European Food Research and Technology (2026) 252:200
https://doi.org/10.1007/s00217-026-05130-8

ORIGINAL PAPER

Lachancea thermotolerans as a tool for enhancing aroma complexity in sparkling wine secondary fermentation

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Received: 8 January 2026 / Revised: 2 March 2026 / Accepted: 29 March 2026 / Published online: 28 April 2026

Abstract

In recent years, there has been an increased interest in non-*Saccharomyces* yeasts for their use in the production of sparkling wines to obtain products with distinctive flavor and increased aromatic complexity. Despite this, there are few studies focused on understanding the effects of using non-*Saccharomyces* yeasts in the secondary fermentation of sparkling wine production. In this study, two strains of *Lachancea thermotolerans* selected for primary fermentation were evaluated in secondary fermentation '*prise de mousse*' using two base wines from different vintages. The pressure-kinetic analysis showed that one strain of *L. thermotolerans* exhibited limited secondary fermentation aptitude, while the other exhibited secondary fermentation kinetics comparable to those of the *Saccharomyces cerevisiae* strain. The resulting sparkling wines, although presenting comparable main analytical characteristics, differed in aromatic composition and sensory profiles. Based on these characters, beyond the different base wines, the strains of *L. thermotolerans* showed a clear different contribution to the resulting sparkling wines. The influence of *L. thermotolerans* compared with *S. cerevisiae* it was found in the production of higher alcohols and acetate esters and appeared to be strictly dependent on the specific strain used and the chemical composition of base wine. *L. thermotolerans* DiSVA322 exhibited the best score in the sensory analysis of resulting sparkling wines, exhibiting, together with a relevant score for citrus and tropical fruit, a significantly high score for softness, structure, and aromatic herbs descriptors. Thus, the use of *L. thermotolerans* in secondary fermentation of sparkling wine could be a suitable strategy to enhance aroma profile and flavor complexity.

Keywords *Lachancea thermotolerans* · Non-*Saccharomyces* · Sparkling wine · Aroma profile · Base wine · Secondary fermentation

Introduction

Effervescent wines containing a significant concentration of carbon dioxide are classified as semi-sparkling wine, which have1–2.5 bars of pressure in the bottle or sparkling wine, with 3–6 bars of pressure in the bottle [1, 2]. Both semi-sparkling and sparkling wine result from two fermentation steps: grape must is first converted into wine that is supplemented of the mixture of base wine and tirage liqueur, which consists of wine and sucrose or grape must or concentrated grape must and yeast cells and re-fermented in a cellar and maturing over a period of 9–12 months [3–5]. In accordance with the OIV International Code of Oenological Practices, sparkling wines are defined by their secondary fermentation vessel: the Traditional Method requires secondary fermentation and lees aging in the same bottle as sold, whereas the Tank (Charmat) Method utilizes pressurized autoclaves to preserve varietal aromatics. Conversely, the Ancestral Method involves bottling partially fermented must to complete a single fermentation without added sugar, while the Transfer Method uses bottles for secondary fermentation before bulk filtering and re-bottling to balance quality and efficiency [6]. Although the sparkling wine market has significantly expanded in recent years, with an increase of over 40% in the last ten years, consumer attention to the quality and safety of fermented beverages requires ever-increasing quality standards and criteria of typicality [7]. The reasons for this growth can be double: sparkling wines are considered products distinct from other categories of wines, endowed

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with a strong symbolic image that makes them particularly suitable for consumption on special occasions and as a tool for social status [8]. Furthermore, recent studies highlight a growing interest among younger consumers in this wine category, primarily due to its affordability and market accessibility [9]. Such emerging trends have also attracted the interest of researchers, leading to in-depth studies of this wine category. The use of modern biotechnologies in the production of sparkling wines enables the achievement of a high-quality end product with reduced production time and costs [10]. The structural and aromatic characteristics of sparkling wines are influenced by yeasts [11]. The main yeasts used in the second fermentation are *Saccharomyces* species, specifically *Saccharomyces cerevisiae* and *Saccharomyces bayanus*, due to their fermentation performance and contribution to the volatile profiles of sparkling wines [12, 13]. Despite the considerable potential of non-*Saccharomyces* yeasts in the production of still wines, research into their possible effects on the second fermentation and on aging are still limited. Instead, the use of selected non-*Saccharomyces* yeasts could be considered as a tool to increase flavor complexity in sparkling wine production. Non-*Saccharomyces* yeast species studied in sparkling wine production are *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, *Schizosaccharomyces pombe* and *Saccharomycodes ludwigii* for the traditional method [13–18]. In the base wine production, *S. pombe* and *S. ludwigii* influenced the acidity, color, and volatile profiles of sparkling wines affecting also biogenic amine content and overall sensory evaluation [13]. *T. delbrueckii*, used in a secondary fermentation as a pure culture exhibited positive and distinctive effects on the overall aroma and sensory characteristics of sparkling wine [14]. Moreover, when inoculated in a sequential fermentation with *S. cerevisiae*, *T. delbrueckii* improved the foaming property [15]. Tofalo et al. [14] studied the impact of *T. delbrueckii* and *Starmerella bacillaris* on traditional sparkling wine production and observed the positive effect of non-*Saccharomyces* yeasts, resulting in distinctive sparkling wines and promising results on the autolytic contribution of *S. bacillaris*. Ivit et al. [15] investigated the chemical and organoleptic characteristics that could occur during the second fermentation and the aging on the lees in the bottle by two non-*Saccharomyces* yeast species: *S. ludwigii* and *S. pombe*. Differences were detected in the sensory profiles; sparkling wines produced with *S. pombe* showed a better aromatic profile than sparkling wines produced with *S. cerevisiae*, with increased clarity, floral, fruity, buttery aromas and color intensity and clarity [18]. The primary contribution of non-*Saccharomyces* yeasts to sparkling wines lies in their ability to modulate the aromatic profile, as different species exert a distinct impact on the concentration of various Volatile Organic Compounds (VOCs) [13]. In this study, we investigated the use of two strains of *L. thermotolerans* in the "*prise de mousse*" of Verdicchio base wine from two different vintages, evaluating their impact on the analytical composition and aromatic profile of the resulting sparkling wine to better understand the role of non-*Saccharomyces* yeasts as a biotechnological tool.

