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ORIGINAL PAPER

# Use of non-*Saccharomyces* yeasts to modulate oenological parameters in Albillo Mayor white wines

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**Abstract**
The white Albillo Mayor grape variety is considered aromatically neutral. Fermentations with non-*Saccharomyces* yeasts could substantially improve the aromatic profile and the acidity of these wines. The aim of this work is to evaluate the use of selected strains of different non-*Saccharomyces* species to improve the quality of Albillo Mayor white wines. Fermentations in duplicate stainless steel barrels of 150 L of volume were carried out at Bodegas Comenge, Ribera del Duero, Spain. All fermentations were sequentially inoculated with *Saccharomyces cerevisiae*. The fermentations were carried out with the species *Hanseniaspora vineae*, *H. opuntiae*, two strains of *Metschnikowia pulcherrima* (M29 and M54) and a mixed fermentation where an inoculum with *M. pulcherrima* and *Lachancea thermotolerans* species was added in a 1:1 ratio. The results indicated an increase in total acidity of 1.8 g/L in the mixed fermentations resulting in 0.2 units of pH reduction. *M. pulcherrima* produced more than twice the 2-phenylethanol content of the control wines. Values for all terpenes were higher in *H. vineae* fermentations, with the exception of nerol, with higher contents in *M. pulcherrima* M54 fermentations. Tasters perceived the M29 wines of *M. pulcherrima* as the most balanced and cleanest. In addition, together with the wines from the mixed fermentations, they were perceived as having the highest varietal aromatic character. It should be noted that the higher acidity, analytically identified in the mixed fermentations, was not perceived by the tasters. The use of non-*Saccharomyces* yeasts can modulate oenological parameters such as acidity or the release of fermentative volatiles in Albillo Mayor wines.

**Keywords** non-*Saccharomyces* · *Hanseniaspora vineae* · *H. opuntiae* · *Metschnikowia pulcherrima* · *Lachancea thermotolerans*

## Introduction

The white grape variety Albillo Mayor is considered aromatically neutral, but with an interesting potential for obtaining quality wines, both young and aged. In this respect, non-*Saccharomyces* yeasts can be used to modulate wine parameters such as the perception of freshness or the formation of aromatic compounds providing aromatic complexity [1]. It should be noted that the fermentative power of some of these species is low, so it is common to use them in both mixed and sequential fermentations with *Saccharomyces cerevisiae* species.

*Hanseniaspora vineae* and *H. opuntiae* from the yeast genus *Hanseniaspora/Kloeckera* could be applied to produce quality white wines. *H. vineae* has some very interesting characteristics for industrial application such as resistance to $SO_2$ and ethanol, exocellular protease and

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β-glucosidase enzymatic activity, fast cell lysis with early release of polysaccharides, DNA and proteins, ideal for aging on lees and other highly produced flavours [2]. Among the aromas produced by this yeast, the most important is 2-phenylethyl acetate [3]. The enhanced formation of 2-phenylethyl acetate in these species is explained by the gene duplications of aromatic amino acid aminotransferases and phenylpyruvate decarboxylases [4]. We have previously investigated the use of *H. vineae* in sequential fermentation with *S. cerevisiae* of Albillo Mayor musts. This yeast modified the aromatic profile by significantly increasing the terpene content, mainly linalool (>×3), β-citronellol (>×4), geraniol (>×2) and α-terpineol (≈×2) [5]. In addition, in other assays with *H. vineae* the powerful aromatic compound safranal was identified in the wines [6]. Finally, when these species were used for the production of rosé wines blend of Albillo and Tempranillo grapes, the results showed 44% more anthocyanins compared to the control wine, resulting in a significant improvement in colour [7]. *H. opuntiae* is also an interesting species for cellar application, in fact *H. opuntiae* can be used with *Lachancea thermotolerans* in warm areas with neutral varieties to enhance freshness and fruitiness [8]. Previous studies show that this yeast produces low volatile acidity [9], increasing the aromas mainly of phenylethanol, 3-methyl-butanol and phenylacetaldehyde, and enhances the production of acetate esters [10]. Other authors used *H. opuntiae* and *S. cerevisiae* in mixed fermentation to make wines from the Sideritis grape variety and noted an increase in most acetate esters, a 2.6-fold increase in 2-phenylethyl acetate, an increase in several ethyl esters and the production of the terpene citronellol [11]. Finally, it should be noted that the main problem of this yeast is its low fermentative power, normally less than 6% vol, which forces its use in sequential fermentation with *S. cerevisiae*.

Another yeast species with interesting oenological application is *Metschnikowia pulcherrima*. This yeast shows the expression of numerous extracellular activities, some of which enhance the release of varietal aroma from the grape by hydrolyzing bound monoterpenes compounds [12, 13]. The low fermentative power of *M. pulcherrima* makes it necessary to use it in mixed or sequential fermentation with *S. cerevisiae*. In this regard, it is noted that the high proteolytic activity of *M. pulcherrima* leads to the release of amino acids that serve as a nutrient for *S. cerevisiae* [14, 15]. Co-inoculations of these two yeasts have produced high contents of acetate esters and β-damascenone with lower levels of C6 alcohols in ice wines made from the Vidal blanc grape variety [16]. Therefore, the application of *M. pulcherrima* can improve aromatic complexity by increasing fermentative esters mainly due to its high extracellular enzymatic activity. Similarly, sequential fermentations with *M. pulcherrima* showed a higher production of higher alcohols, with particularly high concentrations of isobutanol and phenylethanol [17]. Another application of *M. pulcherrima* is its use as a biological control agent as it produces an insoluble red pigment with antimicrobial activity called pulcherrimin which gives *M. pulcherrima* a certain competitive advantage over other non-*Saccharomyces* yeasts [12, 18].

*L. thermotolerans* is a yeast with an interesting application in warm areas since it is a strong producer of lactic acid [19]. This production is made from sugars, so there is also a slight reduction in the alcoholic level (0.7% vol [20]). The lactic acid production of *L. thermotolerans* depends on several factors, not only the inoculated population and the yeast assimilable nitrogen content in must such as the inoculation strategy, the viability or activity of *L. thermotolans* and the species present in the must and during the fermentation. The highest lactic acid productions are observed at yeast assimilable nitrogen contents between 150 and 200 mg/L [21]. The acidification strongly influences pH being possible to reduce more than 0.5 pH units or more during fermentation. Also, an improvement in the production of 2-phenylethanol has been described [21]. *L. thermotolerans* has a medium fermentative power so it must be used in sequential or mixed inoculations with *S. cerevisiae* to get dry wines.

The main objective of this work was to investigate the application of these yeasts to improve the quality of Albillo Mayor grape variety wines from Ribera del Duero, Spain. The study was carried out on a semi-industrial scale, and the profile of the wines was determined analytically.

## Materials and methods

### Yeast strains used in fermentation of albillo white wines

The yeast strain of T02/5A *H. vineae* (HV) were isolated by Professor Francisco Carrau (Facultad de Química, Universidad de la República, Montevideo, Uruguay). The yeasts strains of *M. pulcherrima* M29 and M54, *L. thermotolerans* L31 strain (L31) and A56 *H. opuntiae* (HO) were isolated and selected by enotecUPM (Chemistry and Food Technology Department of Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, UPM, Madrid, Spain). The yeast strain used as control was *Saccharomyces cerevisiae* (SC) CTPL14, also isolated in Ribera del Duero, Spain by enotecUPM. Yeasts were stored at 4 °C in tubes containing YPD agar (10 g/L of yeast extract, 20 g/L of peptone, and 20 g/L of glucose) until used for inoculum preparation. Viability was maintained by regular subculturing. The cultures were scaled up from test tubes to 250 mL flasks and then to 5 L carboys in YPD medium. All incubations

were carried out at 20 °C until the inoculum was ready for the winery.

## Must and fermentation conditions

The must was obtained by pressing of Albillo variety (*Vitis vinifera* L.) from the Ribera de Duero region in Spain (2023 vintage), in a pneumatic press at less than 2 bar and subsequently settled at a low temperature (8 °C) for 24 h. $SO_2$ was added during pressing at a dose of 4 g/hL, in the form of potassium metabisulfite ($K_2S_2O_5$). No enzymes were added to the must, neither was it acidified with tartaric acid. Before inoculation with the different yeasts studied, the Albillo must showed a sugar content of 205 g/L, a pH of 3.3, 63 nephelometric turbidity units, 36 and 14 mg/L of total and free $SO_2$, respectively. The musts were fortified with 100 mg/L of Nutrient Vit Green (Lallemand Inc., Montreal, QC, Canada).

