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Enhancing the aromatic profile and sensory properties of wine via interactions between *Zygosaccharomyces bailii* and *Saccharomyces cerevisiae*

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ABSTRACT

Zygosaccharomyces bailii is a non-conventional wine yeast, traditionally recognized for its spoilage potential in food and beverages. However, strain-level variability within this species presents an opportunity to identify efficient, non-spoilage strains with promising applications as wine starter cultures. The aim of this study was to investigate the interaction between indigenous strains of *Saccharomyces cerevisiae* and *Z. bailii* used as fermentation starters within the wine environment, emphasizing their impact on the aromatic profile and sensory characteristics of the final product. In an initial fermentation assay, pure and mixed cultures of *Z. bailii* and two *S. cerevisiae* strains were separately inoculated, while fermentation kinetics and population dynamics were monitored. Furthermore, in order to evaluate the impact of different inoculation strategies, the most efficient *S. cerevisiae* strain was selected for a second fermentation assay, where sequential and simultaneous co-inoculations were compared to pure cultures of *Z. bailii* and *S. cerevisiae*. Fermentation kinetics, microbial dynamics, and oenological properties were analyzed. The wine's aromatic profile was assessed via Gas Chromatography-Mass Spectrometry (GC-MS), while sensory analysis provided a holistic evaluation of inoculation modality effect. Overall, the mixed cultures of *Z. bailii* and *S. cerevisiae* enhanced the fermentation rate and promoted the growth of *Z. bailii* population, suggesting cooperative behavior. Moreover, *Z. bailii* revealed interesting oenological properties such as, tolerance to alcohol, fructophilic behavior, ability to consume malic acid, and liberation of varietal thiols. In conclusion, co-inoculation modality proved to be the most effective inoculation strategy, producing wines with enhanced citrus, passion fruit, and floral notes.

1. Introduction

Microorganisms are essential contributors for the final chemical and sensory composition of wine (Barata et al., 2012; Comitini et al., 2017; Fleet, 2003; Ribéreau-Gayon et al., 2006; Romano et al., 2019). The first important microbial step of winemaking, alcoholic fermentation (AF), is a complex process where sugars, primarily glucose and fructose, are transformed into ethanol, carbon dioxide and a plethora of secondary metabolites. Spontaneous AF involves a dynamic succession of yeast communities and intricate interactions that influence wine's final aromatic and sensory profile (Fleet, 2003; Querol et al., 2018; Ribéreau-Gayon et al., 2006; Swiegers and Pretorius, 2005). Initially, non-*Saccharomyces* yeast mostly from the *Hanseniaspora* and *Candida* genera dominate, comprising up to 90 % of the total yeast population. Key species at this stage include *Hanseniaspora uvarum*, *Candida stellata*,

and *Starmerella bacillaris*, while *Metschnikowia pulcherrima* may also appear in significant but less consistent abundance. Additionally, various minor species such as *Torulaspota delbrueckii*, *Pichia kluyveri*, *Hanseniaspora guilliermondii*, *Issatchenkia orientalis*, *Zygosaccharomyces bailii*, and *Pichia anomala* have been identified. As fermentation progresses, *S. cerevisiae* rapidly becomes predominant due to its superior ethanol tolerance and competitive advantage, which enable this species to efficiently drive and finalize the fermentation process (Dimopoulou et al., 2023; Jolly et al., 2006; Povhe Jemec et al., 2001; Zott et al., 2010). Inoculation with selected *S. cerevisiae* strains is the most well-established strategy to ensure the normal progression and completion of AF (Bely et al., 2008; Á. Benito et al., 2015; Ciani and Comitini, 2015; Dimopoulou et al., 2020; Marullo and Dubourdieu, 2010). Although *S. cerevisiae* remains the main microorganism involved in AF, interest in the use of non-*Saccharomyces* yeasts has grown due to

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their potential to enhance aromatic complexity and impart desirable sensory attributes to the produced wines (Albertin et al., 2017; Jolly et al., 2014; Padilla et al., 2016; Tofalo et al., 2022; Englezos et al., 2022; Tzamourani et al., 2024a). Whatsoever, the use of non-*Saccharomyces* wine yeasts as fermentation starters has demonstrated both advantages and disadvantages. In the past, non-*Saccharomyces* yeasts were considered to be of secondary importance or as spoilage species (Padilla et al., 2016). Actually, these yeasts may produce high levels of compounds such as acetic acid, ethyl acetate, acetaldehyde, exhibit low fermentation capacity, slow fermentation rates and poor resistance to SO₂. These traits are typically considered to be negative attributes for starter cultures (Comitini et al., 2011; Domizio, et al., 2011; Marullo and Dubourdieu, 2010; Tzamourani et al., 2023). However, it is now well-established that, through careful selection and screening, specific non-*Saccharomyces* strains can play a beneficial role in winemaking (Domizio et al., 2011; Mas and Portillo, 2022; Padilla et al., 2016; Sadoudi et al., 2012; Zott et al., 2011). Recent studies highlight their contributions to the metabolic profile and aroma complexity of the final wine product (Domizio et al., 2011; Sadoudi et al., 2012; Renault et al., 2015, 2016; González, 2018; Benito et al., 2019; Binati et al., 2019; Pourcelot et al., 2023; Chasseriaud et al., 2023). However, due to their lower fermentative capacity, non-*Saccharomyces* yeasts with desirable biotechnological traits are usually inoculated in combination with *S. cerevisiae* as mixed starter cultures.

Among non-conventional yeasts, *Z. bailii* is a non-*Saccharomyces* yeast species traditionally recognized as a spoilage microorganism, due to elevated volatile acidity production during AF (Romano et al., 2019), remarkable resistance to common wine preservatives like sulfur dioxide, and a general tolerance to stresses (Domizio et al., 2011; Escott et al., 2018; Kuanyshhev et al., 2021; Martorell et al., 2007; Rodrigues et al., 2012; Schuller et al., 2000; Thomas and Davenport, 1985). However, the promising role of certain *Z. bailii* strains was recently highlighted in the winemaking of the white Greek variety Assyrtiko (Tzamourani et al., 2024b), showing that the spoilage effect of the species is highly strain dependent. By the same means, *Z. bailii* strains have also been proposed as alternative or complementary starter cultures to *S. cerevisiae* in winemaking and brewing (Capece et al., 2022; Garavaglia et al., 2015; Solieri, 2021).

Different *S. cerevisiae* and *Z. bailii* have been previously isolated from the same sample of various spontaneously fermented wines from the Greek terroir, all exhibiting no off-flavor characteristics (Tzamourani et al., 2023). This observation suggests a potentially beneficial co-existence between the two species, which has not yet been investigated. The objective of the current study is to explore the opportunity to combine indigenous strains of *S. cerevisiae* and *Z. bailii* and to assess their impact on wine's aromatic profile and sensory properties. Fermentation kinetics, microbial dynamics analyses, chemical composition, oenological properties and sensory attributes of wines obtained after co-inoculation and sequential inoculation will be carefully examined and discussed.

2. Material and methods

2.1. Yeast strains and pre-cultures

In this study, two *S. cerevisiae* strains (S10-A6Y10 and S14-K33Y14) and one *Z. bailii* strain (Zb-A19Y5), previously isolated from Greek wines in the end of AF, were used (Tzamourani et al., 2023). The strains were preserved at -20 °C in YPD broth containing 30 % glycerol (composition per liter: 10 g yeast extract, 20 g bacteriological peptone, 20 g dextrose). Before starting the experiments, the cultures were incubated on YPD agar at 28 °C for 48 h. Afterwards, yeasts were pre-cultured in Erlenmeyer flasks filled with half diluted grape juice at 24 °C for 24 h for both yeast strains. The precultures were used to inoculate the fermentations. Cell concentration and viability were determined using flow cytometry with a Cell Lab Quanta™ (Beckman Coulter, Brea, CA, USA), featuring

a 488 nm laser. Samples were diluted in Mc Ilvain buffer (0.2 M sodium phosphate, 0.1 M citric acid, pH = 4) with 0.3 % (v/v) propidium iodide, which stains dead cells.

2.2. Fermentation medium

The medium for laboratory fermentations was a commercial Ugni-blanc grape juice (Nos Régions ont du Talent, France), with a pH of 3.5, a sugar concentration of 210 g/L, and available nitrogen adjusted to and available nitrogen adjusted to 200 mg N/L using an amino acid stock solution (Marullo et al., 2006) (amino acids: 105 mg N/L) and ammonium phosphate ((NH₄)₂SO₄) (ammonia: 95 mg N/L). The malic acid concentration was 2.6 g/L. Prior to yeast inoculation, the must was sterilized by filtration using a 0.45 µm nitrate cellulose membrane (Millipore, Molsheim, France).

