Isolation and characterization of autochthonous *Saccharomyces cerevisiae* from "Pago" Merlot wines of Utiel-Requena (Spain) origin

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Abstract

Background and Aims: a) to investigate *S. cerevisiae* yeast diversity in a spontaneous "Pago" Merlot fermentation from the Utiel-Requena region (Spain); b) to characterize *S. cerevisiae* isolates by a holistic procedure using the same Merlot grape must from which they were isolated.

Methods and Results: Yeast identification and typing were performed by ITS and the HinfI mDNA restriction analysis, respectively. Growth and metabolic characteristics were determined by laboratory-scale Merlot must fermentations. Wines were obtained by microvinifications (50 L), and their polyphenolic and volatile compound compositions and sensorial attributes were determined. Twelve *S. cerevisiae* strains were isolated and characterized. Strains 2E, 4A, 7A and 7F showed better growth abilities (AUC). Strains 9C and 7F conferred wines good intensity and colour quality, marked intensity and aroma quality, fruity character and better overall quality. Strain 9C displayed poor growth abilities.

Conclusions: Strain 7F combined good growth aptitudes and is able to confer Merlot wines the best colour, aroma and flavour characteristics during microvinifcations.

Significance of the Study: *S. cerevisiae* characterization made entirely in Merlot grape must allowed the influence of yeast strains on the final characteristics of industrial-scale Merlot "Pago" wines to be more accurately deduced.

Keywords: *Aroma compounds, colour parameters, Saccharomyces cerevisiae, sensorial evaluation, yeast characterization*

1. Introduction

In today´s globalized market, apart from high quality, wines must exhibit personality and originality, and be clearly distinguished from others from the same grape variety or region. Many factors influence wine characteristics: geography, climate, soil composition, viticultural and enological practices, and grapevine and fermentation-associated microorganisms. The grapevine phyllosphere holds diverse microbes that affect grapevine health, growth, and grape and wine production (Liu et al. 2019|). Fermentation-associated microorganisms modulate the flavour and aroma of final wines (Swiegers et al. 2005). Given this scenario, spontaneous fermentations provide wines with more distinctive traits than inoculated fermentations.

Spontaneous fermentation is performed by genotypically different yeast strains expressing distinctive phenotypic characteristics, which confer wines distinct sensorial characteristics (Capozzi et al. 2015). However, performing fermentation with spontaneous microbiota, changing every year, hinds the fermentation management and results in wines with very different characteristics year after year (Ciani et al. 2010, Pretorius 2000). These drawbacks can be overcome by inoculating commercially selected yeasts. The predominance of *Saccharomyces* species, and their special relevance in the winemaking process, have led companies to produce wine yeast starters to focus their efforts on selecting strains *Saccharomyces cerevisiae* (Petruzzi et al. 2017). Although these companies have extensive yeast catalogs that help to obtain the winemakers' desired wine profile, the generalized use of selected cultures is a simplification of microbial fermentation communities, which leads to the standardization of sensorial wine properties. The use of starters consisting in selected mixed non-*Saccharomyces*/*Saccharomyces* or multiple *Saccharomyces* strains could be a valid alternative for minimizing the microbial spoilage risk and maintaining wine typicity/distinctiveness (Capozzi et al. 2015, Chambers and Pretorius 2010, Roudil et al. 2019). Native yeasts have been naturally adapted to the environmental and soil-climatic characteristics of the "terroir" for centuries, and are better prepared to cope with specific fermentation conditions than commercial cultures (Aponte et al. 2016, Blanco et al. 2012, Viramontes and Pérez Lea 2014). Native yeasts also provide wines with characteristic profiles that enhance "terroir" distinctiveness. Their use helps to maintain the biodiversity of each viticultural area, and ensures better implantation given better adaptation to the habitat where they were isolated from. The use of autochthonous yeasts is also interesting for organic wines production, whose vinification is based on reducing exogenous additives or exogenous microorganisms during fermentation (Berbegal et al. 2017).

Many yeast species are naturally present in grape must, but the most abundant are non-*Saccharomyces* strains. These yeasts could play a beneficial role by adding aroma and flavour complexity, but also a detrimental one depending on the yeast type present and its relative abundance. However, the selective pressures prevailing during winemaking processes favor the dominance the most efficient fermentative yeast, *S. cerevisiae*, from the few first hours of fermentation. Hence this yeast greatly modulates wine chemico-sensorial characteristics. A vast *S. cerevisiae* genetic diversity has been recorded by many studies (Khan et al. 2000, Tristezza et al. 2013, Vigentini et al. 2015), which translates into variable amounts of fermentative by-products with desirable or undesirable effects on wine bouquet (Capozzi et al. 2015). Selecting appropriate strains from spontaneous wine fermentation requires a proper characterization programme. This characterization is directed to check good fermentative abilities (technological properties like growth or fermentation kinetics, sugar exhaustion and low volatile acidity) and good sensorial properties in yeasts (quality traits like aroma compounds production, colour stability and sensorial quality) (Belda et al. 2014, Krieger-Weber 2017). The selection of proper strains is also conditioned by the wine style defined by consumer preferences or winemakers' desires (Goold et al. 2017, Quirós et al. 2014). To enhance wines' "terroir" character, the isolation of *S. cerevisiae* strains from the spontaneous fermentation of wines seems the best strategy. This approach has been applied to search for native *S. cerevisiae* strains from: Montepulciano d`Abruzo, Moscato de Saracena, Nero d'Avola and Grillo de Marsala fermentations in Italy (Aponte et al. 2016, Capece et al. 2010, Settanni et al. 2012, Suzzi et al. 2012); Devín, Pálava, Moravian Muscat and Dunaj, Pinot Gris and Pinot Noir fermentations in Czech Republic and Slovakia (Ďurčanská et al. 2019, Schvarczová et al. 2017, Sůranská et al. 2016); Monastrell, Treixadura, Godello and Albariño (Blanco et al. 2012, Mateo et al. 1992) fermentations in Spain.

"Pago" wine is a wine category, and is actually the highest category to exist in the Spanish wine law (Law 6/2015, D.O.&G.I.). The Vineyard and Wine Act 24/2003 of 10 July states that a "Pago" is "a rural site with particular edaphic and microclimate characteristics which differentiate it from its environment and where wines of singular features and qualities are obtained". The existence of a microbiota in vineyards and cellars confers these wines additional distinctive characteristics. Hence the use of autochthonous yeasts is especially relevant for "Pago" wines. The grape varieties of "Pago" wines must be native to the area geographical area, or adapted to the "Pago" habitat. One of the most appreciated variety grape to produce "Pago" quality wines is Merlot. Originally from Bordeaux, it is one of the most widespread varieties worldwide, and has perfectly adapted to many Spanish areas, including

the region Utiel-Requena where this "Pago" is located. Requena Merlot grape provides well-structured wines with intense colour, and a powerful, complex and elegant aroma when cultivated under suitable conditions and harvested at the optimum maturity time. Currently, interest in exploring the biodiversity of specific "terroirs" or "Pago" has increased to find better fitting yeast to ferment and confer distinctive characteristics to the wines produced in these places (Capozzi et al. 2015, Fleet 2008, Suarez Lepe et al. 2012).

This work aims to investigate the *S. cerevisiae* diversity associated with the spontaneous Merlot grape must fermentation of "Pago" wines in the Utiel-Requena region, and to select the most appropriate strains to achieve a high quality and consistent product. The novelty of this research lies in applying a holistic procedure that includes not only the study of yeasts' growth and fermentative behavior, but also the analysis of yeasts' influence on aroma and polyphenol composition, ands on sensorial wine characteristics.

As far as we know, this is the first research work to illustrate the selection, production and a realistic validation of authochthonous *S. cerevisiae* starter cultures that can be adopted for the vinification of "Pago" Merlot wines from the Utiel-Requena origin.

2. Material and Methods

2.1. Winery characteristics and yeast isolation

The "Pago" winery has a 30.89-hectare vineyard, of which 4.19 ha are used for the Merlot variety. This "Pago" produces approximately 100,000 kg of grapes/year, of which 10,360 kg correspond to the Merlot variety. Wine fermentation is exclusively performed by indigenous yeasts, and commercial yeasts have never been used. Yeasts were isolated from spontaneous fermentation (10000 L vats) of Merlot grape must (20.50º Brix; 5.90 g/L total acidity; pH 3.53). Samples were taken at three different times during the winemaking process: from grape must before fermentation (M), halfway (MAF) and at the end of spontaneus alcoholic fermentation (EAF). Having appropiately diluted samples in saline solution, they were spread on Yeast extract, Peptone, Dextrose (YPD) plates, and incubated at 28ºC for 48-72 h. The colonies that appeared on plates were counted and isolated in the same medium. After ensuring purity, they were grown in YPD broth and stored glycerinated at -20ºC in equal 30% glycerol volumes.

2.2. Yeast identification and typing

Isolates were identified by the ITS length analysis. The ITS1 and ITS4 primers, described by Esteve-Zarzoso et al. (1999), were used to amplify a region of the rRNA gene repeat unit,

151 which includes internal transcribed spacers ITS1 and ITS2, and the 5.8S rRNA gene. The
152 DNA source was a cellular suspension made by dissolving one yeast colony in 25 µL of
153 sterile Milli-Q water. The ITS fragment amplification was performed in a 50 µL total reaction
154 volume containing 5 µL of the reaction buffer, 2 µL of $MgCl_2$ (50 mM) and 0.5 µL of
155 EuroTaq Taq Polymerase (5 U/µL) of the kit provided by EuroClone (Milan), 1 µL of both
156 primers ITS1 and ITS4 (50 µM), 1 µLof DNTPs (40 µM) from Roche, 14.5 1 µL of Milli-Q
157 water and 25 µL of a cellular suspension made as previously described. ITS fragments were
158 separated by electrophoresis on 2% agarose gel in 0.5X TBE buffer (44.5 mM Tris-borate, 1
159 mM EDTA, pH 8) at 90 V for 4 h and 15 minutes, and were then stained with ethidium
160 bromide. Sequencing of ribosomal fragments was performed at the Servei Central de Suport a
161 la Investigació Experimental (SCSIE) of the Universitat de Valencia.

162 All the isolates identified as *S. cerevisiae* were typed by a mitochondrial DNA digestion
163 (mDNA) analysis using HinfI as the restriction enzyme under the conditions described by
164 Querol et al. (1992), with some modifications. DNA extraction was performed on 5 mL YPD
165 (1% yeast extract, 2% peptone, 2% glucose) overnight yeast culture. After recovering cells by
166 centrifugation, they were washed with 1 mL of Milli-Q water and centrifuged again. Cells
167 were resuspended in 0.5 mL of 0.9 M sorbitol, 0.1 M EDTA pH 7.5, to which 0.03 mL of
168 1.15 mg/mL of freshly made Zymolyase 20T solution (Seikagaku Corporation, Tokyo) were
169 added. Tubes were incubated at 37°C for 60 min and then centrifuged. The sediment was
170 dissolved in 0.5 mL of 50 mM Tris-HCl, 20 mM EDTA, pH 7.4, to which 13 µL of SDS
171 10%, pH 7.2, were added and then gently stirred. The mixture was incubated at 65°C for 10
172 min. After incubation, 0.2 mL of 5 M potassium acetate were added. After gently mixing,
173 tubes were placed on ice for 5 min and were then centrifuged at maximum speed in a
174 microfuge for 10 min. The supernatant was transferred to a new microfuge tube and DNA was
175 precipitated by adding 1 vol. of isopropanol cooled at -20°C. After stirring by inversion and
176 incubation at room temperature for 5 min, the mixture was centrifuged for 10 min. Once the
177 supernatant had been discarded, the DNA sediment was resuspended in 0.5 mL of 70%
178 ethanol and then centrifuged in a microfuge for 2 min. Finally, the sediment was vacuum
179 dried and dissolved in 0.03 mL of Tris-EDTA at pH 8.

180 HinfI restriction digestion was performed using 10 µL of the extracted DNA, 2 µL of reaction
181 buffer R and 1 µL of HinfI (10 U/µL) from Sigma, 1 µL RNAase (4 mg/mL) from Roche and
182 6 µL Milli-Q water. The reaction mixture was incubated at 37°C overnight. The restricted
183 DNA was electrophoresed on 0.8% agarose gel in 0.5X TBE buffer at 20 V for 16 h before

being stained with ethidium bromide. Gels were digitalized and Hinf mDNA restriction profiles were compared to one another to classify isolates based on similarities. To do so, the BioNumerics 5 software (Applied Maths, Kortrijk, Belgium) was used. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was selected as the comparison method yb employing the Pearson´s Product-Moment Coefficient. The isolates belonging to the same mDNA retriction profile were considered to be the same strain. One representative isolate of each mDNA restriction profile was chosen to be characterized as described below.

2.3. Yeast characterization

The yeast characteristics considered for yeast evaluations were growth and fermentation kinetics, and ability to produce secondary fermentative products (glycerol, acetic acid). These characteristics were determined in the same Merlot must from which yeasts were isolated.

Merlot must was pretreated to eliminate any existing microorganisms before yeast inoculation. Merlot must was centrifuged (Beckman coulter Avanti J-E, JA10 rotor) at 10000 rpm and 4ºC for 40 min to eliminate solids and most native microorganisms. The supernatant was sterilized by adding 0.25 g/L of Velcorin® (Lanxess, Germany). Antiseptic was added to must and left to act at room temperature for 5-6 h before yeast inoculation. Yeasts were grown in YPD broth at 28ºC for 48 h and yeast concentrations were determined by microscopic counting in a Thoma chamber and by inoculating YPD plates. Yeasts were inoculated in Merlot must at a final concentration of $2x10^5$ CFU/mL. Inoculated musts were incubated at 28ºC for 14 days. Fermentation was done in triplicate. Samples were taken on days 1, 4, 7 and 14. A must sample before inoculation (time 0) was analyzed. Yeast growth was monitored by plate counting the samples that were recovered during fermentation. The parameters considered for characterizing yeast growth were maximum growth rate (μ_{max}), maximum cell count (MCC), Final Cell Count (FCC) at 14 days, and Area Under the Curve (AUC). The μ_{max} values were calculated as the rate between the increased viable cell counts and time in the exponential growth phase (Δ CFU/mL/h). MCC was the highest yeast CFU/mL during growth. The FCC was expressed as CFU/mL when the experiment ended (day 14). The AUC measures the whole two-dimensional area underneath the entire growth curve (Lucio 2014) considering two growth times, from 0 to 14 days in our case. Glucose, fructose, ethanol, glycerol and acetic acid concentrations were established during fermentation by high-performance liquid chromatography (HPLC) and the procedure described by Frayne (1986).

