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PII: S0023-6438(20)31172-5

DOI: https://doi.org/10.1016/j.lwt.2020.110183

Reference: YFSTL 110183

To appear in: *LWT - Food Science and Technology*

Received Date: 17 April 2020

Revised Date: 6 August 2020

Accepted Date: 5 September 2020

Please cite this article as: Parpinello, G.P., Ricci, A., Folegatti, B., Patrignani, F., Lanciotti, R., Versari, A., Unraveling the potential of cryotolerant *Saccharomyces eubayanus* in Chardonnay white wine production, *LWT - Food Science and Technology*, https://doi.org/10.1016/j.lwt.2020.110183.

# Unraveling the potential of cryotolerant *Saccharomyces eubayanus* in Chardonnay white wine production

**Giuseppina Paola Parpinello[a,b], Arianna Ricci[a*], Barbara Folegatti[a], Francesca Patrignani[a,b], Rosalba Lanciotti[a,b], Andrea Versari[a,b]**

[a] Department of Agricultural and Food Sciences, University of Bologna, Piazza Goidanich 60, Cesena (FC), 47521, Italy

[b] Interdepartmental Centre for Agri-Food Industrial Research, University of Bologna, Via Quinto Bucci 336, Cesena (FC), 47521, Italy

[*]**Corresponding author**:

**Arianna Ricci**, Department of Agricultural and Food Sciences - University of Bologna, Piazza Goidanich 60, Cesena (FC), 47521 – Italy

E–mail: arianna.ricci4@unibo.it

## ABSTRACT

This work investigated the suitability of the cryotolerant yeast *Saccharomyces eubayanus* to ferment Chardonnay must at different temperatures (10°C, 12°C, 16°C, and 26°C) over two vintages (2013 and 2014). The effect of added nitrogen was also evaluated. The strain's fermentation parameters (maximum growth rate, lag phase, and asymptotic maximum) and cell growth were compared to the values for two reference *Saccharomyces cerevisiae* strains. *Saccharomyces eubayanus* showed its best fermentation performance at low temperatures (10°C and 12°C), with optimal kinetic parameters and high sugar consumption. Moreover, wines from the cryotolerant yeast showed a volatile acidity reduction of approximately 50%, and a 9% increase in total polyphenols, compared to the reference yeasts. At 16°C the cryotolerant and control yeasts performed quite similarly, whereas at 26°C the former displayed stuck fermentation. For both yeasts, at 10°C the nitrogen content did not affect maximum growth rate, whereas it did at 16°C. Sensory analyses were run on the 2014 trials, showing differences in color, sweetness, and overall liking among wines obtained at 10°C, whereas wines obtained at 16°C differed only in color. Results demonstrate for the first time the *Saccharomyces eubayanus* suitability for low-temperature fermentation in white wine production, potentially enriching yeast biodiversity in winemaking.


**Keywords**: low-temperature, wine fermentation, *Saccharomyces eubayanus*, kinetic parameters, sensory analysis

## Introduction

Sensory properties of wine depend on grape variety, technology, and secondary metabolites produced by microbiota during fermentation (Andorrà, Berradre, Mas, Esteve-Zarzoso, & Guillamón, 2012; Englezos, Rantsiou, Cravero, Torchio, Giacosa, Ortiz-Julien, et al., 2018; Molina, Swiegers, Varela, Pretorius, & Agosin, 2007). There are two approaches to exploiting the role of microbiota to improve wine's sensory characteristics. In the first approach, low temperatures are applied during wine fermentation to improve the stability and excretion of volatile compounds (Killian & Ough, 1979; Torija, Beltran, Novo, Poblet, Guillamón, Mas, et al., 2003), enhancing the aroma complexity of white wines (Beltran, Novo, Guillamón, Mas, & Rozès, 2008; Deed, Fedrizzi, & Gardner, 2017; Pérez, Assof, Bolcato, Sari, & Fanzone, 2018; Rollero, Bloem, Camarasa, Sanchez, Ortiz-Julien, Sablayrolles, et al., 2015; Torija, et al., 2003). The second approach is based on selecting yeasts capable of providing great complexity in terms of aroma, taste, and structure (Maturano, Lerena, Mestrea, Casassa, Toro, Vazquez, et al., 2018; Patrignani, Montanari, Serrazanetti, Braschi, Vernocchi, Tabanelli, et al., 2017; Pretorius, 2000). Combining both approaches represents an opportunity worthy of investigation. S*accharomyces cerevisiae* is the species that has been selected for the starter culture, due to its resistance to the stressful conditions of fermentation (high sugar concentration, ethanol, and temperature and low pH) (Degre, 1993; Reed & Nagodawithana, 1988). However, its inability to lead fermentation at low temperatures can cause delayed (and, sometimes, stuck) sugar consumption. As a result, interest in cold-tolerant yeasts which also improve wine characteristics such as aroma and taste has been growing. Of particular interest is *Saccharomyces eubayanus* CBS 12357 (hereafter referred to as EU), isolated from natural sources in Patagonia (Libkind, Hittinger, Valério, Gonçalves, Dover, Johnston, et al., 2011). Studies performed on EU in the grape juice and brewing sectors showed incomplete sugar consumption (Alonso-del-Real, Lairón-Peris, Barrio, & Querol, 2017; Gibson, Storgårds, Krogerus, & Vidgren, 2013) owed to fermentation stressors (Origone, del Mónaco, Avila, González Flores, Rodríguez, & Lopes, 2017). However, successful apple juice fermentation and cider production

have been reported (González Flores, Rodríguez, Oteiza, Barbagelata, & Lopes, 2017). Other studies demonstrate the ability of a closely related strain, *Saccharomyces eubayanus* NPCC 1285, to ferment under low-nitrogen and low-temperature (i.e. 12°C) conditions (Magalhães, Krogerus, Castillo, Ortiz-Julien, Dequin, & Gibson, 2017a; Su, Origone, Rodríguez, Querol, Guillamón, & Lopes, 2019). However, these studies mostly investigated on nitrogen requirements or selected new interspecies hybrid strains and were often performed on synthetic must only.

In this study, we evaluated the potential of *Saccharomyces eubayanus* CBS 12357 (EU) to enlarge yeast availability in winemaking. The evaluation consisted of fermenting Chardonnay musts at different temperatures (10°C, 12°C, 16°C, and 26°C), with and without nitrogen supplement, in two vintages (2013 and 2014). The performance of EU was compared to two commercial strains *Saccharomyces cerevisiae bayanus and Saccharomyces cerevisiae* used in winemaking worldwide.

## 2. Materials and Methods

### Grape and Yeast Strains

The study was carried out on two vintages of Chardonnay grape must: a 2013 vintage from Cesena (44°14'W, 12°15'S) and a 2014 from Riolo Terme (44°29'W, 11°71'S), both in Emilia Romagna (Italy). The grapes were harvested manually and immediately transported to the winery for destemming and pressing. The juice was settled at 4°C for 24 h. The 2013 must's chemical characteristics were: Babo (soluble solids) 18.0 g/100g, pH 3.25, titratable acidity 8.3 g/L, and assimilable nitrogen 140 mg/L (no nitrogen was added). Babo is a unit measurement to express soluble solids (g/100 g). Soluble solids in must are ~95% sugars. The measurement allows to monitor the fermentation by the reduction of density (from sugar to alcohol). It is the main method used in the small/medium size wineries. The chemical characteristics of the 2014 must were: Babo (soluble solids g/100g) 16.7, pH 3.37, titratable acidity 7.5 g/L, total sulfur dioxide 40 mg/L, and assimilable nitrogen 110 mg/L (supplemented to achieve 160 mg/L before fermentation).

For the 2013 vintage, we compared the cryotolerant *Saccharomyces eubayanus* CBS 12357 (*EU*: Westerdijk Fungal Biodiversity Institute, Utrecht, Nederlands) to S*accharomyces cerevisiae bayanus* QA23 (*CB*: Lalvin, Canada), which optimally ferments white must between 15°C and 26°C. For the 2014 vintage, being the temperatures of investigation restricted at 10°C and 16°C, we compared EU to *Saccharomyces cerevisiae* VIN13 (*CE*: Anchor, South Africa), a relatively cryotolerant strain with an optimal temperature range of 12–16°C and nitrogen requirements.