2.3. Fermentation assays and kinetics

First five different modalities were conducted in 150 ml sterile bioreactors filled with 125 mL of juice at 18 °C under controlled constant stirring (300 rpm): i) pure culture of *Z. bailii* strain Zb-A19Y5, ii) pure culture of *S. cerevisiae* strain S10-A6Y10, iii) pure culture of *S. cerevisiae* strain S14-K33Y14, iv) co-culture of *Z. bailii* strain Zb-A19Y5 and *S. cerevisiae* strain S10-A6Y10 and v) co-culture of *Z. bailii* strain Zb-A19Y5 and *S. cerevisiae* strain S14-K33Y14. In all trials, both the single and mixed cultures were inoculated at a concentration of 10⁶ viable cells/mL. Table 1 and Fig. 1 illustrate the coding of inoculation modalities and outline the experimental design of this study respectively.

Additionally, in larger bioreactors of 410 mL filled with 375 ml of juice, two fermentations with pure cultures Zb-A19Y5 and *S. cerevisiae* S10-A6Y10 and two with mixed cultures, were performed at 18°C under controlled constant stirring (300 rpm). The mixed cultures included two types: a simultaneous mixed modality, where Zb-A19Y5 and *S. cerevisiae* were co-inoculated, and a sequential mixed modality, where *Z. bailii* was inoculated 30 h before *S. cerevisiae*. Both pure and mixed cultures were inoculated with 1 × 10⁶ viable cells/mL of each species (Fig. 1).

All experiments were conducted in triplicate. Fermentations kinetics were monitored by weighing the bioreactors twice daily or more. The CO₂ released (g/L), CO₂ production rate (V) (g/L/h) and other fermentation parameters (lag phase, V_{max}, time at V_{max}, fermentation time, CO₂ max.) were calculated by R software (R Development Core Team, version 4.0.5). Evaporation-related weight loss was below 2 %.

2.4. Population dynamics

Samples were withdrawn throughout fermentation and serially diluted appropriately. Yeast growth in single cultures was assessed using plating onto YPD agar medium. Plates were incubated at 28 °C for 2 days before colony enumeration (CFU/mL). In mixed cultures, yeast growth was monitored by plating onto two different agar media. Each analysis was conducted twice. In order to differentiate *S. cerevisiae* and *Z. bailii*, a selective agar medium (ZM) was modified, with the following composition per liter: 40 g TSA, 10 g fructose, 10 g dextrose, 5 g yeast extract, and 5 g agar (Schuller et al., 2000). After sterilization, the medium was

Table 1
Inoculation modalities and coding assessed in the present study.

Code	Inoculation modalities
S1	Monoculture <i>S. cerevisiae</i> S6-A6Y10
S2	Monoculture <i>S. cerevisiae</i> S14-K33Y14
Z	Monoculture <i>Z. bailii</i> Zb-A19Y5
S1/Z	Simultaneous co-inoculation of <i>S. cerevisiae</i> S6-A6Y10 and <i>Z. bailii</i> Zb-A19Y5
S2/Z	Simultaneous co-inoculation of <i>S. cerevisiae</i> S14-K33Y14 and <i>Z. bailii</i> Zb-A19Y5
Z + S1	Sequential inoculation of <i>Z. bailii</i> Zb-A19Y5 and <i>S. cerevisiae</i> S6-A6Y10

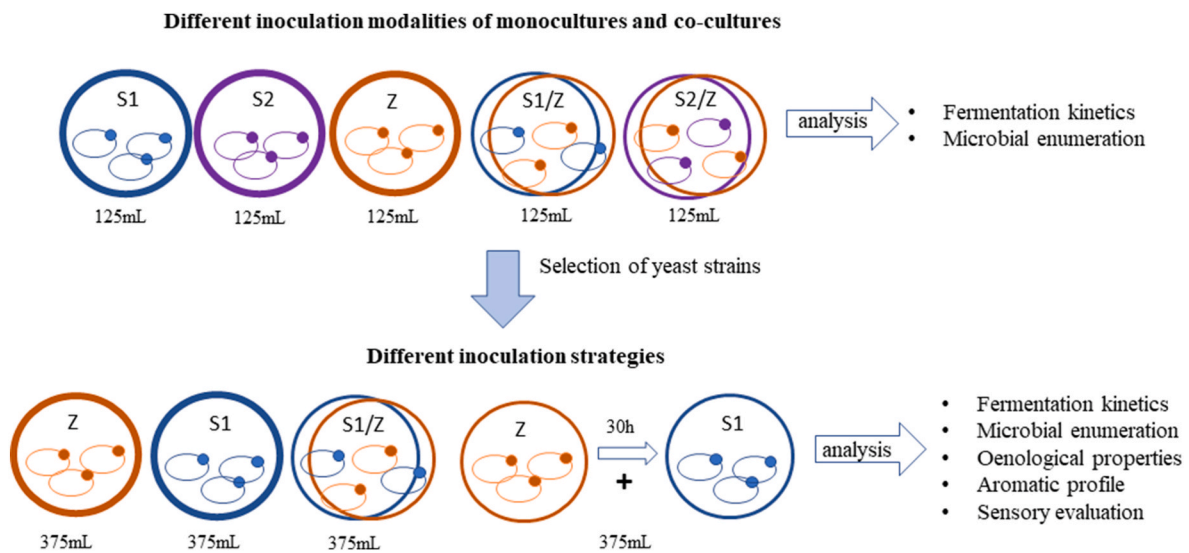


Fig. 1. Schematic overflow of the experimental design.

supplemented with 0.05 % (v/v) filtered formic acid and 0.5 mg/L bromocresol green (pH = 4.6). *S. cerevisiae* and *Z. bailii* colonies developed as green and blue, respectively, after 2 days of incubation at 28 °C. The microbiological analysis of the filtered grape juice showed no viable, culturable cells on YPD and MRS (Difco, France) agar, confirming the effectiveness of sterilization. Furthermore, no bacterial growth was detected during the final stage of fermentation, indicating the absence of bacterial communities. All data correspond to the average values recovered from triplicate fermentations.

2.5. Chemical analysis

2.5.1. Classical oenological parameters

The concentrations of malic acid, fructose, and glucose at the end of AF were determined using an automated enzymatic analyzer (Rhoche-Biopharm, Germany). pH measurements were performed with a pH meter (HI2210, Hanna Instruments, Smithfield, VA, USA). Ethanol concentrations (% vol.) and volatile acidity (expressed as g acetic acid/L) were measured using Fourier transform infrared spectroscopy (FT-IR) (Foss France, Nanterre). These analyses were conducted at the Oenoteam laboratory (Libourne, France).

Carbohydrates, lactic acid, acetic acid, glycerol, succinate and trehalose concentrations in the supernatant were quantified by anion exchange chromatography (Aminex HPX87H column, Bio-Rad) using a Waters system (Milford, USA), comprising a Waters 600 pump, Waters 717 injector, and Waters 2414 refractometer. The eluent used was 5 mM H₂SO₄ at a constant flow rate of 0.5 mL/min at room temperature.

2.5.2. Volatile compounds analysis

2.5.2.1. Ester and higher alcohol analysis. Ester concentrations were measured using headspace solid-phase microextraction (HS-SPME) followed by Gas Chromatography-Mass Spectrometry (GC-MS), as described (Antalick et al., 2010). Briefly, a polydimethylsiloxane (PDMS-100) coated fiber was used for extraction, and internal standards were added to the samples. The mixture was prepared in vials with sodium chloride, sealed, and processed in an autosampler for extraction and desorption. GC-MS analysis was performed using an Agilent system under specified conditions, including a temperature program and helium as the carrier gas. Six major higher alcohols and thirty-two esters were identified by comparing their retention indices and mass spectra with known standards.

2.5.2.2. Thiols analysis. Volatile thiol quantification was performed as described previously (Thibon et al., 2024). 4MSP, 3SH, and 3SHA were extracted by this method which represents an optimization and automation of the one described by Thibon et al. (2008).

2.6. Sensory descriptive analysis

2.6.1. General conditions

The samples were evaluated at controlled room temperature (20 °C), in individual booths, using covered ISO glasses (ISO 3591, 1977), containing about 15 mL of the produced wines. The samples were labeled with random three-digit codes to ensure anonymity and arranged in a randomized sequence. The wines were served at room temperature in a ventilated and air-conditioned tasting room set at approximately 20 °C. The findings of the sensory analyses were confirmed by repeat experiments.

