bc	0.25±0.02a	0.27±0.01a	0.42±0.02bc	0.38±0.01b	0.46±0.02c
Ethyl-2-methyl butyrate	0.05±0.01a	0.05±0.01a	0.09±0b	0.12±0.01c	0.04±0a	0.04±0a	0.05±0a
Ethyl caproate	0.69±0.04a	0.73±0.06a	0.62±0.13a	0.74±0.01a	0.71±0.06a	0.72±0.07a	0.72±0.01a
Ethyl caprylate	0.39±0.02a	0.38±0.02a	0.65±0.35ab	0.97±0.02b	0.37±0a	0.29±0.01a	0.45±0.02ab
Ethyl decanoate	0.16±0.03a	0.13±0.02a	0.12±0.04a	0.14±0.02a	0.16±0.06a	0.10±0.01a	0.13±0.03a
Totalesters	9.34±0.29b	9.64±0.41b	7.27±0.52a	7.28±0.01a	8.97±0.66ab	9.56±0.70b	7.26±0a

Values with different superscript roman letters (a-e) in the same row are significantly different according to Tukey's post hoc test ( $p < 0.05$ ).

the mixed culture with *S. pastorianus* strain 1, while the two pure independent cultures of *S. pastorianus*, Sp1 and Sp2, did not produce significantly different total amounts.

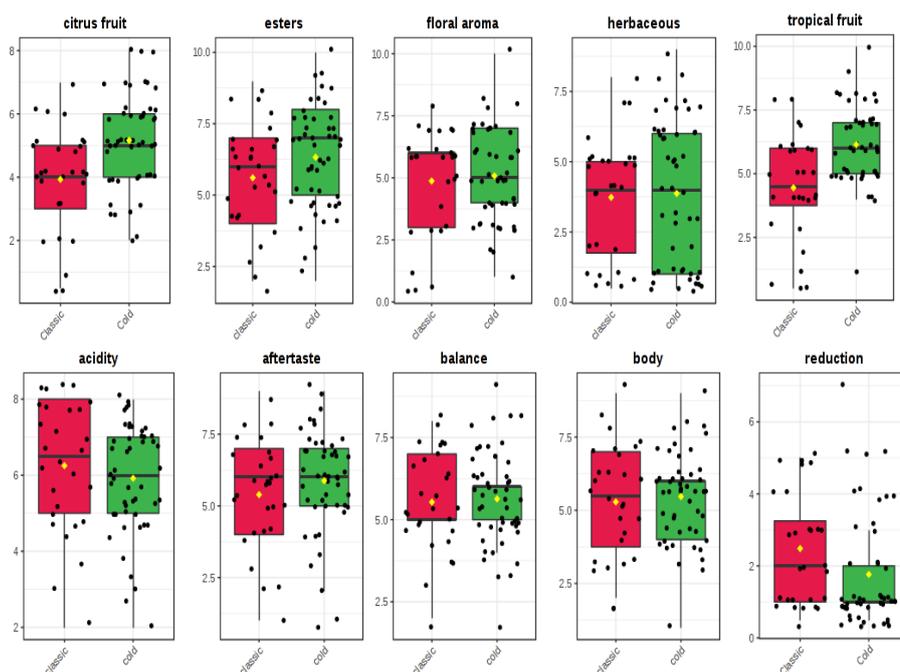
The concentration of higher alcohols in the final wines ranged from  $24.75 \pm 0.16$  mg/L (CldSc1) to  $29.08 \pm 1$  mg/L (CldSp1). The schemes of pure cultures of *S. pastorianus* strains 1 and 2, as well as those of *S. cerevisiae* strains 1 and 2, were significantly different. Conversely, when both strains of *S. pastorianus* were inoculated with the strain of *S. bayanus*, the produced wines did not differ in alcohol level. No differentiation was shown between the different fermentation schemes for both 2-methyl-1-butanol and hexanol-1.