**Inoculum preparation**

Yeasts were grown in 1-L flasks containing 300 mL YPD growth medium (1% yeast extract, 2% peptone, and 2% dextrose; Thermo Fisher Scientific, Monza, Italy). The flasks were incubated overnight at 28°C and shaken at 200 rpm. Before inoculation, the precultures were centrifuged at 6000 g for 10 min and the resulting pellet was resuspended in Chardonnay must, previously heat-treated at 60°C for 15 min. A cell concentration of 6.0 Log CFU/mL was used to inoculate all vinification trials.

**Fermentation conditions**

In 2013, each strain was tested at three temperatures (12°C, 16°C, and 26°C), in either 3-L capacity flasks containing 2 L (for EU: *Saccharomyces eubayanus*) or 1-L capacity flasks containing 0.65 L (for CB: S*accharomyces cerevisiae bayanus*) of must: 2 EU trials and 1 CB trial for each temperature were carried, for a total of 9 trials (**Table 1**). In 2014, the temperature was either 10°C or 16°C and the effect of added assimilable nitrogen (N: di-ammonium phosphate, 50 mg/L) on strain fermentation performance was studied. Fermentations were performed in duplicate in 5-L flasks containing 3 L of must for both EU and CE: 2 strains x 2 temperatures x 2 N conditions were carried out in duplicate for a total of 16 trials (**Table 1**). The N source was added when the must density was 1060 (ca. 3% alcohol). In both vintages, the must was flash-pasteurized (60°C for 15 min), then inoculated to a concentration of 6.0 Log CFU/mL. The flasks were equipped with Müller

valves for $CO_2$ release without oxygen intake. The fermentations, monitored daily by measuring the weight loss of each flask, were considered completed when the weight was unchanged for four consecutive days. Kinetic parameters ($\mu_{max}$: maximum specific growth rate (day $^{-1}$), $\lambda$: lag phase period (day), and $Y_{end}$: asymptotic maximum (g/L)) were calculated from each fermentation curve, which was created by relating weight loss (g/L) to fermentation time (days) and fitted by a non-linear regression (Baranyi & Roberts, 1994). With the exception of the 2013 CB trials, which were carried out singly due to the yeast's well-documented technological performance (Blanco, Mirás-Avalos, Pereira, & Orriols, 2013), all of the trials were run in duplicate; data are presented as average values. After fermentation, the wines were cold–settled (4°C) for a week, then racked. Potassium metabisulphite was added to achieve 100 mg/L of total sulphure dioxide. After bottling, wines were stored at 4°C until chemical, sensory, and aroma analyses were performed.

**Sampling yeast growth kinetic**

Throughout the fermentation, serially diluted samples from each flask were used to inoculate YPD agar plate (1% yeast extract, 2% peptone, 2% dextrose, 1.8% agar; Thermo Fisher, Monza, Italy) for cell growth the number of viable yeast cells was determined by counting colony-forming units (CFU/mL) after the plates were incubated at 25°C for 48 h. Starting at inoculation, one mL from each flask was sampled every few days (vintage 2013: days 0, 4, 6, 11, and 18; vintage 2014: days 0, 1, 5, 9, 12, 15, 19, and 26). Three repetitions for each sampling were carried out on both vintages for the trials without nitrogen. The kinetic parameters $\mu_{max}$: maximum specific growth rate (day $^{-1}$), $\lambda$: lag phase period (day), and $Y_{end}$: growth/asymptotic maximum (Log CFU/mL) were calculated by relating cell load (Log CFU/mL) versus time (days) and fitting a non-linear regression (Baranyi & Roberts, 1994).

**Wine parameters analysis**

The wines were analyzed for alcohol content (AC), pH, total acidity (TA), dry matter (DM), volatile acidity (VA), color at 420 nm (CO), and total polyphenols at 280 nm (TP), using official European methods (EU, 1990). Moreover, total ($SO_{2T}$) and free ($SO_{2F}$) sulphur dioxide (Ripper & Schmitt, 1896) as well as residual sugars (RS) (Lane & Eynon, 1923) were quantified.

**Wine volatile molecule profiles**

The volatile profiles of the wines were analyzed by headspace solid phase microextraction (HS−SPME, CAR/PDMS, 65 µm, SUPELCO, Bellefonte, PA), coupled with gas chromatography mass spectrometry (GC−MS, Agilent 7890 A, Agilent Technologies, PA) equipped with a Varian 50m x 0.25 µm column (Bruker Optics Inc., Billerica, MA). The internal standard was 4-methyl 2-penthanol (100 mg/L). The analysis followed the method proposed by Patrignani et al. (2017). Volatile molecules were identified by referencing NIST 2.0 (US National Institute of Standards and Technology) and Wiley 7 libraries.

**Wine sensory analysis**

For the evaluation of the 2014 vintage wines, twenty-two judges (10 women, 12 men) were recruited from students trained in winemaking and sensory evaluation who were enrolled in the Viticulture and Enology course, University of Bologna (Cesena, Italy). The number of judges was consistent with the minimum suggested for the two selected sensory tests (Lawless and Heymann 1998; Heymann, Machado, Torri & Robinson, 2012). First, a triangle test (ISO, 2004) was performed to disclose any significant differences between replicates. Afterwards, a descriptive analysis of color (intensity, likeability), aroma (flower, fruit, alcohol, overall aroma), taste (sweet, sour, bitter/astringent, alcohol, overall taste), body, persistence, and overall likeability was carried out (Lawless and Heymann 1998). Thirty-mL coded samples were presented in fully randomized order at room temperature (20°C) in 170-mL tulip glasses (ISO, 1977) covered with plastic dishes to preserve aroma. Transparent glasses for color evaluation and black glasses for other descriptors'

evaluation were used. Judges were allowed to rinse their mouths by drinking water between samples. In the descriptive analysis, samples were scored on a 10-point scale anchored with "absent" and "high" intensity. Tasting sessions took place in a facility equipped with individual sensory booths illuminated with daylight lamps (ISO, 2007); two sessions were set up, with eight wines assessed during each session.

**Statistical analysis**

Values of weight loss parameters and chemical data (from analyses of vinifications carried out in duplicate), and cell growth parameters (from three repetitions of trials without nitrogen supplementation), are presented as mean±standard deviation (SD). The one-way and two-way analyses of variance (ANOVA; significance $p \leq 0.05$), Fisher *post-hoc* test, and spider plot representations of sensory profiles were performed using XLSTAT version 2011.1.05 (Addinsoft, Anglesey, UK). The DMFit package (Baranyi & Roberts, 1994) was used for the regression analyses of fermentation kinetics and yeast cell growth data. For the sensory triangle test, the data were analyzed with 95% confidence intervals.

## 3. Results and Discussion

### *3.1. 2013 Vintage*

#### *3.1.1. Fermentation Kinetics and Yeast Cell Growth*

At each temperature (12°C, 16°C, and 26°C), the fermentation kinetics (represented by daily weight loss) and the cell growth data for the two strains (EU and CB) were fitted by non-linear regression (see **Figure 1**). Both curves were considerably affected by temperature. At 12°C, EU showed less weight loss and a higher cell load than CB (**Figure 1a**), whereas at 26°C the CB strain recorded more weight loss then EU, but similar cell load (**Figure 1c**). The EU strain's cell numbers were lower at 26°C than at 12°C, which could be related to impaired cell membrane functionality caused by a decrease of unsaturated fatty acids and an accumulation of ethanol (Torija, et al., 2003;

Beltran et al., 2008; Pérez et al., 2018). The sensitivity of *Saccharomyces eubayanu* to ethanol has, in fact, been shown to increase with temperature (Magalhães et al., 2017a). At the intermediate temperature of 16°C, the cell loads were similar and the weight losses achieved the minimum difference (**Figure 1b**).

In both strains (see **Figure 1**), the stationary phase began after 23 days at 12°C and after 12 days at 16°C. At 26°C, EU stopped losing weight after seven days due to sluggish/stuck fermentation and there was a slight decrease in yeast viability after 11 days; however, CB did not stop losing weight until the 21st day.

