Non-Saccharomyces yeast and lactic acid bacteria in Co-inoculated fermentations with two Saccharomyces cerevisiae yeast strains: A strategy to improve the phenolic content of Syrah wine

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ABSTRACT

Syrah must was co-inoculated with mixed cultures of Saccharomyces + O. oeni/Lb. plantarum and Saccharomyces + non-Saccharomyces + O. oeni/Lb. plantarum to evaluate the effect on phenolics and sensory attributes. Reference wines were produced by S. cerevisiae. Malvidin-3-O-glucoside, flavan-3-ols, flavonols and phenolic acids were quantified using a RP-HPLC technique. Physicochemical characteristics and sensory attributes were measured. Total acidity and alcohol in mixed co-inoculations were different from reference wines. The concentration of l-malic acid was 7-times less in mixed co-inoculations. Mixed co-inoculations had ca. 1.3-times more malvidin-3-O-glucoside and phenolic acids than reference wines. Flavan-3-ols and flavonols were not different between mixed co-inoculations and reference wines. Acidity and astringency were least in mixed co-inoculations. Mouthfeel and bitterness least in S. cerevisiae wines. Tasters preferred mixed co-inoculated wines. Mixed co-inoculation is a strategy to contemplate for Syrah vinification but the modalities of inoculation need further investigation. Success depends on a suitable combination of yeast/bacteria and consideration of strain variation.

1. Introduction

Alcoholic fermentation (AF) is an essential step in the production of red wine (Costello, Francis, & Bartowsky, 2012). Single yeast cultures such as Saccharomyces cerevisiae are usually inoculated into grape must to initiate AF. Phenolic compound concentrations can be modified during AF through enzymatic reactions or metabolic activities of yeasts (Ribéreau-Gayon, Glories, Maujean, & Du Bourdieu, 2006). β-glucosidase is an enzyme responsible for catalysing the hydrolysis of glycosidic linkages in alkyl and aryl-β-D-glucosides to release phenolic aglycone moieties. Wine quality can be assessed by a combination of sensory (colour, aroma, astringency, bitterness, acidity, body, complexity, structure and mouthfeel) and chemical (flavonoids, non-flavonoids, volatile compounds and flavour profiles) analyses. Red wine made with different S. cerevisiae strains resulted in decreased anthocyanin concentrations (Morata, Gomez-Cordoves, Suberviola, Bartolome, & Saurez, 2003). The decrease may have been due to yeast-anthocyanin interaction.

Most non-Saccharomyces yeasts have limited fermentation potential, i.e. low fermentation rates as well as low tolerance for SO₂ and pH (Du
Plessis et al., 2017). Non-Saccharomyces yeasts are therefore used in combination with S. cerevisiae in mixed culture fermentations to finish AF and to ensure that the wines ferment to dryness (Benito, Calderón-Fernandez, Palomero, & Benito, 2015; Varela, Barker, Tran, Borneman, & Curtin, 2017).

The effect of different non-Saccharomyces yeasts species on wine quality has been evaluated by Viana, Belloch, Vallés, and Manzanares (2011). Medina et al. (2013) found that certain positive sensory attributes of Chardonnay wines and quality were correlated with increased phenolic concentrations in spontaneous fermentations and co-fermulations with Hanseniaspora vinaeae. Domízio, Liu, Bisson, and Barile (2014) reported that mixed fermentations of non-Saccharomyces yeasts in complex metabolite matrices can result in increased aroma and flavour diversity with improved wine quality. However, due to incomplete sugar catabolism, such fermentation lacks predictability.

Tempranillo grape must which was inoculated with S. cerevisiae in combination with Metschnikowia pulcherrima added after 48 h, resulted in wines with improved mouthfeel, compared to S. cerevisiae reference wines (Belda et al., 2016). However, the above-mentioned wines were not significantly different in colour (Belda et al., 2016).

