Received: 10 Juillet 2020 | Accepted: 2 October 2020 | Published: 18 November 2020
DOI:10.20870/oeno-one.2020.54.4.4018

OENO One
VINE AND WINE OPEN ACCESS JOURNAL

Combination of pre-fermentative and fermentative strategies to produce Malbec wines of lower alcohol and pH, with high chemical and sensory quality

Martín L. Fanzone[1,*,#], Santiago E. Sari[1,#], María V. Mestre[2,3,#], Anibal A. Catania[1], María J. Catelén[4], Viviana P. Jofré[1], María L. González-Miret[5], Mariana Combina[1,3], Fabio Vazquez[3] and Yolanda P. Maturano[2,3]

[1]Estación Experimental Agropecuaria Mendoza, Instituto Nacional de Tecnología Agropecuaria (EEA Mendoza INTA), San Martín 3853, 5507 Luján de Cuyo, Mendoza, Argentina
[2]Instituto de Biotecnología, Universidad Nacional de San Juan (UNSJ), Av. San Martín 1109 (O), San Juan, Argentina
[3]Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Godoy Cruz 2290 (C1425FQB) CABA, Argentina
[4]Trapiche Winery, Grupo Peñaflor S.A., Nueva Mayorga S/N, (M5513), Maipú, Mendoza, Argentina
[5]Food Colour & Quality Laboratory, Department of Nutrition and Food Science, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain

[#]Authors contributed equally to this work

*corresponding author: fanzone.martin@inta.gob.ar

ABSTRACT

The climatic conditions in some wine regions imply in many cases the important delay in the harvest date to achieve an appropriate phenolic maturity in red grape varieties, leading to wines with high pH and alcohol content. This problem, associated with low acceptation for some consumers and chemical/microbial instability, can be addressed from a viticultural, oenological, or microbiological level. The present study aimed to provide a technological alternative, combining vintages blending and inoculation of native yeasts, for reducing simultaneously the alcohol content and the pH of Malbec wines without altering the chemical and sensory quality. Sauvignon blanc grapes harvested at the beginning of ripening (2017 season) were employed to obtain a wine with high acidity and low alcohol content (LW), that was blending with Malbec grapes of full phenolic maturity. Malbec grapes were harvested at two different moments (1H, 13.0 % v/v; 2H, 14.5 % v/v, probable alcohol), and elaborated following a standard protocol. From 2H, a part of the grape juice was removed and replaced with LW as a strategy for alcohol reduction (2RW). Consequently, it was obtained nine wines by triplicate combining 1H, 2H, 2RW musts with three fermentative strategies: CI, co-inoculation of *Hanseniaspora uvarum* BHu9/*Saccharomyces cerevisiae* BSc114 native yeasts; NS, *S. cerevisiae* BSc114 native yeast; and CS, *S. cerevisiae* EC 1118 commercial yeast. We found that 2RW wines, fermented with different yeast strains, showed similar levels of total phenols, tannins, anthocyanins, and polymeric pigments concerning control wines (2H). In all cases, those wines presented greater phenolic potential compared to wines from 1H. At the same time, the pre-fermentative strategy of vintages blending produced wines with 1.4 % v/v less alcohol than 2H wines, also achieving a pH decrease of 0.3 units. Combined treatments with native strains, especially as single inoculum (2RW–NS), were the most efficient in reducing both parameters, showed higher tannins, anthocyanins levels, and colour saturation, without affecting the sensory quality, in terms of aromas and mouthfeel. In conclusion, the strategies proposed could be simple and economic tools, for red wines production with low alcohol content, and high chemical and organoleptic quality, capable of competing and satisfying market needs.

KEYWORDS

low-alcohol wines, native yeasts, vintages blending, phenolic compounds, sensory analysis

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/4018

INTRODUCTION

In the last decades, the alcohol levels of wines have increased in the most producing wine-regions (Longo *et al.*, 2017; Schultz, 2010). This fact can be associated with current consumer preferences for well-structured, full-bodied wines rich in ripe-fruit flavours. To achieve this purpose, it is required to efficiently extract phenolic and aromatic compounds, especially, from the skins during maceration, with grape maturity as a key factor. Under this scenario, winemakers tend to delay harvest searching the full maturity, leading to the increasing of sugar accumulation in the grape and, therefore, the production of wines with high alcohol concentration (Rolle *et al.*, 2018).

Moreover, global warming has emphasized the imbalance between the sugar concentration and phenolic maturity of grape berries, considerably increasing the alcohol levels, reducing the acidity, and modifying the varietal aromatic profile of wines (Mira de Orduña, 2010).

High sugar in grapes and resulting in excessive ethanol content in wines can conduce to several microbiological, technological, sensorial and economic problems. Increased sugar concentrations may alter the growth of microorganisms (increasing spoilage microbial proliferation, enhancing risks of starvation during the fermentative process, and increasing the content of toxic compounds released by undesired microorganisms, e.g., mycotoxins), and cause sluggish and stuck alcoholic fermentation (Coulter *et al.*, 2008). Moreover, it can produce osmotic stress in yeasts, and consequently affect wine quality (Ferreira *et al.*, 2006). For his part, high ethanol content can alter the sensory attributes of wines by increasing the perception of hotness, bitterness, astringency, and sourness, while modifying aroma and flavours (Kutyna *et al.*, 2010). It is also considered a negative factor for human health, generating some rejection by consumers (Mira de Orduña, 2010).

Increased pH values at early stages of fermentation, before higher alcohol concentrations, lead to increasing the microbial contamination (e.g., lactic bacteria, spoilage yeasts) (Renouf *et al.*, 2007). Furthermore, low acidity promotes oxidative reactions and enzyme activity affecting wine colour, taste and aroma, and it reduces the antiseptic effectiveness of sulphur dioxide (Mira de Orduña, 2010).

In this context, the scientific and professional communities of the wine sector have proposed several possible approaches to reduce the alcohol levels in wines. According to different authors (Varela *et al.*, 2015), they can be grouped in viticultural practices, pre-fermentation and winemaking practices, microbiological strategies, and post-fermentation technologies on membrane-based.

As a background to our work team, in response to the above drawbacks mentioned, 111 native and selected non-Saccharomyces yeasts were thoroughly evaluated to conform a sequential co-culture with *Saccharomyces cerevisiae* (Mestre Furlani *et al.*, 2017). Then, we determined the optimum fermentative conditions for two co-inoculation strategies, followed by the validation and sensory characterization of the wines obtained (Maturano *et al.*, 2019). Moreover, the *Hanseniaspora uvarum–Saccharomyces cerevisiae* strategy was implemented to lab-scale fermentations to evaluate the impact on the sensory and aromatic profile of Malbec wines (Mestre *et al.*, 2019).

Furthermore, another technological alternative easy-to-adopt, flexible and cost-effective, for reducing simultaneously the alcohol content and the pH of red wines (Malbec, Bonarda, and Syrah from Mendoza, Argentina) was assessed. This strategy, previously proposed by other authors (Kontoudakis *et al.*, 2011; Piccardo *et al.*, 2019; Schelezki *et al.*, 2018), consisted of blending wines obtained from grapes of different ripeness. Specifically, we use a low-ethanol and highly acidic wine to replace an equal volume of well-ripened grape juice, prior to fermentation, without altering the chemical and sensory quality of the wines.

There is extensive literature about diverse applications of sequential co-culture of native yeasts and blending of different matrices. Both techniques were separately used to improve flavours profile, acidity and colour in wines (Escudero-Gilete *et al.*, 2010; Hopfer *et al.*, 2012; Loira *et al.*, 2015; Maturano *et al.*, 2015b), and, in recent years, to reduce ethanol (Canonico *et al.*, 2016; Englezos *et al.*, 2016; Longo *et al.*, 2017).

To our knowledge, there is to date no published information on the interactive effect of the

techniques above mentioned. Based on these considerations, we propose to combine both strategies, previously tested separately, to reduce the pH and ethanol levels of Malbec wines: vintages blending and inoculation of native yeasts under optimized conditions. In addition, we consider it crucial to evaluate their impact on the phenolic composition and sensory attributes of the wines.

MATERIALS AND METHODS

1. Microorganisms

In this study, *Hanseniaspora uvarum* BHu9 and *Saccharomyces cerevisiae* BSc114, isolated from oenological environmental, were used. These yeasts have been molecularly identified in previous studies (Maturano *et al.*, 2015a) and were selected according to their fermentative performance and respiratory characteristics, to be used in sequential inoculation, to obtain wines with reduced ethanol content (Mestre Furlani *et al.*, 2017). The commercial yeasts EC1108 (Lallemand, Montreal, Canada) were employed as control. Three treatments were implemented and defined as fermentative strategies (FS): CI, co-inoculation of *H. uvarum* BHu9/*S. cerevisiae* BSc114 native yeasts; NS, *S. cerevisiae* BSc114 native yeast; and CS, *S. cerevisiae* EC 1118 commercial yeast. For CI treatment, BHu9 strain was inoculated at moment 0 in a concentration of $5*10^6$ cells/mL, then $2*10^6$ cells/mL of BSc114 was sequentially inoculated (after 48 h). This inoculation strategy was optimized and validated under laboratory conditions to reduce the ethanol content in wines (Maturano *et al.*, 2019). For NS and CS treatments, the corresponding yeast strains were inoculated at time 0 with $2*10^6$ cells/mL. Before the fermentative process, native yeasts inocula were prepared according to the procedure proposed in Maturano *et al.* (2019).

2. Winemaking procedure and experimental conditions

Grapes from two cultivars, Sauvignon blanc and Malbec (*Vitis vinifera* L.), were obtained from commercial vineyards located in Agrelo (68°51′W and 33°06′S), Luján de Cuyo, Mendoza, Argentina, during the 2017 season.

Sauvignon blanc grapes were manually collected at the beginning of ripening to obtain a juice with a very low sugar concentration (around 6.2 % v/v of potential alcohol degree) and a high acidity (>20 g/L). One hundred kilograms of grapes were transported to the experimental winery of INTA (Mendoza, Argentina). Upon reception, were pressed in a manual screw press to obtain 60 L of an unripe grape juice (115 g/L of sugar, 21.1 g/L of titratable acidity, pH 2.53). The must was immediately sulphited (100 mg $K_2S_2O_5$/L), settled overnight at 4 °C, racked to a 50-L stainless steel tank, and inoculated with the commercial yeast EC1118 (Lallemand, Montreal, Canada). Alcoholic fermentation was carried out at 17 ± 2 °C. When finished, wine were sulphited (100 mg $K_2S_2O_5$/L) and kept at 2–4 °C until implementation in the blending assay with well-ripened Malbec grapes. This low-ethanol wine (LW) registered 6.5 % v/v of ethanol, titratable acidity of 19.5 g tartaric acid/L, pH of 2.65, and the absence of colour and herbaceous aromas.

