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Application of *Hanseniaspora vineae* Yeast in the Production of Rosé Wines from a Blend of Tempranillo and Albillo Grapes

Juan Manuel Del Fresno [1], Iris Loira [1], Carlos Escott [1], Francisco Carrau [2], Carmen González [1], Rafael Cuerda [3] and Antonio Morata [1,*]

[1] enotecUPM, Chemistry and Food Technology Department, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Avenida Complutense S/N, 28040 Madrid, Spain; juanmanuel.delfresno@upm.es (J.M.D.F.); iris.loira@upm.es (I.L.); carlos.escott@gmail.com (C.E.); carmen.gchamorro@upm.es (C.G.)
[2] Área Enología y Biotecnología de Fermentaciones, Facultad de Química, Universidad de la Republica, Gral. Flores 2124, Montevideo 11800, Uruguay; fcarrau@fq.edu.uy
[3] Comenge Bodegas y Viñedos SA, Curiel de Duero, 47316 Valladolid, Spain; cuerda@comenge.com
* Correspondence: antonio.morata@upm.es

Abstract: *Hanseniaspora vineae* is an apiculate yeast that can be used for the production of interesting commercial wines, due to its contribution of fermentative volatiles. This paper presents a detailed comparative study of the use of *H. vineae*, compared to pure fermentations of *S. cerevisiae* in Tempranillo and Albillo rosé wines. Fermentations were carried out in oak barrels and stainless steel barrels. The results indicated that fermentation with *H. vineae* resulted in wines with residual sugars below 3.4 g/L and similar general characteristics, compared to *S. cerevisiae*. However, *H. vineae* wines contain up to 44% more total anthocyanins, resulting in an appreciable improvement in colour. In addition, *H. vineae* produced up to 65% more 2-phenylethyl acetate in stainless steel barrels and 2.5 times more terpene alcohols in oak barrels. Therefore, the use of *H. vineae* results in a more attractive colour, as well as fruity and floral organoleptic characteristics of rosé wines.

Keywords: *Hanseniaspora vineae*; alcoholic fermentation; non-*Saccharomyces*; rosé wines; polysaccharides; colour parameters; anthocyanins; polysaccharides; volatile compounds

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1. Introduction

Rosé wines are made from red grape varieties, in order to extract the amount of pigments that give them their characteristic colour. However, these wines are very similar to white wines, both in terms of the technology used, which consists of separating the must by a rapid bleeding or pressing directly on the grapes [1]; and in terms of their sensory characteristics, as white and rosé wines are usually consumed young with floral and fruity sensory characteristics. Rosé wines are difficult to make, as short macerations lead not only to a low extraction of anthocyanins but also to a poor extraction of volatile compounds from the skins; therefore, the generation of volatile compounds during fermentation plays an important role in the production of quality rosé wines [2]. Rosé wines are mainly a type of fresh wine, and in this respect the use of non-*Saccharomyces* yeasts is an important tool to obtain fresh wines of differentiated quality [3]. Yeasts live on the surface of the grape skins, and the population varies constantly due to climatic conditions and the ripening stage of the berry. At harvest time there is a complex yeast population where *S. cerevisiae* is not the most abundant [4]. For this reason, non-*Saccharomyces* yeasts are dominant at the beginning of alcoholic fermentation, and play an important role in the organoleptic complexity of the wine obtained. The main non-*Saccharomyces* yeasts associated with grapes are the genus *Hanseniaspora* and its asexual anamorph *Kloeckera*, these yeasts are apiculate with bipolar budding [5]. These apiculate yeasts are normally characterised by their low fermentation capacity compared to *S. cerevisiae*, as these species generally do

not tolerate ethanol concentrations higher than 5–7% vol [6], however *H. vineae* strains are capable of producing in up to a 9% ethanol volume [7].

The choice of *H. vineae* as a yeast in rosé winemaking is not limited to its adequate fermentative capacity. Different authors have investigated its application in white winemaking conditions and achieved interesting results. Medina et al., 2013 [7] used a selected strain of *H. vineae* for the production of high quality Chardonnay white wines, fermented in oak barrels. These wines showed a fruity and intense flavour character, with an increased full body and a relatively long palate length, compared to pure *S. cerevisiae* treatments. In addition, other authors have obtained significant organoleptic improvements when using *H. vineae*, based mainly on the production of certain volatile compounds during alcoholic fermentation. In particular, several authors have reported the increase in fruity aromas, due to the high production of 2-phenylethyl acetate and ethyl acetate [8]. 2-Phenylethyl acetate is an acetic ester that gives wines fruity and floral aromas with honey notes [9]. This compound has an important aromatic repercussion, with a 0.25 mg/L of odour threshold [10]. The esters in wines are formed as a consequence of the amino acid catabolism by the Ehrlich transamination pathway, and the higher alcohol formed in this process can be esterified with acetic acid. In addition, *H. vineae* can produce 2-phenylethyl acetate from sugars, following the chorismate-prephenate-(S)-mandelate/phenylalanine pathway [3]. In fact, [11] identified up to 50 times more concentration of 2-phenylethyl acetate in *H. vineae* wines than in *S. cerevisiae* wines in Macabeo white must fermentation. We have also obtained similar results in white winemaking of the Albillo grape must, with significant higher concentrations of 2-phenylethyl acetate compared to *S. cerevisiae* wines [12]. Another important characteristic of *H. vineae* is the synthesis and glucosidase and the capacity that it has for increasing free terpenes and sesquiterpenes compounds after fermentation [5]. In this respect, in previous studies we identified a significant effect of this yeast on terpene contents, where we obtained up to three times higher contents of linalool, β-citronellol, geraniol and α-terpineol compared to *S. cerevisiae* fermentations [13].

The aim of this work is to study the application of *H. vineae* yeast for the production of rosé wines from Tempranillo and Albillo grape varieties. Tempranillo is the dominant variety in Ribera del Duero (Spain), and accounts for 95% of the surface area [14]. This variety produces red wines with a strong violet colour, medium acidity and well-structured tannin. Aromas of blackberry and black forest fruits are typical of wines made from Tempranillo grapes [15]. The main white grape variety in this D.O. is Albillo Mayor. This variety is well adapted to this area and ripens early, producing wines with medium acidity and aromas of pome and stone fruits, apple and peach [15]. The production of rosé wines from the blend of these two grape varieties is a practice of growing interest in this D.O. It is therefore interesting to evaluate the application of yeasts that improve the aromatic profile of this type of wine. Similarly, winery scale studies provide more realistic information on the potential industrial application of the yeast under study. Information about the characteristics of the wines fermented in oak barrels is also interesting because it is a common method in the production of high-quality wines. For this purpose, pure fermentations have been carried out using *H. vineae* or *S. cerevisiae* fermentations at a winery scale. All fermentations have been carried out in both stainless steel and oak barrels. Final wines have been analysed in order to obtain different data of importance on the quality of rosé wines. Analyses carried out were as follows: ethanol, pH, glucose-fructose, volatile acidity, colour parameters, anthocyanins profile, polysaccharide content, volatile compounds and sensory analysis.