The influence of yeast on the polyphenol composition, aroma characteristics and sensorial attributes of Merlot wines was determined by microvinification in the Merlot grape added with SO_2 g/L as described below.

2.4. Microvinifications

The identified *S. cerevisae* strains were tested by microfermentation assays conducted with Merlot grape must ("Pago" Chozas Carrascal) at the experimental winery of the Universitat Politècnica de València (Spain). Vinifications were done in triplicate. Grapes were harvested manually in boxes (10 kg), destemmed and crushed, and mixed and divided into 42 closed glass 2-kilogram pots. Immediately 200 mg/kg of Velcorin® were added to eliminate the autochthonous microbiota of grapes before being subsequently sulphited with potassium bisulphite at a rate of 50 mg per grape kilogram.

The 12 isolated *S. cerevisiae* strains were inoculated 24 h later at the 2.10^5 CFU/mL concentration. Alcoholic fermentation was performed at 25-26ºC. Manual punching down was done twice daily to favor the extraction of polyphenolic compounds. Fermentation was monitored by determining temperature and density to check for adequate fermentation kinetics and lack of fermentation stucks. Wines were left in skins for 10 days and devated when sugar levels went below 2 g/L. When alcoholic fermentation finished, *Oenococcus oeni* strain OE104 (Agrovin, Spain) was inoculated and malolactic fermentation was conducted at room temperature (approx. 20ºC). Wines ended malolactic fermentation between 15 and 20 days. Potassium metabisulphite was added at 100 mg/L before bottling. Wines were stored at room temperature (about 15±2ºC) for 1-2 months. Then polyphenolic, aromatic composition and sensorial characteristics were determined.

2.5 Analytical methods

The common parameters (density, ethanol, pH, total and volatile acidity) in musts and wines were determined according to EU Regulation Official Methods (2676/1990). Total soluble solids (ºBrix) were determined by refractometry and reducing sugars by the Fehling method (Blouin 1992).

Spectrophotometric and chromatographic analyses were undertaken in an UV-Visible JASCO V-530 spectrophotometer, equipped with a JASCO MD-2010 Plushigh-performance liquid chromatography instrument coupled to a diode array detector (DAD)(JASCO LC-NetII/ADC, Tokyo, Japan). Both devices took phenolic measurements. Colour intensity, hue value and

ethanol index (that measures the tannin concentration of polysaccharide-linked molecules) were analyzed according to Glories (1984). The Ribéreau-Gayon and Stronestreet (1965) method was followed to determine the bisulphite non-bleached anthocyanins (coloured anthocyanins). Catechins were quantified by the method reported by Sun et al. (1998). Total condensed tannins were assessed after heat transformation into anthocyanidins in acidic medium (Ribéreau-Gayon 1979). The PVPP (anthocyanin-tannin complexes) and DMACH (tannin degree of polymerization) indices were calculated according to Vivas et al. (1995).

High-performance liquid chromatography was utilized to quantify the individual phenolic compounds via the method reported by Jensen et al. (2007). Total anthocyanins were calculated as the sum of anthocyanidin-3-glucosides and derivated anthocyanins. In each phenolic group, compounds were identified based on their intrinsic spectral features and retention times. Commercial standards were employed to build the calibration curves for phenolic quantifications: flavan-3-ols (Fluka, Milwaukee, WI, USA) and malvidine-3-glucoside (Sigma-Aldrich, St Louis, MO, USA) for anthocyanins. After centrifugation (5000 rpm) and filtration (0.45 mm membrane Millipore filter), 20 mL of the wine sample were injected twice. Separation was performed in a Gemini NX (Phenomenex, Torrance, CA, USA) 5 mm, 250 mm x 4.6 mm i.d. column at 40ºC. Acetonitrile and *o*-phosphoric acid were used as solvents. Solvent composition and the elution gradient are reported elsewhere (Jensen et al. 2007).

Wine volatile composition was analyzed by a HP-6890 gas chromatograph. Extraction of volatile compounds was done following the procedure proposed by Ortega et al. (2001) with slight modifications. A 2.7 mL volume of samples was transferred to a 10 mL screw-capped centrifuge tube containing 4.05 g of ammonium sulfate to which the following compounds were added: 6.3 mL MilliQwater, 20 µL standard internal solution (2-butanol, 4-methyl-2-pentanol and 2-octanol, at 140 µg/mL each), and 0.25 mL dichloromethane. The tube was mechanically shaken for 120 min and centrifuged at 4000 rpm for 15 min. The dichloromethane phase was recovered with a 0.5 mL syringe, transferred to the autosampler phial and analyzed. A chromatographic analysis was run in a Phenomenex ZB-Wax plus column (60 m x 0.25 mm x 0.25 µm). The column temperature was initially set at 40ºC and was left at this temperature for 5 min before being raised to 102ºC at a rate of 4ºC/min; to 112ºC at a rate of 2ºC/min; to 125ºC at a rate of 3ºC/min and left at this temperature for 5 min; then raised to 160ºC at a rate of 3ºC/min; to 200ºC at a rate of 6ºC/min and then left at this temperature for 30 min. The carrier gas was helium and was fluxed at a rate of 3 mL/min. Injection was done in the split mode 1:20 (injection volume 2 µL) with a flame-ionization-

detector (FID detector). Volatile compounds were identified by comparing the retention time with that of the commercial standards.

The sensory analysis of the fermented wines with the different *Saccharomyces cerevisiae* strains was tasted by a panel of 10 expert tasters, previously submitted to selection and training. Tasting took place under standardized conditions in a tasting room with standard cabins (UNE EN ISO 8589). Firstly, Triangular Tests (ISO 4120) were undertaken for the three repetitions of each wine to ascertain whether there were sensorial differences between them before obtaining the average of the sensory analysis values. The descriptive and quantitative scalar sensory analysis (QDA)(ISO 8589, ISO 3591, ISO 11035) was performed during a single session to avoid the influence of tasters' different physical conditions on wine appreciations.

2.6. Statistical analysis

All the analyses were submitted in triplicate for each fermentation replicate. The results are expressed as mean values±standard deviation. To know if yeast significantly affected the physico-chemical, polyphenol and volatile aromatic composition of wines, a simple ANOVA analysis was run by taking a 95% confidence level. The existence of significant differences between yeasts was studied for each parameter. The statistical Statgraphics Centurion XVI software was used for this processing.

Spearman correlation analyses were performed between growth parameters (μ_{max}, MCC, FCC and AUC), glucose and fructose consumptions and ethanol, glycerol and acetic acid production on days 4, 7 and 21. Calculations were done with the GraphPad 5 software.

In order to simplify the results, a principal component analysis (PCA) and orthogonal projections to the latent structures discriminant analysis were performed with SIMCA, version 10. With the PCA, we transform a set of intercorrelated variables with another set of uncorrelated variables, called principal Components, which are a linear combination of the original variables The first main component extracted in the analysis is that which best summarises the information contained in the original data matrix. That is, it is the one which best explains total variance. The second component best summarises the remaining variance and is independent of the first one. The sequence continues to extract factors until total variance is explained.

3. Results and Discussion

317 3.1 Yeast isolation and identification

318 The grape must obtained from an industrial fermenting vat had a total yeast count of $4.6x10^5 \pm$
319 $4.6x10^4$ CFU/mL. The microbiota was composed mainly of *Hanseniaspora uvarum* (53.4%)
320 and *Saccharomyces cerevisiae* (43.6%), whereas low percentages of *Torulaspora delbrueckii*
321 and *Metscnikowia pulcherrima* were detected (1.5% and 1.4%, respectively). The yeast
322 population grew to reach $4.1x10^7 \pm 4.2x10^6$ CFU/mL at MAF, and diminished slightly to
323 $1.3x10^7 \pm 7.1x10^5$ CFU/mL at the end of fermentation. At MAF, most yeasts belonged to *S.*
324 *cerevisiae* (95%), but *H. uvarum* was still present (5%). At EAF, the only remaining yeast
325 was *S. cerevisiae* (100%).

326 Of the 40 isolated obtained from grape must, and the MAF and FAF samples, 37 were
327 identified as *S. cerevisiae* based on their ITS fragment length (850 bp, Fig. 1), *H. uvarum*
328 (760 bp, Fig. 1), *T. delbrueckii* (800 bp, Fig. 1) and *M. pulcherrima* (390 bp, Fig. 1) and
329 sequence (data not shown).

330 The mDNA analysis results showed that the 37 isolates were grouped in 12 different patterns
331 at the 91.2% cutoff level (Fig. 2). The isolates grouped in the same profile were considered to
332 belong to the same strain. The most representad patterns (strains) in the Merlot fermentations
333 were patterns 3 and 4, respectively consisting of eight and twelve isolates. The other groups
334 contained one, two or three isolates (Table 2). The number of different strains (patterns)
335 isolated at different AF times were: eight in grape must, seven at MAF and seven at EAF.
336 Some strains were isolated only at one of the three assayed fermentation times: patterns 10
337 and 11 were exclusively present in grape must (represented by isolates 2F, and 4A), pattern 8
338 (represented by isolate 7D) at MAF, and patterns 2 and 12 (represented by isolate 9C and
339 isolate 10B, respectively) when AF ended. Other patterns were isolated throughout the
340 fermentation process as numbers 3, 4 and 9 (represented by isolates 7F, 2A and 7A,
341 respectively).

342 A similar scenario was reported by Sabate et al. (1998) after analyzing two industrial
343 vinifications for 2 consecutive years in the Priorat region (Spain). They found 60 and 86
344 different strains from 400 isolates recovered for 2 consecutive years, of which only two
345 strains were present throughout the fermentation time, whereas the rest were present only at
346 one fermentation time or two. A similar percentage of different strains and an alike
347 dominance scenario were herein found. The dominance of one *S. cerevisiae* strain or two is a
348 frequent situation in spontaneous fermentations, as Ribéreau-Gayon et al. (2000) reported.

349

350 3.2. *S. cerevisiae* yeast characterization

351 The growth kinetics and fermentative characteristics of the 12 *S. cerevisiae* strains were
352 evaluated in the same Merlot grape must used for industrial vinification to obtain results that
353 could be directly extrapolated to such wines. According to Pereira et al. (2020), the rapid
354 capacity of transforming sugars into ethanol and this efficiency transformation are two of the
355 main selection criteria in the alcoholic beverage industry, which were contemplated herein
356 along with others, such as growth abilities or secondary product production.

357 Yeast strains showed different abilities to grow in terms of their μ_{max}, MCC, FCC and AUC
358 (Fig. 3). The faster growing strains (with higher μ_{max}) were 7D, 10B, 7E , 7A and 7F, whereas
359 the slower ones were 2G, 9C, 4A and 2A (Fig. 3A). Higher MCC were attained by strains 4A,
360 7A, 2F, 7F, 7D, 7I, and lower MCC by 2G, 2E, 10B, 2A and 9C (Fig. 3B). The yeast showing
361 the higher FCC were 4A, 7F and 2F, whereas those exhibiting the lower FFC were 2G, 9C
362 and 10B (Fig. 3C). Considering the AUC, which as a mesure of overall growth, the yeasts
363 with higher AUC values (better growth abilities) were 2F, 7A, 4A, 7D and 7E. Those with
364 lower values were 2G, 2E, 10B y 9C (poor growth) (Figs. 3 D and E).

365 The AUC values and efficiencies in sugar exhaustion (glucose and fructose), and in ethanol,
366 glycerol and acetic acid production, were estimated after 4, 7, and 14 days from the beginning
367 of AF (Fig. 4). The differences in the AUC values of the strains remained at the three
368 sampling times, with some exceptions; strain 7F had comparatively higher AUC at the end
369 than at the beginning of AF, which meant that growth began slowly for the first days 7 days,
370 but then remained at a high cell concentration longer than others, such as 7D and 7E (Fig.
371 4A). When considering the fermenting must's chemical composition, the biggest differences
372 between strains appeared on day 4. On the 4 first days, the yeasts that consumed the highest
373 glucose quantities were 7D, 7E, 7A and 7I, and those that degraded lesser glucose were 2G,
374 and 2A (Fig. 4B). As AF progressed, differences in sugar comsumption diminished. After 14
375 days, all the strains had consumed the same quantities of glucose, except for the strain 2G
376 (Fig. 4B). Bigger differences were found in fructose consumption: the strains that consumed
377 larger fructose quantities were 7D and 7A, 9C and 7I, and those that consumed the smallest
378 were 2G and 2A (Fig. 4C). The strains that produced the largest ethanol quantities on the first
379 4 days were 7E, 7D, and 7I, which were the faster degrading glucose strains. One of these
380 yeasts, strain 7E, was the highest ethanol producer throughout fermentation, and generated
381 0.7% (v/v) more ethanol by the end of the process than strain 2A, which was the second best
382 producer despite being a moderate sugar consumer on the first 4 days. The strains that
383 produced less ethanol after 21 days were 2G, 4A and 9C (Fig. 4D). The strains that yielded

more glycerol were the same on days 4, 7 and 14 (7D, 7I, 7F, 7E, 7I), although the relative order between them varied with time. The lesser glycerol producers after 14 days were 2G, 2E and 9C, with lower AUC values during the experiment (Fig. 4E). The differences in glycerol production between strains could be due to distinct activities or the concentration of the key enzyme triosephosphate isomerase, which catalyzes the triosephosphates interchange (Rodicio and Heinisch 2017). The strains that yielded more acetic acid on the first 4 days were 7D, and 7I, but other strains became the biggest producers after 14 days: 7A, 10B and 9C. Strains 2A and 4A yielded the lowest acetic concentrations on days 4 and 7, but the lowest producers after 14 days were 2A, 7F, 4A and 2F. Differences in acetic acid production were possibly related to the different acetyl-CoA synthetase capacities of strains. Thus poor activities of this enzyme caused acetate overflow (Rodicio and Heinisch 2017).