The Albillo white must was fermented in duplicate in 150 L stainless steel barrels at room temperature (18 °C). The barrels were inoculated with 5 L (≈3%) at a population of 7-log CFU/mL of liquid inoculum of each yeast studied prepared in YPD medium. SC fermentations were inoculated at time zero and used as controls in this study. All fermentations were sequentially inoculated with SC on day six, including the control barrels. For the M54 + L31 treatment, in these barrels a mixed fermentation of M54 and L31 was carried out in a 1:1 ratio. Fermentations were carried out for a period of 20 days. Upon completion, samples were removed from each barrel and immediately refrigerated at 4 °C until all wine analyses were performed. Fermentation was monitored daily by densimetry. The decrease in density indicated the correct progress of the alcoholic fermentation until its completion.

## General oenological parameters analyses

The SO2 analysis of the initial musts was carried out by the Paul-Rankine method. The analysis of general oenological parameters was carried out by direct measurement using Fourier Transform Infrared Spectroscopy (FTIR) with an OenoFoss instrument (FOSS Iberia, Barcelona, Spain). The parameters analysed included: total acidity (g/L, expressed as tartaric acid), pH, volatile acidity (g/L, expressed as acetic acid), glucose/fructose content (g/L), and Total Polyphenol Index (TPI).

## Ethanol, malic acid and lactic acid content Analyses

Liquid chromatography with refractive index detection (LC-RI) was used to determinate de ethanol content, using an Agilent 1200 series HPLC apparatus (Agilent Technologies, Palo Alto, CA, USA) equipped with a Refractive Index Detector. The column used was a Phenosphere XDB C18 column (4.6 × 150 mm, 5-µm particle size) (Phenomenex, Torrance, CA, USA). Milli Q water in isobaric mode at 0.8 mL/min was the solvent of the method. The column and the detector temperature was 30 °C. The injection volume of the sample was 2 µL previously filtrated through 0.45-µm cellulose methyl ester membrane filters (Tecknokroma, Barcelona, Spain). Quantification was carried out using ethanol (99.5% purity) (Panreac, Spain) as an external standard, with four calibration levels: 5, 10, 15, and 20% v/v ($r^2$ = 0.9998).

L-Malic acid and L-Lactic acid were determined using the enzymatic analyzer Y25 (Biosystems, Barcelona, Spain).

## Colour parameters analyses

The colour parameters were measured in 1 mm plastic cuvette using a Smart Analysis (DNA Phone s.r.l, Parma, Italy) spectrophotometer. The parameters colour intensity, tonality and CIELab coordinates were measured directly by this instrument.

## Fermentation volatile compounds analysis

The analysis of fermentative volatiles was carried out using an Agilent Technologies 6850 gas chromatograph, equipped with an integrated flame ionisation detector (GC-FID) and DB-624 column (60 m × 250 µm × 1.40 µm). The temperatures of the detector and the injector were 300 and 250ºC, respectively. External standards were used for 5 levels calibration according to the method described by [22]. The following compounds were employed as external standards: acetaldehyde, methanol, 1-propanol, 1-butanol, 2-butanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-phenylethyl alcohol, diacetyl, ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl butyrate, and ethyl lactate. 4-methyl-2-pentanol was used as the internal standard. All compounds were sourced from Sigma–Aldrich Corp., Buchs SG, Switzerland.

The column temperature program started at 40 °C for 5 min, followed by a linear ramp of 10 °C/min to 250 °C, which was held for 5 min. Hydrogen, supplied by a generator (LNI Schmidlin SA, Geneva, Switzerland), served as the carrier gas. The GC system employed a 1:10 split injection, a column flow rate of 2.2 L/min, and a detection limit of 0.1 mg/L. For sample preparation, 100 µL of internal standard (500 mg/L) was added to 1 mL of each test sample. Samples were then filtered through 0.45 µm syringe filters (Teknokroma, Barcelona, Spain) and transferred to 1,5 mL glass vials. One microliter of the filtered sample was injected into the GC for analysis.

### Terpenes compounds

Terpene compounds were analysed by gas chromatography–mass spectrometry (GC–MS). Calibration was performed using five concentration points with the following external standards: geraniol, α-terpineol, linalool, and nerol (Sigma–Aldrich Corp., Buchs SG, Switzerland). The equipment used was an Agilent Technologies 6890 N-MSD-5973 N gas chromatography–mass spectrometer. Dichloromethane as liquid extraction was used before the chromatographic separation. 250 µL volume of dichloromethane with 2.5 mL of white wine and 25 µL of 3,4-dimethylphenol solution as internal standard (10 mg/L) (Merck, Hohenbrunn, Germany) were mixed with 0.37 g of NaCl and vortexed for 5 min. After centrifugation at 7500 rpm for 15 min at 4 °C, the dichloromethane phase was extracted and injected into the chromatograph (1 µL). Chromatographic separation was performed with the DB-WAX column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness) (J&W Scientific, Folsom, CA, USA). The split ratio used was 20:1. The oven temperature programme was 60 °C for 1 min, followed by a 3 °C $min^{-1}$ ramp until 150 °C, followed by a 10 °C $min^{-1}$ ramp to 260 °C, which was maintained for 2 min. The helium flow rate was 1 ml $min^{-1}$.

### Sensory analysis

The sensory analysis was carried out one month after the end of fermentation. During this period, the wine was stored at 4 °C in 0.75 L glass bottles sealed with natural cork stoppers. The sensory analysis was carried out by an expert panel of eight judges constituted of the Chemistry and Food Technology Department of Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas (UPM, Madrid, Spain). The age range of the judges varied between 30 and 58 years old with judges of both genders. The attributes evaluated were clarity, tonality, aromatic intensity, varietal aroma, fermentation aroma, oxidation, reduction, aromatic quality, acidity, sweetness, bitterness, astringency, body, balance and the global perception (indicates the general quality in terms of all the attributes evaluated) of the wine. Attributes were assessed on a scale from 1 (low perception) to 5 (high perception), with the exception of the tonality parameter which was evaluated from 1 (purple tones) to 5 (yellow tones). All samples were served in the same order to all judges in standardized tasting glasses. The tasters were provided with a white surface to appreciate the differences in colour. Each glass was identified with a code that the tasters used to complete cards with the attributes to be scored. At no time did the tasters know which fermentation each code corresponded to.

### Statistical analysis

Means, standard deviations, analysis of variance, the least significant difference test, and the principal component analysis (PCA) were done using PC Statgraphics v.19 software (Graphics Software Systems, Rockville, MD, USA). The significance was set at $P \leq 0.05$.

## Results and discussion

### General oenological parameters

The effect of non-*Saccharomyces* yeasts on basic oenological parameters, such as volatile acidity or residual sugars, has been widely studied [23] as well as the effect on the polyphenol fraction in wines [24] and of course, the use of these yeasts to modulate the acidity and freshness of wines [1] Table 1. shows the basic oenological parameters measured by FTIR with the exception of TPI which was measured by UV-visible spectrophotometry. The control samples (SC) showed the lowest total acidity with mean values of 4.38 g/L expressed as tartaric acid. The rest of the fermentations showed higher acidities, especially the mixed fermentations (M54 + L31) possibly due to the formation of lactic acid by *L. thermotolerans*. The mixed metabolism of these yeasts resulted in total acidity values of around 6.18 g/L. This resulted in a significant drop in the pH of these wines with values of 3.28. Significant pH reductions by *L. thermotolerans* have been widely published [21] identified pH

**Table 1** Total acidity (g/L) as tartaric acid, pH, volatile acidity as acetic acid (g/L), glucose and Fructose (g/L) and TPI (Total polyphenol Index) of Albillo mayor wines produced with different yeasts; *H. vineae* (HV), M29 yeast strain of *M. pulcherrima* (M29), M54 yeast strain of *M. pulcherrima* (M54), *H. opuntiae* (HO), mixed fermentation of *M. pulcherrima* and *L. thermotolerans* (M54 + L31) and *S. cerevisiae* (SC)

| Fermentations | Total Acidity (g/L) | pH | Volatile Acidity (g/L) | Glucose and Fructose (g/L) | TPI |
|---|---|---|---|---|---|
| SC | $4.35 \pm 0.11^{a}$ | $3.48 \pm 0.03^{bc}$ | $0.46 \pm 0.02^{b}$ | $3.65 \pm 0.07^{ab}$ | $11.63 \pm 1.17^{a}$ |
| M29 | $5.29 \pm 0.11^{c}$ | $3.41 \pm 0.04^{b}$ | $0.37 \pm 0.07^{a}$ | $3.10 \pm 2.55^{ab}$ | $12.28 \pm 0.04^{a}$ |
| HV | $5.09 \pm 0.09^{c}$ | $3.44 \pm 0.01^{bc}$ | $0.46 \pm 0.01^{b}$ | $4.80 \pm 0.28^{b}$ | $12.17 \pm 0.00^{a}$ |
| HO | $5.15 \pm 0.02^{c}$ | $3.45 \pm 0.01^{bc}$ | $0.43 \pm 0.04^{ab}$ | $5.20 \pm 0.00^{b}$ | $12.41 \pm 0.01^{a}$ |
| M54 | $4.80 \pm 0.15^{b}$ | $3.44 \pm 0.02^{bc}$ | $0.49 \pm 0.01^{b}$ | $3.85 \pm 0.35^{ab}$ | $11.89 \pm 0.50^{a}$ |
| M54 + L31 | $6.18 \pm 0.13^{c}$ | $3.28 \pm 0.05^{a}$ | $0.43 \pm 0.00^{ab}$ | $2.15 \pm 0.21^{a}$ | $11.73 \pm 0.19^{a}$ |

