

THE OENOLOGICAL POTENTIAL OF HANSENIASPORA VINEAE YEAST STRAINS TO INDUSTRIAL WINE PRODUCTION

Valentina Martin¹, Laura Fariña¹, Karina Medina¹, María J. Valera¹, Eduardo Boido¹, Eduardo Dellacassa^{2*}, Albert Mas³, Francisco Carrau¹

¹Area Enología y Biotecnología de Fermentaciones, Facultad de Química, Universidad de la República, Montevideo, Uruguay

e-mail: vmartin@fq.edu.uy, lfarina@fq.edu.uy, kmedina@fq.edu.uy, mariajose_valera_martinez@hotmail.com, eboido@fq.edu.uy

²Laboratorio de Biotecnología de Aromas, Facultad de Química, Universidad de la República, Montevideo, Uruguay

e-mail: edellac@fq.edu.uy

³Departament Bioquímica i Biotecnologia, Facultat d'Enologia, Universitat Rovira i Virgili, Tarragona, España
e-mail: albert.mas@urv.cat

Introduction

The challenge of product style differentiation is always more competitive and difficult to attract consumers attention. It is considered that regional consumption will be the main alternative for the many new brands that appear in the market. The use of non-conventional yeasts is a strategy to create unique wine profiles within an extensive market, where region identity is the challenge. The “terroir” concept, which consents wines differentiations has been applied by many winemakers during the last 20 years. Many practices are involved (soil selection, minimal fertilizers application, irrigation control, old vines recovery) in order to produce differential wines searching for complexity, but probably one of the key components of the terroir concept are the native yeasts associated with the grapes of a particular region.

However, actually most of the wine fermentation technology uses commercial yeast strains, mainly belonging to the genus *Saccharomyces*, as they allow an adequate development of the fermentation process. However, exists a great diversity of native yeasts that is not reflected in the availability of commercial yeasts.

Several researchers have studied the contribution of non-*Saccharomyces* yeasts on the organoleptic characteristics of wines at laboratory level (Medina et al., 2012; Padilla et al., 2016; Varela, 2016). Although there are still little experiences at industrial level, some strains of non-*Saccharomyces* yeasts are starting to be available in the commercial wine starters market. The apiculate yeast group *Hanseniaspora/Kloeckera* is the most abundant genus present in grapes and other berry fruits.

This group usually dominates the natural epiphytic grape flora with proportions above 70% at harvest time in different wine regions (Barata et al., 2012). However, very limited reports of *Hanseniaspora/Kloeckera* application at industrial level are found in the literature (Masneuf-Pomarede et al., 2015).

H. vineae was successfully applied for commercial barrel fermented white wines in Chardonnay and Petit Manseng (Medina et al., 2013), and *H. uvarum* was applied for Negroamaro grape must (Tristezza et al., 2016).

The contribution is mostly associated to the increase sensorial complexity of wines compared to *S. cerevisiae* alone fermentations (Medina et al, 2013). Extracellular enzymatic reactions that occur in grape juice during the early stages of alcoholic fermentation, resulting in increased levels of free monoterpenes and other isoprenoids was proved in grape juice. In addition, many compounds associated to flavour and wine colour are known to be yeast strain-dependent. Because these metabolites are important for the sensory quality of wines, it is important to understand that winemaking is favoured by increased yeast diversity (Martin et al., 2016ab). This strategy is the ideal way to create unique wine profiles within a massive market.

In this work, we present the results obtained in the study of the oenological potential of 10 different strains of *H. vineae* isolated from Uruguayan vineyards selected according to flavour performance in a low yeast assimilable nitrogen synthetic medium. The results were compared with the commercial available strain T02/5AF that is being used in our region in order to better understand diversity within the species.

Material and methods

Yeasts. The eleven strains of *Hanseniaspora vineae* in this study belong to the yeast culture collection of Area Enología y Biotecnología de Fermentaciones (Facultad de Química, Universidad de la República, Uruguay). T02.05F was isolated from Tannat grapes in 2002 being currently utilized in some regional wineries at industrial scale. The other strains were selected from our yeast culture collection by sensory positive characteristics in a synthetic grape juice medium of low assimilable nitrogen (Carrau et al., 2015). Two reference wine commercial strains of *S. cerevisiae* were used, Montrachet 522 (UCDavis, USA) and QA23 (Lallemand Inc, Canada).

Molecular identification

H. vineae yeasts were differentiated at the strain level by a previously described genetic characterisation method (Barquet et al., 2012). The PCR products were separated at low voltage (50V) in a 1.8% or 2% agarose gel. The amplifications made from DNA isolates from independent experiments always gave the same profile. All of the experiments were performed in duplicate with independent DNA isolations.