Materials and methods

Yeast strains

L. thermotolerans strains DiSVA 322, DiSVA 1066, and *S. cerevisiae* strain DiSVA 527, were used in the secondary fermentation for the sparkling wine production. These strains belong to the Yeast Collection of the Department of Life and Environmental Sciences (DiSVA) at the Polytechnic University of Marche (Italy) and were already used as starter cultures in previous studies [19, 20]. For short-term storage, the yeast strains were cultivated and maintained on YPD agar (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 18 g/L agar) at 4 °C, while for long-term storage, YPD broth supplemented with 40% (w/v) glycerol at −80 °C was used.

Sparkling wine production

Verdicchio base wine coming from two different vintages (2021–2022), used for secondary fermentation, was provided by Terre Cortesi Moncaro s.r.c.l, Montecarotto, Ancona, Italy. Base wine was stabilized and filter-sterilized using 0.45 µm membranes. The main analytical characteristics of the Verdicchio base wines are reported in Table 1.

The base wine of each vintage was immediately subjected to the elaboration of sparkling wines. These base wines were supplemented with 24 g/L sucrose (expected pressure ca. 6 bar = 600 kPa), 10 mg/L diammonium phosphate, Tanniperle style untreated oak tannin (to provide complete structure and prevent reduction notes) (Enartis, Novara Italy), and with Actiperle cuve specified fermentation activator for

Table 1 Main analytical properties of the Verdicchio base wine coming from two vintages

Base wine vintage	Total acidity (g/L tartaric acid)	Volatile acidity (g/L acetic acid)	pH	Ethanol (%v/v)	Residual sugar (g/L)	Total SO_2 (mg/L)
2021	7.34 ± 0.02	0.16 ± 0.01	3.13 ± 0.01	9.30 ± 0.10	1.20 ± 0.10	20 ± 0
2022	7.25 ± 0.05	0.33 ± 0.05	3.03 ± 0.01	9.66 ± 0.09	2.40 ± 0.08	8 ± 0

'*prise de mousse*' (Station Oenotechnique De Champagne, France), both used following the manufacturer's instructions. The yeast strains were pre-cultured in flasks containing a mixture of 50 mL of pasteurized grape juice and 50 mL Verdicchio wine for 72 h at 18–20 °C. After this period, 150 mL of wine was added and after 4–5 days the preculture was used to inoculate the base wines. The inoculations were designed for an initial yeast concentration of approximately 1×10^6 cells/mL. The yeast cell numbers were counted using a Thoma Zeiss cell chamber under an optical microscope (Leitz mod HM-LUX 3). Each single bottle starting from a bulk of biomass for each strain. The final volume of each bottle was 750 ml. For each sparkling wine yeast trial ten bottles were set up and crown corked. Two bottles per trial were plugged with an amphometer (Oenoitalia Group S.r.l. Brescia, Italy), to monitor the evolution of pressure during the bottle fermentation phase. The temperature of the secondary fermentation was of 15 °C in a thermostat, and storage (12 months) at 12 °C. Following 12 months of lees aging, the sparkling wines were disgorged and subjected to analytical and sensory evaluation. Three bottles for each strain were analysed for the analytical and sensorial analysis.

Biomass evolution

During the secondary fermentation (0, 7, 14, 120 days), 3 of the bottles collected were used to monitor the biomass evolution during the "*prise de mousse*". The viable cell count (CFU/mL) was determined by plating out on Wallerstein Nutrient agar (Oxoid, Hampshire, UK).

Analytical determinations

Ethanol content, pH, and volatile acidity were determined according to the official methods established by the International Organization of Vine and Wine (OIV) [21]. The sugar concentration (glucose and fructose) was determined using specific enzyme activity kits (K-FRUGL, Megazyme, Ireland), following the manufacturer's instructions. Acetaldehyde, ethyl acetate and the higher alcohols were quantified by direct injection into a gas chromatography system (GC-FID 2014; Shimadzu, Kyoto, Japan) as reported by Canonico et al. [19]. The main volatile compounds were analysed using the headspace solid phase microextraction (HS-SPME) method using a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber (Sigma-Aldrich, St. Louis, MI, USA). The procedure consists of taking 5mL of each sample and putting it in a vial with 1 g NaCl closed with a septum-type cap. HS-SPME was carried out under magnetic stirring for 10 min at 25 °C. After this, 3-octanol as the internal standard (1.6 mg/L) was added, and the solution was heated to 40 °C and extracted with a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber for 30 min by insertion into the headspace vial. The fiber was inserted into a Shimadzu gas chromatograph GC injector for 5 min [19]. The fiber was inserted in split–splitless mode. The compounds were identified and quantified by comparisons with calibration curves for each compound. Each analytical determination was repeated three times.

