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Impact of *Hanseniaspora Vineae* in Alcoholic Fermentation and Ageing on Lees of High-Quality White Wine

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Abstract: *Hanseniaspora vineae* is an apiculate yeast that plays a significant role at the beginning of fermentation, and it has been studied for its application in the improvement of the aromatic profile of commercial wines. This work evaluates the use of *H. vineae* in alcoholic fermentation compared to *Saccharomyces cerevisiae* and in ageing on the lees process (AOL) compared to *Saccharomyces* and non-*Saccharomyces* yeasts. The results indicated that there were not significant differences in basic oenological parameters. *H. vineae* completed the fermentation until 11.9% *v/v* of ethanol and with a residual sugars content of less than 2 g/L. Different aroma profiles were obtained in the wines, with esters concentration around 90 mg/L in *H. vineae* wines. Regarding the AOL assay, the hydroalcoholic solutions aged with *H. vineae* lees showed significantly higher absorbance values at 260 (nucleic acids) and 280 nm (proteins) compared to the other strains. However, non-significant differences were found in the polysaccharide content at the end of the ageing process were found compared to the other yeast species, with the exception of *Schizosaccharomyces pombe* that released around 23.5 mg/L of polysaccharides in hydroalcoholic solution. The use of *H. vineae* by the wineries may be a viable method in fermentation and AOL to improve the quality of white wines.

Keywords: *Hanseniaspora vineae*; alcoholic fermentation; non-*Saccharomyces*; ageing on lees; polysaccharides; white wines

1. Introduction

The inoculation of commercial *S. cerevisiae* yeast strains is the most common practice in the industrial elaboration of commercial wines. However, nowadays, winemakers are trying to obtain quality wines with different organoleptic characteristics. In this regard, the use of different species of yeast could be interesting. Many studies have been done with respect to obtaining differentiated quality products and the use of non-*Saccharomyces* yeasts for this purpose [1–3]. The use of *H. vineae* in wineries could be a good alternative to the traditional *Saccharomyces* fermentations. This yeast and

others apiculate yeast of the genus *Hanseniaspora/Kloeckera* are the main species present on mature grapes and play a significant role at the beginning of fermentation, producing enzymes and aroma compounds that expand the diversity of wine colour and flavor [4]. Normally, *H. vineae* appears in the first stages of the fermentation but it is quickly dominated by *S. cerevisiae* [5]. The main interest in this yeast is due to the aromatic profile of the wines obtained [6]. This yeast produces a fruity and floral aroma due to the increased amounts of acetate esters, primarily 2-phenylethyl acetate [7] and benzyl acetate. Other authors [8,9] investigated the potential of to the genus *Hanseniaspora* to produce acetate esters. In the same way, the modulation of the aeration during the growing stage of these yeasts can increase the aromatic diversity and quality of the wine obtained [10]. In addition, the *H. vineae* species can be used in pure culture because this yeast might reach about 10% of the alcohol by volume of fermentative capacity under winemaking conditions [4]. In this respect, we conducted a semi-industrial assay in this study using *H. vineae* in pure culture compared to *S. cerevisiae* in the control.

Additionally, in this study, the use of *H. vineae* in aging on lees (AOL) has been assayed in comparison with other yeast species. The AOL technique consists of a long contact of the yeast lees with the wine. During this contact, the yeast autolysis is produced with the breakdown of cell membranes, the release of intracellular constituents, the liberation of hydrolytic enzymes and the hydrolysis of intracellular biopolymers into low molecular weight products [11]. Among these compounds, the polysaccharides have an effect on the physico-chemical properties of the wine, as well as on the sensory properties [12]. The AOL improves the aromatic and gustatory complexity of wine, mainly by improving its body and reducing its astringency [13]. The main problem of this technique is that the AOL is a slow process, many studies have been done trying to accelerate the cell lysis like the use of emerging physical technologies such as high hydrostatic pressures and ultrasounds [14]. Another technique to reduce the ageing time is the use of yeast species that have a high capacity to release polysaccharides into the wine. In previous studies, [15] certain wine spoilage yeasts like *Saccharomycodes ludwigii, Zygosaccharomyces bailii,* and *Brettanomyces bruxellensis* were shown to produce a greater quantity of polysaccharides compared to *S. cerevisiae* strains. In the same way, these authors classified the released polysaccharides according to their composition. Therefore, the AOL may depend on the yeast used and its cell wall polysaccharide composition.

The main objective of this work is to obtain information about the use of *H. vineae* in alcoholic fermentation as well as in the AOL technique.

2. Materials and Methods

2.1. Yeast Species Used in Alcoholic Fermentation

The *H. vineae* yeast strain used in this study was isolated by Professor Francisco Carrau (Facultad de Química, Universidad de la República, Montevideo, Uruguay) and it is currently under evaluation by "Oenobrands SAS, France".

The yeast strain Fermivin 3C (*S. cerevisiae*) used as control in this study is a selected yeast marketed by "Oenobrands SAS, France".