2.6.2. Sensory panel

The panelists were selected from the research laboratory staff at ISVV (Bordeaux, France), and all participants provided written and signed informed consent. The panel consisted of 12 trained judges, 7 females and 5 males aged 34.2 ± 6.4 [mean ± standard deviation (SD)], chosen for their expertise in evaluating aromas in white wines. Participants were not given any information regarding the nature of the samples.

2.6.3. Descriptive analysis

The panel performed a descriptive sensory profile analysis to assess the intensity of twelve specific descriptors: aromatic intensity, fruitiness, citrus, tropical, passion fruits, floral, off-flavor, acidity, bitterness, aromatic aftertaste, body-texture, total perception, as described by Tzamourani et al. (2024b). These descriptors were selected through a discussion with the panelists, based on the sensory vocabulary they had used for describing freely the produced wines of the first fermentation assay (125 mL). For the sample evaluation, panelists were presented with four glasses of wine, and each sample was assessed using an unstructured 0–10 cm scale, where 0 represented the absence of any detectable odor while 10 indicated the highest level of intensity. A computerized data collection program was used (FIZZ, Software Solutions for Sensory Analysis and Consumer Tests, Biosystemes, Couternon, France).

2.7. Statistical analysis

Statistical significance was determined using One-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for pairwise comparisons, with a significance threshold of $p < 0.05$. The homogeneity of the data variance had previously been tested using Levene's test. These analyses were conducted using Statgraphics Centurion 18 software (Statgraphics Technologies, Inc., The Plains, VA, USA).

Principal component analysis (PCA) was used to explore the aroma and flavor profiles of the wines. Additionally, a second PCA was performed to visualize the degree of correlation of z-scores between the intensity of aromatic descriptors and concentrations of chemical compounds (R 6.3 software).

3. Results and discussion

3.1. Co-inoculated fermentations of *Z. bailii* with two *S. cerevisiae* strains in 125 mL trials

The variation in CO₂ production rate over time is presented in Fig. 2. AF was successfully completed in almost all trials, with durations ranging from 290 to 330 h, except for the *Z. bailii* monoculture, which did not complete AF after 382 h. Fermentation time was reduced in the co-inoculated fermentations compared to the *S. cerevisiae* monocultures in both strains. In terms of fermentation parameters, the lag phases ranged from 8 to 9 h, with no significant differences observed. In the co-inoculated trials, the maximum CO₂ production rate (V_{max}) was high, with an acceleration (slope of the curve up to V_{max}) similar to that of the *S. cerevisiae* monocultures. More specifically, fermentations inoculated with *S. cerevisiae* monocultures (S1, S2), and mixed cultures (S1/Z) showed no statistically significant differences in maximum CO₂ production rates (approximately V_{max} = 1.21–1.27 gCO₂/L/h). However, the fermentation rate was significantly higher in the co-inoculated S2/Z modality (V_{max} = 1.38 ± 0.03 gCO₂/L/h), while the *Z. bailii* pure culture (Z) exhibited the lowest rate (V_{max} = 0.66 ± 0.12 gCO₂/L/h). V_{max} was reached between 39 and 40 h in all tested conditions, except for the *Z. bailii* pure culture, where it occurred at 68 h. By opposition, the CO₂ rate curve for the *Z. bailii* (Z) monoculture displayed notable differences compared to the other fermentations modalities. These results are in agreement with previous observations, which noted that V_{max} is generally lower in non-*Saccharomyces* yeasts and obtained later compared to *S. cerevisiae* (Renault et al., 2016).

The evolution of the microbial consortia in the distinct trials is illustrated in Fig. 3. Maximum microbial populations (logCFU/mL)

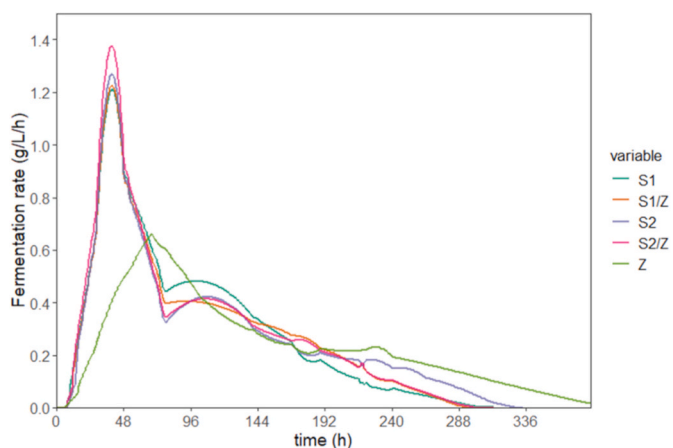


Fig. 2. Fermentation rate expressed in released CO₂ (g/L/h) over time in pure cultures of S1 (*S. cerevisiae* S6-A6Y10), S2 (*S. cerevisiae* S14-K33Y14), Z (*Z. bailii* Zb-A19Y5) and mixed (S1/Z, S2/Z) cultures. Average values of three experiments, standard deviation < 5 %.

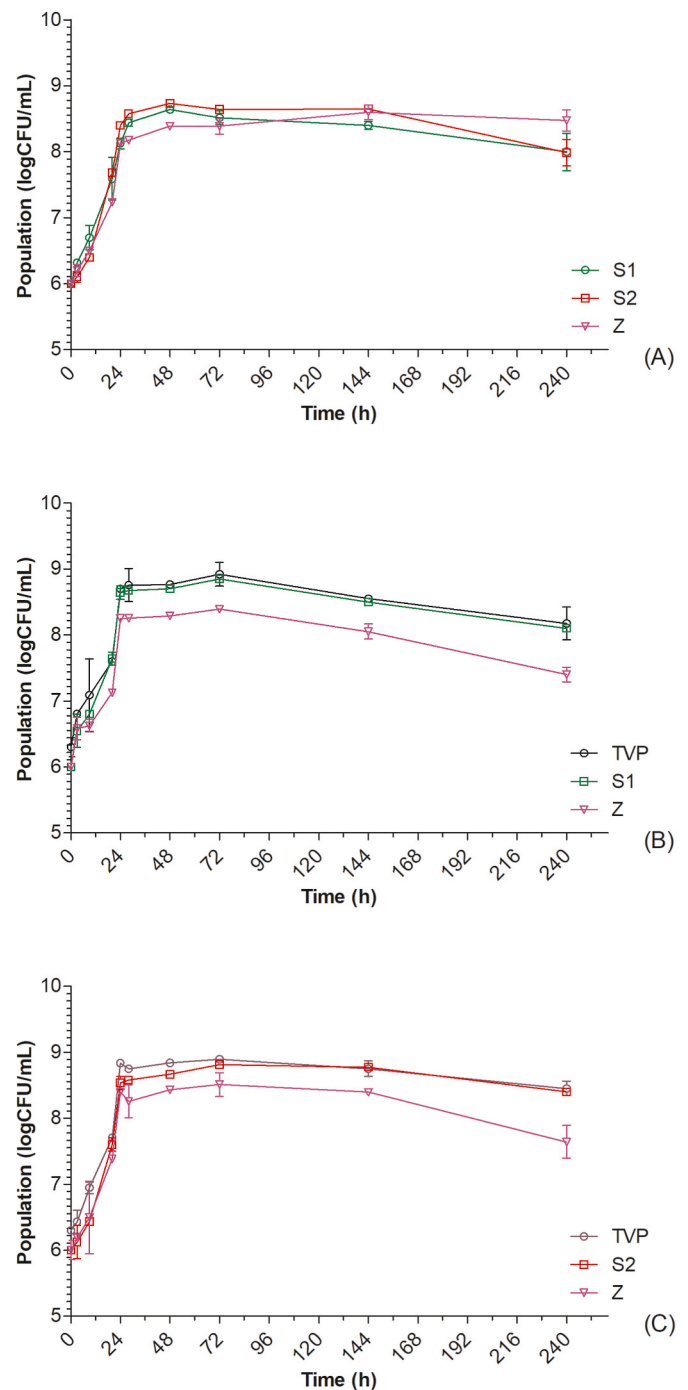


Fig. 3. Yeast growth kinetics: (A) Pure culture populations: S1 (*S. cerevisiae* S6-A6Y10), S2 (*S. cerevisiae* S14-K33Y1) and Z (*Z. bailii* Zb-A19Y5); (B) Populations in co-culture (S1/Z): total viable population (TVP) (S1 + Z), S1 and Z; (C) Populations in co-culture (S2/Z): total viable population (TVP), (S2+Z), S2 and Z. Average value of three experiments ± standard deviation.

ranged from 8.39 ± 0.02 to 8.92 ± 0.08 across all modalities. Moreover, total viable population (TVP) were slightly lower when species were inoculated individually compared to mixed cultures, with maximum populations of 8.6 ± 0.01 (in pure cultures) and 8.9 ± 0.1 (in co-inoculation modalities) logCFU/mL, respectively. It is important to note that in the co-inoculated modalities, the total number of inoculated cells is 2×10^6 (logCFU/mL), which may explain the slightly higher population. Furthermore, at the final stages of AF, *S. cerevisiae* populations were noted higher in co-inoculation mode compared to

monocultures, whereas *Z. bailii* populations exhibited the opposite trend. In addition, in both co-inoculated fermentations, *S. cerevisiae* strains dominated AF, while *Z. bailii* strain persisted at lower concentrations throughout. These findings, therefore, align closely with results from previous studies investigating co-inoculation between *S. cerevisiae* and *Z. bailii* in different matrices (Capece et al., 2021, 2022; Xu et al., 2017). Intriguingly, *Z. bailii* population reached the stationary phase significantly faster (24 h) in co-inoculated fermentations with *S. cerevisiae* compared to monoculture AF (48 h). This accelerated growth suggests that *Z. bailii* strain Zb-A19Y5 benefits from the presence of *S. cerevisiae*, which appears to remain unaffected (neither helped nor harmed) by the interaction.

