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Consuelo Ceppi De Lecco , Wendy Franco , Alejandra Urtubia , Reynier Baez , Sergio Benavides-Valenzuela *

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Article

Exploratory Assessment of Native *Starmerella bacillaris* and *Hanseniaspora uvarum* Under Different Fermentation Strategies in Chilean Sauvignon Blanc

Consuelo Ceppi De Lecco [1], Wendy Franco [2,3], Alejandra Urtubia [4], Reynier Baez [5] and Sergio Benavides-Valenzuela [6,*]

[1] Departamento de Fruticultura y Enología, Pontificia Universidad Católica de Chile, Ave. Vicuña Mackenna 4860, Santiago, Chile.
[2] Departamento de Ingeniería Química y Bioprocesos, Pontificia Universidad Católica de Chile, Ave. Vicuña Mackenna 4860, Santiago, Chile.
[3] Departamento de Ciencias de la Salud, Carrera de Nutrición, Pontificia Universidad Católica de Chile, Ave. Vicuña Mackenna 4860, Santiago, Chile.
[4] Departamento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María, Ave. España 1680, Valparaíso, Chile.
[5] Núcleo de Investigación de Agroalimentos y Nutrición Aplicada, Universidad Adventista de Chile, Cam. Las Mariposas km 12, Chillán, Chile
[6] Escuela de Nutrición y Dietética, Facultad de Ciencias para el Cuidado de la Salud, Universidad San Sebastián, Lientur 1457, Concepción, Chile.
* Correspondence: sergio.benavides@uss.cl

Abstract

Non-Saccharomyces yeasts (NSY) are increasingly investigated as biotechnological tools to diversify wine profiles and modulate fermentation outcomes. This study evaluated the enological behavior of two Chilean isolates, *Starmerella bacillaris* (SB) and *Hanseniaspora uvarum* (HU), in Sauvignon Blanc must from the Casablanca Valley under monoculture and sequential inoculation (NSY → *Saccharomyces cerevisiae*) at laboratory (500 mL) and microvinification (10 L) scales. In synthetic medium (150 g/L sugars), SB and HU showed incomplete sugar consumption, producing 4.25% and 8.50% v/v ethanol, respectively, compared with 9.16% v/v for *S. cerevisiae*. In laboratory-scale fermentation in real must, both strains completed fermentation in monoculture, with moderate reductions in ethanol production relative to the control. At the microvinification scale, monocultures yielded lower ethanol concentrations (11.90–12.50% v/v) than *S. cerevisiae* (13.50% v/v), whereas sequential fermentations converged toward control values. NSY treatments showed higher relative abundances of medium-chain ethyl esters associated with fruity and floral sensory attributes while maintaining acetic acid concentrations ≤0.50 g/L. These findings indicate that the effects of SB and HU depended primarily on fermentation strategy and process scale under the evaluated conditions.

Keywords: non-Saccharomyces fermentation; sequential inoculation; Sauvignon Blanc; native yeast isolates; aroma modulation

1. Introduction

One of the current frontiers in modern oenology is the exploration of microbial resources capable of diversifying wine styles and modulating fermentation outcomes. In this context, non-Saccharomyces yeasts (NSY) have gained increasing attention as promising biotechnological tools that expand metabolic possibilities and influence the sensory and chemical properties of wines [1]. Their controlled application has been proposed as a strategy to enhance aromatic complexity and,

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under specific conditions, to contribute to moderate ethanol reduction [2,3], although these effects are strongly dependent on strain identity, fermentation conditions, and fermentation design.

Unlike *Saccharomyces cerevisiae* (SC), many NSY species exhibit distinct carbon flux distributions and metabolomic profiles, frequently leading to altered production of esters, higher alcohols, organic acids, and other secondary metabolites of sensory relevance [4,5]. Among them, *Starmerella bacillaris* is commonly associated with high glycerol production, fructophilic behavior, and comparatively low ethanol yields, whereas *Hanseniaspora uvarum* has been linked to early-stage fermentation activity and the production of acetate esters and other aroma-active compounds, despite its limited ethanol tolerance. Over the past decade, several NSY species—including *Starmerella bacillaris*, *Hanseniaspora uvarum*, *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, and *Torulaspora delbrueckii*—have been investigated for their potential to modulate acidity, influence glycerol production, and enhance fruity and floral attributes, particularly when applied in sequential or mixed fermentations with SC [6,7].

At the strain level, NSY display considerable metabolic variability, which may be influenced by ecological origin and local viticultural conditions. This variability has contributed to the broader concept of microbial terroir, in which indigenous microbial communities are considered potential contributors to wine differentiation [8]. Nevertheless, most experimental evaluations remain focused on commercial starters or isolates from temperate Northern Hemisphere regions, while fewer studies have examined the fermentation-related behavior of Chilean NSY isolates at laboratory scale under controlled conditions [9]. In addition, the extent to which fermentation strategy influences NSY metabolic outputs in defined must systems remains incompletely understood.

Chile offers a wide diversity of viticultural environments and native microbial resources, creating favorable conditions to investigate strain-specific fermentation responses in locally relevant systems [10]. Nevertheless, studies addressing Chilean isolates of *Starmerella bacillaris* (SB) and *Hanseniaspora uvarum* (HU) under monoculture and sequential inoculation with *S. cerevisiae* remain scarce, particularly those integrating physiological, chemical, and sensory responses within a unified experimental approach [11].