For cell growth, all quantitative parameters achieved good fit (see $R^2$ values, **Table 2**). The lowest value for the $\mu_{max}$ of EU (0.32 $day^{-1}$) was recorded at 26°C and for that of CB (0.20 $day^{-1}$) at 12°C; the highest $\mu_{max}$ value for EU (0.43 $day^{-1}$) occurred at 12°C and for CB (0.50 $day^{-1}$) at 16°C. These results support the findings that characterize EU as cryotolerant (Libkind et al., 2011; Peris et al., 2016). No lag phase was detected in any trial, perhaps due to the three-day interval between the first two samplings. For the EU strain, the asymptotic maximum ($Y_{end}$) was proportional to $\mu_{max}$, with higher values at 12°C (7.7 Log CFU/mL) and lower values at 26°C (7.1 Log CFU/mL), confirming the suitability of this yeast for low-temperature fermentation.

For weight loss, wide differences were found in the quantitative parameters, depending on the growth temperature (**Table 2**). A direct proportional relationship between $\mu_{max}$ and temperature was observed for EU; in fact, the former rose from 3.6 to 15.1 $day^{-1}$ when the latter increased from 12°C to 26°C. However, at 26°C, the $Y_{end}$ was low (59.1 $day^{-1}$) due to stuck fermentation (**Table 2)**. At 16°C, the $\mu_{max}$ of the EU strain (8.2 $day^{-1}$) was similar to that of CB (9.9 $day^{-1}$), which demonstrated higher $Y_{end}$ at any temperature. At 12°C, EU fermentation was characterized by a lower $\mu_{max}$ (3.6 $day^{-1}$) and $Y_{end}$ (79.7 g/L) compared to CB. However, EU adapted quickly to the low growth temperature, while CB showed a 1.3-day lag phase.

*3.1.2. Wine Characteristics*

The final compositions of the EU and CB wines are shown in **Table 3**. No significant differences were found for free sulphur dioxide or pH. However, all EU wines, regardless of temperature, had more residual sugars (range: 6.0−15.6 vs 1.0–1.3 g/L) and consequently, lower alcohol content (range: 10.0−10.7 vs 11.9−13.0 %) than CBs. Highest residual sugar and lowest alcohol content occurred in one of the two 26°C replicates due to stuck fermentation. Low temperature determined a significant decrease in volatile acidity (VA) in EU wines compared to CB wines. At all temperatures, the color intensity was lower for EU than CB wines.

*3.2. 2014 Vintage*

*3.2.1. Fermentation Kinetics and Yeast Cell Growth*

The combined effects of temperature (10°C, 16°C) and nitrogen supplementation were compared for the EU strain and a commercial *Saccharomyces cerevisiae* strain (CE) (**Figure 2**).

Interestingly, the weight loss and cell load were similar at both temperatures for the EU strain; however, at 16°C the fermentation was faster and the stationary phase was reached five days earlier. Compared to the commercial yeast CE, at 10°C the EU strain showed faster sugar consumption (correlated with a higher cell load) and entered the stationary phase earlier (**Figure 2a**). At 16°C, the two yeasts' weight losses were similar (**Figure 2b**)—as in the 2013 vintage; however, EU entered the stationary phase after ten days, two days before CE. The maximum cell load was not affected by temperature for either strain, but EU reached a higher population than CE. Note that the nitrogen supplement did not affect weight loss for any fermentations, indicating that the must's initial nitrogen concentration (110 mg/L) was enough to permit suitable development of both yeasts: specifically, nitrogen was not a limiting factor.

For cell growth, the maximum growth rate ($\mu_{max}$) and the asymptotic maximum ($Y_{end}$) were significantly higher for EU at both temperatures **(Table 2**). In particular, the highest $\mu_{max}$ was recorded for EU at 10°C (2.8 $day^{-1}$) and 16°C (2.9 $day^{-1}$), whereas CE was almost 3.5 slower regardless of temperature. Satisfactory model fitting ($R^2$) was achieved for every model.

Weight loss **(Table 2)**, quantified by $\mu_{max}$ and $Y_{end}$, was unaffected by strain or nitrogen supplement at 10°C, whereas at 16°C nitrogen did increase the $\mu_{max}$, but not the $Y_{end}$, of both strains. Thus, nitrogen would improve the maximum growth rate of EU at 16°C, but not at 10°C, confirming its suitability for low temperatures. A decrease in the amount of ammonium required for low-temperature fermentation has previously been reported (Beltran et al., 2008; Pérez et al., 2018); possible causes include a diminution of permease activity due to decreased membrane fluidity or a change in nitrogen catabolite repression. For CE strain at 10°C a lag phase (λ) of 1.1 and 1.3 days with or without nitrogen supplementation, respectively, was recorded.

*3.2.2. Wine Characteristics*

At both temperatures, the alcohol content, pH, and dry matter were similar for both yeasts **(Table 4)**; however, volatile acidity, a parameter related with impaired wine quality, was almost twice as high for CE. For both UE and CE, the free sulphur dioxide content was similar, while total sulphur dioxide was higher in EU, probably due to its combination with aldehydes (Frivik & Ebeler, 2003)., Total polyphenols were significantly predominant in all EUs, regardless of temperature; previous research has reported yeast-specific variations in polyphenol concentrations (Le Bourvellec & Renard, 2012; Nguela, Poncet-Legrand, Sieczkowski, & Vernhet, 2016). At 10°C, the total acidity, residual sugars, and color were similar. In EUs, residual sugars were higher at 16°C than at 10°C, confirming the yeast's ability to ferment better at low temperatures. The color of EU was also higher at the highest temperature. Nitrogen had no effect on any parameter at either temperature.

*3.2.3. Quantification of Volatile Compounds*

The EU wines presented a high concentration of total alcohols and esters, whereas the CE wines had a high content of volatile acids (**Table 5**); moreover, the concentration was unaffected by fermentation temperature or nitrogen supplement. No significant interaction between strain and nitrogen was detected; this result is supported by a previous study (Magalhães et al., 2017a)

281 performed on Sauvignon blanc wine produced with the interspecies hybrid *Saccharomyces*
282 *cerevisiae x Saccharomyces eubayanus*. The differences in detected volatile compounds for EU and
283 CE yeasts fermenting at 10°C and 16°C are reported in **Table 5**. The yeast had a significant effect
284 on the synthesis of most of the compounds, whereas nitrogen addition (N) and interactions
285 (YS*TM, TM*N, YS*N) affected to a less extent. Among the detected compounds with sensory
286 thresholds available in the literature, sixteen were present with an odor activity value (OAV:
287 concentration divided by sensory threshold) ≥1. In the EU wines, 2-phenethyl alcohols (rose aroma)
288 reached the highest OAV value compared to other aromatics, regardless of the temperature. Among
289 esters, the ethyl nonanoate (nut, rose) was detected in EU wine only, with a higher OAV in the wine
290 fermented at 16°C. The ethyl decanoate (fruit, grape) displayed an opposite trend in EU and CE
291 wine at both temperatures. Ethyl myristate (floral) was detected only in EU wine produced at 10°C,
292 whereas methyl myristate (floral, orris) was produced at the highest concentrations by both yeasts at
293 16°C. As expected from manufacturer information, CE wines reached high concentrations of ethyl
294 hexanoate (apple, pineapple) and octanoic acid (fruit) with OAV in the interval 6237–10471.
295 Isoamyl octanoate, (fruit) was found only in EU and CE wines at 10°C. At 10°C the EU strain
296 produced less ethyl acetate (which has negative aroma characteristics) than the CE strain.
297 Interestingly, EU wines were also characterized by a higher concentration of phenethyl acetate
298 (rose) than CE wines at both temperatures; this aromatic compound is considered a marker in
299 fermentation performed with *Saccharomyces eubayanus* (Magalhães et al., 2017a; Magalhães,
300 Krogerus, Vidgren, Sandell, & Gibson, 2017b). Isoamyl acetate (banana, fruit) was the dominant
301 compound for CE wines at both temperatures; this result was expected, since the strain has been
302 genetically selected to prioritize ester production (reaching highest level at 16°C). For EU wines,
303 the concentrations of this compound were comparable to CE wines at 10°C (regardless the nitrogen
304 supplementation) and at 16°C without nitrogen supplementation, supporting previous findings that
305 high isoamyl acetate production occurs at low temperatures (Killian & Ough, 1979; Molina et al.,
306 2007).

