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2	Article Sub- Title		
3	Article Copyright - Year	**Springer-Verlag and the University of Milan 2011 (This will be the copyright line in the final PDF)**	
4	Journal Name	Annals of Microbiology	
5	Corresponding Author	Family Name	**Combina**
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67	Schedule	Received	17 January 2011
68		Revised	
69		Accepted	28 April 2011

70 Abstract

The aim of this work was to select native *Saccharomyces cerevisiae* strains to conduct the alcoholic fermentation of red must at low temperature (15°C), thus producing volatile compounds that enhance the aromatic profile of young red wines. Native yeast strains were isolated from red musts and characterized using different oenological and technological criteria. The selection procedure included evaluating the yeasts' characteristics in order to efficiently transform grape sugars into alcohol and carbon dioxide at a controlled rate and without development of off-flavors. The selection procedure also considered another set of oenological properties, namely: SO_2 resistance, killer activity, low foam production, volatile acidity, high ethanol production and tolerance, sugar exhaustion, growth at low temperature, growth at high sugar concentration, formation of H_2S, β-glycosidase activity and volatile compound synthesis in synthetic media. The pre-selected native *S. cerevisiae* strains were evaluated in microvinifications of Malbec must at 15°C, which were then evaluated to volatile compound composition and subjected to a sensorial descriptive analysis. The complete selection procedure was carried out over 2 years. This study provides a complete description of techniques for obtaining validated scientific results that can be used by oenologists and researchers in the selection of specific yeasts.

71 Keywords separated by ' - ' *Saccharomyces cerevisiae* - Low temperature - Fermentation - Red wine - Aromatic profile

72 Foot note information

Ann Microbiol
DOI 10.1007/s13213-011-0271-0

ORIGINAL ARTICLE

Selection of indigenous *Saccharomyces cerevisiae* strains to ferment red musts at low temperature

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Received: 17 January 2011 / Accepted: 28 April 2011

Abstract The aim of this work was to select native *Saccharomyces cerevisiae* strains to conduct the alcoholic fermentation of red must at low temperature (15°C), thus producing volatile compounds that enhance the aromatic profile of young red wines. Native yeast strains were isolated from red musts and characterized using different oenological and technological criteria. The selection procedure included evaluating the yeasts' characteristics in order to efficiently transform grape sugars into alcohol and carbon dioxide at a controlled rate and without development of off-flavors. The selection procedure also considered another set of oenological properties, namely: SO_2 resistance, killer activity, low foam production, volatile acidity, high ethanol production and tolerance, sugar exhaustion, growth at low temperature, growth at high sugar concentration, formation of H_2S, β-glycosidase activity and volatile compound synthesis in synthetic media. The pre-selected native *S. cerevisiae* strains were evaluated in microvinifications of Malbec must at 15°C, which were then evaluated to volatile compound composition and subjected to a sensorial descriptive analysis. The complete selection procedure was carried out over 2 years. This study provides a complete description of techniques for obtaining validated scientific results that can be used by oenologists and researchers in the selection of specific yeasts.

Keywords *Saccharomyces cerevisiae* · Low temperature · Fermentation · Red wine · Aromatic profile

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Introduction

As far as consumers are concerned, aroma is one of the main characteristics that determine the quality and value of a wine (Swiegers et al. 2005). This is due to the combined effects of several volatile compounds such as alcohols, aldehydes, esters, acids, monoterpenes and other minor components that are already present in the grapes or are formed during the fermentation and maturation process (Lambrechts and Pretorius 2000; Verzera et al. 2008). This great variety of volatile compounds has different polarities and volatilities and a wide range of concentrations which are responsible for the complexity of wine flavor and ensure its specificity and character (Mauriello et al. 2009; Bovo et al. 2011). The nature and amount of volatile compounds that make up the wine flavor depend on multiple factors such as the nitrogen content of the must, the fermentation temperature and the yeast strain (Lambrechts and Pretorius 2000; Swiegers et al. 2006; Cavazza et al. 2011).

JnlID 13213_ArtID 271_Proof# 1 - 07/05/2011

Winemakers recognize that low temperature fermentation (10–15°C) results in a product with improved flavor (Bardi et al. 1997). In this way, white and rosé wines of greater aromatic complexity can be produced (Lambrechts and Pretorius 2000; Llauradó et al. 2002; Torija et al. 2003). This technique has also been proposed to enhance the aromatic profile in young red wine vinification.

Temperature affects both yeast growth and fermentation rate, with lower temperatures giving longer fermentations and increased risk of stuck and sluggish fermentations (Fleet and Heard 1993; Bisson 1999). Moreover, changes in the fermentation rate may also modify yeast and bacterial ecology, ethanol sensitivity and yeast metabolism (Fleet and Heard 1993). Some indigenous non-*Saccharomyces* species grow faster than *Saccharomyces cerevisiae* at low temperatures, meaning there is greater competition for nutrients between these species and the inoculated yeast (Fleet 1997). However, inoculation with selected yeasts could allow faster imposition and control of alcoholic fermentation (AF) (Fleet and Heard 1993). Wine yeasts are usually selected from the species *S. cerevisiae* (the most important species in winemaking) according to a set of physiological features (criteria) that indicate their potential usefulness for industrial wine production (Rainieri and Pretorius 2000). The importance of the additional yeast characteristics differs according to the type and style of wine to be made and the winery's technical requirements. Generally, oenological characters are evaluated for all yeast isolates by carrying out small-scale fermentations in synthetic or semi-synthetic media (Vaughan-Martini and Martini 1998; Vazquez et al. 2000; Lopes et al. 2007a). The selection procedure includes evaluating the yeast characteristics that efficiently transform grape sugars into alcohol and carbon dioxide at a controlled rate and without the development of off-flavors. The yeasts are also selected on the basis of SO_2 resistance, killer activity, low foam production, volatile acidity, high ethanol and tolerance production, sugar exhaustion, growth at low temperature, growth at high sugar concentration and formation of H_2S (Regodon et al. 1997; Martínez-Rodríguez et al. 2001; Grieco et al. 2011).

Whereas alcoholic fermentation at low temperature is a common practice for white and rosé wines, it is a new concept for red wines and there are few examples of yeasts being selected for this purpose (Argiriou et al. 1996; Llauradó et al. 2002). In Argentina, several native *S. cerevisiae* strains have been selected for red must fermentation, but these fermentations have been carried out at the temperatures traditionally used for this process (22–28°C) (Lopes et al. 2007b).

The aim of this study was to select native *S. cerevisiae* strains to conduct the AF of red must at low temperature (15°C), thus producing volatile compounds that enhance the aromatic profile of young red wines. Native yeast strains were isolated from red musts and characterized using different oenological and technological criteria. The pre-selected native *S. cerevisiae* strains were evaluated in microvinifications of Malbec must at 15°C. The complete selection procedure was carried out over 2 years.

Materials and methods

Isolation of yeast strains from spontaneous fermentations

Spontaneous fermentations were conducted using red grape varieties (Malbec, Cabernet Sauvignon, Tempranillo, Bonarda and Syrah) from vineyards located in different areas in the province of Mendoza (Argentina). The grapes were crushed aseptically and the must obtained was placed in 5-L tanks and administered with 50 mg L^{-1} of total SO_2 and 30 g hL^{-1} of yeast nutrient Fermaid K (Lallemand, Montreal, Canada). The fermentations were carried out spontaneously following the Mendoza wineries' standard vinification practices for red must. The only change in the standard vinification protocol was that the AF was conducted at 15°C.