The patterns of bands obtained were analysed with the GelCompare program version 4.2 (Applied Maths BVBA, Belgium), using the UPGMA method (Unweighted Pair Group Method using arithmetic Average, Unweighted Method of group with average arithmetic), cluster clustering and the Jaccard coefficient.

Extracellular enzymatic activity characterization

Protease activity. To determine acid protease activity, skim milk medium at pH 6 and 4.5 was prepared according to Mostert (2013). *M. pulcherrima* was present as a positive control and *S. cerevisiae* M522 as a negative control on each plate. The plates were incubated at 25°C for 5 days and observed for any halo formation. A clear halo surrounding the colony was taken as positive protease activity.

β -Glucosidase activity. The screening method for β -glucosidase activity used Esculin Glycerol Agar (EGA) medium. Screening was carried out according to Pérez et al. (2011) on agar plates with esculin as a substrate. A non-inoculated plate served as a control. Strains with β -glucosidase activity hydrolysed the substrate and a dark brown colour developed in the agar. The diameter of the brown halo was measured in millimetres.

Microvinifications. Chemically-defined fermentation medium (nutrient components of grape juice) was prepared as described previously (Carrau et al., 2005a), but modified as follows: the total nitrogen content was adjusted to a basic amount of 100mgN/L with each amino acid and ammonium component added in the same proportions as indicated previously (Henschke and Jiranek, 1993). The YAN amounts were not a limiting factor for the complete fermentation of sugars by the yeast strains used. Ergosterol was added as the only supplemented lipid at a final concentration of 10 mg/L. YAN, at a concentration of 100 mgN/L, was chosen as the limiting condition for selecting good flavour strains under low YAN experimental conditions that could ensure a successful performance at an industrial level (Carrau et al., 2008). Once a day, samples were taken to measure cell growth in an improved Neubauer chamber. Samples for sensory and GC-MS analysis were taken 2 days after the end of fermentation, filtered through 0.45-

µm pore membranes and SO₂ was added as 50 mg/L of sodium metabisulphite (Fariña et al., 2012).

Glycerol production. The glycerol production of each yeast strain was determined during fermentation using Enzytec™ Glyceroenzymatic kit (I. R- Biopharm).

Resistance to SO₂. Sulphite tolerance was determined according to Mauriello et al. (2009), replacing the agarised grape must by the chemically-defined fermentation medium in Section 2.4. Different concentrations of potassium metabisulphite, K₂S₂O₅, were added releasing a total SO₂ at concentrations of 0 (control), 50, 75 and 100 mg/L at the pH of the medium of 3.5. This technique allowed to compare SO₂ resistance capacity between *H. vineae* strains and the control. The commercial strain *S. cerevisiae* Lalvin QA23 (Lallemand R) was used as the control. The OD was measured after 24 h at 27°C, by a microplate reader (Omega PolarStar, BMG Labtech).

Polysaccharide determination. Polysaccharide release capacity during fermentation was analysed at the middle and end of the process (days 5 and 10) in the chemically-defined medium (see the microvinifications section). The content of polysaccharides in the samples was determined in accordance with the protocol described by Fanzone et al. (2012) using an Agilent 1200 series liquid chromatograph equipped with a G1362A refractive index detector (RID), a G1311A quaternary pump, a G1316A column furnace and the G1329A automatic injector (Agilent Technologies, Palo Alto, CA). The molecular weight distribution of the fractions of the samples was determined from a standard calibration of pullulans and dextrans of different molecular weights.

Extraction and identification of aromatic compounds. Fermentation volatiles were quantified after adsorption and separate elution from an Isolute ENV+ cartridge packed with 1 g of highly cross-linked styrene-divinyl benzene (SDVB) polymer (40–140 µm, cod. no. 915-0100-C), as previously reported by Carlin (1998) and adapted by Boido et al. (2003).

The identification and quantification were conducted by GC-MS using a Shimadzu QP 2020 instrument. The working conditions were those reported by Boido et al. (2013). The identification was carried out through the use of libraries of reference spectra and the library of our laboratory, made with standard injections and data reported in the literature.

Sensory evaluation. The evaluation of aromas was carried out by an expert panel of ten winemakers, where the "check all that apply" methodology was used. Each judge analysed the samples one by one and marked in a list (composed of 67 typical aromatic descriptors of wines) all of the perceived aromas that they considered adequate to describe them. With all of the samples analysed, the frequency of use of each descriptor selected for each sample was determined and a map of the isolates was generated by correspondence analysis (Campo et al., 2008). From the coordinates of the samples in the correspondence analysis, a cluster analysis was performed to identify groups of isolates with similar characteristics.

All statistical analyses were performed using the Statistica 7.0 software (StatSoft Inc., USA, 2000).

Results and discussion

Molecular identification. Identification was performed following the protocol described in the materials and methods section. Figure 1 shows a dendrogram with the cluster analysis that allowed patterns of bands obtained on gels and between gels to be compared. Strains isolations from 2002 and 2011 were grouped together, while those of

2012 were divided into two groups. It was demonstrated that all of the yeast strains were different.