The seven fermentation schemes could be differentiated regarding the concentration of fermentation esters in the final wines. When the

two strains of *S. pastorianus* were inoculated in pure cultures (Sp1 and Sp2), the concentration of the ten ester compounds was significantly lower than the corresponding mixed cultures with *S. bayanus*. More precisely, the strain *S. pastorianus* 1 produced 2.07 mg/L more total esters in the CldSp1Sb1 scheme than in CldSp1, and *S. pastorianus* 2 produced 2.36 mg/L more aromatic esters in the CldSp2Sb1 scheme than in CldSp2. The fermentation of wines with both *S. cerevisiae* strains resulted in significantly different concentrations of fermentation esters being produced.

### 5. Specific trends in wine sensory properties under different inoculation schemes

The Sauvignon blanc wines produced using the different fermentation schemes described above were all evaluated by the sensory expert panel. Figure 4 shows the mean scores of ten sensory



**FIGURE 4.** Box-plots of the sensory evaluation of ten sensory descriptors, shown as the mean values of Sauvignon blanc wines produced using classic and cold inoculation schemes with different *Saccharomyces* species in pure and mixed cultures. Red: classic inoculation conditions. Green: cold inoculation conditions. Median: • in yellow; panel dispersion• in black; whiskers: ±minimum-maximum ratings (without outliers); box: percentiles 25 %-75 %.

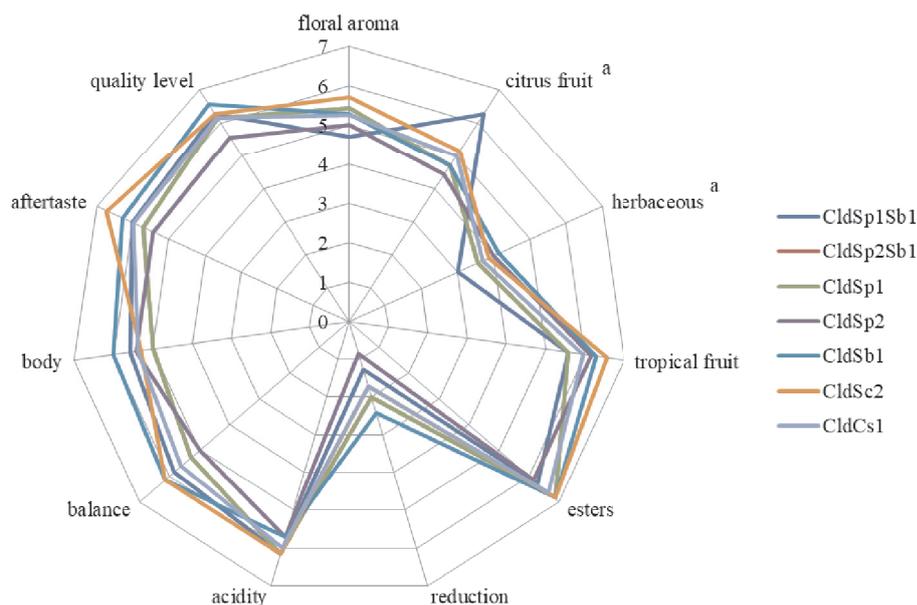
characteristics, with a maximum score of 10, of the wines produced with cold and classic inoculation schemes, either in mixed or pure cultures. The statistical analysis (ANOVA) indicated a significant difference ( $p < 0.05$ ) between the two inoculation schemes for citrus and tropical fruit aromas, as well as for the reduction level of the produced wines. Specifically, when the inoculation temperature was 13 °C (cold), the produced wines fermented using the *Saccharomyces* species expressed the fruity aromas of citrus and tropical fruits more. Furthermore, this fermentation scheme minimised the undesirable notes of wine reduction.

Figure 4 shows the effects of the different fermentation schemes in the cold inoculation conditions on the sensory profile of Sauvignon blanc wines, which were evaluated using ten descriptors. All the fermentation schemes expressed similar profiles, except for the mixed culture of *S. pastorianus* strain 1 co-inoculated with *S. bayanus* strain 1. According to the ANOVA, the wine inoculated with CIdSp1Sb1 showed a significant increase ( $p < 0.05$ ) in notes of citrus fruits and decrease in herbaceous notes

in comparison with all the other fermentation schemes.