2. Materials and Methods

2.1. Yeast Strains Used in Fermentation of Rosé Wines

The yeast strain T02/5A *H. vineae* was used for the fermentation of Tempranillo and Albillo must. This yeast strain is currently under evaluation by "Oenobrands SAS, France", and it was isolated by Francisco Carrau (Facultad de Química, Universidad de la República,

Montevideo, Uruguay). *H. vineae* was inoculated at a population of 7 log CFU/mL after being grown in YPD medium.

The yeast strain Fermivin 3C (*S. cerevisiae*), used as a conventional control in this study, is a selected commercial yeast (Oenobrands SAS, Montpellier, France). *S. cerevisiae* was inoculated as active dried yeast after hydration, according to the manufacturer's instructions at a dosage of 20 g/hL.

2.2. Must and Fermentation Conditions

The rosé must was obtained by direct pressing of a mixture of 50% Tempranillo and 50% Albillo grapes from the Ribera de Duero region in Spain. Only at this stage of the production process was the liquid in contact with the skins, so there was no maceration prior to the fermentation process. This must showed the following characteristics before inoculation with the two yeast species studied: 1093 g/L of density; pH = 3.34; 4.05 g/L of total acidity expressed as tartaric acid and 15.05 mg/L of total SO_2 content.

The fermentations with *H. vineae* were carried out in stainless steel barrels with 150 L capacity in triplicate (HV), and in oak barrels with 225 L capacity in duplicate (HVW). The *H. vineae* yeast strain that was used can ferment until 9% vol of ethanol content. The end of the fermentation process was likely carried out by a *S. cerevisiae* yeast strain, possibly from the winery population or from the grape skin population.

The *S. cerevisiae* fermentations were carried out in the same way (SC and SCW). All fermentations were carried out in "Bodegas Comenge" (Curiel de Duero, Valladolid, Spain), in a room at a controlled temperature (18 °C). The fermentation process was controlled by density measurement. Population monitoring of both yeast species was carried out by periodic sampling and microscopic observation.

2.3. General Oenological Parameters Analyses

Ethanol (% *v*/*v*), volatile acidity (g/L) expressed as acetic acid and glucose/fructose content (g/L) were analysed using an OenoFoss instrument (FOSS Iberia, Barcelona, Spain). This instrument provides a great number of oenological parameters by Fourier transform infrared spectroscopy (FTIR).

2.4. Colour Parameters Analyses

A Smart Analysis (DNA Phone s.r.l, Parma, Italy) spectrophotometer was used to measure the colour parameters with a 1 mm plastic cuvette. This instrument allows the direct measurement of absorbance at 420, 520 and 620 nm, the parameters colour intensity, tonality and CIELab coordinates.

2.5. Anthocyanins Analysis

Pigment content was determined by HPLC-DAD according to [16]. The equipment used was an Agilent Technologies (Palo Alto, CA, USA) series 1100 HPLC chromatograph, equipped with a diode array detector and a quadrupole mass spectrometer with an electrospray interface and a column RP Kinetex C18 100 Â (100 × 4.6 mm; 2.6 µm) (Phenomenex, Torrance, CA, USA). The solvents were: Solvent A (water/formic acid 95:5 *v*/*v*) and Solvent B (methanol/formic acid 95:5 *v*/*v*).

2.6. Polysaccharide Analysis

The polysaccharide analysis was carried out according to the method described by [17]. The technique used for the analysis was HPLC-RI by means of an 1100 HPLC chromatograph (Agilent Technologies, Palo Alto, CA, USA), equipped with a refractive index detector with Ultrahydrogel 250 molecular exclusion column (Waters).

The following standards of pullulan (polymaltotriose) (Shodex, Showa Denko K.K, Tokyo, Japan) were used for the calibration curve: 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). The eluent used for this method was 0.1 M $NaNO_3$ in deionised water (MilliQ, Merck KGaA, Darmstadt, Germany).

2.7. Fermentation Volatile Compounds Analysis

The analysis of volatile compounds of fermentative origin were analysed according to the method described by [18]. The measurement 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 detector temperature was 300 °C, and the injector temperature was 250 °C. The carrier gas was hydrogen. External standards were used for the calibration of the method.

2.8. GC-MS Analysis

Terpene alcohols and volatile compounds from wood were measured only in oak barrel fermented wines by gas chromatography−mass spectrometer (GC−MS). The equipment used was an Agilent Technologies 6890N-MSD-5973N gas chromatography−mass spectrometer. The column used to perform the chromatographic separation was a DB-WAX column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness) (J&W Scientific, Folsom, CA, USA).

Prior to injection into the equipment, an extraction with dichloromethane was carried out. A 2.5 mL volume of rosé wine was mixed with 250 µL of dichloromethane and 25 µL of 3.4-dimethylphenol solution (10 mg/L) (Merck, Hohenbrunn, Germany) as the internal standard; 0.37 g of NaCl was added and stirred in a vortex for 5 min. After centrifugation at 7500 rpm for 15 min at 4 °C, 1 µL of the dichloromethane phase was injected into the chromatograph. The following external standards were used for the calibration of the method: furfural, linalool, 5-methyl furfural, furfuryl alcohol, α-terpineol, guaiacol, *cis*-oak lactone, β-ionone, *trans*-oak lactone, eugenol and 4-ethylphenol.

2.9. Sensory Analysis

All sensory analysis parameters were rated on a scale from 1 (low perception) to 5 (high perception), except for the tonality parameter which was evaluated from 1 (purple tones) to 5 (yellow tones). The parameters assessed were colour intensity, tonality, aromatic intensity, aromatic quality, floral, fruity, wood, yeasty aroma, sweetness, bitterness, acidity and freshness. The global impression was also evaluated. The assessments were made by an expert panel of seven judges of both genders, with ages from 27 to 50 years old. The panel was constituted of members of the Chemistry and Food Technology Department of Escuela Técnica Superior de Ingenería Agronómica, Alimentaria y de Biosistemas (UPM, Madrid, Spain).

2.10. Statistical Analysis

Means, standard deviations, analysis of variance and the least significant difference test ($p < 0.05$) were calculated using PC Statgraphics v.5 software (Graphics Software Systems, Rockville, MD, USA).

3. Results and Discussion

3.1. General Oenological Parameters Obtained after the Alcoholic Fermentation

The general oenological parameters are shown in Table 1. The ethanol content was between 12.7% and 13.27% vol in all wines studied, without significant differences for the yeast strain used. In the same way, the material of the stainless steel did not have an effect on the ethanol content. Some differences were found regarding the pH's values, as the wines fermented in oak barrels showed lower pH values.