Sensory analysis

After 12 months of maturation at 12 °C, the resulting sparkling wines were evaluated by a panel of ten tasters (7 males and 3 females aged 24–50 years) with experience in sparkling wine sensory analysis. Sensory evaluation was performed using a 10-point scale to assess a specific set of descriptors, encompassing both aromatic profiles and key structural characteristics. Sparkling wines were stored at the appropriate light (UV-filtered lighting), humidity (50%-60%), and temperature (18–20 °C) conditions. Samples were tasted in a randomized order and presented to the panelists in tasting glasses at 20–22 °C. Water was provided to rinse the palate between tastings. For this tasting, Standard ISO glasses were used. The volume was 50 ml per glass to ensure a consistent sensory evaluation and to maintain the wine's temperature during the session. The wine was served at a controlled temperature of 8–10 °C to preserve its organoleptic profile and CO_2 solubility. The data were combined, and the means were subjected to statistical analysis. Two bottles for each sample were tested.

Statistical analysis

The main analytical characters and volatile compounds of the sparkling wines were evaluated by analysis of variance (ANOVA). Duncan's test was used to determine the significant differences. The results were considered significant if associated with a probability (P) value < 0.05. Volatile compounds were also evaluated by using principal component analysis (PCA) to discriminate between the means and the variability of the inoculated strains and different base wines used for sparkling wine production. Statistical software package JMP 11® (SAS, Cary, NC, USA) was used for statistical analysis. The data coming from the sensory analysis were processed according to Fisher 's ANOVA, to determine the significant differences ($P < 0.05$).

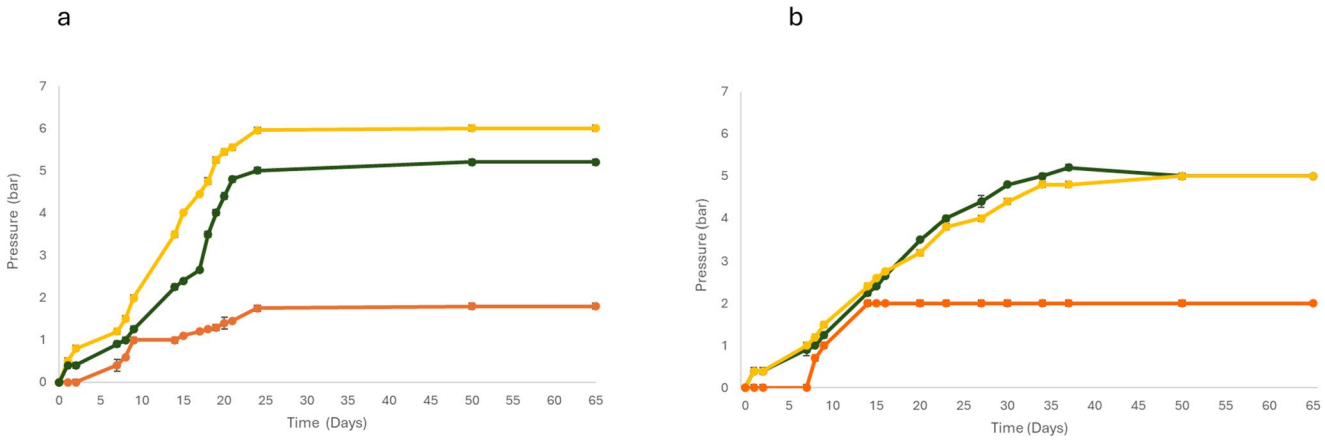

Fig. 1 Fermentation kinetics of sparkling wines produced with different yeasts during two vintages. **a** Vintage 2021, **b** Vintage 2022. Pure fermentations of *S. cerevisiae* DiSVA 527 (Yellow line), *L. thermotolerans* DiSVA 1066 (Orange line) and *L. thermotolerans* DiSVA 322 (Green line)