In this context, we hypothesized that the enological behavior of NSY changes according to fermentation strategy and process scale, leading to differential effects on ethanol production, metabolite formation, and aromatic expression within a controlled experimental system. Accordingly, this study evaluates Chilean NSY isolates under two fermentation strategies (monoculture and sequential inoculation) in Sauvignon Blanc must from Casablanca Valley.

This study characterizes strain-associated responses under defined experimental conditions at laboratory and microvinification scales by integrating fermentation kinetics, metabolite production, volatile profiles, and sensory attributes. Although dynamic population monitoring was not performed, the combined physiological, chemical, and sensory approach provides a controlled framework for interpreting strain-dependent oenological behavior.

2. Materials and Methods

2.1. Yeast Strains and Stabilization

SB and HU were obtained from the microbiological culture collection of the Food Fermentation Laboratory (Pontifical Catholic University of Chile). Both strains were originally isolated from grapes harvested in the Maule Region, Chile (Figure 1), and identified by partial sequencing of the 26S rDNA [6].

Strain identification was performed through sequencing of the D1/D2 domain of the 26S rRNA gene, and sequence similarity (≥99%) was confirmed by comparison with reference sequences available in the NCBI GenBank database, ensuring reliable species-level taxonomic assignment. Considering the known intraspecific variability among NSY, the metabolic behavior observed in this study should be interpreted as strain-specific rather than representative of the species as a whole.

The yeasts were maintained in YMA (Yeast and mould agar) medium (Oxoid, UK) and freeze-dried for stabilization and storage prior to inoculation [7]. Cultures were grown in Sabouraud broth

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(Biokar, Spain) at 28 °C for 24 h, centrifuged (5,660 × g; 5 min), washed with 0.9% saline solution, frozen at -40 °C for 48 h, and freeze-dried (Liobras L108, Brazil) at -50 °C and 100 μm Hg for 24 h. The dried yeast was stored at room temperature, protected from light, until use.

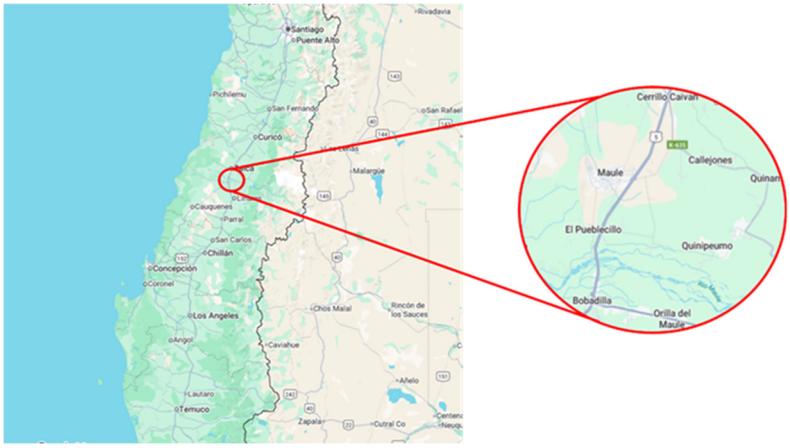

Figure 1. Geographical origin of the native yeast collection: 35°21′50.0″S, 71°30′05.0″W.

2.2. Preliminary Evaluation of Fermentative Viability

2.2.1. Activation of Yeast Strains

Lyophilized *Starmerella bacillar*is (SB) and *Hanseniaspora uvarum* (HU) cultures were rehydrated in sterile saline solution (0.9% w/v) at 25 °C for 30 min. Cell suspensions were then streaked onto YMA agar plates and incubated at 28 °C for 24 h to obtain isolated colonies [7]. Single colonies were transferred into 100 mL of Sabouraud broth (Biokar, France) and incubated under the same temperature conditions for 24 h. Cell concentration was initially estimated spectrophotometrically at 600 nm (Mecasys Optizen POP, Korea) and adjusted to approximately 10^7 cells/mL, followed by verification by direct counting using a Neubauer hemocytometer. Cultures were centrifuged (6,810 × g, 15 min), and the biomass obtained was resuspended in 100 mL of glucose/fructose solution (150 g/L each) to prepare the starter inocula. Final inocula were standardized by OD600 and verified by direct counting prior to fermentation.

2.2.2. Synthetic Fermentation Medium

A synthetic medium was used as a simplified fermentation system prior to grape must experiments in order to evaluate fermentative performance under controlled conditions. The medium contained yeast extract (7 g/L) as nitrogen source and glucose/fructose (75 g/L each) as carbon source, with pH adjusted to 3.5 [12]. After sterilization at 121 °C for 15 min, pH was readjusted to 3.5 using 1 M HCl. Fermentations were carried out in triplicate, and final ethanol concentration, ethanol yield, and residual sugar were determined at the end of fermentation.

2.3. Laboratory-Scale Fermentations

2.3.1. Grape Must Preparation and Fermentation Conditions

Sauvignon Blanc grapes harvested in the Casablanca Valley, Chile (Table 3), were used for laboratory-scale fermentations. Grapes were manually cleaned, pressed, and filtered through fine mesh to remove pomace [13]. Initial must characteristics were density 1.088 g/mL, 21.1 °Brix, and expected alcohol content (EAC) 12.01%. Yeast assimilable nitrogen (YAN) was adjusted to 250 mg/L using diammonium phosphate (DAP; Sigma-Aldrich, USA), while sodium metabisulfite was added at 30 mg/L to reduce native microbial load; free SO_2 was verified by titration (HI 84500 Hanna Instruments, USA). Thermal sterilization was intentionally avoided to preserve volatile composition.