Mean ± SD for two replicates. Means in the same column followed with the same letter are not significantly different ($p < 0.05$)

reductions of 0.3 units from the formation of 3 g/L of lactic acid. Other studies similarly reported increases in lactic acid when using *L. thermotolerans* in mixed fermentation with *M. pulcherrima* compared to *L. thermotolerans* only [25]. Regarding volatile acidity, all fermentations showed low contents in this parameter (0.37–0.49 g/L). Although significant differences were identified between wines, in all cases these are acceptable values for young wines. Interestingly, wines fermented by *Hanseniaspora* yeasts showed higher reducing sugar contents than the rest of the samples. Possibly due to competition between yeasts of the genus *Hanseniaspora* and the *S. cerevisiae* inoculated on the sixth day, this competition may have made it difficult for the total consumption of sugars at the end of alcoholic fermentation [26]. However, SC was the yeast that finished all fermentations and should have finished the sugars in all samples.

The PCA (Fig. 1) allowed the splitting of mixed fermentations (M54+L31), corresponding to the highest total acidity values. The first two components explain 84.256% of the variability of the data, with the variables 'volatile acidity' and 'pH' contributing the most importance to component 1. It is noted that yeasts of *Hanseniaspora* genus (HV and HO) could be grouped into a single cluster. The volatile acidity parameter contributed positively to component 1, with M54 fermentations and SC controls clustered in the positive zone of this component with the highest volatile acidity values.

## Ethanol, malic acid and lactic acid content

Figure 2 also shows the malic acid content of the fermentations, the control wines exhibited the lowest malic acid content, as all the wines were made from the same Albillo Mayor must. SC almost completely performed malolactic fermentation (MLF), while *M. pulcherrima* M54 partially completed it, leaving less than 0.1 and 1.0 g/L of malic acid, respectively (Fig. 2). Controlling MLF helps maintain the freshness of white wines through pH effects. SC fermentations exhibited higher pH values (Table 1). Furthermore, the use of *L. thermotolerans* can help protect malic acidity due to its inhibitory effect on lactic acid production during MLF [28]. M29, HV, HO and M54+L31 fermentations kept the malic acid in the grapes with values around 1.5 g/L, which is beneficial for both the overall freshness and the retention of the green and crisp acidity associated with malic acid.

Lactic acid is an acid of fermentative origin, stable throughout the winemaking process and with a good sensory perception [29]. The content of lactic acid is shown in Fig. 2. The highest contents were identified in the M54+L31 fermentations, due to the metabolism of *L. thermotolerans*. It should be noted that the lactic acid concentrations in this study were not as high as those identified in other investigations, where lactic acid yields of up to 16 g/L have been published [30]. Despite this, the amounts of lactic acid produced by *L. thermotolerans* influenced pH since these samples resulted in the lowest pH values and the highest total acidity content (Table 1). Also, the protective effect of low pH as a result of lactic acid produced by *L. thermotolerans* should be highlighted, enhancing the contents of free and molecular $SO_2$ which help to avoid oxidations in white wines.

Figure 2 shows the ethanol content of the different fermentations carried out. Many of the non-*Saccharomyces* yeasts have a low fermentative power, in the case of the species used, *H. vineae* and *L. thermotolerans* around 10% vol [1] and *M. pulcherrima* 4% vol [27]. However, all the wines studied had similar ethanol contents, between 11.6

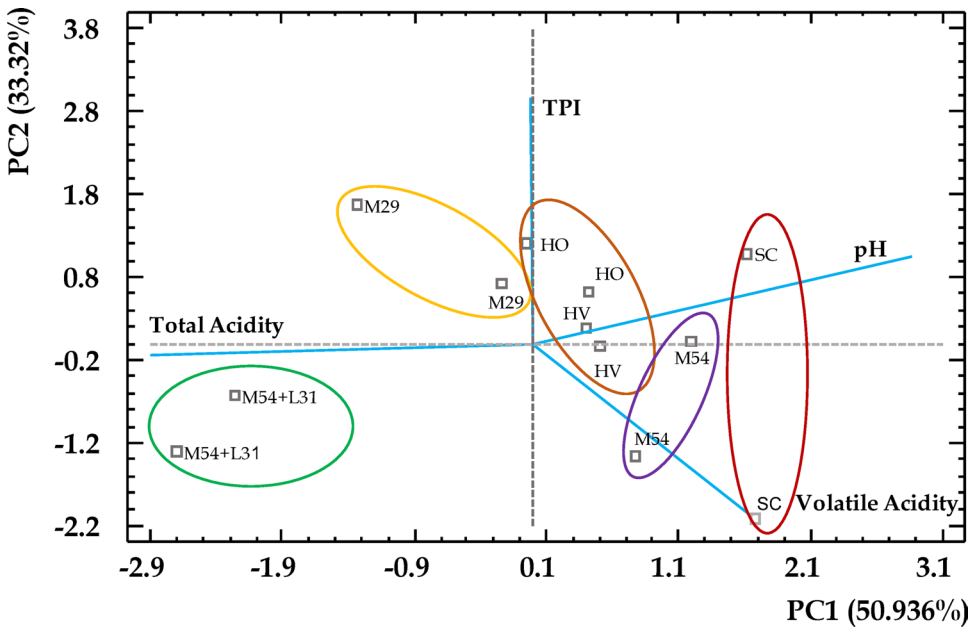


**Fig. 1** Principal component analysis (PCA) for Total acidity, pH, volatile acidity and TPI (Total Polyphenol Index) of Albillo Mayor wines produced with different yeasts; *H. vineae* (HV), M29 yeast strain of *M. pulcherrima* (M29), M54 yeast strain of *M. pulcherrima* (M54), *H. opuntiae* (HO), mixed fermentation of *M. pulcherrima* and *L. thermotolerans* (M54+L31) and *S. cerevisiae* (SC)

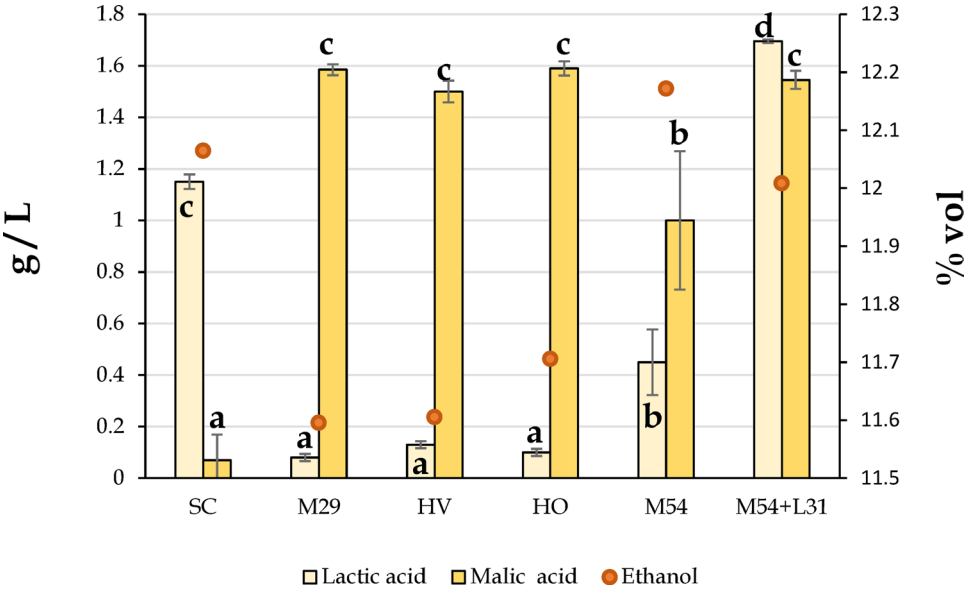


**Fig. 2** Lactic acid and malic acid (g/L) measured by enzymatic analysis and ethanol content (% vol) measured by HPLC-RI of Albillo Mayor wines produced with different yeasts; *H. vineae* (HV), M29 yeast strain of *M. pulcherrima* (M29), M54 yeast strain of *M. pulcherrima* (M54), *H. opuntiae* (HO), mixed fermentation of *M. pulcherrima* and *L. thermotolerans* (M54+L31) and *S. cerevisiae* (SC). Different letters indicate significant difference between means ($p<0.05$)