The yeasts were isolated by taking wine samples from each tank during fermentation (at 3/4 AF and when the AF was completed). Aliquots (0.1 mL each) of several decimal dilutions in 0.1% peptone-water were spread onto WL Nutrient Agar (Oxoid, Basingstoke, UK) that had been treated with chloramphenicol (50 mg L^{-1}) and erythromycin (70 mg L^{-1}). Plates were incubated at 28°C for 2 days. Plates containing between 30 and 300 colonies were examined. WL Nutrient Agar allows the presumptive identification of the yeast species according to colony morphology and color (Pallmann et al. 2001). Putatives colonies of *Saccharomyces* spp. were isolated for identification.

Yeast species identification

Initially, isolated yeasts were identified according to certain phenotypic criteria (Kurtzman and Fell 1998). To distinguish between *Saccharomyces* and non-*Saccharomyces* yeasts, every isolate was evaluated according to its ability to grow in L-lysine medium (Oxoid). All isolates that were not able to grow using L-lysine as the sole nitrogen source were regarded as *Saccharomyces* spp. Also evaluated was the ability of the isolated yeasts to produce ascospores with 4 spores on acetate agar. The identification of each yeast was confirmed by the restriction patterns generated from the region spanning the internal transcribed spacers (ITS1 and ITS2 primers) and the 5.8S rRNA gene.

Total DNA was extracted following the method described by Hoffman and Winston (1987). The region between the 18S rRNA and 28S rRNA genes was amplified using specific internal transcribed spacers (i.e. the ITS1 and ITS4 primers) (White et al. 1990). The polymerase chain reaction

(PCR) conditions and the methodology used to digest the PCR products were similar to those described by Fernández-Espinar et al. (2000) when they differentiated the species into the *Saccharomyces* sensu stricto complex. The PCR products were digested with the restriction enzymes *Hae* III, *Hpa* II y *Scr*F I (New England BioLabs, Hanover, USA) according to the supplier's instructions. The PCR products and their restriction fragments were separated on 1.4 and 3% agarose gels (Invitrogen, Carlsbad, USA), respectively, with 0.5× TBE buffer. After electrophoresis, the gels were stained with 0.5 μg mL^{-1} ethidium bromide, visualized under UV light and photographed with a camera coupled to Gel Doc XR software (Bio Rad Laboratorios, Hemel Hempstead, UK.). The molecular marker 100-bp DNA ladder (Invitrogen) was used as the molecular size standard.

Saccharomyces strain level identification

S. cerevisiae isolates were subsequently differentiated at strain level by PCR interdelta element analysis and mitochondrial DNA restriction fragment length polymorphism (mtDNA-RFLP). For the PCR interdelta analysis, the total DNA was extracted as described above. The oligonucleotide primers delta12 (5′-TCAACAATGGAATCCCAAC-3′) and delta21 (5′-CATCTTAACACCGTATATGA-3′) were used to amplify the total genomic DNA between the repeated interspersed delta sequences (Legras and Karst 2003). Amplification reactions were performed with a Mastercycler Gradient Eppendorf thermocycler (Eppendorf, Hamburg, Germany) using the following program: initial denaturation at 95°C (5 min); 35 cycles of denaturing at 94°C (1 min), annealing at 50°C (1 min), extension at 72°C (1 min) and a final extension at 72°C (10 min). PCR products were separated onto 1.5% agarose gels in 0.5× TBE buffer. After electrophoresis, gels were stained and visualized as described above. The molecular marker 100 bp DNA ladder (Promega, Madison, USA) served as the size standard. For the mitochondrial DNA restriction fragment length polymorphism the total extracted DNA was digest with the restriction enzyme *Hinf* I (New England BioLabs) as described by Querol et al. (1992). Restriction fragments were separated by electrophoresis onto 1% agarose gel, and then stained and visualized following the procedure described above for the PCR products. The molecular patterns of *S. cerevisiae* native strains were compared with each other and with the molecular patterns obtained from 33 commercial *S. cerevisiae* strains frequently used in the Mendoza region.

Oenological fermentations

Small-scale fermentations were carried out in concentrated red must diluted to 240 g L^{-1} with reducing sugar (RS) (Vazquez et al. 2000). The yeast assimilable nitrogen concentration (YAN) was adjusted to 200 mg L^{-1} with ammonium sulphate, and NaOH was added in order to increase the medium pH to 3.5 (Lopes et al. 2007b). The assay was done in 500-mL Erlenmeyer flasks containing 300 mL of the culture media. After sterilization, the Erlenmeyer flasks were inoculated with 10^6 CFU mL^{-1} of each indigenous *S. cerevisiae* strain. The flasks were plugged with glass fermentation traps containing sulphuric acid so that only CO_2 could evolve from the system, and they were kept at 15°C without agitation (Vaughan-Martini and Martini 1998). The weight loss of the fermentation was monitored daily until the same weight was obtained for two consecutive measures. To determine the vigor of each strain's fermentation (FV), a similar assay was carried out with 300 g L^{-1} of initial RS concentration (Vazquez et al. 2000). The fermentations were done in triplicate in separate trials. Two commercial yeast strains, the native yeast strain INTA-MZA (Lallemand Inc.) and the foreigner strain VL3 (Lallemand.), were used as controls in all the oenological characterizations and in the following selection steps. When the AF had finished, the fermented media were racked and centrifuged 5 min at 2,133*g*. The clear fermented media were assayed for ethanol, RS and volatile acidity (VA) as described in "Analytical methods". These data were used to calculate the following oenological parameters proposed by Vazquez et al. (2000) for strain characterization: (1) fermentative efficiency (FE) is measured as the amount of RS (g L^{-1}) needed to produce 1% of ethanol. FE (initial RS concentration – final RS concentration)/ethanol concentration; and (2) fermentation rate (FR) is calculated as the amount of CO_2 produced after 3 days of fermentation (CO_2 day^{-1}). This parameter is measured during the exponential phase of fermentation; and fermentation vigor (FV) indicates the maxim ethanol yield that a yeast strain can produce by fermentation in the presence of a high initial RS concentration (300 g L^{-1}).

Ethanol and SO_2 tolerance assays

Ethanol and SO_2 tolerance was determined using the tests proposed by Vazquez et al. (2000) with modifications. Different concentrations of ethanol (2, 10, 12, 13, 14, 15 and 16%) and SO_2 (25, 50, 100, 150, 200, 250 and 300 mg L^{-1}) were added to the fermentation media before the yeast inoculation. Fermentation tubes without ethanol or SO_2 were used as the control. All the tubes were simultaneously inoculated with 10^6 CFU mL^{-1} of active yeast strain from the overnight culture. Turbidity and gas production were considered a positive result.

Killer assay

The K2-type killer toxin character (negative, neutral or positive) was determined using the seeded-agar-plate

technique on YEPD medium (g L^{-1}): yeast extract, 10; glucose, 20; peptone, 20; agar, 20. The YEPD medium was treated with 3 mg L^{-1} methylene blue and buffered at pH 4.5 with 0.1 M phosphate-citrate following the protocol described by Vazquez et al. (2000).

Hydrogen sulfite production

H_2S production by *S. cerevisiae* strains on BigGy agar (Difco), a commercially available bismuth-containing agar, was determined following the methodology proposed by Jiranek et al. (1995) and Mendes-Ferreira et al. (2002). The color of the colonies growing on the indicator media provides a visual measure of the genetically determined maximal activity of sulfite reductase and its potential to produce H_2S under permissive conditions (Jiranek et al. 1995). Reactions on indicator media were determined by streaking yeasts for single colonies onto BigGy agar and incubating at 25°C (48–72 h). The color of the isolated yeast colony (white, pale hazel, hazel, dark hazel, black) was observed. The darkness of the color on BigGy agar is in direct proportion to H_2S production (Mendes-Ferreira et al. 2002).

Foam production

Foam height produced by *S. cerevisiae* strains was measured as previously described by Lopes et al. (2007b). Yeasts were classified into three categories on the basis of the maximum foam height reached: lower (foaming lower than 2 mm), middle (foaming between 2 and 4 mm) and higher (foaming greater than 4 mm), in accordance with Martínez-Rodríguez et al. (2001).

