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SPECIAL ISSUE

Pilot-scale assessment of native Uruguayan yeast strains for Tannat wine fermentation

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ABSTRACT

In recent decades, there has been renewed interest in studying and utilising indigenous yeast strains to preserve the unique characteristics of the terroir, thus producing wines that truly reflect their place of origin. A key challenge is the identification of non-conventional yeasts capable of both completing fermentation and enhancing a wine's uniqueness, complexity, and sensory appeal. In this study, we evaluated the oenological potential of three selected native strains, *Saccharomyces cerevisiae* 3FS, *Saturnispora diversa* 1FS, and *Starmerella bacillaris* 3MS, through pilot-scale fermentations of Tannat must in 200-L stainless steel tanks. We also explored the influence of these strains on the volatile compound profiles of the resulting wines. The native strains exhibited notable kinetic fermentative similarities to the reference strain *S. cerevisiae* RX60, thus demonstrating their comparable robust fermentative power, which is crucial for ensuring reliable fermentation in a commercial setting. This confirms that these strains can independently conduct fermentation. All three native strains consumed approximately 99 % of the must sugars within 11 days of alcoholic fermentation, achieving ethanol concentrations in the range of 9.65–11.58 %. Pilot-scale trials highlighted the pronounced fructophilic nature of *S. bacillaris* 3MS, which left very low levels of residual fructose (0.06 ± 0.05 L^{-1}). This strain fermented glucose at a significantly lower rate than it did fructose, resulting in a final glucose-to-fructose (G/F) ratio of 1.83, which is considerably higher than that observed for the other strains (0.41–0.50). Moreover, fermentation with *S. bacillaris* 3MS reduced ethanol content by 2 % (*v/v*) compared to the *Saccharomyces cerevisiae* strains; such behaviour is advantageous for producing wines with lower alcohol levels. Fermentations using native strains produced wines with more pronounced floral and fruity aromas: specifically, *S. bacillaris* 3MS significantly increased the total concentrations of volatile esters, norisoprenoids, and terpenes, as detected by GC-MS, relative to the other species. The approach used here can enable winemakers to utilise non-*Saccharomyces* yeasts, such as *S. bacillaris* 3MS and *S. diversa* 1FS, to enhance wine aroma, while promoting biodiversity, fostering innovation, and creating wines with greater authenticity and uniqueness.

KEYWORDS: *Starmerella bacillaris*, *Saturnispora diversa*, wine fermentation, pilot-scale, aroma, Macrowine 2025

INTRODUCTION

The microbiology of wine fermentation has been extensively studied, revealing the intricate complexity of fermentation ecology (Romano *et al.*, 2019.). Traditional wine production relies on spontaneous fermentation, with indigenous yeast strains naturally present on grape surfaces and winery equipment driving the process and imparting unique organoleptic characteristics. However, due to challenges linked to reproducibility, quality control, and traceability, spontaneous fermentation has declined in modern wineries (Pinto *et al.*, 2020).

In light of the aforementioned issues, many winemakers use selected *Saccharomyces cerevisiae* strains as commercial starter cultures, thus ensuring more controlled and predictable fermentations. While these starter cultures offer practical benefits, their widespread use across different regions leads to the standardisation of fermentative flora. This homogenisation reduces the complexity of wine, masking the distinctive aromas and terroir-specific attributes that define regional identity (Binati *et al.*, 2019; Rainieri & Pretorius, 2000). In recent decades, there has been a renewed interest in the study and use of indigenous yeast strains to preserve the uniqueness of the terroir, and thus produce wines that truly reflect their place of origin (Lappa *et al.*, 2020). The connection between a wine's microbiota and its terroir highlights the relationship between the native microorganisms and the environmental conditions of a vineyard. Numerous studies highlight that, when co-fermented with *Saccharomyces cerevisiae*, *non-Saccharomyces* (NS) strains enhance fermentation dynamics and enrich the aromatic profiles of wine, thus reflecting a wine's regional identity and terroir (Jolly *et al.*, 2014; Padilla *et al.*, 2016). There are many reports on the properties and advantages of NS yeasts; however, these yeasts are generally developed within a microvinification framework at the laboratory scale, and the results are rarely validated at an industrial or semi-industrial scale, thus calling into question their applicability (Jolly *et al.*, 2014). Finding new *Saccharomyces* and NS strains with promising oenological properties for use as starter cultures represents a major challenge today. Yeasts selected for their specific fermentative and technological capabilities play a vital role in optimising winemaking processes, enhancing the expression of terroir, and crafting wines with unique sensory profiles. Validating these strains at a semi-industrial scale is essential to ensure their effectiveness and applicability in producing high-quality wines.