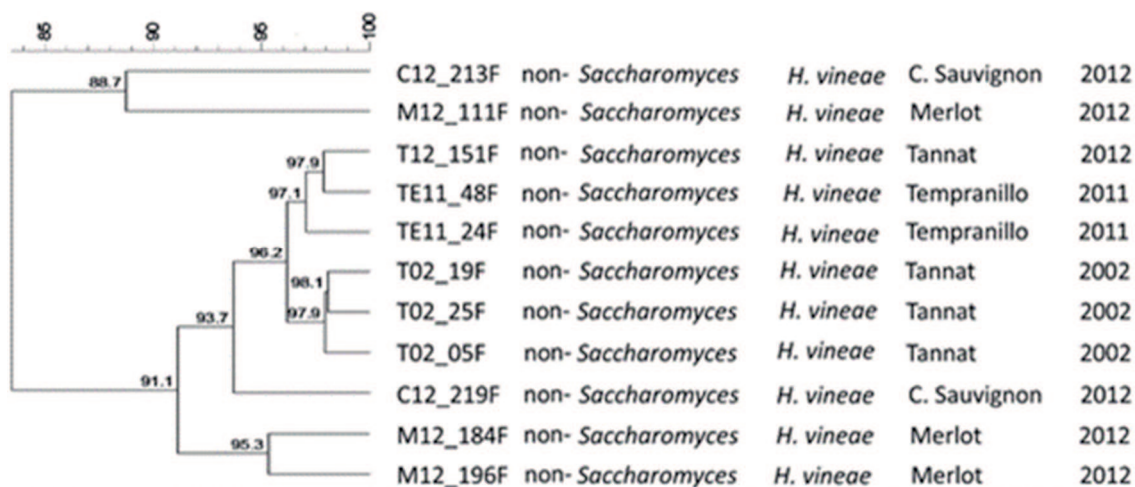


Figure 1. Phylogenetic analysis showing the differentiation of yeasts in 11 different strains. The columns on the right indicate the grape variety from which they were isolated and the year of harvest

Enzymatic characterization. Plate tests were carried out using specific substrates to evaluate the different enzymatic activities: protease and β -glucosidase. All *H. vineae* strains were active at pH 6.0 with these tests compared to the negative controls of *S. cerevisiae* M522 and QA23.

Proteolytic activity. Although no proteolytic evidence was found at pH 4.0, a significant proteolytic activity was observed at pH 6.0 for all of the studied strains of the genus, with the strains T02_05F, T02_25F and M12_111F producing the highest enzymatic activity (data not shown). From an oenological point of view, the proteolytic activity is relevant because it is responsible for the hydrolysis of proteins and peptides that affect wine protein stability (Waters et al., 2005), being a possible solution to address the problems associated with clarification, stabilisation and filtration of wines.

β -Glucosidase activity. β -glucosidase activity was evidenced and results were in agreement with results previously reported for two strains by Pérez et al. (2011) and for other strains of *H. vineae* (López et al., 2015). Although this activity was not evidenced in plates at pH 4.5 (data not shown), it is known that many of these enzymes are active against glycosylated aroma compounds in real grape wine fermentations as it was shown for *Hanseniaspora vineae* and *Metschnikowia* species (Perez et al, 2011). In our work, esculin, a β -glucoside, was the substrate used, as it is known that the plating enzymatic activity phenotype correlates with active enzymes against bound Muscat monoterpenes in real wine conditions (Perez et al, 2011).

Fermentative capacity and growth performance. In order to study the fermentation kinetics, micro-fermentations were carried out in the chemically-defined grape defined medium until no variation in weight loss was observed (13 days).

The fermentation kinetics for each yeast is shown in Figure 2, expressed as CO₂ released in g/100mL (these data include 3g/L of water vapor losses through cotton plugs, 3% of the volume). All of the strains were able to ferment at low assimilable nitrogen levels (100 mgN/L), although 150 mgN/L is considered the desired concentration at which to carry out fermentation processes (Ribereau-Gayon et al., 2003). The results showed different behaviours for the yeast fermentative capacity: the strain T02_25F presented the greatest release of carbonic gas during fermentation (11.52 gCO₂/100mL),

followed by a group including the control strain of *S. cerevisiae* (M522), and strains TE11_24F, T02_19F, T02_05F, M12_196F, C12_219F.