β-glucosidase activity

β-glucosidase activity was screened on agar plates containing arbutine as the carbon source (Strauss et al. 2001). The composition of the medium was (g L^{-1}): yeast nitrogen base with aminoacids (Sigma), 6.7; arbutine (Sigma), 5; agar (Britania), 20. The pH was adjusted to 4.0 prior to sterilization. Immediately after sterilization, 2 mL of ferric ammonium citrate solution (10 g L^{-1}) was added to 100 mL of medium. The plates were incubated for 7 and 15 days at 25 and 15°C, respectively. The appearance of a dark brown color in the colonies indicated β-glucosidase activity.

Volatile compound production at laboratory scale

Small-scale fermentations were carried out to characterize the volatile compounds produced by the yeast strains. A chemically defined fermentation medium (resembling grape juice) was made as follows: 240 g L^{-1} of RS (120 g L^{-1} glucose and 120 g L^{-1} fructose) and 250 mg L^{-1} of YAN without lipids or anaerobic factors. The final pH of each medium was adjusted to 3.5. Fermentations were carried out in 250-mL Erlenmeyer flasks containing 125 mL of medium and closed with cotton wool plugs to achieve micro-aerobic conditions. Static batch fermentations were conducted in triplicate at 15°C to simulate winemaking conditions. The volatile compounds obtained were evaluated by gas chromatography as described below in "Gas chromatography analysis". The AF was monitored as previously described in the fermentation experiments.

Microvinifications

The fermentations were carried out in 6-L flasks containing 5 L of Malbec must (244 g L^{-1} of reducing sugars, pH 3.9) supplemented with potassium metabisulphite at a final concentration of 50 mg L^{-1}. The YAN concentration was adjusted to 230 mg L^{-1} with ammonium sulfate. The flasks were inoculated with 10^6 CFU mL^{-1} of each indigenous and control *S. cerevisiae* strains. Fermentation was conducted at 15°C and were considered complete once the RS had depleted (<1.8 g L^{-1}). The imposition of the inoculated strain was checked by PCR interdelta analysis at 3/4 of the AF. At the end of the AF, the wines were settled and racked. After that, the wines were physically and chemically stabilized, bottled and stored at 18°C. The chemical characteristics and volatile compounds of the wines were then determined.

Analytical methods

Yeast assimilable nitrogen (YAN) evaluated as initial free α-amino nitrogen in must was calculated by formol titration (Aerny 1996). The volatile acidity, pH, ethanol and sugar concentrations, color index (CI) and tint were determined by standard methods (Organisation Internationale de la Vigne et du Vin 2007). The microvinification progress of the AF was monitored daily using density gravimetric method (Iland et al. 2000). The chromatic characteristics of the wines were also determined by CIELab measures. The Commission Internationale de l'Eclairage (1986) tristimulus values (X, Y, Z), and CIELab rectangular (L^*, a^*, b^*) and cylindrical (L^*, C^*, h) coordinates (illuminant/standard observer conditions: D65/CIE 1964 10°) were calculated using the simplified method described by Ayala et al. (2001). The software MSCV for Windows 95/98 (http://www.unizar.es/nequeruela/html/grupo_color.htm) was used to obtain these values. Color differences (ΔE^*) in CIELab units between two wines (r and s) were calculated with the following equation: $\Delta E^*_{r,s} = \left[(\Delta L^*_{r,s})^2 + (\Delta a^*_{r,s})^2 + (\Delta b^*_{r,s})^2\right]^{1/2}$.

Two wines had color differences which could be perceived by the human eye when the ΔE^* value was more than 2.7 CIE-Lab units (Negueruela et al. 2001).

Reagents and standards

R-(−)-2-octanol (Fluka) was used as the internal standard (IS). Water was obtained from an Elix3/Sinergy-185 purification system (Millipore, Brazil). Sodium chloride (ACS-ISO quality) and methanol (Lichrosolv grade) were purchased from Merck (Darmstadt, Germany).

Solid phase microextraction (SPME) sampling conditions

Samples were obtained by extracting 15 mL of synthetic medium or wine from each treatment and were conserved at −18°C until analysis. Samples were defrosted at room temperature and centrifuged at 2,133*g* for 5 min (Rolco, Argentine). Five milliliters of the samples and 4,975 μL of pure water (Millipore, Brazil) were placed in a 20-mL glass sample vial. R-2-octanol was used as the internal standard (25 μL of a methanolic solution of 25 ng μL^{-1}) and 3 g NaCl was added. The vial was sealed with a Teflon-faced septum cap and put on a magnetic stirrer (IKA, USA) at 1,100*g*. The sample was pre-conditioned for 15 min at the extraction temperature (40°C). The SPME fiber used was a 65-μm polydimethylsiloxane-divinylbenzene (PDMS/DVB) fiber coating (Supelco, USA). Before using, the fiber was conditioned according to the manufacturer's instructions. After the sample had been pre-conditioned, the SPME fiber was exposed (2 cm) to the headspace for 15 min at a controlled temperature (40°C) during the extraction process and then immediately inserted for 20 min into the GC injector port (230°C) for thermal desorption of the volatile compounds.

Gas chromatographic conditions

Volatile compounds were determined by gas chromatography. This analysis was performed using a Varian CP-3800 gas chromatograph with an ion trap mass detector (MS) Saturn 2200 (Varian, CA, USA). The column used was 30 m×0.25 mm Factor Four VF5 with a 0.25-μm film thickness (Varian). The column temperature was initially set at 40°C (5 min), programmed to ramp to 100°C at a rate of 1.5°C min^{-1}, then raised at 3 C min^{-1} up to 215°C for 5 min. Helium was used as a carrier gas at a constant flow rate of 1.0 mL min^{-1}. The injection port temperature was 230°C. Splitless injections were made. An electron impact (EI) at energy of 70 eV was used for ionization, and the temperature of the transferline and the ion trap was 200°C. The identification and the quantification of volatile compounds were identified by comparing them with the retention times of standard solutions and with the mass spectra from the Nist 2.0 library. They were quantified using relative areas related to the internal standard.

Sensorial descriptive analysis

Sensorial descriptive analysis (SDA) was carried out to explore the differences between the Malbec wines which had each been fermented at low temperature with a different yeast strain. Sensory descriptive analysis was carried out 4 months after bottling by 13 trained panelists from the Stable Sensorial Analysis Group of the Oenological Research Centre at the Instituto Nacional de Tecnología Agropecuaria (National Institute of Agricultural Technology). Panelists from this group are continuously trained in monthly sessions and in the Annual Sensory Descriptive Training Course. Wines were equilibrated at room temperature (22°C) and 50-mL samples were poured into wine glasses ISO 3591 International Standards Organization 3591 (1977). Sensory descriptive analysis was performed on anonymous samples. The order of the samples was assigned randomly for each panelist. The first session evaluated the wine descriptors for SDA that are associated with typical Malbec wine flavors, these being: color intensity, violet tint, aroma intensity, red fruits, balsamic flavor, bitterness, astringency and concentration. In addition, two descriptors related to defects in the wine were included: nail polish and rotten egg aromas. In the following session, the intensity of each descriptor was measured using a no-structured scale (Reynolds et al. 2001). All the panelists' average ratings for each wine and each descriptor were obtained. Replicates were done separately on different days.

Statistical analysis

Statistical data analyses were done using Statgraphics Plus (version 5.1). The data normality and variance homogeneity in the residuals were verified. Analysis of variance (ANOVA) followed by an LSD Fisher Test was used to evaluate the significance of variation between means. All significance tests were conducted at levels of $p \leq 0.05$. The Kruskal–Wallis test was used for nonparametric data. The oenological parameters and sensorial analyses also were investigated by principal component analysis (PCA) using the software InfoStat/Professional version 1.5 (Estadística y Diseño, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina).