307

308 *3.2.4. Sensory Analysis*

309 A preliminary discriminatory triangle test demonstrated that the replicates were not significantly
310 different. Consequently, one replicate of each wine was randomly selected for sensory descriptive
311 analysis; the results are represented as spider plots. At 10°C **(Figure 3a)**, the highest color intensity
312 scores, positively correlated to color liking, were obtained in CE wines. These wines also scored
313 higher than EUs for sweetness and overall liking. There were no significant differences for the
314 remaining olfactory and taste descriptors.

315 At 16°C (**Figure 3b**), CE wines were again scored higher than EUs for color liking. Unlike at 10°C,
316 the sweetness was similar for both wines. Moreover, overall liking and all other sensory descriptors
317 were not significantly different; the EU wines were comparable to the CEs. However, the fruit notes
318 differed: the exotic fruit notes (banana, apple) were the key sensory aromas of CE wines, whereas
319 the EUs were scored higher for floral notes (e.g., rose). This result reflects the predominant OAVs.

320

321

322 **4. Conclusions**

323 *Saccharomyces eubayanus* demonstrated an ability to adapt to low-temperature fermentation
324 environments, obtaining the best kinetic parameters at 10°C and 12°C, whereas at 26°C stuck
325 fermentation are possible. At 16°C it performed similarly to *Saccharomyces cerevisiae/bayanus*
326 *c*ommercial yeasts.

327 From a biotechnological point of view, wines obtained using this cryotolerant yeast showed good
328 chemical characteristics in fermentation carried at 10°C, 12 and 16°C, whereas the main differences
329 were disclosed for sensory characteristics, especially in fermentation carried out at the lowest
330 temperature (10°C). Nitrogen addition slightly boosted the maximum growth rate at 16°C, but did
331 not affect the wines' other kinetic parameters or chemical characteristics, thus demonstrating this

yeast's low nitrogen requirement. In *Saccharomyces eubayanus* wines a higher concentration of volatile compounds, responsible for floral and white fruit notes, were detected.

In conclusion, *Saccharomyces eubayanus* adapted to the stress of low temperatures and started fermentation quickly. For most of the available commercial yeasts, 10–12°C is an extremely low fermentation temperature range.

The chemical and sensory characteristics of the obtained wines make this yeast worthy of further investigation for production of wine with low pH and light color, such as base wine for sparkling wines. This development would expand the biodiversity of winemaking yeasts capable of producing quality wines.

## ACKNOWLEDGMENTS

We acknowledge Kristina Mayberry for language revision.

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## FIGURE CAPTIONS

**Figure 1**–Cell growth and weight loss for 2013 vintage: (a) 12°C, (b) 16°C, and (c) 26°C. Error bars represent the standard deviation. EU: *Saccharomyces eubayanus* CBS 12357; CB: *Saccharomyces cerevisiae bayanus*; A: 2013 vintage; C: cell growth; W: weight loss; F: fitting curves.

**Figure 2**–Time course experiments at (a) 10°C and (b) 16°C in 2014 vintage. Error bars represent the standard deviation. EU: *Saccharomyces eubayanus* CBS 12357; CE: *Saccharomyces cerevisiae*; 10, 16: growth temperature; N: nitrogen supplement; B: 2014 vintage; C: cell growth; W: weight loss; F: fitting curves.

**Figure 3**–Sensory profile of EU and CE wines produced at (a) 10°C and (b) 16 °C in 2014. EU: *Saccharomyces eubayanus* CBS 12357; CE: *Saccharomyces cerevisiae*; 10, 16: growth temperatures (°C); N: nitrogen supplement; B: 2014 vintage; T: sensory taste; O: sensory olfaction; * $p \leq 0.10$; ** $p \leq 0.05$; *** $p \leq 0.001$.

Table 1 – Yeasts and fermentation conditions in vintages 2013 and 2014.

| Vintage | Trial | Must (L) | Yeast | Temperature (°C) | $SO_2$ at crushing (mg/L) | N supplement (mg/L) |
|---|---|---|---|---|---|---|
| 2013 | EU−12A | 2.00 | *S. eubayanus* | 12 | *none* | *none* |
| | CB−12A | 0.65 | *S. cerevisiae bayanus* | 12 | *none* | *none* |
| | EU−16A | 2.00 | *S. eubayanus* | 16 | *none* | *none* |
| | CB−16A | 0.65 | *S. cerevisiae bayanus* | 16 | *none* | *none* |
| | EU−26A | 2.00 | *S. eubayanus* | 26 | *none* | *none* |
| | CB−26A | 0.65 | *S. cerevisiae bayanus* | 26 | *none* | *none* |
| 2014 | EU−10B | 3.00 | *S. eubayanus* | 10 | 50 | *none* |
| | EU−10NB | 3.00 | *S. eubayanus* | 10 | 50 | 50 |
| | CE−10B | 3.00 | *S. cerevisiae* | 10 | 50 | *none* |
| | CE−10NB | 3.00 | *S. cerevisiae* | 10 | 50 | 50 |
| | EU−16B | 3.00 | *S. eubayanus* | 16 | 50 | *none* |
| | EU−16NB | 3.00 | *S. eubayanus* | 16 | 50 | 50 |
| | CE−16B | 3.00 | *S. cerevisiae* | 16 | 50 | *none* |
| | CE−16NB | 3.00 | *S. cerevisiae* | 16 | 50 | 50 |

*Legend*: EU: *Saccharomyces eubayanus* CBS12357*;* CB: *Saccharomyces cerevisiae bayanus* QA23*;* CE*: Saccharomyces cerevisiae* VIN13; N*: di*−ammonium phosphate addition in the must before fermentation; A, B: refer as for vintage 2013 and 2014, respectively.

**Table 2**−ANOVA of parameters for cell growth and weight loss obtained by non-linear model.

| | | Strain/ Temperature | $\mu_{max}$ ($day^{-1}$) | $\lambda$ (day) | $Y_{end}$ (*Log CFU/mL – g/L) | $R^2$ |
|---|---|---|---|---|---|---|
| **Vintage 2013** | **Cell Growth** | EU−12AC | $0.43\pm0.00_{a}$ | nd | $7.7\pm0.1_{a}$ | 0.9875±0.00 |
| | | CB−12AC | $0.20\pm0.04_{b}$ | nd | $7.3\pm0.1_{ab}$ | 0.9768±0.01 |
| | | EU−16AC | $0.38\pm0.05_{a}$ | nd | $7.6\pm0.3_{ab}$ | 0.9746±0.00 |
| | | CB−16AC | $0.50\pm0.19_{a}$ | nd | $7.6\pm0.3_{ab}$ | 0.9773±0.03 |
| | | EU−26AC | $0.32\pm0.02_{ab}$ | nd | $7.1\pm0.0_{b}$ | 0.9250±0.00 |
| | | CB−26AC | $0.38\pm0.05_{a}$ | nd | $7.5\pm0.2_{ab}$ | 0.9532±0.00 |
| | **Weight Loss** | EU−12AW | $3.6\pm0.2_{c}$ | nd | $79.7\pm2.0_{a}$ | 0.9897±0.00 |
| | | CB−12AW | 5.4 | 1.3 | 101.8 | 0.9870 |
| | | EU−16AW | $8.2\pm0.4_{b}$ | nd | $77.3\pm3.5_{a}$ | 0.9425±0.00 |
| | | CB−16AW | 9.9 | nd | 95.1 | 0.9809 |
| | | EU−26AW | $15.1\pm0.7_{a}$ | nd | $59.1\pm0.2_{b}$ | 0.9430±0.03 |
| | | CB−26AW | 7.9 | nd | 154.9 | 0.8684 |
| **Vintage 2014** | **Cell Growth** | EU−10BC | $2.8\pm0.0_{a}$ | nd | $8.6\pm0.0_{a}$ | 0.9762±0.02 |
| | | CE−10BC | $0.8\pm0.1_{b}$ | nd | $8.3\pm0.1_{b}$ | 0.9224±0.00 |
| | | EU−16BC | $2.9\pm0.1_{a}$ | nd | $8.7\pm0.0_{a}$ | 0.9898±0.00 |
| | | CE−16BC | $0.8\pm0.1_{b}$ | nd | $8.4\pm0.0_{b}$ | 0.8958±0.03 |
| | **Weight Loss** | EU−10BW | 5.6±0.4 | nd | 71.2±0.5 | 0.9677±0.00 |
| | | CE−10BW | 4.7±0.9 | 1.3±1.3 | 69.7±1.2 | 0.9857±0.00 |
| | | EU−10NBW | 5.3±0.1 | nd | 73.3±0.4 | 0.9594±0.00 |
| | | CE−10NBW | 4.1±1.0 | 1.1±1.1 | 65.0±8.2 | 0.9912±0.00 |
| | | EU−16BW | $9.3\pm0.0_{c}$ | nd | $74.9\pm0.2_{c}$ | 0.9805±0.00 |
| | | CE−16BW | $9.4\pm0.1_{c}$ | nd | $79.6\pm0.4_{a}$ | 0.9963±0.00 |
| | | EU−16NBW | $10.1\pm0.0_{a}$ | nd | $76.2\pm1.0_{bc}$ | 0.9840±0.00 |
| | | CE−16NBW | $9.9\pm0.0_{b}$ | nd | $79.1\pm1.2_{ab}$ | 0.9915±0.00 |