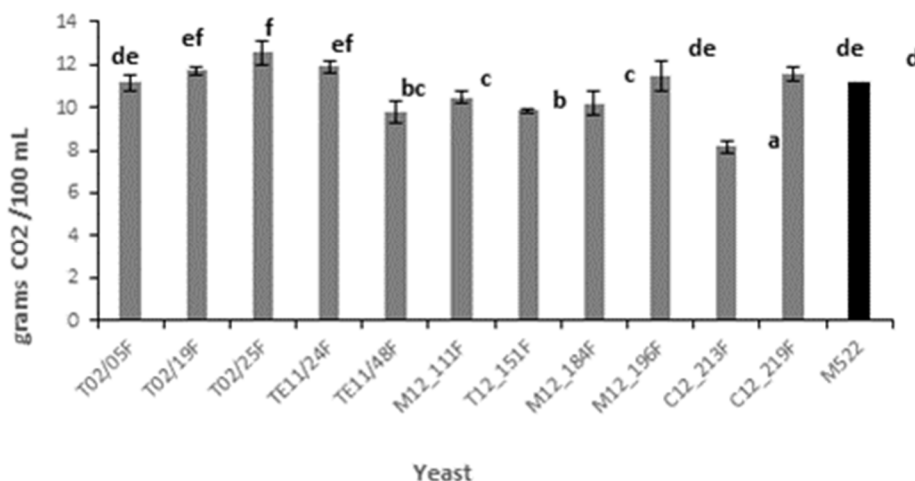


Figure 2. Final values of CO₂ released at the end of the fermentation for each yeast strain. Samples with the same letters do not present differences to a 95% confidence level

The fact that some *H. vineae* strains had a better fermentation performance than the control strain could be a consequence of the relatively low concentration of assimilable nitrogen in the chemically-defined medium (100 mgN/L). It is known that strain M522 is considered a high nitrogen demand yeast (Carrau et al., 2008) that might be affected by this condition.

At the end of the fermentation, the viable cell count was significantly different between some of the yeasts (Figure 3), behaviours which cannot be directly correlated to their fermentation kinetics of each strain. However, the yeast showing the lowest CO₂ yield during fermentation (strain C12_213F) presented the higher cell count, consistent with the fact that the weak fermentation capacity implied an increase in growth metabolism resulting in higher biomass production.

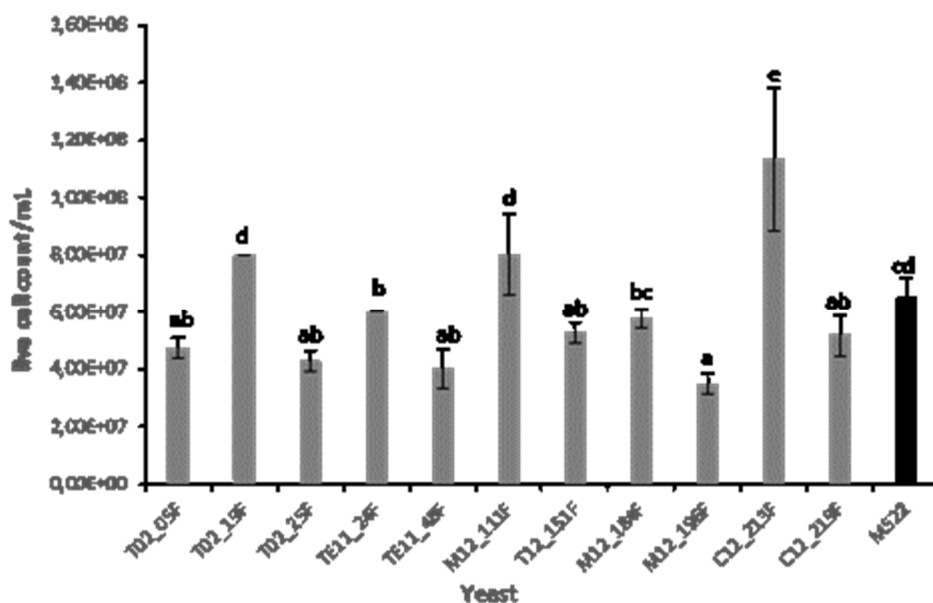


Figure 3. Live cells count at the end of the fermentation. Data with the same letter, do not differ from each other (LSD Test, 95% confidence level)

Glycerol production. The two strains responsible for a significantly higher production of glycerol belonged to the *H. vineae* species, with C12_213F followed by M12_196F being the main producer strains.

The work of Ciani and Comitini (2011) demonstrated that glycerol production is associated with the number of cells in the medium, which is in agreement with our results for C12_213F, where the higher living cell count at the end of fermentation resulted in higher glycerol levels.

SO₂ resistance. All *H. vineae* strains showed a good performance under 75 mg/L SO₂, which is a considerably higher level than that applied at an industrial level, where 50 mg/L is considered sufficient to protect grape juice processes before fermentation at pH 3.5. Yeast strains TE11_48F, M12_184F and *S. cerevisiae* (QA23) showed greater resistance to SO₂ levels, presenting significant differences above 75 mg/L of SO₂.