Overall, the current findings demonstrate that mixed cultures of *Z. bailii* strain Zb-A19Y5 and *S. cerevisiae* not only enhanced the fermentation rate but also accelerated the growth of the non-*Saccharomyces* species population, indicating a clear benefit for the *Z. bailii* strain in the co-culture environment. This result is particularly novel as it highlights a previously uncharacterized ecological interaction between *S. cerevisiae* and *Z. bailii*, offering valuable insights into their cooperative behavior during fermentation processes indicating a commensal type of interaction (Fleet, 2003; Ivey et al., 2013; Torres-Guardado et al., 2021). To elucidate the mechanism driving the observed interactions, one hypothesis involves nutrients and metabolic resources sharing. It is possible that the two investigated yeast species employ distinct nutrient utilization strategies and/or produce bioactive metabolites, ultimately leading to a commensal relationship (Comitini et al., 2021; Shekhawat et al., 2019). Positive interactions under conditions of substrate limitation or depletion have been documented in previous studies (Ciani et al., 2016; Ivey et al., 2013). For instance, Gobert et al. (2017) demonstrated that non-*Saccharomyces* yeasts preferentially consume specific yeast-assimilable nitrogen (YAN) sources over others. Additionally, during co-inoculation trials, metabolites produced by one yeast species have been shown to serve as substrates for another species, thereby influencing microbial growth and modifying the volatolome of the resulting wine (Englezos et al., 2018; Padilla et al., 2016; Renault et al., 2016). Alternatively, the observed interaction could result from direct interspecies actions, such as physical cell-to-cell contact or quorum-sensing mechanisms. Cell-to-cell contact is known to influence yeast metabolic behavior, potentially triggering or suppressing the production of specific chemical compounds that affect wine aroma characteristics (Chasseriaud et al., 2023; Englezos et al., 2019; Renault et al., 2013). Recent studies provide preliminary evidence suggesting that interactions between *S. cerevisiae* and certain non-*Saccharomyces* species involve direct cell-to-cell contact mechanisms (Chasseriaud et al., 2023; Nissen and Arneborg, 2003a; Nissen et al., 2003; Pietrafesa et al., 2020). Conversely, quorum-sensing (QS) mechanisms, which regulate communication and growth, have been proposed as a potential interaction pathway (Avbelj et al., 2015; Samanta et al., 2011). Although some QS molecules have been shown to affect the growth of non-*Saccharomyces* yeasts (González, 2018), further research is required to confirm their role under oenological conditions (Bordet et al., 2020).

3.2. Different inoculation modalities of *Z. bailii*-ZbA19Y5 and *S. cerevisiae*- S10-A6Y10 in 375 mL trials

To further investigate the impact of this microbial interaction on oenological traits, aromatic profiles, and the sensory attributes of the resulting wines, *S. cerevisiae* strain S10-A6Y10 (S1) underwent a second laboratory-scale fermentation in a larger volume (375 mL). The two strains investigated in the previous section exhibited similar behavior towards *Z. bailii* strain Zb-A19Y5 in terms of fermentation performance and population dynamics. However, *S. cerevisiae* S10-A6Y10 (S1) demonstrated slightly shorter fermentation duration and was therefore selected. Subsequent experiments explored the effects of different inoculation strategies (simultaneous co-inoculation and sequential inoculation) (Fig. 1).

3.2.1. Fermentation and yeast growth kinetics

The impact of different inoculation modalities on fermentation kinetics is summarized in Table 2. No significant differences were noticed in the lag phases across the various assays. Fermentation with *Z. bailii* strain Zb-A19Y5 alone showed the lowest maximum CO₂ production rate ($V_{max} = 0.7 \pm 0.1$ gCO₂/L/h). On the contrary, fermentations with *S. cerevisiae* pure culture and co-inoculation exhibited higher V_{max} values (1.2–1.3 gCO₂/L/h), resulting in the shortest fermentation durations (337 h and 316 h, respectively). Interestingly, the AF durations in both fermentation modalities, S1 and S1/Z, are slightly longer than in the first test. This can be attributed to the change in fermenters between sessions 3.2 and 3.3, which differ slightly in their air/liquid volume ratio, which can impact oxygen availability and therefore AF duration. As expected, when S1 was added sequentially, the fermentation kinetics resembled those of the *Z* monoculture but with a slightly higher V_{max} and a shorter fermentation duration. Additionally, most fermentative parameters, including lag phase, maximum fermentation rate (V_{max}), and time at V_{max} , were consistent across the same modalities (S1, S1/Z, Z) with those observed in the previous section. The only notable difference was a prolonged fermentation duration especially in the *Z* monoculture during the second assay. However, in cases of stuck fermentation, accurately determining the exact cessation of AF is challenging.

The changes in the population dynamics of the two yeast species driving AF are presented in Fig. 4. The evolution of the total viable population (TVP) reveals a longer lag phase when *Z. bailii* was inoculated alone, followed by the sequential inoculation trials. The maximum total population during the stationary phase fluctuated from 8.00 ± 0.03 logCFU/mL to 8.80 ± 0.04 logCFU/mL in all modalities. In more detail, during AF where *S. cerevisiae* and *Z. bailii* were co-inoculated simultaneously, *S. cerevisiae* emerged as the predominant species. However, *Z. bailii* also showed significant growth, maintaining an average population that was 0.6 logCFU/mL lower than *S. cerevisiae*. Under the sequential inoculation, the pure culture of *Z. bailii* exhibited an increase in viable cell count from 6.00 ± 0.00 to 7.50 ± 0.04 logCFU/mL within 30 h, initiating fermentation. *S. cerevisiae* showed substantial growth up to 8.32 ± 0.02 logCFU/mL but was prevented from dominating the process. In this modality, the non-*Saccharomyces* species dominated *S. cerevisiae*, which maintained a slightly lower population (approximately 0.4 logCFU/mL). This phenomenon has not been reported in previous research on the sequential inoculation of non-*Saccharomyces* yeasts (Capece et al., 2022; Ciani et al., 2006; Liu et al., 2017; Renault et al., 2016).

3.2.2. Physicochemical parameters of wine

The main oenological characteristics and produced metabolites of the fermentations are summarized in Table 3 and Fig. 5. In the pure culture of Zb-A19Y5, residual glucose levels were measured at 11.1 ± 1.0 g/L, indicating that AF remained incomplete. Consequently, while the final ethanol concentrations in all wine samples ranged between 12.4 and 12.5 % vol, in the samples with pure *Z. bailii* cultures, reached only 11.4 % vol. The consumption kinetics of glucose and fructose highlighted the fructophilic behavior of *Z. bailii* metabolism. During co-inoculated fermentations, both sugars were consumed but glucose was preferred. In conditions where *Z. bailii* dominated (Z + S1 and Z trials), fructose was rapidly depleted, with complete consumption observed within approximately 192 h. Glucose consumption accelerated after fructose depletion. During alcoholic fermentation processes, the preferential consumption of glucose by *S. cerevisiae* may lead sometimes to undepleted fructose in the media (Berthels et al., 2004). In such cases, fructophilic yeasts can metabolize the residual fructose, restoring the glucose-fructose equilibrium and resulting to complete fermentation process (Romano et al., 2019). This approach demonstrates considerable potential for managing fermentation challenges prevalent in the wine industry (Jolly et al., 2006; Pina et al., 2004). Indeed, *Z. bailii* preferentially consumes fructose than glucose, a trait that has been

Table 2

Kinetic parameters in pure (S and Z), co-inoculated (S1/Z) and sequentially inoculated (Z + S1) fermentations. Average values of three experiments, standard deviation < 5 %. Different letters uppercase in the same column indicate the significance of One-way Analysis of Variance (ANOVA), which was followed by Tukey's post hoc test ($p < 0.05$).