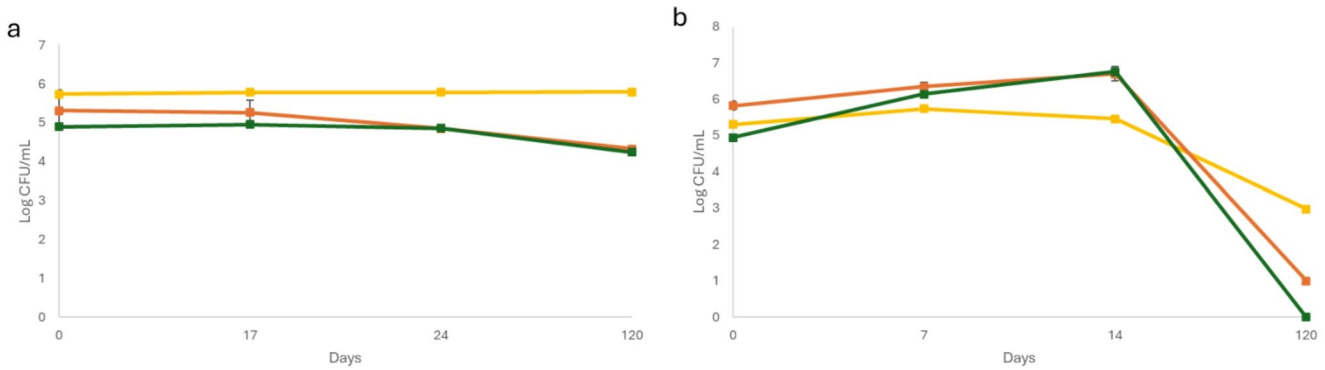

Fig. 2 Biomass evolution of the sparkling wines produced during two vintages. **a** Vintage 2021, **b** Vintage 2022. Pure fermentations of *S. cerevisiae* DiSVA 527 (Yellow line), *L. thermotolerans* DiSVA 1066 (Orange line) and *L. thermotolerans* DiSVA 322 (Green line)

Results

Evolution of the pressure

The evolution of the pressure (Fig. 1) was different among the strains tested in the two base wines regarding vintages 2021 and 2022. In base wine of the 2021vintage (Fig. 1a), *S. cerevisiae* exhibited the fastest fermentation kinetics, reached 6 bars in 25 days of fermentation. The two strains of *L. thermotolerans* exhibited different trends: *L. thermotolerans* DiSVA 322 reached approximately 5 bars in 25 days, while *L. thermotolerans* DiSVA 1066 showed the lowest secondary fermentation activity (2 bars) after 65 days.

In base wine of the 2022 vintage (Fig. 1b), *S. cerevisiae* and *L. thermotolerans* DiSVA 322 showed overlapping fermentation kinetics, highlighting a similar behaviour observed in base wine 2021vintage. *L. thermotolerans* DiSVA 1066 showed a lower performance comparable to that exhibited in the previous year, confirming its lower aptitude for secondary fermentation in sparkling wine.

Biomass evolution during secondary fermentation during two vintages

The biomass evolution during secondary fermentation in the two base wines is reported in Fig. 2. During this phase, in the base wine of the 2021 vintage (Fig. 2a) the three strains *S. cerevisiae*, *L. thermotolerans* DiSVA 322 and DiSVA1066 maintained the same cell concentration until 17th day during the secondary fermentation, then *L. thermotolerans* DiSVA322 and DiSVA 1066 exhibited a slight reduction. (1 log CFU/mL). In secondary fermentation trials of vintage 2022 (Fig. 2b) the trend of biomass kinetics was comparable to that of vintage 2021 for *S. cerevisiae*, while an increase in biomass was detected for *L. thermotolerans* Disva322 and DiSVA 1066, except for the cell concentration at the point 122th day. Indeed, no viable cells of *L. thermotolerans* DiSVA 322 were detected and a significant reduction in biomass concentration in *S. cerevisiae* and *L. thermotolerans* DiSVA 1066 was observed.

The main analytical characteristics of sparkling wine

The main analytical characteristics of the sparkling wines were analysed at the end of the secondary fermentation phase (Table 2). Regarding the pH value and volatile acidity, the results did not show significant differences among the different strains and the vintages. The residual sugar content reflects the fermentation trend shown in Fig. 1. *S. cerevisiae* DiSVA 527 consumed almost all the sugars present, while *L. thermotolerans* DiSVA 322 wines contained some residual sugars (4–8 g/L), and *L. thermotolerans* DiSVA1066 wines exhibited significantly higher sugar residues.

The main volatile compounds of sparkling wines

The data on the main volatile compounds of sparkling wine coming from the two vintages are reported in Table 3. The effect of *L. thermotolerans* strains on the volatile compounds varied over the two vintages. Indeed, in the 2021 vintage base wine, *L. thermotolerans* strains DiSVA 1066 and DiSVA322 exhibited a significant increase in ethyl acetate and β-phenyl ethanol (OAV < 1) compared to *S. cerevisiae* DiSVA 527. In addition, *L. thermotolerans* DiSVA 322 wines showed a significant increase in acetaldehyde and phenyl ethyl acetate content, while *L. thermotolerans* DiSVA 1066 displayed a significant increase in linalool (OAV < 1). In the base wine vintage 2022, only *L. thermotolerans* DiSVA 1066 exhibited a significant increase in n-propanol, isobutanol amyl alcohol, and isoamyl alcohol in comparison with the other strains. To be noted that among these higher alcohols, only isoamyl alcohol showed an OAV > 1. No significant differences were observed in the concentrations of other volatile compounds between the 2021 and 2022 sparkling wine vintages.

Table 2 The main analytical characteristics of sparkling wine produced during the 2021, 2022 vintages

Inoculated strain	Volatile acidity (g/L acetic acid)	pH	Residual sugar (g/L)
Vintage 2021			
S. cerevisiae DiSVA 527	0.30 ± 0.02^{a}	3.21 ± 0.02^{a}	0.90 ± 0.14^{c}
L. thermotolerans DiSVA 1066	0.34 ± 0.02^{a}	3.2 ± 0.02^{a}	13.37 ± 0.24^{a}
L. thermotolerans DiSVA 322	0.40 ± 0.04^{a}	3.22 ± 0.01^{a}	8.37 ± 0.99^{b}
Vintage 2022			
S. cerevisiae DiSVA 527	0.27 ± 0.05^{b}	3.32 ± 0.02^{a}	0.90 ± 0.08^{c}
L. thermotolerans DiSVA 1066	$0.38 \pm 0.06a^{b}$	3.21 ± 0.02^{a}	16.90 ± 1.05^{a}
L. thermotolerans DiSVA 322	0.44 ± 0.02^{a}	3.22 ± 0.01^{a}	4.8 ± 0.23^{b}