3.3.Correlation analysis

The Spearman correlation analysis applied to the data obtained on day 7, when an average of 70% sugar had been consumed. It showed that μ_{max} did not correlate with the other growth parameters (Table 2), which were deduced from the yeast growth kinetics (Figure 3D), such as: cell concentration, maximum cell concentration (MCC) and AUC value. However, the MCC correlated with AUC.

The correlation analysis performed between the growth parameters and the yeast metabolism-related parameters showed that μ_{max} correlated positively and significantly with glucose consumption, ethanol and glycerol, but not with fructose consumption or acetic acid production (Table 2). The 7-day cell concentration and AUC correlated with ethanol and glycerol production, whereas MCC did so only with glycerol production. A positive correlation was expected between μ_{max} and both glucose exhaustion and ethanol production because *S. cerevisiae* obtains energy from sugar fermentation for growth (two ATP moles per glucose mole) (Rodicio and Heinisch 2017). Hence the higher both alcohol production and glucose consumption are, the faster cell growth is. Pereira et al. (2020) stated that μ_{max} affected both sugar consumption and efficiency ethanol production in a sugary substrate. So this parameter should be considered to be one of the main criteria for selecting a starter for alcoholic beverage industries. However, despite some strains having a high μ_{max}, they were neither the highest glucose consumer nor the biggest ethanol producer.

The correlation analysis run between the yeast metabolism-related parameters revealed that glucose depletion correlated positively and significantly with fructose degradation, ethanol

and glycerol production, but not with acetic acid production (Table 2). Fructose degradation correlated with glucose consumption, and ethanol and acetic acid production, but not with glycerol. Ethanol production correlated with all the yeast metabolism-related parameters, except for acetic acid production. Finally, acetic acid production only correlated with fructose depletion.

The different correlation results among the considered parameters appeared at several fermentation time points (Tables S1 and S2). Glucose and fructose consumption correlated positively for 14 fermentation days, while residual fructose was higher than the glucose concentrations in the finished wines. Berthel et al. (2004) indicated that ethanol had a stronger inhibitory effect on fructose than on glucose utilization. Theoretically, the synthesis of glycerol from sugars occurs mainly at the beginning of alcoholic fermentation when enzymes pyruvate decarboxylase and alcohol dehydrogenase are not fully expressed (Goold et al. 2016, Rodicio and Heinisch 2017). So larger amounts of glycerol are expected to be generated at the beginning of AF, as in our experiments (Fig. 4E). Glycerol is synthesized as a way to re-oxidise the NADH produced during glycolysis. Thus dihydroxyacetone phosphate is reduced to glycerol (Rodicio and Heinisch 2017). Unexpectedly, the strains that produced more ethanol did not generate less glycerol and the correlations between these products were always positive instead of negative whatever the fermentation time (Suplementary Tables S1 and S2). We stress that the above-presented results were obtained from the fermentation performed with the sterile Merlot grape must. Under these conditions, the inoculated strain was the only one to perform alcoholic fermentation. Different results can be obtained when microvinification is performed with incompletely sterile grape must, in which competition between inoculated and native yeasts certainly took place.

3.3 Physico-chemical parameters of Merlot microvinifications

Table 3 contains the mean and standard deviation values and the ANOVA of the wine physico-chemical parameters obtained from microvinifications. All the tested yeasts completely consumed sugars; the residual sugars in wines ranged between 1.7 and 2.5 g/L, which fall in line with those usually reported in wines (Figueiredo-Gonzalez et al. 2013). Volatile acidity ranged from 0.32 to 0.65 g/L, which are usual in industrial wines (Vigentini et al. 2017). pH values hardly differed, only by 0.08 units. The wines with the lowest (3.47) and highest (3.55) pH values were those fermented with strain 7I and strain 2G, respectively. Wine pH affects taste, colour, oxidation degree, among other factors (Schvarczová et al. 2017). The pH values of the resulting wines were low enough to avoid physico-chemical and

451 microbial alterations (Forino et al. 2020). The total acidity and alcoholic degree of wines
452 varied from 6.38 to 6.97 g/L, and from 12.53 to 13.43% vol/vol, respectively. The wines
453 fermented with 7I, 7A and 2F had higher total acidity values (6.97-6.81 g/L), whereas those
454 fermented with strains 7E, 2A and 2G had lower ones (6.25-6.38 g/L). A 0.90% difference in
455 the ethanol degree was found between the wines fermented with the highest and lowest
456 ethanol producer yeasts; the wines with higher alcoholic degrees were those fermented with
457 7D, 7A, 7I and 7E (13.43-13.37%), whereas lower contents (12.53-12.67%) were for those
458 fermented with 2A, 2G and 10B. Yeasts providing high acid and low ethanol contents are
459 recommended for fermenting low acidity and high sugar content meridional grape must,
460 which present an imbalanced composition because of the climate change (Gobbi et al. 2013).

461 Other authors approached a similar *S. cerevisiae* selection programme as we did to choose the
462 appropriate strains for fermenting grape must from different varieties (Callejón et al. 2010,
463 Nikolau et al. 2006, Schvarczová et al. 2017), but our procedure provides more consistent
464 results because was performed using the same grape must in which yeast will be inoculated.

465

466 3.4 Polyphenolic composition of Merlot microvinifications

467 Table 4 shows the values for the polyphenol parameters in the wines fermented with different
468 yeasts. From the colour-related parameters, strains 10B, 7I and 9C best maintained wine
469 colour (10.98-10.74), while strains 2E, 7E and 2G led to less coloured wines (8.87-9.36). A
470 2.11 difference (19%) in colour intensity appeared between the least and most coloured
471 wines. Differences in hue were slight. In the wines made with strains 2G and 7F, hue values
472 were higher (57.41-55.9), but lower (50.75-51.58) in those made with strains 7I and 9C,
473 which coincides with the highest colour intensity. The total and coloured anthocyanins
474 concentrations were higher in the wines fermented with strains 9C, 10B and 7I (494.24-483.9
475 mg/L) and (392.6-383.88 mg/L), respectively. In those fermented with strain 2G, the total and
476 coloured anthocyanins concentrations were lower (431.8 and 350.33 mg/L, respectively). The
477 strains conferring high colour intensity, low hue values, and high total and coloured
478 anthocyanins (i.e. 7I, 9C, 10B), are preferred for red winemaking because they provide a
479 stabler colour (Pérez-Lamela et al. 2007).

480 Regarding tannins (compounds responsible for structure and astringency) composition, the
481 higher concentrations were for the wines fermented with strains 7D, 7F and 9C (1.25-1.19
482 g/L) with the lowest ones in the wines fermented with 7E and 2G (1.07-1.08 g/L). With all the
483 polyphenolic compounds (total polyphenols/IPT index), the higher concentrations (3.42-3.39

484 and 40.89-39.87 g/L, respectively) were for the wines fermented with strains 7D, 9C and 10B,
485 and the lower ones for those fermented with strain 2G (2.92 and 35.47 g/L).

486 Wine bitterness, astringency and colour stability depend on the quantity of tannins and on the
487 state in which they are found in wine. Tannins can join to one another, and also with
488 anthocyanins or macromolecules as polysaccharides. The tannin polymerization degree is
489 estimated by the concentration of condensed tannins, and by the DMACH (an index inversely
490 proportional to the tannin polymerization degree). The wines fermented with strains 7F, 9C
491 and 7D had lower DMACH values (67.33-68.28%), whereas those made with strain 7E had
492 the highest DMACH (84.74%). As the DMACH index lowered (i.e. polymerization
493 increased), the catechin concentration also dropped as catechin molecules joined together to
494 form polymers. The ethanol index reports the tannin polymerization degree with
495 polysaccharides. The wines fermented with strains 7E, 10B and 7D presented lower ethanol
496 index values (41.14-43.60%), whereas those fermented with strains 2F, 2A and 2G had higher
497 ones (56.13-54.01%).

498 The wines fermented with strains 9C and 7F displayed lower catechin and higher
499 concentrations of condensed tannins and a lower DMACH index. Using these strains for
500 winemaking guarantees a more agreeable wine mouthfeel.

501 From the results herein obtained, we deduce that yeast strains notably influence colour and
502 the taste of "Pago" Merlot wines. The differences in polyphenolic composition result from the
503 different yeast strain activities (distinct abilities to extract phenolic compounds from grape
504 skins, distinct capacities for adsorbing tannins or coloured compounds on their cell walls, and
505 varying metabolic or enzymatic activities (Bindon et al. 2019, Caridi et al. 2004, 2017,
506 Morata et al. 2003, Rivas-Gonzalo et al. 1995, Sharma et al. 2012). The ability to adsorb
507 anthocyanins and polyphenols (tannins) is a yeast strain-dependent character (Bautista-Ortín
508 et al. 2007, Medina et al. 2005, Morata et al. 2016) and it is related to biomass, membrane
509 composition and cell wall/membrane integrity of each strain (Echeverrigaray et al. 2020, Holt
510 et al. 2013, Rinaldi et al. 2016). The presence of β-glucosidase enzymes in yeasts causes β-
511 glucosidic links between anthocyanin and sugars to break down, which leads to the release of
512 free anthocyanins that are more oxidizable compounds, with the consequent loss of colour
513 quality (Hernández et al. 2003). Different metabolites production by yeasts, like pyruvic acid
514 and acetaldehyde, leads them to react with anthocyanins or to mediate adducts formation
515 between flavanols and anthocyanins, which entails stabler colour (Morata et al. 2016). The
516 polymerization of tannins or tannins with polysaccharides, as respectively measured by the

517 DMACH and Ethanol indices is related to wine mouthfeel and astringency. Fermentative
518 yeasts influence both concentration of wine polyphenolic compounds, as well as the reactivity
519 of these compounds toward salivary proteins that is responsible for wine astringency (Rinaldi
520 et al. 2016). The yeasts possessing β-glucanase activity show higher autolysis percentages,
521 which result in the release of glucans and mannans, and also of mannoproteins from their cell
522 walls (Walker 1998). The binding of these macromolecules to anthocyanins and tannins by
523 their free radicals decreases tannin reactivity and astringency, protects them from
524 precipitation and increases wine smoothness and volume in the mouth (Del Barrio-Galán et al.
525 2012, 2015, Rinaldi et al. 2016, Sacchi et al. 2005) .

526

527 3.5 Aromatic composition of Merlot microvinifications

528 Twenty-three volatile compounds deriving from yeast metabolism, and belonging to five
529 chemical families, were identified in wines: five higher alcohols, seven esters, one lactone,
530 seven acids and three aldehydes (Table 5). Different studies reveal that wine aroma is more
531 affected by odorant families than by individual compounds. The effect of each component of
532 a family of aromas is additive or synergistic. Thus aroma groups are considered instead of
533 individual compounds (Ferreira et al. 2004).

534 The wines fermented with strains 9C, 2G, 2A and 2E had larger amounts of the analyzed
535 alcohols (194.95-184.11 mg/L), wheras those fermented with strains 7A and 4A had lower
536 concentrations (93.55-96.31 mg/L). Higher alcohols are quantitatively the largest group of
537 volatile compounds in wine. The contribution of alcohols to the wine aromatic profile can be
538 beneficial or detrimental depending on the total concentration of alcohol species. If the
539 alcohol concentration does not exceed 350 mg/L, it positively contributes to wine aroma
540 (Ciani and Comitini 2015) by providing fruity or floral notes, depending on their
541 concentration and compound type (Ribéreau-Gayon et al. 1998). 2-phenylethanol is
542 particularly interesting. This compound is related to the aroma of rose petals (Francis and
543 Newton 2005) and was the most abundant in the studied wines. However, excessive
544 concentrations of higher alcohols can confer wine chemical aromas.

545 Although esters are usually found at lower concentrations than higher alcohols in wine, they
546 are a group of compounds with a qualitatively relevant impact on aroma because their
547 concentration in wine generally exceeds its sensory threshold (Ivit et al. 2018, Lambrechts
548 and Pretorius 2000, Torrens et al. 2008). They confer to wine floral and fruit aromas.
549 Although not all esters are beneficial for quality, ethyl and methyl acetate confer an

550 unpleasant solvent aroma at high concentrations, and are considered a defect in wine.
551 However, they provide fruit aromas at low concentrations. The yeast strains herein isolated
552 produced small amounts ethyl and methyl acetate, which ranged between 26.45 mg/L in the
553 wines fermented with strain 2F, and 4.16 mg/L in those fermented with strain 2A. These
554 amounts are below the concentration considered to be detrimental for wines (Gómez-Mínguez
555 et al. 2007). Regarding the other esters herein considered, the wines with higher
556 concentrations were those fermented with strains 9C and 7F (8.43-8.18 mg/L), whereas those
557 fermented with strains 7I had lower concentrations (4.59 mg/L). The higher 2-phenylethyl
558 acetate values, an ester that confers wine fruity, honey and rose aromas (Moreno-Arribas et al.
559 2009), were recorded in the wines fermented with strains 7F and 9C (7.11-6.82 mg/L),
560 whereas lower values were obtained in those fermented with 7I and 4A (3.26-3.75 mg/L).
561 Higher butyrate, octanoate, decanoate and ethyl succinate contents were recovered in the
562 wines fermented by 9C and 7F (8.41-8.11 mg/L), whereas lower concentrations were for
563 those fermented by 7I and 4A (4.53-4.89 mg/L). Strain 9C gave rise to the highest ethyl
564 decanoate and ethyl octanoate concentrations, whereas strain 7F produced more 2-phenylethyl
565 acetate and significant amounts of ethyl decanoate in wines, which all confer wine fruity and
566 floral aromas (Loscos et al. 2007).