## DISCUSSION

Sauvignon blanc has become an international variety with distinctive sensory attributes, which is cultivated all over the world and makes great financial profits for the wine industry. Obtaining high quality wines which will keep their varietal typicity could be directly associated with the microorganisms and the fermentation processes used in winemaking. As most wild yeasts are not able to ferment all the sugars in the must, and as their co-existence with *S. cerevisiae* strains is not always compatible, the use of other species has become much sought-after. Our work investigated the use of non-conventional yeast strains from different *Saccharomyces* species in the production of Sauvignon blanc wine. At the beginning, one strain of *S. pastorianus* and one of *S. bayanus* were chosen to ferment Sauvignon blanc must at the classic inoculation temperature (18 °C). However, the quality of the wines produced using the different fermentation schemes (including a mixture of both strains) was found to compete well with the wine fermented using the commercial *S. cerevisiae*



**FIGURE 5.** Means of the sensory characteristics of the Sauvignon blanc wines produced with different fermentation schemes in cold inoculation conditions.

*Saccharomyces* strains were inoculated in mixed cultures (CldSp1Sb1: 70 % *S. pastorianus* 1 and 30 % *S. bayanus* 1 and CldSp2Sb1: 70 % *S. pastorianus* 2 and 30 % *S. bayanus* 1) as well as in pure cultures (CldSp1: *S. pastorianus* 1, CldSp2: *S. pastorianus* 2, CldSb1: *S. bayanus* 1, CldSc1: *S. cerevisiae* 1, CldSc2: *S. cerevisiae* 2). Significant differences among samples are indicated with a ( $p < 0.05$ ).

strain; the experiment was therefore expanded to include an additional strain of *S. pastorianus* in pure culture and in co-inoculation with *S. pastorianus*, as well as a second control strain of *S. cerevisiae*. Moreover, the inoculation temperature for all the trials was decreased to 13 °C to assess the impact of cold conditions on the aromatic intensity of wine.

Under classic inoculation conditions (18 °C), the mixed culture of *S. pastorianus* and *S. bayanus* were of intermediate oenological interest compared to the wines fermented with the pure cultures of the species. The values for the analysed parameters of the mixed culture were closer to the ones of the pure culture of *S. bayanus*. This was expected as a DNA fingerprint analysis has revealed *S. bayanus* to be predominant in the co-inoculation scheme. For instance, the inoculum ratio has been shown to have a decisive influence on wine characteristics in the case of mixed cultures of *Saccharomyces* and non-*Saccharomyces* species (Domizio *et al.*, 2011; Satora *et al.*, 2018).

Compared to the tested commercial *S. cerevisiae* strain in all the fermentation trials, the presence of both of these non-conventional yeast strains, either in pure or in mixed cultures, contributed

interesting properties to the produced wines, such as significantly lower volatile acidity and medium de-acidification via malic acid degradation. *Torulasporadel brueckii* is the most reported species able to reduce the volatile acidity of wine in mixed cultures with *S. cerevisiae* (Bely *et al.*, 2008; Renault *et al.*, 2009, Ciani *et al.*, 2010; Ciani and Comitini, 2015). *Saccharomyces* stricto sensu strains have been reported to be able to degrade malic acid via the maloethanolic pathway (Redzepovic *et al.*, 2003; Morata *et al.*, 2019). *S. cerevisiae* can hardly metabolise malic acid, especially compared to the relative enzymatic activity of the species of *Schizosaccharomyces pombe*. In contrast with our results, it has been previously reported that *S. bayanus* strain EC1118 can only degrade 8 % of the malic acid during alcoholic fermentation, while *S. pastorianus* can mainly synthesise malic acid rather than degrade it (Redzepovic *et al.*, 2003). Substantially more strains of each species should be tested under various vinification conditions for their ability to perform maloethanolic fermentation.