In previous studies, we isolated, identified, and conducted physiological and biochemical characterisations of native yeasts from Tannat grapes in the Maldonado vineyard (Morera *et al.*, 2022). Three isolated strains; *Saccharomyces cerevisiae* T19-3FS, *Saturnispora diversa* T19-1FS, and *Starmerella bacillaris* T19-3MS demonstrated high performance in laboratory conditions. In this study, we assessed the oenological potential and reproducibility of these native strains at a pilot scale in 200-L stainless tanks during the vinification of a Tannat must with an expected ethanol content of 12.9 %; we then explored the influence of the volatile compounds on the resulting wines.

MATERIALS AND METHODS

1. Yeast strains

Saccharomyces cerevisiae T19-3FS (*S. cerevisiae* 3FS), *Starmerella bacillaris* T19-3MS (*S. bacillaris* 3MS), and *Saturnispora diversa* T19-1FS (*S. diversa* 1FS) strains had been previously isolated from grapes of Tannat variety in the vineyard of Maldonado-Uruguay (Morera *et al.*, 2022). The commercial *S. cerevisiae* yeast strain used as a reference was Zymaflore™ RX60 from Laffort (France). All the strains were maintained at 4 °C for short-term storage on a YPDA medium (20 g L^{-1} bacteriological peptone, 10 g/L yeast extract, 20 g/L glucose, 15 g/L agar, Oxoid) in Petri dishes and preserved at 20 % glycerol in YPD (10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose) at –80 °C.

2. Obtaining biomass for semi-industrial-scale purposes

The yeast cultures were grown in the laboratory by refreshing each strain on YPDA plates and then they were incubated at 28 °C for 48 h. Then, 500 mL flasks were filled with 50 mL of YPD broth, which was inoculated with about 2×10^6 cell/mL of each strain for 12 h at 28 °C and 180 rpm (preculture). For the fermentation trials in the Uruguayan winery Bodega Garzón, the starter culture for fermentation was prepared using the preculture in 5 L of YPD medium and incubated for 48 h at 28 °C and 180 RPM. The cells of each strain were then collected by centrifugation (5000 g, 15 min) and exhaustively washed with distilled water. Cell concentration in the pellet was determined by microscope in the Neubauer chamber. The collected biomass was preserved at 4°C for a restricted duration until utilised.

3. Semi-industrial fermentations

S. cerevisiae 3FS, *S. bacillaris* 3MS, and *S. diversa* 1FS strains were used in pilot scale fermentations in the Bodega Garzon Winery, Departamento of Maldonado-Uruguay. Each strain biomass was suspended and mixed in 1 L of Tannat grape must at room temperature, and then inoculated at a density of about 2×10^6 cell/mL in a container with 200 L of Tannat grape must (200 g/L sugars, pH of 3.46, 7.2 g/L tartaric acid, and 120 mg/L yeast assimilable nitrogen) at 21 ± 2 °C. Grape must was supplemented with 50 mg/L of sulfur dioxide in potassium metabisulfite form and 20 g/L of Thiazote (Laffort, France) once the fermentation had started. The inoculum of each strain in the tanks was carried out in duplicate. The fermentation was monitored daily by measuring density, temperature, and pH.

4. HPLC analysis of fermentation metabolites

Fermentable sugars (fructose and glucose) and fermentation metabolites (ethanol and glycerol) were quantified by High-Pressure Liquid Chromatography (HPLC Waters 510). A volume of 20 µL of sample diluted 1/10 (or 1/100 for must) was injected into a ROA-Organic Acid H+ 8 % column (300 × 7.8 mm) (Phenomenex) with a flow of 0.5 mL/min using 1.5 mM sulfuric acid at 25 °C. A coupled infrared (IR)

(WATERS R401) detector was used and data processing was performed using the Empower software. All data are reported as the average of three replicates ± standard deviation. Quantification of compounds was carried out by the external standard method using the pure standard (HPLC quality) of each.

5. Analytical determination of volatile compounds

The extraction of free fraction (volatile) was carried out by solid phase extraction (SPE) in Manifold equipment using commercial cartridges Isolute ENV+ (Biotage) (Boido *et al.*, 2003). For the quantification, 0.1 mL of internal standard (1-heptanol at 230 mg/L in a 50 % hydroalcoholic solution) was added to each sample before extraction.