When compared with previous data from the literature, our results indicated that the concentration of SO₂ in the medium under 50 mg/L is an acceptable level for most of the strains of *H. vineae* analysed. Some contrasting reports described for other *Hanseniaspora* sp. (Fleet, 1993) suggest that species such as *H. uvarum* have a very weak resistant capacity to SO₂.

Release of polysaccharides during alcoholic fermentation. Figure 4 shows the high diversity of polysaccharides released by each yeast at the middle (dark grey bars) and end of fermentation (light grey bars).

The release of polysaccharides into the medium is strain-dependent and it might be significantly higher for some strains such as T02_19F at the end of fermentation compared to that for *S. cerevisiae* (Martí-Raga et al., 2016). From these results, it was observed that the release of polysaccharides from the yeasts to the medium during alcoholic fermentation was higher during the first days of fermentation (exponential phase). However, further research into the lysis process after fermentation may be required to determine whether the increased body of *H. vineae* wines (Medina et al., 2013) might be due to this other phenomenon.

Production of aroma compounds. The main results obtained are presented in Figures 4 and 5 by each yeast strain. *H. vineae* strains showed a consistent tendency towards higher production of acetate esters, benzenoids, acetoin/2,3 butanediol (Figure 5B), terpenes (citronellol and linalool) and sesquiterpenes (E)-nerolidol and (E,E)-farnesol). In contrast, it shows a significantly low production of ethyl esters, acids (Figure 5) and tyrosol/3-methylthio-1-propanol (Figure 5A) compared to *S. cerevisiae* M522. The lower production of acids by the yeasts *H. vineae* might explain the lower production of ethyl esters and weak ethanol resistance of this species compared to *S. cerevisiae*. Interestingly, due to the artificial fermentation medium utilized, de novo synthesis of isoprenoids such as citronellol (sweet and floral, threshold value 40µg/L), linalool (floral and lavender, threshold value 10µg/L) and sesquiterpenes (fruity) that contribute to increase fruity and flowery described flavours was demonstrated for *H. vineae*.

This is the first report to prove this capacity for this species where some strains (Figure 4) produced significant higher concentrations compared to *S. cerevisiae*, and above the sensory threshold of these compounds. We have report previously some little concentrations of these compounds for *H. uvarum*, but under the threshold odour levels (Carrau et al., 2005). Furthermore, the significantly higher formation of acetoin and 2,3-butanediol was detected in the *H. vineae* strains (Figure 5B), which might contribute to the buttery and honey sensory notes.