Subsequently, Malbec grapes were hand-picked at two different ripening stages. The first harvest (1H) was carried out when the potential degree of alcohol was approximately 13.0 % v/v (around 22 °Brix, 215 g/L of sugar, 6.0 g/L of titratable acidity, pH 3.40). The second harvest (2H) took place when the grapes reached optimum phenolic maturity (around 24 °Brix, 245 g/L of sugar, 5.6 g/L of titratable acidity, pH 3.82) with 14.5–15.0 % v/v of potential degree of alcohol. Grapes (1H, 225 kg; 2H, 450 kg) were crushed, destemmed (Metal Liniers model MTL 12, Mendoza, Argentina), sulphited (100 mg $K_2S_2O_5$/kg), and the musts (skins, seeds, flesh and juice) placed into 25-L food-grade plastic tanks.

The experimental design consisted of three treatments with the selected yeasts, previously proposed, and applied by triplicate to the fruit of each harvest: 1H-CS, 1H-NS, 1H-CI, 2H-CS, 2H-NS and 2H-CI. Furthermore, from the second harvest, a part of the total volume of the grape juice was removed and replaced with the same volume of Sauvignon blanc low-ethanol wine (LW), as a strategy for alcohol reduction in wines (2RW). In this matrix, the different selected yeasts were also applied, obtaining the treatments 2RW-CS, 2RW-NS and 2RW-CI. This substitution volume was calculated, for each experimental unit, to reproduce the probable alcoholic degree of the corresponding wine from the first harvest (22 °Brix), following the equation proposed by Kontoudakis *et al.* (2011). This substitution represented around 20 % of the weight of the destemmed and crushed grapes.

Consequently, 27 vinifications [3 pre-fermentative treatments (PFS) × 3 fermentative treatments (FS) × 3 replicates] were conducted at 25 ± 2 ºC, with a maceration length of 14 days. For cap management, two daily punch-downs (morning and afternoon, 1 min each) were applied. All tanks were daily controlled through the measurement of temperature and the weight loss of the fermenting systems. The monitoring of the growth of yeast populations was carried out by the periodically withdrawn samples from all fermentations. These samples were serially diluted, spread onto WLN-Agar medium, and incubated during 5–7 days at 28 ± 1 °C (Pallmann *et al.*, 2001). Once fermentation-maceration was completed, free-run wines were collected into 10 L glass carboys fitted with airlocks. Malolactic fermentation (MLF) was induced with a commercial *Oenococcus oeni* culture (VP-41, Lallemand, Montreal, Canada). After MLF finished, the wines were racked off the lees, adjusted to 35 mg/L of free SO_2, and stored at 1–3 °C for 30 days to allow tartaric stabilization. Finally, wines were bottled and stored in a dark cellar at 12–15 °C until analysis. In all cases, the analyses were completed in about two months, starting from the second month after bottling.

3. Grape and wine general analytical parameters

For grape analysis, one hundred berries were randomly selected, from each cultivar and corresponding harvest time, and used to measure the sugar concentration, pH, and titratable acidity (OIV, 2012). For wine analysis, standard parameters including titratable acidity (tartaric acid, g/L), volatile acidity (acetic acid, g/L), pH, residual sugar (g/L), and alcohol content (% v/v) were determined as described by International Organization of Vine and Wine (2012), using an ALPHA FT-IR Wine Analyzer (Bruker Optics, Ettlingen, Germany), and alcohol tester (Alcolyzer Wine®; Anton-Paar GmbH, Austria). The ethanol yield metabolic parameter was calculated from the following analytical data: the ratio between sugar required (initial sugars - final sugars) in g/L and the percentage of ethanol produced (% v/v).

4. Phenolic composition and colour parameters

Wine samples were centrifuged (11,000 g × 5 min) and filtered through with 0.22-mm membranes (Microclar, Buenos Aires, Argentina) before analysis. Absorbance measurements were made with a Perkin-Elmer UV–visible Spectrophotometer Model Lambda 25 (PerkinElmer, Hartford, CT).

Tannins were analysed by protein precipitation (Harbertson *et al.*, 2003). Anthocyanins, small polymeric pigments (SPP), large polymeric pigments (LPP), and total polymeric pigments (SPP + LPP) were measured as previously described (Harbertson *et al.*, 2003). Iron reactive phenolics (total phenols) were analysed following the method described by Heredia *et al.* (2006).

CIELAB parameters [L*(lightness, 0 black and 100 white), C^*_{ab} (chroma, saturation), h_{ab} (tone; red, green, yellow) and the a*b* (red/green; yellow/blue) coordinates], were calculated from the absorption spectra by using the CromaLab® software (Heredia *et al.*, 2004), following the recommendations of the Commission Internationale de L'Eclairage (CIE, 2004). Colour difference (ΔE^*_{ab}) was calculated as the Euclidean distance between two points (1 and 2) in three-dimensional (L*a*b*) space. ΔE^*_{ab} $(L^*_1, a^*_1, b^*_1; L^*_2, a^*_2, b^*_2) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where $\Delta L^* = L^*_1 - L^*_2$; $\Delta a^* = a^*_1 - a^*_2$ and $\Delta b^* = b^*_1 - b^*_2$.

5. Anthocyanins and derived pigments profile

The chromatographic system employed for wine anthocyanin identification and quantification was a Perkin-Elmer Series 200 high-performance liquid chromatograph equipped with a diode array detector, a quaternary pump, and an autosampler (HPLC-DAD; PerkinElmer, Shelton, CT). Separation was performed on a reversed-phase Chromolith Performance C18 column (100 mm × 4.6 mm I.D., 2 µm; Merck, Darmstadt, Germany) with a Chromolith guard cartridge (10 mm × 4.6 mm) at 25 °C. A gradient consisting of solvent A (water/formic acid, 90:10, v/v) and solvent B (acetonitrile) was applied at a flow rate of 1.1 mL/min from 0 to 22 min and 1.5 mL/min from 22 to 35 min as follows: 96–85 % A and 4–15 % B from 0 to 12 min, 85–85 % A and 15–15 % B from 12 to 22 min, 85–70 % A and 15–30 % B from 22 to 35 min; followed by a final wash with 100 % methanol and re-equilibration of the column. Two millilitres of wine samples were filtered (0.45-µm pore size nylon membrane; Microclar, Buenos Aires, Argentina), and then 100 µL-

aliquot was injected onto the column. Diode array detection was performed from 210 to 600 nm, and the quantification was carried out by peak area measurements at 520 nm. Anthocyanin amount was expressed by using malvidin-3-glucoside chloride as standard for a calibration curve (R^2 = 0.99). Identification and confirmation of anthocyanin pigments were performed by HPLC-DAD/ESI-MS as described by Blanco-Vega *et al.* (2011).

6. Sensory analysis

All the wines obtained were evaluated three months after bottling by a trained panel of 12 volunteers (8 males and 4 females) aged 26–48 years, including researchers and technicians (EEA Mendoza INTA) with the previous wine tasting experience. To determine if wine replicates from each treatment were perceptibly different, we performed first discrimination tests (Triangle tests). Because no significant differences were found between the replicates, we performed a descriptive sensory analysis (DSA) with nine wines, one of each treatment (Heymann and Lawless, 2010). Consensus terminology, reference standards (Table 1), and scale practice were developed over four training sessions (of 1-hour each) held over two weeks. Panellists selected two colour attributes (colour saturation and violet hue), four taste and mouthfeel descriptors (acidity, bitterness, astringency, and fullness) and five aromas (fruity, floral, herbaceous, spicy, and balsamic). Following training, panellists were required to evaluate the nine wines by triplicate during three tasting sessions. Each session began with the assessment of the aroma standards. The William Latin Square design was used to control for carryover effects. Approximately 30–40 mL of wine was served, at 16–18 °C, in clear wine tasting glasses (ISO 3591, 1977) labelled with three-digit code. For each descriptor, panellists had to rate the intensity of each wine on a scale from 1 to 10. To collect data from the panellists we used Smartphones with SOLDESA software (ISETA, Buenos Aires, Argentina). Panel performance was monitored by assessing the correlation of panellists with the panel mean and by their contribution to the panellist × wine interaction for each attribute. No information about the nature of the study was provided to reduce bias.

6. Data analysis

All chemical analyses were carried out in duplicate. Statistical analysis was assessed with Statgraphics Centurion XVI software (Statistical Graphics Corp., Warrenton, VA, 2009) and R (R Core, 2018). All results (chemical and sensory) were tested for homogeneity of variance using Cochran's test, and analysed by two-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$). A $p < 0.05$ was considered to be statistically significant. Principal component analysis (PCA) was performed on all the sensory attributes using the correlation matrix. Confidence ellipses indicating 95 % confidence intervals were based on the multivariate distribution of the Hotelling's test for $p < 0.05$ and were constructed using SensoMineR panellipse function on R (Husson *et al.*, 2005).

RESULTS AND DISCUSSION

To assess the impact of the simultaneous reduction of alcohol content and pH on the

TABLE 1. Descriptors generated by the panel to describe wines with reference formulations.