**Table 2** Colour parameter values and CIELab coordinates of Albillo mayor wines produced with different yeasts; *H. vineae* (HV), M29 yeast strain of *M. pulcherrima* (M29), M54 yeast strain of *M. pulcherrima* (M54), *H. opuntiae* (HO), mixed fermentation of *M. pulcherrima* and *L. thermotolerans* (M54+L31) and *S. cerevisiae* (SC)

| Fermentations | Colour intensity (absorbance units) | Tonality (adimensional) | Chroma | Hue (°) | L | a | b |
|---|---|---|---|---|---|---|---|
| SC | $0.06\pm0.01^{a}$ | $3.81\pm0.39^{a}$ | $3.91\pm0.06^{a}$ | $87.09\pm0.00^{a}$ | $99.20\pm0.14^{a}$ | $0.21\pm0.01^{ab}$ | $3.90\pm0.06^{a}$ |
| M29 | $0.11\pm0.03^{a}$ | $2.96\pm0.76^{a}$ | $4.88\pm0.53^{ab}$ | $86.81\pm2.03^{a}$ | $98.20\pm0.85^{a}$ | $0.28\pm0.18^{b}$ | $4.87\pm0.52^{ab}$ |
| HV | $0.12\pm0.02^{a}$ | $2.62\pm0.27^{a}$ | $4.81\pm0.21^{ab}$ | $88.24\pm0.81^{ab}$ | $97.85\pm0.49^{a}$ | $0.13\pm0.07^{ab}$ | $4.81\pm0.21^{ab}$ |
| HO | $0.08\pm0.00^{a}$ | $3.78\pm0.31^{a}$ | $4.51\pm0.26^{ab}$ | $89.95\pm0.00^{b}$ | $98.90\pm0.14^{a}$ | $-0.01\pm0.01^{a}$ | $4.51\pm0.26^{ab}$ |
| M54 | $0.13\pm0.03^{a}$ | $2.94\pm0.85^{a}$ | $5.57\pm0.54^{b}$ | $88.24\pm0.81^{ab}$ | $97.85\pm1.06^{a}$ | $0.17\pm0.07^{ab}$ | $5.56\pm0.54^{b}$ |
| M54+L31 | $0.12\pm0.06^{a}$ | $2.85\pm0.43^{a}$ | $5.42\pm1.37^{ab}$ | $86.81\pm0.40^{a}$ | $97.90\pm1.13^{a}$ | $0.30\pm0.12^{b}$ | $5.41\pm1.37^{ab}$ |

Values in the same column with the same letter are not significantly different ($p<0.05$)

and 12.1% vol ith no significant differences among them. This is because in all cases the sugars were consumed by the metabolism of *S. cerevisiae* CTPL14 which was inoculated in all samples to finish the alcoholic fermentation.

## Colour parameters

Table 2. shows the mean values of the colourimetric parameters measured spectrophotometrically. No significant differences were observed in the colour intensity of the wines, with values between 0.06 and 0.12 absorbance units. Similarly, no significant differences were observed in the tonality parameter between the wines. The high paleness, along with low levels of polyphenols (11.6–12.4, Table 1), contributes to the wine's resistance to browning.

Table 2 also shows the coordinates L*, a* and b* representing the three-dimensional CIELab space and Fig. 3 graphically represents these coordinates. The values obtained for lightness (L*) were between 97.85 and 99.2, which means that all fermentations were very transparent, the highest values were identified in the SC control wines but without significant differences between them. In this respect, it can be seen in Fig. 3 that a large zoom of the luminosity representation is necessary to be able to separate the different samples graphically. Figure 3 also shows the hue circle, coordinate a* represent green/red colour component (a* >0, red, and a* < 0, green) and b* blue/yellow colour component (b* >0, yellow, and b* < 0 blue) [31]. The mixed M54+L31 and sequential M29 fermentations resulted in statistically higher a* coordinate values compared to the HO fermentations, which were identified as slightly more greenish. The highest b* coordinate values were identified in the M54 fermentations, making these samples slightly more yellowish, but this difference was only significant when compared to the SC fermentations. Chroma is the attribute which allows to determine for each hue its degree of difference in comparison to a grey colour with the same lightness, so it is considered the quantitative attribute of colourfulness [32]. In all cases, low Chroma values were obtained, so they are not brightly coloured wines, only significant differences were identified between SC, with the lowest values, and M54 with the highest values. Hue is a qualitative attribute

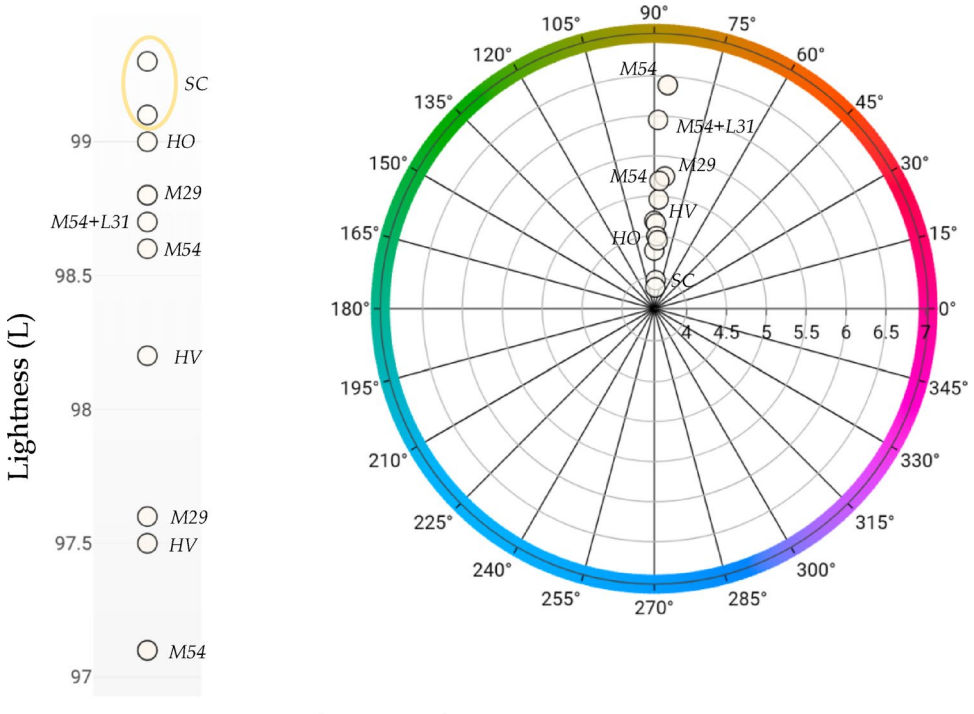


**Fig. 3** Graphic presentation of CIELab coordinates of Albillo Mayor wines produced with different yeasts; *H. vineae* (HV), M29 yeast strain of *M. pulcherrima* (M29), M54 yeast strain of *M. pulcherrima* (M54), *H. opuntiae* (HO), mixed fermentation of *M. pulcherrima* and *L. thermotolerans* (M54+L31) and *S. cerevisiae* (SC)

according to which colours have been traditionally defined as reddish, greenish, etc. It is the attribute which allows to distinguish a colour with reference to a grey colour with the same lightness [32]. All fermentations showed hue between 86.81 and 89.95º with HO samples having a significantly higher value compared to SC, M29 and M54+L31.

## Fermentative volatile compounds

Table 3 shows the concentration of volatiles of fermentation origin measured by GC-FID. Regarding the sum of identified volatiles, no significant differences were found between the fermentations, with total values between 1190 and 1410 mg/L. Acetaldehyde is an important aromatic compound that accounts for 90% of the aldehydes present in wine [33]. The highest values of this compound were identified in HO fermentations and SC controls, but with values around 30 mg/L reported as average values in wines [34]. Other compounds such as diacetyl were not identified in the fermentations except for HV and M54, but with values around 1.5 mg/L below their threshold of perception (8 mg/L according to [33]). Values around 14 mg/L acetoin were identified in *Hanseniaspora* fermentations (HV and HO) together with M29. In relation to the 2,3-Butanediol content, the *Hanseniaspora* fermentations had the lowest values of this compound, between 493 and 648 mg/L.

The total content in higher alcohols was similar in all samples, with only significant differences between the SC control samples and the M29 and M54+L31 fermentations. It is convenient to note that in no case the total value of 400 mg/L, considered unfavourable [35], was exceeded. Among these compounds, the most important are 2-methyl-1-butanol and 3-methyl-1-butanol identified by a pungent odour with a perception threshold of 40 mg/L [36]. No significant differences in the concentrations of 3-methyl-1-butanol were identified. In the case of 2-methyl-1-butanol, the SC samples showed the lowest concentrations with values around 20 mg/L. 2-phenylethanol is a compound that contributes positively to wines as it is associated with to rose and honey aromas [37]. All the wines fermented by non-*Saccharomyces* yeasts presented more than twice the concentration of 2-phenyl-ethanol than the control samples and always above the perception threshold of 10 mg/L [37].