Modality	Lag phase (h)	Time at Vmax (h)	Vmax (g/L/h)	CO ₂ max (g/L)	Fermentation time (h)
S1	8.0 ± 0.6 ^a	39 ± ^a	1.2 ± 0.1 ^b	95.4 ± 0.2 ^b	337 ± 2 ^b
S1/Z	9.0 ± 1.5 ^a	40 ± ^a	1.3 ± 0.0 ^b	94.8 ± 0.2 ^b	316 ± 2 ^a
Z + S1	10.0 ± 1.0 ^a	68 ± ^b	0.9 ± 0.0 ^{ab}	92.8 ± 0.4 ^{ab}	453 ± 5 ^c
Z	9.0 ± 0.7 ^a	70 ± ^b	0.7 ± 0.1 ^a	87.7 ± 0.9 ^a	557 ± 8 ^d

investigated for applications such as restarting fermentation in order to reduce residual sugars in stuck fermentations (Englezos et al., 2018; Jolly et al., 2006; Pina et al., 2004; Zuehlke et al., 2015). The gene associated with this phenotype in *Z. bailii* was found to encode fructose-specific membrane transporters, characterized by FFZ-like genes (fructose facilitator *Zygosaccharomyces*) (Cabral et al., 2015; Pina et al., 2004).

Additionally, volatile acidity was below the detection threshold in all modalities (<0.1 g/L). Typically, *Z. bailii* strains are known to produce high levels of acetic acid, a trait that often characterizes them as spoilage species in winemaking (Palma et al., 2015; Rodrigues et al., 2012; Solieri, 2021). However, in our study, acetic acid was not detected (<0.2 g/L), aligning with findings for other *Z. bailii* strains reported in the literature (Capece et al., 2022; Domizio et al., 2011; Jolly et al., 2006).

Malic acid concentration was significantly lower at the end of AF in fermentations where *Z. bailii* strain Zb-A19Y5 was predominant. Additionally, glycerol and lactic acid production were higher, with additional 1.2–2.9 g of glycerol/L and 0.4–0.8 g of lactic acid/L, in both monoculture and sequential inoculation of *Z. bailii*. Malic acid degradation accompanied by lactic acid production, occurred in cases where *Z. bailii* had a longer period of independent activity (in sequential inoculation and monoculture conditions). It is important to note that the medium was sterilized, and no bacterial communities were detected on MRS agar plates, either in the initial grape juice or at the final stages of fermentation. Therefore, the possibility of malolactic fermentation by lactic acid bacteria is eliminated. In recent years, studies have demonstrated that malic acid decomposition by yeasts can proceed through either the malic enzyme pathway, producing ethanol, or via fumarase, yielding succinate (Redzepovic et al., 2003; Su et al., 2014). The capacity of yeast to degrade malic acid relies on the presence of an efficient malate utilization system and specific malate transport enzymes, such as the malolactic enzyme, malic enzyme, malate dehydrogenase, or fumarase (Su et al., 2014). Previous research has also documented malic acid degradation by *Z. bailii* strains (Jolly et al., 2006; Romano and Suzzi, 1993). In our study, however, we did not observe the production of succinate. One possible hypothesis is that malic acid degradation may have contributed to ethanol production via the malate utilization system and the malolactic transport enzyme. Whatsoever, the remaining sugars and the already lower alcohol level in *Z. bailii* monocultures, fail to confirm this assumption. Notably, the malic acid-degrading capacity has been observed to be higher in strains of *Schizosaccharomyces pombe*, *Z. bailii*, and *Issatchenkia orientalis* compared to *S. cerevisiae* (Su et al., 2014). Furthermore, lactic acid production can occur via pyruvate produced by carbon catabolism through glycolysis and the pentose phosphate pathway, as observed with *Lachancea thermotolerans* (Tzamourani et al., 2024a). To the best of our knowledge, this is the first report monitoring both malic and lactic acid concentrations during AF using *Z. bailii* as fermentation starter. Hence, no definitive assumptions can be outlined and further investigation is necessary to clarify these findings.

3.2.3. Aromatic profile of produced wines

Principal component analysis (PCA) was used to identify the specific volatile compounds (esters, higher alcohols and thiols) best discriminating among the four Ugniblanc wines studied. PCA was applied to the

concentrations of the 37 volatile compounds determined by GC–MS and detected in our samples (Fig. 6). The first two principal components, PC1 and PC2, accounted for 75 % of the total variance (63.3 % and 11.7 %, respectively). In this way, wines inoculated with pure cultures of *S. cerevisiae* (S1) and co-inoculated with *Z. bailii* were clustered at negative PC1 and positive PC2 scores, thus containing high relative correlations. Wines inoculated with *Z. bailii* monocultures were associated to positive values of both PC1 of PC2 and were associated with ethyl dihydrocinnamate, ethyl isobutanoate, ethyl propanoate (esters), and 4-methyl-4-sulfanylpentan-2-one (thiol). Notably, these three esters have been described previously as activity markers of *T. delbrueckii* in mixed fermentations with *S. cerevisiae* (Renault et al., 2015). Finally, the sequential inoculation that clusters at positive PC1 and negative PC2 contained high relative correlations of the varietal thiol 3-sulfanylhexanol (3SH) and esters, including ethyl 2-methylbutyrate, 2-methylpropan-1-ol, propan-1-ol, propyl acetate, ethyl trans-2-hexanoate, and 2-methylbutan-1-ol.

3.2.3.1. Esters and higher alcohols. The current study focused on quantifying various esters and major volatile alcohols known for contributing fruity aromas to wine, such as ethyl propanoate (strawberry), ethyl isobutanoate (strawberry, kiwi), propyl acetate (pear), isobutyl acetate (banana), ethyl butyrate (kiwi, strawberry), ethyl 2-methylbutyrate (kiwi), and others like isoamyl acetate (banana), hexyl acetate (pear), and ethyl dihydrocinnamate (fruit, pineapple, almond) (Antalick et al., 2010).

The final higher alcohols and ester concentrations at the end of fermentation are detailed in Supplementary Table 1. Higher alcohol concentrations showed no statistically significant differences among the modalities, except for propan-1-ol, which was found in higher concentrations in sequential inoculation and Zb-A19Y5 pure culture. Therefore, in the present study the interest was focused on the 30 reported esters. Regarding pure cultures, *Z. bailii* showed lower concentrations of acetate esters, methyl esters, and isoamyl acetates by 7-, 2-, and 6-fold, respectively, than *S. cerevisiae*, while aliphatic acid esters were much higher in *Z. bailii* fermentations ($31.7 \pm 11.9 \mu\text{g/L}$) compared to *S. cerevisiae* ($9.1 \pm 0.9 \mu\text{g/L}$). Mixed cultures, either co- or sequentially inoculated, showed a midway composition showing that each species participated in the aroma profile. These observations highlight the importance of the inoculation protocol in mixed fermentations, as it influences the growth kinetics of each yeast species, ultimately altering the final ester profile (Renault et al., 2015). The production of esters, such as ethyl acetate, geranyl acetate, isoamyl acetate and 2-phenylethyl acetate (Rojas et al., 2001, 2003), during AF is closely linked to the specific yeast species or strains used as inoculum (Romano et al., 2019). The current results are in line with literature, outlining that non-*Saccharomyces* pure cultures in winemaking may produce less overall esters than *S. cerevisiae*, while released some minor esters in significantly higher concentrations up to 6-fold more (Renault et al., 2015).