2.2. Alcoholic Fermentation Conditions

The Albillo grape variety (*Vitis vinifera L.*) was fermented at "Comenge Bodegas y Viñedos SA" (Curiel de Duero, Spain). The white must was fermented in triplicate in 120 L stainless steel barrels. The fermentation process was monitored following the daily variation of density and temperature. The samples were taken once at the end of the fermentation.

2.3. Yeast Species Used in Ageing on Lees

Two strains of *S. cerevisiae* were used as controls in the AOL assay, the strains 7VA and G37 (SC7VA, SCG37), both yeasts were isolated by the Chemistry and Food Technology Department of ETSI Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid.

Three species of non-*Saccharomyces* yeasts were used, the same *H. vineae* strain that had been previously used in the alcoholic fermentation trial, as well as *Lachancea thermotolerans* L31 strain (L31) isolated and selected by enotecUPM (Food Technology Department, ETSIAAB, Universidad Politécnica de Madrid) and *Schizosaccharomyces pombe* 938 (SP938, IFI, CSIC, Madrid, Spain).

The yeast lees biomass used for the AOL assay was obtained by growing in 2 L of YEPD medium enriched with 100 g/L of glucose. The growth was carried out at 25 °C for three days. Then, the biomass was washed three times with deionized water, discarding the supernatant after each centrifugation, at 1200 rcf, for 3 min.

2.4. Ageing on Lees Conditions

The AOL was done in hydroalcoholic solution (13.5% *v*/*v*) sulphited to 60 mg/L with $K_2S_2O_5$ and the pH was adjusted to 3.5 with phosphoric acid. The samples were prepared in triplicates, using ISO flasks of 0.5 L. The dosage of yeast lees was 6 g/L and the ageing process was done at 16 °C in a dark room for 156 days. The samples were mixed once a week to simulate a *bâtonnage* process.

2.5. Basic Oenological Parameters Analysis

The values of ethanol (% *v*/*v*), pH, total acidity (g/L) expressed as tartaric acid, volatile acidity (g/L) expressed as acetic acid, malic acid (g/L), lactic acid (g/L) and glucose/fructose content (g/L) were obtained by Fourier transform infrared spectroscopy (FTIR), using an OenoFoss™ instrument (FOSS Iberia, Barcelona, Spain).

2.6. NMR Spectroscopy

NMR spectra of a triplicate set of Albillo white wines fermented with *H. vineae* and *S. cerevisiae* yeast strains, were carried out on a Bruker 600 Avance III HD spectrometer, equipped with a 5-mm 1H/D TXI probehead equipped with a z-gradient at 298 ± 0.1 K of temperature. The following set of NMR experiments were conducted:

(a) Standard 1H-one-dimensional NMR experiment was carried out as step for calibration of the water-to-ethanol multi-presaturation module: with 4 transients of 32,768 complex points, having recycling delays of 5 s and with acquisition times of 1700 milliseconds, produced an experimental time of 26 s. No apodization function was applied during Fourier Transform.

(b) {1Hwater_presat NMR}: 1D single pulse NOESY experiment with a homemade shaped-pulse water-to-ethanol presaturation during both the relaxation delay (5 s) and mixing times (100 milliseconds), with a 8.18×10^{-4} W power irradiation level for the solvent signals' elimination, centering the transmitter frequency at 4.7 ppm and shifting the decoupler frequency between 3.55 ppm (CH2-ethanol) and 1.08 ppm (CH3-ethanol) for accurate multi-presaturation of all signals [16,17] were acquired for each sample as follows: a total of 128 transients were collected into 32,768 complex data points, with a spectral width of 9615.4 Hz and acquisition times of 1700 ms, produce experimental times of 10'58''.

(c) NMR post-processing was carried out as follows: ppm calibration and manual phase corrections were conducted with the use of Bruker TopSpin 4.0.8 software. Global and soft baseline corrections, least-squares NMR alignments, variable size bucketing and data matrix normalization were carried out with NMRProcFlow [18]. Scaling and statistical analysis workflow for obtaining the Principal Component Analysis to determine relationships between *H. vineae* and *S. cerevisiae* wine samples, from the constant sum normalized NMR data matrix, were developed with the BioStatFlow 2.9.2 software. Identified metabolites were quantified (Table 1) through qNMR methods [19,20] routinely used in oenology [21,22].

Table 1. Targeted metabolites concentration (mg/L) of *Saccharomyces cerevisiae* and *Hanseniaspora vineae* wine samples obtained with the PULCON-NMR method [21].