In relation to the total ester content, no significant differences were identified between all the wines studied. Possibly due to the fact that there were no differences in the majority ester (ethyl acetate). These levels correspond to the levels of volatile acidity and demonstrate how the use of selected non-*Saccharomyces* yeasts, including apiculate yeasts, can enhance the formation of fruity esters while maintaining low levels of off-flavors [38], such as the undesirable 'barn-like' ester, ethyl acetate. It is interesting to note the importance of the compound 2-Phenylethyl acetate, an ester associated with a fruity, floral and honey aroma [39]. In addition, it has a strong aromatic power with 0.25 mg/L of perception threshold [40]. The highest concentrations of this compound were identified in the HV samples but without significant differences compared to the HO and SC samples.

Figure 4 represents the PCA performed for the sum of total volatiles, higher alcohols, esters, acetaldehyde,

**Table 3** Concentration of volatile compounds produced by fermentation (mg/L) measured by GC-FID of Albillo mayor wines produced with different yeasts; *H. vineae* (HV), M29 yeast strain of *M. pulcherrima* (M29), M54 yeast strain of *M. pulcherrima* (M54), *H. opuntiae* (HO), mixed fermentation of *M. pulcherrima* and *L. thermotolerans* (M54+L31) and *S. cerevisiae* (SC)

| | SC | M29 | HV | HO | M54 | M54+L31 |
|---|---|---|---|---|---|---|
| Acetaldehyde | 38.87±0.85$^{c}$ | 28.57±4.66$^{ab}$ | 30.01±2.50$^{ab}$ | 37.98±0.71$^{c}$ | 33.94±0.10$^{bc}$ | 26.87±0.18$^{a}$ |
| Methanol | 47.25±0.81$^{ab}$ | 54.79±9.27$^{ab}$ | 71.52±22.37$^{b}$ | 61.24±5.18$^{ab}$ | 45.09±1.43$^{a}$ | 43.68±4.20$^{a}$ |
| 1-Propanol | 35.69±1.79$^{ab}$ | 33.60±0.10$^{ab}$ | 41.31±2.33$^{c}$ | 36.36±0.73$^{b}$ | 32.65±1.60$^{a}$ | 35.87±1.14$^{ab}$ |
| Diacetyl | nd | nd | 1.55±0.01$^{b}$ | nd | nd | 1.58±0.04$^{b}$ |
| Ethyl acetate | 71.52±1.17$^{a}$ | 58.18±6.60$^{a}$ | 85.44±33.42$^{a}$ | 89.88±8.40$^{a}$ | 81.06±0.49$^{a}$ | 75.64±7.31$^{a}$ |
| 2-butanol | nd | nd | nd | nd | nd | nd |
| Isobutanol | 34.04±0.73$^{a}$ | 53.04±9.16$^{c}$ | 42.52±1.87$^{ab}$ | 38.90±1.08$^{a}$ | 42.99±0.82$^{ab}$ | 48.74±2.37$^{bc}$ |
| 1-butanol | nd | nd | nd | nd | nd | 4.31±0.07$^{b}$ |
| Acetoin | 12.49±0.86$^{a}$ | 13.98±0.73$^{bc}$ | 14.19±0.34$^{bc}$ | 14.67±0.15$^{c}$ | 12.92±0.62$^{ab}$ | 12.55±0.33$^{a}$ |
| 3-Methyl-1-butanol | 137.81±22.28$^{a}$ | 145.66±33.96$^{a}$ | 133.92±5.49$^{a}$ | 119.16±5.57$^{a}$ | 115.45±1.47$^{a}$ | 142.62±7.94$^{a}$ |
| 2-Methyl-1-butanol | 19.91±28.16$^{a}$ | 53.45±10.74$^{b}$ | 50.78±4.49$^{ab}$ | 46.55±0.66$^{ab}$ | 45.72±2.26$^{ab}$ | 50.13±4.83$^{ab}$ |
| Isobutyl acetate | nd | nd | nd | 1.35±0.03$^{b}$ | nd | nd |
| Ethyl butyrate | 1.73±0.47$^{a}$ | 3.08±0.57$^{a}$ | 4.03±5.70$^{a}$ | nd | 1.43±0.09$^{a}$ | 1.36±0.02$^{a}$ |
| 2,3-Butanediol | 830.42±78.41$^{b}$ | 846.44±54.78$^{b}$ | 493.31±51.56$^{a}$ | 648.36±43.83$^{ab}$ | 744.47±128.03$^{b}$ | 760.33±111.44$^{b}$ |
| Isoamyl acetate | 2.70±0.05$^{a}$ | 2.93±0.43$^{a}$ | 3.33±4.72$^{a}$ | 16.10±0.42$^{b}$ | 3.29±0.30$^{a}$ | 3.92±0.17$^{a}$ |
| Hexanol | 4.42±0.03$^{c}$ | nd | 4.06±0.05$^{b}$ | 3.89±0.14$^{b}$ | 3.91±0.01$^{b}$ | 4.05±0.18$^{b}$ |
| 2-Phenylethanol | 9.66±0.38a | 27.40±5.66$^{c}$ | 25.02±0.72$^{bc}$ | 21.45±0.22$^{b}$ | 22.06±0.09$^{bc}$ | 25.04±1.08$^{bc}$ |
| 2-Phenylethyl acetate | 7.44±0.16$^{ab}$ | 6.98±1.63$^{a}$ | 8.91±0.20$^{b}$ | 7.78±0.07$^{ab}$ | 7.18±0.39$^{a}$ | 6.53±0.07$^{a}$ |
| Total esters | 83.40±0.59$^{a}$ | 71.17±7.24$^{a}$ | 101.71±43.64$^{a}$ | 115.11±8.92$^{a}$ | 92.95±0.31$^{a}$ | 87.46±7.57$^{a}$ |
| Total higher alcohols | 241.53±2.95a | 313.15±59.62$^{b}$ | 297.62±5.97$^{ab}$ | 266.31±7.08$^{ab}$ | 262.79±1.70$^{ab}$ | 310.76±17.60$^{b}$ |
| Total volatiles | 1,410.45±287.44$^{a}$ | 1,340.49±0.69$^{a}$ | 1,190.00±159.96$^{a}$ | 1,273.32±90.15$^{a}$ | 1,203.30±131.22$^{a}$ | 1,268.75±136.23$^{a}$ |

Mean±standard deviation of two replicates. Different letters in the same row indicate significant differences between means ($p<0.05$)

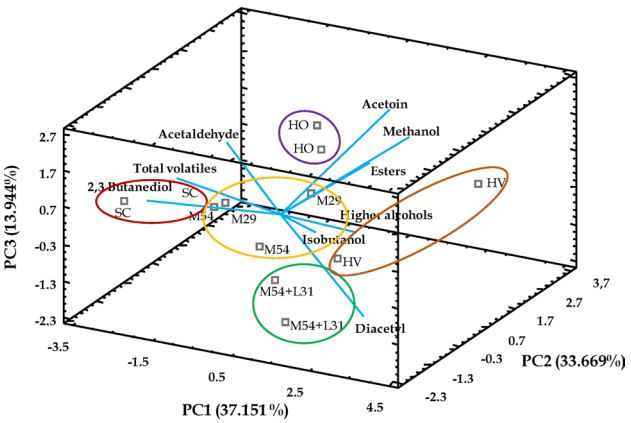


**Fig. 4** Principal component analysis (PCA) for the fermentative volatile compounds of Albillo Mayor wines produced with different yeasts; *H. vineae* (HV), M29 yeast strain of *M. pulcherrima* (M29), M54 yeast strain of *M. pulcherrima* (M54), *H. opuntiae* (HO), mixed fermentation of *M. pulcherrima* and *L. thermotolerans* (M54+L31) and *S. cerevisiae* (SC)

diacetyl, acetoin, 2,3-butanediol, isobutanol and methanol. The distribution includes the first 3 components that explain 84.76% of the variability of the original data. The analysis allowed us to separate the fermentations of *M. pulcherrima* into a single cluster, and the results indicated that both strains (M54+M29) gave rise to wines with a similar aromatic profile. Interestingly, the fermentations of the *Hanseniaspora* strains gave rise to two separate clusters, one for HO and one for HV, possibly due to the fact that they are different species, resulting in different fermentative volatile profiles. A cluster was also identified for mixed fermentations (M54+L31) and another for SC control wines.