The presence of some enzymes, such as esterases and acetyltransferases, between the two species, may account for the differing concentrations of esters observed (Marullo et al., 2021). Additionally, *Z. bailii* has been previously proposed as a co-starter with *S. cerevisiae* to enhance the production of ethyl esters (Garavaglia et al., 2015), while *Zygosaccharomyces* sp. have been reported for providing high levels of 2-

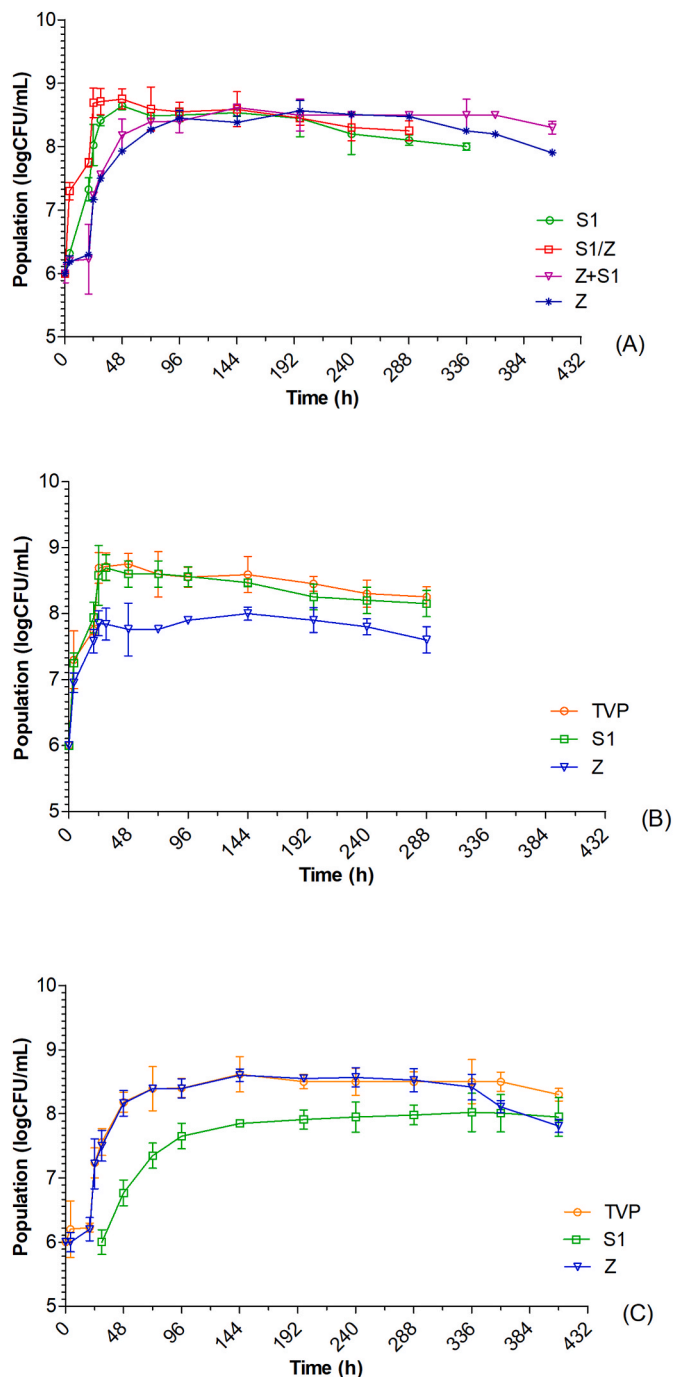


Fig. 4. Yeast growth kinetics: (A) Total viable population in pure cultures S1 (*S. cerevisiae* S6-A6Y10) and Z (*Z. bailii* Zb-A19Y5), in co-culture (S1/Z) and sequential culture (Z + S1); (B) Populations in co-culture (S1/Z): total viable population (TVP), S1 and Z; (C) Populations in sequential culture (S1+Z): total viable population (TVP), S1 and Z. Average value of three experiments \pm standard deviation.

phenyl ethyl acetate, isoamyl acetate, ethyl acetate and ethyl esters (Rojas et al., 2001), and are capable to produce these esters through the esterification reaction with ethanol (Plata et al., 2003; Rojas et al., 2003; Solieri, 2021).

3.2.3.2. Thiols. GC-MS analysis results, presented in Table 4, show that wines fermented by with *Z. bailii* (Z) produced with higher concentrations of 3SH and 4MSP (294.7 ± 43.7 ng/L and 1.9 ± 0.2 ng/L, respectively) compared to wines fermented by *S. cerevisiae* (S1) (173.9

Table 3

Average values (\pm standard deviation) of final oenological parameters of the produced wines inoculated with pure and mixed *Z. bailii* (Z)/*S. cerevisiae* (S1) cultures, inoculated simultaneously (S1/Z) and sequentially (Z + S1). Different letters uppercase in the same row indicate the significance of the one-way Analysis of Variance (ANOVA), which was followed by Tukey's post hoc test ($p < 0.05$).

	S1	S1/Z	Z + S1	Z
D-Glucose (g/L)	0.7 ± 0.1^a	0.7 ± 0.0^a	1.9 ± 0.0^b	11.1 ± 1.0^c
D-Fructose (g/L)	1.9 ± 0.0^a	1.8 ± 0.0^a	0.5 ± 0.1^a	1.2 ± 0.0^a
Alcoholic Degree % (vol)	12.5 ± 0.1^b	12.5 ± 0.1^b	12.4 ± 0.1^b	11.4 ± 0.1^a
Volatile Acidity (g/L)	<0.1	<0.1	<0.1	0.2 ± 0.0
Malic Acid (g/L)	2.6 ± 0.0^b	2.4 ± 0.0^b	1.6 ± 0.0^c	0.5 ± 0.0^a
pH	3.3 ± 0.1^a	3.5 ± 0.1^a	3.3 ± 0.2^a	3.5 ± 0.1^a
Glycerol (g/L)	5.0 ± 0.0^{ab}	4.6 ± 0.1^a	6.2 ± 0.2^b	7.5 ± 0.3^b
Lactic acid (g/L)	0.2 ± 0.0^a	0.2 ± 0.0^a	0.6 ± 0.0^b	1.0 ± 0.0^c
Succinate (g/L)	nd	nd	nd	nd

± 26.5 ng/L and 0.8 ± 0.2 ng/L, respectively), indicating distinct differences in thiol production between the two species. The investigated *S. cerevisiae* strain (S6-A6Y10) has been shown in previous research to release 4MSP in concentrations above perception threshold in Assyrtiko wines (Tzamourani et al., 2024a); therefore, the low production of this thiol may be due to the limited availability of precursors (Cys-4MSP or Glut-4MSP) in the Ugni-Blanc grape juice. Additionally, the presence of *Z. bailii* alone at the onset of AF, as observed in Z and Z + S1 modalities, appear to enhance 3SH synthesis (Table 4). However, concerning the co-inoculated cultures, 3SH production was similar to that of the pure *S. cerevisiae* culture. To the best of our knowledge, the role of *Z. bailii* in volatile thiol formation in both pure and mixed cultures has not been previously investigated. Among non-*Saccharomyces* yeasts, certain species have been noted for their ability to enhance the release of volatile thiols in the wine matrix (Cordente et al., 2019; Renault et al., 2016; Sadoudi et al., 2012). Moreover, *S. cerevisiae* pure cultures, along with mixed co-inoculation and sequential inoculation cultures, exhibited significantly higher (12-fold) 3SHA production compared to Zb-A19Y5 pure cultures. Several factors can impact volatile thiol release, including the yeast strain (Tominaga and Dubourdieu, 2006). One possible explanation could be the absence of a specific gene in *Z. bailii* required for 3SH acetylation, such as *ATF1* in *S. cerevisiae* (Swiegers et al., 2006). Under similar experimental conditions, another non-*Saccharomyces* yeast, *T. delbrueckii* produced no 4MSP and only very small amounts of 3SHA, yet exhibited high 3SH levels in both pure *T. delbrueckii* fermentations and sequential *T. delbrueckii*/*S. cerevisiae* cultures, compared to fermentations with *S. cerevisiae* alone. In the same study, through monitoring 3SH and its precursors (Glut-3SH and Cys-3SH) throughout AF revealed that *T. delbrueckii* exclusively assimilates the glutathionylated precursor, whereas *S. cerevisiae* metabolizes both precursor forms (Renault et al., 2016). A similar metabolic pattern may occur in the studied *Z. bailii* strain, though further investigation is needed.