Data with different superscript letters ($^{a, b,c}$) within each column are significantly different. (Duncan tests; p -value < 0.05)

Principal component analysis (PCA) elaborated on the main volatile compounds in sparkling wines obtained from the two vintages using the *L. thermotolerans* strains and the *S. cerevisiae* control strain explaining a total variance of 66.8% (PC1 49.1% and PC2 17.7%) (Fig. 3).Indeed, the sparkling wines produces in 2022 vintage were placed in the right quadrants while those of the 2021 vintage were placed in the left quadrants. Regarding volatile composition, the sparkling wines were clearly discriminated by the strains used for refermentation, with *L. thermotolerans* DiSVA 322 showing an intermediate volatile profile between *S. cerevisiae* and *L. thermotolerans* DiSVA 1066. To better detect the influence of the starter strain on sparkling wine aromatic profiles, the PCA was conducted for each vintage (Supplementary materials Fig. 1s).

The results of PCA of 2021 vintage base wine (variance explained 100%) showed a clear differentiation between sparkling wines of *S. cerevisiae* DiSVA 527 and those of *L. thermotolerans* (PC1) (Fig. 1aS). The two *L. thermotolerans* sparkling wines were further distinguished by PC2 placing *L. thermotolerans* DiSVA322 in the upper quadrant, and *L. thermotolerans* DiSVA1066 in the lower quadrant mainly due to production of higher alcohols. PCA results of the 2022 vintage revealed the same explained total variance (PC1 59.4%; PC2 40.6%), confirming a clear distinction between *S. cerevisiae* DiSVA 527 and *L. thermotolerans* DiSVA 322 and *L. thermotolerans* DiSVA1066 (Fig. 1bS). It is interesting to note that the secondary fermentations of sparkling wines carried out by the *L. thermotolerans* strains were characterized by a broad production of volatile compounds as acetate esters and higher alcohols.

Sensorial analysis

Sensory analysis of the sparkling wines revealed significant differences across several aromatic and taste descriptors, as illustrated in Fig. 4.Regarding the vintage 2021 (Fig. 4a), sparkling wines produced with *L. thermotolerans* DiSVA 322 exhibited a significant increase in aromatic herbs and softness, while *L. thermotolerans* DiSVA 1066 sparkling wines were characterized by cooked vegetables (olfactory) and structure, softness and balance notes. *S. cerevisiae* sparkling wines displayed a phenolic character. In the vintage 2022 (Fig. 4b), the olfactory perception of the sparkling wines produces with L. *thermotolerans*, DiSVA 1066, was different from vintage 2021. This strain exhibited a significant increase in spicy, herbal and phenolic character with a perception of cooked vegetables. Moreover, regarding the taste, these sparkling wines were less soft and structured unlike the *L. thermotolerans* DiSVA 322 sparkling wines which confirmed the high score in the descriptors aromatic herbs and structure. The overall results of sensory analysis

Table 3 The main volatile compounds (mg/L) of sparkling wine of two vintages

Fermentation trials 2021 (odor threshold value)*	*S. cerevisiae* DiSVA 527	OAV	*L. thermotolerans* DiSVA 1066	OAV	*L. thermotolerans* DiSVA 322	OAV
Ethyl Butyrate 0.02	1.82 ± 0.32[a]	91.0	2.03 ± 0.48[a]	101.50	2.04 ± 0.24[a]	102.00
Ethyl Acetate 7.5	9.48 ± 0.65[b]	1.26	13.91 ± 1.55[a]	1.85	16.69 ± 0.67[a]	2.22
Phenyl ethyl Acetate 0.250	0.1 ± 0.02[b]	0.40	0.33 ± 0.05[ab]	1.32	0.66 ± 0.25[a]	2.64
Ethyl Hexanoate 0.014	0.25 ± 0.01[a]	17.80	0.38 ± 0.19[a]	27.14	0.87 ± 0.26[a]	62.14
Isoamyl Acetate 0.03	1.36 ± 0.38[a]	45.30	1.92 ± 0.27[a]	64.00	1.41 ± 0.05[a]	47.00
n-Propanol 306	33.27 ± 0.76[a]	0.12	28.59 ± 1.59[a]	0.09	37.18 ± 1.47[a]	0.12
Isobutanol 40	8.84 ± 0.89[a]	0.22	6.77 ± 0.94[a]	0.17	6.89 ± 0.68[a]	0.17
Amyl Alcohol 64	9.80 ± 0.25[a]	0.15	5.76 ± 0.15[b]	0.09	6.33 ± 0.49[b]	0.09
Isoamylic Alcohol 3.0	87.88 ± 1.38[a]	29.30	42.71 ± 1.67[b]	14.20	77.47 ± 1.40[ab]	25.8
ß-Phenyl ethanol 14	50.70 ± 0.40[b]	0.25	82.90 ± 0.20[a]	0.04	82.10 ± 0.50 [a]	0.04
Hexanol 8.0	0.01 ± 0.01[a]	0.001	0.03 ± 0.00[a]	0.003	0.02 ± 0.01[a]	0.002
Acetaldehyde 0.5	3.68 ± 1.56[b]	7.36	8.83 ± 0.29[ab]	17.70	11.33 ± 2.38[a]	22.60
Linalool 0.025	0.00 ± 0.00[b]	0.00	0.05 ± 0.00[a]	2.00	0.00 ± 0.00[b]	0.00
Nerol 0.015	0.004 ± 0.00[a]	0.26	0.004 ± 0.00[a]	0.026	0.01 ± 0.00[a]	0.66
Fermentation trials 2022 (odor threshold value)	*S. cerevisiae* DiSVA 527	OAV	*L. thermotolerans* DiSVA 1066	OAV	*L. thermotolerans* DiSVA 322	OAV
Ethyl Butyrate 0.02	0.80 ± 0.06[a]	40.00	0.84 ± 0.01[a]	42.00	0.85 ± 0.06[a]	42.50
Ethyl Acetate 7.5	6.26 ± 0.19[a]	0.83	7.69 ± 2.17[a]	1.02	10.85 ± 1.33[a]	1.44
Phenyl ethyl Acetate 0.250	0.37 ± 0.10[a]	1.48	0.37 ± 0.08[a]	1.48	0.26 ± 0.08[a]	0.17
Ethyl Hexanoate 0.014	0.19 ± 0.33[a]	13.60	0.21 ± 0.32[a]	15.00	0.21 ± 0.24[a]	15.00
Isoamyl Acetate 0.03	1.14 ± 0.11[a]	38.00	1.14 ± 0.31[a]	38.00	1.22 ± 0.14[a]	40.00
n-Propanol 306	31.49 ± 0.92[b]	0.10	40.68 ± 1.75[a]	0.13	28.25 ± 3.75[b]	0.09
Isobutanol 40	12.05 ± 0.79[b]	0.30	16.89 ± 0.07[a]	0.42	9.27 ± 2.39[b]	0.23