As far as the control trials were concerned, both *S. cerevisiae* strains exhibited high intra-species variability for almost all the performed analyses, a phenomenon not observed with either of the

*S. pastorianus* strains. Even if our results come from a very low number of tested strains, this observation could be explained by the high genome plasticity of *S. cerevisiae* reflected in the genotypic and phenotypic differentiation between the species (Albertin *et al.*, 2015). Indeed, the species has a remarkable capacity for adaptation to selective pressures deriving mainly from environmental factors (Legras *et al.*, 2018).

Masneuf-Pomarède *et al.* (2006) showed that fermentation temperature plays an essential role in the concentration of released thiols, which for all tested *S. cerevisiae* strain swas found to be higher at a fermentation temperature of 20 °C than that of 13 °C. Moreover, when the development of *S. cerevisiae* was delayed and the wild yeast were dominant in the must of Sauvignon blanc, the concentration of thiols increased, especially that of 3-mercaptohexan-1-ol (3MH) (Zott *et al.*, 2011). However, the effect of inoculation temperature had never been tested before. In our work, we have shown for the first time that a cold inoculation temperature can increase the production of the examined thiols in all tested fermentation schemes with strains from different *Saccharomyces* species. This significant difference between the two inoculation modes was also confirmed by the sensory analyses, with all wines inoculated at cold temperature containing more citrus and tropical fruits notes than the wine inoculated at classic temperature. Additionally, this work is also the first to show that non-conventional yeast species can increase the aromatic typicity of Sauvignon blanc. It would be interesting in the future to study the activity of  $\beta$ -lyase (one of the enzymes involved in the release of thiols (Belda *et al.*, 2016) at different inoculation and fermentation temperatures.

## CONCLUSIONS

In the present study, we investigated the impact of the two non-conventional yeast species, *S. pastorianus* and *S. bayanus*, in mixed and/or pure cultures on the fermentation and sensory profiles of a Sauvignon blanc wine. We evidenced that the applied temperature at the inoculation phase can significantly affect the varietal notes of Sauvignon blanc for all tested fermentation schemes at both the chemical and sensory levels. Furthermore, when the Sauvignon blanc must was co-inoculated with *S. pastorianus* and *S. bayanus* cultures, the produced wines expressed higher citrus fruit

notes. Our results show the promising oenological potential of the non-conventional yeast strains. In the future, it would be interesting to study more strains of *S. bayanus* and *S. pastorianus* in pure or mixed cultures with different inoculum support.

## REFERENCES

- Albertin, W., Masneuf-Pomarède, I., Galeote, V., & Legras, J.-L., (2019). New insights into wine yeast diversities, in Romano, P., Ciani, M., and Fleet, G. H. (Eds.), *Yeasts in the Production of Wine*, New York, NY, Springer, pp. 117–163. doi: 10.1007/978-1-4939-9782-4\_4.
- Albertin, W., Setati, M. E., Miot-Sertier, C., Mostert, T. T., Colonna-Ceccaldi, B., Coulon, J., Girard, P., Moine, V., Pillet, M., Salin, F., Bely, M., Divol, B., & Masneuf-Pomarède, I., (2015). *Hanseniaspora-rouvareum* from winemaking environments show spatial and temporal genetic clustering, *Frontiers in Microbiology*, 6, 1569. doi: 10.3389/fmicb.2015.01569.
- Belda, I., Ruiz, J., Navascués, E., Marquina, D., & Santos, A., (2016). Improvement of aromatic thiol release through the selection of yeasts with increased  $\beta$ -lyase activity, *International Journal of Food Microbiology*, 225, 1–8. doi: 10.1016/j.ijfoodmicro.2016.03.001.
- Bely, M., Stoeckle, P., Masneuf-Pomarède, I., & Dubourdieu, D., (2008). Impact of mixed *Torulasporadelbrueckii-Saccharomyces cerevisiae* culture on high-sugar fermentation, *International Journal of Food Microbiology*, 122, 3, 312–320. doi: 10.1016/j.ijfoodmicro.2007.12.023.
- Benito, S., Hofmann, T., Laier, M., Lochbühler, B., Schüttler, A., Ebert, K., Fritsch, S., Röcker, J., & Rauhut, D., (2015). Effect on quality and composition of Riesling wines fermented by sequential inoculation with non-*Saccharomyces* and *Saccharomyces cerevisiae*, *European Food Research and Technology*, 241, 5, 707–717. doi: 10.1007/s00217-015-2497-8.
- Branco, P., Viana, T., Albergaria, H., & Arneborg, N., (2015). Antimicrobial peptides (AMPs) produced by *Saccharomyces cerevisiae* induce alterations in the intracellular pH, membrane permeability and culturability of *Hanseniaspora-guilliermondii* cells, *International Journal of Food Microbiology*, 205, 112–118. doi: 10.1016/j.ijfoodmicro.2015.04.015.
- Ciani, M. & Comitini, F., (2015). Yeast interactions in multi-starter wine fermentation, *Current Opinion in Food Science*, (Food Chemistry and Biochemistry - Food Bioprocessing), 1, 1–6. doi: 10.1016/j.cofs.2014.07.001.