Quantification and identification of isolated volatiles were performed using a gas chromatograph (GC) coupled to a Shimadzu QP 5050 mass spectrometer (GC-MS) equipped with reference libraries (Adams, 2001; Marais *et al.*, 1992; Strauss *et al.*, 1987). The chromatographic column used was Carbowax (25 mx 0.25 mm id) coated with polyethylene glycol (0.25 µm phase thickness). The programme was 60 °C (1 min) with a ramp from 60 °C to 240 °C (from 3 °C/min) and maintenance at 240 °C for 1 min. The injector temperature was 250 °C, the injection mode was splitless and the injected volume was 1.0 µL using He as carrier gas, at 92.6 kPa (55.9 cm/s), an interface temperature of 250 °C at 70 eV, and a masses range of 40–400 amu. Compounds were identified by their MS spectra and confirmed by the determination of their Kovats indices. The amounts were calculated as equivalents of the internal standard (Boido *et al.*, 2003).

RESULTS AND DISCUSSION

1.Fermentation kinetics and major metabolites

The strains *S. cerevisiae* 3FS, *S. bacillaris* 3MS, and *S. diversa* 1FS, which had shown desirable analytical profiles in laboratory fermentations, were used in pilot scale fermentations of naturally processed grape Tannat must at the premises of a commercial winery. From previous experience and preliminary internal trials conducted on this must we knew that the applied conditions prevent spontaneous fermentation. The aim of our study was to evaluate the fermentation performance of selected native yeast strains and compare them under conditions that reflect standard winery practices, using the commercial *S. cerevisiae* strain RX60 as a reference. The results of this pilot scale experiment show that these strains are not only capable of completing the fermentation process within a similar time frame (11 days) to the commercial *S. cerevisiae* strain RX60, but they could also provide insights into their fermentative characteristics and potential for producing high-quality wines. As was expected and consistent with Morera *et al.* (2022), the three native strains showed similar fermentation behaviour to the reference strain *S. cerevisiae* RX60 (Figure 1). The kinetic similarities between the native strains and the reference strain *S. cerevisiae* RX60 are noteworthy, as they suggest that the native strains possess the robust fermentative power essential for successful fermentation in a commercial setting, and that they can thus be used independently for fermentation. The most surprising finding is the remarkable fermentative capacity of the NS strains *S. bacillaris* 3MS and *S. diversa* 1FS, which can complete fermentations independently.

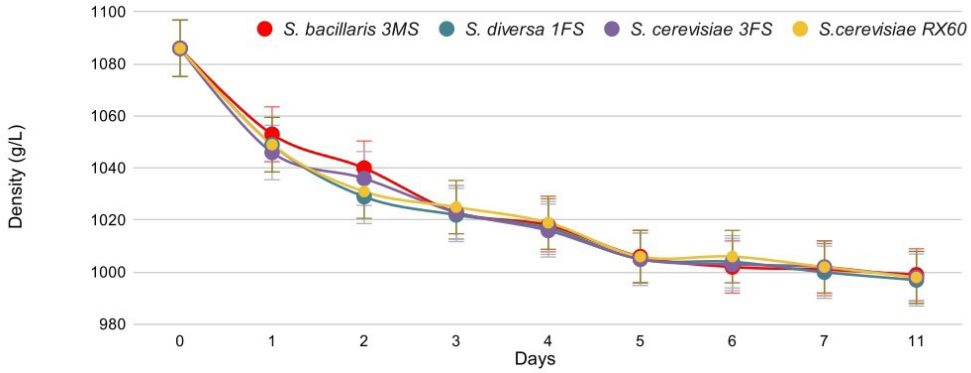

FIGURE 1. Density measures of fermentation kinetics in Tanat must tank inoculated with the three native strains *S. bacillaris* 3MS, *S. diversa* 1FS, and *S. cerevisiae* 3FS, and the commercial *S. cerevisiae* strain RX60.

In addition to confirming the high fermentative power of the native strains *S. cerevisiae* 3FS, *S. bacillaris* 3MS, and *S. diversa* 1FS in pilot-scale fermentations, it is crucial to assess the metabolites generated after fermentation and their potential benefits for commercial winemaking. Fermentation metabolites can directly impact the organoleptic characteristics and quality of wine; therefore, their evaluation in wine research and optimisation is key to providing insights into their applicability in larger-scale vinification processes.