Mixed fermentation cultures can modulate the chemical profiles of wine (Benito et al., 2015; Minnaar et al., 2017). Grape phenolic compounds, such as anthocyanins, flavonols, flavan-3-ols and phenolic acids contribute to wine quality by the amelioration of colour, astrignency, bitterness, body, mouthfeel, fullness, complexity and structure (Rodríguez-Montalegre, Romero-Peces, Chacón-Vozmediano, Martínez-Gascueña, & García-Romero, 2006). Viana and co-workers (2011) found that mixed fermentations of S. cerevisiae and Hanseniaspora uvarum (anamorph: Kloekera apiculate) using Muscat grape must have the ability to improve wine flavour, however, excessive growth of H. uvarum can result in wines with increased volatile acidity. Hranilovic et al. (2017) reported that Shiraz wines made with S. cerevisiae and M. pulcherrima had less flavan-3-ols and anthocyanins, compared to Saccharomyces wines. Medina, Boido, Dellecasse, and Carrau (2018) reported increased concentrations of colour intensities (anthocyanins) in Tannat wines made with mono cultures of M. pulcherrima and Hanseniaspora opuntiae than wines made with S. cerevisiae. Tannat wines made with S. cerevisiae and H. uvarum had increased colour intensity but less total anthocyanins than S. cerevisiae wines.

Red wine can also undergo a secondary fermentation called malolactic fermentation (MLF) using lactic acid bacteria (LAB), which can be induced at the beginning or during the final stages of AF or post AF (Costello et al., 2012). Malolactic fermentation leads to the enzymatic conversion of dicarboxylic l-malic acid to mono-carboxylactic l-lactic acid, which results in de-acidification of wine (Pérez-Martín, Seseña, Izquierdo, & Palop, 2013). Certain LAB can however cause stuck fermentations or wines with increased acetic acid. Oenococcus oeni (O. oeni) is associated with MLF due to its tolerance to an acidic pH and increased alcohol content (Hernández et al., 2007). Tempranillo wines made with a combination of S. cerevisiae yeasts (indigenous strains) and Lactobacillus plantarum in sequential inoculations had increased concentrations of flavonoids and phenolic acids, compared to Saccharomyces wines (Hernández et al., 2007).

Lactic acid bacteria can cause secondary metabolic activities during MLF that modulate the sensory attributes of wine with negative or positive effects on colour and mouthfeel (López et al., 2011). The effect of LAB on wine sensory attributes showed that it can enhance body, complexity, structure and mouthfeel of Cabernet Sauvignon wine (Costello et al., 2012). Syrah wines made with yeast and Lb. plantarum had more intense mouthfeel, when compared to Saccharomyces reference wines (Minnaar et al., 2017).

Co-inoculation of Malbec grape must with H. uvarum and S. cerevisiae yeast with O. oeni, resulted in wines with more phenolic aroma intensity than wines without MLF (Mendoza, Merin, Morata, & Farias, 2011). López et al. (2011) demonstrated that the esterase and glycosidase activities of O. oeni resulted in Tempranillo wines with increased total phenolics and anthocyanins, compared to wines that did not undergo MLF.

Malolactic fermentations in combination with non-Saccharomyces yeast can result in increased phenolic concentrations of Cabernet Sauvignon, Nero di Troia and Syrah wines (Costello et al., 2012; Suriano, Ceci, & Tamborra, 2012). Abrahamse and Bartowsky (2012) reported that mixed inoculations after MLF of Chardonnay, Malbec and Syrah grape musts, were not different in the final chemical composition, compared to wines without MLF. Burns and Osborne (2015) reported increased anthocyanin concentrations in Pinot noir wines that underwent MLF, when compared to wines made with S. cerevisiae. Chescheir, Philbin, and Osborne (2015) reported increased concentrations of phenolic acids in Pinot noir wines made with S. cerevisiae in combination with O. oeni as opposed to S. cerevisiae reference wines. Hranilovic et al. (2017) reported that colour density increased in Syrah wines of sequentially inoculated grape must with M. pulcherrima and S. cerevisiae, compared to wines made with mixed cultures of S. cerevisiae, Torulaspora delbrueckii and Lb. plantarum. Wines made with a combination of Saccharomyces, non-Saccharomyces and O. oeni in sequential inoculations, when compared to S. cerevisiae reference wines, had increased anthocyanin concentrations (Minnaar et al., 2017). Increased concentrations of phenolic acids were also reported in sequentially inoculated Syrah grape must with S. cerevisiae, M. pulcherrima and H. uvarum after MLF.