Table 1. General oenological parameters in rosé wines measured by FTIR.

Rosé Wines	Ethanol (% *v*/*v*)	pH	Glucose-Fructose (g/L)	Volatile Acidity (g/L Acetic Acid)
SC	12.90 ± 0.53 ^{a,b}	3.51 ± 0.03 ^{c}	3.45 ± 0.45 ^{a}	0.44 ± 0.11 ^{a,b}
HV	12.70 ± 0.10 ^{a}	3.43 ± 0.04 ^{b}	2.95 ± 1.75 ^{a}	0.42 ± 0.01 ^{a,b}
SCW	13.27 ± 0.12 ^{b}	3.39 ± 0.03 ^{a,b}	2.10 ± 0.10 ^{a}	0.52 ± 0.02 ^{b}
HVW	13.20 ± 0.10 ^{a,b}	3.36 ± 0.02 ^{a}	3.35 ± 0.15 ^{a}	0.40 ± 0.01 ^{a}

SC (fermentations with *S. cerevisiae* in stainless steel barrels); HV (fermentations with *H. vineae* in stainless steel barrels); SCW (fermentations with *S. cerevisiae* in oak barrels); HVW (fermentations with *H. vineae* in oak barrels). Values in the same column with the same letter are not significantly different ($p < 0.05$).

All of the wines completed the fermentation process with a residual sugar content below 4 g/L, similar to typical dry wines [19] without significant differences among them (Appendix A). In all of the wines the volatile acidity was well below 0.7 g/L, considered as the sensory limit, above which it could be perceived as a wine odour defect. Only significant differences were identified between the wines produced in oak barrels, as an increase of more than 0.1 g/L was obtained in the SCW treatments compared to the HVW wines. These low volatile acidity values in *H. vineae* wines are interesting because other authors have reported an increase in this parameter after the use of other *Hanseniaspora*/*Kloeckera* yeast species [20–22].

3.2. Colour Parameters

Figure 1a shows the different spectrophotometric parameters measured in the different rosé wines treatments. Colour intensity is the sum of the absorbance at 420, 520 and 620 nm. For this chromatic parameter, no significant differences were observed between the two yeast strains, but an increase in colour intensity was identified in oak barrel wines. Regarding the tonality parameter, significant differences were identified that can be seen visually (Figure 1b). *S. cerevisiae* wines showed a higher tonality compared to *H. vineae* wines, both in stainless steel and oak barrel fermentations, although all of the samples showed a low tonality between 0.76–0.82. These are considered standard values in young rosé wines. These differences identified in the tonality parameter could be due to the formation of more orange pigments, such as vitisins, when fermenting with *S. cerevisiae.* Similarly, the production of bluer pigments during the fermentation by *H. vineae* could also modify the tonality parameter, and this might be the case for the formation of acylated anthocyanins.

In an attempt to obtain more exact colorimetric results, the CIELab coordinates were used. The CIELab is a uniform three dimensional space, defined by colorimetric coordinates L*, a* and b* [23]. In Figure 1c, a graphic representation of these coordinates can be observed. L* is a measure of lightness, from completely opaque (0) to completely transparent (100). The bar in Figure 1c shows the representation of this parameter, and two groups of samples can be clearly identified. In addition, these two groups are statistically different. The samples fermented in stainless steel barrels showed higher L* values (around 85), while SCW and HVW wines resulted in lower values (around 80). It is possible that the extraction of certain compounds from the barrel by the wine modifies the lightness of it. Therefore, the yeast strain used did not influence the L* value.

In the hue circle in Figure 1c, a* is a measure of redness (or −a* of greenness) and b* of yellowness (or -b* of blueness). In general, all samples are grouped in the same area of the graphic representation. Coordinate a* values were between 17.18 and 19.48, the wines SCW and HVW presented higher values, but without significant differences with SC and HV wines. Therefore, the yeast strain used and the material of the fermentation tank have not influenced the a*coordinate value. In relation to the b* coordinate, the yeast strain used had a notable influence. Wines fermented by *S. cerevisiae* resulted in statistically higher values than *H. vineae*, thus, *S. cerevisiae* wines had more yellowness, corresponding to the tonality values obtained.

a

Rosé wines	Colour intensity (absorbance units)	Tonality (adimensional)	Chroma	Hue (°)	L	a	b
SC	0.59±0.02^{a}	0.82±0.01^{b}	21.22±0.90^{ab}	32.09±0.99^{b}	85.33±0.81^{b}	17.95±0.56^{a}	11.30±0.81^{b}
HV	0.59±0.01^{a}	0.80±0.02^{ab}	19.22±2.27^{a}	26.36±1.51^{a}	84.73±0.23^{b}	17.18±1.82^{a}	8.60±1.45^{a}
SCW	0.78±0.02^{b}	0.82±0.01^{b}	22.67±0.51^{b}	32.66±0.00^{b}	80.50±0.71^{a}	19.09±0.48^{a}	12.23±0.20^{b}
HVW	0.75±0.00^{b}	0.76±0.02^{a}	21.41±0.17^{ab}	24.64±0.81^{a}	80.50±0.00^{a}	19.48±0.28^{a}	8.88±0.23^{a}

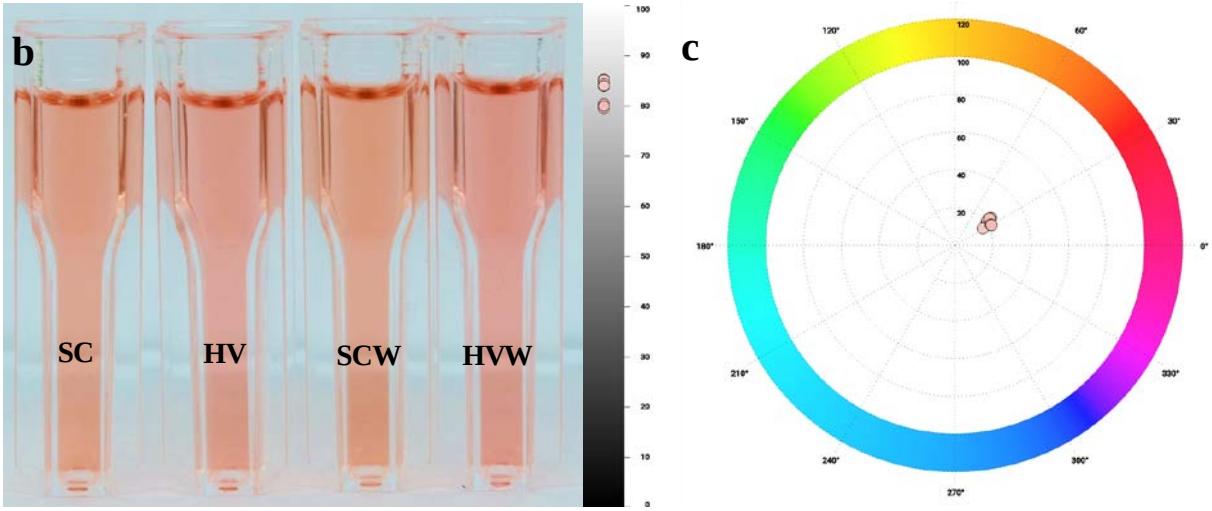

Figure 1. Colour parameter values and CIELab coordinates (**a**); visual colour aspect of rosé wines (**b**); graphic presentation of CIELab coordinates (**c**). SC (fermentations with *S. cerevisiae* in stainless steel barrels); HV (fermentations with *H. vineae* in stainless steel barrels); SCW (fermentations with *S. cerevisiae* in oak barrels); HVW (fermentations with *H. vineae* in oak barrels). Values in the same column with the same letter are not significantly different ($p < 0.05$).