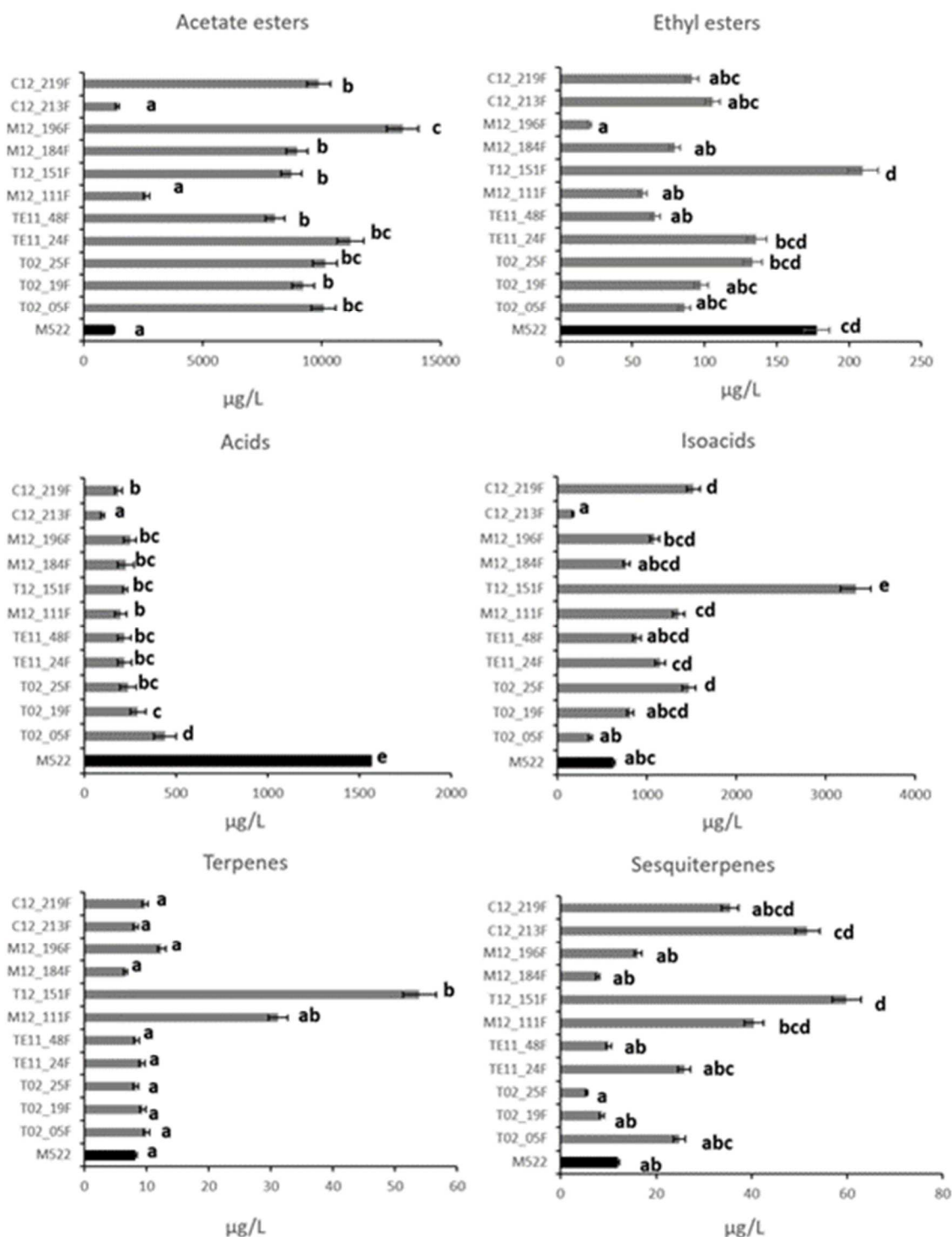


Figure 4. Aroma compounds produced by each strain of *H. vineae* in the chemical defined grape medium. Results showed the sum of different aromatic compounds families. MS22 is the *S. cerevisiae* control strain. The data with the same letter do not differ from each other (LSD Test, 95% confidence level).

In the case of tyrosol, a compound usually associated with a sensory character of bitterness or chemical notes, it was found at a significantly lower level in *H. vineae* strains (Figure 5A), probably due to the capacity of this species to form p-hydroxy benzenoids from tyrosine, as discussed by Martin et al. (2016a), with other benzenoid compounds

not being produced in *S. cerevisiae*. A similar behaviour was found for 3-methylthio-1-propanol.

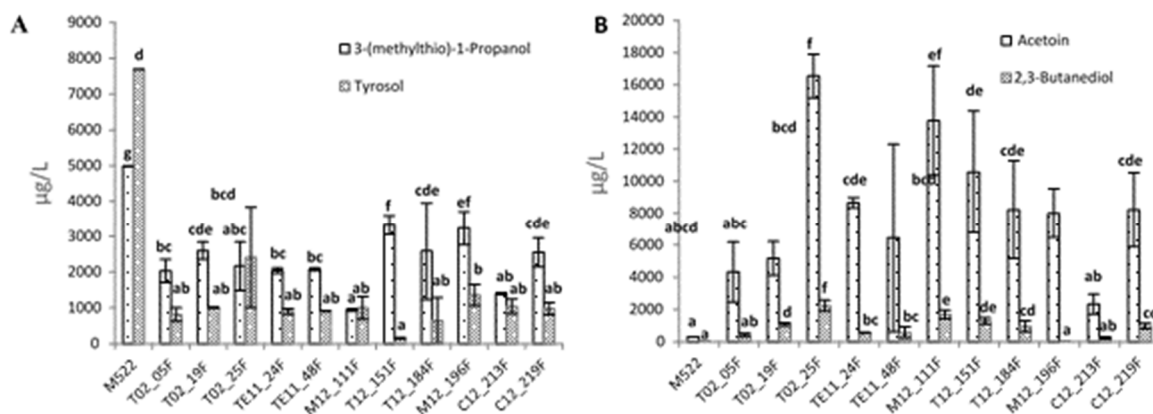


Figure 5. Tyrosol and 3-(methylthio)-1-propanol (A), and acetoin and 2,3-Butanediol (B) produced by each strain of *H. vineae* in the chemical defined grape medium. Results showed the average of these aromatic compounds. M522 is the *S. cerevisiae* control strain. The data with the same letter (for each compound) do not differ from each other (LSD Test, 95% confidence level)

H. vineae differed from *S. cerevisiae* in that it has the capacity to produce benzyl alcohol, since this compound was not detected in M522 and confirmed the recent report with other strains of *S. cerevisiae*, where low assimilable nitrogen levels contribute for their synthesis (Martin et al, 2016b). Furthermore, *H. vineae* produced significantly higher concentration of acetate esters compared to *S. cerevisiae*, which confirms that this is a particular characteristic of this species (Medina et al., 2013). In this aroma group, the major compound was β -phenylethyl acetate, exceeding approximately 12 times that produced by M522, highlighting the strain M12_196F.