Results

For many years, different procedures have been applied to selecting yeast strains to find the most appropriate strains for fermenting different wine styles. Tests to evaluate and select the best strain for a specific kind of fermentation differ depending on the wine style that is desired. Fermentation at a low temperature is common practice in white and rosé wine production but its application in red wine production is a new development. In the present study,

native *S. cerevisiae* strains have been characterized and selected to ferment red must at 15°C and thus obtain aromatic young red wines.

Isolation and identification of yeast strains

More than 100 yeasts were isolated from the red must fermented at 15°C. Of these, 34 isolates yeasts were evaluated. These yeast isolates were selected considering the different musts and sampling points during AF (3/4 and the end), and to avoid duplicate isolates, colonies with different morphologies and colors in WL Nutrient Agar were selected (Oxoid). Phenotypic criteria were used to presumptively identify the yeasts as *Saccharomyces* spp., which were then confirmed as *S. cerevisiae* by molecular methods (data not shown). Of the 34 strains tested, 9 and 8 isolates showed the same interdelta PCR molecular pattern as the two commercial yeast strains ICV D254 and EC1118 (Lallemand), respectively. These commercial yeast strains are the most frequently used to wine fermentation in the Mendoza region. Of the 17 remaining isolates, only 14 molecular patterns differed from the commercial strains evaluated (Fig. 1). These molecular data were confirmed by mtDNA-RFLP (data not shown). Fourteen different *S. cerevisiae* strains were submitted to the following oenological characterization (Table 1).

Oenological characterization of S. cerevisiae native strains

Small-scale fermentation was conducted with 14 *S. cerevisiae* native strains at 15°C. The wines obtained were chemically analyzed and fermentative parameters were calculated. Nine native yeast strains performed well when fermenting red musts at low temperature (Fig. 2). These strains consumed all the residual sugars and produced less volatile acidity than the control strains under the same conditions. In addition, the native yeasts strains showed a higher tolerance to stressful situations than the commercial strains. They were able to start the AF with a high RS concentration (300 g L^{-1}) and exhibited high fermentation vigor (FV) at a low temperature (Fig. 2). Table 2 summarizes the results of the tests carried out to evaluate the oenological properties of 9 different native yeast strains. Most of the native strains showed high tolerance to ethanol and SO_2 concentrations and low genetic potential to produce H_2S. BLA-39, MaB-2 C, MaE-1 C, and Bo-1 C strains showed a positive killer phenotype, whereas a neutral killer phenotype was observed in All-9, BBT-27, and UBA-21 strains. Only MaB-2 C strain presented low foam production whereas other native strains presented a foam production similar to that of commercial strains. The strains used in this trial did not show β-glucosidase activity at 15°C, although All-9, UBA-21, Bo-1 C, and MaE-1 C strains show this enzyme activity at 25°C (Table 2). Considering all data obtained, 3 of the 9 initial native strains were eliminated. The A11-8 and M11-13 strains were eliminated because showed a negative killer phenotype. Also, A11-8 was the least resistant to ethanol, and M11-13 showed the greatest genetic potential to produce H_2S. The BBT-27 strain formed a high quantity of foam during fermentation and was therefore also excluded from the following selection step.

Production of volatile compounds in synthetic medium

The evaluated All-9, BLA-39, UBA-21, MaB-2 C, MaE-1 C, Bo1C strains produced fermentative volatile compounds related to fruity, floral and spicy aromas. Fermentations were carried out in a synthetic medium without free monoterpenes and glycoconjugate precursors. In this medium, the synthesis of most of the fermentative compounds differed significantly according to the yeast strain (Table 3). Some volatile compounds could not be produced in sufficient concentrations to allow detection by the analytical method employed.

Most of the esters detected in the synthetic medium were related to desirable aromas in wines and were present at

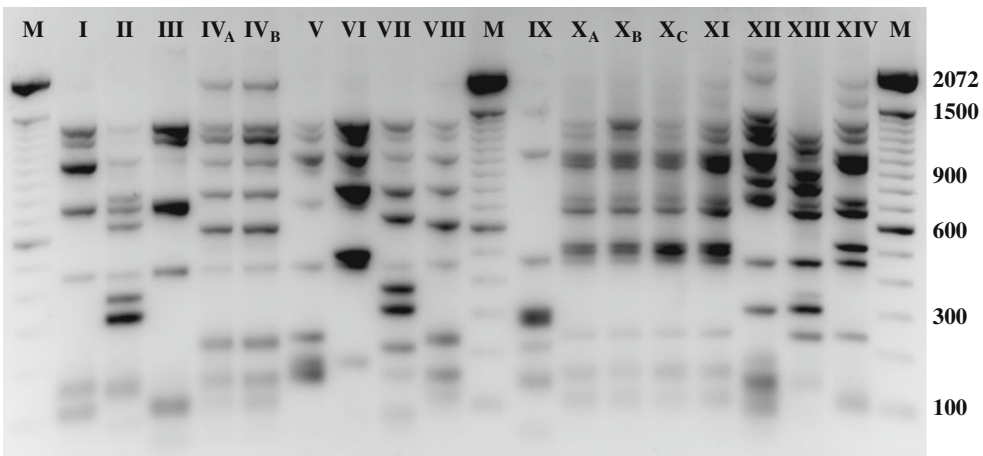

Fig. 1 Molecular patterns obtained by interdelta PCR of 17 *Saccharomyces cerevisiae* native isolates. The *Roman numbers* indicate the identity of different isolates. *Lane M* corresponds to the 100-bp DNA ladder. Sizes of the markers in base pairs are indicated on the *right*

Q6t1.1 **Table 1** Name and isolation origin of 14 yeast native strains included in the oenological characterization

Strain	Isolation origin	Molecular pattern[a]	
A11-8	Malbec (2000) – Luján de Cuyo, Mendoza	I	t1.3
A11-9	Malbec (2000) – Luján de Cuyo, Mendoza	II	t1.4
BBT-27	Bonarda (2000) – Maipú, Mendoza	III	t1.5
BLA-39	Bonarda (2000) - Maipú, Mendoza	IV	t1.6
M11-13	Malbec (2000) – Luján de Cuyo, Mendoza	V	t1.7
SBB-11	Syrah (2000) – Maipú, Mendoza	VI	t1.8
SLB-5	Syrah (2000) – Maipú, Mendoza	VII	t1.9
UBA-21	Ugni Blanc (2000) – Maipú, Mendoza	VIII	t1.10
ULA-16	Ugni Blanc (2000) – Maipú, Mendoza	IX	t1.11
MaB-2C	Malbec B(2006) – La Consulta, Mendoza	X	t1.12
MaE-1C	Malbec E (2006) – Tunuyán, Mendoza	XI	t1.13
MaS-1B	Malbec S (2006) – La Consulta, Mendoza	XII	t1.14
MaS-1C	Malbec S (2006) – La Consulta, Mendoza	XIII	t1.15
Bo-1C	Bonarda (2006) – Tupungato, Mendoza	XIV	t1.16

[a] Molecular patterns obtained by PCR interdelta and confirmed by RFLP mtDNA.