mg/L	Fermentation with *Saccharomyces cerevisiae*	Fermentation with *Hanseniaspora vineae*
Furfural	1.47 ± 1.14 [a]	3.29 ± 2.82 [a]
Formiate	2.44 ± 0.66 [a]	3.05 ± 0.84 [a]
Shikimic	1.54 ± 0.21 [a]	1.85 ± 0.19 [a]
Fumaric	0.58 ± 0.44 [a]	0.53 ± 0.17 [a]
Sorbic	1.63 ± 1.44 [a]	2.73 ± 3.33 [a]
β-Glucose	500.02 ± 58.39 [b]	365.60 ± 37.23 [a]
Fructose	695.11 ± 146.39 [a]	803.69 ± 238.53 [a]
Citrate	244.05 ± 25.82 [a]	255.38 ± 7.52 [a]
Succinate	291.47 ± 28.40 [a]	233.36 ± 25.83 [a]
Glutamine	54.03 ± 10.14 [a]	59.20 ± 5.41 [a]
Acetate	289.70 ± 18.64 [a]	274.73 ± 22.25 [a]
Proline	34.17 ± 7.66 [a]	42.29 ± 6.35 [a]
γ-Aminobutyric acid	67.68 ± 5.11 [a]	73.61 ± 7.32 [a]
Arginine	28.43 ± 11.10 [a]	44.00 ± 26.46 [a]
Alanine	119.82 ± 42.98 [a]	150.20 ± 78.16 [a]
Lactic	156.56 ± 31.04 [a]	174.25 ± 44.30 [a]
Threonine	188.38 ± 70.77 [a]	230.49 ± 78.09 [a]
Valine	52.72 ± 18.84 [a]	37.63 ± 17.85 [a]
Isoleucine	29.33 ± 9.08 [a]	37.18 ± 7.66 [a]

[a] Means with the same letter are not significantly different ($p < 0.05$).

2.7. Volatile Compounds from the Alcoholic Fermentation Analysis

The volatile compounds of the wines obtained in fermentation assay were measured using an Agilent Technologies 6850 gas chromatograph, equipped with an integrated flame ionization detector (GC-FID) and DB-624 column (60 m × 250 µm × 1.40 µm). Analyses were performed according to the method described by [23]. The injector temperature was 250 °C, and the detector temperature was 300 °C. The column temperature was 40 °C for the first 5 min, rising linearly by a 10 °C/min until reaching 250 °C; this temperature was maintained for 5 min. Hydrogen was used as the carrier gas. The flow rate was 22.1 L/min. The injection split ratio was 1:10. The detection limit was 0.1 mg/L.

Calibration was performed using the following external standards: acetaldehyde, metanol, 1-propanol, diacetyl, ethyl acetate, 2-butanol, isobutanol, 1-butanol, acetoin, 2-methyl-1-butanol, 3-methyl-1-butanol, isobutyl acetate, ethyl butyrate, ethyl lactate, 2.3-butanediol, 3-ethoxy-1-propanol, isoamyl acetate, hexanol, 2-phenyl ethanol and 2-phenylethyl acetate.

2.8. Proteins and Nucleic Acids Estimation by Absorbance at 260 and 280 nm

The absorbance measurements were done through the ageing after centrifugation (1200 rcf for 3 min) using a 1-cm path-length quartz cuvette. All spectrometric measurements were obtained using an 8453 spectrophotometer from Agilent Technologies™ (Palo Alto, CA, USA).

2.9. Polysaccharides Analysis (HPLC-RI)

The polysaccharides content was measured after 156 days of ageing in the AOL assay, using an HPLC-RI technique. An 1100 HPLC chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a refractive index detector with Ultrahydrogel 250 molecular exclusion column (Waters) was used, according to the method described by [24]. The eluent was 0.1 M $NaNO_3$ in deionized water (MilliQ). A calibration curve constructed from the following pullulan standards (polymaltotriose) (Shodex, Showa Denko K.K, Japan) were used to determine the concentration of polysaccharides in the samples: P-800 (788 kDa), P-400 (404 kDa), P-200 (212 kDa), P-100 (112 kDa), P-50 (47.3 kDa), P-20 (22.8 kDa), P-10 (11.8 kDa) and P-5 (5.9 kDa).

2.10. Statistical Analysis

Statgraphics v.5 software (Graphics Software Systems, Rockville, MD, USA) was used to calculate means, standard deviations, analysis of variance (ANOVA), least-significant difference (LSD) test and principal component analysis (PCA). The LSD test was used to detect significant differences between means. Significance was set at $p < 0.05$.

3. Results and Discussion

3.1. Basic Oenological Parameters

In general, no significant differences were found in the wines fermented by *H. vineae* compared to conventional wines fermented by *S. cerevisiae*, with the exception of the total acidity parameter. The *S. cerevisiae* wines showed 0.5 g/L more total acidity than the *H. vineae* wines (Table 2). However, no differences in lactic acid, malic acid and volatile acidity content were found, therefore, the decrease of total acidity may be due to the precipitation of tartaric acid during the alcoholic fermentation. It is important to mention that these differences were not reflected in the pH values, since the pH was similar in all the wines studied.

Table 2. Ethanol content (% *v/v*), pH, total acidity (g/L) as tartaric acid, volatile acidity as acetic acid (g/L), malic acid (g/L), lactic acid (g/L) and glucose and fructose (g/L) after fermentation process. Mean ± SD for three replicates.

	Fermentation with *Saccharomyces cerevisiae*	**Fermentation with *Hanseniaspora vineae***
Ethanol (% *v/v*)	11.93 ± 0.15 [a]	11.90 ± 0.00 [a]
pH	3.17 ± 0.03 [a]	3.21 ± 0.02 [a]
Total Acidity (g/L)	6.30 ± 0.10 [b]	5.80 ± 0.17 [a]
Volatile Acidity (g/L)	0.45 ± 0.07 [a]	0.36 ± 0.02 [a]
Malic Acid (g/L)	2.00 ± 0.10 [a]	1.87 ± 0.06 [a]
Lactic Acid (g/L)	0.10 ± 0.10 [a]	0.00 ± 0.00 [a]
Gluc and Fruc (g/L)	1.67 ± 0.60 [a]	1.07 ± 0.38 [a]

[a] Means with the same letter are not significantly different ($p < 0.05$).