Hue angle (H*) and chroma (C*) values are obtained from L*, a* and b* coordinates [24]. Changes in C* in red wine reflect a bias towards the dominant colour component (a* or b*) [25]. In all wines studied, (C*) values varied between 19.22 and 21.41. These values are under 50, therefore it is expected that these wines do not show vivid colours. In the same way, C* value was not related to the yeast strain or the tank material used. Hue angle (h*) is defined in relation to the +a* axis and is expressed in degrees; 0º would be +a* (red), 90º would be +b* (yellow), 180º would be -a* (green) and 270º would be -b* (blue) [26]. In Figure 1c, it can be seen that all wine samples are grouped between 24º and 60º, but wines fermented by *S. cerevisiae* resulted in higher values of h*. In light of this, we can conclude that the use of *H. vineae* results in wines with a tone closer to red, while fermentations with *S. cerevisiae* result in a more yellow tone. Therefore, it seems that the use of different yeast species for the fermentation process leads to the formation of pigments, with absorbances at different wavelengths.

3.3. Anthocyanins

The anthocyanins are the main agents responsible for the colour of red wines. They are made up of a monoglucosides anthocyanin (malvidin, delphinidin, peonidin, petunidin or cyanidin), substituted at position three by a molecule of glucose. The glucosides, in turn, can be acylated at position six of the sugar, with either acetic, *p*-coumaric or caffeic acid [27]. Figure 2 shows the comparison of chromatograms for the different wines studied, measured by HPLC-DAD where seven different pigments can be observed. Considering the total content of anthocyanins identified, it can be observed that wines fermented by *H. vineae* showed statistically higher contents of pigments than the wines fermented by *S. cerevisiae*. This increase was intensified when wines were fermented in oak barrels, obtaining up to a 44% increase in total anthocyanins in HVW samples compared to SCW samples (Table 2). It

is interesting to note that these pigments only come from the Tempranillo red grape variety, and values around 50 mg/L have been identified by other authors when making rosé wines from Cabernet Sauvignon grapes [28]. Other authors have obtained lower amounts of total pigments, around 22 mg/L [29]. The colour of rosé wines depends not only on the grape variety utilised, but also on other factors, such as the maceration time during winemaking. Since the same rosé must was used for this study, the variation in total anthocyanin content could be due to the precipitation of colouring matter, and, therefore, *H. vineae* was able to maintain a higher amount of stable pigments.

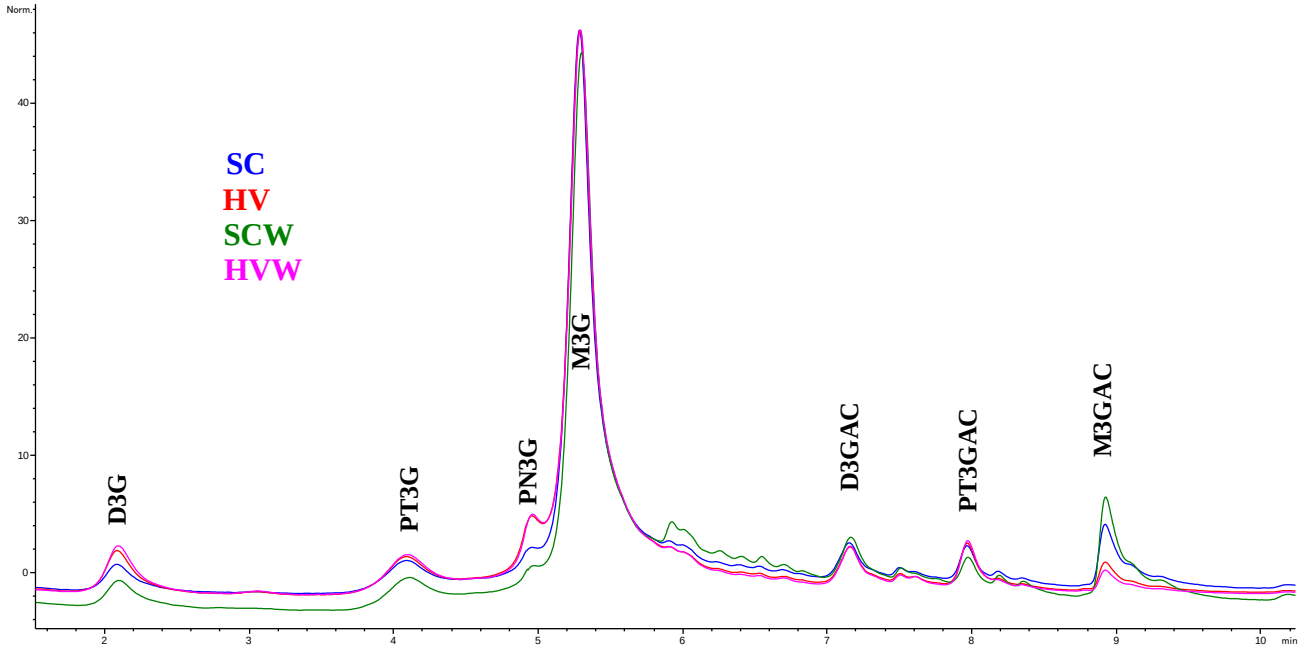

Figure 2. Chromatograms for anthocyanin identification by HPLC-DAD. SC (fermentations with *S. cerevisiae* in stainless steel barrels); HV (fermentations with *H. vineae* in stainless steel barrels); SCW (fermentations with *S. cerevisiae* in oak barrels); HVW (fermentations with *H. vineae* in oak barrels).

Table 2. Anthocyanins content (mg/L) measured by HPLC-DAD.