Attribute	Reference
Colour saturation	NA*. It refers to the overall colour saturation of wine observed when tilting the glass 45° against a white background
Violet hue	NA. It refers to the overall amount of violet hue of wine observed when tilting the glass 45° against a white background
Fruity	20 g/L of plum jam (Emeth, Buenos Aires, Argentina)
Floral	1 mL rose water (Laborit, Buenos Aires, Argentina)
Herbaceous	1 mL asparagus cooking water + 2 g fresh chopped green peppers (distributed by Carrefour, Buenos Aires, Argentina)
Spicy	1 g ground allspice + 1 g ground cinnamon (distributed by Carrefour Buenos Aires, Argentina)
Balsamic	5 eucalyptus seed capsules
Acidity	2 g/L L-(+)-tartaric acid (Derivados Vínicos, Buenos Aires, Argentina) in water
Astringency	0.43 g/L aluminium ammonium sulphate (Anedra Research AG S.A., Buenos Aires, Argentina) in water
Bitterness	1 g/L caffeine (Sigma Aldrich, Buenos Aires, Argentina) in water
Fullness	1.5 g/L carboxymethylcellulose sodium salt (Sigma Aldrich, Buenos Aires, Argentina) in water

NA: not applicable

phenolic composition and sensory attributes of Malbec wines, we have imposed two strategies, pre-fermentative treatments (PFS, vintages blending) and fermentative treatments (FS, yeast strains). We found that wines made blending ripe must with a proportion of low-ethanol wine (2RW), fermented with native *S. cerevisiae* strain (NS), showed markedly chemical differences and higher phenolic potential, without affecting the sensory quality, in terms of colour, aromas and mouthfeel.

1. Yeast growth and fermentative kinetic

As to be shown in previous work (Mestre Furlani *et al.*, 2017), both native yeasts, *S. cerevisiae* BSc114, and *H. uvarum* BHu9 tolerate high concentrations of SO_2. These yeasts started without difficulty the fermentative process at SO_2 levels applied at grape must, as can be observed in the short phase lag (Figure 1). Wild yeasts were detected the first stage of all trials, but at significantly lower proportions than inoculated starter yeasts. Resistance degree to SO_2 is variable at strain level and several non-*Saccharomyces* yeast species can be tolerant at commonly employed concentrations in winemaking (Mendoza *et al.*, 2009; Padilla *et al.*, 2016).

As expected, the fermentation times were closely related with the sugar concentration of the grape musts, that is the treatments that started the process with around 22 °Brix (1H-CS, 1H-NS, 1H-CI, 2RW-CS, 2RW-NS and 2RW-CI) finished the fermentative process in a range of 6–9 days. While 2H-CS, 2H-NS and 2H-CI fermentations were completed in 8–11 days (24 °Brix, initial grape must). In all cases, the treatments that included sequential co-inoculation, 1H-CI, 2H-CI and 2RW-CI, lasted more time demonstrating the lower fermentative ability of non-*Saccharomyces* yeast (Figure 1). Others authors employed co-cultures and obtaining similar results concluding that longer fermentations reduce the flavour lost or high energetic demand for refrigeration (Lleixà *et al.*, 2016).

During the fermentation process, it was evident that all experimental units inoculated with the BSc114 yeast at 48 h (CI treatments) increased significantly your population size and CO_2 production. Furthermore, these exhibited the highest CO_2 production rates and population sizes during the first 4–5 days reaching maximum populations above 8 Log CFU/mL and then slowly declined as fermentation progressed to completion (Figure 1c,f,i). The results of population dynamics indicated that *S. cerevisiae* BSc114 was able to overcome the autochthonous microbiota of the grape musts from the beginning. All these facts denote that this native yeast achieved rapid implantation.

Hanseniaspora uvarum is one of the predominant species at the beginning of the process and has been frequently reported by others authors (Lleixà *et al.*, 2016; Tristezza *et al.*, 2016), therefore sequential co-inoculation with *S. cerevisiae* is one of the most feasible strategies to mimic what happens during spontaneous fermentation and to improve the quality of the wine. In this study, all co-cultures registered very low fermentative activities during the period before the BSc114 yeasts inoculation compared with the pure cultures of *S. cerevisiae* (Figure 1). Other authors confirmed an initial reduced fermentative rate in *H. uvarum*/*S. cerevisiae* co-cultures (Ciani *et al.*, 2006; Du Plessis *et al.*, 2019; Tristezza *et al.*, 2016). Despite the low fermentation activity, consumption of reducing sugars was 23.5 % (1H-CI), 20.4 % (2H-CI) and 34.2 % (2RW-CI) during this period. The highest consumption of sugars was detected in 2RW-CI, that can be attributed to the acidity affinity of the grape juice (pH 3.6, 6.5 g/L titratable acidity) by this yeast, as reported by other authors (Çelik *et al.*, 2017; Ciani *et al.*, 2006). This sugar consumption was reflected in the increase in biomass favoured by the oxygen levels still present, as can be seen in Figure 1. From the beginning, *H. uvarum* BHu9 was present at populations higher than 6.0 Log UFC/mL; it reached a maximum (between 6.86 and 7.73 Log CFU/mL) in three days and then decreased. This apiculate yeast´s behaviour was previously reported, which indicates that non-*Saccharomyces* yeasts dominate during the first days of fermentation up to an ethanol concentration of about 4–7 % v/v, and then they decline your growth (Fleet, 2003). BHu9 yeast population remained until Day 4 (1H-CI) and Day 6 (2H-CI and 2RW-CI). On the other hand, it is a relevant highlight that this early consumption diminishes the fermentable sugars for the *S. cerevisiae* starter. Therefore, it could be inferred that it reduced the impact of osmotic stress on this starter yeast. Furthermore, it no presented lag phase after its inoculation (at 48 h) and rapidly started to increase your population

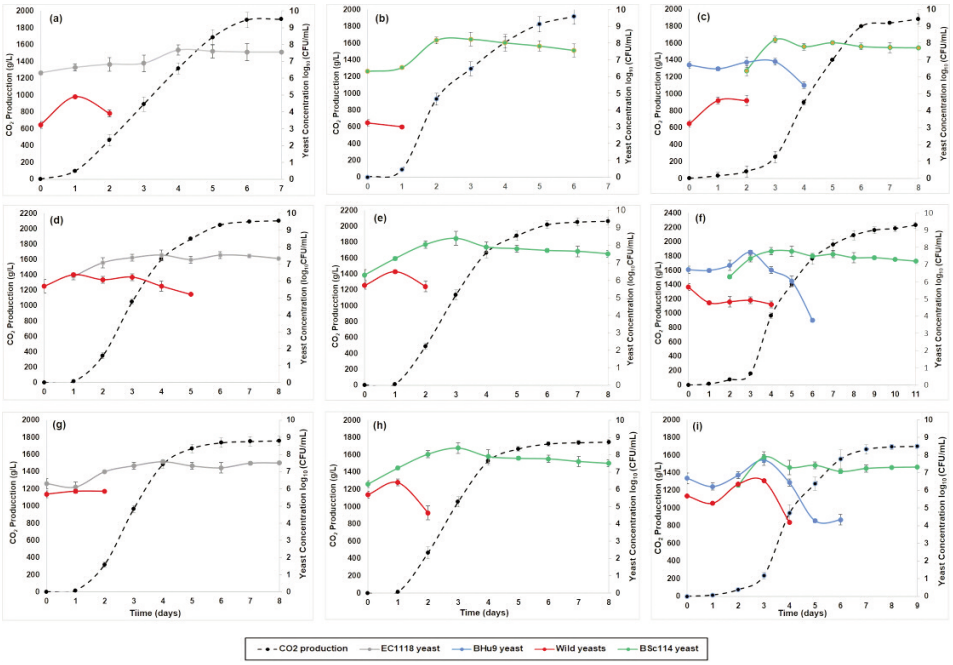

FIGURE 1. Evolution of the yeast populations (Log CFU/mL) and CO_2 production (g/L) during fermentation of Malbec wines obtained applying pre-fermentative and fermentative strategies of winemaking: (a) 1H-CS, (b) 1H-NS, (c) 1H-CI, (d) 2H-CS, (e) 2H-NS, (f) 2H-CI, (g) 2RW-CS, (h) 2RW-NS and (i) 2RW-CI.

and CO_2 production. It is important to emphasize that the total populations reached by the co-cultures did not significantly exceed those found in vinifications conducted by BSc114 under pure conditions (data not shown).

Fermentation assays of the commercial yeast EC1118 (1H-CS, 2H-CS and 2RW-CS) always reached population sizes lower than fermentation with native yeasts. Furthermore, fermentations conducted by EC1118 registered CO_2 production higher than native yeasts (Figure 1). These results could indicate that the strain EC1118 presented a high sugar/ethanol conversion, that is, it requires less sugar to produce more ethanol.

2. General analytical parameters of Malbec wines

The sequential inoculation of *H. uvarum*/*S. cerevisiae* yeasts was successfully implemented by researchers in others oenological regions, achieving good quality wines (Canonico *et al.*, 2016; Ciani *et al.*, 2006; Mendoza *et al.*, 2009; Tristeza *et al.*, 2016). The co-inoculation strategy applied in the present work was previously optimized and it achieved promising results on a laboratory scale (Maturano *et al.*, 2019; Mestre *et al.*, 2019). It is important to note that all the wines from the different treatments fermented to dryness (< 3 g/L of total residual sugars, Table 2).

The final amounts of ethanol, pH, titratable and volatile acidity of the wines obtained are summarized in Table 2. As expected, the ethanol content of the wines elaborated from grapes harvested at technological maturity (2H) was significantly higher than wines from the first harvest (1H) and those made blending ripe must with a proportion of low-ethanol wine (2RW). Overall, regardless of the yeast strain used (FS), 2RW wines had 1.4 % v/v less alcohol than 2H wines, and levels close to 1H wines. Ethanol yield was also considered an important factor. As can be observed in Table 2, the fermentative strategy 2RW–CI registered the highest values, demonstrating its low efficiency in the conversion of sugars into ethanol. Likewise, the pH values of the wines showed similar behaviour, achieving a reduction of 0.3 units by applying the 2RW treatments; while the titratable acidity only increased by 4 %. Conversely, comparable pH and acidity levels were obtained between H1 and H2 wines; probably due to high potassium levels observed in grapes since the beginning of ripening (H1, 1500 mg/L; H2, 1700 mg/L).

TABLE 2. General analytical parameters of Malbec wines obtained applying pre-fermentative (PFS) and fermentative strategies (FS) of winemaking.