Regarding the residual sugar content, both yeasts have been able to ferment all the sugar, with final concentrations in the wine below 2 g of residual sugar per litre. These results are in line with those obtained by other authors that compared both yeast species in Macabeo and Merlot grape wines [25]; nevertheless, [26] found 0.5 g/L of glucose and frutose more in *H. vineae* wines than in *S. cerevisiae* wines before the malolactic fermentation. This fact is linked to the glycolytic power—all wines showed similar ethanol contents around 11.9% *v/v*. These results indicate that both yeast species may produce wines with similar basic oenological parameters.

Targeted NMR analysis allowed the identification and quantification (Table 1) of typical wine metabolites in both *H. vineae* and *S. cerevisiae* samples: furfural (9.64 ppm), formiate (8.41 ppm), shikimic acid (6.87 ppm), fumaric acid (6.4 ppm), β-glucose (4.55 ppm), fructose (4.04 ppm), citrate (2.84 ppm), succinate (2.66 ppm), glutamine (2.25 ppm), acetate (2.01 ppm), proline (2.05 ppm), γ-aminobutyric acid (1.96 ppm), arginine (1.70 ppm), alanine (1.55 ppm), threonine (1.28 ppm), valine (1.1 ppm) and isoleucine (0.91 ppm). With the results obtained by NMR, a principal component analysis (PCA) was performed. Using the 2D-projections (PC1 = 43.1%, PC2 = 24.2%), slight overlaps were observed amongst groups (Figure 1A). The distribution was better explained with the first three components (PC1 = 43.1%, PC2 = 24.23% and PC3 = 13.59%). Even though the results were not statistically significant between the two yeasts studied (Table 1), the PCA made it possible to differentiate the wines studied into two independent clusters corresponding with the two target yeasts (Figure 1). Chemical shift loading plots (Figure 1B) show a set of relevant resonances that permits the discrimination between yeasts by PCA: formiate (8.4123 ppm, PC1 [+], PC2 [+]); shikimic (6.8740 ppm, PC1 [−], PC2 [−]); β-glucose (4.5395 ppm, PC1 [−], PC2 [−]); fructose (4.0375 ppm, PC1 [+], PC2 [−]); citrate (2.8415 ppm, PC1 [−], PC2 [−]); succinate (2.6655 ppm, PC1 [+], PC2 [−]); all amino acids present positive PCA 2

(glutamine 2.2465 ppm, PC1 [+], PC2 [+]; alanine 1.551 ppm, PC1 [+], PC2 [+], valine 1.0595 ppm, PC1 [+], PC2 [+] and isoleucine 0.9140 ppm, PC1 [−], PC2 [+]) and acetate (2.0925 ppm, PC1 [−], PC2 [−]). These results allow us to differentiate the metabolism of both yeasts, even though these differences were not quantitatively observed. It is noted that we identified the same separation between the must fermented by *H. vineae* and *S. cerevisiae* when the PCA was done on fermentative volatile compounds (Figure 2).

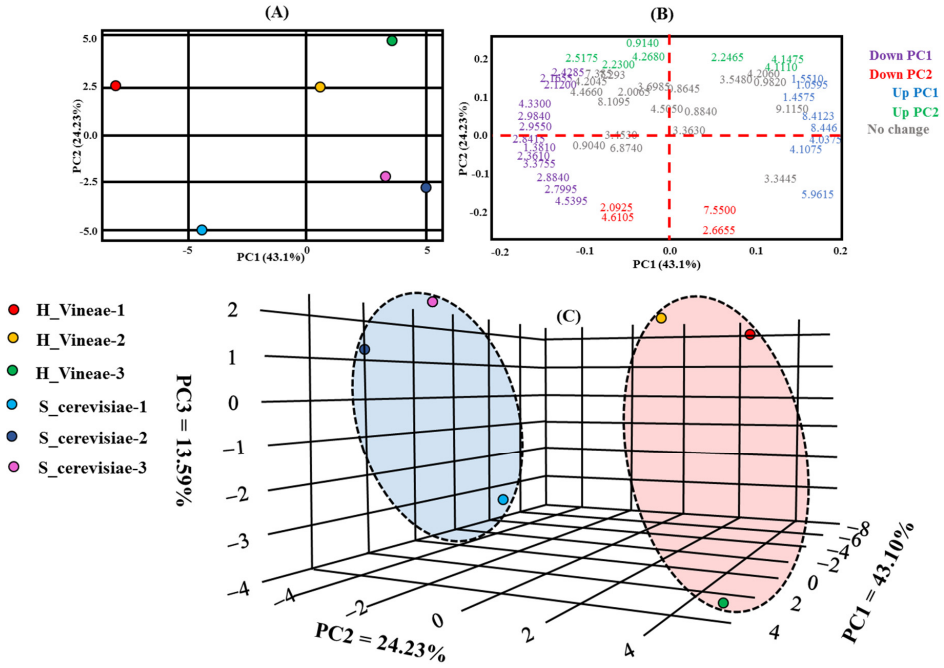

Figure 1. Principal component analysis (PCA) score plots comprising the 67.33% variance (**A**) and 80.92% variance (**C**) and chemical shift loading plots (**B**) obtained by a variable NMR bucketing procedure) of the data-reduced NMR fingerprints of Albillo white wines fermented at two different conditions. Red and blue ovals (89% confidence intervals) represent respectively *H. vineae* and *S. cerevisiae* fermentation groups, each analyzed in triplicate.