- Ciani, M., Comitini, F., Mannazzu, I. & Domizio, P., (2010). Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking, *FEMS yeast research*, 10, 2, 123–133. doi: 10.1111/j.1567-1364.2009.00579.x.
- Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I. & Ciani, M., (2011). Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*, *Food Microbiology*, 28, 5, 873–882. doi: 10.1016/j.fm.2010.12.001.
- Domizio, P., Romani, C., Lencioni, L., Comitini, F., Gobbi, M., Mannazzu, I. & Ciani, M., (2011). 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, *International Journal of Food Microbiology*, 147, 3, 170–180. doi: 10.1016/j.ijfoodmicro.2011.03.020.
- Dubourdieu, D., Tominaga, T., Masneuf, I., Gachons, C. P. des & Murat, M. L., (2006). The Role of Yeasts in Grape Flavor Development during Fermentation: The Example of Sauvignon blanc, *American Journal of Enology and Viticulture*, 57, 1, 81–88.
- Dujon, B.A., & Louis, E.J., (2017). Genome Diversity and Evolution in the Budding Yeasts (*Saccharomycotina*), *Genetics*, 206, 717–750. doi: 10.1534/genetics.116.199216.
- Englezos, V., Cocolin, L., Rantsiou, K., Ortiz-Julien, A., Bloem, A., Dequin, S., & Camarasa, C., (2018). Specific phenotypic traits of *Starmere-llabacillaris* related to nitrogen source consumption and central carbon metabolite production during wine Fermentation, *Applied and Environmental Microbiology*, 84, 16. doi: 10.1128/AEM.00797-18.
- Englezos, V., Rantsiou, K., Giacosa, S., Río Segade, S., Rolle, L., & Cocolin, L., (2019). Cell-to-cell contact mechanism modulates *Starmere-llabacillaris* death in mixed culture fermentations with *Saccharomyces cerevisiae*, *International Journal of Food Microbiology*, 289, 106–114. doi: 10.1016/j.ijfoodmicro.2018.09.009.
- Fleet, G. H., (2008). Wine yeasts for the future, *FEMS Yeast Research*, 8, 7, 979–995. doi: 10.1111/j.1567-1364.2008.00427.x.
- Hart, R. S., Ndimba, B. K., & Jolly, N. P., (2017). Characterisation of thiol-releasing and lower volatile acidity forming intra-genus hybrid yeast strains for Sauvignon blanc Wine, *South African Journal of Enology and Viticulture*, 38, 2, 144–155.
- He, Y., Dong, J., Yin, H., Chen, P., Lin, H., & Chen, L., (2014). Monitoring of the production of flavour compounds by analysis of the gene transcription involved in higher alcohol and ester formation by the brewer's yeast *Saccharomyces pastorianus* using a multiplex RT-qPCR assay, *Journal of the Institute of Brewing*, 120, 2, 119–126. doi: 10.1002/jib.120.