The aim of this study was to investigate the effect of mixed culture co-inoculation fermentations using two S. cerevisiae strains and two non-Saccharomyces yeasts with two lactic acid bacteria on the phenolic concentrations of Syrah wines. Additionally, the effect of treatment on selected sensory attributes were also reported on.

2. Material and methods

2.1. Yeast strains and lactic acid bacteria

Two commercial S. cerevisiae yeast strains (VIN13 and NT202, Anchor Wine Yeast, South Africa), one H. uvarum yeast strain (ARC Infruitec-Nietvoorbij culture collection, Y0858), one M. pulcherrima yeast strain (ARC Infruitec-Nietvoorbij culture collection, Y0839) and two LAB strains, i.e. O. oeni (Vinflora® oenos, Chr. Hansen, Denmark) and Lb. plantarum (Enoferm V22, Lallemand, France) were used to inoculate Syrah grape must. The following abbreviations were used: S. cerevisiae VIN13 (Sc1), S. cerevisiae NT202 (Sc2), H. uvarum (Hu), M. pulcherrima (Mp), O. oeni (LAB1) and Lb. plantarum (LAB2).

2.2. Fermentation process

Handpicked Syrah grapes from vines planted to a northwest-southeast row orientation and trained to a vertical shoot position trellis system on the Nietvoorbij research farm near Stellenbosch (~33.914865, 18.861047) South Africa were utilised for vinification at the ARC’s experimental wine cellar in Stellenbosch. Grapes were mechanically destemmed and crushed. Grape skins and grape pulp were separated, homogenised and reconstituted in equal ratios into 70 L fermentation bins. Yeast assimilable nitrogen (YAN) of the grape juice was measured using a Foss® Winescan (IWB, Stellenbosch University, Stellenbosch). The YAN was 133.0 mg/L which was considered insufficient. Therefore, a standard addition of 50 g/L diammonium hydrogen phosphate (DAP) was added to the juice (Minnaar, Ntushelo, Ngqumba, Van Breda, & Jolly, 2015). Fermentations were conducted in a temperature-controlled room at ca. 24 °C using a standardised winemaking protocol as described by Minnaar et al. (2015). Treatments included S. cerevisiae (Sc1 or Sc2) on its own (reference wines), S. cerevisiae (Sc1 or Sc2) in combination with LAB (LAB1 or LAB2), and non-Saccharomyces yeasts (H. uvarum or M. pulcherrima) in combination with S. cerevisiae (Sc1 or Sc2) and LAB (O. oeni or Lb. plantarum). All treatments were repeated independently in three fermentation bins.
Metschnikowia pulcerrima and H. uvarum were inoculated as wet cultures on day 0 at a concentration of $8.4 \times 10^5$ and $6.4 \times 10^5$ cells/mL, respectively. Rehydrated commercial S. cerevisiae (0.3 g/L active dry yeast) was added 24 h later (day 1) to complete AF in the mixed culture co-inoculation fermentations, whereas 0.3 g/L of the active dry yeast was added on day 0 for the reference wines. Lactic acid bacteria were added to the ferments after 25 h (Day 1) and all ferments that will undergo MLF were inoculated according to the supplier’s recommendations, before the alcoholic fermentation became tumultuous.

The fermentation caps were punched down twice each day and all treatments were subjected to the same grape-pomace contact time. Wines were racked off the lees and the total SO$_2$ adjusted to ca. 85 mg/L after completion of MLF. Malolactic fermentation was considered complete for the experimental wines when l-malic acid concentrations were below 0.3 g/L. All wines fermented to dryness (< 0.2 g/L residual sugar). Wines were stored at 15°C until required for analysis. The yeast and LAB populations were monitored throughout the duration of fermentation to ensure yeast and bacteria multiplication, which was reported by Du Plessis et al. (2019).