*Legend:* $\mu_{max}$: maximum growth rate; $\lambda$: lag phase duration; $Y_{end}$: growth/asymptotic maximum; $R^2$: coefficient of determination. Values are given as mean±SD for the replicated trials, which are coded as follows: EU: *Saccharomyces eubayanus* CBS 12357; CE: *Saccharomyces cerevisiae*; CB: *Saccharomyces cerevisiae bayanus*; N: nitrogen supplement; 10 ,12, 16, 26: growth temperature (°C); A: vintage 2013; B: vintage 2014; C: cell growth; W: weight loss; nd: not detected; CFU: colony-forming unit Value followed by different letters in the same column are significantly different according to the *Fisher* LSD test (α: 0.05). * Data were expressed as Log CFU/mL for cell count, whereas are expressed as g/L for weight loss.

**Table 3**–Qualitative parameters (mean±SD) in wines fermented at 12, 16 and 26°C with EU (*Saccharomyces eubayanus)* or CB (*Saccharomyces cerevisiae bayanus)* in the 2013 vintage.

| Wine | $SO_{2F}$ (mg/L) | $SO_{2T}$ (mg/L) | AC (%) | VA (g/L) | pH | CO (AU) | RS (g/L) |
|---|---|---|---|---|---|---|---|
| EU−12A | 13±0.6 | $54±0.6_{a}$ | 10.7±0.1 | $0.36±0.03_{c}$ | 3.4±0.0 | $0.075±0.0_{b}$ | 6.6±2.8 |
| CB−12A | 14 | 45 | 13.0 | 0.6 | 3.4 | 0.081 | 1.0 |
| EU−16A | 11±3.2 | $41±3.8_{ab}$ | 10.7±0.2 | $0.49±0.01_{b}$ | 3,4±0.0 | $0.058±0.0_{c}$ | 6.0±2.2 |
| CB−16A | 12 | 27 | 12.5 | 0.5 | 3.5 | 0.210 | 1.3 |
| EU−26A | 12±0.6 | $33±4.5_{c}$ | 10.0±0.3 | $0.76±0.01_{a}$ | 3.4±0.0 | $0.097±0.0_{a}$ | 15.6±13.0 |
| CB−26A | 10 | 23 | 11.9 | 0.6 | 3.5 | 0.147 | 1.1 |

*Legend*: $SO_{2F:}$ free sulphur dioxide; $SO_{2T:}$ total sulphur dioxide; AC: alcohol content; VA: volatile acidity; CO: color at 420 nm; RS: residual sugars. Trials are coded as follows: EU: *Saccharomyces eubayanus* CBS 12357; CB: *Saccharomyces cerevisiae bayanus*; 12, 16, 26: growth temperature (C°); A: vintage 2013. Value followed by different letters in the same column are significantly different according to the *Fisher* LSD test (α: 0.05).

**Table 4**–Wine characteristics (mean±SD) in fermentations carried out by *Saccharomyces eubayanus* or *Saccharomyces cerevisiae* at 10°C and 16°C with or without nitrogen supplementation in vintage 2014.

| Wine | AC (%) | TA g/L | pH | VA (g/L) | CO (AU) | $SO_{2F}$ (mg/L) | $SO_{2T}$ (mg/L) | DM g/L | TP mg/L | RS (g/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| EU−10B | 10.3±0.0 | 7.9±0.1 | 3.8±0.0 | $0.25±0.0_{b}$ | 0.107±0.0 | 12±1.3 | $92±3.8_{a}$ | 23±0.6 | $240±1.3_{a}$ | 1.9±0.1 |
| CE−10B | 10.0±0.2 | 7.3±0.1 | 3.8±0.0 | $0.51±0.0_{a}$ | 0.106±0.0 | 18±2.6 | $51±4.5_{b}$ | 23±1.7 | $222±1.1_{b}$ | 1.9±0.1 |
| EU−10NB | 10.2±0.0 | 7.9±0.4 | 3.8±0.0 | $0.25±0.0_{b}$ | 0.107±0.0 | 13±0.6 | $94±3.2_{a}$ | 23±0.3 | $239±1.1_{a}$ | 1.8±0.0 |
| CE−10NB | 10.2±0.0 | 7.3±0.2 | 3.8±0.0 | $0.49±0.1_{a}$ | 0.091±0.0 | 19±0.1 | $46±0.6_{b}$ | 21±0.0 | $225±0.7_{b}$ | 2.0±0.0 |
| EU−16B | 10.1±0.1 | $7.0±0.1_{ab}$ | 3.7±0.0 | $0.30±0.0_{b}$ | $0.173±0.0_{a}$ | $13±2.5_{ab}$ | $83±1.3_{a}$ | 22±0.1 | $270±3.1_{a}$ | $3.9±0.1_{a}$ |
| CE−16B | 10.5±0.0 | $6.8±0.0_{b}$ | 3.7±0.0 | $0.51±0.0_{a}$ | $0.107±0.0_{b}$ | $19±1.9_{ab}$ | $36±3.8_{b}$ | 22±0.2 | $237±3.7_{b}$ | $1.5±0.2_{b}$ |
| EU−16NB | 10.1±0.1 | $7.2±0.1_{a}$ | 3.7±0.0 | $0.30±0.0_{b}$ | $0.151±0.0_{ab}$ | $12±0.1_{b}$ | $80±3.2_{a}$ | 22±0.1 | $269±0.9_{a}$ | $3.4±0.3_{a}$ |
| CE−16NB | 10.5±0.2 | $6.7±0.0_{b}$ | 3.7±0.0 | $0.49±0.0_{a}$ | $0.108±0.0_{b}$ | $22±0.0_{a}$ | $30±1.9_{b}$ | 22±0.4 | $241±1.6_{b}$ | $1.8±0.1_{b}$ |

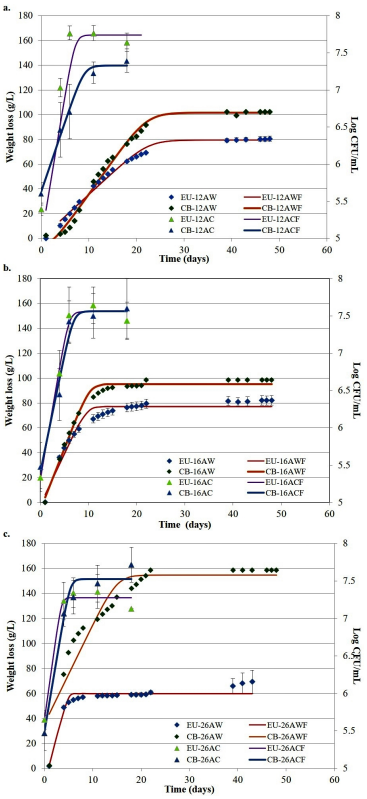
*Legend*: AC: alcohol content; TA: titratable acidity; VA: volatile acidity; CO: color at 420 nm; $SO_{2F:}$ free sulphur dioxide; $SO_{2T:}$ total sulphur dioxide; DM: dry matter; TP: total polyphenols; RS: residual sugars. Each value is the mean of two independent vinifications. Trials are coded as follows: EU: *Saccharomyces eubayanus* CBS 12357; CE: *Saccharomyces cerevisiae*; N: nitrogen supplement; 10, 16: growth temperature; B: vintage 2014. Value followed by different letters in the same column are significantly different according to the *Fisher* LSD test (α: 0.05).