567 The wines fermented by strains 9C, 7E, 2F and 7F presented the most γ-butyrolactone (7.54-
568 6.96 mg/L) and those fermented with strain 7A contained the least (3.96 mg/L). This lactone
569 is produced by yeasts from glutamic acid and is most abundant in wines (Wanikawa et al.
570 2001). Its perception threshold is low and it improves aromatic complexity because it is
571 associated with dairy notes. It also contributes to the peach aroma observed in some red wines
572 (Ferreira et al. 2004, Jarauta 2004).

573 Regarding the volatile fatty acids group, the wines showing higher contents of these
574 compounds were those fermented with strains 9C, 7D and 7I (2.87-2.79 mg/L), while that
575 fermented with strain 2A had the lowest values (1.44 mg/L). Volatile fatty acids are related to
576 negative properties, e.g. rancid, fatty or cheese notes, but are important for aromatic balance
577 and wine complexity (Callejón et al. 2010). We highlight their importance because they are
578 precursors of fruity esters. The aromatic influence of these compounds is not as important as
579 that of ethyl esters, but some (hexanoic acid, octanoic acid, decanoic acid, isovaleric acid)
580 have been recently identified as compounds with a strong aromatic impact on wine (Aznar et
581 al. 2001, Komes et al. 2006, Li 2008). These acids have low perception thresholds. When
582 medium-chain fatty acids are below 10 mg/L, they positively contribute to wine aroma by

583 mainly providing dairy notes, but become off-flavours beyond 20 mg/L (Zhang et al. 2013).
584 The concentration of these acids in the wines herein produced is certainly not detrimental.

585 Of the compounds included in the aldehyde group, acetaldehyde is the most abundant. It is
586 produced by pyruvate decarboxylation during the carbohydrate metabolism of yeast. At low
587 concentrations, it provides a fruity aroma of ripe apple and dried fruit, but has a pungent and
588 irritating odor at high concentrations (Arslan et al. 2018, Moreno-Arribas el al. 2009). The
589 yeasts under study are low acetaldehyde producers as the concentration of this compound in
590 the wines ranges from 57.5 to 8.82 mg/L. The diacetyl concentrations in the wines are very
591 low, between 0.05 mg/L in the wines fermented with strain 2G and 0.01 mg/L for those made
592 with strains 7D, 7E and 7F. This compound provides dairy and butter notes, but is undesirable
593 at high concentrations (Jackson 2008). 5-methylfurfural is a furan derivative that confers wine
594 a roasted almond aroma. It is formed mainly during wine barrel ageing and stems mostly from
595 the barrel-toasting process as a consequence of the Maillard reaction of wood carbohydrate
596 compounds (Towey et al. 1996), but can also be synthesized or degraded by yeast during
597 fermentation (Gül et al. 2011). Strains 7I, 10 B and 9C produced higher contents of this
598 compound (0.25-0.23 mg/L), which went undetected in the wines fermented with strains 2E
599 and 2G.

600 From our results, we deduce that yeast strain considerably influences the aromatic
601 composition of Merlot wines. Differences in sugar and amino acid metabolism of yeasts result
602 in differences in higher alcohols, esters, volatile fatty acids and aldehydes (Álvarez-Pérez et
603 al. 2012). Hence studying yeast's ability to produce aromatic compounds is crucial for
604 selecting an appropiate yeast strain (Suárez-Lepe and Morata 2012).

605 The wines fermented with strain 9C had the most beneficial esters (2-phenylethyl acetate,
606 ethyl octanoate, and ethyl decanoate) γ-buyrolactone, fatty acids (isopentanoic and hexanoic
607 acids), 2-phenylethanol and 2-butanediol, whereas those fermented with strain 7F scored the
608 second ones with large amounts of esters and lactones. None of these strains produced wines
609 with high concentrations of undesirable compounds, such as acetaldehyde and diacetyl,
610 among others.
611

612 3.6 Sensory profile of Merlot microvinifications

613 The sensory analysis highlighted that some descriptors were significantly influenced by yeast
614 strains (Table 5). The wines that obtained the highest sensorial scores were those fermented
615 with strain 9C in terms of colour (intensity and quality, 8.8 points out of 10 in both cases),

intensity and aromatic quality (8 and 8.3 points, respectively), red fruit aroma (4.8) and overall quality (7.7). The wines produced with strain 7F were the second most preferred by the sensorial panel, and had similar intensity and quality (8.8 points for both) and intensity of aromas (7.8) scores than those fermented with 9C, and were slightly lower for aromatic quality (7.7), red fruit aroma (4.6) and overall quality (7.3). The colour intensity and colour quality of the wines fermented with strains 2G, 7E and 7D, the aroma intensity and aroma quality of those fermented with strains 2F, 7A, 7D and 7E, and the overall quality of the wines made with 7A and 7D, were also highly rated. No significant differences in the colour intensity between wines were observed, probably because the ability of the human eye to distinguish similar anthocyanin concentrations is limited. However, differences in colour quality were more noticeable. This parameter is related mainly to the coloured anthocyanins concentration, colour absorbance at 520 nm and the hue value.

Regarding aroma, significant differences appeared in aroma intensity, aroma quality and red fruits aroma between the wines fermented by distinct yeast strains. Aromatic quality discriminates those compounds that are organoleptically favorable, e.g. ethyl esters, 2-phenylethanol, γ-buyrolactone, among others, which are normally related to fruit and flower descriptors. These compounds were possibly responsible for the differences in the scores of wine red fruit aromas, just as Antonielli et al. (1999) and Campo et al. (2005) reported. Significant differences were found only in colour quality, and in two out of the 20 aroma and taste attributes considered in the sensory analysis, namely aroma intensity and aroma quality. Indeed lots of the differences observed in the wine volatile aroma composition were undetectable.

The sensory analysis revealed that the highest ranked wines were those fermented with strains 9C and 7F, based on good intensity and colour quality, higher aroma intensity, aroma quality and overall quality. The high olfactory analysis scores reflected the higher concentration of esters that conferred the wines fermented with these two strains a fruity character. Strains 9C and 7F are good candidates for improving the flavour complexity of industrial Merlot wines and could contribute to improve the distinctiveness of this “Pago” wine.

3.7 Multivariate data analysis of Merlot wines

A holistic approach was applied to correlate the physico-chemical, polyphenol and aroma compound contents and sensory parameters of wines with the yeast used for fermentation. A PCA analysis was performed on the 36 wines and 62 variables (6 physico-chemical

parameters, 10 polyphenolic measurements, 23 aromatic compounds, 23 sensory profiles). The bi-plot showed that the first two main components explained 91.2% of the explained variance (PC1 = 66.4% and PC2 = 24.8%) of the dataset (Fig. 5). PC1 positively correlated with the concentration of polyphenols and anthocyanins, wine colour parameters, ethyl decanoate, ethyl succinate and decanoic acid contents, and negatively with acetaldehyde, diacetyl and butyric acid concentrations. PC2 positively correlated with red fruit aroma, aroma quality and ethyl octanoate parameters, and negatively with 2-phenylethyl acetate, alcoholic degree, unctuousness and hue values.

The score plot shows the distribution of yeast strains (Figure 5A), while the loading plot, which indicates the weight of variables, depicts the arrangement of the different chemico-sensory parameters in the plane formed by Components 1 and 2 (Fig. 5B). In the score, we see that strains 9C, 7F and 10B lie in the centre of the coordinate axis, and PC1 has a very important weight in differentiating these three strains from the rest. PC1 and PC2 separate strains 9C, 7F and 7D from the rest. When we look at the loading plot, we see that the wines fermented with strains 9C and 7F are separate from others based on their hue values, total and coloured anthocyanins, polyphenols, tannins, ethyl octanoate, ethyl decanoate, 2-phenylethyl acetate, 2-phenyletanol, γ-butyrolactone and hexanoic acid concentrations, and other attributes like intensity and quality of aroma, red fruit aroma and overall quality. These attributes appeared in high quality wines, and strains 9C and 7F are the best choice to improve the "Pago" Merlot wine quality.

4. Conclusions

A wide diversity of characteristics was found in the *S. cerevisiae* strains isolated from Merlot "Pago" wines. From the growth-related and metabolic characteristics, strain 7F was one of the four best growing yeasts, and was one of the three highest sugar consumers and ethanol and glycerol producers, whereas was the second one produced lower acetic acid behind strain 2G, in the lab-scale experiments. Wines fermented with strains 9C, 7F showed excellent colour intensity, a high concentration of total and coloured anthocyanins, tannins and polyphenols, and a high tannin polymerization degree. In addition, the wines fermented with strains 9C and 7F presented a high concentration of compounds with a pleasant aroma, such as esters, higher alcohols, and especially 2 phenylethanol, and γ-butyrolactone. Both strains 9C and 7F were low producers of acetaldehyde and diacetyl, compounds that confer a negative impact on wine aroma. The wines scoring higher overall quality marks in the sensorial analysis were those

fermented with strains 9C and 7F. These wines showed good intensity, colour quality, higher intensity, aroma quality and an intense fruity character.

Of these two yeast, strain 7F combined adequate growth and metabolic-related parameters and could, hence, be a valuable tool to improve the distinctiveness of Merlot "Pago" wines produced in a particular microclimate and soil composition.

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Figure legends:

Figure 1: ITS fragments of the isolated yeast species. Lane P: 1 Kb Plus DNA ladder (Invitrogene). Lane 1: *Saccharomyces cerevisiae*. Lane 2: *Torulaspora delbrueckii*. Lane 3: *Hanseniaspora uvarum*. Lane 4: *Metschnikowia pulcherrima*.

Figure 2: Dendrogram based on the similarities of the mDNA HinfI restriction profiles built using the Pearson Product-Moment Correlation Coefficient and the Unweighted Pair Group Method with Arithmetic Mean. (UPGMA). Cutoff level set at 91.2% similarity.

Figure 3: Growth parameters and kinetics recorded for the different *S. cerevisiae* strains grown in sterile grape Merlot must. A: The maximum growth rate expressed as Δ CFU/mL/h; B: The maximum cell concentration (MCC) expressed as CFU/mL achieved during growth; C: The final cell concentration (FCC) on day 14 of growth, expressed as CFU/mL; D: Growth kinetics of the different yeast strains; E: Area under the curve (AUC) calculated from the growth kinetics data.

Figure 4: Growth, sugars consumed and fermentation products generated by the different *S. cerevisiae* strains grown in sterile grape Merlot must. A: Area under the curve (AUC) expressed as arbitrary units; B: Glucose consumed expressed as g/L; C: Fructose consumed expressed as g/L; D: Ethanol produced expressed as % (v/v); E: Glycerol produced expressed as g/L; F: Acetic acid produced expressed as g/L; Blue bars: data corresponding to fermentation day 4; Red bars: data corresponding to fermentation day 7; Green bars: data corresponding to fermentation day 14.

Figure 5: Score plot (A) and loading plot (B) on the first (PC1) and second (PC2) principal components corresponding to the PCA of the chemico-sensorial parameters of Merlot wines

Table 1: *S. cerevisiae* HinfI restriction mDNA patterns with isolates from different spontaneous fermentation times of the grouped Merlot grape must (strains). The right column describes the isolate that represents each pattern. M: Grape must; MAF: Middle alcoholic fermentation; EAF: End alcoholic fermentation.

Pattern number (Strains)	**Isolates**	**Isolated from**	**Representative pattern isolate**
1	2G	M	2G
	7H	MAF	
2	9C	EAF	9C
3	2B, 2H	M	
	7B, 7F,7J,	MAF	7F
	9F, 9G, 9I,	EAF	
4	2A, 2D	M	2A
	4B, 7C, 7G, 8A, 8B	MAF	
	9B, 9D, 9J, 10A, 10D	EAF	10A
5	2C	M	
	7E	MAF	7E
6	2E	M	2E
	9A	EAF	
7	7I	MAF	7I
	9H	EAF	
8	7D	MAF	7D
9	2I	M	
	7A	MAF	7A
	9E	EAF	
10	2F	M	2F
11	4A, 4C	M	4A
12	10B	EAF	10B

Table 2: Correlation values among the maximum growth rate (μ_{max}), consumed glucose and fructose and produced ethanol, glycerol, acetic acid, maximum cell concentrations, cell concentration and AUC values on day 7. [a]: the maximum growth rate was measured the first 24 h and expressed as CFU/mL/h; [b]: Cons. gluc. is glucose consumed expressed as g/L; [c]: Cons. Fruc. is fructose consumed expressed as g/L; [d]: Ethan. is ethanol produced expressed as % (v/v); [e]: Glyc. is glycerol produced expressed as g/L; [f]: Acetic ac. is acetic acid produced expressed as g/L; [g]: Cell conc. is cell concentration on day 7 expressed as CFU/mL; [g]: MCC is the maximum cell concentration found along the growth; [i]:AUC is the area under the curve on day 7 expressed as arbitrary units; [ns]: non-significant ($p>0.05$).