concentrations above their odor threshold, with the exception of ethyl acetate which was synthesized at concentrations below its odor threshold. The hexanoate and octanoate ethyl esters were present at greater concentrations. Statistical differences in the total concentration of esters were detected among the strains evaluated. The A11-9, UBA-21 and VL3 strains showed the highest concentrations of total esters; in contrast, the BLA-39, MaE-1 C, Bo-1 C and INTA-MZA strains produced significantly fewer total esters (Table 3). We evaluated the two most important higher alcohols for all the yeasts which synthesized different concentrations of 2-phenylethanol above its odor threshold. Among these, the MaB-2 C strain showed the lowest 2-phenylethanol value (0.57 mg L^{-1}) and the VL3 strain produced the highest concentration (1.54 mg L^{-1}). The 3-(methylthio)-1-propanol concentrations for all the strains were below its odor threshold. The MaB-2 C strain synthesized the smallest concentration of total higher alcohols (0.58 mg L^{-1}) whereas the VL3 strain the largest, 1.54 mg L^{-1} (Table 3). The yeast strains synthesized terpenes (linalool and/or citronellol) in the synthetic medium at 15°C in concentrations near or above their odor threshold (Table 3). Bo-1 C strain showed the lowest values of linalool (5 μg L^{-1}) whereas BLA-39 and MaB-2 C showed the higests, 17 μg L^{-1} and 14 μg L^{-1}, respectively. On the other hand, the BLA-39 and Bo-1 C strains exhibited the maximum concentrations of citronellol 55 μg L^{-1} and 34 μg L^{-1}, respectively. The control strain INTA-MZA showed the lowest citronellol concentration, this being below the odor threshold of this terpene. Furthermore, the native strain MaE-1 C did not synthesize citronellol in detectable concentrations. MaE-1 C synthesized the lowest concentration of total terpenes whereas BLA-39 synthesized

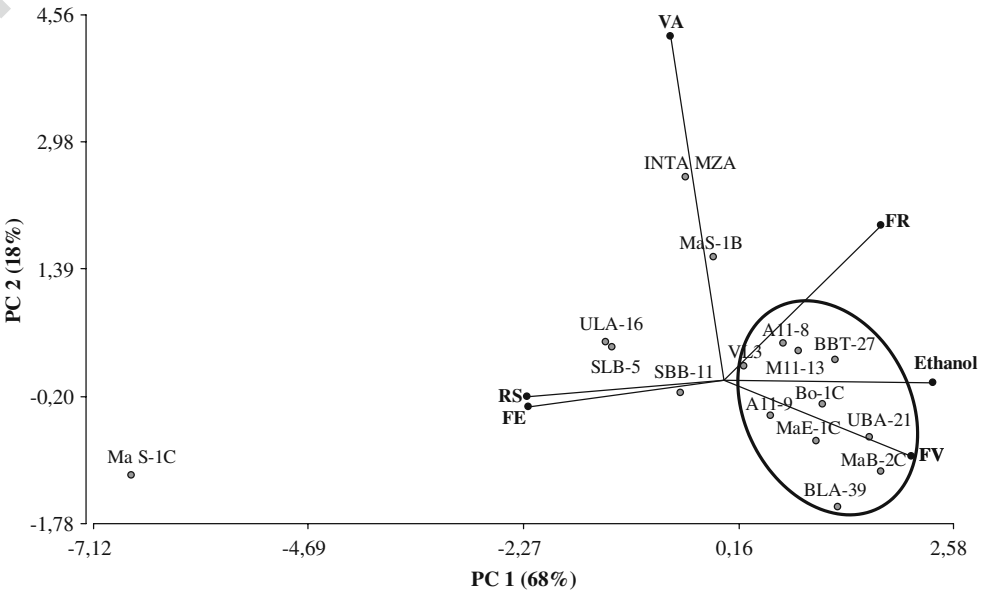

Fig. 2 Principal component analysis (PCA) of wine chemical compositions (*RS* reducing sugar, *VA* volatile acidity and ethanol) and fermentative parameters (*FE* fermentation efficiency, *FR* fermentation rate, *FV* fermentation vigor) of the *Saccharomyces cerevisiae* native and commercial (*VL3* and *INTA-MZA*) strains in fermentations carried out at laboratory scale. The *circle* indicates the native *S. cerevisiae* strains that were pre-selected at this stage

JnlID 13213_ArtID 271_Proof# 1 - 07/05/2011

t2.1 **Table 2** Oenological characterization of native and commercial (VL3 and INTA MZA) yeast strains.

Strain	Ethanol tolerance (%)	SO_2 tolerance (mg L^{-1})	Foam	H_2S[a]	Killer character[b]	β-Glucosidase[c]
A11-8	12	300	Middle	+	K^-R^-	−
A11-9	14	300	Middle	+	K^-R^+	+
BBT-27	14	300	High	++	K^-R^+	−
BLA-39	14	300	Middle	+/−	K^+R^+	−
M11-13	13	300	Middle	++	K^-R^-	−
UBA-21	13	300	Middle	+/−	K^-R^+	+
MaB-2C	15	300	Low	+	K^+R^+	−
MaE-1C	13	300	Middle	+	K^+R^+	+
Bo-1C	14	250	Middle	+	K^+R^+	+
VL3	14	300	Middle	+++	K^-R^+	+
INTA MZA	14	300	Middle	++	K^-R^+	+

[a] Genetic potential to product H_2S: null (−), weak (+/−), low (+), media (++), high (+++)

[b] Character to K2-type killer toxin: negative (K^-R^-), neutral (K^-R^+), positive (K^+R^+)

[c] β-glucosidase activity determinate at 25°C: negative (−), positive (+)

the highest (Table 3). The synthesized octanoic and decanoic acids by the *S. cerevisiae* strains are below the odor threshold. Under the conditions of the assay, we could not detect for the Bo-1 C strain octanoic acid production, and the commercial strain VL3 showed the highest concentration of both acids (Table 3). The control yeasts (VL3 and INTA-MZA) differed from each other in terms of the total production of odorant compounds. In the assay conditions, VL3 strain produced significantly higher concentrations of all the groups of volatile compounds (Table 3).

Microvinifications

Six native yeast strains were tested in microvinifications of Malbec must (5 L) conducted at 15°C. The wines were considered to be "dry" and AF concluded when the RS concentration was below 1.8 g L^{-1}. AF was completed between 14 and 18 days depending on the strain. Implantation control of inoculated strains was performed in each vinification. In all cases, more than 93% of the strain isolates at the end of fermentation showed a PCR interdelta molecular pattern corresponding to the inoculated strain (data not shown). The MaB-2 C and MaE-1 C strains were statistically fastest at completing the AF whereas the A11-9 strain and the control strain VL3 were the slowest. The BLA-39 and UBA-21 native strains could not finish the AF (Table 4). The final ethanol concentration was very similar for all the wines. Under the test conditions, all the native strains produced significantly less volatile acidity (around 0.1 g L^{-1}) than the control strains VL3 and INTA-MZA, 0.32 g L^{-1} and 0.44 g L^{-1}, respectively (Table 4). The wines produced with both commercial strains showed the highest tint values which indicated a higher proportion of yellow pigments (Table 4). The wine made with MaB-2 C had the highest IC whereas both the wines made with commercial strains showed the lowest IC. The wines produced with both commercial strains showed the highest tint values which indicated a higher proportion of yellow pigments (Table 4). The ΔE^* parameter was calculated using the CIE-Lab coordinates. All wines made with native strains showed ΔE^* values greater than 2.7 units when compared with commercial strains wines. The values obtained from this parameter indicated that consumers could perceive the color differences between the wines. The wine produced with MaB-2 C showed the highest ΔE^* values when it was compared with the commercial strains wines (data not shown). SDA was carried out to evaluate wines obtained with the 6 native and 2 commercial strains (Fig. 3). Wines fermented with the MaB-2 C, MaE-1 C, Bo-1 C and UBA- 21 native strains were related to descriptors of color intensity, violet tint, concentration and astringency by the panelists. Wines made with the MaE-1 C and Bo-1 C native strains were noted for their aromatic intensity and red fruit notes. On the other hand, wine made with MaB-2 C was related to the balsamic descriptor mainly associated with greater aromatic complexity. The A11-9 and BLA-39 wines showed intermediate values for the descriptors used. Juries found lower color intensity, violet tint, aromatic intensity, concentration and astringency in the commercial strain wines (Fig. 3). Sensory descriptive analysis showed that wines made with native strains were more related to intense color descriptors and violet tint than the commercial strains wines were. All the yeasts tested were able to produce esters related to desirable aromas in wines. The isolated strains used in this study produced wines with statistically different concentrations of most volatile compounds (Table 5). The esters, ethyl hexanoate and ethyl octanoate, were present in the greatest concentration (Table 5). Under mirovinification conditions, the yeasts synthesized ethyl acetate in concentrations below its odor threshold (Table 5). This result was consistent with the tasters' descriptions, which did not associate the wines with the ethyl acetate descriptor (nail polish) (Fig. 3). In general, the range of concentrations for different esters quantified in