Treatments	Ethanol (% v/v)	pH	Titratable acidity (g/L)	Volatile acidity (g/L)	Residual sugar (g/L)	Ethanol yield
1H-CS	13.33* ± 0.23	3.95 ± 0.02c	4.77 ± 0.07a	0.45 ± 0.02	1.47 ± 0.12cd	16.02 ± 0.29cde
1H-NS	13.83 ± 0.23cd	3.91 ± 0.01bc	5.04 ± 0.03b	0.45 ± 0.01	1.21 ± 0.11bc	15.46 ± 0.27bcd
1H-CI	12.20 ± 0.35a	3.91 ± 0.02b	5.16 ± 0.12b	0.37 ± 0.03a	1.03 ± 0.03abc	17.55 ± 0.51f
2H-CS	15.07 ± 0.21e	3.91 ± 0.03d	4.78 ± 0.04a	0.57 ± 0.02c	1.78 ± 0.10d	14.15 ± 0.20a
2H-NS	14.00 ± 0.10d	3.91 ± 0.02bc	5.59 ±0.06c	0.56 ± 0.03c	1.32 ± 0.21cd	15.26 ± 0.11bc
2H-CI	14.20 ± 0.20d	3.91 ± 0.03bc	5.58 ± 0.07c	0.54 ± 0.05c	0.74 ± 0.21ab	15.09 ± 0.20b
2RW-CS	13.23 ± 0.35bc	3.91 ± 0.01a	5.19 ± 0.06b	0.52 ± 0.04bc	1.48 ± 0.22cd	16.14 ± 0.41de
2RW-NS	12.93 ± 0.12bc	3.91 ± 0.03a	5.76 ± 0.03c	0.52 ± 0.01bc	1.25 ± 0.26c	16.53 ± 0.16e
2RW-CI	12.87 ± 0.12b	3.91 ± 0.03a	5.62 ± 0.14c	0.52 ± 0.03bc	0.72 ± 0.12a	16.65 ± 0.14e
1H	13.12 ± 0.76A	3.91 ± 0.04B	4.99 ± 0.19A	0.42 ± 0.04A	1.28 ± 0.48A	14.84 ± 0.54B
2H	14.42 ± 0.51B	3.91 ± 0.12C	5.32 ± 0.41B	0.56 ± 0.03C	1.15 ± 0.38A	16.44 ± 0.33A
2RW	13.01 ± 0.26A	3.91 ± 0.04A	5.52 ± 0.27C	0.52 ± 0.03B	1.24 ± 0.21A	16.34 ± 0.99B
CS	13.88 ± 0.92χ	3.91 ± 0.19ß	4.91 ± 0.21α	0.51 ± 0.06ß	1.58 ± 0.20χ	15.44 ± 1.00α
NS	13.59 ± 0.52ß	3.91 ± 0.12a	5.46 ± 0.33ß	0.51 ± 0.05 ß	1.26 ± 0.18ß	15.75 ± 0.61α
CI	13.09 ± 0.91a	3.91 ± 0.10a	5.46 ± 0.24ß	0.48 ±0.09α	0.83 ± 0.20α	16.43 ± 1.11ß
Two-way ANOVA						
PFS	<0.0001	<0.0001	<0.0001	<0.0001	0.2893	<0.0001
FS	<0.0001	<0.0001	<0.0001	0.0246	<0.0001	<0.0001
Interaction (PFS x FS)	<0.0001	<0.0001	0.0001	0.0983	0.0652	<0.0001

* Mean ± SD (n = 3). Different Roman lowercase letters in the same column indicate significant differences among treatments (Tukey HSD test, $p < 0.05$). Different Roman uppercase letters indicate statistical differences ($p < 0.05$) between wines from pre-fermentative strategies. Different Greek letters indicate statistical differences ($p < 0.05$) between wines from fermentative strategies. Pre-fermentative strategies (PFS): 1H, 1st harvest wines (22°Brix); 2H, 2nd harvest wines (24 °Brix); 2RW, reduced alcohol wines. Fermentative strategies (FS): CS, *S. cerevisiae* commercial strain (EC1118); NS, *S. cerevisiae* native strain (BSc114); CI, co-inoculation *S. cerevisiae/Hanseniaspora* (BHu9/BSc114).

These results are in agreement with previous experiments (Kontoudakis *et al.*, 2011; Piccardo *et al.*, 2019; Schelezki *et al.*, 2018), in which the substitution of the grape juice with unripe must/wine determined a decrease in sugar content and an increase in acidity. Specifically, a huge effect of the pre-fermentative factor (60.2 % and 74.3 %) and a slight PFS x FS interaction effect (19.0 % and 9.0 %) on alcohol and pH, respectively, was observed. While, in the case of titratable acidity, the impact of the fermentative factor (PF) was greater than the rest (51.5 %). Therefore, the 2RW-CI and 2RW-NS combinations were the most effective treatments in reducing alcohol and pH of wines (Table 2).

As mentioned previously, the active participation of the non-*Saccharomyces* native yeast as initial inoculum influences the fermentation course and the quality of the final product. Some species of non-*Saccharomyces* yeasts are recognized as potential acidifying, such as *Lanchacea thermotolerants*, *Schizosacchamyces pombe*, *Starmerella Bacillaris* and *Candida zemplinina* (Benito *et al.*, 2015; Gobbi *et al.*, 2013). These yeasts are capable of inducing biological acidification due to their physiological features and genetic determinants associated with the production of organic acids (Berbegal *et al.*, 2019). Hong and Park (2013) reported to *H. uvarum* strains as a good producer of organics acids in Korean wines.

Finally, while the volatile acidity increased with the fruit ripeness levels, all treatments presented acetic acid values lower to 0.6 g/L (Table 2), considered acceptable according to the regulations in force (Instituto Nacional de Vitivinicultura, Argentina).

3. Global phenolic parameters of wines

Figure 2 shows the global phenolic parameters of Malbec wines obtained applying pre-fermentative and fermentative strategies of winemaking to reduce alcohol and pH. First, it is well known that grape maturity significantly

influences on colour and phenolic composition of red wines (Bindon *et al.*, 2013). Wines from the riper grapes (2H) presented higher total concentrations of phenols (~47 %), anthocyanins (~26 %), and to a lesser extent of tannins (~19 %) than their corresponding first harvest wines (1H), confirming trends previously observed for this variety (Fanzone *et al.*, 2011) and other red cultivars (Fournand *et al.*, 2006). The main differences were observed in total phenols, measured in reaction with iron chloride, counting all phenolics containing vicinal dihydroxyls (Harbertson and Spayd, 2006). Therefore, these measurements include not only tannins but also flavan-3-ols and flavonols, compounds that increase their concentration and possibly extractability during grape ripening (Fanzone *et al.*, 2011; Liang *et al.*, 2012).

However, when each reduced alcohol wine (2RW) were compared with controls (2H), no significant differences in these parameters were found, considered as satisfactory results concerning wine quality (Figure 2). These findings agree with those obtained in similar experiments carried out by other authors (Kontoudakis *et al.*, 2011; Piccardo *et al.*, 2019; Rolle *et al.*, 2018; Schelezki *et al.*, 2018; Schelezki *et al.*, 2020), and confirmed that changes (reduction) in alcohol levels (around 1.5–2 % v/v) in the wine matrix did not affect the extractability of anthocyanins and tannins from the skins. Furthermore, the replacement of some of the juice, before maceration, by S. blanc low-ethanol wine did not involve losses of anthocyanins extracted during the crushing process, but even a possible supply of copigments, which protects anthocyanins against oxidation (Boulton, 2001). These factors must be added to low pH values, which slow down this reaction and favour the extraction of these compounds during winemaking (Canals *et al.*, 2005; Kontoudakis *et al.*, 2011).

Table 3 shows the probability values of each factor, when the two-way ANOVA was applied using «pre-fermentative (PFS)» and «fermentative (FS)» strategies as factors. From this analysis, a significant effect of the FS factor (yeast strain used) was also observed, indicating in all cases higher levels of tannins (~34 %) and anthocyanins (~12 %) in wines fermented with native yeasts (*S. cerevisiae* BSc114, NS) than those fermented with the commercial yeast (CS) (Figure 2). The higher extraction of these compounds could be explained by a positive effect of the lower pH levels obtained with the native strain (Table 2). It is well known that the yeast´s polysaccharides retain anthocyanins and tannins, thereby preventing their precipitation. Therefore, colour stability is improved and astringency diminished (Escot *et al.*, 2001). These molecules are released from the yeast cell wall during alcoholic fermentation and wine ageing processes. The amount released is intraspecific and depends on the number of cells formed and their physiological conditions (Domizio *et al.*, 2014). In this study, as can be seen in Figure 1, the native *S. cerevisiae* yeast reached high populations throughout the fermentation process, therefore a higher concentration of polysaccharides could be expected, although this was not determined in this work.

Polymeric pigments formed during winemaking provide stable colour and positive mouthfeel properties. Their production is modulated by the relative molar amount of anthocyanins and tannins (Kilmister *et al.*, 2014). Total polymeric pigments result from the summation of small polymeric pigments (SPP; including pyranoanthocyanins and flavanol-anthocyanin ethyl-bridged adducts, that cannot precipitate proteins and are resistant to SO_2 bleaching) and large polymeric pigments (LPP; covalent adducts between tannins and anthocyanins, of relatively high molecular weight, that can precipitate proteins and are resistant to SO_2 bleaching) (Adams *et al.*, 2004). The total polymeric content (TPP) of the wines made from riper grapes (2H and 2RW) was higher than wines from less mature grapes (1H). LPP formation followed the trend observed regarding the tannin content in wines, while the rate of SPP showed a direct correlation with the level of anthocyanins (Figure 2). Similar results were reported by other authors in Syrah (Garrido-Bañuelos *et al.*, 2019), Cabernet-Sauvignon (Bindon *et al.*, 2013) and Merlot wines (Casassa *et al.*, 2019) obtained from full-ripe fruit harvested between 23 and 25 °Brix.