		μ_{max}[a]	Cons. gluc.[b]	Cons. fruc.[c]	Ethan.[d]	Glyc.[e]	Acetic ac.[f]	Cell conc.[g]	MCC[h]	AUC[i]
μ_{max}[a]	rho		0.6103	0.2965[ns]	0.6118	0.6018	0.3169[ns]	0.0013[ns]	0.3699[ns]	0.4551[ns]
	P value		0.0351	0.3493	0.0345	0.0384	0.3155	0.9967	0.2367	0.1372
Cons. gluc.[b]	rho			0.8731	0.8605	0.7700	0.5535[ns]	0.4163[ns]	0.3619[ns]	0.2503[ns]
	P value			0.0002	0.0003	0.0034	0.0619	0.1782	0.2476	0.4326
Cons. fruc.[c]	rho				0.6228	0.5039[ns]	0.6287	0.2658[ns]	0.1562[ns]	0.1507[ns]
	P value				0.0305	0.0949	0.0285	0.4036	0.6278	0.6401
Ethan.[d]	rho					0.9077	0.5407[ns]	0.6007	0.5798	0.4048[ns]
	P value					0.0000	0.0695	0.0389	0.0481	0.1917
Glyc.[e]	rho						0.5652[ns]	0.6950	0.6782	0.4161[ns]
	P value						0.0555	0.0121	0.0153	0.1785
Acetic ac.[f]	rho							0.2677[ns]	0.3247[ns]	0.1720[ns]
	P value							0.4002	0.3031	0.5930
Cell conc.[g]	rho								0.7610	0.3855[ns]
	P value								0.0040	0.2158
MCC[h]	rho									0.8244
	P value									0.0010
AUC[i]	rho									

Table 3: Physico-chemical parameters of the Merlot wines fermented with the selected yeast strains

STRAIN	Density	Volatile acidity (g/L acetic acid)	pH	Total acidity (g/L tart. acid)	Alcoholic degree (%vol/vol)	Sugar (g/L)
2A	992 ± 0.0^{a}	0.32 ± 0.05^{a}	3.52 ± 0.03^{b}	6.34 ± 0.25^{ab}	12.53 ± 0.51^{a}	2.38 ± 0.11^{a}
2E	992 ± 0.0^{a}	0.43 ± 0.02^{b}	3.48 ± 0.01^{a}	6.63 ± 0.11^{b}	12.90 ± 0.20^{b}	2.27 ± 0.26^{a}
2F	993 ± 0.0^{a}	0.41 ± 0.02^{b}	3.48 ± 0.03^{a}	6.81 ± 0.43^{cd}	12.97 ± 0.12^{b}	2.38 ± 0.19^{a}
2G	993 ± 0.0^{a}	0.43 ± 0.02^{b}	3.55 ± 0.04^{b}	6.38 ± 0.38^{ab}	12.67 ± 0.12^{ab}	1.92 ± 0.08^{a}
4A	992 ± 0.0^{a}	0.39 ± 0.02^{b}	3.54 ± 0.01^{b}	6.65 ± 0.17^{b}	13.22 ± 0.31^{c}	1.69 ± 0.31^{a}
7A	993 ± 0.0^{a}	0.59 ± 0.10^{c}	3.51 ± 0.02^{b}	6.85 ± 0.34^{d}	13.37 ± 0.12^{cd}	2.06 ± 0.21^{a}
7D	992 ± 0.0^{a}	0.50 ± 0.13^{bc}	3.52 ± 0.01^{b}	6.51 ± 0.22^{b}	13.43 ± 0.31^{d}	2.17 ± 0.13^{a}
7E	992 ± 0.0^{a}	0.37 ± 0.00^{ab}	3.50 ± 0.02^{b}	6.25 ± 0.22^{a}	13.30 ± 0.00^{c}	2.51 ± 0.33^{a}
7F	993 ± 0.0^{a}	0.46 ± 0.06^{b}	3.54 ± 0.02^{b}	6.75 ± 0.24^{c}	13.23 ± 0.12^{cd}	1.79 ± 0.19^{a}
7I	993 ± 0.0^{a}	0.65 ± 0.06^{d}	3.47 ± 0.03^{a}	6.97 ± 0.22^{d}	13.37 ± 0.12^{cd}	2.18 ± 0.06^{a}
9C	992 ± 0.0^{a}	0.40 ± 0.06^{ab}	3.54 ± 0.02^{b}	6.58 ± 0.27^{b}	12.87 ± 0.83^{b}	2.28 ± 0.16^{a}
10B	993 ± 0.0^{a}	0.43 ± 0.08^{b}	3.48 ± 0.02^{a}	6.63 ± 0.22^{bc}	12.67 ± 0.12^{ab}	1.92 ± 0.18^{a}
F-Ratio	*1.99*	*9.47*	*1.50*	*4.39*	*6.87*	*0.61*
P-Value	*0.3534*	*0.0000*	*0.0454*	*0.0000*	*0.0087*	*0.0642*

Different letters in the same column mean significant differences ($p<0.05$) between fermented wines

Table 4: Polyphenols parameters of the Merlot wines made with the selected yeast strains

STRAIN	Colour Intensity (CI)	Hue	Total anthocyanins (mg/L)	Coloured anthocyanins (mg/L)	Catechins (g/L)	Condensed tannins (g/L)	Total polyphenols (g/L)	Total Polyphenol Index (IPT)	DMACH Index (%)	Ethanol Index (%)
2A	10.18 ± 0.45^{bc}	53.16 ± 1.12^{ab}	469.62 ± 21.33^{b}	377.06 ± 21.11^{c}	0.13 ± 0.01^{b}	1.13 ± 0.03^{b}	3.36 ± 0.24^{bc}	39.12 ± 1.56^{b}	71.65 ± 8.65^{b}	54.65 ± 3.19^{cd}
2E	8.87 ± 0.94^{a}	53.75 ± 0.87^{b}	480.61 ± 9.54^{c}	371.85 ± 20.94^{b}	0.15 ± 0.02^{b}	1.15 ± 0.10^{b}	3.02 ± 0.24^{a}	38.34 ± 1.18^{b}	72.20 ± 3.98^{b}	53.14 ± 5.26^{cd}
2F	9.77 ± 0.80^{b}	53.65 ± 0.55^{b}	473.56 ± 15.16^{b}	372.6 ± 15.34^{b}	0.14 ± 0.02^{b}	$1.10 \pm 0.0b^{a}$	3.06 ± 0.21^{a}	38.21 ± 2.50^{bc}	69.4 ± 3.35^{ab}	56.13 ± 1.87^{d}
2G	9.36 ± 0.53^{b}	57.41 ± 2.25^{c}	431.8 ± 11.49^{a}	350.33 ± 13.68^{a}	0.13 ± 0.01^{b}	1.09 ± 0.08^{a}	2.92 ± 0.15^{a}	35.47 ± 1.58^{a}	69.53 ± 3.80^{ab}	54.01 ± 6.39^{cd}
4A	10.65 ± 0.77^{bc}	52.23 ± 1.20^{ab}	481.63 ± 7.14^{bc}	386.67 ± 12.88^{d}	0.12 ± 0.01^{ab}	1.17 ± 0.06^{b}	3.28 ± 0.12^{bc}	39.92 ± 0.89^{b}	75.15 ± 5.69^{c}	51.79 ± 6.68^{bc}
7A	10.51 ± 0.3^{2bc}	52.45 ± 1.36^{ab}	472.1 ± 20.93^{b}	368.76 ± 19.61^{b}	0.11 ± 0.01^{a}	1.11 ± 0.07^{ab}	3.18 ± 0.16^{ab}	38.52 ± 1.50^{b}	76.84 ± 6.60^{cd}	52.35 ± 6.69^{c}
7D	10.02 ± 0.77^{b}	52.81 ± 1.07^{b}	481.02 ± 5.44^{bc}	378.36 ± 22.51^{cd}	0.14 ± 0.01^{b}	1.25 ± 0.08^{d}	3.42 ± 0.14^{d}	40.89 ± 1.86^{c}	68.28 ± 6.61^{a}	43.60 ± 2.55^{ab}
7E	9.26 ± 0.19^{ab}	52.68 ± 1.33^{ab}	474.42 ± 11.33^{b}	369.28 ± 11.71^{bc}	0.14 ± 0.01^{b}	1.08 ± 0.06^{a}	3.23 ± 0.04^{bc}	37.12 ± 1.48^{b}	84.74 ± 6.02^{e}	41.14 ± 6.70^{a}
7F	9.95 ± 0.47^{bc}	55.9 ± 1.67^{bc}	481.17 ± 19.27^{c}	381.61 ± 23.45^{cd}	0.11 ± 0.01^{a}	1.24 ± 0.09^{d}	3.22 ± 0.18^{bc}	38.95 ± 1.68^{bc}	67.33 ± 6.81^{a}	44.64 ± 5.60^{ab}
7I	10.86 ± 0.64^{d}	50.75 ± 2.44^{a}	483.9 ± 36.17^{c}	388.65 ± 15.47^{d}	0.14 ± 0.0^{b}	1.15 ± 0.07^{b}	3.28 ± 0.12^{bc}	38.63 ± 1.74^{bc}	77.2 ± 6.76^{d}	45.12 ± 1.65^{ab}
9C	10.74 ± 0.58^{cd}	51.58 ± 1.05^{a}	494.24 ± 14.13^{d}	392.61 ± 13.37^{d}	0.11 ± 0.01^{a}	1.19 ± 0.10^{cd}	3.41 ± 0.22^{cd}	40.06 ± 1.09^{c}	67.43 ± 5.16^{a}	50.83 ± 5.04^{cd}
10B	10.98 ± 0.80^{d}	52.04 ± 0.76^{b}	488.59 ± 10.69^{cd}	383.88 ± 10.01^{cd}	0.13 ± 0.01^{ab}	1.16 ± 0.07^{c}	3.39 ± 0.16^{c}	39.87 ± 1.58^{bc}	69.69 ± 3.61^{ab}	43.13 ± 3.95^{a}
F-Ratio	*6.60*	*6.67*	*15.33*	*9.24*	*1.99*	*9.47*	*7.50*	*4.39*	*6.87*	*6.61*
P-Value	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0434*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*

Different letters in the same column mean significant differences ($p<0.05$) between fermented wines

Table 5: Aromatic compounds of the Merlot wines made with the selected yeast strains