It is known that the high synthesis capacity of β -phenylethyl acetate ester is particular of *H. vineae* strains within *Hanseniaspora* species compared to *guilliermondii*, *opuntiae*, *uvarum* and *clermontiae* that produced higher ethyl acetate levels (Medina, 2013). These results are in agreement with the high copy numbers of putative acetyl transferase genes found in the *H. vineae* genome (Giorello et al., 2014; Martin et al., 2016a), and that are not found in other *Hanseniaspora* species that are currently sequenced in data banks.

Sensory evaluation. The fermentations carried out from the different yeasts were divided into 5 groups according to the descriptors selected and a cluster analysis. Figure 6 shows the groups and sensory descriptors obtained from the tasting panel. Interestingly, the commercial strain T02_5F is included in the same cluster of strain T12_151F that was the best isoprenoids producer of this species.

Conclusions

We have already obtained positive contributions at the winery level from this yeast species for strain T02_5F. The yeast strains were evaluated to determine the extracellular protease and β -glucosidase enzyme activities, the fermentative capacity at low nitrogen levels, glycerol production, polysaccharide release, SO₂ resistance and the formation of aromatic compounds. Fermentation products were studied to determine the sensory properties related to consumer preferences. The results obtained enabled the verification of variability between strains of this species for some key attributes among the 11 strains, such as high protease and β -glucosidase enzymatic activity, and comparable to conventional wine yeasts of SO₂ resistance. The accumulation of some key aroma compounds was also consistent, including the low production of short chain fatty acids, ethyl esters and tyrosol, and the high production of acetate esters,

sesquiterpenes and decanoic acid compared to *Saccharomyces cerevisiae* strains. The most outstanding characteristic of the species *H. vineae* was the two orders of magnitude higher production of benzenoids and phenylpropanoid esters. From the sensory analysis point of view, 5 groups of strains were obtained, each identified by a set of aromatic descriptors. From these, four groups with the best sensory attributes were identified, highlighting aromatic descriptors such as flowers, fruits and honey.

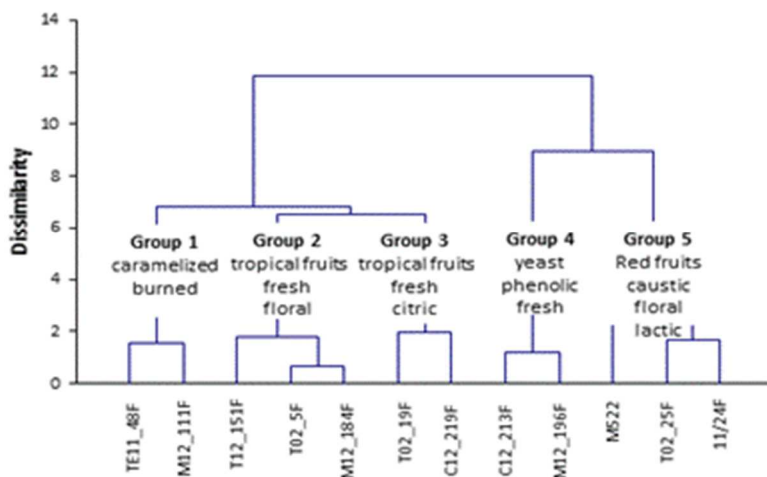


Figure 7. Aroma characterization of the groups derived from the sensory panel evaluation.

References

Barata, A., Malfeito-Ferreira, M., Loureiro, V. (2012). The microbial ecology of wine grape berries. *Int J Food Microbiol.* 153, 243-259.

Barquet, M., Martín, V., Medina, K., Pérez, G., Carrau, F., and Gaggero, C. (2012). Tandem repeat-tRNA (TRtRNA) PCR method for the molecular typing of non-*Saccharomyces* subspecies. *Appl. Microbiol. Biotechnol.* 93, 807–814.

Campo, E., Do, B. V., Ferreira, V., and Valentin, D. (2008). Aroma properties of young Spanish monovarietal white wines: a study using sorting task, list of terms and frequency of citation. *Aust. J. Grape Wine Res.* 14, 104–115.

Carrau, F., Gaggero, C., and Aguilar, P. S. (2015). Yeast diversity and native vigor for flavor phenotypes. *Trends Biotechnol.* 33, 148–154.

Carrau, F. M., Medina, K., Boido, E., Farina, L., Gaggero, C., Dellacassa, E., et al. (2005). De novo synthesis of monoterpenes by *Saccharomyces cerevisiae* wine yeasts. *FEMS Microbiol. Lett.* 243, 107–115.

Carrau, F., Medina, K., Farina, L., Boido, E., Henschke, P. A., and Dellacassa, E. (2008). Production of fermentation aroma compounds by *Saccharomyces cerevisiae* wine yeasts: Effects of yeast assimilable nitrogen on two model strains. *FEMS Yeast Res.* 8, 1196–1207.