Two-factor ANOVA showed a significant pre-fermentative × fermentative treatments interaction for LPP and SPP (Table 3). This suggests that the effect of yeast strains employed (FS) did depend upon pre-fermentative strategy. In other words, the native strains (NS and CI treatments) applied to control wines from ripe grapes (2H) and reduced alcohol wines (2RW) produced contrasting effects on polymeric

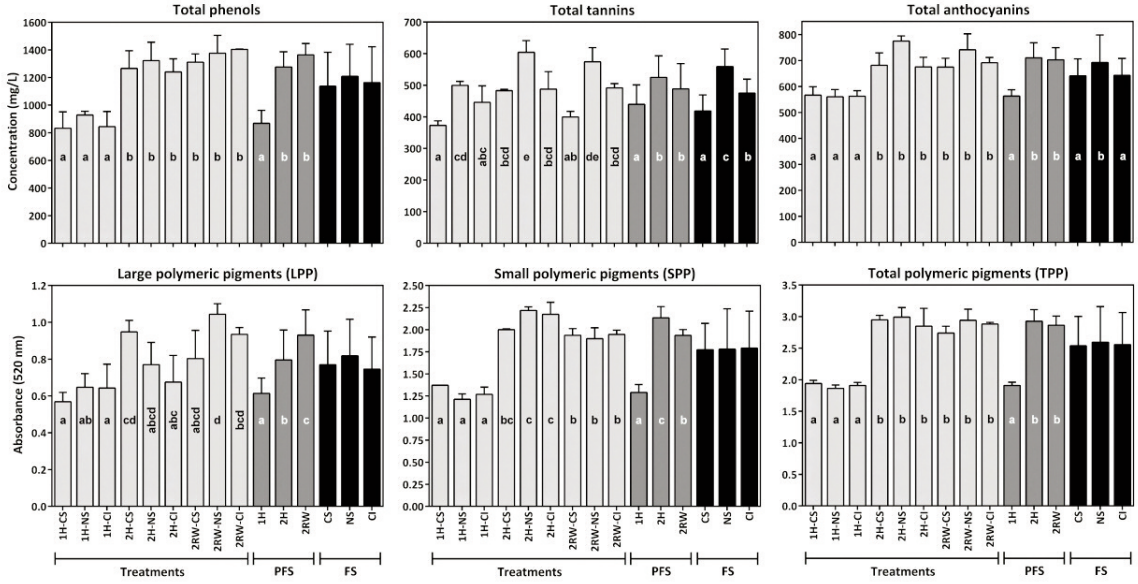

FIGURE 2. Global phenolic parameters of Malbec wines obtained applying pre-fermentative and fermentative strategies of winemaking.
Different letters for each group of bars indicate significant differences (Tukey HSD test, $p < 0.05$).

pigments of different molecular weight, without affecting the summation (TPP).

4. Wine colour

For further understanding of the colour composition of wines, we analysed them by the CIELAB colour space. Figure 3 shows the location of wines from the different treatments in the colour plane (a*b*) of CIELAB space and L* (lightness) values. Data obtained from the two-way ANOVA (Table 3), show a significant effect ($p < 0.05$) of pre-fermentative and fermentative strategies in the final colour of Malbec wines, in both quantitative (C^*_{ab} and L*) and qualitative (h_{ab}) terms; and significant interaction PFS × FS in chroma (C^*_{ab}) and hue (h_{ab}).

Regardless of the yeast strain used, it was found that the colour of first harvest wines (1H) was less vivid and lighter (lowest C^*_{ab}), and showed a slight decrease of violet hues, given a markedly decrease of a* and b* values. Conversely, the colour of the wines from riper grapes displayed high intensity due to an increase of C^*_{ab} (2H, 47 %; 2RW, 78 %), and a shift towards a violet hue (decrease in h_{ab} of 5° and 8° for 2H and 2RW, respectively) (Figure 3A). Furthermore, C^*_{ab} presents a negative correlation with L* and, as expected, when the former increased, the latter decreased (Figure 3B). The L* value is the vertical axis and defines the lightness, the property according to which each colour can be considered as equivalent to a member of the greyscale, between black and white, taking values within the range of 0−100, respectively (Gordillo *et al.*, 2014). This parameter showed significant differences between wines, presenting a relative

TABLE 3. Probability values (two-way ANOVA) for pre-fermentative (PFS) and fermentative (FS) strategies to the global phenolic and colour parameters in Malbec wines.

Parameter	*p* value[a] for factor		
	PFS	FS	PFS x FS[b]
Total phenols	<0.0001	0.3221	0.8014
Total tannins	0.0002	<0.0001	0.1805
Total anthocyanins	<0.0001	0.0111	0.1232
LPP	<0.0001	0.3541	0.0101
SPP	<0.0001	0.7426	0.0066
TPP	<0.0001	0.3672	0.2759
L*	<0.0001	0.0047	0.1156
C^*_{ab}	<0.0001	<0.0001	0.0111
h_{ab}	<0.0001	<0.0001	<0.0001

[a] Considered to be significant when $p < 0.05$.
[b] Interaction effect between pre-fermentative (PFS) and fermentative (FS) strategies.

percentage decrease (on average) of 16 % in 2H and 19 % in 2RW, in relation to 1H wines. These results can be explained by the higher levels of phenolic compounds, especially anthocyanins and polymeric pigments, in 2H and 2RW wines (Figure 2).

Additionally, when each reduced alcohol wine (2RW) were compared with controls (2H), significant differences were also found in these parameters, 2RW wines had a deeper colour (higher C^*_{ab}, ~20 %; lower L*, ~4 %). The determinant factor possibly is the lower pH, which displaces the balance between the different forms of the anthocyanins towards the flavylium cation. Once again, these findings coincide with those obtained by other authors (Kontoudakis *et al.*, 2011; Piccardo *et al.*, 2019) in similar trials.

As mentioned above, it was observed a significant influence of the yeast strains and the PFS × FS interaction on the colour parameters of wines (Table 3). The inoculation of *S. cerevisiae* native strain (BSc114), especially as a single inoculum (NS), for each pre-fermentative treatment, generated greater colour intensity in wines (higher C^*_{ab}), with a tendency towards violet-blue hues (lower h_{ab}) (Figure 3A). Likewise, the L* values in NS wines were lower than those of CI and CS treatments, for each pre-fermentative strategy (Figure 3B). These results are consistent with the higher levels of anthocyanins and tannins in the wines obtained with this native yeast (Figure 2).

Finally, we also evaluated the total colour difference (ΔE^*_{ab}) between Malbec wines obtained by applying different pre-fermentative treatments and fermented with different yeast strains. It is not possible to establish the concrete value for the colour discrimination because many factors are conditioning this limit, such as viewing geometry, surroundings, even the colour region and the ability, visual capacity, and training of observers. Some studies performed under specific conditions have established the visual discrimination threshold in wines between 3 and 5 CIELAB units (Martínez *et al.*, 2001, Pérez-Magariño and González-SanJosé, 2003), although these values must be considered in relative character. For all yeast studied, the highest colour difference values (ΔE^*_{ab}) were found between first harvest wines (1H) and reduced alcohol wines (2RW) (mean 23.6 CIELAB units), and the least ones between second harvest control wines (2H) and 2RW wines (mean 7.6 u), although in all cases they were visually distinguished (Figure 4). Taking into account that both 2H and 2RW wines come from grapes with the same degree of ripeness, the implementation of the alcohol reduction strategy significantly changed the colour, mainly in quantitative terms ($\%\Delta^2C^*_{ab} > 82$).

Analysing the influence of yeast strains, the native inoculum applied (*S. cerevisiae* BSc114, NS) generated the highest colour difference (mean 18.0 u) in the wines obtained from all pre-fermentative treatments (PFS). While the commercial strain (CS) showed the least colour differences (mean 13.8 u) in all the wines. Following the same trend indicated above, the microbiological strategy of alcohol reduction applied also modified the colour of the wines in mainly quantitative terms ($\%\Delta^2C^*_{ab} > 60$) (Figure 4).

5. Anthocyanin profile of Malbec wines

The identified and quantified compounds in the wine samples are summarizes in Tables S1-S5 (Supplementary data). They were grouped in non-acylated glucosides (5), acetyl-glucosides (5), cinnamoyl-glucosides (6), and low molecular anthocyanin-derived pigments (8 pyranoanthocyanins, and 2 flavanol-

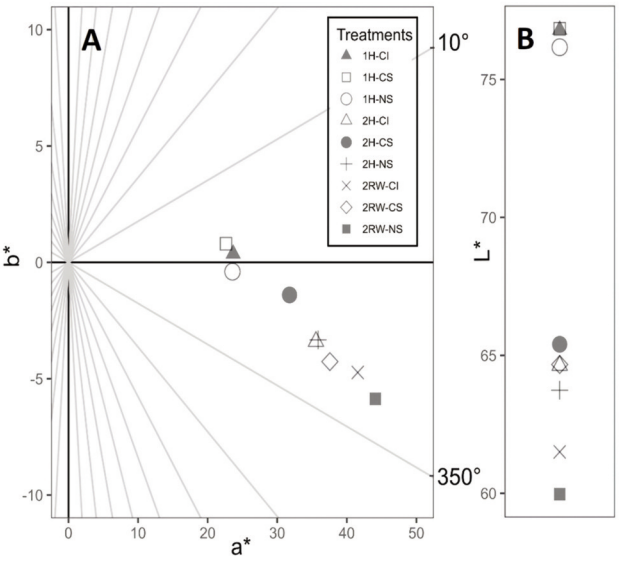

FIGURE 3. CIELAB parameters of Malbec wines. (A) (a*b*) Diagram; chroma (C^*_{ab}) is a vector that links the dot (wine location) with the origin of coordinates; hue (h_{ab}) is the angle of this vector. (B) Lightness (L*).

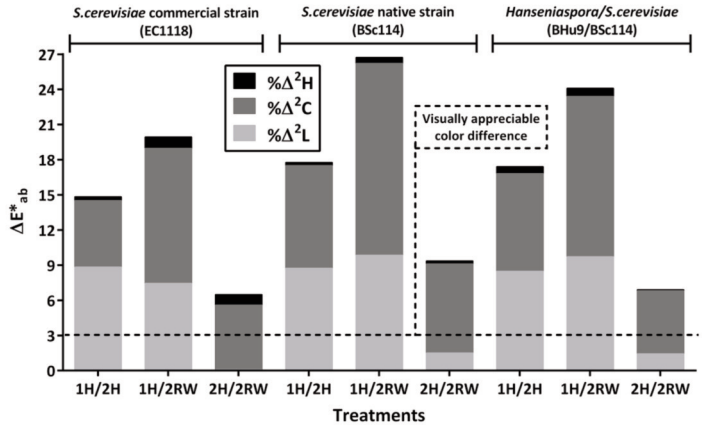

FIGURE 4. Colour differences (ΔE^*_{ab}), with the relative contribution of lightness, chroma and hue ($\%\Delta^2L$, $\%\Delta^2C$, $\%\Delta^2H$), between Malbec wines obtained applying different pre-fermentative treatments and fermented with different yeast strains.

anthocyanin adducts). All identified compounds were detected in all of the wines studied. Figure 5 shows the results obtained in wines grouped by families. The simple glucosides represented the highest proportion of all anthocyanins in the samples (68.8 %), followed by acetylglucosides (15.8 %), cinnamoylglucosides (7.4 %), and pyranoanthocyanins (6.2 %), in accordance with the results previously published by our group for the same cultivar (Fanzone *et al.*, 2012).