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Monoculture fermentation was conducted in 500 mL Erlenmeyer flasks equipped with airlocks and sampling probes. Each flask contained must inoculated with SB or HU at a final concentration of 10^6 CFU/mL. *Saccharomyces cerevisiae* (Actiflore® 1118, Laffort, France) was used as control and inoculated according to the manufacturer's instructions at an equivalent final concentration. Flasks were aerated for 2 min and incubated at 15 °C under orbital agitation (200 rpm; JS Research JSSI-100C, Korea) [13]. Fermentation progress was monitored by density changes throughout the process. Mid-fermentation samples were plated on YMA agar to verify the absence of contaminant colonies, although no quantitative microbiological monitoring was performed.

2.4. Microvinification Setup

Two fermentation strategies were evaluated: monoculture and sequential inoculation. Sauvignon Blanc grapes from the Casablanca Valley were used (Figure 2; Table 3). Grapes were visually inspected to exclude *Botrytis sp.* contamination, refrigerated at 15 ± 1 °C, destemmed, and pressed. Potassium metabisulfite (Sigma-Aldrich, USA) was added to obtain 10–20 ppm free sulfite, and YAN was adjusted to 250 mg/L using DAP. Musts were clarified for 24 h at 4 ± 1 °C until turbidity reached 120–150 NTU. Fermentations were conducted in 15 L glass fermenters containing 10 L of must equipped with fermentation locks [13].

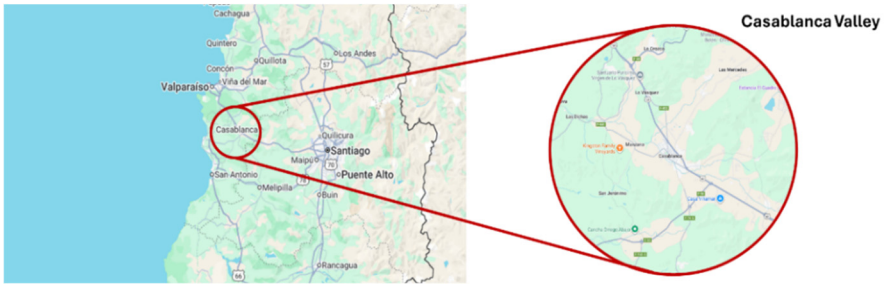

Figure 2. Geographical origin of Sauvignon Blanc must. 33°19'00''S, 71°25'00''W.

For monoculture fermentation, SB or HU were inoculated at 10^6 CFU/mL and maintained throughout fermentation at 18 ± 1 °C. In sequential fermentations, SB or HU were inoculated first at the same concentration, followed by SC inoculation at approximately 50% sugar depletion, also at 10^6 CFU/mL. Fermentation completion was defined as stable density for more than 48 h.

2.5. Analytical Methods

2.5.1. Fermentation Parameters

All determinations were performed in triplicate using independent fermentation replicates. Ethanol was quantified by HPLC (Agilent Infinity 1260, Germany) using an Aminex HPX-87H column (Bio-Rad, USA) coupled to diode array detection at 210 nm. The mobile phase consisted of 0.005 M H_2SO_4 at 0.6 mL/min, with column temperature maintained at 55 °C and injection volume of 20 µL. Quantification was performed using external calibration curves ($R^2 \geq 0.99$). Glucose, fructose, glycerol, and organic acids were determined using enzymatic kits (Megazyme, Ireland) with spectrophotometric detection at 340 nm.

2.5.2. Volatile Compound Analysis and Multivariate Evaluation

Volatile compounds were extracted in 20 mL headspace vials containing 3 mL of sample and 1 mL of saturated NaCl solution. Samples were incubated at 50 °C for 10 min under stirring (300 rpm) and extracted using a DVB/CAR/PDMS SPME fiber (50/30 µm). GC–MS analysis was performed using a Thermo Triplus 1310 gas chromatograph coupled to an ISQ LT mass spectrometer equipped with an RTX-5MS column (30 m × 0.25 mm × 0.25 µm). The oven program started at 40 °C for 5 min,

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increased at 10 °C/min to 250 °C, and was maintained for 10 min. Compounds were identified using Chromeleon 7.3 software and the NIST 2017 library, and results were expressed as relative peak area percentages.

Principal component analysis (PCA) was performed using Minitab Statistical 21 based on the correlation matrix of volatile compounds showing relative abundance >3%, selected to retain the dominant aroma-related variables and reduce noise from minor peaks. Components with eigenvalues >1 were retained according to the Kaiser criterion, and the first two principal components explaining at least 80% of total variance were used for graphical representation.

2.6. Sensory Evaluation

A trained sensory panel (n = 11; 25–50 years old), previously trained according to ISO 8586:2012 and OIV 332A/2009 standards, evaluated wines under controlled conditions (22 ± 2 °C) using ISO tasting glasses. Descriptive attributes included aromatic intensity, fruity, floral, tropical notes, acidity, and overall balance, using a 9-point structured scale. Samples were evaluated in duplicate under randomized coded conditions. All panelists participated voluntarily and provided informed consent.