	Delphinidin 3-*O*-Glucoside (D3G) (mg/L)	Petunidin 3-*O*-Glucoside (PT3G) (mg/L)	Peonidin 3-*O*-Glucoside (PN3G) (mg/L)	Malvidin 3-*O*-Glucoside (M3G) (mg/L)	Delphinidin 3-*O*-(6″-*O*-Acetyl) Glucoside (D3GAC) (mg/L)	Petunidin 3-*O*-(6″-*O*-Acetyl) Glucoside (PT3GAC) (mg/L)	Malvidin 3-*O*-(6″-*O*-Acetyl) Glucoside (M3GAC) (mg/L)	Total Anthocyanin Content (mg/L)
SC	3.78 ± 0.20 ^{a}	4.28 ± 0.22 ^{b}	3.61 ± 0.10 ^{a}	31.69 ± 1.27 ^{b}	4.06 ± 0.07 ^{b}	3.82 ± 0.07 ^{b}	4.64 ± 0.02 ^{c}	55.87 ± 1.69 ^{b}
HV	4.79 ± 0.32 ^{b}	4.96 ± 0.13 ^{c}	4.84 ± 0.40 ^{b}	35.98 ± 0.27 ^{c}	4.25 ± 0.03 ^{c}	4.24 ± 0.00 ^{c}	3.63 ± 0.09 ^{b}	62.70 ± 0.40 ^{c}
SCW	3.63 ± 0.16 ^{a}	3.80 ± 0.02 ^{a}	3.33 ± 0.15 ^{a}	23.60 ± 2.35 ^{a}	3.78 ± 0.02 ^{a}	3.39 ± 0.11 ^{a}	4.76 ± 0.05 ^{c}	46.29 ± 2.71 ^{a}
HVW	5.54 ± 0.15 ^{c}	4.98 ± 0.03 ^{c}	5.16 ± 0.14 ^{b}	38.56 ± 0.42 ^{d}	4.41 ± 0.02 ^{d}	4.46 ± 0.02 ^{d}	3.50 ± 0.03 ^{a}	66.61 ± 0.60 ^{d}

SC (fermentations with *S. cerevisiae* in stainless steel barrels); HV (fermentations with *H. vineae* in stainless steel barrels); SCW (fermentations with *S. cerevisiae* in oak barrels); HVW (fermentations with *H. vineae* in oak barrels). Values in the same column with the same letter are not significantly different ($p < 0.05$).

Malvidin 3-*O*-glucoside (M3G) is the main pigment, and, therefore, followed the same trend as the total anthocyanins content obtaining values up to 38.56 mg/L in HVW wines. However, when this compound is acylated (M3GAC), the highest concentrations are found in the samples fermented by *S. cerevisiae*. Regarding the other pigments identified, all samples fermented by *H. vineae* resulted in significantly higher concentrations of anthocyanins, and this matched with the colour measurements (Figure 1a). In this respect, the higher concentrations of acylated pigments, except M3GAC, in HV and HVW wines are

noteworthy. These compounds are bluer than the non-acylated ones, and this was reflected in the b*value of the CIELab coordinates (Figure 1a). The variations in the concentration of these acylated pigments may also be due to the fermentation kinetics of the two yeasts studied. The rate of ethanol formation in the medium can influence the solubility of the pigments; slower fermentations usually result in a higher amount of colouring matter in the solution.

3.4. Polysaccharides Released during the Fermentation Process

The polysaccharide content identified in the studied wines can be seen in Figure 3a. The comparison of the different chromatograms by HPLC-DAD that were obtained in the fermentations can also be observed in Figure 3b. The double peak corresponds to the wine polysaccharides in a retention time of 6.5–10 min. It is interesting to note that the different yeast strains gave rise to different peak shapes without appreciable differences, depending on the material of the barrel in which they were fermented.

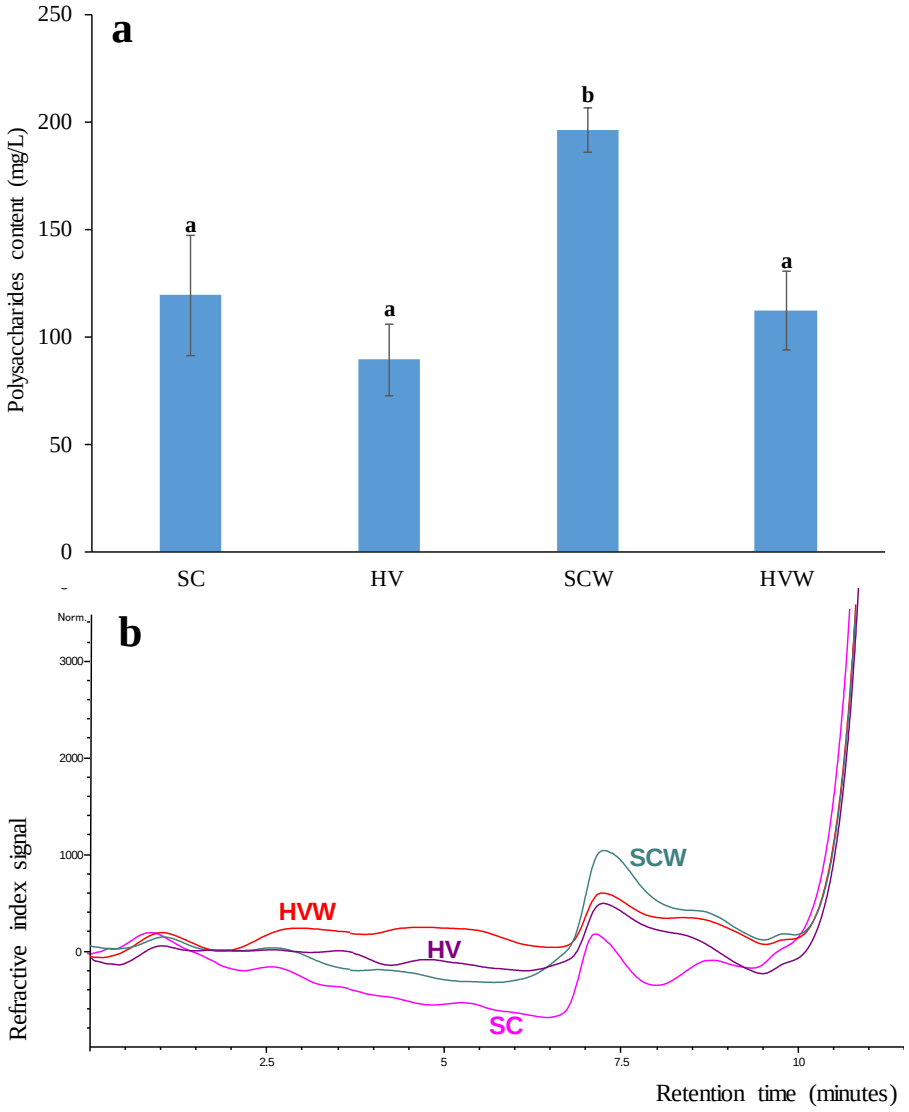

Figure 3. Polysaccharides (mg/L) (**a**) and chromatogram (**b**) measured by molecular exclusion liquid chromatography-refractive index detection (LC-RID). SC (fermentations with *S. cerevisiae* in stainless steel barrels); HV (fermentations with *H. vineae* in stainless steel barrels); SCW (fermentations with *S. cerevisiae* in oak barrels); HVW (fermentations with *H. vineae* in oak barrels). Bars with the same letter are not significantly different ($p < 0.05$).