Table 1 provides the chemical compositions of the wines produced at a commercial winery using the three selected native strains at the end of each alcoholic fermentation and 11 days after inoculation with the yeast strains.

We assessed the oenological potential of these strains at a pilot scale during the vinification of a Tannat must with an expected ethanol content of 12.9 %. All three native strains were able to consume nearly all the fermentable sugars

TABLE 1. HPLC analysis of Tannat alcoholic fermentations using three selected native strains *S. bacillaris* 3MS, *S. diversa* 1FS, and *S. cerevisiae* 3FS, and the commercial strain *S. cerevisiae* RX60. The data are shown as ranges between the minimum and maximum values obtained between the duplicates.

Sample ID	Glucose (G) g L^{-1}	Fructose (F) g L^{-1}	Glycerol g L^{-1}	Ethanol		G/F ratio
				% (*v/v*)	g L^{-1}	
S. bacillaris 3MS	0.11 ± 0.01 (A)	0.06 ± 0.05 (A)	9.15 ± 0.18 (C)	9.65 ± 0.44 (A)	7.62 ± 0.35 (A)	1.83
S. diversa 1FS	0.07 ± 0.00 (B)	0.14 ± 0.01 (B)	8.19 ± 0.18 (A)	10.69 ± 0.78 (AB)	8.45 ± 0.62 (AB)	0.50
S. cerevisiae 3FS	0.06 ± 0.02 (B)	0.15 ± 0.01 (B)	8.71 ± 0.16 (BC)	11.91 ± 0.57 (B)	9.41 ± 0.45 (B)	0.40
S. cerevisiae RX60	0.07 ± 0.01 (B)	0.17 ± 0.01 (B)	8.49 ± 0.42 (AB)	11.58 ± 0.33 (B)	9.15 ± 0.26 (B)	0.41

An ANOVA was performed, followed by Tukey's *post hoc* test. The letters correspond to the grouping with an alpha of 0.05.

(glucose and fructose) present in the must after 11 days of alcoholic fermentation. *S. bacillaris* 3MS, *S. diversa* 1FS, and *S. cerevisiae* 3FS consumed around 99 % of the must sugars, resulting in robust ethanol production in the range of 9.65 to 11.58 % (Table 1). The *S. bacillaris* 3MS and *S. diversa* 1FS strains exhibited markedly different behaviour to other non-Saccharomyces (NS) strains as previously reported, highlighting the novelty and relevance of our findings - particularly considering that NS species are typically employed in co-fermentation with Saccharomyces to ensure successful fermentation (Binati *et al.*, 2019; Canonico *et al.*, 2016). Nevertheless, our results are consistent with previous reports, further indicating the robustness and fermentative potential of certain NS strains under specific conditions (Englezos *et al.*, 2015; Bagheri *et al.*, 2018).

Most of the analysed strains exhibited a preference for glucose (G) over fructose (F), confirming the glucophilic character of *S. cerevisiae* 3FS and *S. diversa* 1FS, except for *S. bacillaris* 3MS. The G/F ratios values were < 1 for *S. diversa* 1FS (0.50) and both of the *S. cerevisiae* strains (0.40-0.41) (Table 1). The exception was *S. bacillaris* 3MS (1.83), showing a significant preference for fructose rather than glucose; this is consistent with Magyar and Tóth (2011). The present study highlights the strong fructophilic character of this strain, which left very low levels of fructose residues (0.06 ± 0.05 g/L) in comparison with the other species studied. Glucose and fructose consumption profiles were considerably influenced by the yeast strains used as starters, and selecting strains with different glucose/fructose preferences could be very useful for the wine industry. Yeasts with a higher capacity for fructose consumption are of interest to the industry, as they help avoid undesirable sweetness in dry wines–given that fructose is approximately twice as sweet as glucose–and could also improve wine stability by minimizing residual fructose and reducing the risk of secondary fermentation (Rossouw & Bauer, 2016; Magyar & Tóth, 2011).