2.7. Statistical Analysis

All experiments were performed in triplicate under a completely randomized design. Data was analyzed by ANOVA followed by Tukey's HSD test ($p \leq 0.05$) using Statgraphics Centurion XIX v15.2.05. Sensory variables were analyzed independently using the same statistical model, and results are expressed as mean ± standard deviation.

3. Results and Discussion

3.1. Fermentation in Synthetic Glucose–Fructose Medium

The oenological potential of native NSY from the Maule Valley (Chile), specifically SB and HU, was initially evaluated in synthetic glucose/fructose medium and compared with the SC (control). Fermentations were performed under controlled conditions to assess fermentative behavior prior to grape must experiments.

Table 1 summarizes the fermentative performance of SB and HU compared with SC in synthetic glucose/fructose medium. Neither SB nor HU fully consumed the available sugars, reaching approximately 90% utilization, whereas SC consumed more than 99.9%. This incomplete substrate consumption was reflected in final ethanol concentrations: SC produced 9.16% v/v, HU 8.50% v/v, and SB 4.25% v/v. These results are consistent with previous reports describing the limited fermentative capacity of several non-Saccharomyces yeasts under defined conditions.

Ethanol yields further highlighted differences among treatments. SC exhibited a yield of 0.48 g ethanol/g sugar, HU 0.44 g/g, whereas SB showed a markedly lower yield of 0.22 g/g. In practical terms, HU and SC required approximately 2.3 g of fermentable sugar to produce 1 g of ethanol, while SB required nearly 4.5 g.

The reduced conversion efficiency observed in SB suggests a distinct carbon allocation pattern, potentially involving alternative metabolic pathways beyond ethanol production. This behavior has been previously associated with *Starmerella bacillaris*, particularly its fructophilic metabolism and redox-balancing mechanisms linked to glycerol formation and biomass development [17]. Although metabolic fluxes were not directly quantified in the present study, the observed trend is consistent with the metabolic profile commonly described for this species.

The simplified composition of the synthetic medium, which lacks the complexity of grape must (including organic acids, micronutrients, and buffering capacity), likely contributed to the metabolic differences observed among strains. Therefore, the results should be interpreted as strain responses under controlled conditions rather than as direct predictors of winemaking performance.

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For HU, the relatively high ethanol yield despite incomplete sugar consumption suggests fermentative efficiency closer to SC under the evaluated conditions, although fermentation completion may have been limited by species-specific tolerance during later stages. Overall, these findings agree with previously reported trends for both species while highlighting the variability associated with native isolates.

The observed differences in carbon-to-ethanol conversion indicate strain-dependent metabolic tendencies whose practical relevance should be interpreted within the scope of the synthetic fermentation system.

Table 1. Fermentative performance of *Starmerella bacillaris* and *Hanseniaspora uvarum* in synthetic glucose/fructose medium (150 g/L total sugars).

Yeast	Ethanol (% v/v)	Yield (g ethanol/g sugar)	Sugar intake (%)
Starmerella bacillaris	4.25±0.35a	0.22±0.03a	90.30±2.77a
Hanseniaspora uvarum	8.50±0.73b	0.44±0.02b	90.40±5.75a
Saccharomyces cerevisiae	9.16±0.25b	0.48±0.04b	99.92±1.11b

Data are presented as mean ± standard deviation (n = 3). Different lowercase letters within a column indicate significant differences ($p < 0.05$).

3.2. Laboratory-Scale Fermentations (Monoculture)

Following the preliminary tests in synthetic medium, a laboratory-scale evaluation was performed using Sauvignon Blanc must from the Casablanca Valley (1.097 g/mL; 22.9 °Brix) under monoculture conditions. The results are presented in Table 2.

Laboratory-scale fermentations revealed differentiated behavior relative to the control and between NSY treatments. SB exhibited performance partially comparable to SC in several parameters. Residual sugar concentrations remained below 2.6 g/L for both treatments, consistent with dry wine classification. Likewise, ethanol yield (Yp/s) did not differ significantly between SB (0.374 g/g) and SC (0.368 g/g). Glycerol concentrations were also comparable, reaching 10.61 g/L for SB and 11.27 g/L for SC.

Table 2. Fermentative potential of the NSY *Starmerella bacillaris* and *Hanseniaspora uvarum* in Sauvignon Blanc must monoculture (Casablanca Valley 1.097 g/mL at a laboratory scale).

Oenological Parameter		Yeast strains		
		Saccharomyces cerevisiae (control)	Non-Saccharomyces yeast	
			Starmerella bacillaris	*Hanseniaspora uvarum*
Fermentation duration (days)		13	20	15
Specific growth rate μ (1/h)		0.021±0.002a	0.014±0.001b	0.019±0.002a
Final sugar concentration (g/L)		2.50±0.17a	2.59±0.90a	4.70±0.90b
Yp/s (g ethanol/ g sugars)		0.37±0.00a	0.37± 0.01a	0.41±0.01b
Ethanol production (%v/v)		13.49±0.06a	13.04±0.35b	12.36±0.14c
Glycerol (g/L)		11.27±0.46a	10.61±1.00a	5.85±0.54b
Organic acids (g/L)	Acetic	0.20±0.20a	0.66±0.01b	0.34±0.14a
	Malic	2.68±0.07a	1.82±0.09a	2.34±0.05a
	Tartaric	3.15±0.36a	3.57±0.31a	2.87±0.95a

Data are presented as mean ± standard deviation (n = 3). Different lowercase letters within a column indicate significant differences ($p < 0.05$).