- König, H., & Berkelmann-Löhnertz, B., (2017). Maintenance of wine-Associated Microorganisms, in König, H., Uden, G., and Fröhlich, J. (Eds.), *Biology of microorganisms on grapes, in must and in wine*, Cham, Springer International Publishing, pp. 549–571. doi: 10.1007/978-3-319-60021-5\_23.
- Legras, J.-L., Galeote, V., Bigey, F., Camarasa, C., Marsit, S., Nidelet, T., Sanchez, I., Couloux, A., Guy, J., Franco-Duarte, R., Marcet-Houben, M., Gabaldon, T., Schuller, D., Sampaio, J. P., & Dequin, S., (2018). Adaptation of *S. cerevisiae* to fermented food environments reveals remarkable genome plasticity and the footprints of domestication, *Molecular Biology and Evolution*, Edited by P. Wittkopp, 35, 7, 1712–1727. doi: 10.1093/molbev/msy066.
- Legras, J.-L., & Karst, F., (2003). Optimisation of interdelta analysis for *Saccharomyces cerevisiae* strain characterisation, *FEMS microbiology letters*, 221, 2, 249–255. doi: 10.1016/S0378-1097(03)00205-2.
- Libkind, D., Hittinger, C. T., Valério, E., Gonçalves, C., Dover, J., Johnston, M., Gonçalves, P., & Sampaio, J. P., (2011). Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast, *Proceedings of the National Academy of Sciences of the United States of America*, 108, 35, 14539–14544. doi: 10.1073/pnas.1105430108.
- López-Malo, M., Querol, A., & Guillamon, J. M., (2013). Metabolomic comparison of *Saccharomyces cerevisiae* and the cryotolerant species *S. bayanus* var. *uvarum* and *S. kudriavzevii* during wine fermentation at low temperature, *PLoS ONE*, 8, 3. doi: 10.1371/journal.pone.0060135.
- Martini, A. V., & Martini, A., (1987). Three newly delimited species of *Saccharomyces sensu stricto*, *Antonie Van Leeuwenhoek*, 53, 2, 77–84. doi: 10.1007/bf00419503.
- Masneuf-Pomarède, I., Mansour, C., Murat, M.-L., Tominaga, T., & Dubourdieu, D., (2006). Influence of fermentation temperature on volatile thiols concentrations in Sauvignon blanc wines, *International Journal of Food Microbiology*, 108, 3, 385–390. doi: 10.1016/j.ijfoodmicro.2006.01.001.
- Mira de Orduña, R., (2010). Climate change associated effects on grape and wine quality and production, *Food Research International*, (Climate Change and Food Science), 43, 7, 1844–1855. doi: 10.1016/j.foodres.2010.05.001.
- Morata, A., Escott, C., Bañuelos, M. A., Loira, I., Fresno, J. M. D., González, C., & Suárez-Lepe, J. A., (2019). Contribution of con-*Saccharomyces* yeasts to wine freshness. A Review, *Biomolecules*, 10, 1. doi: 10.3390/biom10010034.