t3.1 **Table 3** Volatile compounds produced by *Saccharomyces cerevisiae* native and commercial (VL3 and INTA MZA) strains in synthetic media fermented at 15°C

t3.2 / t3.3	Volatile compounds (mg L^{-1})	Odor threshold (mg L^{-1})	*S. cerevisiae* strains							
			A11-9	BLA-39	UBA-21	MaB-2 C	MaE-1 C	Bo-1 C	VL3	INTA MZA
t3.4	Ethyl acetate	7.5	0.45±0.24 a	0.80±0.25 ab	0.60±0.18 ab	ND	0.53±0.12 ab	0.59±0.09 ab	1.02±0.38 b	0.65±0.64 ab
t3.5	Ethyl butanoate	0.02	0.03±0.02 a	0.05±0.02 a	0.04±0.01 a	0.04±0.01 a	0.07±0.01 a	0.04±0.01 a	0.07±0.04 a	0.07±0.06 a
t3.6	Ethyl hexanoate	0.005	2.51±1.59 b	0.90±0.84 a	0.91±0.40 a	ND	1.18±0.68 ab	0.88±0.49 a	1.92±1.43 ab	ND
t3.7	Ethyl octanoate	0.58	1.72±0.65 abcd	1.18±0.16 abc	3.45±1.65 d	3.24±0.58 cd	1.04±0.20 a	1.05±0.22 a	2.40±1.37 bcd	1.10±0.23 ab
t3.8	Isoamyl acetate	0.03	0.13±0.06 a	0.23±0.09 a	0.14±0.09 a	0.15±0.02 a	0.22±0.09 a	0.12±0.04 a	0.30±0.20 a	0.24±0.22 a
t3.9	2-phenylethyl acetate	0.25	0.28±0.09 abc	0.43±0.16 bc	0.37±0.29 abc	0.13±0.02 a	0.14±0.01 a	0.17±0.03 ab	0.53±0.26 c	0.29±0.13 abc
t3.10	Hexil acetate	0.002	0.012±0.010 a	ND	ND	0.024±0.008 b	0.014±0.002 a	0.015±0.002 a	ND	ND
t3.11	Total esters[a]		4.68±1.07 cd	2.80±0.48 ab	4.91±1.33 d	3.58±1.30 bc	2.66±0.52 ab	2.28±0.46 a	5.23±1.03 d	1.71±0.42 a
t3.12	2-phenilethanol	0.3	0.92±0.34 abc	1.31±0.46 bc	1.07±0.69 abc	0.57±0.08 a	0.73±0.12 ab	0.77±0.16 ab	1.54±0.69 c	0.73±0.23 ab
t3.13	3-(methylthio)-1-propanol	0.5	0.015±0.008 a	0.018±0.007 ab	0.009±0.002 a	0.010±0.006 a	0.018±0.004 ab	0.027±0.006 b	ND	0.015±0.011 a
t3.14	Total alcohols		0.94±0.34 abc	1.33±0.47 bc	1.08±0.68 abc	0.58±0.08 a	0.75±0.13 ab	0.80±0.16 ab	1.54±0.69 c	0.74±0.24 ab
t3.15	Linalool	0.015	0.011±0.004 ab	0.017±0.007 b	0.010±0.007 ab	0.014±0.002 b	0.010±0.003 ab	0.005±0.002 a	0.013±0.005 ab	0.011±0.005 ab
t3.16	Citronellol	0.015	0.025±0.008 ab	0.055±0.026 c	0.016±0.011 ab	0.019±0.007 ab	ND	0.034±0.003 bc	0.025±0.016 ab	0.008±0.003 a
t3.17	Total terpenes		0.036±0.010 bc	0.071±0.027 d	0.026±0.004 abc	0.033±0.003 bc	0.010±0.007 a	0.039±0.021 c	0.038±0.009 c	0.019±0.002 ab
t3.18	Octanoic acid	10	1.18±0.76 a	1.81±0.53 a	1.20±0.59 a	1.04±0.10 a	1.20±0.20 a	ND	1.97±1.06 a	1.44±0.81 a
t3.19	Decanoic acid	15	0.17±0.04 a	0.60±0.29 b	0.42±0.23 ab	0.18±0.02 a	0.19±0.07 a	0.12±0.04 a	0.73±0.40 b	0.16±0.08 a
t3.20	Total acids		1.35±0.72 b	2.41±0.86 cd	1.62±0.55 bc	1.22±0.60 b	1.39±0.72 b	0.12±0.04 a	2.71±0.88 d	1.60±0.90 bc

Numbers located in the same row having different letters differ at $p<0.05$ level (Fisher LSD´s test). Values are means of three replicates±standard deviation

ND Not detected

[a] Ethyl acetate was not considered in calculating total esters because this compound is not desired in high concentrations in wine. Ethyl acetate produces a smell of solvent or nail polish in the wine when its concentration exceeds the perception threshold

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Table 4 Characteristics of the Malbec wines produced by native and commercial (VL3 and INTA MZA) *Saccharomyces cerevisiae* strains

Strain	AF duration (days)	Color characteristics		Ethanol (%)	Chemical characteristics	
		Color index	Tint		Reducing sugar (g L^{-1})	Volatile acidity (g L^{-1} acetic acID)
A11-9	17.7±0.6 b	0.98±0.12 ab	0.68±0.03 ab	14.8±0.3 a	<1.80	0.08±0.01 a
BLA-39	16.3±1.0 ab	0.97±0.03 ab	0.67±0.02 ab	15.6±0.3 b	2.38±0.82 a	0.18±0.03 c
UBA-21	16.0±1.2 ab	0.99±0.06 ab	0.66±0.05 a	15.3±0.7 ab	5.12±1.86 b	0.14±0.03 abc
MaB-2C	14.0±0.6 a	1.07±0.04 b	0.67±0.01 ab	15.3±0.3 ab	<1.80	0.10±0.01 ab
MaE-1C	14.3±0.6 a	0.92±0.16 ab	0.72±0.01 abc	15.3±0.4 ab	<1.80	0.13±0.05 abc
Bo-1C	15.3±0.6 ab	0.74±0.22 ab	0.74±0.08 bcd	15.6±0.1 b	<1.80	0.16±0.01 bc
VL3	17.3±0.6 b	0.76±0.03 a	0.82±0.01 d	15.7±0.1 b	<1.80	0.32±0.06 d
INTA-MZA	15.7±0.6 ab	0.79±0.04 a	0.78±0.01 cd	15.5±0.1 ab	<1.80	0.44±0.05 e