These glycerol values were slightly above the range commonly reported for *S. cerevisiae* under standard winemaking conditions (typically 5–10 g/L), suggesting that laboratory fermentation

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conditions may have favored glycerol accumulation, likely due to increased oxygen transfer associated with orbital agitation [12,13].

No significant differences were detected in tartaric or malic acid concentrations. However, SB displayed distinct kinetic characteristics, with a lower specific growth rate (0.014 h^{-1}) than SC (0.021 h^{-1}), resulting in a longer fermentation time (20 versus 13 days). Although ethanol yield remained similar, final ethanol concentration was slightly lower in SB (13.04% v/v) than in SC (13.49% v/v), indicating moderate ethanol reduction under the evaluated conditions.

SB-associated fermentations also showed higher acetic acid concentrations (0.66 g/L) than SC (0.20 g/L). Although this value approached commonly reported sensory thresholds, it remained within acceptable enological limits and may reflect differences in oxygen transfer linked to the higher surface-to-volume ratio of laboratory fermenters.

HU showed a specific growth rate (0.019 h^{-1}) close to SC and completed fermentation in 15 days. Residual sugar remained slightly higher than in the control (4.7 versus 2.5 g/L), while glycerol production was markedly lower (5.85 versus 11.27 g/L), suggesting a distinct carbon allocation pattern.

Considering the freshness-oriented style of Sauvignon Blanc, moderate glycerol production by HU may remain compatible with the expected sensory profile of this wine [18].

3.3. Microvinifications (Sequential and Monoculture)

For microvinification, Sauvignon Blanc must from Casablanca Valley (central zone of Chile) was used (Figure 2), and their physicochemical characteristics are presented in Table 3.

Table 3. Characteristics of the Sauvignon Blanc must used in the microvinifications.

Harvest	Density g/mL	°Brix	EAC*	pH	Titratable acidity g/L**
May/21	1.097	22.9	13.2-13.4	3.0	8.64

*EAC: Expected alcohol content (theoretical). **Titratable acidity was reported as Tartaric acid YAN adjusted to 250 mg/L prior to inoculation.

The enological behavior of SB and HU was evaluated under monoculture and sequential fermentations using must from the Casablanca Valley. Fermentation trends are presented considering the experimental conditions applied, including controlled inoculation without dynamic microbiological monitoring. Figures 3 and 4 show the evolution of substrate consumption, monitored through density changes over time, for each yeast strain and fermentation strategy.

Microvinification trials showed generally similar fermentation kinetics among SB, HU, and the SC (control) under both monoculture and sequential inoculation strategies (Figure 3). In monoculture fermentations, all treatments exhibited a progressive density decline, reaching fermentation completion between 16 days (SC and HU) and 18 days (SB).

These fermentation times differed from those observed at laboratory scale, likely reflecting the combined influence of temperature, fermentation volume, and matrix complexity. Microvinifications were conducted at 18 °C, whereas laboratory fermentations were performed at 15 °C, and even moderate temperature increases are known to accelerate yeast metabolic activity.

Under these conditions, SB and HU displayed kinetic profiles broadly comparable to SC when fermentable sugars and nitrogen were not limiting, although this does not imply equivalent metabolic behavior.

Sequential inoculation did not substantially modify the overall fermentation pattern compared with monoculture fermentations. Approximately 50% of sugars were consumed by day 5, and fermentation completion occurred within a similar timeframe, indicating that prior NSY activity did not markedly delay sugar depletion after SC inoculation.

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Overall, both inoculation strategies maintained stable fermentation progression during microvinification, suggesting that the selected NSY strains were compatible with SC implantation and did not compromise fermentation completion within the tested system.

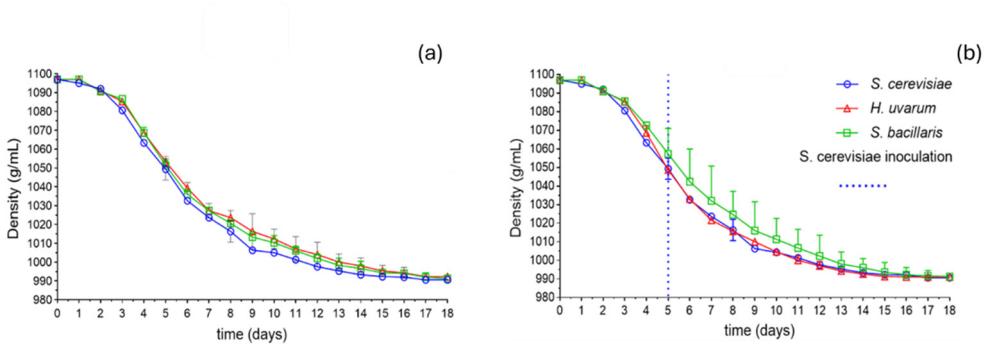

Figure 3. Evolution of substrate consumption during monoculture (a) and sequential inoculation (b) fermentations in Casablanca Sauvignon Blanc must.

Ethanol production during microvinification varied according to fermentation strategy (Table 4).

In monoculture fermentations, SB (11.90% v/v) and HU (12.50% v/v) yielded lower ethanol concentrations than the *S. cerevisiae* control (13.50% v/v), accompanied by lower ethanol yields (0.410 g/g for SB and 0.429 g/g for HU versus 0.471 g/g in SC). These values are consistent with previous reports describing lower ethanol formation by SB under monoculture conditions, frequently associated with fructophilic metabolism and alternative carbon allocation toward glycerol and biomass [19,20].