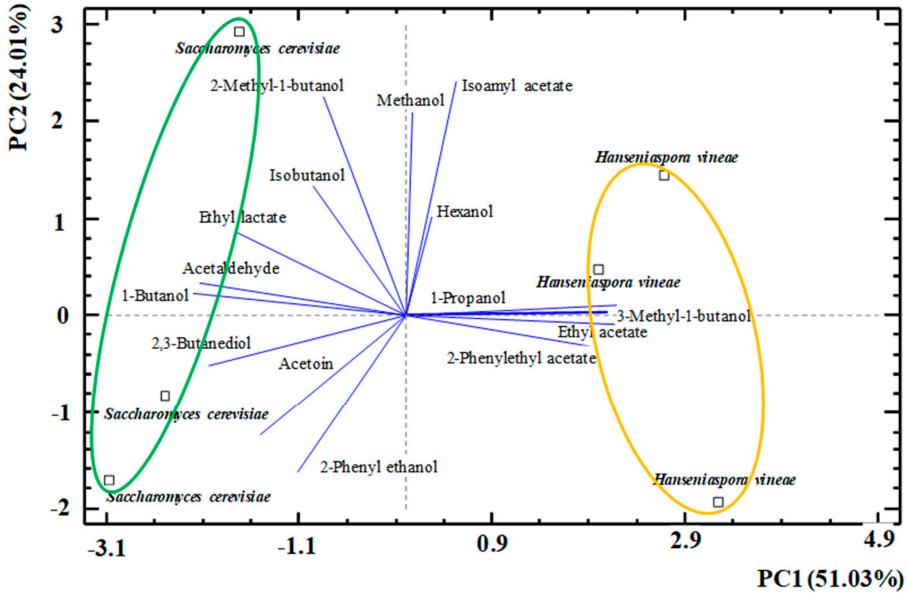

Figure 2. Principal component analysis (PCA) of the fermentative volatile compounds.

3.2. Volatile Compounds from the Alcoholic Fermentation

Considering the total volatile compounds identified, *S. cerevisiae* produced a larger amount of volatile compounds (Table 3) with around 1200 mg/L. In this regard the concentration of acetaldehyde and 2,3-butanediol have a special importance. The amount of these compounds was significantly higher in the wines from *S. cerevisiae*. Similar results were obtained after the fermentation of artificial red must [27].

Both yeast species did not show significant differences in the sum of higher alcohols. It interesting to point out that other authors reported a decrease in higher alcohols after the fermentation of the Chardonnay grapes must have with *H. vineae* compared with that of *S. cerevisiae* [5].

The fermentation with *H. vineae* resulted in increases in acetate esters and some ethyl esters, like ethyl acetate with concentrations around 79 mg/L. These results are similar to the results obtained by [5].

2-Phenylethyl acetate is an ester with strong aromatic power and its perception threshold reported is 250 μg/L [28]. This compound is associated with fruity, floral and honey aromas [25]. The 2-phenylethyl acetate concentration was significantly higher in *H. vineae* wines than in *S. cerevisiae* wines (Table 3). This fact has been reported by several authors [25,29] who identified up to 50 times more abundance of this compound in wines fermented by *H. vineae*. However, no significant differences in 2-phenylethanol content were found. This can be due to the fact that there are significant differences between these two yeast species in the acetylation step due to an increase in the copy number of the acetyl transferases genes in *H. vineae* [29].

In addition, the "odour activity values" (OAV) were calculated (see Table 3). It allows us to estimate the contribution of a specific compound to the aroma of the wine [30]. Among the compounds that have been identified, only ethyl acetate, 2-methyl-1-butanol, 2.3-butanediol, isoamyl acetate, hexanol and 2-phenylethyl acetate have obtained an OAV greater than one. It must again be emphasized the importance of the 2-phenylethyl acetate. This compound had 31.84 OAV and statistically higher concentrations in *H. vineae* than in *S. cerevisiae* wines. In this regard, the concentration identified as 2-phenylethyl acetate had an important organoleptic repercussion in the wines obtained by the fermentation of *H. vineae*, providing fruity, floral and honey aromas to these wines.