## Terpenes compounds

Terpenes are volatile compounds, derived from isoprene, which contribute significantly to the aromatic profile of wines, providing floral and fruity notes. Grapes produce terpenes during ripening and their concentration in wines can vary depending on a variety of factors such as pH, β-glucosidase enzyme activity of the yeasts involved in fermentation, fermentation conditions and the ageing process [41]. Figure 5 shows the concentrations of 4 terpenes measured by GC-MS in the fermentations.

Geraniol and linalool are considered the most important terpenes, as they are found in higher concentrations and have lower flavour thresholds than other monoterpenes in wine [42]. The highest geraniol concentrations were identified in HV wines with average contents of 577 µg/L, followed by 377 µg/L in the mixed M54+L31 fermentations. Higher geraniol values with the same strain of *H. vineae* compared to *S. cerevisiae* were identified in previous research [6]. These contents are high, as in terpenic varieties such as Gewürztraminer, values of around 221 µg/L have been identified [43]. The same trend has been identified for

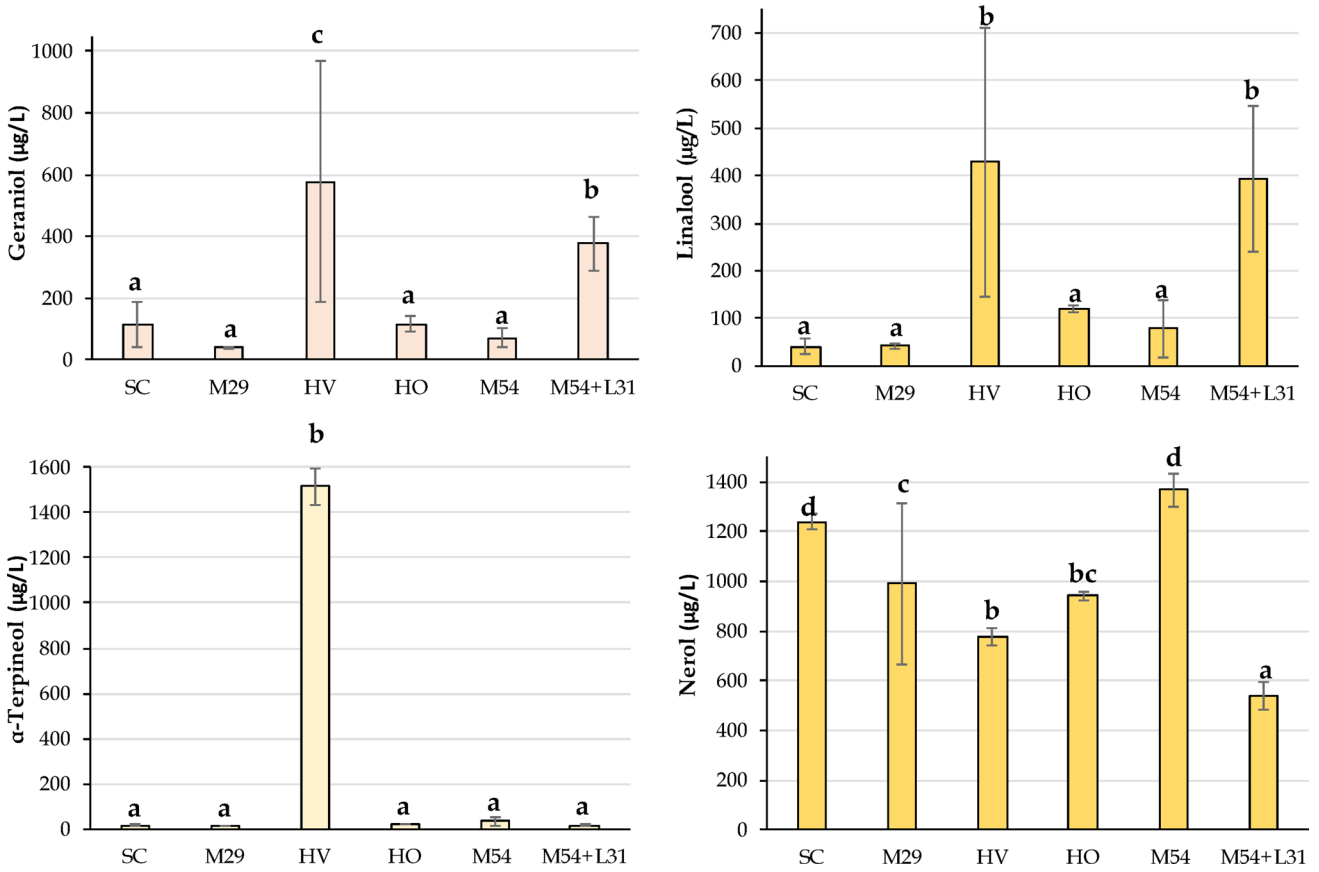


**Fig. 5** Concentrations of terpenes (µg/L) of Albillo Mayor wines produced with different yeasts; *H. vineae* (HV), M29 yeast strain of *M. pulcherrima* (M29), M54 yeast strain of *M. pulcherrima* (M54), *H. opuntiae* (HO), mixed fermentation of *M. pulcherrima* and *L. thermotolerans* (M54+L31) and *S. cerevisiae* (SC). Values are means ± standard deviations ($n=3$). A different letter means significant differences ($p<0.05$)

linalool, showing concentrations above its threshold of perception in HV fermentations with values around 428 µg/L. The perception threshold for this compound is 25.2 µg/L in model wine [44]. Linalool is identified with a citrus, floral and lavender aroma and its concentration in muscatel wines ranges from 14.7 to 102.9 µg/L [45]. In regard to the α-terpineol content, it is interesting to note that in all samples low concentrations of this compound were obtained, between 14 and 33 µg/L, with the exception of the HV samples with concentrations of around 1500 µg/L. The aroma of α-terpineol is described as floral, lilac and pine, its threshold of perception is 250 µg/L [44] and concentrations in muscat wines range from 60.3 to 146.1 µg/L. Finally, it is worth noting the high concentrations identified in all samples for the nerol content, being significantly higher in the SC and M54 fermentations with values of around 1200 µg/L. The aroma of nerol is described as floral, fruity and rose, with a perception threshold of 300 µg/L in water [44].

## Sensory evaluation

Figure 6 shows the sensory evaluation carried out by 8 tasters. The wines fermented by M29 were the cleanest, while the SC control samples were the most turbid. Enhanced clarity in specific non-*Saccharomyces* wines can be attributed to the release of cell wall mannoproteins with sedimentation and stabilization capabilities [46]. The tasters also identified the M29 wines as having the highest tonality, it is possible that the perception of this parameter is affected by the turbidity of the sample, as no significant differences for tonality were identified by spectrophotometric analysis.

In relation to the aromatic parameters evaluated, it should be underlined that the samples with the highest aromatic intensity were the control samples, these wines were also the ones with the highest aromatic quality score, but without significant differences with the fermentations of the *Hanseniaspor*a genus and with M54 fermentations. The wines identified as most varietal were M29 and M54+L31. The reason for this may be that the M54+L31 fermentations had high amounts of linalool (Fig. 5). The fermentative aroma