For HU, ethanol production remained closer to the control than commonly expected for non-Saccharomyces species, supporting the known strain-dependent variability reported for HU, particularly among native grape-associated isolates adapted to sugar-rich fermentation matrices [21–23]. In sequential inoculation, ethanol concentrations converged toward control values (13.68% and 13.50% v/v for SB–SC and HU–SC, respectively), consistent with fermentation completion after S. cerevisiae became metabolically predominant, partially masking the contribution of early NSY metabolism [24,25]. Overall, these results indicate that ethanol modulation by SB and HU during microvinification depended primarily on fermentation strategy, with the clearest differences observed in monoculture fermentations.

Organic acid and glycerol production further illustrated treatment-dependent metabolic differences during microvinification (Table 4).

In monoculture fermentations, SB and HU resulted in lower acetic acid concentrations (0.40 and 0.30 g/L, respectively) than their corresponding sequential fermentations (0.50 g/L in both SB–SC and HU–SC treatments). Although all values remained below commonly reported sensory perception thresholds (0.7–0.9 g/L), the increase observed during sequential inoculation suggests that fermentation progression and yeast succession may influence acetate accumulation within the evaluated system.

While previous studies have reported reductions in volatile acidity with certain sequential or co-inoculation strategies [25,26], the present results indicate that this response remains strongly dependent on strain combination and inoculation timing.

Lactic acid concentrations showed only minor variation among treatments, suggesting limited involvement of non-Saccharomyces strains in major acid transformations in this experimental context. Overall titratable acidity remained within the typical range for Sauvignon Blanc, indicating that NSY application did not induce destabilizing shifts in acid balance.

Glycerol production also differed slightly according to fermentation strategy. The *S. cerevisiae* monoculture produced 7.26 g/L glycerol, whereas SB and HU monocultures yielded slightly lower concentrations (6.65–6.69 g/L). Sequential fermentations showed similar values (6.51–6.74 g/L), indicating limited variation associated with inoculation strategy at this scale.

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A consistent difference was observed between laboratory-scale and microvinification trials, with laboratory fermentations showing higher glycerol concentrations across treatments. This contrast likely reflects differences in process conditions, particularly temperature, agitation, and oxygen transfer.

The higher glycerol values observed at laboratory scale may be associated with increased oxygen availability and surface-to-volume ratio, conditions known to affect redox balance and promote glycerol synthesis. In contrast, microvinification conditions likely favored a more reductive environment, with greater carbon allocation toward ethanol formation.

Overall, these results indicate that glycerol production was more strongly influenced by fermentation conditions and system scale than by yeast treatment alone.

Table 4. Fermentative potential of *Starmerella bacillaris* and *Hanseniaspora uvarum* (microvinifications).

Fermentation strategy	Yeast	Ethanol (%v/v)	Residual Sugar (g/L)	Yp/s	Organic acids (g/L)				Glycerol (g/L)
					Tartaric	Lactic	Acetic	Malic	
Control	†*Saccharomyces cerevisiae*	13.50±0.18a	1.00±0.10a	0.471±0.03a	3.38±0.09a	0.09±0.03a	0.29±0.12a	2.42±0.28a	7.26±0.27a
Monoculture	*Starmerella bacillaris*	11.90±0.12b	1.80±0.40b	0.410±0.03b	3.51±0.04a	0.25±0.00b	0.40±0.01b	2.17±0.04a	6.74±0.11b
	Hanseniaspora uvarum	12.50±0.17c	1.50±0.35b	0.429±0.01c	3.42±0.14ab	0.19±0.01b	0.30±0.06a	2.23±0.04a	6.51±0.24b
Sequential	*Starmerella bacillaris*	13.68±0.12a	1.10±0.35a	0.470±0.02a	3.31±0.09b	0.18±0.16b	0.50±0.10b	2.27±0.05a	6.65±0.21b
	Hanseniaspora uvarum	13.50±0.10a	0.90±0.00a	0.464±0.02a	3.39±0.09b	BDL*	0.50±0.00b	2.18±0.01a	6.69±0.20b

Data represents the mean ± standard deviation of three replicates. Different lowercase letters within each fermentation treatment indicate significant differences ($p < 0.05$). †The same *Saccharomyces cerevisiae* control was used as reference for both fermentation strategies.

3.4. Volatile Compounds and Aromatic Differentiation During Microvinification

Given the differences observed in fermentation performance and metabolite production, volatile compound formation was further evaluated to assess their potential contribution to aroma differentiation. Wine aroma results from the interaction of multiple volatile compounds derived from both grape matrix composition and yeast metabolism, whose relative abundance contributes to aromatic quality and sensory identity. Figure 5 summarizes the predominant volatile compounds detected in Sauvignon Blanc microvinifications from the Casablanca Valley.