- Ness, F., Lavallée, F., Dubourdieu, D., Aigle, & M., Dulau, L., (1993). Identification of yeast strains using the polymerase chain reaction, *Journal of the Science of Food and Agriculture*, 62, 89–94. doi: 10.1002/jsfa.2740620113.
- OIV (2015). Oenological practices: Wines. oiv.int.
- Petitgonnet, C., Klein, G. L., Roullier-Gall, C., Schmitt-Kopplin, P., Quintanilla-Casas, B., Vichi, S., Julien-David, D., & Alexandre, H., (2019). Influence of cell-cell contact between *L. thermotolerans* and *S. cerevisiae* on yeast interactions and the exometabolome, *Food Microbiology*, 83, 122–133. doi: 10.1016/j.fm.2019.05.005.
- Rainieri, S., Zambonelli, C. & Kaneko, Y., (2003). *Saccharomyces sensu stricto* : Systematics, Genetic Diversity and Evolution. *J Biosci Bioeng* 96, 1–9. doi.org/10.1016/S1389-1723(03)900 89-2.
- Redzepovic, S., Orlic, S., Majdak, A., Kozina, B., Volschenk, H., & Viljoen-Bloom, M., (2003). Differential malic acid degradation by selected strains of *Saccharomyces* during alcoholic fermentation, *International Journal of Food Microbiology*, 83, 1, 49–61. doi: 10.1016/s0168-1605(02)00320-3.
- Renault, P., Miot-Sertier, C., Marullo, P., Hernández-Orte, P., Lagarrigue, L., Lonvaud-Funel, A., & Bely, M., (2009). Genetic characterization and phenotypic variability in *Torulasporadelbrueckii* species: Potential applications in the wine industry, *International Journal of Food Microbiology*, 134, 3, 201–210. doi: 10.1016/j.ijfoodmicro.2009.06.008.
- Sadoudi, M., Tourdot-Maréchal, R., Rousseaux, S., Steyer, D., Gallardo-Chacón, J.-J., Ballester, J., Vichi, S., Guérin-Schneider, R., Caixach, J., & Alexandre, H., (2012). 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 Microbiology*, 32, 2, 243–253. doi: 10.1016/j.fm.2012.06.006.
- Salvadó, Z., Arroyo-López, F. N., Guillamón, J. M., Salazar, G., Querol, A., & Barrio, E., (2011). Temperature adaptation markedly determines evolution within the genus *Saccharomyces*, *Applied and Environmental Microbiology*, 77, 7, 2292–2302. doi: 10.1128/AEM.01861-10.
- Satora, P., Semik-Szczurak, D., Tarko, T., & Bułdys, A., (2018). Influence of selected *Saccharomyces* and *Schizosaccharomyces* strains and their mixed cultures on chemical composition of apple wines, *Journal of Food Science*, 83, 2, 424–431. doi: 10.1111/1750-3841.14042.
- Schuller, D., Valero, E., Dequin, S., & Casal, M., (2004). Survey of molecular methods for the typing of wine yeast strains, *FEMS Microbiology Letters*, 231, 19–26. doi:10.1016/S0378-1097(03)00928-5
- Šuklje, K., Antalick, G., Buica, A., Coetzee, Z. A., Brand, J., Schmidtke, L. M., & Vivier, M. A., (2016). Inactive dry yeast application on grapes modify Sauvignon blanc wine aroma, *Food Chemistry*, 197, 1073–1084. doi: 10.1016/j.foodchem.2015.11.105.
- Tempère, S., Marchal, A., Barbe, J.-C., Bely, M., Masneuf-Pomarède, I., Marullo, P., & Albertin, W., (2018). The complexity of wine: clarifying the role of microorganisms, *Applied Microbiology and Biotechnology*, 102, 9, 3995–4007. doi: 10.1007/s00253-018-8914-8.
- Tominaga, T., Murat, M.-L., & Dubourdieu, D., (1998). Development of a method for analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. Cv. Sauvignon Blanc, *Journal of Agricultural and Food Chemistry*, 46, 3, 1044–1048. doi: 10.1021/jf970782o.
- Xufre, A., Albergaria, H., Gírio, F., & Spencer-Martins I., (2011). Use of interdelta polymorphisms of *Saccharomyces cerevisiae* strains to monitor population evolution during wine fermentation, *Journal of Industrial Microbiology and Biotechnology*, 38, 127–132. doi:10.1007/s10295-010-0837-z.
- Zott, K., Thibon, C., Bely, M., Lonvaud-Funel, A., Dubourdieu, D. & Masneuf-Pomarède, I., (2011). The grape must non-*Saccharomyces* microbial community: Impact on volatile thiol release, *International Journal of Food Microbiology*, 151, 2, 210–215. doi: 10.1016/j.ijfoodmicro.2011.08.026.