A principal component analysis (PCA) was done for the 15 volatile compounds identified after the fermentation process (Figure 2) and it allowed to differentiate the aromatic profile between the yeasts studied. The distribution was explained with the first two components. The compounds 2-phenylethyl acetate, ethyl acetate, 3-methyl-1-butanol, 1-propanol, hexanol, isoamyl acetate and methanol are associated positively with the PC1. A cluster including the wines fermented by *H. vineae* was found in the positive values of the PC1 with the highest concentration of these volatiles. It is noteworthy the contribution of the 2-phenylethyl acetate produced by the metabolism of this yeast species; on the contrary, on the negative values of the principal component PC1, a cluster composed of the wines fermented by *S. cerevisiae* was identified, including the contribution of 2-phenyl ethanol and indicating the difference between the two yeast species in the acetylation of this compound.

Table 3. Concentration of volatile compounds produced by fermentation (mg/L), measured by GC-FID. Mean ± standard deviation of three replicates. Different letters indicate values with statistical significant differences ($p < 0.05$).

	Fermentation with *Saccharomyces cerevisiae*	**Fermentation with *Hanseniaspora vineae***	**OAV *Saccharomyces cerevisiae***	**OAV *Hanseniaspora vineae***	**Odor Character**	**Perception Threshold (mg/L)**
Acetaldehyde	26.34 ± 2.19 [b]	19.86 ± 2.29 [a]	0.263	0.198	pungent, fruity, suffocating, fresh, green [4]	100–125 [31]
Methanol	43.03 ± 2.76 [a]	42.42 ± 1.56 [a]	0.059	0.063	pungent odor [6]	668 [32]
1-Propanol	20.49 ± 1.72 [a]	28.68 ± 0.52 [b]	0.041	0.057	alcohol, ripe fruit [7]	500 [1] [33]
Diacetyl	nd	nd	-	-	pleasant, buttery [4]	0.2 [33]
Ethyl acetate	45.99 ± 2.72 [a]	79.26 ± 3.31 [b]	3.832	6.605	fruity, sweet, fingernail polish, etherous [4]	12 [2] [34]
2-Butanol	nd	nd	-	-	medicinal, wine-like [7]	150 [2] [33]
Isobutanol	23.80 ± 0.57 [a]	22.64 ± 3.49 [a]	0.595	0.566	Coca [5]	40 [2] [33]
1-Butanol	3.97 ± 0.10 [b]	0.00 ± 0.00 [a]	0.026	0	Medicinal [7]	150 [2] [33]
Acetoin	5.66 ± 0.21 [a]	5.50 ± 0.09 [a]	0.037	0.036	from buttery to plastic [6]	150 [1] [33]
2-Methyl-1-butanol	22.96 ± 0.90 [a]	25.29 ± 0.15 [b]	0.574	0.632	pungent odor [6]	40 [1] [35]
3-Methyl-1-butanol	112.83 ± 17.57 [a]	101.43 ± 17.53 [a]	2.821	5.536	pungent odor [6]	40 [1] [35]
isobutyl acetate	nd	nd	-	-	sweet, ester, medicinal [4]	1.6 [3] [33]
Ethyl butyrate	nd	nd	-	-	strawberry, apple, banana [7]	0.02 [1] [35]
Ethyl lactate	7.97 ± 0.13 [a]	7.15 ± 0.81 [a]	0.569	0.511	Floral [5]	14 [1] [35]
2.3-Butanediol	892.38 ± 97.06 [b]	643.99 ± 28.48 [a]	5.94	4.293	creamy, buttery [8]	150 [1] [36]
3-ethoxy-1-propanol	nd	nd	-	-	-	0.1 [1] [37]
Isoamyl acetate	3.63 ± 0.44 [a]	3.71 ± 0.22 [a]	121	123.66	banana, fresh [4]	0.03 [2] [33]
Hexanol	4.64 ± 0.05 [a]	4.75 ± 0.32 [a]	1.16	1.187	green [5]	4 [1] [33]
2-Phenyl ethanol	9.67 ± 0.33 [a]	9.52 ± 0.30 [a]	0.976	0.976	rose talc, honey [8]	10 [32]
2-Phenylethyl acetate	6.33 ± 0.45 [a]	7.96 ± 0.34 [b]	25.32	31.84	Flowery [7]	0.25 [1] [33]
Higher Alcohols	189.76 ± 18.63 [a]	187.55 ± 21.03 [a]				
Esters	55.96 ± 2.73 [a]	90.93 ± 3.13 [b]				
Total Volatiles	1.229.71 ± 77.42 [b]	1.002.14 ± 33.29 [a]				

Different letters indicate values with statistical significant differences ($p < 0.05$). [1] In wines; [2] In hydroalcoholic solution 10% *v/v*; [3] In beer; [4] from [38]; [5] [39]; [6] [34]; [7] [34]; [8] [32].

3.3. Intracellular Components and Polysaccharides Content Measured in the Ageing on Lees

The relative measurement of the intracellular components release has been done by the UV absorbance at 260 and 280 nm [40,41]. These measurements correspond to the relative amount of nucleic acids and proteins, respectively [42].

Regarding the monitoring at 260 nm, the samples with HV yeast lees showed the highest values during the entire ageing period. However, the SCG37 samples showed the lowest absorbance values without significant differences with SP938 through the AOL stage. It is also interesting to note the difference between the two *Saccharomyces* strains studied, the SC7VA samples showed absorbance values around 0.4–1 AU, while the lees of the yeast SCG37 resulted in lower values, around 0.1–0.2 AU. These results may indicate that the same yeast species can show different capacities for releasing cellular compounds depending on the strain used.