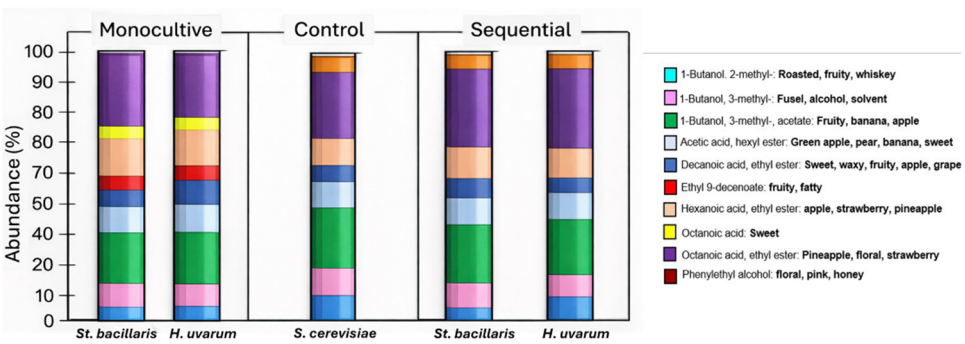

Figure 5. Relative abundance of predominant volatile compounds in Sauvignon Blanc microvinifications from the Casablanca Valley.

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Volatile analysis revealed differentiated aromatic tendencies associated with fermentation strategy and yeast treatment. Monoculture fermentations with SB and HU showed higher relative abundances of medium-chain ethyl esters, particularly ethyl hexanoate and ethyl octanoate, compounds commonly associated with fruity and floral descriptors in Sauvignon Blanc wines. These esters are formed through the condensation of ethanol with medium-chain fatty acids and are strongly influenced by yeast metabolic activity during fermentation [4,19].

Their enrichment in NSY monocultures suggests differences in ester-forming metabolism within the evaluated system, consistent with previous reports describing altered ester production in NSY fermentations compared with SC [27].

Aromatic differentiation among treatments was particularly evident in monoculture fermentation, where NSY fermentations generated more distinct volatile profiles relative to the SC (control).

Sequential fermentations showed intermediate volatile profiles between NSY and SC monocultures. This pattern is consistent with the expected overlap between early NSY metabolism and later dominance of SC, where part of the ester profile generated during early fermentation may remain detectable after fermentation completion. Although yeast population dynamics were not monitored, the persistence of NSY-associated esters suggests that early metabolic activity contributed to volatile differentiation during sequential fermentation. Higher alcohols were generally more prominent in SC monocultures, in agreement with the stronger fermentative activity commonly associated with this species and its amino acid catabolism. Although amino acid consumption was not directly quantified, no excessive accumulation of fusel alcohols was observed in NSY treatments, indicating that volatile differentiation did not compromise aromatic balance under the evaluated conditions.

Overall, the volatile profile indicates that SB and HU influenced ester formation during fermentation, with the magnitude of this effect depending primarily on inoculation strategy. These results reinforce the view that aroma modulation by NSY emerges from the interaction between microbial metabolism and fermentation dynamics.

3.5. Sensory–Chemical Correlation

PCA Analysis

To explore whether volatile differentiation among treatments followed consistent multivariate trends, principal component analysis (PCA) was applied to the dominant aroma-related compounds. Two principal components explained 89.7% of the total variance (Figure 6), with PC1 mainly associated with ethyl decanoate and PC2 with isoamyl alcohol.

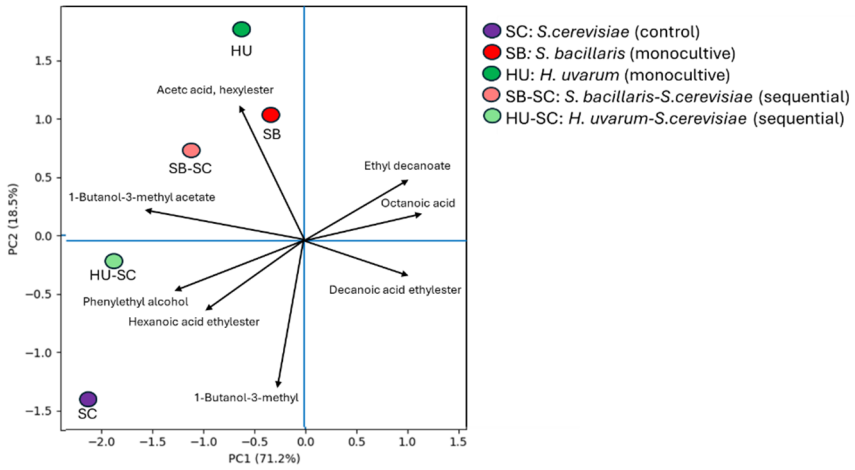

Figure 6. Principal component analysis (PCA) score plot based on dominant volatile compounds under different fermentation strategies.

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The PCA provided an exploratory overview of relationships among dominant volatile compounds and fermentation treatments, including yeast treatment and inoculation strategy. NSY fermentations (SB and HU, under both monoculture and sequential strategies) clustered in regions associated with medium-chain ethyl esters, compounds commonly related to fruity and floral aroma descriptors and widely reported as characteristic metabolites in fermentations involving NSY.

In contrast, the SC monoculture showed a tendency to associate with higher alcohol-related variables, consistent with the stronger fermentative activity typically observed for this species.

Sequential fermentations generally occupied intermediate positions between NSY monocultures and the SC (control), consistent with the combined metabolic contribution expected when early NSY activity is followed by SC implantation. This intermediate positioning suggests that part of the volatile signature generated during early NSY metabolism remained detectable after fermentation completion.

Overall, PCA trends were coherent with univariate volatile analysis and support the existence of treatment-associated differences in aromatic expression (Figure 6).