Volatile compounds (mg/L)	2A	2E	2F	2G	4A	7A	7D	7E	7F	7I	9C	10B	F-ratio	P-value
Alcohols														
Isoamyl alcohol	36.5 ± 4.07^{bc}	36.5 ± 4.07^{bc}	18.3 ± 8.06^{a}	27.6 ± 9.3^{ab}	18.8 ± 14.61^{a}	28.4 ± 7.2^{ab}	51.4 ± 4.56^{de}	37.5 ± 6.71^{bc}	34.7 ± 4.23^{bc}	42.4 ± 7.88^{cd}	34.1 ± 9.49^{cd}	34.2 ± 5.92^{bc}	*16.87*	0.0000
2,3-butanediol	40.6 ± 0.27^{cd}	42.1 ± 0.35^{cd}	51.2 ± 0.38^{f}	53.1 ± 0.31^{e}	31.5 ± 0.25^{c}	12.1 ± 0.16^{a}	23.4 ± 0.21^{b}	26.5 ± 0.30^{b}	10.7 ± 0.19^{a}	13.2 ± 0.10^{a}	46.7 ± 0.22^{df}	43.2 ± 0.39^{d}	*81.76*	0.0000
1-heptanol	nd	nd	0.00 ± 0.00^{a}	nd	nd	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.00 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.16 ± 0.03^{d}	0.04 ± 0.00^{c}	*8.56*	0.0000
Benzyl alcohol	0.01 ± 0.01^{b}	0.02 ± 0.01^{bc}	0.00 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.02 ± 0.00^{bc}	0.04 ± 0.00^{d}	0.03 ± 0.01^{d}	0.02 ± 0.00^{bc}	0.03 ± 0.01^{d}	0.03 ± 0.01^{d}	0.03 ± 0.01^{d}	0.02 ± 0.00^{bc}	*21.67*	0.0000
2-phenylethanol	107 ± 15^{c}	108 ± 12^{c}	42 ± 5.9^{a}	111 ± 8.2^{cd}	46 ± 2.6^{a}	53 ± 3.9^{ab}	111 ± 10^{c}	89 ± 6.8^{abc}	72 ± 4.2^{ab}	85 ± 6.5^{bc}	114 ± 12^{d}	73 ± 2.9^{ab}	*19.76*	0.0000
Esters														
Methyl acetate	0.06 ± 0.02^{bcd}	0.06 ± 0.01^{abc}	0.15 ± 0.01^{fg}	0.26 ± 0.13^{h}	0.02 ± 0.00^{ab}	0.04 ± 0.03^{abc}	0.07 ± 0.02^{cde}	0.01 ± 0.00^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{ab}	0.08 ± 0.05^{cde}	0.11 ± 0.04^{ef}	45.78	0.0000
Ethyl acetate	6.11 ± 0.2^{ab}	14.0 ± 2.5^{cd}	26.3 ± 2.2^{g}	22.1 ± 1.6^{ef}	9.7 ± 1.8^{bc}	23.6 ± 3.41^{fg}	13.5 ± 2.1^{cd}	4.32 ± 0.1^{a}	17.1 ± 1.4^{de}	19.4 ± 1.7^{def}	10.2 ± 0.8^{bc}	22.3 ± 1.4^{ef}	27.64	0.0000
Ethyl butirate	0.08 ± 0.07^{ab}	0.18 ± 0.03^{bc}	0.08 ± 0.02^{ab}	0.16 ± 0.40^{b}	0.10 ± 0.040^{abc}	0.12 ± 0.05^{abc}	0.15 ± 0.05^{abc}	0.14 ± 0.03^{abc}	0.10 ± 0.04^{ab}	0.20 ± 0.05^{bc}	0.10 ± 0.10^{abc}	0.03 ± 0.01^{a}	17.48	0.0000
Ethyl octanoate	0.37 ± 0.17^{ab}	0.60 ± 0.17^{f}	0.64 ± 0.33^{d}	0.27 ± 0.08^{a}	0.78 ± 0.12^{def}	0.22 ± 0.01^{a}	0.24 ± 0.08^{a}	0.71 ± 0.06^{de}	0.56 ± 0.26^{bcd}	0.61 ± 0.19^{cd}	0.92 ± 0.45^{f}	0.26 ± 0.10^{a}	*127.54*	0.0000
Ethyl decanoate	0.34 ± 0.08^{cd}	0.33 ± 0.02^{cd}	0.32 ± 0.02^{bc}	0.24 ± 0.03^{a}	0.31 ± 0.06^{bcd}	0.35 ± 0.03^{cd}	0.21 ± 0.14^{a}	0.32 ± 0.08^{bcd}	0.39 ± 0.01^{d}	0.31 ± 0.07^{bcd}	0.43 ± 0.06^{d}	0.29 ± 0.13^{abc}	*86.74*	0.0000
Ethyl succinate	nd	nd	0.02 ± 0.00^{b}	0.00 ± 0.00^{a}	nd	0.02 ± 0.00^{b}	nd	nd	0.02 ± 0.03^{b}	0.21 ± 0.03^{e}	0.16 ± 0.02^{d}	0.11 ± 0.01^{c}	*72.41*	0.0000
2-phenylethyl acetate	6.60 ± 0.13^{f}	4.96 ± 0.19^{cd}	3.92 ± 0.15^{bc}	6.56 ± 0.93^{ef}	3.75 ± 0.14^{c}	6.31 ± 0.82^{ef}	6.63 ± 0.29^{f}	5.23 ± 0.49^{de}	7.11 ± 0.32^{g}	3.26± 0.14^{a}	6.82 ± 0.45^{g}	6.22 ± 0.11^{ef}	*39.16*	0.0000
Lactones														
γ-buyrolactone	7.13 ± 0.86^{cd}	6.91 ± 1.06^{cd}	7.25 ± 0.64^{cd}	6.58 ± 1.02^{c}	6.34 ± 0.84^{c}	5.15 ± 0.23^{a}	5.73 ± 0.89^{b}	7.43 ± 0.92^{d}	6.96 ± 0.63^{cd}	6.92 ± 0.73^{cd}	7.54 ± 0.12^{d}	5.75 ± 0.44^{bc}	61.65	0.0000
Acids														
Butyl acid	0.08 ± 0.03^{a}	0.14 ± 0.01^{d}	0.77 ± 0.06^{f}	0.78 ± 0.04^{f}	0.14 ± 0.01^{d}	0.10 ± 0.01^{ab}	0.19 ± 0.02^{b}	0.01 ± 0.01^{ab}	0.09 ± 0.01^{ab}	0.11 ± 0.01^{b}	0.13 ± 0.03^{cd}	0.11 ± 0.03^{bc}	35.87	0.0000
Isopentanoic acid	0.29 ± 0.04^{a}	0.26 ± 0.03^{a}	0.54 ± 0.04^{ef}	0.33 ± 0.25^{ab}	0.46 ± 0.04^{de}	0.47 ± 0.05^{de}	0.50 ± 0.11^{ef}	0.46 ± 0.07^{cde}	0.36 ± 0.06^{abc}	0.46 ± 0.11^{cde}	0.62 ± 0.08^{f}	0.40 ± 0.08^{bcd}	13.76	0.0000
Hexanoic acid	0.41 ± 0.06^{bc}	0.71 ± 0.14^{e}	0.38 ± 0.15^{b}	0.37 ± 0.13^{b}	0.39 ± 0.21^{bc}	0.48 ± 0.10^{cd}	0.74 ± 0.07^{fg}	0.51 ± 0.07^{cd}	0.48 ± 0.04^{bcd}	0.78 ± 0.15^{fg}	0.84 ± 0.06^{g}	0.55 ± 0.05^{d}	48.97	0.0000
Ethylhexanoic acid	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.02 ± 0.00^{c}	0.02 ± 0.00^{c}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.00 ± 0.00^{a}	0.03 ± 0.01^{c}	0.02 ± 0.00^{bc}	0.02 ± 0.01^{bc}	0.05 ± 0.00^{d}	0.01 ± 0.00^{ab}	4.65	0.0078
Octanoic acid	0.24 ± 0.01^{a}	0.77 ± 0.18^{g}	0.35 ± 0.12^{bc}	0.39 ± 0.13^{cd}	0.39 ± 0.19^{cd}	0.46 ± 0.11^{cde}	0.85 ± 0.06^{gh}	0.54 ± 0.06^{ef}	0.52 ± 0.10^{def}	0.79 ± 0.17^{gh}	0.74 ± 0.06^{g}	0.91 ± 0.09^{h}	32.54	0.0000
Decanoic acid	0.25 ± 0.17^{b}	0.15 ± 0.03^{a}	0.42 ± 0.88^{d}	0.39 ± 0.17^{cd}	0.31 ± 0.10^{c}	0.19 ± 0.03^{ab}	0.25 ± 0.04^{b}	0.14 ± 0.04^{a}	0.20 ± 0.07^{ab}	0.27 ± 0.07^{c}	0.28 ± 0.02^{c}	0.28 ± 0.05^{b}	29.64	0.0000
Isobutyl acid	0.16 ± 0.07^{ab}	0.14 ± 0.01^{a}	0.23 ± 0.01^{bc}	0.33 ± 0.01^{d}	0.15 ± 0.02^{ab}	0.30 ± 0.08^{d}	0.26 ± 0.07^{cd}	0.17 ± 0.06^{ab}	0.27 ± 0.09^{cd}	0.36 ± 0.02^{d}	0.21 ± 0.02^{bc}	0.43 ± 0.07^{e}	16.23	0.0000
Aldehydes														
Acetaldehyde	14.3 ± 1.ç25^{b}	28.6 ± 3.11^{ef}	36.3 ± 4.16^{ef}	57.5 ± 8.77^{g}	32.8 ± 2.63^{f}	24.5 ± 1.62^{de}	19.9 ± 2.34^{bc}	13.6 ± 1.82^{ab}	14.2 ± 8.67^{b}	13.1 ± 5.28^{ab}	8.82 ± 1.65^{a}	19.2 ± 1.9^{bc}	*12.76*	0.0000
Diacetyl	0.03 ± 0.02^{ab}	0.03 ± 0.00^{ab}	0.03 ± 0.00^{ab}	0.05 ± 0.02^{b}	0.02 ± 0.01^{ab}	0.02 ± 0.00^{ab}	0.01 ± 0.0^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.03 ± 0.02^{ab}	0.04 ± 0.01^{ab}	0.02 ± 0.00^{a}	*4.63*	0.0132
5-methylfurfural	0.20 ± 0.07^{bc}	nd	0.03 ± 0.00^{a}	Nd	0.03 ± 0.00^{a}	0.04 ± 0.03^{a}	0.15 ± 0.00^{b}	0.03 ± 0.08^{a}	0.22 ± 0.09^{c}	0.25 ± 0.10^{c}	0.24 ± 0.04^{c}	0.23 ± 0.04^{c}	*87.56*	0.0000

Different letters within the same column mean significant differences ($p<0.05$) between fermented wines

Table 6: Sensory attributes of the Merlot wines made with the selected yeast strains

Sensory attributes	2A	2E	2F	2G	4A	7A	7D	7E	7F	7I	9C	10B	F-ratio	P-value
Colour														
Colour quality	8.5 ± 0.95^{b}	8.8 ± 0.99^{c}	8.3 ± 0.95^{a}	8.8 ± 1.03^{c}	8.6 ± 0.97^{b}	8.3 ± 0.82^{a}	8.8 ± 1.03^{c}	8.8 ± 1.03^{c}	8.8 ± 0.95^{c}	8.7 ± 1.03^{c}	8.8 ± 0.97^{c}	8.5 ± 1.08^{b}	6.65	0.0453
Colour intensity	8.7 ± 1.03^{a}	8.6 ± 1.03^{a}	8.7 ± 1.03^{a}	8.8 ± 1.03^{a}	8.8 ± 1.03^{a}	8.6 ± 0.95^{a}	8.8 ± 1.03^{a}	8.8 ± 1.03^{a}	8.8 ± 1.03^{a}	8.8 ± 1.03^{a}	8.8 ± 1.03^{a}	8.7 ± 1.03^{a}	1.63	0.3423
Aroma														
Aroma intensity	7.4 ± 0.92^{b}	7.1 ± 1.52^{a}	7.9 ± 1.06^{d}	7.3 ± 0.95^{ab}	7.2 ± 1.81^{a}	7.7 ± 1.10^{cd}	7.6 ± 1.58^{c}	7.8 ± 1.14^{d}	7.8 ± 1.26^{d}	7.6 ± 1.58^{d}	8.0 ± 0.72^{e}	7.4 ± 1.77^{b}	11.68	0.0000
Aroma quality	7.4 ± 0.82^{b}	7.8 ± 0.99^{c}	7.6 ± 1.25^{bc}	7.1 ± 1.37^{a}	8.3 ± 1.03^{e}	7.6 ± 0.97^{c}	7.8 ± 1.14^{d}	7.8 ± 1.14^{c}	7.9 ± 1.64^{d}	7.3 ± 2.08^{ab}	8.3 ± 0.95^{e}	7.4 ± 1.05^{b}	8.18	0.0376
Red fruits aroma	3.4 ± 0.88^{a}	4.1 ± 0.11^{bc}	4.5 ± 0.70^{d}	3.9 ± 0.77^{ab}	4.2 ± 0.67^{bc}	4.5 ± 1.12^{bc}	4.7 ± 0.90^{d}	4.8 ± 0.67^{d}	4.6 ± 0.45^{d}	3.6 ± 0.89^{b}	4.8 ± 0.78^{d}	3.4 ± 1.02^{a}	3.84	0.0462
Black fruits aroma	5.9 ± 2.82^{a}	5.8 ± 3.01^{a}	6.1 ± 3.01^{a}	5.6 ± 2.22^{a}	7.2 ± 2.10^{a}	6.1 ± 3.00^{a}	5.9 ± 2.33^{a}	6.6 ± 2.22^{a}	5.7 ± 3.06^{a}	6.8 ± 2.32^{a}	6.8 ± 2.44^{a}	5.9 ± 1.42^{a}	2.62	0.0786
Floral aroma	2.0 ± 1.60^{a}	1.7 ± 0.89^{a}	1.7 ± 0.54^{a}	2.0 ± 0.80^{a}	1.6 ± 0.67^{a}	1.7 ± 0.23^{a}	2.0 ± 0.56^{a}	1.6 ± 0.43^{a}	1.6 ± 0.12^{a}	1.6 ± 0.61^{a}	1.8 ± 0.56^{a}	1.9 ± 0.43^{a}	1.16	0.1214
Balsamic aroma	5.3 ± 0.51^{a}	3.9 ± 1.69^{a}	5.0 ± 0.70^{a}	4.4 ± 1.84^{a}	4.3 ± 0.47^{a}	5.0 ± 0.50^{a}	4.7 ± 0.72^{a}	5.7 ± 1.90^{a}	4.7 ± 0.89^{a}	5.4 ± 0.63^{a}	4.4 ± 0.13^{a}	5.3 ± 0.76^{a}	0.86	0.2653
Spicy aroma	1.6 ± 0.43^{a}	2.3 ± 0.23^{a}	2.3 ± 0.31^{a}	2.2 ± 0.78^{a}	2.2 ± 0.56^{a}	2.3 ± 0.21^{a}	2.7 ± 0.50^{a}	2.0 ± 0.8^{a}	1.5 ± 0.30^{a}	2.1 ± 0.40^{a}	2.1 ± 0.21^{a}	1.6 ± 0.46^{a}	1.75	0.0875
Lactic aroma	1.7 ± 0.70^{a}	1.7 ± 0.94^{a}	1.7 ± 0.95^{a}	1.9 ± 1.23^{a}	1.7 ± 0.76^{a}	1.7 ± 0.76^{a}	2.1 ± 1.73^{a}	1.5 ± 1.08^{a}	1.6 ± 0.97^{a}	2.3 ± 0.70^{a}	2.3 ± 0.65^{a}	1.7 ± 0.67^{a}	1.64	0.2624
Vegetable aroma	1.2 ± 0.32^{a}	2.1 ± 0.90^{a}	1.7 ± 0.42^{a}	1.6 ± 0.33^{a}	1.2 ± 0.42^{a}	1.7 ± 0.56^{a}	1.4 ± 0.84^{a}	1.7 ± 0.78^{a}	1.1 ± 0.50^{a}	1.5 ± 0.97^{a}	1.5 ± 0.76^{a}	1.2 ± 0.34^{a}	3.16	0.0658
Aromatic herbs	1.5 ± 0.98^{a}	2.2 ± 0.75^{a}	1.8 ± 0.45^{a}	1.9 ± 0.78^{a}	1.9 ± 0.99^{a}	1.8 ± 0.87^{a}	1.7 ± 0.54^{a}	2.0 ± 0.85^{a}	1.5 ± 0.69^{a}	2.4 ± 1.20^{a}	1.4 ± 0.80^{a}	1.5 ± 0.96^{a}	0.55	0.5434
Chocolate aroma	3.6 ± 0.87^{a}	3.8 ± 1.23^{a}	3.6 ± 1.50^{a}	2.8 ± 0.98^{a}	3.1 ± 1.78^{a}	3.6 ± 0.68^{a}	2.3 ± 0.98^{a}	2.9 ± 0.78^{a}	3.5 ± 1.23^{a}	3.0 ± 0.65^{a}	3.0 ± 0.64^{a}	3.6 ± 0.72^{a}	0.49	0.5823
Taste														
Taste intensity	7.6 ± 2.47^{a}	7.7 ± 1.25^{a}	7.3 ± 1.33^{a}	7.2 ± 1.14^{a}	7.7 ± 0.95^{a}	7.3 ± 1.34^{a}	7.7 ± 1.16^{a}	7.7 ± 1.25^{a}	7.3 ± 1.48^{a}	7.3 ± 1.65^{a}	7.7 ± 0.92^{a}	7.6 ± 1.17^{a}	1.83	0.0862
Taste quality	6.8 ± 0.99^{a}	7.2 ± 0.92^{a}	7.0 ± 1.26^{a}	7.1 ± 0.88^{a}	7.4 ± 0.84^{a}	7.0 ± 1.33^{a}	7.1 ± 0.99^{a}	7.1 ± 1.10^{a}	7.3 ± 1.30^{a}	7.2 ± 1.17^{a}	7.5 ± 0.71^{a}	6.8 ± 1.37^{a}	3.86	0.0521
Acidity	5.9 ± 0.82^{a}	6.0 ± 0.94^{a}	6.0 ± 0.71^{a}	5.7 ± 0.82^{a}	5.9 ± 0.74^{a}	6.0 ± 1.25^{a}	6.0 ± 0.94^{a}	5.5 ± 1.78^{a}	6.0 ± 1.05^{a}	5.9 ± 1.41^{a}	5.9 ± 0.88^{a}	5.9 ± 1.40^{a}	0.54	0.7227
Sweetness	1.3 ± 0.65^{a}	1.1 ± 0.32^{a}	1.1 ± 0.71^{a}	1.1 ± 0.32^{a}	1.1 ± 0.32^{a}	1.1 ± 0.32^{a}	1.2 ± 0.42^{a}	1.1 ± 0.32^{a}	1.1 ± 0.32^{a}	1.1 ± 0.67^{a}	1.1 ± 0.32^{a}	1.3 ± 0.63^{a}	0.24	0.6324
Unctuousness	5.3 ± 1.65^{a}	4.7 ± 1.83^{a}	4.6 ± 1.97^{a}	4.7 ± 1.49^{a}	5.1 ± 1.46^{a}	4.6 ± 1.58^{a}	4.9 ± 1.73^{a}	4.2 ± 1.80^{a}	4.9 ± 1.93^{a}	5.4 ± 1.66^{a}	5.4 ± 1.20^{a}	5.3 ± 1.56^{a}	1.15	0.2624
Structure	4.4 ± 1.70^{a}	4.3 ± 1.68^{a}	4.3 ± 1.57^{a}	4.2 ± 1.93^{a}	4.4 ± 1.65^{a}	4.3 ± 1.34^{a}	4.3 ± 1.77^{a}	3.9 ± 1.79^{a}	4.3 ± 1.87^{a}	4.2 ± 1.62^{a}	4.2 ± 1.54^{a}	4.4 ± 1.90^{a}	1.78	0.4565
Astringency	4.1 ± 1.45^{a}	4.5 ± 1.54^{a}	4.2 ± 1.91^{a}	3.9 ± 1.23^{a}	4.2 ± 1.32^{a}	4.2 ± 1.18^{a}	3.8 ± 1.05^{a}	4.3 ± 1.20^{a}	4.0 ± 1.58^{a}	3.9 ± 1.11^{a}	3.9 ± 0.98^{a}	4.1 ± 1.78^{a}	0.22	0.3218
Bitterness	2.2 ± 0.97^{a}	2.3 ± 0.67^{a}	2.5 ± 0.63^{a}	2.2 ± 0.51^{a}	2.3 ± 0.78^{a}	2.5 ± 0.88^{a}	2.5 ± 0.67^{a}	2.3 ± 0.56^{a}	2.2 ± 0.89^{a}	2.0 ± 0.49^{a}	2.0 ± 0.78^{a}	2.2 ± 0.87^{a}	0.11	0.6856
Taste persistence	6.2 ± 2.49^{a}	6.2 ± 2.39^{a}	5.1 ± 2.44^{a}	6.5 ± 2.27^{a}	5.5 ± 2.51^{a}	5.1 ± 2.47^{a}	6.6 ± 2.27^{a}	5.9 ± 2.47^{a}	6.5 ± 2.51^{a}	6.4 ± 2.62^{a}	6.5 ± 2.22^{a}	6.2±2.30^{a}	1.76	0.1275
Overall Quality	6.6 ± 0.82^{a}	6.6 ± 0.56^{a}	7.1 ± 0.70^{b}	7.1 ± 0.98^{ab}	7.0 ± 0.64^{ab}	7.2 ± 0.45^{bc}	7.2 ± 0.74^{cd}	7.1 ± 0.94^{ab}	7.3 ± 0.81^{c}	7.1 ± 0.54^{b}	7.6 ± 0.75^{d}	6.5 ± 1.03^{a}	16.34	0.0011