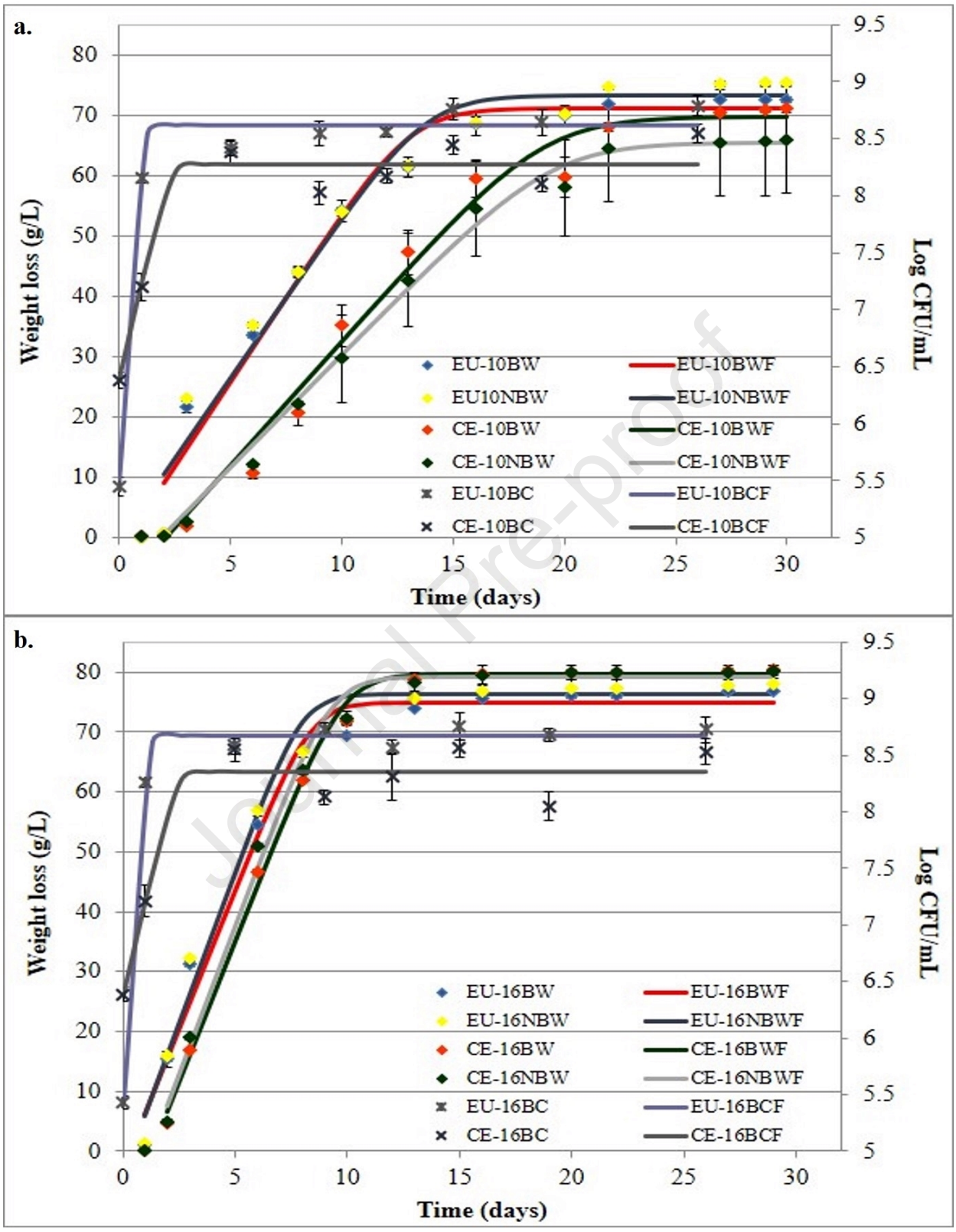
**Table 5**−Volatiles composition, one way ANOVA and interactions (yeast strain, temperature, nitrogen supplement) of EU and CE white wines (mg/L) produced during 2014 harvest (mean value±SD of two vinifications) at different fermentation temperatures (10°C and 16°C) with or without nitrogen (N) supplement.