In relation to the amount of polysaccharides identified, it is important to note that these polysaccharide values correspond to the sum of both polysaccharides from the grape and yeast cell wall. The grape polysaccharides include polysaccharides, rich in arabinose, and galactose and polysaccharides, rich in rhamnogalacturonans, which both come from the pectocellulosic cell walls of the grape berries. The cell wall polysaccharides include the mannoproteins, and these compounds are released by yeasts during fermentation and the lees ageing process [30]. Wines fermented in stainless steel barrels showed a polysaccharide content between 89 and 119 mg/L, with no significant differences between the different yeasts studied. However, wines fermented by *S. cerevisiae* in oak barrels showed a higher concentration of these compounds of around 195 mg/L. Therefore, fermentation tank material may have an influence on the release of these compounds from yeast cell walls. As these differences were not found in stainless steel tanks, we cannot be sure that there is a higher polysaccharide release by *S. cerevisiae* in all fermentation conditions. Other authors have found higher amounts of polysaccharides (425–605 mg/L) after HPLC-RI analysis of red wines [31]. It is possible that these differences are due to the fact that maceration of red wines with grape skins resulted in a greater transfer of polysaccharides from the pectocellulosic cell walls of grape berries. Finally, it is interesting to note that as these are young wines, hence the contribution of polysaccharides yielded by the yeast cell lysis process is low. Since the process of cell lysis is slow, in previous investigations the release of these compounds in model medium was between 12–13 mg/L after 4.5 months of lees ageing assisted with ultrasounds [32]. Similarly, other authors identified amounts of 16–22 mg/L in model mediums after 7 months of ageing [33].

3.5. Volatile Compounds Produced by Fermentation

Table 3 shows aroma compounds produced during fermentation, measured by GC-FID. The wines fermented by *S. cerevisiae* in stainless steel barrels (SC) resulted in a significantly higher concentration of total volatiles than other treatments. In relation to the concentration of acetaldehyde, higher amounts were found in the wines aged in oak barrels (SCW and HVW), but without significant differences depending on the yeast strain identified. However, HVW samples resulted in lower diacetyl concentrations than the other wines. There were no significant differences in acetoin formation for all of the wines studied. Finally, 2,3-butanediol content was 536 mg/L for SC, a higher concentration than the amount identified in the other samples.

Table 3. Volatile compounds produced by fermentation (mg/L), measured by GC-FID. Mean ± standard deviation of three replicates.

Compound	SC	HV	SCW	HVW
Acetaldehyde	63.25 ± 4.63 ^{a}	70.23 ± 2.76 ^{a,b}	74.75 ± 0.65 ^{b,c}	79.08 ± 6.57 ^{c}
Methanol	26.64 ± 0.84 ^{b}	27.21 ± 2.07 ^{b}	22.84 ± 2.86 ^{a}	25.31 ± 0.68 ^{a,b}
Diacetyl	1.55 ± 0.07 ^{b}	1.60 ± 0.12 ^{b}	1.56 ± 0.01 ^{b}	0.49 ± 0.84 ^{a}
Acetoin	5.50 ± 0.05 ^{a}	6.46 ± 1.32 ^{a}	5.74 ± 1.66 ^{a}	5.70 ± 0.40 ^{a}
2,3-butanediol	536.22 ± 80.81 ^{b}	381.94 ± 41.65 ^{a}	360.03 ± 13.82 ^{a}	394.49 ± 89.51 ^{a}
Isobutanol	21.05 ± 0.85 ^{b}	17.73 ± 0.40 ^{a}	26.24 ± 1.04 ^{c}	18.21 ± 0.40 ^{a}
1-propanol	24.23 ± 0.63 ^{b}	20.23 ± 1.59 ^{a}	24.41 ± 0.49 ^{b}	20.22 ± 1.67 ^{a}
2-methyl-1-butanol	24.17 ± 0.57 ^{b,c}	19.44 ± 1.07 ^{a}	25.46 ± 1.47 ^{c}	22.58 ± 0.91 ^{b}
3-methyl-1-butanol	120.25 ± 0.72 ^{c}	93.13 ± 4.75 ^{a}	125.40 ± 2.97 ^{c}	108.64 ± 1.34 ^{b}
Hexanol	4.46 ± 0.18 ^{b}	4.17 ± 0.22 ^{a,b}	4.10 ± 0.29 ^{a,b}	3.86 ± 0.03 ^{a}
2-phenyl-ethanol	16.46 ± 0.96 ^{a}	15.21 ± 0.27 ^{a}	17.08 ± 1.63 ^{a}	16.16 ± 1.47 ^{a}
∑ **Higher alcohols**	210.61 ± 1.29 ^{c}	169.91 ± 7.56 ^{a}	222.69 ± 4.97 ^{d}	189.67 ± 4.80 ^{b}
Ethyl lactate	15.25 ± 1.60 ^{b}	14.50 ± 3.75 ^{a,b}	10.11 ± 0.83 ^{a}	15.08 ± 3.19 ^{b}
Ethyl acetate	70.96 ± 4.09 ^{b}	57.55 ± 4.62 ^{a}	85.08 ± 2.99 ^{c}	63.30 ± 3.20 ^{a}
Isoamyl acetate	4.63 ± 0.11 ^{a,b}	4.52 ± 0.37 ^{a}	5.08 ± 0.22 ^{b}	4.89 ± 0.39 ^{a,b}
2-phenylethyl acetate	9.28 ± 1.63 ^{b}	15.38 ± 1.22 ^{c}	6.66 ± 0.60 ^{a}	7.35 ± 0.40 ^{a,b}
∑ **Esters**	100.12 ± 6.34 ^{a,b}	93.45 ± 5.37 ^{a}	106.93 ± 3.16 ^{b}	90.62 ± 5.76 ^{a}
∑ **Total volatiles**	943.90 ± 78.80 ^{b}	750.80 ± 28.39 ^{a}	794.54 ± 22.04 ^{a}	785.37 ± 100.17 ^{a}

SC (fermentations with *S. cerevisiae* in stainless steel barrels); HV (fermentations with *H. vineae* in stainless steel barrels); SCW (fermentations with *S. cerevisiae* in oak barrels); HVW (fermentations with *H. vineae* in oak barrels). Values in the same row with the same letter are not significantly different ($p < 0.05$).