Climate change is driving an increase in the alcohol content of wines due to the rapid accumulation of sugars in grapes, posing significant challenges for winegrowers. To mitigate these effects, producers are adopting viticultural, technological, and microbiological strategies for producing balanced wines in the new climatic conditions, thus maintaining their quality and ensuring consumer satisfaction. NS yeasts in mixed or sequential fermentations with *Saccharomyces cerevisiae* are therefore gaining in popularity due to their ability to reduce ethanol production in wines besides providing aromatic compounds (Basso *et al.*, 2016; Englezos *et al.*, 2015). Some authors have proposed the potential use of *S. bacillaris* strains in combination with *S. cerevisiae* in wine fermentations (Capece *et al.*, 2022); for instance, Giaramida *et al.* (2013) and Zara *et al.* (2014) demonstrated an increase in glycerol content and a decrease in alcoholic degree when *S. Bacillaris* was used in combination with *S. cerevisiae* at a pilot scale. Fermentation trials showed that *S. bacillaris* 3MS produced a significantly lower ethanol concentration (9.65 ± 0.18 %) than *S. cerevisiae* 3FS (11.91 ± 0.57 %) and *S. cerevisiae* RX60 (11.58 ± 0.33 %), confirming its lower fermentative capacity. Inoculation of Tannat must with *S. bacillaris* 3MS not only highlighted its marked fructophilic behaviour but also resulted in an approximate 2 % (*v/v*) ethanol reduction compared to the evaluated *S. cerevisiae* strains - which is of interest for the production of reduced-alcohol wines. The *S. bacillaris* strain produced a significantly higher glycerol concentration (9.15 ± 0.18 g/L) than *S. diversa* 1FS (8.19 ± 0.18 g/L) and *S. cerevisiae* RX60 (8.49 ± 0.42 g/L).

Different behaviour in the fermentation of NS and *S. cerevisiae* strains could significantly affect subtle differences in aromatic profiles and the overall complexity of the wine. As discussed extensively by several authors, native yeasts could enhance the expression of terroir characteristics by contributing to unique aromatic compounds, thus enriching the regional identity of the wine (Morera *et al.*, 2022; Jolly *et al.*, 2014). In the present study, volatile compounds were identified and quantified after the alcoholic fermentation of Tannat must from Uruguay. The Tannat variety, emblematic of the country, produces a robust wine known for its strong character, consistently standing out for producing high-quality wines with a unique identity. Thus, an SPE/GC-MS analysis was conducted on Tannat wines fermented with *S. cerevisiae* 3FS, *S. diversa* 1FS, and *S. bacillaris* 3MS, which were compared to a commercial yeast strain usually used in the winery, thus allowing volatile metabolites produced during yeast metabolism to be evaluated.

TABLE 2. SPE/GC-MS Analysis of volatile compounds in wines produced using three native strains. Values with different superscript letters (a, b, c) within a row indicate significantly different concentrations between strains (ANOVA, $p < 0.05$). (part 1/2)