Numbers located in the same column having different letters differ at $p<0.05$ level (Fisher LSD´s test). Values are means of three replicates ± standard deviation

wine was similar to that found in the synthetic medium. Ethyl butanoate and isoamyl acetate concentrations in wine were 20 and 4 times higher than in the synthetic medium, respectively (Tables 3 and 5). When the total ester concentrations were estimated, statistical differences were observed between the yeast strains. The native strains A11-9, BLA-39 and MaB-2 C and the control strain MZA-INTA synthesized the highest concentration of total esters in the wine (Table 5). The yeast strains produced 2-phenylethanol concentrations above the odor threshold, whereas this did not happen with 3-(methylthio)-1-propanol (Table 5). MaE-1 C was the lowest producer of 2-phenylethanol whereas BLA-39, UBA-21 and Bo-1 C were the highest. Most of the strains tested did not synthesize 3-(methylthio)-1-propanol in concentrations that the analytical method could detect. Similar or statistically greater concentrations of total higher alcohols were found in both the wine and the synthetic media (Tables 3 and 5). All the yeast strains were able to produce both terpenes at concentrations above their odor thresholds. Significant differences in citronellol production were found between the wines produced with the *S. cerevisiae* strains. The isolates A11-9 and VL3 showed citronellol concentrations significantly higher than those of the control strain INTA-MZA. The remaining native strains synthesized intermediate concentrations of this odorant compound (Table 5). The range of the total terpenes in the wines was between 66 and 125 μg L^{-1} and showed statistical differences between wine samples. The A11-9, BLA-39 and VL3 strains were the largest terpene producers whereas INTA-MZA was the smallest (Table 5). Generally, the Malbec wines analyzed in this study showed total terpene concentrations that were statistically higher than those quantified in the synthetic medium mainly because grape free terpenes were present in the wines. However, if the lowest concentration of total

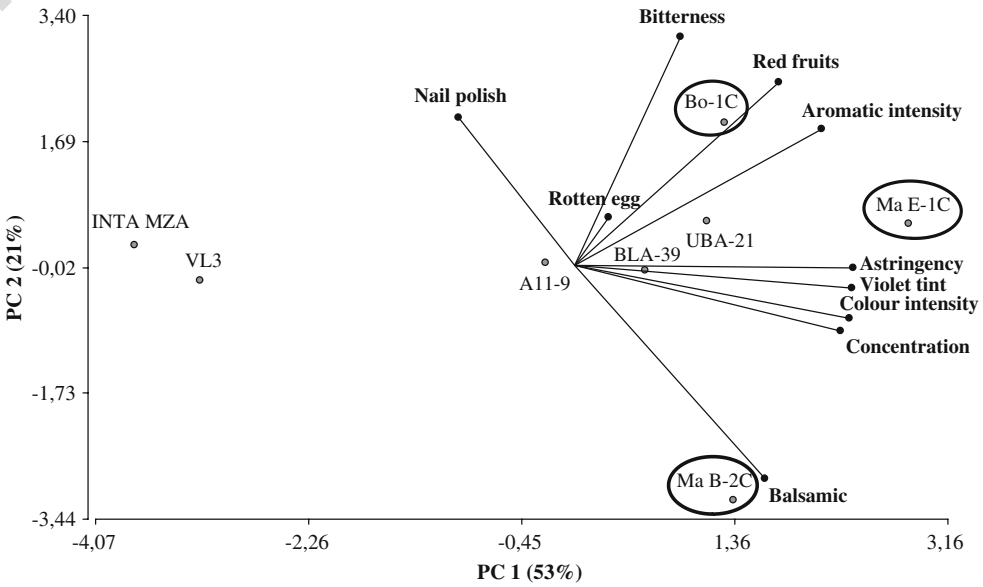

Fig. 3 Principal component analysis of the sensorial analysis of Malbec wines obtained by microvinifications (5 L) using with *Saccharomyces cerevisiae* native and commercial (*VL3* and *INTA-MZA*) strains. The *circle* indicates the native *S. cerevisiae* strains that were selected at this stage

t5.1 **Table 5** Volatile compounds in Malbec wines obtained at 15°C

t5.2 Volatile compounds (mg L^{-1})	Odor threshold (mg L^{-1})	*S. cerevisiae* strain							
t5.3		A11-9	BLA-39	UBA-21	MaB-2 C	MaE-1 C	Bo-1 C	VL3	INTA-MZA
t5.4 Ethyl acetate	7.5	0.38±0.17 a	0.69±0.04 a	0.56±0.06 a	0.40±0.24 a	0.46±0.14 a	0.36±0.02 a	0.51±0.05 a	0.68±0.21 a
t5.5 Ethyl butanoate	0.02	0.119±0.003 abcd	0.097±0.009 abc	0.085±0.016 a	0.134±0.003 d	0.111±0.016 abcd	0.088±0.003 ab	0.123±0.013 bcd	0.132±0.033 cd
t5.6 Ethyl hexanoate	0.005	1.11±0.71 a	0.98±0.24 a	0.87±0.61 a	1.59±1.86 a	0.57±0.53 a	0.77±0.28 a	0.67±0.27 a	1.16±0.48 a
t5.7 Ethyl octanoate	0.58	2.77±0.51 a	1.73±0.65 a	1.00±0.48 a	2.41±3.32 a	0.44±0.17 a	0.88±0.25 a	1.50±0.27 a	2.01±0.63 a
t5.8 Isoamyl acetate	0.03	0.49±0.24 a	1.53±0.75 a	0.84±1.17 a	0.72±0.40 a	0.79±0.61 a	0.45±0.02 a	0.81±0.28 a	1.65±0.48 a
t5.9 2-phenylethyl acetate	0.25	0.13±0.02 a	0.21±0.02 b	0.19±0.02 b	0.13±0.01 a	0.14±0.04 a	0.14±0.03 a	0.17±0.02 ab	0.18±0.01 ab
t5.10 Hexil acetate	0.002	0.005±0.004 a	0.020±0.006 b	0.017±0.004 ab	0.011±0.001 ab	0.010±0.011 ab	0.004±0.002 a	0.012±0.001 ab	0.020±0.010 b
t5.11 Total esters[a]		4.63±0.96 b	4.56±0.10 b	3.01±0.12 a	5.00±1.07 b	2.05±0.03 a	2.34±0.07 a	3.28±0.29 a	5.15±0.39 b
t5.12 2-phenilethanol	0.3	1.49±0.23 ab	1.84±0.17 b	1.50±0.05 b	1.35±0.06 ab	1.00±0.49 a	1.74±0.16 b	1.40±0.15 ab	1.40±0.02 ab
t5.13 3-(methylthio)-1-propanol	0.5	ND	0.011±0.004 b	ND	0.004±0.005 a	ND	ND	0.007±0.005 ab	ND
t5.14 Total alcohols		1.49±0.23 ab	1.85±0.18 b	1.50±0.05 b	1.35±0.06 ab	1.00±0.49 a	1.74±0.16 b	1.40±0.16 ab	1.40±0.02 ab
t5.15 Linalool	0.015	0.017±0.002 a	0.055±0.058 a	0.017±0.005 a	0.015±0.001 a	0.015±0.007 a	0.015±0.001 a	0.020±0.002 a	0.019±0.002 a
t5.16 Citronellol	0.015	0.094±0.018 b	0.067±0.032 ab	0.083±0.004 ab	0.081±0.021 ab	0.078±0.030 ab	0.075±0.004 ab	0.105±0.013 b	0.048±0.002 a
t5.17 Total terpenes		0.110±0.020 b	0.122±0.026 b	0.100±0.001 ab	0.095±0.020 ab	0.093±0.023 ab	0.090±0.005 ab	0.125±0.011 b	0.066±0.004 a
t5.18 Octanoic acid	10	0.74±0.01 bc	0.40±0.16 a	0.52±0.11 ab	0.77±0.16 c	0.85±0.10 c	0.86±0.07 c	0.76±0.10 bc	0.71±0.02 bc
t5.19 Decanoic acid	15	0.33±0.06 ab	0.15±0.05 a	0.24±0.07 ab	0.26±0.08 ab	0.42±0.09 b	0.38±0.16 b	0.26±0.10 ab	0.22±0.04 ab
t5.20 Total acids		1.07±0.06 bc	0.55±0.21 a	0.76±0.17 ab	1.03±0.24 bc	1.28±0.20 c	1.24±0.23 c	1.02±0.21 bc	0.93±0.03 abc