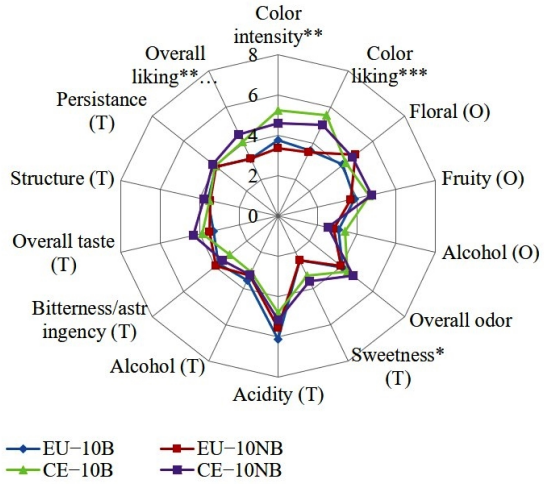
| **Compound (mg/L)** | **Wine** | | | | | | | | | ***p-value* for each variable** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **EU−10B** | **CE−10B** | **EU−10NB** | **CE−10NB** | **EU−16B** | **CE−16B** | **EU−16NB** | **CE−16NB** | **ST** | **TM** | **YS** | **N** | **YS*TM** | **TM*N** | **YS*N** |
| ***Alcohols*** | | | | | | | | | | | | | | | |
| *1*−Butanol | 0.4±0.4 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.5±0.4 | 0.6±0.4 | 0.6±0.3 | 0.0±0.0 | 150^ | 0.246 | 0.414 | 0.421 | 0.903 | 0.914 | 0.791 |
| *t*−3−Hexen−1−ol** | $0.0±0.0_{c}$ | $0.3±0.1_{a}$ | $0.0±0.0_{c}$ | $0.4±0.0_{a}$ | $0.1±0.0_{bc}$ | $0.4±0.1_{a}$ | $0.2±0.0_{ab}$ | $0.2±0.0_{ab}$ | 0.4# | 0.388 | **0.001** | 0.960 | 0.201 | 0.555 | 0.466 |
| 2−Methyl−1−propanol | $3.0±0.2_{a}$ | $1.3±0.5_{b}$ | $2.2±0.8_{ab}$ | $1.7±0.2_{ab}$ | $2.2±0.1_{ab}$ | $3.1±0.4_{a}$ | $2.3±0.2_{ab}$ | $2.8±0.5_{a}$ | 40§ | 0.131 | 0.503 | 0.774 | **0.020** | 0.872 | 0.560 |
| 3−Methyl−1−butanol | 19.3±19.3 | 0.0±0.0 | 0.1±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.2±0.1 | 0.0±0.0 | 30# | 0.347 | 0.338 | 0.350 | 0.347 | 0.341 | 0.350 |
| 3−Methyl−1−pentanol*** | $0.0±0.0_{b}$ | $0.0±0.0_{b}$ | $0.3±0.0_{a}$ | $0.0±0.0_{b}$ | $0.3±0.1_{a}$ | $0.0±0.0_{b}$ | $0.3±0.0_{a}$ | $0.1±0.0_{b}$ | 0.8−1.2~ | **0.017** | **0.000** | **0.050** | 0.161 | **0.101** | **0.095** |
| 2−Phenethyl alcohol*** | $211±13_{b}$ | $51±5_{c}$ | $204±9_{b}$ | $36±5_{c}$ | $225±13_{ab}$ | $38±4_{c}$ | $240±6_{a}$ | 46±4c | 1.4§ | **0.067** | **< 0.0001** | 0.967 | **0.044** | **0.074** | 0.534 |
| ***Total*** | *234±85* | *52±20* | *207±83* | *38±15* | *228±92* | *42±15* | *244±98* | *49±19* | | *0.385* | ***0.019*** | *0.541* | *0.398* | *0.208* | *0.864* |
| ***Esters*** | | | | | | | | | | | | | | | |
| Diethyl malonate*** | $2.2±0.5_{a}$ | $0.1±00_{b}$ | $2.2±0.2_{a}$ | $0.2±0.1_{b}$ | $2.8±0.3_{a}$ | $0.0±0.0_{b}$ | $2.4±.04_{a}$ | $0.0±0.0_{b}$ | n.a | 0.510 | **< 0.0001** | 0.694 | 0.157 | 0.563 | 0.650 |
| Ethyl 4-decenoate* | $15.0±8.0_{a}$ | $0.0±0.0_{b}$ | $5.0±2.0_{ab}$ | $0.0±0.0_{b}$ | $2.0±0.5_{b}$ | $6.2±2.0_{ab}$ | $1.8±0.9_{b}$ | $8.3±3.0_{ab}$ | n.a | 0.880 | 0.410 | 0.475 | **0.019** | 0.295 | 0.282 |
| Ethyl acetate | $13.6±5.2_{b}$ | $33.7±3.8_{a}$ | $13.6±0.3_{b}$ | $21.4±3.8_{ab}$ | $15.2±0.5_{b}$ | $30.3±3.9_{ab}$ | $16.2±1.0_{ab}$ | $13.1±8_{b}$ | 198± | 0.621 | **0.025** | **0.085** | 0.318 | 0.814 | **0.072** |
| Ethyl butyrate | 2.2±0.2 | 3.3±0.1 | 1.9±0.1 | 3.4±0.0 | 2.0±0.2 | 3.9±0.1 | 1.8±0.1 | 1.7±1.7 | 0.02# | 0.441 | **0.039** | 0.178 | 0.650 | 0.247 | 0.420 |
| Ethyl decanoate*** | $110±25_{a}$ | $7.5±2.6_{b}$ | $86.3±37.7_{a}$ | $7.0±0.6_{b}$ | $72.9±1.9_{a}$ | $10.8±2.5_{b}$ | $81.5±4.5_{a}$ | $14.0±4.3_{b}$ | 0.2§ | 0.496 | **< 0.0001** | 0.780 | 0.272 | 0.438 | 0.697 |
| Ethyl hexanoate*** | $14.0±0.0_{d}$ | $52.4±8.4_{a}$ | $10.3±3.9_{d}$ | $40.2±1.8_{ab}$ | $17.9±0.2_{cd}$ | $36.6±4.3_{b}$ | $19.5±1.5_{cd}$ | $31.2±8.4_{bc}$ | 0.005# | 0.380 | **< 0.0001** | 0.152 | **0.015** | 0.364 | 0.256 |
| Ethyl laurate | 2.5±2.0 | 2.2±1.8 | 20.2±20.2 | 0.0±0.0 | 0.0±0.0 | 0.9±0.7 | 0.0±0.0 | 0.0±0.0 | n.a | 0.266 | 0.360 | 0.492 | 0.320 | 0.443 | 0.332 |
| Ethyl myristate*** | $24.6±2.3_{a}$ | $0.0±0.0_{b}$ | $25.5±0.4_{a}$ | $0.0±0.0_{b}$ | $0.0±0.0_{b}$ | $0.0±0.0_{b}$ | $0.0±0.0_{b}$ | $0.0±0.0_{b}$ | 4.0~ | **< 0.0001** | **< 0.0001** | 0.667 | **< 0.0001** | 0.667 | 0.667 |
| Ethyl nonanoate* | $44.9±4.6_{ab}$ | $0.0±0.0_{b}$ | $49.0±0.3_{ab}$ | $0.0±0.0_{b}$ | $67.5±1.5_{a}$ | $0.0±0.0_{b}$ | $71±0.6_{a}$ | $0.0±0.0_{b}$ | 12~ | 0.106 | **0.011** | 0.287 | 0.848 | 0.351 | 0.4121 |
| Ethyl octanoate | 0.0±0.0 | 52.3±4.6 | 9.0±9.0 | 20.2±2.0 | 31.7±31.7 | 44.9±0.1 | 46.3±5.9 | 35.7±35.7 | 0.005§ | 0.133 | 0.192 | 0.715 | 0.226 | 0.558 | 0.199 |
| Heptyl acetate*** | $0.0±0.0_{c}$ | $0.2±0.1_{a}$ | $0.0±0.0_{c}$ | $0.2±0.1_{ab}$ | $0.0±0.0_{c}$ | $0.0±0.0_{c}$ | $0.1±0.2_{b}$ | $0.0±0.0_{c}$ | 0.32~ | **0.004** | **0.004** | 0.530 | **< 0.0001** | **0.035** | **0.035** |
| Hexyl acetate | 0.7±0.7 | 1.9±0.8 | 0.8±0.9 | 2.5±0.3 | 2.0±1.0 | 0.2±0.0 | 0.5±0.5 | 0.3±0.1 | 1.5^ | 0.222 | 0.731 | 0.746 | **0.059** | 0.340 | 0.388 |
| Hexyl formate | 3.7±0.4 | 5.2±1.2 | 3.6±0.0 | 4.1±0.1 | 3.4±0.5 | 3.5±0.1 | 4.3±0.1 | 3.2±0.3 | n.a | 0.140 | 0.424 | 0.677 | **0.059** | 0.221 | 0.141 |
| Isoamyl acetate* | $19.6±0.7_{b}$ | $38.0±4.1_{ab}$ | $26.9±8.6_{b}$ | $43.5±0.5_{ab}$ | $17.9±1.4_{b}$ | $40.3±7.8_{ab}$ | $19.5±0.6_{b}$ | $80.7±34.7_{a}$ | 0.002−0.043~ | 0.463 | **0.015** | 0.199 | 0.251 | 0.479 | 0.376 |
| Isoamyl formate | 35.7±35.7 | 53.9±13.1 | 54.6±13.8 | 36.9±0.2 | 30.9±30.9 | 51.6±4.5 | 0.0±0.0 | 50.6±2.2 | n.a | 0.388 | 0.208 | 0.585 | 0.215 | 0.539 | 0.912 |
| Isoamyl hexanoate | 2.2±0.7 | 10.7±10.7 | 1.9±0.5 | 0.0±0.0 | 0.4±0.1 | 0.0±0.0 | 0.3±0.0 | 0.0±0.0 | n.a | 0.221 | 0.601 | 0.324 | 0.511 | 0.335 | 0.364 |
| Isoamyl laurate*** | $1.6±0.2_{a}$ | $0.0±0.0_{b}$ | $1.5±0.3_{a}$ | $0.0±0.0_{b}$ | $0.0±0.0_{b}$ | $0.0±0.0_{b}$ | $0.0±0.0_{b}$ | $0.0±0.0_{b}$ | n.a | **< 0.0001** | **< 0.0001** | 0.755 | **< 0.0001** | 0.755 | 0.755 |
| Isoamyl octanoate* | $7.5±1.4_{ab}$ | $5.7±5.7_{abc}$ | $9.6±0.7_{a}$ | $0.0±0.0_{c}$ | $0.0±0.0_{c}$ | $0.0±0.0_{c}$ | $1.6±1.6_{bc}$ | $0.0±0.0_{c}$ | 0.125§ | **0.007** | **0.066** | 0.750 | 0.150 | 0.417 | 0.160 |
| Isobutyl acetate | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.3±0.0 | 0.0±0.0 | 0.2±0.0 | 0.0±0.0 | 0.1±0.1 | 0.065−0.88~ | 0.898 | **0.039** | 0.274 | 0.955 | 0.226 | 0.355 |