The overall amount of anthocyanins were similar to those obtained by spectrophotometry (Figure 2), although the values were significantly lower. However, the increasing trend detected between the first (1H) and second harvest (2H), by the mentioned technique, was not observed in the individual quantification by HPLC-DAD, presenting similar levels, mainly in the malvidin derivatives. The only compounds that increased at the second harvest (2H) were petunidin-3-glucoside, peonidin-3-glucoside, delphinidin-3-(6''-acetyl)-glucoside, cyanidin-3-(6''-acetyl)-glucoside, delphinidin-3-(6''-*p*-coumaroyl)-glucoside, and malvidin-3-(6''-caffeoyl)-glucoside (Tables S1-S3, supplementary data). This behaviour could be explained by the fact that HPLC-DAD analysis only detects free anthocyanins, whereas spectrophotometric analysis overestimates their total amount including other red pigments (Canals *et al.*, 2008). Furthermore, the substitution of part of the juice of ripe grapes by low-alcohol wine (2RW) did not imply losses of anthocyanins, coinciding with the results presented above (Figure 2).

It should be noted that the concentration of pyranoanthocyanins and flavanol-anthocyanin adducts in the wines was significantly affected by the prefermentative strategy applied, and in the case of vitisins also by the yeasts used (Figure 5, Table 4). In addition, data obtained from the two-way ANOVA, show a significant interaction PFS × FS ($p < 0.05$) only for hydroxyphenyl derivatives. Pyranoanthocyanins are anthocyanin-derived pigments formed by the reaction of monomeric anthocyanins with acetaldehyde, acetoacetic acid, pyruvic acid and other carbonyl compounds (Blanco-Vega *et al.*, 2011; Rentzsch *et al.*, 2007). These compounds are of interest for winemakers because they have high stability during the ageing of red wines, are more resistant to elevated pH values and bisulphite bleaching than anthocyanins, and express more colour than other pigments at the typical pH of wine (Rentzsch *et al.*, 2007). Meanwhile, ethylene-bridged flavanol-anthocyanin adducts often disappear during wine ageing, maybe due to the reported instability of the ethylidene linkage (Escribano-Bailón *et al.*, 2001).

In our study, the Malbec wines from ripe grapes (2H and 2RW) fermented with native or commercial yeasts (NS and CS) contained the highest levels of all derived pigments, which support the greater proportion of small polymeric pigments obtained by spectrophotometry (Figure 2).

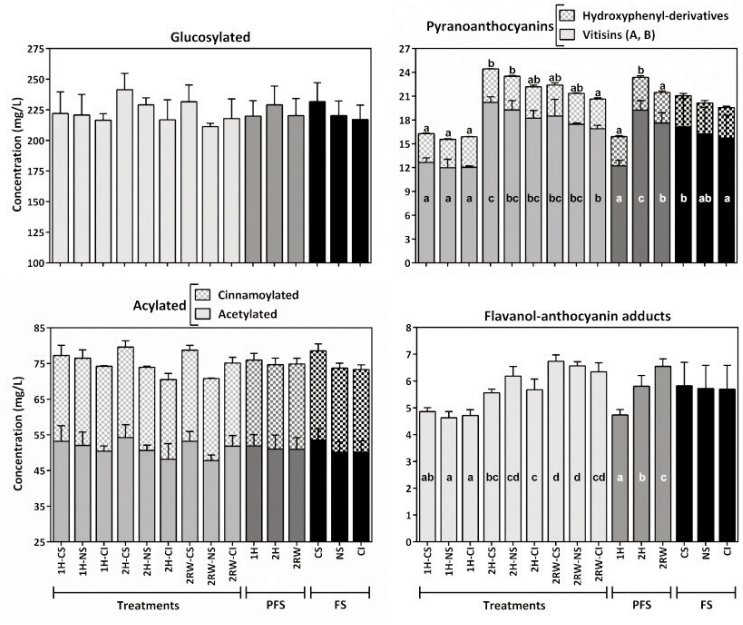

FIGURE 5. Anthocyanins and derived pigments profile of Malbec wines obtained applying pre-fermentative and fermentative strategies of winemaking.
Different letters for each group of bars indicate significant differences by two-way ANOVA (Tukey HSD test, $p < 0.05$).

TABLE 4. Probability values (two-way ANOVA) for pre-fermentative (PFS) and fermentative (FS) strategies to the anthocyanins, pyranoanthocyanins (PA) and direct adducts in Malbec wines.

Parameter	*p* value[a] for factor		
	PFS	FS	PFS x FS[b]
Glucosylated	0.2604	0.0710	0.5488
Acetylated	0.7776	0.0510	0.3518
Cinnamoylated	0.8456	0.0727	0.4699
Vitisins	<0.0001	0.0259	0.8125
Hydroxyphenyl-PA	<0.0001	0.3128	0.0232
Flavanol-anthocyanin adducts	<0.0001	0.2136	0.0525
Total anthocyanins	0.1750	0.0505	0.5177

[a]Considered to be significant when $p < 0.05$.
[b]Interaction effect between pre-fermentative (PFS) and fermentative (FS) strategies.

6. Descriptive sensory analysis

Descriptive analysis with a trained panel was conducted to determine the specific sensory effects of both pre-fermentative and fermentative strategies. A fixed-effect two-way ANOVA with interaction was performed on the sensory data (Table S6, supplementary data). There was no interaction between pre-fermentative and fermentative treatments in any of the wine's attributes, showing there were not any synergistic or antagonistic sensory effects of both strategies. The effect of the pre-fermentative strategy prevailed in all the sensory attributes. In brief, the descriptive analysis showed significant pre-fermentative effects for colour saturation, violet hue, fruity, spicy, balsamic, bitterness, and fullness. Although there were chemical differences in total acidity, these differences were not perceived by the sensory panel. 2H and 2RW wines showed higher colour saturation and violet hue than 1H wines. Contrary to the results obtained in CIELAB parameters (Figure 3), there were no significant differences in visual appreciation of colour saturation when reduced alcohol wines

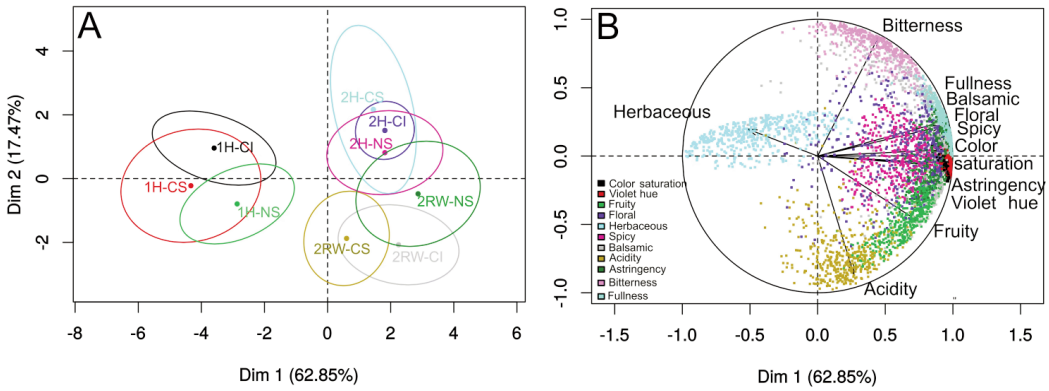

FIGURE 6. Principal component analysis (PCA) of descriptive sensory data of Malbec wines evaluated by a trained panel (n = 12). (A) wine factor map. (B) sensory attributes loadings with 95 % confidence ellipses based on the multivariate distribution of Hotelling's test ($p < 0.05$).

(2RW) were compared with controls (2H). This result provided evidence that the use of low-alcohol wine in the Malbec wine matrix did not negatively affect the perception of colour.

The sensory aromatic profile of the wines revealed that the main differences were found between 1H wines and both treatments coming from the second harvest. According to the DSA, producing Malbec wines that were up to 1.4 % v/v less alcohol by employing reduced alcohol wine (2RW) did not cause significant changes in the perceptions of any aroma when comparing with wines from the same harvest time (2H). Schelezki *et al.* (2020) reported similar results in Cabernet Sauvignon and Shiraz wines from Australia, and Piccardo *et al.* (2019) in Pinot noir and Tannat wines from Uruguay.

The wines behaved in a similar way for fullness and astringency attributes, being both wines from second harvest (2H and 2RW) the ones with a higher intensity. In the case of astringency, there was also a significant fermentative effect, CS wines showed fewer levels of astringency than NS and CI. In both cases, the perception of this mouthfeel attribute was in agreement with the measurement of tannin content by protein precipitation showed in Figure 2. There is considerable evidence (Kutyna *et al.*, 2010) to support that wines with high alcohol content can be more bitter than control ones. This situation is evident in our experiment in which 2H wine is bitterer than the others are.

To further explore the comparative influence of both pre-fermentative and fermentative strategies on the sensory profile of the wines, the full dataset was submitted to principal component analysis (PCA). The PCA biplot and confidence ellipses were constructed with 95 % certainty according to the Hotelling's test (Husson *et al.*, 2005), which provides significance testing. The sizes of the confidence ellipses are related to the variability of each wine (Figure 6A), while the colour dots in the loading plot showed the variability around the sensory attributes (Figure 6B). Dimensions 1 showed the wines were separated mainly by pre-fermentative strategies, with the first principal components explaining ~63 % of the total variance (Figure 6). The 1H wines clustered in the negative region of dimension 1, whereas 2H and 2RW wines grouped on the positive region of dimension 1 (Figure 6A). The factor map showed significant overlap between wines from the first harvest time (1H) indicating no differences between fermentative strategies applied to that matrix. A similar situation occurred with the ellipses of wines 2H and 2RW wines except for wines 2H-NS and 2RW-CI that appear to be significantly different wines. The corresponding loading plot (Figure 6B) showed the separation of the wines in the first principal components could be attributed to the herbaceous descriptor negatively loaded in the first dimension with almost the rest of the attributes positively loaded. The exception was acidity and bitterness characters that were positively and negatively loaded in the second dimension.