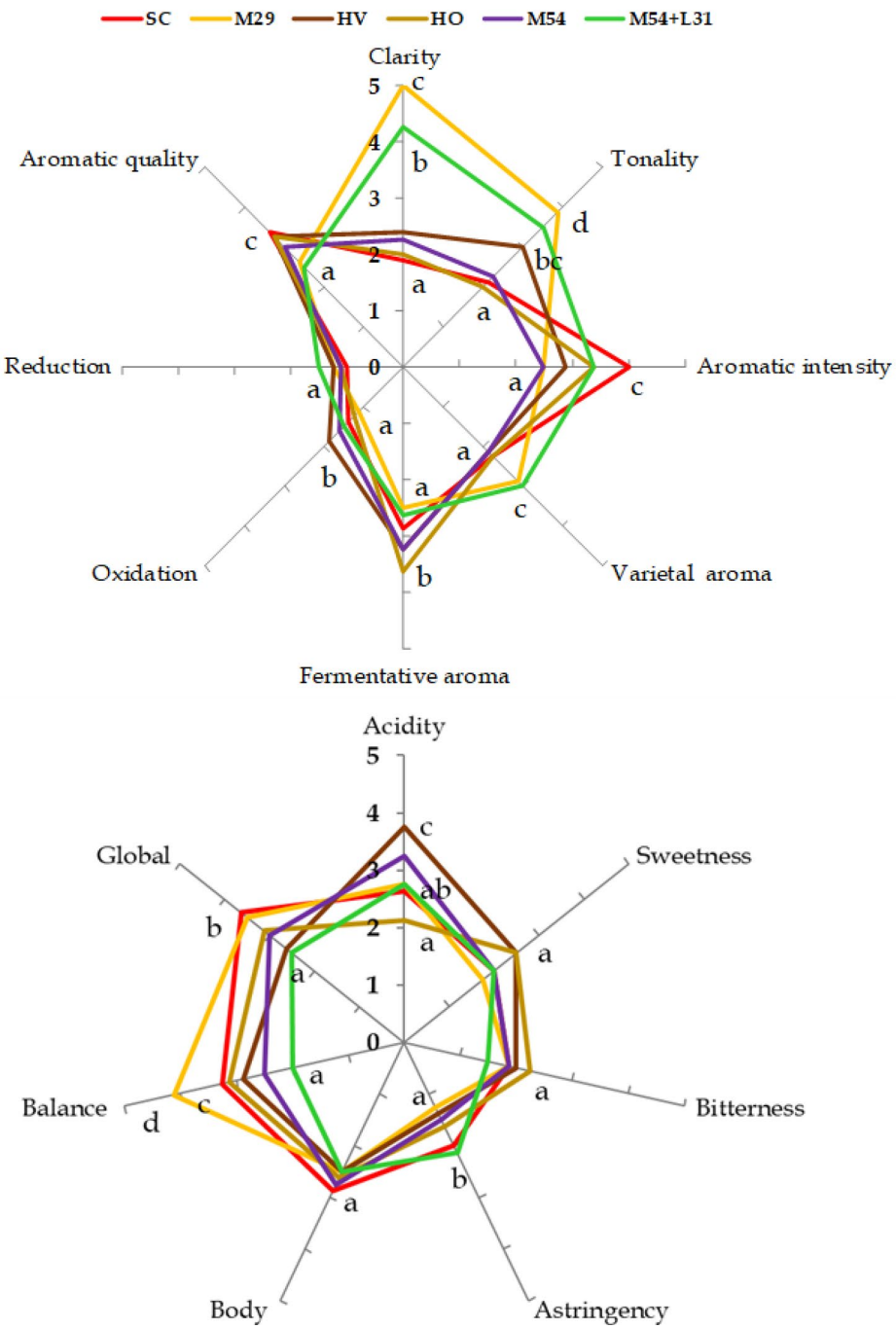


**Fig. 6** Spider plot showing the sensory evaluation of Albillo Mayor wines produced with different yeasts; *H. vineae* (HV), M29 yeast strain of *M. pulcherrima* (M29), M54 yeast strain of *M. pulcherrima* (M54), *H. opuntiae* (HO), mixed fermentation of *M. pulcherrima* and *L. thermotolerans* (M54+L31) and *S. cerevisiae* (SC). Means in the same axis with the same letter are not significantly different ($p<0.05$)

was scored similarly for all wines and this is in agreement with the GC-FID data, as no significant differences in the sum of total volatiles were observed (Table 3).

Acidity was perceived as higher in HV samples, although the most acidic wines were those from M54+L31 (Table 1). No differences in sweetness, body and bitterness were perceived. SC and M54+L31 wines were perceived as the most astringent. Finally, it should be noted that the tasters considered that the most balanced wine was the M29, which together with the control wines was considered to be the best quality.

## Conclusions

The use of non-*Saccharomyces* yeast species resulted in wines with higher total acidity compared to controls. This is particularly the effect of mixed fermentations, due to the formation of lactic acid by *L. thermotolerans* which had a

direct influence on the pH of the wines. All the wines studied had similar ethanol contents. Similarly, the colorimetric parameters were also similar, with only small differences in the a* and b* CIElab coordinates.

The metabolism of the non-*Saccharomyces* yeasts resulted in a higher concentration of higher alcohols, producing higher concentrations of the compound 2-phenylethanol in all these wines. Likewise, yeasts of the *Hanseniaspora* genus produced more 2-phenylethyl acetate. Each yeast species was separated into a cluster in the PCA, including the two *M. pulcherrima* strains. The *H. vineae* metabolism resulted in increased amounts of the terpenes geraniol, linalool and α-terpineol.

The most balanced wines were those produced by *M. pulcherrima* M29 strain. The M54+L31 wines were not perceived by the tasters as the most acidic in contrast to the analytical results. The use of non-*Saccharomyces* yeasts in the production of Albillo Mayor white wines allows the modulation of important organoleptic parameters such as acidity, some positive fermentative volatiles and even the presence of terpenic compounds in the wines.

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**Author contributions** J.M.D.F. performed the analysis of colour parameters and fermentation volatile compounds, and drafted the manuscript; Y.R. performed the analysis of terpenes compounds; M.S. revised and corrected the manuscript; C.E. performed the analysis of the general oenological parameters, ethanol, malic acid and lactic acid content; F.P. revised and corrected the manuscript; R.C. performed the fermentation assays at Bodegas Comenge; A.M. undertook the study's conceptualisation, coordinated the investigation and revised the manuscript.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The research for this study was approved by the ethics committee of the Universidad Politécnica de Madrid.

**Consent to participate** The sensory evaluation of the wines was conducted with the informed consent of the participants and in accordance with the World Medical Association's Declaration of Helsinki.



## References

1. Morata A, Escott C, Bañuelos MA, Loira I, Del Fresno JM, González C, Suárez-Lepe JA (2020) Contribution of Non-*Saccharomyces* yeasts to wine freshness. Rev Biomolecules. https://doi.org/10.3390/biom10010034
2. Carrau F, Dellacassa E, Boido E, Medina K, Valera MJ, Fariña L, Pérez G, Martín V, Álvarez- Valín F, Balestrazzi L (2023) Biology and physiology of *Hanseniaspora vineae*: metabolic diversity and increase flavour complexity for food fermentation. FEMS Yeast Res 23. https://doi.org/10.1093/femsyr/foad010
3. Martin V, Valera MJ, Medina K, Dellacassa E, Schneider R, Boido E, Carrau F (2021) In: Morata A (ed) White wine technology, 1rd edn. Academic, New York
4. Giorello F, Valera MJ, Martin V, Parada A, Salzman V, Camesasca L, Fariña L, Carrau F (2019) Genomic and transcriptomic basis of *Hanseniaspora vineae*'s impact on flavor diversity and wine quality. Appl Environ Microbiol. https://doi.org/10.1128/AEM.01959-18
5. Del Fresno JM, Escott C, Loira I, Carrau F, Cuerda R, Schneoder R, Bañuelos MA, González C, Suárez-Lepe JA (2021) The impact of *Hanseniaspora vineae* fermentation and ageing on lees on the terpenic aromatic profile of white wines of the Albillo variety. Int J Mol Sci. https://doi.org/10.3390/ijms22042195
6. Del Fresno JM, Escott C, Carrau F, Herbert-Pucheta JE, Vaquero C, González C, Morata A (2022) Improving Aroma Complexity with *Hanseniaspora* spp.: Terpenes, Acetate Esters, and Safranal. Fermentation. https://doi.org/10.3390/FERMENTATION8110654
7. Del Fresno JM, Loira I, Escott C, Carrau F, González C, Cuerda R, Morata A (2021) Application of Hanseniaspora vineae yeast in the production of Rosé wines from a blend of Tempranillo and Albillo grapes. Fermentation. https://doi.org/10.3390/fermentation7030141
8. Morata A, Escott C, Loira I, Del Fresno JM, Vaquero C, Bañuelos MA, Palomero F, López C, González C (2021) In: Morata A, Loira I, González C (eds) Grapes and wine, 1rd edn. Intechopen, London
9. Luan Y, Zhang BQ, Duan CQ, Yan GL (2018) Effects of different pre-fermentation cold maceration time on aroma compounds of *Saccharomyces cerevisiae* co-fermentation with *Hanseniaspora opuntiae* or *Pichia kudriavzevii*. LWT. https://doi.org/10.1016/J.LWT.2018.02.004
10. Bourbon-Melo N, Palma M, Rocha MP, Ferreira A, Bronze MR, Elias H, Sá-Correia I (2021) Use of *Hanseniaspora guilliermondii* and *Hanseniaspora opuntiae* to enhance the aromatic profile of beer in mixed-culture fermentation with *Saccharomyces cerevisiae*. Food Microbiol. https://doi.org/10.1016/j.fm.2020.103678
11. Filippousi ME, Chalvantzi I, Mallouchos A, Marmaras I, Banilas G, Nisiotou A (2024) The use of *Hanseniaspora opuntiae* to