Numbers located in the same row having different letters differ at $p<0.05$ level (Fisher LSD´s test). Values are means of three replicates ± standard deviation

ND Not detected

[a] Ethyl acetate was not considered in calculating total esters because this compound is not desired in high concentrations in wine. Ethyl acetate produces a smell of solvent or nail polish in the wine when its concentration exceeds the perception threshold

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terpenes found in wine (66 μg L^{-1}) comes from free terpenes present in the must, the differences found between the wines can be attributed to yeast strains. The results obtained in both assays (synthetic medium and Malbec wines) showed that the S. cerevisiae strains included in this study were able to synthesize terpenes during fermentation at 15°C. Fatty acids identified in this study were synthesized by yeasts at concentrations below their odor threshold (Table 5). However, strains differed statistically in the production of both octanoic and decanoic acids. The native strains MaE-1 C and Bo-1 C synthesized the highest concentrations of them, 1.28, and 1.24 mg L^{-1}, respectively, whereas BLA-39 synthesized the lowest concentration, 0.55 mg L^{-1} (Table 5). Most of the strains in this study produced more total fatty acid concentrations in synthetic media than in wine (Tables 3 and 5).

Discussion

Wine flavor is a combination of taste and aroma and is important for consumers when defining their preferences. Fermentation at low temperatures (10–15°C) is used to increase or retain the volatile compounds of white and rosé wines but is a new concept in red wine fermentation. Little research has been published regarding the selection of yeasts for this purpose. The present study shows a process for selecting the most suitable native *S. cerevisiae* strains to carry out the AF of red musts at a low temperature (15°C). The protocol proposed to find a native *S. cerevisiae* strain suitable for conducting low temperature fermentations was successful. As described by other authors (Lopes et al. 2002; Mercado et al. 2007), and as shown by molecular analysis, different *S. cerevisiae* strains were involved in the spontaneous AF at 15°C, which meant there is a good source of genetic diversity. Some of the yeast isolates displayed the same molecular pattern as the commercial yeast strains that are widely used in the Mendoza region, which indicates that this yeast should be present in the vineyards. Various authors have suggested that commercial strains are transmitted from the cellar to the vineyards (Valero et al. 2005; Martínez et al. 2007; Schuller et al. 2004; Cubillos et al. 2009). Regardless of the purpose for which a yeast strain is selected, it must be well adapted to the vine-growing practices, winemaking techniques and must compositions of its particular area. Several authors suggest that native yeasts are more competitive than foreign commercial yeasts because the former are better adapted to the ecological and technological conditions of their wine areas (Lopes et al. 2007a; Grieco et al. 2011). Selecting native yeast strains favors the implantation of native inoculated strains in fermentations, thus diminishing the risk of deviations in the process, as our results demonstrated (Grieco et al. 2011). The commercial strains tested in this study were able to ferment red must at 15°C. However, the selected native strains were faster and produced wines with better color intensity and flavor than the commercial strains. The two commercial strains (INTA-MZA and VL3) used in this study were able to perform AF under the conditions (temperature and must type) for which they were selected, but they had problems carrying out the AF at different conditions, as our results showed. This demonstrates the importance of selecting native yeasts that are capable fermenting red musts at 15°C.

During this selection process, all the native strain isolates were subjected to selection pressure from the beginning of the process (red must and low temperatures), which allows the number of strains to be reduced as the selection steps progressed. The fermentation parameters and chemical data obtained from semi-synthetic medium (diluted grape concentrate) on a laboratory scale were correlated with those obtained from Malbec wines fermented at 15°C. In both samples the native strains produced a similar or greater concentration of ethanol and less volatile acidity than the commercial strains. These results support the use of a semi-synthetic medium (diluted must concentrate) during the screening protocols for yeast selection as proposed other authors (Vazquez et al. 2000; Lopes et al. 2007b). The color of the Malbec wines made at low temperatures with the native strains was similar to that previously reported for red wines produced by traditional maceration (Casassa and Sari 2007). However, the wines fermented at 15°C with different strains of *S. cerevisiae* showed differences in color. These yeasts could affect the color of red wines in different ways. Some strains could favor the extraction of anthocyanins from the grapes during maceration and fermentation, depending on the activity of their extracellular enzymes and their ability to produce ethanol. Furthermore, levels of acetaldehyde produced by different yeast strains promote the formation of anthocyanin-ethylflavanol adducts which are more stable to pH and to SO_2 decoloration than monomeric anthocyanins (Escribano-Bailón et al. 2001). Caridi et al. (2004) found a correlation between the yeast strain used for winemaking and the phenolic composition of the wine. They highlighted the ability of the strain used to modify the wine's color, antioxidant power and phenolic compound profile.

An important factor to consider when selecting a yeast strain is its ability to produce aromatic compounds, this consideration being driven by consumer demand for aromatic wines. Numerous works have shown that yeasts involved in vinification possess β-glucosidase activity, and that this activity is greater in non-*Saccharomyces* yeast strains than in *S. cerevisiae* (Rosi et al. 1994; Strauss et al. 2001; Rodríguez et al. 2004; Fia et al. 2005). Unexpectedly, 44% of the yeast strains tested in this work showed this enzymatic activity at 25°C. A high percentage of *S.*

cerevisiae strains with β-glucosidase activity has been only reported by Spagna et al. (2002) and Fia et al. (2005) with 12 and 25%, respectively. Rodríguez et al. (2004) found one *S. cerevisiae* with this activity over 73 isolates (1%) from north Patagonia (Argentina) tested. These data suggest that this character may not be homogeneously distributed in the environment. More studies are needed to confirm these observations. On the other hand, the native strains were able to produce fermentative volatile compounds related to fruity, floral and spicy aromas. Similar concentrations of different volatile compounds were produced by yeast strains in synthetic media and wine, which validates the synthetic media as a selection protocol for making a preliminary evaluation of yeast's aroma production. The *S. cerevisiae* strains selected were able to synthesize monoterpenes (linalool and citronellol) in a synthetic medium and in Malbec grape juice fermented at 15°C. The terpene concentration obtained in both arrays depended on the strain used. The terpene concentration was above the odor thresholds in most of the conditions evaluated in our study. Although GC-MS was used to evaluate several volatile compounds related to yeast metabolism, the resulting analytical profile not did allow the wine aroma to be predicted with precision. Both the sensory descriptive analysis and the fermentative volatile compound composition obtained by GC-MS found differences between the strains evaluated. However, volatile compounds associated with pleasant notes are not always present in wines in high enough concentrations to be detected by tasters. Here, it is important to consider the balance between the different compounds that shape wine aroma because an aromatic compound found in the same concentration in two different wines might not be perceived in the same way or may result in different flavors as a result of its interactions with other compounds present in wine (Cabredo-Pinillos et al. 2006). This could explain the difficulties in establishing a relationship between a wine's odorant compound profile as determined by GC-MS on the one hand and an SDA conducted by a panel of tasters on the other hand. Consequently, SDA remains a very useful tool when taking a final decision in the yeast selection procedure.

Conclusion

The MaB-2 C, MaE-1 C and Bo-1 C native strains were selected to ferment red wines at low temperatures. These strains carried out a good fermentation profile and displayed attributes desirable in oenological yeast strains such as killer character, low foam formation, low genetic potential for SH_2 production, elevated ethanol and SO_2 tolerance, and β-glucosidase activity (MaE-1 C and Bo-1 C). In addition, these strains were able to synthesize linalool and citronellol in concentrations above their odor thresholds during AF at 15°C. Furthermore, the tasters described the wines obtained with these three native strains as having the most intense colors and aromas.

Acknowledgement This work was supported by the Viticulture Regional Project MZA-SJ 51007 of the Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina.

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