3.6. Sensory Analysis

Sensory evaluation performed by a trained panel (n = 11) revealed that wines produced with SB (monoculture) and HU (both monoculture and sequential strategies) exhibited significantly higher aromatic intensity than the SC (control) ($p \leq 0.05$). HU fermentations were predominantly associated with citrus, fresh tropical fruit, and floral descriptors, whereas SB wines showed a profile characterized by spicy nuances accompanied by citrus and tropical notes (Figure 7).

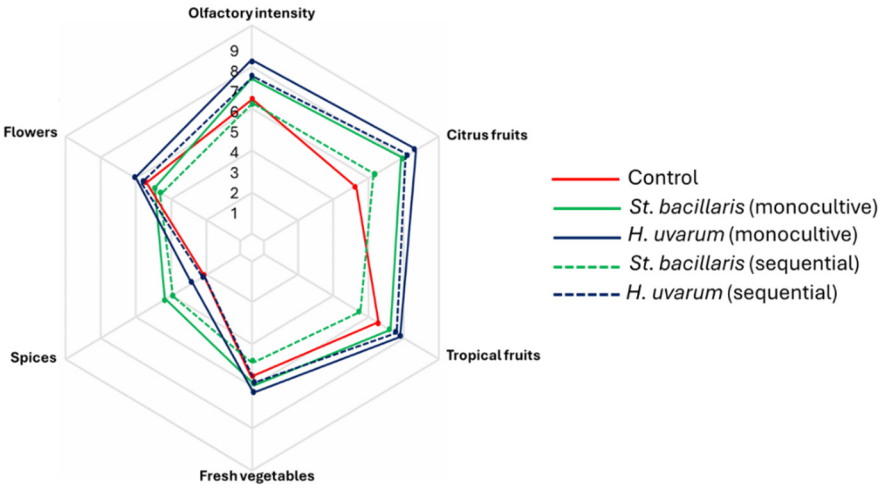

Figure 7. Sensory evaluation results.

These sensory trends were broadly consistent with the volatile composition observed (Figure 5), where NSY treatments displayed higher relative abundances of medium-chain ethyl esters, including ethyl octanoate, ethyl decanoate, and hexyl acetate, compounds widely recognized for their contribution to fruity and floral aromas in white wines. Based on odor thresholds reported in the literature [16], esters such as isoamyl acetate and ethyl octanoate are commonly present above their perception thresholds in Sauvignon Blanc wines, supporting their plausible contribution to the observed sensory differences. Although volatile compounds were expressed as relative abundances, the consistency between analytical and sensory trends supports their relevance under the evaluated conditions. Collectively, these findings indicate that NSY treatments influenced aromatic perception, particularly by enhancing fruity and floral attributes, with effects modulated primarily by fermentation strategy.

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4. Limitations

The present study should be interpreted within the scope of the experimental conditions applied. First, although inoculation conditions were designed to favor implantation of the selected non-Saccharomyces strains, dynamic microbiological monitoring was not performed during fermentation; therefore, the persistence and relative contribution of inoculated versus indigenous yeast populations cannot be fully resolved throughout the process.

Second, fermentations were conducted at laboratory and microvinification scales in controlled systems that do not fully reproduce industrial winery environments, particularly with respect to oxygen transfer, vessel geometry, and process dynamics.

Third, the study was based on Sauvignon Blanc must from a single harvest season within a specific experimental framework, which limits direct extrapolation to other vintages, grape varieties, or production regions.

Consequently, the results should be interpreted as context-specific evidence of strain behavior in controlled fermentation systems rather than as broadly generalizable responses across winemaking systems. These limitations should be considered when extrapolating the present findings to industrial winemaking scenarios.

5. Conclusions

This study provides a multi-scale evaluation of two Chilean isolates of *Starmerella bacillaris* (SB) and *Hanseniaspora uvarum* (HU) using monoculture and sequential fermentation strategies in Sauvignon Blanc must under controlled experimental settings. The results indicate that strain performance depended primarily on fermentation design and scale, with distinct responses observed across synthetic, laboratory, and microvinification systems.

In synthetic medium, both strains showed incomplete sugar consumption and lower ethanol yields than SC, confirming differentiated metabolic behavior in a simplified system. At laboratory scale, both strains completed fermentation in monoculture, with SB showing moderate ethanol reduction and HU lower glycerol production relative to the control.

During microvinification, monoculture fermentations produced lower ethanol concentrations than SC, whereas sequential fermentations converged toward control values while partially preserving aromatic differentiation. Volatile and sensory analyses consistently showed that NSY treatments were associated with higher relative abundances of medium-chain ethyl esters linked to fruity and floral attributes.

Overall, SB and HU represent promising native biotechnological resources for controlled aroma modulation in Sauvignon Blanc, although their practical performance remains dependent on fermentation strategy and process conditions. Further studies including molecular monitoring, multiple vintages, and broader production conditions are needed to define industrial applicability more precisely.

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Institutional Review Board Statement: This study involved voluntary adult participants (⩾18 years old) and consisted exclusively of a non-invasive sensory evaluation (taste, aroma, and texture), representing a minimal-risk procedure. All participants provided written informed consent prior to participation, and no personal or sensitive data were collected or recorded in identifiable form. The study was conducted in accordance with the principles of the Declaration of Helsinki.

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

Data Availability Statement: The datasets generated during the current study are available from the corresponding author upon reasonable request.

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Abbreviations

The following abbreviations are used in this manuscript:

NSY	Non-Saccharomyces Yeasts
SC	*Saccharomyces cerevisiae*
SB	*Starmerella bacillaris*
HU	*Hanseniaspora uvarum*

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