3.2.4. Sensory evaluation

Ten of the panelists generated olfactory attributes to describe the produced white wines. The free descriptors provided by the panelists were organized into general aromatic groups, following the structure of the aromatic wheel proposed by (Noble et al., 1987). Wine samples from Zb-A19Y5 monocultures, were considered undesirable due to off-flavors (70 %, 7/10), and more specifically hydrogen sulphide (30 %, 3/10), and chemical odors (10 %). In contrast, co-inoculated modality displayed floral (50 %, 5/10) and fruity characteristics (60 %, 6/10), with a significant percentage of panelists also detecting citrus notes (50 %, 5/10) and thiol-related tropical aromas, such as passionfruit (30 %, 3/10). Notably, wines produced by monocultures of *S. cerevisiae* were

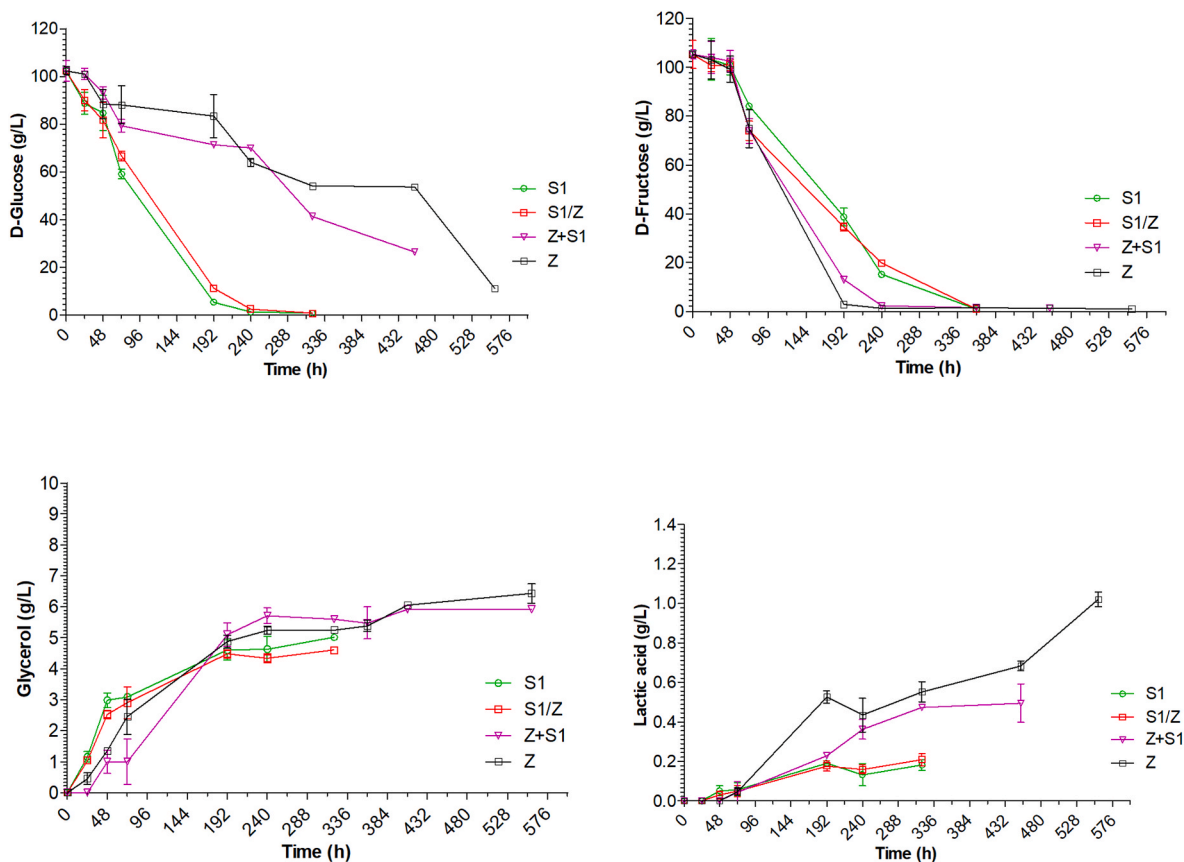


Fig. 5. Changes in the concentration (g/L) of D-glucose, D-fructose, glycerol and lactic acid during the fermentation of grape juice in pure cultures: S1(*S. cerevisiae* S6-A6Y10), S2 (*S. cerevisiae* S14-K33Y1) and Z (*Z. bailii* Zb-A19Y5), co-cultures: S1/Z and sequential culture: Z + S1.

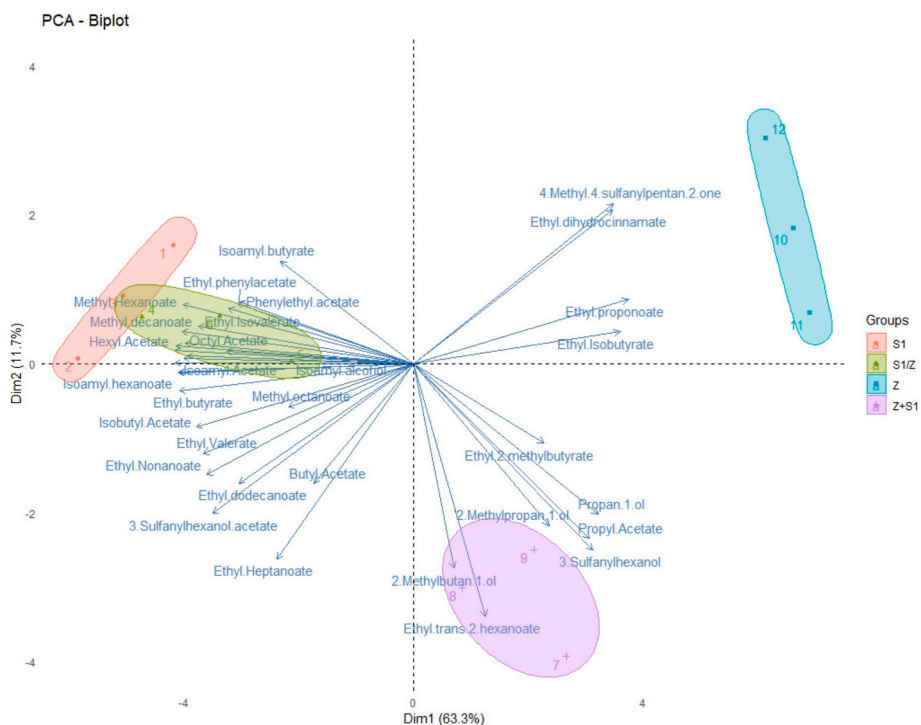


Fig. 6. Principal Component Analysis of fruity esters alcohols, and thiols in pure cultures *Z. bailii* Zb-A19Y5 (Z)/*S. cerevisiae* S6-A6Y10 (S1) cultures, co-cultures (S1/Z) and sequential culture (Z + S1). The average value is represented here. Solid lines represent the dispersion of our values; dashed lines represent the central axis of the ellipses.

Table 4

Volatile thiols (ng/L) of wines produced in pure and mixed *Z. bailii* (Z)/*S. cerevisiae* (S1) cultures, inoculated simultaneously (S1/Z) and sequentially (Z + S1). Different letters in each row indicate the significance of One-way Analysis of Variance (ANOVA), which was followed by Tukey's post hoc test ($p < 0.05$).

Thiols (ng/L)	S1	S1/Z	Z + S1	Z	Threshold (ng/L) ^a	Odor
3-sulfanylhhexanol (3SH)	173.9 ± 26.5 ^a	197.3 ± 29.7 ^a	355.9 ± 59.1 ^b	294.7 ± 43.7 ^b	60	Tropical, grapefruit, passionfruit
3-sulfanylhhexanol acetate (3SHA)	32.4 ± 3.6 ^b	31.4 ± 2.2 ^b	30.0 ± 2.2 ^b	2.6 ± 0.6 ^a	4.2	Tropical, grapefruit, passionfruit
4-methyl-4-sulfanylpentan-2-one (4MSP)	0.8 ± 0.1 ^a	0.9 ± 0.1 ^a	0.9 ± 0.1 ^a	1.9 ± 0.2 ^b	3	Box wood

^a (Tominaga et al., 2000).

described as less intense (40 %, 4/10), pleasant (70 %, 7/10) compared to those produced through co-inoculation modality. Five additional gustatory descriptors (acidity, bitterness, body/texture, and aromatic aftertaste) were introduced to better capture the flavor profile of the wines.

On a 100 mm scale of intensity, the co-inoculated fermentations exhibited higher scores for fruity, citrus, passion fruit, and floral characteristics compared to other fermentation modalities (Fig. 7). Furthermore, in the current experiment, the simultaneous inoculation of *S. cerevisiae* (S1) and *Z. bailii* (Z) resulted in wines with enhanced aromatic aftertaste, body, and overall perception, followed by *S. cerevisiae* (S1) monocultures. However, the organoleptic expression of tropical and passion fruit characteristics in the analyzed wine samples appears inconsistent with the concentrations of 3SH and 3SHA, compounds known to be associated with these aromas according to the bibliography (Tominaga et al., 2000; Capone et al., 2018; Carlin et al., 2022). The complexity of sensory perception in wine analysis remains a significant challenge, as no single volatile organic compound (VOC) or group of VOCs can fully explain a wine's overall aromatic profile (Bordet et al., 2020). While correlations and trends do exist, the relationship between chemical composition and sensorial perception is not always straightforward (Lytra et al., 2014, 2016). This discrepancy can be partially attributed to interactions between volatile aromatic compounds and non-odorous volatile compounds, which influence aroma formation (McKay et al., 2020). Additionally, various factors can contribute to the misalignment between a wine's volatile composition and its perceived aroma, including the masking effects of certain compounds (S. Benito et al., 2015). Furthermore, multiple VOCs often contribute to the same aromatic note (Chambers IV and Koppel, 2013). Organoleptic profiling

therefore remains an efficient tool to qualify the impact of co-inoculation on the properties of wine, however, it cannot explain the interaction mechanisms that may occur (Bordet et al., 2020). Conversely, monocultures of *Z. bailii* were characterized by strong off-flavor attributes, potentially masking other positive sensory characteristics, such as thiols. Indeed, the monocultures of *Z. bailii* resulted in prolonged and incomplete AF which may also be linked to the sulfur-like off-aromas reported by the panelists (Linderholm et al., 2008; Romano et al., 2019).