improve 'sideritis' wine quality, a Late-Ripening Greek grape variety. Foods. https://doi.org/10.3390/foods13071061
12. Morata A, Loira I, Escott C, Del Fresno JM, Bañuelos MA, Suárez Lepe, JA (2019) Applications of Metschnikowia pulcherrima in wine biotechnology. Ferment. https://doi.org/10.3390/FERMENTATION5030063
13. Perpetuini G, Rossetti AP, Quadrani L, Arfelli G, Piva A, Suzzi G, Tofalo R (2023) Sequential inoculation of Metschnikowia pulcherrima and Saccharomyces cerevisiae as a biotechnological tool to increase the terpenes content of Pecorino white wines. Fermentation. https://doi.org/10.3390/fermentation9090785
14. Romano P, Capece A, Jespersen L (2006) In: Querol A, Fleet G (eds) Yeasts in food and beverages, 1rd edn. Springer Berlin, Heidelberg
15. Testa B, Coppola F, Iorizzo M, Di Renzo M, Coppola R, Succi M (2024) Preliminary characterisation of Metschnikowia pulcherrima to be used as a starter culture in red winemaking. Beverages. https://doi.org/10.3390/beverages10030088
16. Zhang BQ, Shen JY, Duan CQ, Yan GL (2018) Use of Indigenous *Hanseniaspora vineae* and *Metschnikowia pulcherrima* Co-fermentation with *Saccharomyces cerevisiae* to improve the aroma diversity of Vidal blanc Icewine. Front Microbiol. https://doi.org/10.3389/fmicb.2018.02303
17. Prior KJ, Bauer FF, Divol B (2019) The utilisation of nitrogenous compounds by commercial non-*Saccharomyces* yeasts associated with wine. Food Microbiol. https://doi.org/10.1016/J.FM.2018.12.002
18. Kregiel D, Nowacka M, Rygala A, Vadkertiová R (2022) Biological activity of pulcherrimin from the Meschnikowia pulcherrima clade. Molecules. https://doi.org/10.3390/molecules27061855
19. Mora J, Barbas JI, Mulet A (1990) Growth of yeast species during the fermentation of musts inoculated with *Kluyveromyces thermotolerans* and *Saccharomyces cerevisiae*. Am J Enol Vitic. https://doi.org/10.5344/ajev.1990.41.2.156
20. Ciani M, Morales P, Comitini F, Tronchoni J, Canonico L, Curiel JA, Oro L, Rodriguez AJ, González R (2016) Non-conventional yeast species for Lowering ethanol content of wines. Front Microbiol. https://doi.org/10.3389/fmicb.2016.00642
21. Morata A, Loira I, Tesfaye W, Bañuelos MA, González C, Suárez-Lepe JA (2018) *Lachancea thermotolerans* Applications in Wine Technology. Ferment. https://doi.org/10.3390/fermentation4030053
22. Abalos D, Vejarano R, Morata A, González C, Suárez-Lepe JA (2011) The use of furfural as a metabolic inhibitor for reducing the alcohol content of model wines. Eur Food Res Technol. https://doi.org/10.1007/S00217-011-1433-9
23. Jolly NP, Augustyn OPR, Pretorius IS (2003) The effect of Non-*Saccharomyces* yeasts on fermentation and wine quality. S Afr J Enol Vitic. https://doi.org/10.21548/24-2-2638
24. Miranda A, Pereira V, Jardim H, Malfeito-Ferreira M, Marques JC (2023) Impact of Non-*Saccharomyces* yeast fermentation in Madeira wine chemical composition. Processes. https://doi.org/10.3390/PR11020482
25. Escott C, Vaquero C, Loira I, López C, González C, Morata A (2022) Synergetic effect of Metschnikowia pulcherrima and lachancea thermotolerans in acidification and aroma compounds in airén wines. Foods. https://doi.org/10.3390/foods11223734
26. Gallo A, Roman T, Paolini M, Cappello N, Castello D, Schiavon S, Guzzon R, Carrau F, Scheneider R, Larcher R (2024) The co-inoculation ratio of *Hanseniaspora* vineae-to-*Saccharomyces cerevisiae* correlates with aroma metabolic features in wine. Eur Food Res Technol. https://doi.org/10.1007/s00217-024-04588-8
27. Comitini F, Gobbi M, Domizio P, Romani C, Lencioni L, Mannazzu I, Ciani M (2011) Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. Food Microbiol. https://doi.org/10.1016/j.fm.2010.12.001
28. Morata A, Bañuelos MA, López C, Song C, Vejarano R, Loira I, Palomero F, Suárez-Lepe JA (2029) Use of fumaric acid to control pH and inhibit malolactic fermentation in wines. Food Addit Contam Part A-Chem. https://doi.org/10.1080/19440049.2019.1684574
29. Morata A, Loira I, Del Fresno JM, Escott C, Bañuelos MA, Tesfaye W, González C, Palomero F, Suárez-Lepe JA (2019) In: Morata A, Loira I (eds) Grape and wine biotechnology, 1rd edn. IntechOpen, London
30. Banilas G, Sgouros G, Nisiotou A (2016) Development of microsatellite markers for *Lachancea thermotolerans* typing and population structure of wine-associated isolates. Microbiol Res. https://doi.org/10.1016/j.micres.2016.08.010
31. Carvalho MJ, Pereira V, Pereira AC, Pinto JL, Marques JC (2015) Evaluation of wine colour under accelerated and Oak-Cask ageing using CIELab and chemometric approaches. Food Bioprocess Technol. https://doi.org/10.1007/s11947-015-1585-x
32. Recamales ÁF, Sayago A, González-Miret ML, Hernanz D (2006) The effect of time and storage conditions on the phenolic composition and colour of white wine. Food Res Int. https://doi.org/10.1016/j.foodres.2005.07.009
33. Styger G, Prior B, Bauer FF (2011) Wine flavor and aroma. J Ind Microbiol Biotechnol. https://doi.org/10.1007/S10295-011-1018-4
34. Liu SQ, Pilone GJ (2000) An overview of formation and roles of acetaldehyde in winemaking with emphasis on Microbiological implications. Int J Food Sci Technol. https://doi.org/10.1046/j.1365-2621.2000.00341.x
35. Rapp A, Versini G (1995) Influence of nitrogen compounds in grapes on aroma compounds of wines. Dev Food Sci. https://doi.org/10.1016/S0167-4501(06)80257-8
36. Vilanova M, Martínez C (2007) First study of determination of aromatic compounds of red wine from Vitis vinifera cv. Castañal grown in Galicia (NW Spain). Eur Food Res Technol. https://doi.org/10.1007/S00217-006-0322-0
37. Martínez-García R, García-Martínez T, Puig-Pujol A, Mauricio JC, Moreno J (2017) Changes in sparkling wine aroma during the second fermentation under CO2 pressure in sealed bottle. Food Chem. https://doi.org/10.1016/J.FOODCHEM.2017.06.066
38. Whitener MEB, Stanstrup J, Carlin S, Divol B, Du Toit M, Vrhovsek U (2017) Effect of non-*Saccharomyces* yeasts on the volatile chemical profile of Shiraz wine. Aust J Grape Wine Res. https://doi.org/10.1111/AJGW.12269
39. Lleixà J, Martín V, Portillo M del, Carrau C, Beltran F, Mas G A (2016) Comparison of fermentation and wines produced by inoculation of *Hanseniaspora vineae* and *Saccharomyces cerevisiae*. Front Microbiol. https://doi.org/10.3389/FMICB.2016.00338
40. Bartowsky EJ, Pretorius IS (2009) Microbial formation and modification of flavor and off-flavor compounds in wine. Biol Microorg Grapes Must Wine. https://doi.org/10.1007/978-3-540-85463-0_11
41. Furdíková K, Khvalbota L, Machyňáková A, Špánik I (2021) Volatile composition and enantioselective analysis of chiral terpenoids in Tokaj varietal wines. J Chromatogr B Anal Technol Biomed Life Sci. https://doi.org/10.1016/j.jchromb.2021.122565
42. Pedersen DS, Capone DL, Skouroumounis GK, Pollnitz AP, Sefton MA (2003) Quantitative analysis of geraniol, nerol, linalool, and α-terpineol in wine. Anal Bioanal Chem 375:517–522. https://doi.org/10.1007/S00216-002-1716-X
43. Guth H (1997) Quantitation and sensory studies of character impact odorants of different white wine varieties. J Agric Food Chem. https://doi.org/10.1021/JF970280A

44. Black CA, Parker M, Siebert TE, Capone DL, Francis IL (2015) Terpenoids and their role in wine flavour: recent advances. Aust J Grape Wine Res. https://doi.org/10.1111/ajgw.12186
45. Piñeiro Z, Palma M, Barroso CG (2004) Determination of terpenoids in wines by solid phase extraction and gas chromatography. Anal Chim Acta. https://doi.org/10.1016/j.aca.2003.12.044
46. Ribeiro T, Fernandes C, Nunes FM, Filipe-Ribeiro L, Cosme F (2014) Influence of the structural features of commercial mannoproteins in white wine protein stabilization and chemical and sensory properties. Food Chem. https://doi.org/10.1016/J.FOODCHEM.2014.02.149