CONCLUSION

The combination of vintages blending and inoculation of native yeast strategies reduced the pH and ethanol levels of Malbec wines and positively impacted on phenolic composition, especially in tannins, anthocyanins, and polymeric pigments, which had repercussions on the final colour and mouthfeel sensations. Therefore, the proposed technologies could be simple and economic tools, for the production of red wines with low alcohol content and high chemical and organoleptic quality, capable of competing and satisfying market needs.

Acknowledgements: This work was supported by the Research Project IDeA 1400-SECITI 0041-2014, CICITCA- UNSJ; and INTA Project PNAIyAV-1130032 (2016-2019). The authors are grateful to Fincas Patagónicas S.A. (Argentina) for providing the grapes for this study, and to the members of the sensory panel (INTA) for their technical assistance.

REFERENCES

Adams, D. O., Harbertson, J. F., & Picciotto, E. A. (2004). Fractionation of Red Wine Polymeric Pigments by Protein Precipitation and Bisulfite Bleaching. *In* A. L. Waterhouse & J. A. Kennedy (Eds.), *Red Wine Color* (Vol. 886, pp. 275–288). American Chemical Society. doi:/10.1021/bk-2004-0886.ch017

Benito, Á., Calderón, F., Palomero, F., & Benito, S. (2015). Combine use of selected *Schizosaccharomyces pombe* and *Lachancea thermotolerans* yeast strains as an alternative to the traditional malolactic fermentation in red wine production. *Molecules*, *20*(6), 9510–9523. doi:/10.3390/molecules20069510

Berbegal, C., Fragasso, M., Russo, P., Bimbo, F., Grieco, F., Spano, G., & Capozzi, V. (2019). Climate changes and food quality: the potential of microbial activities as mitigating strategies in the wine sector. *Fermentation*, *5*(4), 85. doi:/10.3390/fermentation 5040085

Bindon, K., Varela, C., Kennedy, J., Holt, H., & Herderich, M. (2013). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet-Sauvignon 1. Grape and wine chemistry. *Food Chemistry*, *138*(2–3), 1696–1705. doi:/10.1016/j.foodchem.2012.09.146

Blanco-Vega, D., López-Bellido, F. J., Alía-Robledo, J. M., & Hermosín-Gutiérrez, I. (2011). HPLC–DAD–ESI-MS/MS Characterization of Pyranoanthocyanins Pigments Formed in Model Wine. *Journal of Agricultural and Food Chemistry*, *59*(17), 9523–9531. doi:/10.1021/jf201546j

Boulton, R. B. (2001). The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture*, *52*(2), 67–87. https://www.ajevonline.org/content/52/2/67

Canals, R., Llaudy, M. C., Valls, J., Canals, J. M., & Zamora, F. (2005). Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *Journal of Agricultural and Food Chemistry*, *53*(10), 4019–4025. doi:/10.1021/jf047872v

Canals, R., del Carmen Llaudy, M., Canals, J. M., & Zamora, F. (2008). Influence of the elimination and addition of seeds on the colour, phenolic composition and astringency of red wine. *European Food Research and Technology*, *226*(5), 1183–1190. doi:/10.1007/s00217-007-0650-8

Canonico, L., Comitini, F., Oro, L., & Ciani, M. (2016). Sequential fermentation with selected immobilized non-*Saccharomyces* yeast for reduction of ethanol content in wine. *Frontiers in Microbiology*, *7*, 278. doi:/10.3389/fmicb.2016.00278

Casassa, L., Sari, S., Bolcato, E., & Fanzone, M. (2019). Microwave-assisted extraction applied to Merlot grapes with contrasting maturity levels: effects on phenolic chemistry and wine color. *Fermentation*, *5*(1), 15. doi:/10.3390/fermentation 5010015

Çelik, Z., Erten, H., Darici, M., & Cabaroğlu, T. (2017). Molecular characterization and technological properties of wine yeasts isolated during spontaneous fermentation of *Vitis vinifera* L.cv. Narince grape must grown in ancient wine making area Tokat, Anatolia. *In* J. Aurand (Ed.), *BIO Web of Conferences* (p. 02017). EDP Sciences. doi:/10.1051/bioconf/20170902017

Ciani, M., Beco, L., & Comitini, F. (2006). Fermentation behaviour and metabolic interactions of multistarter wine yeast fermentations. *International Journal of Food Microbiology*, *108*(2), 239–245. doi:/10.1016/j.ijfoodmicro.2005.11.012

CIE (2004). Technical report colorimetry. *Commission Internationale de l'Éclairage Central Bureau*. Vienna, Austria.

Coulter, A. D., Henschke, P. A., Simos, C. A., & Pretorius, I. S. (2008). When the heat is on, yeast fermentation runs out of puff. *Australian and New Zealand Wine Industry Journal, 23*, 26-30.

Domizio, P., Liu, Y., Bisson, L. F., & Barile, D. (2014). Use of non-*Saccharomyces* wine yeasts as novel sources of mannoproteins in wine. *Food Microbiology*, *43*, 5–15. doi:/10.1016/j.fm.2014.04.005

Du Plessis, H., Du Toit, M., Nieuwoudt, H., Van der Rijst, M., Hoff, J., & Jolly, N. (2019). Modulation of

wine flavor using *Hanseniaspora uvarum* in combination with different saccharomyces cerevisiae, lactic acid bacteria strains and malolactic fermentation strategies. *Fermentation*, *5*(3), 64. doi:/10.3390/fermentation5030064

Englezos, V., Rantsiou, K., Cravero, F., Torchio, F., Ortiz-Julien, A., Gerbi, V., Rolle, L., & Cocolin, L. (2016). *Starmerella bacillaris* and *Saccharomyces cerevisiae* mixed fermentations to reduce ethanol content in wine. *Applied Microbiology and Biotechnology*, *100*(12), 5515–5526. doi:/10.1007/s00253-016-7413-z

Escot, S., Feuillat, M., Dulau, L., & Charpentier, C. (2001). Release of polysaccharides by yeasts and the influence of released polysaccharides on colour stability and wine astringency. *Australian Journal of Grape and Wine Research*, *7*(3), 153–159. doi:/10.1111/j.1755-0238.2001.tb00204.x

Escribano-Bailón, T., Álvarez-García, M., Rivas-Gonzalo, J. C., Heredia, F. J., & Santos-Buelga, C. (2001). Color and stability of pigments derived from the acetaldehyde-mediated condensation between malvidin 3-o-glucoside and (+)-catechin. *Journal of Agricultural and Food Chemistry*, *49*(3), 1213–1217. doi:/10.1021/jf0010811

Escudero-Gilete, M. L., González-Miret, M. L., & Heredia, F. J. (2010). Implications of blending wines on the relationships between the colour and the anthocyanic composition. *Food Research International*, *43*(3), 745–752. doi:/10.1016/j.foodres.2009.11.004

Fanzone, M., Zamora, F., Jofré, V., Assof, M., Gómez-Cordovés, C., & Peña-Neira, Á. (2011). Phenolic characterisation of red wines from different grape varieties cultivated in Mendoza province (Argentina). *Journal of the Science of Food and Agriculture*, *92*(3), 704–718. doi:/10.1002/jsfa.4638

Fanzone, M., Zamora, F., Jofré, V., Assof, M., & Peña-Neira, A. (2011). phenolic composition of malbec grape skins and seeds from Valle de Uco (Mendoza, Argentina) during ripening. Effect of cluster thinning. *Journal of Agricultural and Food Chemistry*, *59*(11), 6120–6136. doi:/10.1021/jf200073k

Ferreira, J., Toit, M. D., & Toit, W. J. D. (2006). The effects of copper and high sugar concentrations on growth, fermentation efficiency and volatile acidity production of different commercial wine yeast strains. *Australian Journal of Grape and Wine Research*, *12*(1), 50–56. doi:/10.1111/j.1755-0238.2006.tb00043.x

Fleet, G. (2003). Yeast interactions and wine flavour. *International Journal of Food Microbiology*, *86*(1–2), 11–22. doi:/10.1016/s0168-1605(03)00245-9

Fournand, D., Vicens, A., Sidhoum, L., Souquet, J.-M., Moutounet, M., & Cheynier, V. (2006). Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. *Journal of Agricultural and Food Chemistry*, *54*(19), 7331–7338. doi:/10.1021/jf061467h

Garrido-Bañuelos, G., Buica, A., Schückel, J., Zietsman, A. J. J., Willats, W. G. T., Moore, J. P., & Du Toit, W. J. (2019). Investigating the relationship between grape cell wall polysaccharide composition and the extractability of phenolic compounds into Shiraz wines. Part I: Vintage and ripeness effects. *Food Chemistry*, *278*, 36–46. doi:/10.1016/j.foodchem.2018.10.134

Gobbi, M., Comitini, F., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., & Ciani, M. (2013*). Lachancea thermotolerans* and *Saccharomyces cerevisiae* in simultaneous and sequential co-fermentation: A strategy to enhance acidity and improve the overall quality of wine. *Food Microbiology*, *33*(2), 271–281. doi:/10.1016/j.fm.2012.10.004

Gordillo, B., Cejudo-Bastante, M. J., Rodríguez-Pulido, F. J., Jara-Palacios, M. J., Ramírez-Pérez, P., González-Miret, M. L., & Heredia, F. J. (2014). Impact of adding white pomace to red grapes on the phenolic composition and color stability of syrah wines from a warm climate. *Journal of Agricultural and Food Chemistry*, *62*(12), 2663–2671. doi:/10.1021/jf405574x

Harbertson, J. F., Picciotto, E. A., & Adams, D. O. (2003). Measurement of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. *American Journal of Enology and Viticulture*, *54*(4), 301–306. https://www.ajevonline.org/content/54/4/301

Harbertson, J. F., & Spayd, S. (2006). Measuring Phenolics in the Winery. *American Journal of Enology and Viticulture*, *57*(3), 280–288. https://www.ajevonline.org/content/57/3/280

Heredia, F. J., Alvarez, C., González-Miret, M. L., & Ramírez, A. (2004). *CromaLab®. Análisis de color.* (Registro de la Propiedad Intelectual No. SE-1052-04) [Software]. http://www.color.us.es/esp/sexternos/analisis-del-color.htm