Different letters within the same column mean significant differences ($p<0.05$) between fermented wines

Table S1: Correlation values among the maximum growth rate (μ_{max}), consumed glucose and fructose and produced ethanol, glycerol, acetic acid, cell concentration and AUC values on day 4. [a]: the maximum growth rate was measured during first 24 h and expressed as CFU/mL/h; [b]: Cons. gluc. is glucose consumed expressed as g/L; [c]: Cons. Fruc. is fructose consumed expressed as g/L; [d]: Ethan. is ethanol produced expressed as % (v/v); [e]: Glyc. is glycerol produced expressed as g/L; [f]: Acetic ac. is acetic acid produced expressed as g/L; [g]: Cell conc. is cell concentration expressed as CFU/mL; [h]: AUC is the area under the curve expressed as arbitrary units; [ns]: non-significant ($p>0.05$).

		μ_{max}[a]	Cons. gluc.[b]	Cons. fruc.[c]	Ethan.[d]	Glyc.[e]	Acetic ac.[f]	Cell conc.[g]	AUC[h]
μ_{max}[a]	rho		0.8112	0.5175[ns]	0.6993	0.7483	0.6550	0.3427[ns]	0.5664
	P value		0.0022	0.0888	0.0142	0.0070	0.0239	0.2762	0.0591
Cons. gluc.[b]	rho			0.7762	0.9580	0.8881	0.6270	0.3636[ns]	0.6084
	P value			0.0043	0.0000	0.0003	0.0325	0.2464	0.0399
Cons. fruc.[c]	rho				0.6573	0.6154	0.6130	0.3357[ns]	0.4895
	P value				0.0238	0.0373	0.0375	0.2869	0.1098
Ethan.[d]	rho					0.8811	0.4764[ns]	0.2867[ns]	0.5524
	P value					0.0003	0.1191	0.3663	0.0667
Glyc.[e]	rho						0.6900	0.1329[ns]	0.4266[ns]
	P value						0.0157	0.6832	0.1689
Acetic ac.[f]	rho							-0.1296[ns]	0.0876[ns]
	P value							0.6785	0.7875
Cell conc.[g]	rho								0.9161
	P value								0.0001
AUC[h]	rho								
	P value								

Table S2: Correlation values among the maximum growth rate (μ_{max}), consumed glucose and fructose and produced ethanol, glycerol, acetic acid, maximum cell concentration, cell concentration and AUC values on day 14. [a]: the maximum growth rate was measured during the first 24 h and expressed as CFU/mL/h; [b]: Cons. gluc. is glucose consumed expressed as g/L; [c]: Cons. Fruc. is fructose consumed expressed as g/L; [d]: Ethan. is ethanol produced expressed as % (v/v); [e]: Glyc. is glycerol produced expressed as g/L; [f]: Acetic ac. is acetic acid produced expressed as g/L; [g]: FCC is cell concentration at the end of the experiment, expressed as CFU/mL; [h]: MCC is maximum cell concentration along the growth, expressed as CFU/mL; [i]:AUC is the area under the curve, expressed as arbitrary units; [ns]: non-significant ($p>0.05$).

		μ_{max}[a]	Cons. gluc.[b]	Cons. fruc.[c]	Ethan.[d]	Glyc.[e]	Acetic ac.[f]	Cell conc.[g]	MCC[h]	AUC[i]
μ_{max}[a]	rho		0.6830	0.3916[ns]	0.5315[ns]	0.5804[ns]	0.3614[ns]	0.2238[ns]	0.4615[ns]	0.3147[ns]
	P value		0.0171	0.2097	0.0794	0.0521	0.2467	0.4851	0.1340	0.3194
Cons. gluc.[b]	rho			0.7180	0.4623[ns]	0.6340[ns]	0.5817[ns]	0.0245[ns]	0.4098[ns]	0.1891[ns]
	P value			0.0107	0.1314	0.0302	0.0503	0.9433	0.1859	0.5531
Cons. fruc.[c]	rho				0.4476[ns]	0.6923	0.2947[ns]	0.1608[ns]	0.3077[ns]	0.1399[ns]
	P value				0.1474	0.0155	0.3496	0.6192	0.3310	0.6673
Ethan.[d]	rho					0.7203	0.0842[ns]	0.2168[ns]	0.5105[ns]	0.3846[ns]
	P value					0.0106	0.7953	0.4990	0.0936	0.2183
Glyc.[e]	rho						0.0246[ns]	0.6224	0.7972	0.6713
	P value						0.9426	0.0347	0.0029	0.0202
Acetic ac.[f]	rho							-0.2211[ns]	0.1404[ns]	-0.0175[ns]
	P value							0.4797	0.6618	0.9518
Cell conc.[g]	rho								0.7203	0.8531
	P value								0.0106	0.0008
MCC[h]	rho									0.9091
	P value									0.0001
AUC[i]	rho									
	P value									

Figure 1

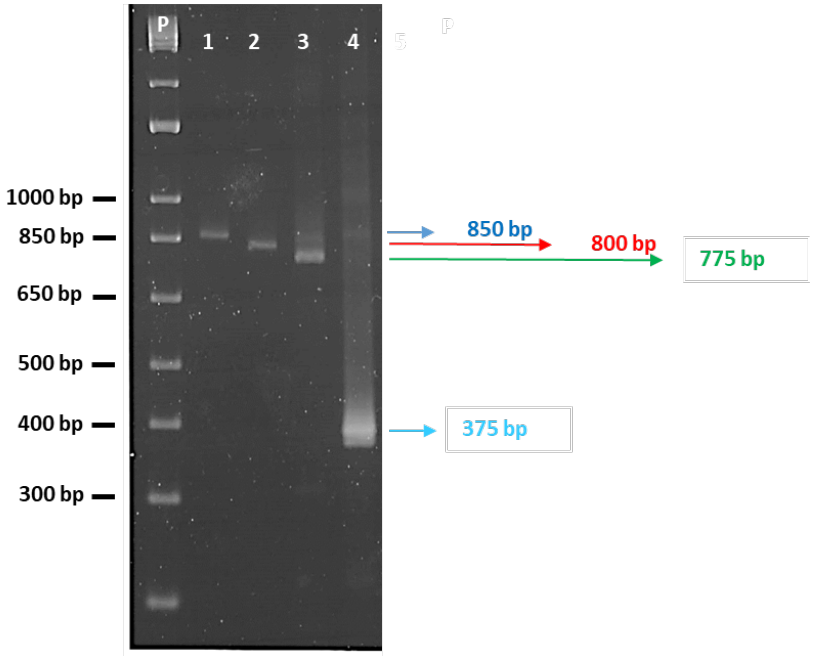

Figure 1: ITS fragments of the isolated yeast species. Lane P: 1 Kb Plus DNA ladder (Invitrogen). Lane 1: *Saccharomyces cerevisiae*. Lane 2: *Torulaspora delbrueckii*. Lane 3: *Hanseniaspora uvarum*. Lane 4: *Metschnikowia pulcherrima*.

Figure 2

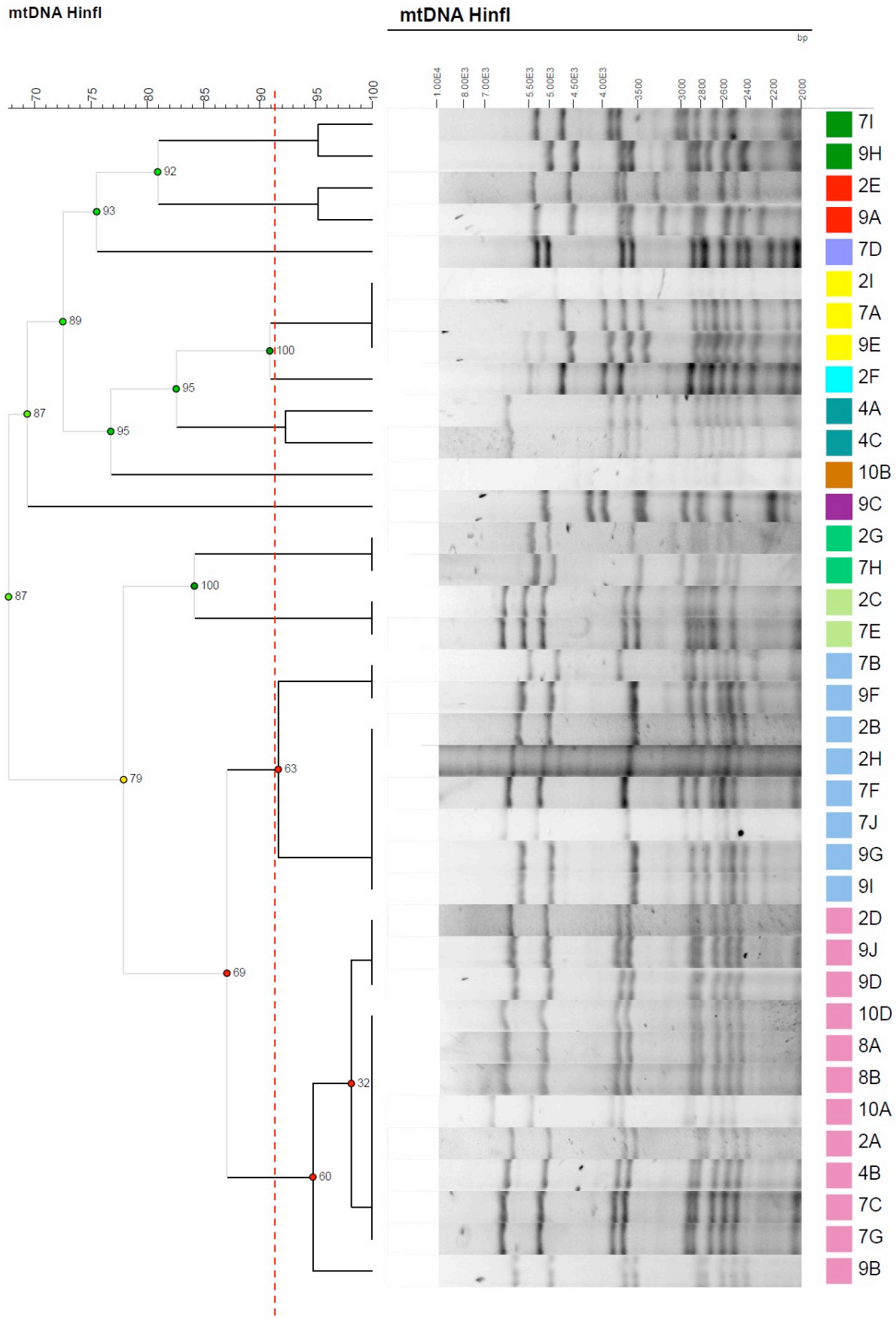

Figure 2: Dendrogram based on the similarities of the mDNA HinfI restriction profiles built using the Pearson Product-Moment Correlation Coefficient and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Cutoff level set at 91.2% similarity.