	Compound	*S. bacillaris* 3MS Conc. (µg L^{-1}) ± S.D.	*S. diversa* 1FS Conc. (µg L^{-1}) ± S.D.	*S. cerevisiae* 3FS Conc. (µg L^{-1}) ± S.D.	*S. cerevisiae* RX60 Conc. (µg L^{-1}) ± S.D.
Acids	Isobutyric acid	70 ± 3 ^{c}	96 ± 5 d	32 ± 5 ^{a}	54 ± 6 ^{b}
	Isovaleric acid	98 ± 0.2 ^{c}	95 ± 7 ^{c}	41 ± 2 ^{b}	n.d. ^{a}
	Butanoic acid	60 ± 5 ^{b}	104 ± 12 ^{c}	40 ± 9 ^{a}	53 ± 5 $^{a, b}$
	Hexanoic acid	130 ± 24	144 ± 25	147 ± 34	135 ± 20
	Octanoic acid	n.d. ^{a}	91 ± 15 ^{c}	54 ± 6 ^{b}	100 ± 12 ^{c}
	Total acids	358 ± 32 ^{a}	530 ± 64 ^{b}	314 ± 56 ^{a}	342 ± 43 ^{a}
Alcohols	3-Ethoxy-1-propanol	18 ± 5 $^{b, c}$	20 ± 1 ^{c}	6 ± 2 ^{a}	10 ± 1 $^{a, b}$
	2,3-butanediol	187 ± 45 ^{b}	379 ±102 ^{c}	n.d. ^{a}	177 ± 14 ^{b}
	3-(Methylthio)-propanol	47 ± 4	50 ± 12	51 ± 9	51 ± 9
	Tyrosol	199 ± 22 ^{a}	554 ± 149 ^{c}	283 ± 8 ^{b}	418 ± 52 ^{c}
	1-Methoxy-2-butanol	n.d. ^{a}	27 ± 8 ^{b}	n.d. ^{a}	n.d. ^{a}
	Benzyl alcohol	n.d. ^{a}	n.d. ^{a}	27 ± 3 ^{b}	n.d. ^{a}
	Total alcohols	451 ± 76 ^{a}	1030 ± 272 ^{b}	367 ± 22 ^{a}	656 ± 76 ^{b}
C6	3-Hexen-1-ol (Z)	n.d. ^{a}	4 ± 0.1 ^{b}	n.d. ^{a}	n.d. ^{a}
	1-Hexanol	n.d. ^{a}	n.d. ^{a}	36 ± 6 ^{b}	74 ± 12 ^{c}
	Total C6	0.0 ^{a}	4 ± 0.1 ^{b}	36 ± 6 ^{c}	74 ± 12 d
Esters	Ethyl hexanoate	21 ± 3 ^{c}	n.d. ^{a}	n.d. ^{a}	5 ± 2 ^{b}
	(R) Ethyl 2-hydroxypropanoate	441 ± 49 ^{b}	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}
	Ethyl octanoate	39 ± 2 ^{c}	26 ± 7 $^{b, c}$	7 ± 3 ^{a}	16 ± 4 $^{a, b}$
	Hexyl methoxyacetate	36 ± 3 ^{c}	n.d. ^{a}	n.d. ^{a}	16 ± 1 ^{b}
	Butanedioic acid, diethyl ester	71 ± 1 ^{a}	108 ± 29 ^{b}	64 ± 10 ^{a}	84 ± 9 ^{a}
	Ethyl octadecanoate	43 ± 4 ^{b}	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}
	Diethyl succinate	482 ± 104 ^{c}	n.d. ^{a}	134 ± 6 ^{b}	n.d. ^{a}
	Propanoic acid, 2-hydroxy-, ethyl ester	n.d. ^{a}	223 ± 45 ^{c}	73 ± 10 ^{b}	148 ± 44 ^{c}
	Methyl 4-hydroxybutanoate	n.d. ^{a}	24 ± 3 ^{c}	9 ± 2 ^{b}	n.d. ^{a}
	Isopentyl hexanoate	n.d. ^{a}	n.d. ^{a}	33 ± 7 ^{b}	n.d. ^{a}
	Valeric acid, 2-methyl-, pentyl ester	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}	22 ± 4 ^{b}
	Butanedioic acid, monomethyl ester	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}	4 ± 1 ^{b}
	Total esters	1133 ± 166 ^{b}	381 ± 84 ^{a}	320 ± 38 ^{a}	295 ± 65 ^{a}
Lactones	Butyrolactone<gamma->	75 ± 11 $^{b, c}$	89 ± 8 ^{c}	56 ± 4 ^{a}	66 ± 9 $^{a, b}$
	Dodecalactone<gamma->	64 ± 13 ^{b}	73 ± 12 ^{b}	n.d. ^{a}	n.d. ^{a}
	Mevalolactone	n.d. ^{a}	17 ± 5 ^{b}	n.d. ^{a}	n.d. ^{a}
	Decalactone<gamma->	n.d. ^{a}	81 ± 14 ^{b}	n.d. ^{a}	66 ± 11 ^{b}
	Heptalactone<gamma->	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}	44 ± 7 ^{b}
	Nonalactone<gamma->	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}	7 ± 2 ^{b}
	Total lactones	139 ± 24 ^{b}	260 ± 39 ^{c}	56 ± 4 ^{a}	183 ± 29 $^{b, c}$

TABLE 2. SPE/GC-MS Analysis of volatile compounds in wines produced using three native strains. Values with different superscript letters (a, b, c) within a row indicate significantly different concentrations between strains (ANOVA, $p < 0.05$). (part 2/2)