Ciani, M., and Comitini, F. (2011). Non-*Saccharomyces* wine yeasts have a promising role in biotechnological approaches to winemaking. *Ann. Microbiol.* 61, 25–32.

Boido, E., Fariña, L., Carrau, F., Dellacassa, E., Cozzolino, D. (2013). Characterization of n Tannat grapes and their prediction by Near Infrared Spectroscopy. *Food Anal. Met.* 6, 100-111

Fanzone, M., Peña-Neira, A., Gil, M., Jofré, V., Assof, M., and Zamora, F. (2012). Impact of phenolic and polysaccharidic composition on commercial value of Argentinean Malbec and Cabernet Sauvignon wines. *Food Res. Int.* 45, 402–414.

Fariña, L., Medina, K., Urruty, M., Boido, E., Dellacassa, E., and Carrau, F. (2012). Redox effect on volatile compound formation in wine during fermentation by *Saccharomyces cerevisiae*. *Food Chem.* 134, 933–939.

Fleet, G. H. (1993). *Wine microbiology and biotechnology*. First. , ed. G. H. Fleet New York: Taylor & Francis.

- Giorello, F. M., Berná, L., Greif, G., Camesasca, L., Salzman, V., Medina, K., et al. (2014). Genome Sequence of the native apiculate wine yeast *Hanseniaspora vineae* T02/19AF. *Genome Announc.* 2, e00530-14.
- Henschke, P. A., and Jiranek, V. (1993). Yeasts-metabolism of nitrogen compounds. *Wine Microbiol. Biotechnol.*, 77-164.
- López, S., Mateo, J., and Maicas, S. (2015). Screening of *Hanseniaspora* Strains for the Production of enzymes with potential interest for winemaking. *Fermentation* 2, 1.
- Martí-Raga, M., Martín, V., Gil, M., Sancho, M., Zamora, F., Mas, A., et al. (2016a). Contribution of yeast and base wine supplementation on sparkling wine composition. *J. Sci. Food Agric.* 96, 4962-4972.
- Martin, V., Giorello, F., Fariña, L., Minteguiaga, M., Salzman, V., Boido, E., et al. (2016b). De novo synthesis of benzenoid compounds by the yeast *Hanseniaspora vineae* increases the flavor diversity of wines. *J. Agric. Food Chem.* 64, 4574-4583.
- Masneuf-Pomarede, I., Bely, M., Marullo, P., Albertin, W. (2015). The genetics of non-conventional wine yeasts: current knowledge and future challenge. *Front Microbiol.* 6, 1563.
- Medina, K., Boido, E., Dellacassa, E., and Carrau, F. (2012). Growth of non-*Saccharomyces* yeasts affects nutrient availability for *Saccharomyces cerevisiae* during wine fermentation. *Int. J. Food Microbiol.* 157, 245–250.
- Medina, K., Boido, E., Fariña, L., Gioia, O., Gomez, M. E., Barquet, M., et al. (2013). Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with *Hanseniaspora vineae*. *Food Chem.* 141, 2513-2521.
- Mostert, T. T. (2013). Investigating the secretome of non *Saccharomyces* yeast in model wine. Unpublished M. Sc. Thesis. South Africa, Stellenbosch Inst. Wine Biotechnol. Fac. Agric. Sci. Stellenbosch University, 98.
- Padilla, B., Gil, J. V, and Manzanares, P. (2016). past and future of non-*Saccharomyces* yeasts: from spoilage microorganisms to biotechnological tools for improving wine aroma complexity. *Front. Microbiol.* 7, 411.
- Pérez, G., Fariña, L., Barquet, M., Boido, E., Gaggero, C., Dellacassa, E., et al. (2011). A quick screening method to identify β -glucosidase activity in native wine yeast strains: application of esculin glycerol agar (EGA) medium. *World J. Microbiol. Biotechnol.* 27, 47–55.
- Ribereau-Gayon, P., Dubourdieu, D., Doneche, B., and Lonvad, A. (2003). *Tratado de Enología: volúmenes I y II*. Ed. Hemisferio Sur-Mundi Prensa, Madrid.
- Tristezza, M., Tufariello, M., Capozzi, V., Spano, G., Mita, G., Grieco, F. (2016). The oenological potential of *Hanseniaspora uvarum* in simultaneous and sequential co-fermentation with *Saccharomyces cerevisiae* for industrial wine production. *Front. Microbiol.* 7, 670.
- Varela, C. (2016). The impact of non-*Saccharomyces* yeasts in the production of alcoholic beverages. *Appl. Microbiol. Biotechnol.* 10, 9861-9874.
- Waters, E. J., Alexander, G., Muhlack, R., Pocock, K. F., Colby, C., O'Neill, B. K., et al. (2005). Preventing protein haze in bottled white wine. *Proteins* 11, 215–225.