The alcohols generated during *S. cerevisiae* fermentation were higher in all cases (SC and SCW), irrespective of the barrel material in which the wines were fermented. None of the fermentations exceeded an alcohol content of more than 400 mg/L, which is considered to negatively affect the wine's quality [34]. In particular, this increased concentration in SC and SCW fermentations was intensified for compounds, such as 2-methyl-1-butanol and 3-methyl-1-butanol. These are the most important higher alcohols, in which their aroma was identified by a pungent odour with a perception threshold of 40 mg/L [35]. The only higher alcohol that contributes positively to the aroma of wine is 2-phenyl-ethanol, which has the olfactory descriptor of rose petals [9]. The obtained concentrations of this compound were between 16.46 and 17.08 mg/L, with no significant differences between the yeast treatments studied. We obtained similar results when comparing both species in fermentations of Albillo grape variety, but with less concentrations of 2-phenyl-ethanol (around 9.6 mg/L) [12]. However, other authors have observed even higher yields of this compound when fermenting *H. vineae* sequentially with *S. cerevisiae*, compared to pure fermentations of *S. cerevisiae* and other non-*Saccharomyces* yeasts [36].

Volatile esters are an important group of aromatic compounds, and are considered to be the main source of the fruity aroma of wines [37]. The most abundant ester in wine is ethyl acetate; the identified amounts of this compound were significantly higher in *S. cerevisiae* fermentations compared to *H. vineae*, with values between 70 and 85 mg/L. It is interesting to note that ethyl acetate concentrations below 80 mg/L provide a positive organoleptic perception [38]. Another important ester is 2-phenylethyl acetate, as this compound has a strong aromatic power and is associated with fruity, floral and honey aromas [11]. Previous studies have linked *H. vineae* yeast to an increase in 2-phenylethyl acetate content [39]. In the present work, an increase in the concentration of this compound was also observed in *H. vineae* wines, particularly when fermentation was carried out in stainless steel barrels (HV). In this case, there was an increase of up to 65 % compared to fermentations with *S. cerevisiae*.

3.6. Volatile Compounds Measured by GC-MS

Different families of compounds were identified by GC-MS. As they are mainly oak-derived compounds, only wines fermented in oak barrels were analysed. The first group of compounds is the sum of norisoprenoids and terpenes alcohols. β-ionone was the only norisoprenoid identified. This is an odorant compound that is generated from β-carotene by thermal degradation, or by photo-oxygenation. β-ionone is a compound widely used in perfumery for its violet odour [40]. *S. cerevisiae* fermentations showed values around 30 μg/L, which is about twice as high as the *H. vineae* samples. The average amount of β-ionone in wines varies considerably, from a few micrograms per litre to more than 60 μg/L [41]. However, based on the sum of norisoprenoids and terpene alcohols, the highest amounts were identified in wines fermented by *H. vineae* (Figure 4). This is because significantly higher amounts of the terpene linalool were identified in *H. vineae*, with values around 1000 μg/L which is about twice higher compared to *S. cerevisiae* wines. The monoterpene linalool is important for its aromatic contribution in aromatic white grape varieties (about 1–4 mg/L), but other grape varieties with lower concentrations, such as Chardonnay might also have a floral impact due to the low sensory threshold of linalool at about 10 μg/L [42].

Regarding the compounds extracted from oak wood, the volatile phenols include eugenol and guaiacol. Both are obtained by the breakdown of lignin during the cooperage toasting process. Guaiacol is the compound responsible for the burnt and smoky aromas of aged wines, and eugenol provides spicy and smoky aromas [43]. The concentrations of total volatile phenols in SCW samples were higher than in HV samples, but without significant differences between them. Other aroma groups analysed were the furanic compounds, which include furfural, 5-methyl furfural and furfuryl alcohol. These compounds are derived from the degradation of cellulose and hemicellulose monosaccharides [44]. Concentrations between 350 and 400 μg/L were obtained with no significant differences

between the yeast strains used. These are not higher values compared to those obtained by other authors after wine ageing in oak barrels [45], with an average value of 4500 and 4700 µg/L of furfural in French and American oak barrels, respectively. No significant differences were identified in the concentration of lactones transferred from the barrel during the fermentation. Values obtained were between 40 and 50 µg/L for both yeasts. These values did not have a significant aromatic impact on the wine as the perception threshold for cis-isomer is 92 µg/L [46]. Finally, small amounts of ethylphenols were found, with no significant differences between the wines and all being below the threshold of perception of this compound, 140 µg/L for 4-ethylguaiacol, according to [47].

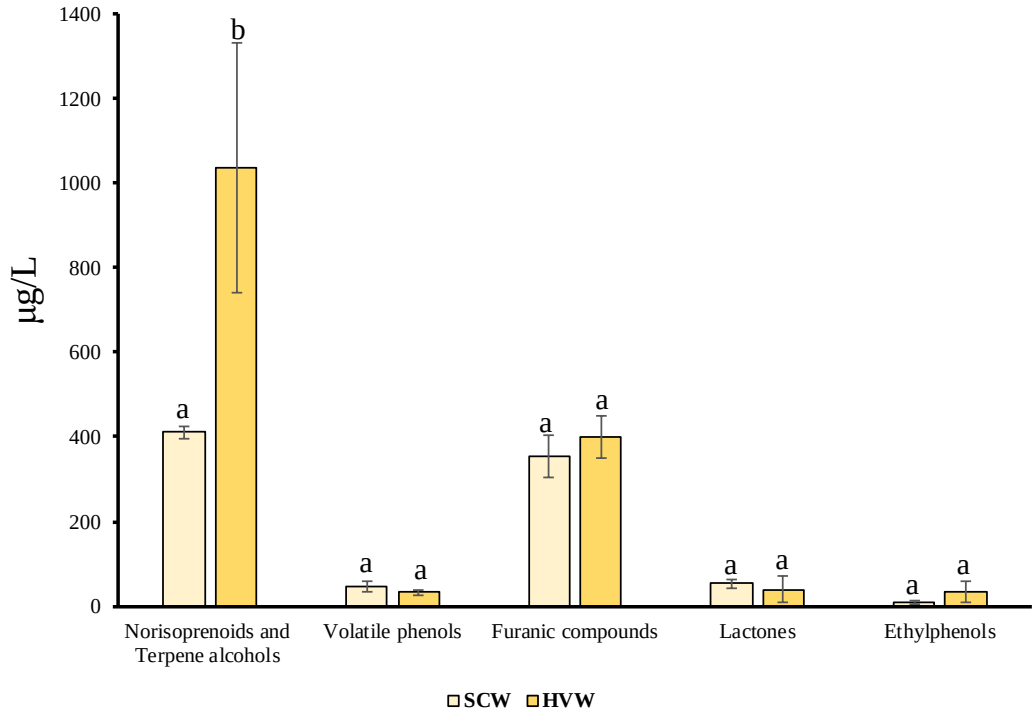

Figure 4. Volatile compounds measured by GC-MS. SCW (fermentations with *S. cerevisiae* in oak barrels); HVW (fermentations with *H. vineae* in oak barrels). Mean ± standard deviation of two replicates. Bars with the same letter are not significantly different ($p < 0.05$).