Phenols	3,5-Di-tert-butylphenol	70 ± 8 ^{b}	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}
	4 Vinyl guaiacol	n.d. ^{a}	2 ± 0.3 ^{b}	n.d. ^{a}	n.d. ^{a}
	Vanillyl alcohol	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}	5 ± 1 ^{b}
	Homovanillyl alcohol	59 ± 2 ^{b}	56 ± 5 ^{b}	n.d. ^{a}	58 ± 2 ^{b}
	Total phenols	129 ± 10 ^{c}	58 ± 5 ^{b}	0.0 ^{a}	63 ± 3 ^{b}
Norisoprenoids	3-Oxo-7,8-dihydro-alpha-ionol	9 ± 0.3 ^{c}	n.d. ^{a}	n.d. ^{a}	6 ± 1 ^{b}
	Alpha-ionol	n.d. ^{a}	n.d. ^{a}	0.4 ± 0.1 ^{b}	n.d. ^{a}
	Total norisoprenoids	9 ± 0.3 ^{d}	0.0 ^{a}	0.4 ± 0.1 ^{b}	6 ± 1 ^{c}
Terpens	Cis-beta-terpineol	6 ± 0.1 ^{b}	n.d. ^{a}	13 ± 5 ^{c}	n.d. ^{a}
	Linalool oxide, dihydro	41 ± 7 ^{b}	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}
	Longipinanol	5 ± 1 ^{b}	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}
	Ethyl linalool	n.d. ^{a}	4 ± 0.1 ^{b}	n.d. ^{a}	n.d. ^{a}
	Iso-Isopulegol	n.d. ^{a}	11 ± 1 ^{b}	n.d. ^{a}	n.d. ^{a}
	Hydroxy-alpha-terpenyl acetate	n.d. ^{a}	9 ± 2 ^{b}	n.d. ^{a}	n.d. ^{a}
	Citronellol epoxide (R or S)	n.d. ^{a}	n.d. ^{a}	2 ± 0.9 ^{b}	6 ± 2 ^{c}
	Tetrahydro-Lavandulol	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}	7 ± 2 ^{b}
	Cedrol	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}	2 ± 0.6 ^{b}
	1,2-dihydro-8-hydroxylinalool	n.d. ^{a}	n.d. ^{a}	11 ± 2 ^{b}	n.d. ^{a}
	Epoxy-linalool oxide	n.d. ^{a}	n.d. ^{a}	n.d. ^{a}	6 ± 2 ^{b}
	Total terpenes	52 ± 8 ^{b}	24 ± 3 ^{a}	26 ± 8 ^{a}	21 ± 7 ^{a}

2. Volatile aromatic compounds

A total of 53 volatile compounds were identified and quantified in Tannat red wine following alcoholic fermentation. (Table 2). The volatile fraction was composed of acids (9 %), aliphatic and aromatic alcohols, including C6 (15 %), esters (23 %), lactones (11 %), phenols (8 %), norisoprenoids (4 %), terpenes (21 %) and other identified compounds (9 %). Regarding the acid fraction, it is well known that acids are formed enzymatically during fermentation and can contribute to floral, cheesy, fatty, and rancid notes. In the resulting Tannat wines, aliphatic acids (C4–C8) were identified and significant differences were found only between the *S. diversa* 1FS fermentation (530 ± 64 µg/L) and the other strains (< 358 ± 32 µg/L), indicating that *S. diversa* 1FS produces the highest amount of acids. Aliphatic and aromatic alcohols are key compounds that enhance the sweet, floral, and fruity notes in the aromas of wines. Regarding these alcohols, significant differences in total alcohols were found between *S. diversa* 1FS (1030 ± 272 µg/L) and the commercial and other native strains. The greatest significant difference was found in the production of 2,3-butanediol, with *S. diversa* 1FS producing the highest amounts (379 ± 102 µg/L). 2,3-butanediol is important as it can enrich the aromatic profile of red wine by adding a layer of sweet and creamy notes. The *Saccharomyces* strains were prominent in the production of C6 alcohols, exhibiting a profile closely resembling this group of compounds, which can evoke vegetal sensations. Esters confer pleasant fruit notes, and therefore these kinds of compounds are desired in wines. The strain that stood out for its total ester production was *S. bacillaris* 3MS (1133 ± 166 µg/L), with highly significant differences found for the ethyl hexanoate (21 ± 3 µg/L), ethyl octanoate (39 ± 2 µg/L), hexyl methoxyacetate (36 ± 3 µg/L) and diethyl succinate (482 ± 104 µg/L) relative to the commercial strain and other native strains. Additionally, fermentation with *S. bacillaris* 3MS led to the formation of ethyl 2-hydroxypropanoate (441 ± 49 µg/L) and ethyl octadecanoate (43 ± 4 µg/L); such concentrations were not