Table 3 (continued)

Fermentation trials 2022 (odor threshold value)	*S. cerevisiae* DiSVA 527	OAV	*L. thermotolerans* DiSVA 1066	OAV	*L. thermotolerans* DiSVA 322	OAV
Amyl Alcohol 64	4.36 ± 2.12[c]	0.06	15.95 ± 0.07[a]	0.24	10.38 ± 0.26[b]	0.16
Isoamylic Alcohol 3	65.55 ± 0.65[c]	21.85	98.60 ± 0.01[a]	32.80	73.14 ± 1.13[b]	24.38
ß-Phenyl ethanol 14	19.20 ± 0.64[a]	0.09	23.70 ± 0.13[a]	0.12	16.50 ± 0.39[a]	0.08
Hexanol 8	0.04 ± 0.10[a]	0.005	0.04 ± 0.00[a]	0.005	0.03 ± 0.00[a]	0.003
Acetaldehyde 0.5	4.88 ± 0.60[a]	9.8	3.95 ± 0.57[a]	7.90	1.42 ± 0.34[b]	2.80
Linalool 0.025	0.042 ± 0.01[a]	1.68	0.025 ± 0.01[ab]	1.00	0.01 ± 0.00[b]	0.40
Nerol 0.015	0.06 ± 0.01[a]	4.00	0.09 ± 0.01[a]	6.00	0.08 ± 0.01[a]	5.30

OAV: odor activity value. OAV = C / OT. C (Concentration): The actual concentration of the individual compound in the sample; OT (Odor Threshold): *The "Odor Threshold Value" (OTV). Data with different superscript letters ([a, b,c]) within each row is significantly different (Duncan tests; p-value < 0.05)