3.7. Sensory Analysis

The sensory analysis of the wines studied showed that there were no significant differences in the colour intensity parameter (Figure 5). However, the tasters identified a clear increase in tonality in *S. cerevisiae* wines. These results are in agreement with spectrophotometric colour measurements, both in tonality and in the CIELab coordinates including the yellow component (Figure 1). These differences may be due to the higher concentrations of acylated anthocyanins identified in *H. vineae* wines (Figure 2).

In relation to aromatic parameters, the tasters did not identify differences in the aromatic intensity of the wines studied. However, *H. vineae* wines were scored higher on the floral parameter. These scores are possibly due to the higher 2-phenylethyl acetate concentrations (Table 3). In the same way, the high linalool content could influence this parameter too (Figure 4). Tasters described *H. vineae* wines as fruitier than *S. cerevisiae* wines, and these results are in the agreement with the higher content of 2-phenylethyl acetate. The wood character was perfectly identified in samples fermented in oak barrels.

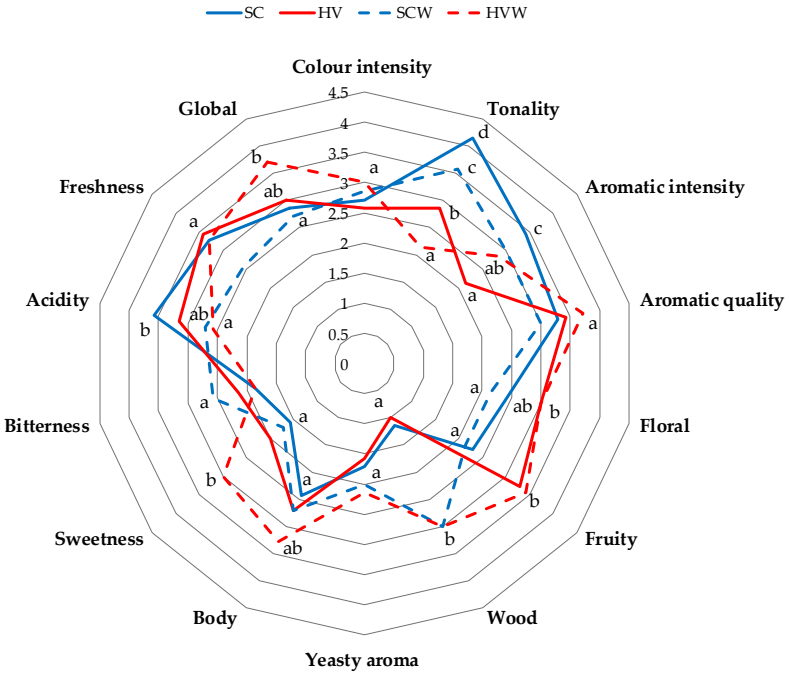

Figure 5. Spider plot showing sensory analysis by eight trained tasters. SC (fermentations with *S. cerevisiae* in stainless steel barrels); HV (fermentations with *H. vineae* in stainless steel barrels); SCW (fermentations with *S. cerevisiae* in oak barrels); HVW (fermentations with *H. vineae* in oak barrels). Means in the same axis with the same letter are not significantly different ($p < 0.05$).

No major differences in mouthfeel were identified, with the exception of the HVW wines which were rated as sweeter than the rest, and SC wines were found to be more acidic than HVW wines. Finally, the global perception was higher in HVW wines.

4. Conclusions

The use of *H. vineae* in rosé winemaking resulted in wines with similar general oenological parameters to those obtained with a commercial *S. cerevisiae*. Wines fermented with *H. vineae* preserved a higher amount of total pigments, and this is reflected by spectrophotometric analysis where *H. vineae* wines showed more reddish and less yellowish tones. These colour differences were visible to the naked eye. There were no clear results on the yeast dependence on the amount of polysaccharides obtained. In terms of the aromatic profile of the wines obtained, significant increases in the 2-phenylethyl acetate compound were identified with *H. vineae* treatments, which were particularly noticeable in stainless steel barrel fermentations. In addition, *H. vineae* produced wines with a lower content of both higher alcohols and ethyl acetate. Higher concentrations of linalool were also identified in *H. vineae* wines, which might also contribute to floral note characteristics with concentrations above the sensory threshold.

H. vineae is a yeast species with an interesting application for the production of Tempranillo and Albillo rosé wines. It is able to produce a good aromatic profile, by increasing the amount of sensory desirable compounds. In addition, it is able to hold a higher amount of pigments, resulting in more attractive colours.

Author Contributions: J.M.D.F. performed the analysis and drafted the manuscript; I.L. revised and corrected the manuscript; C.E. revised and corrected the manuscript; F.C. revised and corrected the manuscript; C.G. revised and corrected the manuscript; R.C. performed the fermentations assays in the winery; and A.M. undertook the study's conceptualisation, coordinated the investigation and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

The fermentation kinetics of the different rosé wines studied are shown in Figure A1. During the first days of fermentation, a higher density loss was identified in *H. vineae* samples than in *S. cerevisiae* samples. The stainless steel barrels finished the fermentation after 33 days. However, the oak barrels completed fermentation more quickly, as no differences in density were detected after 22 days. This fact was observed for both yeast species; therefore, the fermentation tank material seems to have a significant influence on the fermentation kinetics.

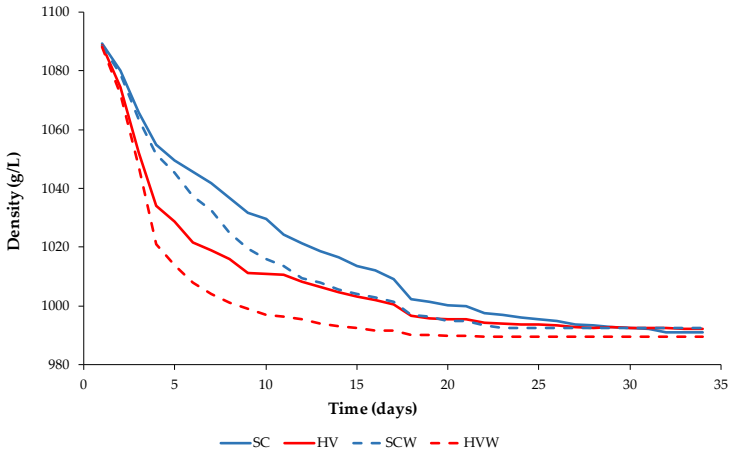

Figure A1. Fermentation kinetics of studied yeast strains. SC (fermentations with *S. cerevisiae* in stainless steel barrels); HV (fermentations with *H. vineae* in stainless steel barrels); SCW (fermentations with *S. cerevisiae* in oak barrels); HVW (fermentations with *H. vineae* in oak barrels).

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