As an overview, the effect of this controversial non-*Saccharomyces* yeast on two different inoculation modalities is summarized in Fig. 8, revealing interesting trends. Principal component analysis (PCA) accounted for 92.4 % of total variance (PC1: 77.2 % and PC2: 15.2 %), revealing a correlation between the intensity of aromatic descriptors and the concentrations of the aromatic compounds that differentiated most the investigated samples, namely esters and thiols. The positive PC1 and PC2 section, where *Z. bailii* monoculture modality is located, reveals the positive correlation of ethyl esters, aliphatic acid esters, and 4-MSP with aromatic intensity, aromatic aftertaste and off flavor characteristics. The sequential inoculation is correlated with the concentrations of 3SH, while the rest modalities were located at the positive side of the PC1 axis and at the negative side of the PC2 axis. In general, the perception of thiols is associated with tropical (passion fruit) and citrus notes (grapefruit) (Fedrizzi et al., 2009; Perez-Coello and Diaz-Maroto, 2009; Tominaga et al., 2000; Capone et al., 2018; Carlin et al., 2022). More specifically, 3-sulfanylhhexan-1-ol (3SH) and its acetate derivative (3SHA), formed by yeast acetylase, contributing grapefruit and passion fruit aromas (Fedrizzi et al., 2009). Despite the higher thiol concentrations observed in the present study, in the Z + S1 and Z fermentation

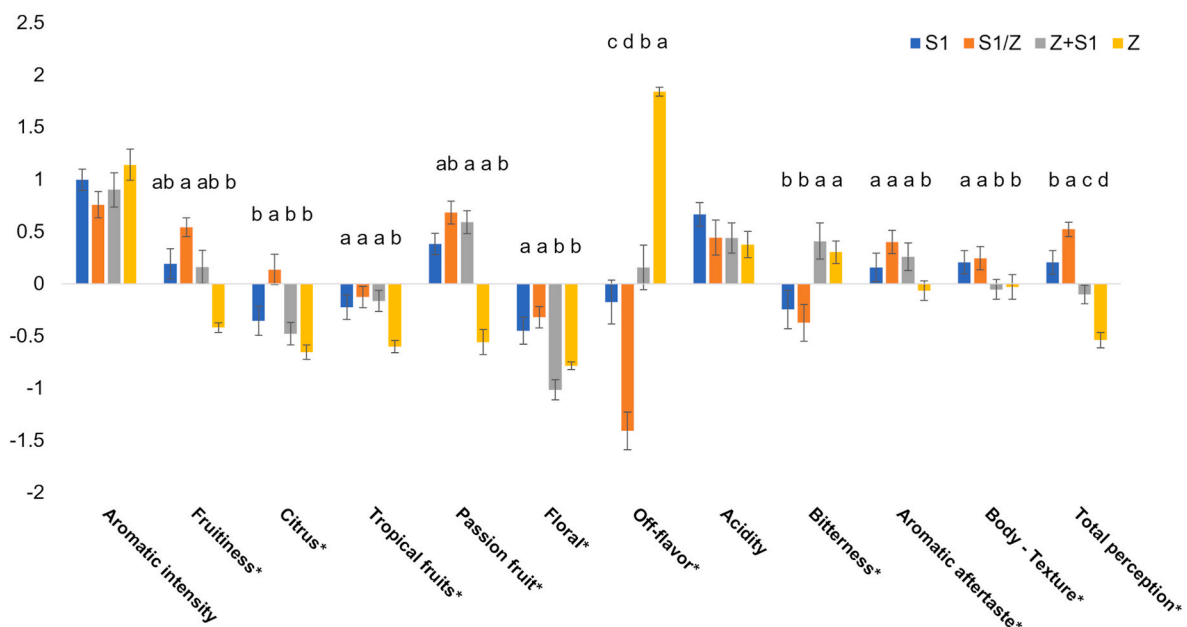


Fig. 7. Mean-centered intensities of descriptors for aromatic profiling wines inoculated with pure and mixed *Z. bailii* Zb-A19Y5 (Z)/*S. cerevisiae* S6-A6Y10 (S1) cultures, simultaneously (S1/Z) and sequentially (Z + S1). The asterisk next to each attribute indicates statistically significant differences between the different wines. Mean-centered values with different letters are significantly different ($p < 0.05$). Error bars indicate the 95 % confidence interval on the mean value.

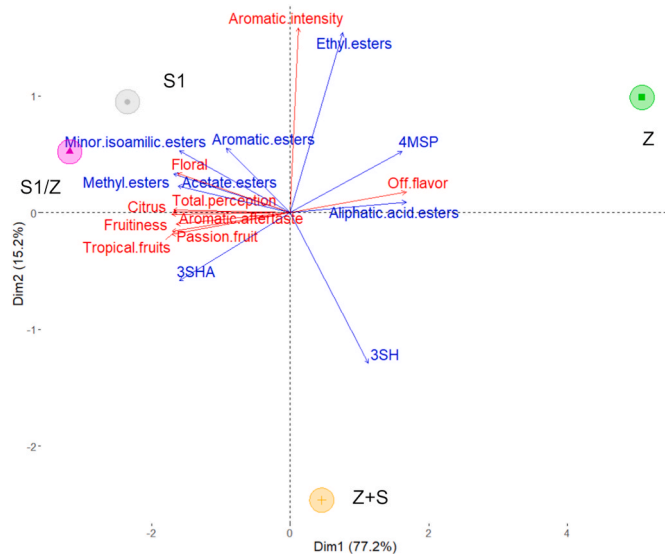


Fig. 8. PCA (principal component analysis) of z-scores of aromatic descriptor intensities (red labels) and chemical compound concentrations (blue labels) of the wines produced in the present study inoculated with pure and mixed *Z. bailii* Zb-A19Y5 (Z)/*S. cerevisiae* S6-A6Y10 (S1) cultures, simultaneously (S1/Z) and sequentially (Z + S1). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

modalities, these aromas were not distinctly perceived by the panelists, potentially due to this masking effect. The loading plots for methyl esters, minor isoamyl esters, and acetate esters were projected on the negative side of the first axis, indicating that these compounds were well-correlated with all floral, and fruity descriptors. Therefore, co-inoculation and *S. cerevisiae* monoculture are grouped together with the co-inoculated wines enhancing the floral, and fruity attributes.

4. Conclusion

This study explores the oenological potential of a promising *Z. bailii* strain in multi-starter fermentations with *S. cerevisiae*, unravelling the nature of their interaction during vinification. Fermentation kinetics and microbial enumeration throughout the process suggest a commensal relationship between *Z. bailii* and *S. cerevisiae*. Notably, the observed tolerance to alcohol, fructophilic behavior, and ability to consume malic acid of this non-*Saccharomyces* strain are unusual and beneficial properties for the wine industry. Furthermore, the sensory and aromatic profiles of the resulting wines are significantly influenced by the performance of *Z. bailii* inoculum. The timing of *S. cerevisiae* addition in mixed cultures emerges as a critical factor. Among the evaluated inoculation strategies, co-inoculation proved to be the most effective, producing wines similar to those inoculated with pure *S. cerevisiae* cultures with no off-flavor attributes, together with more pronounced fruity, citrus, passion fruit, and floral properties. This study also opens up new investigative perspectives on the positive interaction between *Z. bailii* and *S. cerevisiae*. Nutrient sharing or exchange, competition for limiting factors and positive direct species interactions (such as physical cell-to-cell contact or quorum-sensing mechanisms) should also be investigated to unravel the interaction mechanism in more depth.

CRedit authorship contribution statement

Aikaterini Tzamourani: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation. **Marina Bely:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Cecile Thibon: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation. **Marguerite Dols-Lafargue:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Maria Dimopoulou:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Data availability

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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