Heredia, T. M., Adams, D. O., Fields, K. C., Held, P. G., & Harbertson, J. F. (2006). Evaluation of a Comprehensive Red Wine Phenolics Assay Using a Microplate Reader. *American Journal of Enology and Viticulture*, *57*(4), 497–502. https://www.ajevonline.org/content/57/4/497

Heymann, H., & Lawless, H. T. (2010). Descriptive Analysis. In *Sensory Evaluation of Food. Principles and Practices* (Second Edition, pp. 227–258). Springer Publishing. https://www.springer.com/gp/book/9781441964878

Hong, Y.-A., & Park, H.-D. (2013). Role of non-*Saccharomyces* yeasts in Korean wines produced from Campbell Early grapes: Potential use of *Hanseniaspora uvarum* as a starter culture. *Food*

Microbiology, *34*(1), 207–214. doi:/10.1016/j.fm.2012.12.011

Hopfer, H., Ebeler, S. E., & Heymann, H. (2012). How blending affects the sensory and chemical properties of red wine. *American Journal of Enology and Viticulture*, *63*(3), 313–324. https://www.ajevonline.org/content/63/3/313

Husson, F., Lê, S., & Pagès, J. (2005). Confidence ellipse for the sensory profiles obtained by principal component analysis. *Food Quality and Preference*, *16*(3), 245–250. doi:/10.1016/j.foodqual.2004.04.019

Kilmister, R. L., Mazza, M., Baker, N. K., Faulkner, P., & Downey, M. O. (2014). A role for anthocyanin in determining wine tannin concentration in Shiraz. *Food Chemistry*, *152*, 475–482. doi:/10.1016/ j.foodchem.2013.12.007

Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2011). Use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content and pH of wine. *Australian Journal of Grape and Wine Research*, *17*(2), 230–238. doi:/10.1111/j.1755-0238.2011.00142.x

Kutyna, D. R., Varela, C., Henschke, P. A., Chambers, P. J., & Stanley, G. A. (2010). Microbiological approaches to lowering ethanol concentration in wine. *Trends in Food Science & Technology*, *21*(6), 293–302. doi:/10.1016/j.tifs.2010.03.004

Liang, N.-N., He, F., Bi, H.-Q., Duan, C.-Q., Reeves, M. J., & Wang, J. (2012). Evolution of flavonols in berry skins of different grape cultivars during ripening and a comparison of two vintages. *European Food Research and Technology*, *235*(6), 1187–1197. doi:/10.1007/s00217-012-1850-4

Lleixà, J., Martín, V., Portillo, M. del C., Carrau, F., Beltran, G., & Mas, A. (2016). Comparison of fermentation and wines produced by inoculation *of Hanseniaspora vineae* and *Saccharomyces cerevisiae*. *Frontiers in Microbiology*, *7*, 338. doi:/10.3389/fmicb. 2016.00338

Loira, I., Morata, A., Comuzzo, P., Callejo, M. J., González, C., Calderón, F., & Suárez-Lepe, J. A. (2015). Use of Schizosaccharomyces pombe and Torulaspora delbrueckii strains in mixed and sequential fermentations to improve red wine sensory quality. *Food Research International*, *76*, 325–333. doi:/10.1016/j.foodres.2015.06.030

Longo, R., Blackman, J. W., Torley, P. J., Rogiers, S. Y., & Schmidtke, L. M. (2017). Changes in volatile composition and sensory attributes of wines during alcohol content reduction. *Journal of the Science of Food and Agriculture*, *97*(1), 8–16. doi:/10.1002/ jsfa.7757

Martínez, J. A., Melgosa, M., Pérez, M. M., Hita, E., & Negueruela, A. I. (2001). Note. Visual and Instrumental Color Evaluation in Red Wines. *Food Science and Technology International*, *7*(5), 439–444. doi:/10.1106/vfat-5ren-1wk2-5jgq

Maturano, Y. P., Mestre, M. V., Esteve-Zarzoso, B., Nally, M. C., Lerena, M. C., Toro, M. E., Vazquez, F., & Combina, M. (2015a). Yeast population dynamics during prefermentative cold soak of Cabernet Sauvignon and Malbec wines. *International Journal of Food Microbiology*, *199*, 23–32. doi:/10.1016/j.ijfoodmicro.2015.01.005

Maturano, Y. P., Mestre, M. V., Kuchen, B., Toro, M. E., Mercado, L. A., Vazquez, F., & Combina, M. (2019). Optimization of fermentation-relevant factors: A strategy to reduce ethanol in red wine by sequential culture of native yeasts. *International Journal of Food Microbiology*, *289*, 40–48. doi:/10.1016/j.ijfoodmicro.2018.08.016

Maturano, Y. P., Assof, M., Fabani, M. P., Nally, M. C., Jofré, V., Rodríguez Assaf, L. A., Toro, M. E., Castellanos de Figueroa, L. I., & Vazquez, F. (2015b). Enzymatic activities produced by mixed *Saccharomyces* and non-Saccharomyces cultures: relationship with wine volatile composition. *Antonie van Leeuwenhoek*, *108*(5), 1239–1256. doi:/10.1007/ s10482-015-0578-0

Mendoza, L. M., de Nadra, M. C. M., Bru, E., & Farías, M. E. (2009). Influence of wine-related physicochemical factors on the growth and metabolism of non-*Saccharomyces* and *Saccharomyces* yeasts in mixed culture. *Journal of Industrial Microbiology & Biotechnology*, *36*(2), 229–237. doi:/10.1007/s10295-008-0489-4

Mestre Furlani, M. V., Maturano, Y. P., Combina, M., Mercado, L. A., Toro, M. E., & Vazquez, F. (2017). Selection of non-*Saccharomyces* yeasts to be used in grape musts with high alcoholic potential: a strategy to obtain wines with reduced ethanol content. *FEMS Yeast Research*, *17*(2), 1–10. doi:/10.1093/ femsyr/fox010

Mestre, M. V., Maturano, Y. P., Gallardo, C., Combina, M., Mercado, L., Toro, M. E., Carrau, F., Vazquez, F., & Dellacassa, E. (2019). Impact on sensory and aromatic profile of low ethanol malbec wines fermented by sequential culture of *Hanseniaspora uvarum* and *Saccharomyces cerevisiae* Native Yeasts. *Fermentation*, *5*(3), 65. doi:/10.3390/fermentation5030065

Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International*, *43*(7), 1844–1855. doi:/10.1016/j.foodres.2010.05.001

OIV (2012). Compendium of international methods of analysis of wines and musts, vol. 1. *Organisation Internationale de la Vigne et du Vin*, Paris.

Padilla, B., Gil, J. V., & Manzanares, P. (2016). Past and future of non-*Saccharomyces* yeasts: from spoilage microorganisms to biotechnological tools for

improving wine aroma complexity. *Frontiers in Microbiology*, *7*, 411. doi:/10.3389/fmicb.2016.00411

Pallmann, C. L., Brown, J. A., Olineka, T. L., Cocolin, L., Mills, D. A., & Bisson, L. F. (2001). Use of WL medium to profile native flora fermentations. *American Journal of Enology and Viticulture*, *52*(3), 198–203. https://www.ajevonline.org/content/52/3/198

Pérez-Magariño, S., & González-SanJosé, M. L. (2003). Application of absorbance values used in wineries for estimating CIELAB parameters in red wines. *Food Chemistry*, *81*(2), 301–306. doi:/10.1016/s0308-8146(02)00509-5

Piccardo, D., Favre, G., Pascual, O., Canals, J. M., Zamora, F., & González-Neves, G. (2019). Influence of the use of unripe grapes to reduce ethanol content and pH on the color, polyphenol and polysaccharide composition of conventional and hot macerated Pinot noir and Tannat wines. *European Food Research and Technology*, *245*(6), 1321–1335. doi:/10.1007/s00217-019-03258-4

Renouf, V., Claisse, O., & Lonvaud-Funel, A. (2007). Inventory and monitoring of wine microbial consortia. *Applied Microbiology and Biotechnology*, *75*(1), 149–164. doi:/10.1007/s00253-006-0798-3

Rentzsch, M., Schwarz, M., & Winterhalter, P. (2007). Pyranoanthocyanins – an overview on structures, occurrence, and pathways of formation. *Trends in Food Science & Technology*, *18*(10), 526–534. doi:/10.1016/j.tifs.2007.04.014

Rolle, L., Englezos, V., Torchio, F., Cravero, F., Río Segade, S., Rantsiou, K., Giacosa, S., Gambuti, A., Gerbi, V., & Cocolin, L. (2018). Alcohol reduction in red wines by technological and microbiological approaches: a comparative study. *Australian Journal of Grape and Wine Research*, *24*(1), 62–74. doi:/10.1111/ajgw.12301

Schelezki, O. J., Antalick, G., Šuklje, K., & Jeffery, D. W. (2020). Pre-fermentation approaches to producing lower alcohol wines from Cabernet-Sauvignon and Shiraz: Implications for wine quality based on chemical and sensory analysis. *Food Chemistry*, *309*, 125698. doi:/10.1016/j.foodchem.2019.125698

Schelezki, O. J., Smith, P. A., Hranilovic, A., Bindon, K. A., & Jeffery, D. W. (2018). Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet-Sauvignon grapes: Impact on polysaccharide and tannin content and composition. *Food Chemistry*, *244*, 50–59. doi:/10.1016/j.foodchem.2017.10.024

Schultz, H. R. (2010). Climate change and viticulture: Research needs for facing the future. *Journal of Wine Research*, *21*(2–3), 113–116. doi:/10.1080/09571264.2010.530093

Tristezza, M., di Feo, L., Tufariello, M., Grieco, F., Capozzi, V., Spano, G., Mita, G., & Grieco, F. (2016). Simultaneous inoculation of yeasts and lactic acid bacteria: Effects on fermentation dynamics and chemical composition of Negroamaro wine. *LWT - Food Science and Technology*, *66*, 406–412. doi:/10.1016/j.lwt.2015.10.064

Varela, C., Dry, P. R., Kutyna, D. R., Francis, I. L., Henschke, P. A., Curtin, C. D., & Chambers, P. J. (2015). Strategies for reducing alcohol concentration in wine. *Australian Journal of Grape and Wine Research*, *21*, 670–679. doi:/10.1111/ajgw.12187