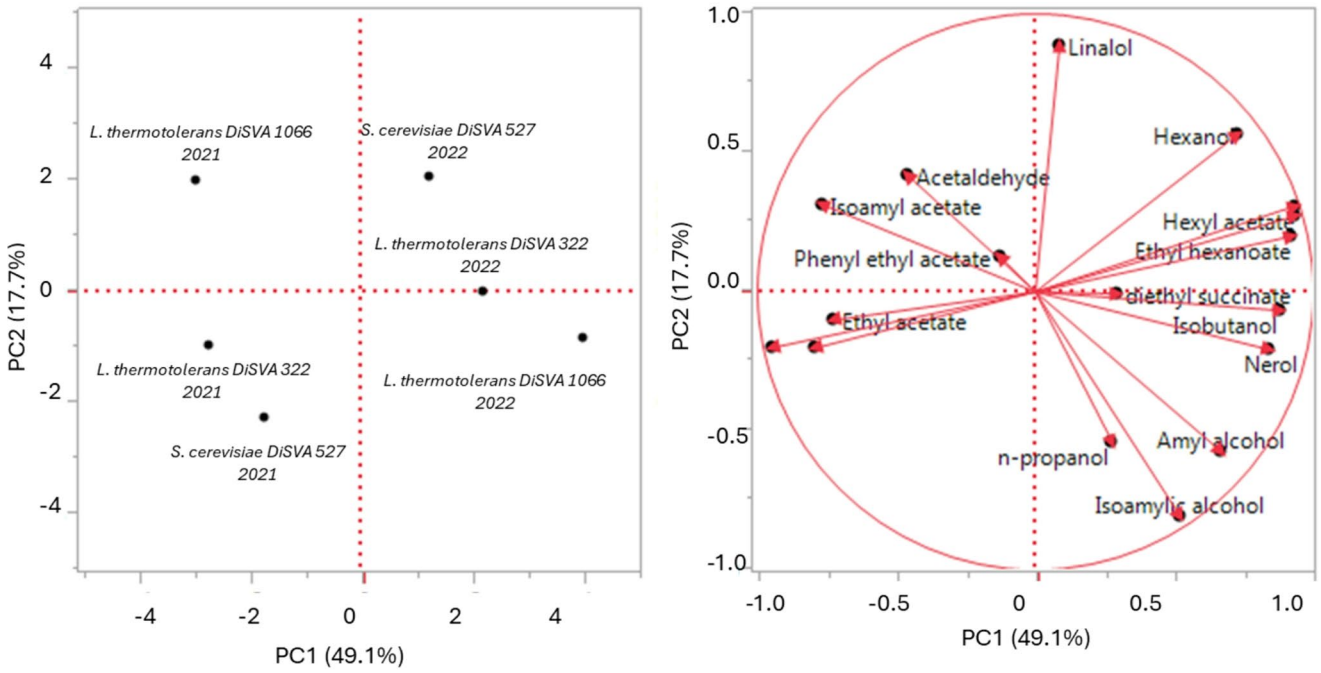

Fig. 3 Principal component analysis (PCA) applied on the main volatile compounds sparkling wine coming from different yeast strains and vintages

of both vintages indicated that the sparking wine's preferences were *L. thermotolerans* DiSVA 322 followed by *S. cerevisiae* and at the end *L. thermotolerans*, DiSVA 1066 that showed in both vintages some defects (data not shown).

Discussion

The use of non-*Saccharomyces* yeasts has been investigated in the production of base wines, few applications have been conducted in secondary fermentation [13, 14, 17, 22, 23]. The discrepancies found suggest that oenological performance is highly dependent on intraspecific strain variability and the specific chemical properties of the base wine.

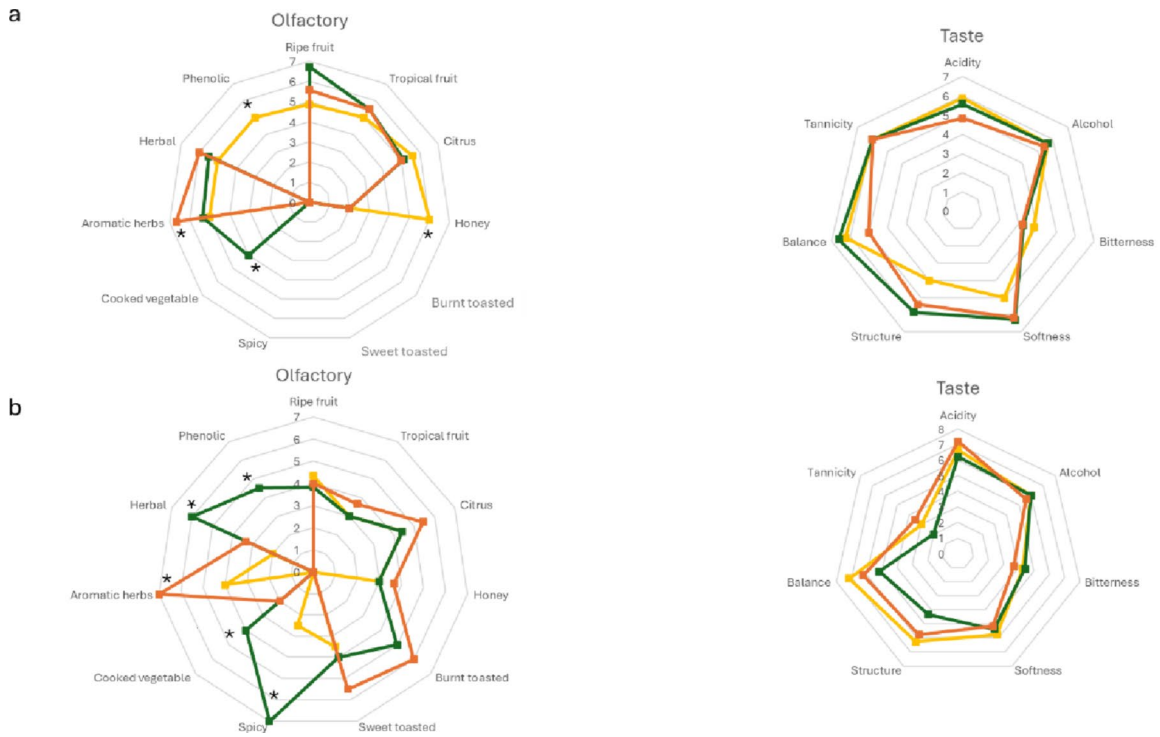

Fig. 4 Sensory analysis of the sparkling wines produced during two vintages. **a** Vintage 2021, **b** Vintage 2022. *S. cerevisiae* DiSVA 527 (Yellow line), *L. thermotolerans* DiSVA 1066 (Green line) and *L. thermotolerans* DiSVA 322 (Orange line). *Significantly different (Fisher ANOVA; p-value < 0.05)