detected in fermentations with the other native or commercial strains. Diethyl succinate is typically formed during alcoholic fermentation and is associated with aromatic notes reminiscent of wine, toffee, and fruits (Mendes *et al.*, 2022). The origin of this compound may be linked to the catabolic pathway of glutamate, commonly known as the GABA shunt. In this pathway, glutamate is decarboxylated to γ-aminobutyric acid (GABA), which is then transaminated to succinate semialdehyde (SSA), and subsequently oxidised to succinate. When SSA is not oxidised, it can instead be reduced to γ-hydroxybutyric acid (GHB), a precursor for lactonisation. This step may explain why *S. bacillaris* also stands out for the production of γ-butyrolactone. The final product of the GABA shunt, succinate, can undergo double esterification with ethanol to form diethyl succinate (Pérez *et al.*, 2022). Another strain that showed a notable production of diethyl succinate was the native *S. cerevisiae*, although it was the lowest producer of γ-butyrolactone. This therefore makes it a promising strain for enhancing these aromas in wines, as these compounds play a crucial role in the aromatic profile of red wines, contributing to their complexity, freshness, and balance. Lactones are among the most important compounds, as they contribute to the sensory characteristics of wines aged in oak wood (Pérez-Olivero *et al.*, 2014). Except for the native *S. cerevisiae*, all of the strains stood out for producing these compounds, with the highest concentrations observed for *S. diversa* 1FS (260 ± 39 µg L^{-1}). Phenols play a crucial role in the aromatic profile of Tannat, a variety known for its intensity, structure, and complexity. *S. bacillaris* 3MS t produced the highest concentration of these compounds (129 ± 10 µg L^{-1}), and *S. diversa* 1FS and *S. cerevisiae* RX60 produced similar amounts (58 *vs* 63 µg L^{-1}, respectively). By contrast, the native *S. cerevisiae* 3FS did not produce any phenols during Tannat must fermentation. In Tannat, volatile phenols (4-vinyl guaiacol) and phenolic compounds related to vanillin are key contributors to specific aromas, such as spicy, smoky, and sweet vanilla notes. Norisoprenoids and terpenes are part of the glycosylated reserve of aromas. These compounds play a key role in wine aroma due to their very low olfactory thresholds and their ability to impart pleasant floral and fruity notes, even at low concentrations. *S. bacillaris* 3MS was the highest producer of norisoprenoids (9 ± 0.3 µg L^{-1}) and terpenes (52 ± 8 µg L^{-1}), underscoring the potential of this strain for enhancing these valuable aromatic compounds. Among the norisopreoinds compounds, 3-Oxo-7,8-dihydro-alpha-ionol sis a key compound in red wines due in particular to contribution to floral and fruity notes. Its presence can enrich the wine's aromatic profile, adding freshness, complexity, and elegance, and it plays a key role in the sensory perception of young and aged wines. Fermentation using *S. cerevisiae* 3FS was the only one to produce alpha-ionone (0.4 ± 0.1 µg L^{-1}), a highly significant compound in red wines due to its contribution to floral and fruity notes, which enrich the wine's aromatic profile. Significant differences in norisoprenoid concentrations were observed between the use of commercial and native strains of *Cerevisiae* in fermentations (0.4 and 6 µg/L, respectively); however, no significant differences were observed between their terpene fractions (26 and 21 µg/L, respectively).

CONCLUSION

The results of the present study align with those of growing research, which highlights the potential of using NS yeasts to improve fermentation outcomes while preserving wine typicity. Selected native yeast strains were evaluated through pilot-scale fermentations, resulting in finished wines that exhibited desirable oenological properties, including distinctive aromatic profiles and an appropriate chemical composition, thereby underscoring their potential for commercial application in regional winemaking. Furthermore, the use of these NS strains as a pure inoculum in must fermentation - without the need for co-fermentation with *Saccharomyces cerevisiae* represents a significant advancement in modern winemaking, offering an alternative to traditional methods that rely solely on commercial starters for their robustness and fermentative power. This approach could enable winemakers to fully harness the unique characteristics of NS yeasts, such as *Starmerella bacillaris* 3MS, and *Saturnispora diversa* 1FS, to enrich the aroma of wines. Not only does this approach promote biodiversity but it also fosters greater innovation and differentiation in winemaking, resulting in wines with enhanced typicity and authenticity. Furthermore, *Starmerella bacillaris* 3MS offers a promising strategy for producing wines with lower alcohol content while enhancing sensory quality. Its ability to reduce ethanol yield, increase glycerol production, and enhance aromatic complexity makes it a valuable tool, particularly in regions with rising grape sugar levels. Although this strain can successfully ferment independently, it would be useful to carry out future research on semi-pilot co-fermentation using *Starmerella bacillaris* and native *S. cerevisiae* 3FS, in order to compare alcohol levels and aromatic profiles.

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