Figure 3

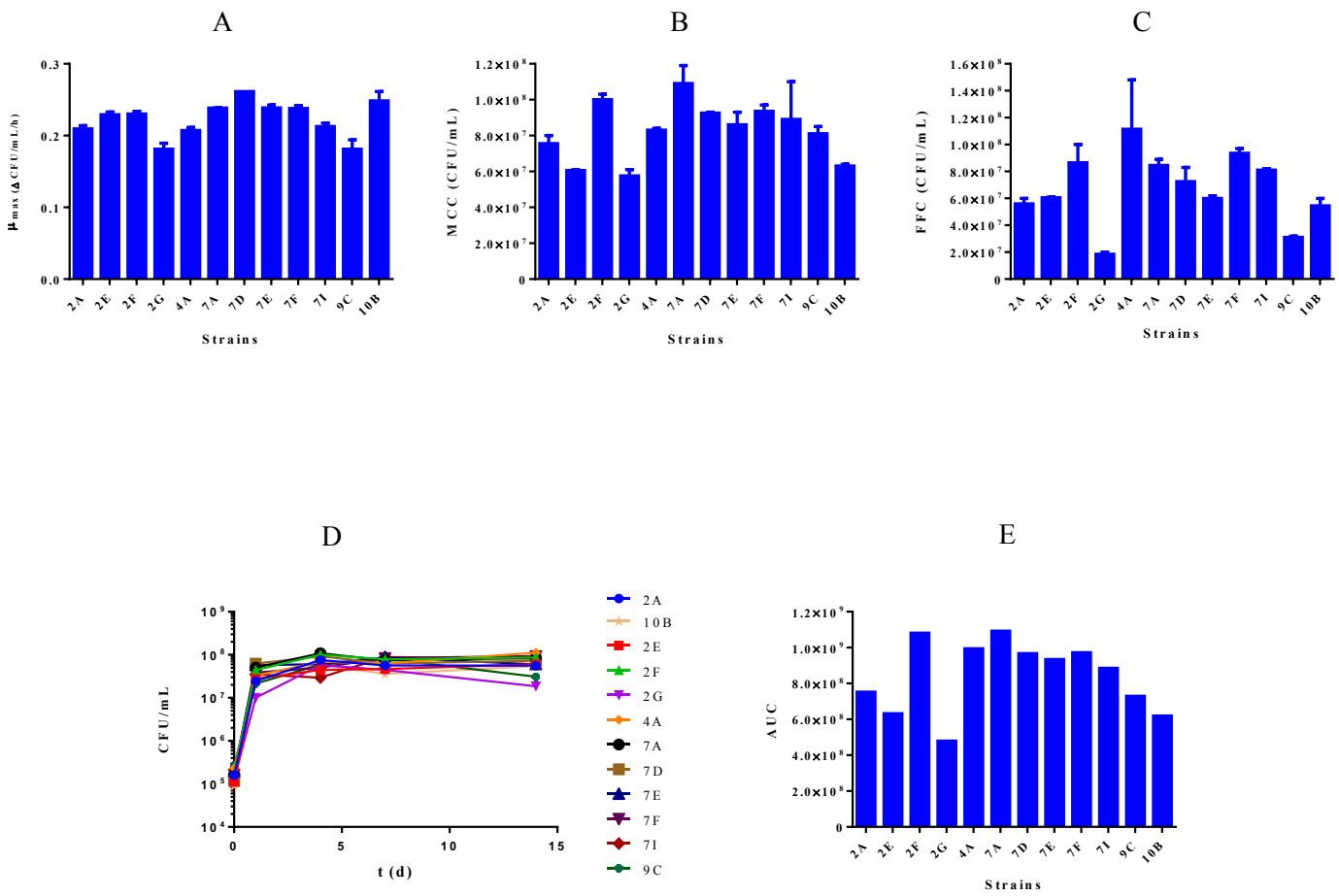

Figure 3: Growth parameters and kinetics recorded for the different *S. cerevisiae* strains grown in sterile grape Merlot must. A: The maximum growth rate expressed as Δ CFU/mL/h; B: The maximum cell concentration (MCC) expressed as CFU/mL achieved during growth; C: The final cell concentration (FCC) at 14 days of growth, expressed as CFU/mL; D: Growth kinetics of the different yeast strains; E: Area Under Curve (AUC) calculated from the growth kinetics data.

8 **Figure 4**

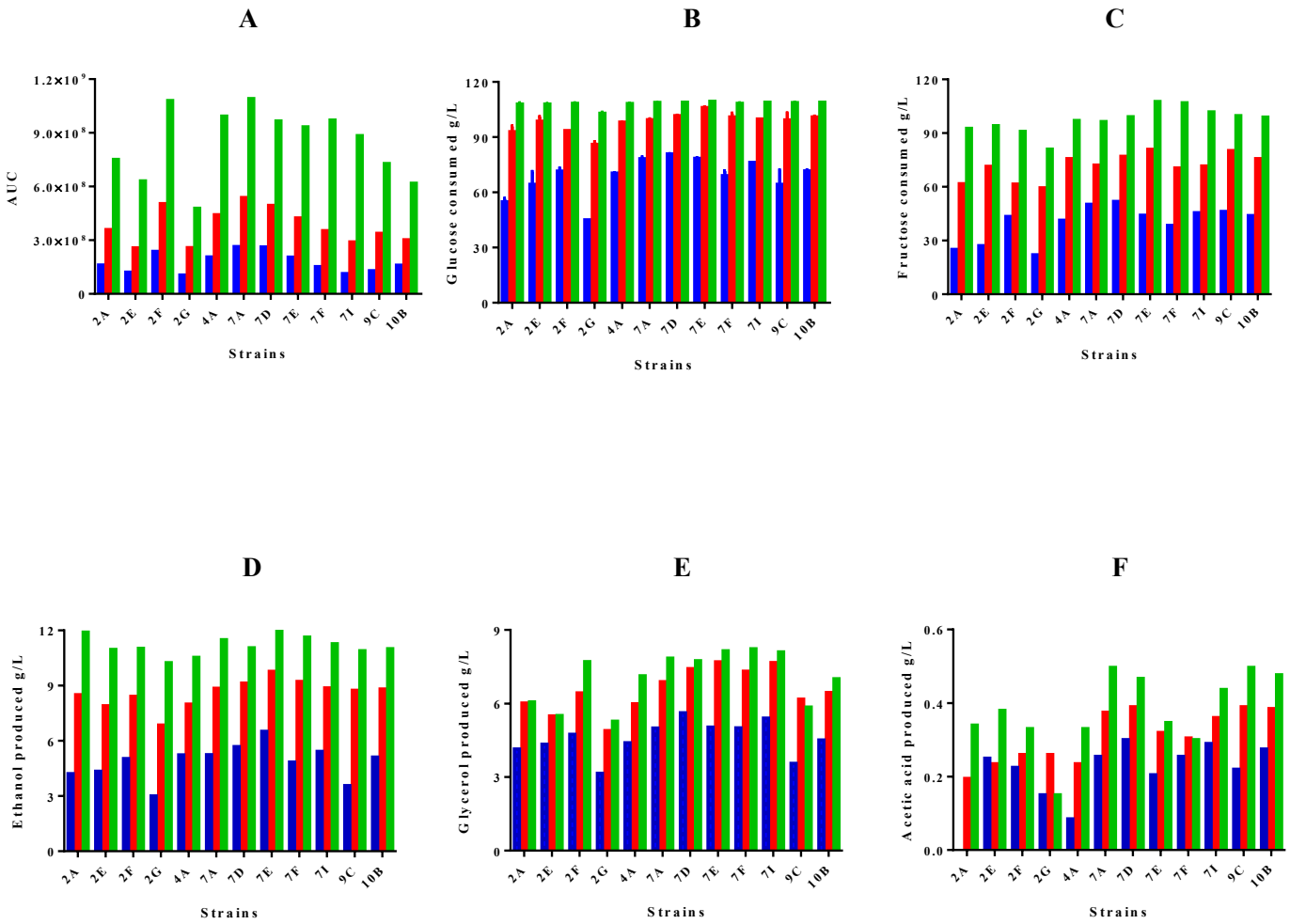
A
B
C
D
E
F
AUC
Glucose consumed g/L
Fructose consumed g/L
Ethanol produced g/L
Glycerol produced g/L
Acetic acid produced g/L
Strains
2A
2E
2F
2G
4A
7A
7D
7E
7F
7I
9C
10B

Figure 4: Growth, sugars consumed and fermentation products generated by the different *S. cerevisiae* strains grown in sterile grape Merlot must. A: Area under the curve (AUC) expressed as arbitrary units; B: Glucose consumed expressed as g/L; C: Fructose consumed expressed as g/L; D: Ethanol produced expressed as % (v/v); E: Glycerol produced expressed as g/L; F: Acetic acid produced expressed as g/L; Blue bars: data corresponding to fermentation day 4; Red bars: data corresponding to fermentation day 7; Green bars: data corresponding to fermentation day 14.

Figure 5

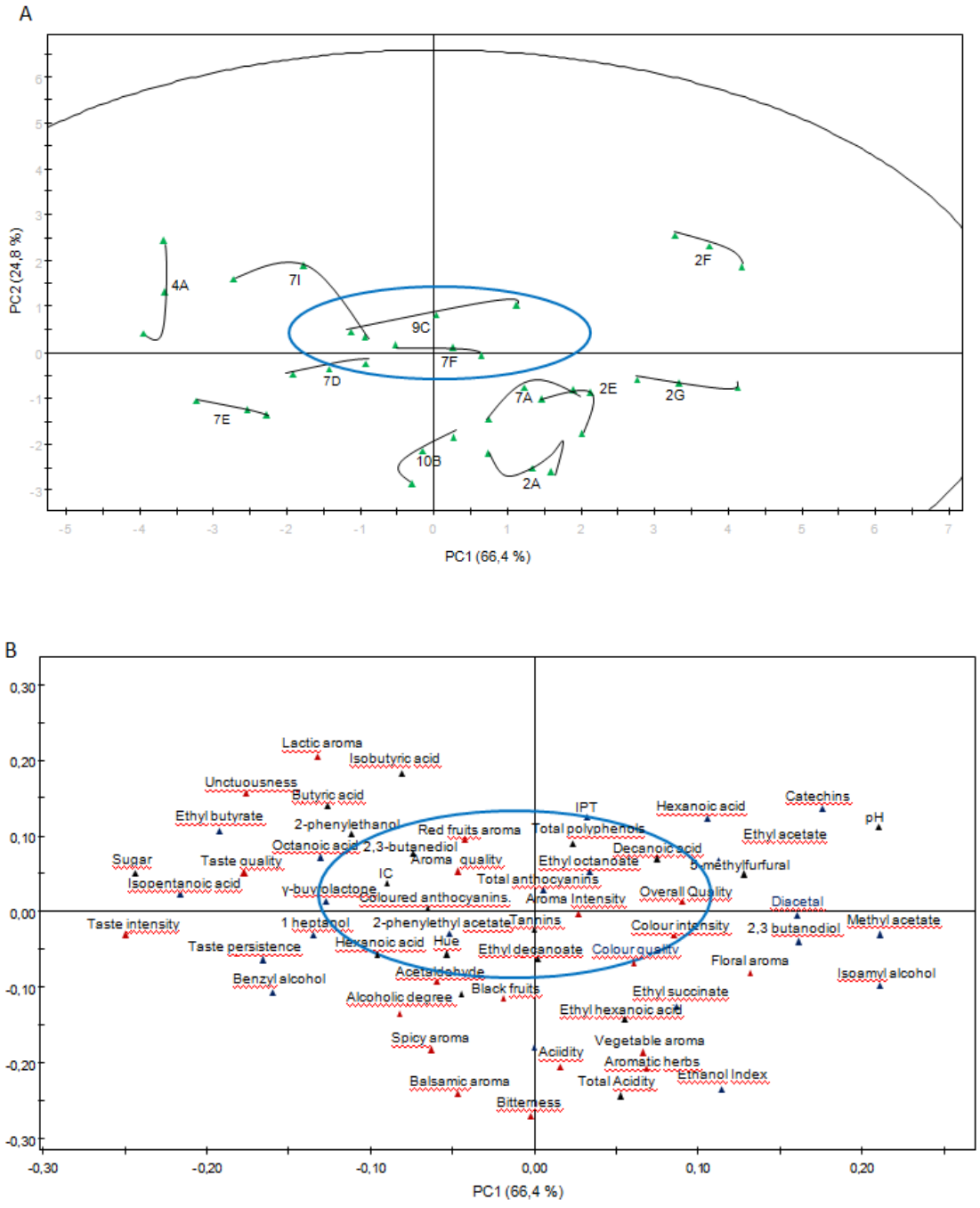

Figure 5: Score plot (A) and loading plot (B) on the first (PC1) and second (PC2) principal components corresponding to the PCA of the chemico-sensorial parameters of Merlot wines