Similar results were obtained in the monitoring of 280 nm absorbance, but in this case no significant differences were obtained between the HV and SC7VA samples during the 91 days of ageing. These values indicated that both yeasts could be used to accelerate the release of cellular compounds. Therefore, the use of HV and SCVA yeast strains could be indicated to perform an AOL process.

The polysaccharides released after the action of glucanases are a good indicator of the autolysis process, being the parietal mannoproteins the majority of these polysaccharides [12]. After 156 days of ageing, the samples on SP938 lees have shown the highest content of polysaccharides with values around 23.5 mg/L. This quick releasing of compounds from the *Schizosaccharomyces* cell wall has already been observed by other authors [12]. It is interesting to stress the fact that the SP938 samples did not show the greatest absorbance values at 260 and 280 nm (Figure 3). This is possibly due to the fact that the high molecular weight polysaccharides do not have absorbance at these wavelengths as nucleic acids and proteins.

The HV samples showed a polysaccharides content of around 11 mg/L; this concentration was not statistically significant with respect to samples aged on the lees of the two *Saccharomyces* yeast strains (Figure 4). In the same way, it was not significantly different from the results obtained in L31 samples. The results obtained in the hydroalcoholic solution of these three yeast species were similar to the result of other assays with *Saccharomyces* previously done [13]. In other words, the yeast *H. vineae* could be an alternative to replace *S. cerevisiae* yeast in an AOL process after the alcoholic fermentation.