Consequently, the selection of tailored strains is essential for consistent product differentiation. The sparkling wine market is in search of new tools to enhance and diversify the sensory properties of the final product [24–25]. In this study, for the first time, the fermentation performance of two *L. thermotolerans* strains, already selected for primary fermentation, was evaluated in secondary fermentation of sparkling wine production. The results showed strain-dependent behavior: while both *L. thermotolerans* strains exhibited analogous viable cell counts, only *L. thermotolerans* DiSVA 322 achieved a pressure level comparable to the *S. cerevisiae* control strain. *L. thermotolerans* DiSVA 1066 showed a lower fermentative activity despite its biomass being comparable to that of DiSVA 322, could be due to a lower specific glycolytic flux or a divergence in metabolic efficiency. Although both strains achieved similar growth rates, DiSVA 1066 may have exhibited a greater degree of metabolic quiescence or a less efficient energy yield per unit of substrate consumption. Furthermore, this discrepancy could be explained by different stress response mechanisms during secondary fermentation. This yeast species has been successfully used in winemaking to enhance acidity, as well as aromatic and sensory characteristics [26, 27]. As expected, the different base wines resulted in substantial variations among the sparkling wines [28–30]. The use of two different base wines had a strong impact on the resulting sparkling wine, although only Verdicchio grape must be used. The vintage (climate condition and other factors) had a major influence on aromatic and sensory profile of sparkling wines. However, a further differentiation was detected on the basis of inoculated strain in secondary fermentation. Analytical profile and sensory analysis revealed significant differences in both chemical and organoleptic properties between *L. thermotolerans* and *S. cerevisiae* sparkling wines. *L. thermotolerans* strains showed an enhancement of some volatile compounds as acetate esters and higher alcohols that showed an OAV > 1 specially in sparkling wine of 2021 vintage highlighting the influence of inoculated yeast species. *L. thermotolerans* DiSVA 322 demonstrated promising characteristics, producing a sparkling wine with peculiar sensory profile. The strain increased the perceived structure and herbal complexity with a good correlation with acetate esters and higher alcohols with and OAV > 1, while maintaining high levels of citrus and tropical fruit aromas. These results align with the findings of Wang et al. (2022) on *S. japonicus*, where non-conventional yeasts were shown to increase ester complexity, thereby defining the "typicality" of the final product. Even when Odor Activity Values (OAV) were below 1, these compounds synergistically contributed to the wine's sensory depth [31]. The results highlight a stark contrast between strains: DiSVA 322 consistently improved "structure," "softness," and "herbal complexity,"

whereas DiSVA 1066 produced off-notes like "cooked vegetables" due to an excessive accumulation of higher alcohols (e.g., isoamyl alcohol with OAV > 1 in 2022). This supports the strategy discussed by Lola et al. [32] which emphasizes that selecting specific autochthonous strains is essential to enhance aroma variability and typicity. The ability of DiSVA 322 to maintain high scores in citrus and tropical fruit descriptors across different vintages suggests it as robust biotechnological tool for high-quality sparkling wine production. *L. thermotolerans* DiSVA 1066, which displayed sluggish fermentation, showed limitations in aromatic and sensory attributes, leading to the lowest preference among the samples. A recent review [20] highlighted that managing fermentation with non-*Saccharomyces* yeasts becomes more difficult due to their reduced ethanol tolerance, which requires co-inoculation to ensure the exhaustion of sugars. Here, using a selected non-*Saccharomyces* yeast, as *L. thermotolerans* DiSVA 322 strain for secondary fermentation in sparkling wine production,, establishes an effective and novel approach to develop an innovative product.

This strain showed a promising ability to produce sparkling wine using two base wines from different vintages. Although the influence of the base wine vintage was greater, the *L. thermotolerans* DiSVA 322 strain consistently and positively characterized the final sparkling wines.

Conclusions

The ability of *L. thermotolerans* in secondary fermentation of sparkling wine production was determined even though strain-dependent ability was shown. The analytical and sensory profile of the resulting sparkling wines indicated some relevant positive influence of *L. thermotolerans*. Further investigations are required to identify strains best suited for the challenging conditions of secondary fermentation. In this regard, mixed inoculation strategies may be explored as an alternative to *S. cerevisiae* single inoculation, to improve the organoleptic quality of sparkling wines while ensuring fermentation security. A single inoculation of non-*Saccharomyces* yeasts in secondary fermentation for sparkling wine required additional exploration to evaluate the influence of different base wines and other factors (species and strain selection, inoculation modality) that could affect the refermentation process to resolve some conflicting results reported in the literature and to further explore the potential of non-*Saccharomyces* yeasts in the production of sparkling wines. At the end, another important aspect worth investigating concerns the cellular release during the maturation phase of the classic/traditional method sparkling wine for the contribution to the analytical sensorial composition of the inoculated strain.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00217-026-05130-8.

Author contributions Laura Canonico: carried out the experimental part, data curation, writing original draft, methodology. Alice Agarbati: data curation, Conceptualization. Francesca Comitini: formal analysis, conceptualization. Maurizio Ciani: validation, supervision, methodology, investigation. All authors have read and agreed to the published version of the manuscript.

Funding Open access funding provided by Università Politecnica delle Marche within the CRUI-CARE Agreement. This research received no external funding.

Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval The study design adhered to the ethical principles of the Declaration of Helsinki and participant privacy and data security were strictly protected. All participants were informed about the study and participated voluntarily. The wine was the same used by the winery. The yeast strains used are GRAS and already used in wine fermentation. In addition, the final wine products were filtered and added with sulphur dioxide, which, together with the alcohol content, provided the microbiological safety of the wines.

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

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