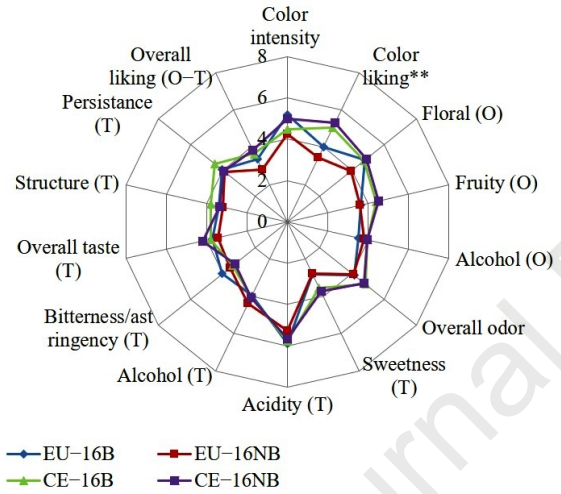
| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methyl myristate** | $2.4\pm2.0_{c}$ | $0.0\pm0.0_{c}$ | $9.6\pm9.6_{bc}$ | 0.0±0.0c | $25.6\pm3.4_{ab}$ | $7.2\pm7.2_{c}$ | $25.9\pm5.6_{a}$ | $14.5\pm2.3_{abc}$ | n.a | **0.002** | **0.016** | 0.317 | 0.238 | 0.974 | 0.982 |
| Methyl octanoate*** | $0.0\pm0.0_{d}$ | $0.0\pm0.0_{d}$ | $0.0\pm0.0_{d}$ | $0.0\pm0.0_{d}$ | $0.1\pm0.0_{cd}$ | $0.2\pm0.0_{ab}$ | $0.1\pm0.0_{bc}$ | $0.2\pm0.1_{a}$ | 0.20−0.87[~] | **< 0.0001** | **0.014** | 0.267 | **0.014** | 0.267 | 0.859 |
| Octyl acetate*** | $0.3\pm0.2_{cd}$ | $1.4\pm0.4_{ab}$ | $0.8\pm0.1_{bc}$ | $1.6\pm0.3_{a}$ | $0.4\pm0.0_{cd}$ | 0.0±0.0d | $0.4\pm0.1_{cd}$ | 0.0±0.0d | 0.023−0.047[~] | **0.000** | **0.088** | 0.222 | **0.001** | 0.282 | 0.422 |
| Phenethyl acetate*** | $144\pm5.8_{a}$ | $29.9\pm1.9_{b}$ | $140\pm12.3_{a}$ | $21.3\pm0.1_{b}$ | $109\pm1.5_{a}$ | $0.0\pm0.0_{b}$ | $134\pm32.4_{a}$ | $0.0\pm0.0_{b}$ | 0.25[#] | **0.023** | **< 0.0001** | 0.727 | 0.783 | 0.291 | 0.409 |
| Phenethyl formate | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.1±0.1 | 0.0±0.0 | 0.2±0.0 | 0.0±0.0 | 0.27[~] | **0.016** | **0.016** | 0.325 | **0.016** | 0.325 | 0.325 |
| Phenethyl hexanoate*** | $62.2\pm13.1_{a}$ | $0.0\pm0.0_{b}$ | $72.0\pm6.5_{a}$ | $0.0\pm0.0_{b}$ | $15.8\pm15.8_{b}$ | $0.0\pm0.0_{b}$ | $0.0\pm0.0_{b}$ | $0.0\pm0.0_{b}$ | n.a | **0.000** | **< 0.0001** | 0.795 | **0.000** | 0.273 | 0.795 |
| Phenethyl octanoate | 1.9±1.9 | 0.0±0.0 | 6.6±4.6 | 0.0±0.0 | 1.7±1.7 | 0.3±0.3 | 4.2±0.8 | 0.0±0.0 | n.a | 0.739 | **0.066** | 0.335 | 0.675 | 0.724 | 0.296 |
| ***Total*** | *511±36* | *299±19* | *551±34* | *203±14* | *419±27* | *237±16* | *482±33* | *20 3±18* | | *0.108* | ***0.024*** | *0.606* | *0.232* | *0.268* | *0.104* |
| ***Acids*** | | | | | | | | | | | | | | | |
| Acetic acid* | $0.0\pm0.0_{b}$ | $2.4\pm0.1_{ab}$ | $0.0\pm0.0_{b}$ | $2.8\pm0.4_{ab}$ | $2.2\pm1.2_{ab}$ | $5.4\pm0.2_{a}$ | $2.4\pm1.1_{ab}$ | $5.7\pm0.2_{a}$ | 10−552[~] | **0.008** | **0.005** | 0.781 | 0.679 | 0.964 | 0.860 |
| Decanoic acid** | $6.5\pm1.0_{a}$ | $4.9\pm4.9_{ab}$ | $8.3\pm0.7_{a}$ | $0.0\pm0.0_{b}$ | $0.0\pm0.0_{b}$ | $0.0\pm0.0_{b}$ | $0.0\pm0.0_{b}$ | $0.0\pm0.0_{b}$ | 1.0[§] | **0.005** | **0.094** | 0.578 | **0.094** | 0.578 | 0.231 |
| Hexanoic acid | 0.0±0.0 | 4.3±1.2 | 0.0±0.0 | 2.3±2.3 | 0.0±0.0 | 1.3±0.8 | 0.1±0.0 | 0.0±0.0 | 0.42[§] | **0.091** | **0.019** | 0.264 | **0.083** | 0.780 | 0.242 |
| Nonanoic acid | 0.0±0.0 | 1.4±0.7 | 0.0±0.0 | 1.1±0.1 | 0.0±0.0 | 0.0±0.0 | 0.3±0.3 | 0.5±0.3 | 3−9[~] | **0.092** | **0.017** | 0.557 | **0.026** | 0.260 | 0.879 |
| Octanoic acid*** | $4.4\pm0.4_{d}$ | $61.9\pm14.3_{a}$ | $8.3\pm0.60_{cd}$ | $52.0\pm3.9_{ab}$ | $31.6\pm1.2_{bc}$ | $55.6\pm11.5_{a}$ | $38.8\pm5.7_{b}$ | $60.0\pm5.9_{a}$ | 0.50[§] | **0.015** | **< 0.0001** | 0.779 | **0.019** | 0.395 | 0.423 |
| ***Total*** | *11±3* | *75±26* | *17±4* | *58±23* | *34±14* | *62±24* | *42±17* | *66±26* | | *0.261* | *0.075* | *0.971* | *0.218* | *0.440* | *0.394* |
| ***Miscellaneous*** | | | | | | | | | | | | | | | |
| *n*-Nonanal | 0.5±0.5 | 1.6±1.4 | 0.0±0.0 | 0.0±0.0 | 0.2±0.1 | 0.0±0.0 | 0.4±0.0 | 0.1±0.1 | 0.001−0.008[~] | 0.438 | 0.690 | 0.311 | 0.346 | 0.180 | 0.483 |
| Thiophene 2−acetic acid, dodec−9−ynyl ester** | $2.8\pm2.2_{bc}$ | $0.0\pm0.0_{c}$ | $7.4\pm2.5_{ab}$ | $0.0\pm0.0_{c}$ | $9.1\pm1.9_{a}$ | $0.0\pm0.0_{c}$ | $8.7\pm1.7_{a}$ | $0.0\pm0.0_{c}$ | n.a | 0.137 | **0.000** | 0.385 | 0.137 | 0.305 | 0.385 |

*Legend:* EU: *Saccharomyces eubayanus* CBS 12357; CE: *Saccharomyces cerevisiae*; 10, 16: growth temperature (°C); N: nitrogen supplement; B: vintage 2014. ST: Sensory threshold; n.a: not available; TM: temperature (10 and 16°C); YS: Yeast strain (EU and CE); The subscript letters represent the results of Fisher's LSD *post hoc* comparison tests: for values with the same letter, different wines have significantly different means; *p≤= 0.10; **p≤= 0.05; ***p≤= 0.001. Number in bold indicate p-value in the range: 0.001≤p≤0.100; [§] (Ferreira, López, & Cacho, 2000), [^](Etiévant, 1991); [#](Guth, 1997); [~] (Burdock, 2005); [±] (Cliff & Pickering, 2006).







- *S. eubayanus* showed good adaptation to low temperature and wines were characterized by low volatile acidity.
- Nitrogen requirements of commercial and cryotolerant strains were similar.
- *S. eubayanus* wines were characterized by 2-phenethyl alcohols (rose aroma) whereas *S. cerevisiae* by ethyl hexanoate (apple, pineapple).
- The cryotolerant yeast *S. eubayanus* is a valuable alternative to conventional yeast in the production of base wine for sparkling wines.

GPP: Conceptualization, Methodology, Writing- Original draft, Software and Validation; AR: Data curation, Writing- Original draft preparation, Software and Validation; BF: Methodology, Investigation. FP: Methodology, Investigation and Validation; RL: Methodology, Writing- Reviewing and Supervision. AV: Conceptualization, Validation, Writing- Reviewing and Editing.

**Declaration of interests**

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