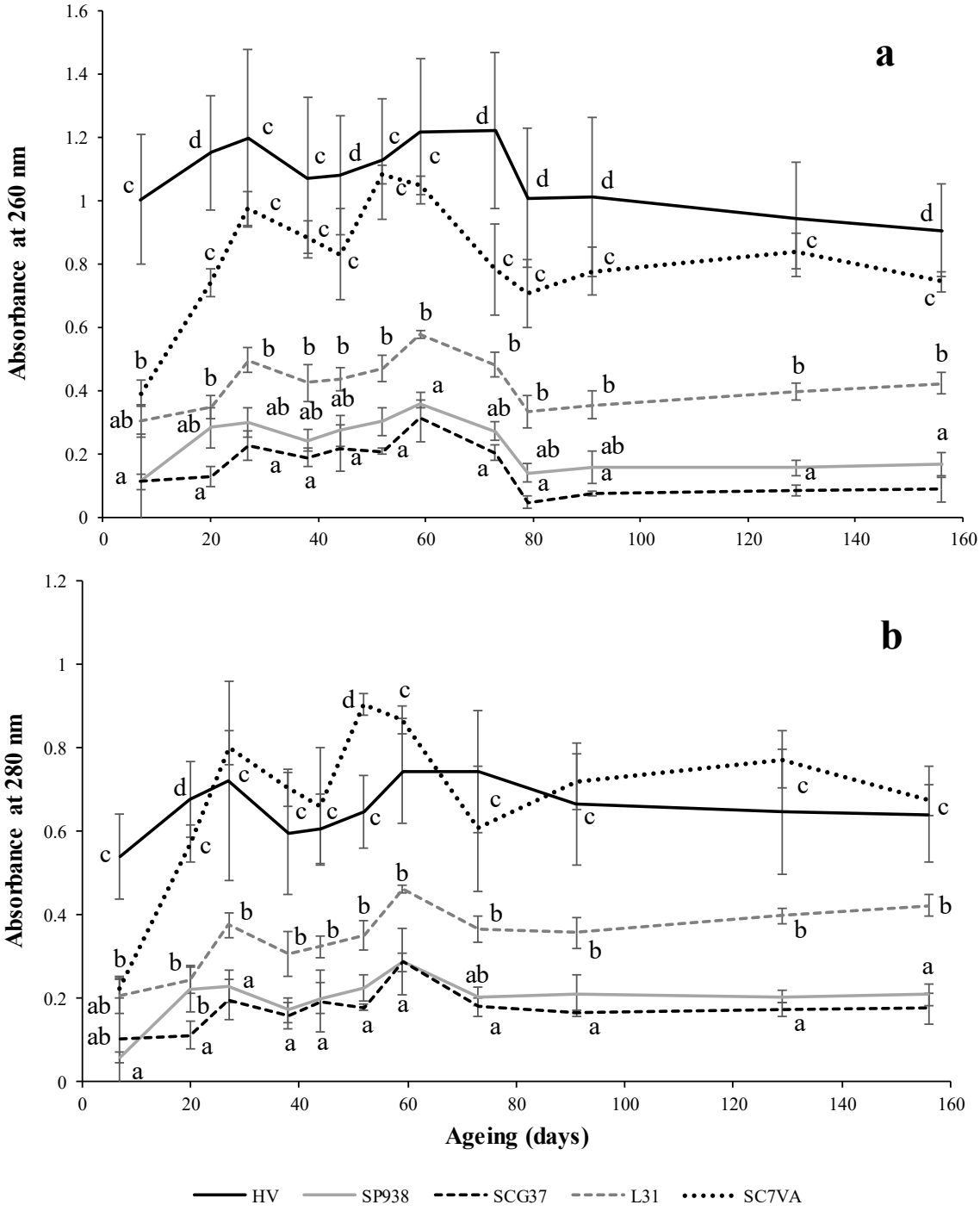

Figure 3. Evolution of the absorbance at 260 nm (**a**) and at 280 nm (**b**) in hydroalcoholic solutions, throughout 156 days of ageing on lees. HV (*Hanseniaspora vineae*); SP938 (*Schizosaccharomyces pombe* strain 938); SCG37 (*Saccharomyces cerevisiae* strain G37); L31 (*Lachancea thermotolerans* strain L31); SC7VA (*Saccharomyces cerevisiae* strain 7VA). Mean ± standard deviation of three replicates. Different letters in the same day indicate values with statistically significant differences ($p < 0.05$).

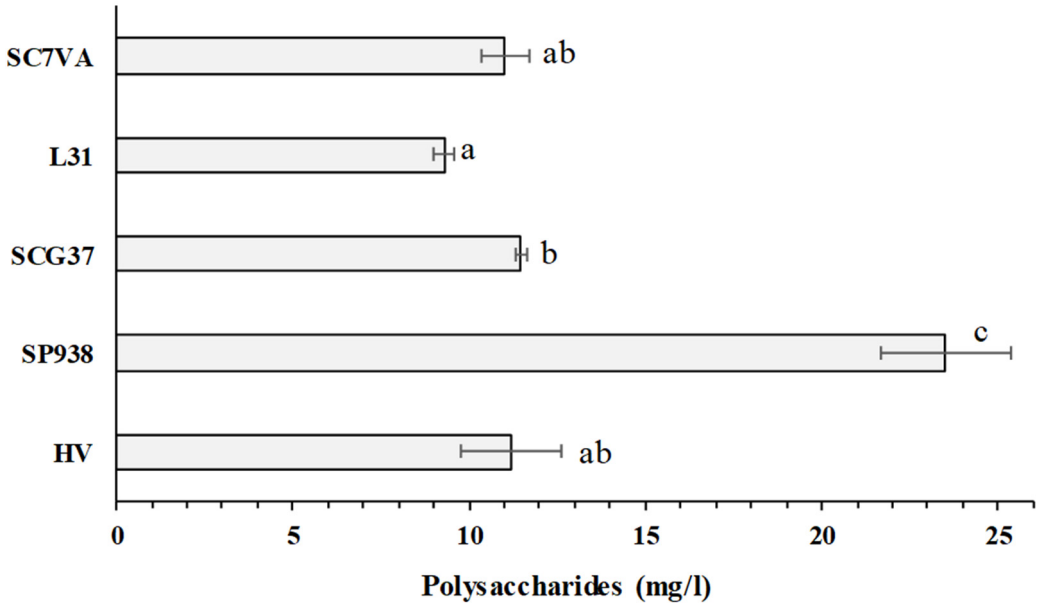

Figure 4. Polysaccharides content (mg/L) after 156 days of ageing on lees in hydroalcoholic solution. HV (*Hanseniaspora vineae*); SP938 (*Schizosaccharomyces pombe* 938 strain); SCG37 (*Saccharomyces cerevisiae* G37 strain); L31 (*Lachancea thermotolerans* L31 strain); SC7VA (*Saccharomyces cerevisiae* 7VA strain). Mean ± standard deviation of three replicates. Different letters indicate values with statistically significant differences ($p < 0.05$).

4. Conclusions

The use of *H. vineae* yeast in alcoholic fermentation resulted in wines with similar basic oenological parameters like the wines obtained by the *S. cerevisiae* fermentation. However, different aromatic profiles were identified by the PCA. Two clusters were shown with more production of acetate esters and ethyl esters by *H. vineae.* This yeast stands out for its higher production of 2-phenylethyl acetate, thus enhancing the fruity character of the wines.

The monitoring of the absorbance at 260 and 280 nm allowed to obtain a relative amount of nucleic acids and proteins released during the AOL process. In this context, the *H. vineae* yeast lees resulted in higher values of absorbance at these wavelengths throughout the ageing process. Nevertheless, the measurement of polysaccharides concentration by HPLC-RI after 156 days of ageing showed that there were no significant differences between the use of *H. vineae* yeast lees and the rest of yeast species studied, with the exception of the *S. pombe* samples.

H. vineae is an interesting yeast species to be used in alcoholic fermentation that can provide wines with more esters. In the same way, this yeast could be used in AOL processes because it is apparently quick to transfer certain cellular compounds. Nevertheless, further studies are necessary to obtain information on the cell wall polysaccharides released by this yeast and their sensory repercussion on aged wines.

Author Contributions: J.M.D.F. performed the analysis of the ageing on lees trial and drafted the manuscript; C.E. performed the analysis of the fermentation trial; I.L. revised and corrected the manuscript; J.E.H.-P. performed the analysis by NMR spectroscopy; R.S. performed the experimental design; F.C. revised and corrected the manuscript; R.C. performed the fermentations assays in the winery; and A.M. undertook the study's conceptualization, coordinated the investigation and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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