2.3. Physicochemical characteristics

Total soluble solids, total acidity (TA), l-malic acid and volatile acidity (VA) were analysed in the Syrah must using a Foss® Winescan (IBWT, Stellenbosch University, Stellenbosch). Residual sugar (RS), l-malic acid, pH, TA, alcohol and VA were determined on the finished wine using an OenoFoss™ analyser (FOSS Analytical A/S, Denmark).

2.4. Phenolic compounds

Phenolics were quantified using a liquid chromatographic method (RP-HPLC-DAD) as described by Waterhouse, Price, and McCord (1999). Malvidin-3-O-glucosides, flavan-3-ols, flavonols and phenolic acids were measured at absorbance wavelengths of 520 nm, 280 nm, 360 nm, and 316 nm, respectively. Quantification of phenolics was performed based on calibration curves using commercially available reference standards and matching ultra-violet absorbance spectra. Wines were filtered through a 0.45 µm nylon membrane syringe filter prior to analysis.

2.5. Sensory evaluation

A panel of twenty-four wine tasters evaluated the wines 16 months after bottling. The panelists were commercial winemakers and/or staff of The Fruit, Vine and Wine Institute of the Agricultural Research Council in Stellenbosch. Panel members had between 2 and 20 years’ experience in wine evaluation.

Wines were evaluated (classical profiling) during three sessions (three days) in a temperature-controlled room at ± 20°C with fluorescent light illumination. Each panelist was allocated to a separate tasting booth and ca. 30 mL of wine was presented in a randomised order, in a standard international wine tasting glass, labelled with a three-digit code. Water and wheat biscuits (neutral taste) were provided to tasters for palate cleansing between sample tastings. The tasters rated the attributes on a 10 cm unstructured line-scale from “low” to “high” (acidity, astringency, preference), “thin” to “full” (mouthfeel) and “undetectable” to “prominent” (bitterness).

2.6. Statistical analysis

Resulting data was subjected to analysis of variance (ANOVA) using SAS version 9.4 (SAS Institute Inc., Cary, USA). Fisher’s significant difference values were calculated at a 5% probability level to facilitate comparisons between treatment means. Means within data sets that differed at a 5% probability level were considered significantly different.

3. Results and discussion

This paper reports on the effect of different treatments, i.e. Saccharomyces/LAB and Saccharomyces/non-Saccharomyces/LAB as strategies on Syrah wine’s physicochemical characteristics, phenolics and selected sensory attributes.

3.1. Yeast development

The naturally occurring Saccharomyces and non-Saccharomyces yeast populations in the Syrah must were reported in Du Plessis et al. (2019). Initial yeast counts of the wines inoculated with H. uvarum and M. pulcerrima at day 0 were below 1 x 10$^6$ CFU/mL, but increased to levels > 10 million CFU/mL after 24 h (Du Plessis et al., 2019). However, this trend changed after inoculation of commercial S. cerevisiae yeasts (day 1), which resulted in the decrease of H. uvarum and M. pulcerrima cells.

3.2. LAB development and progression of MLF

Naturally occurring lactic acid bacteria populations in the Syrah grape must were reported by Du Plessis et al. (2019). The addition of commercial LAB resulted in an expected increase of LAB cells. No notable delays in MLF was found in the inoculated wines, despite the decrease of Lb. plantarum and O. oeni cells.

Mixed culture co-inoculations (Sc1/Sc2) of H. uvarum completed MLF (O. oeni, Lb. plantarum) within 18 days, while S. cerevisiae (Sc1), completed MLF (O. oeni, Lb. plantarum) within 34 days. A delay in MLF (Sc1, Lb. plantarum) can be ascribed to a decrease in LAB cells. This trend was however not observed for S. cerevisiae (Sc1) after MLF (O. oeni), which had LAB cell count of > 1 x 10$^6$ CFU/mL throughout the fermentation (Du Plessis et al., 2019).

3.3. Physicochemical characteristics

The physicochemical characteristics of Syrah must and wine are listed in Table 1. Total acidity and l-malic acid were 7.43 g/L and 3.1 g/L, respectively in grape must with a pH of 3.57, compared to fermented must of 4.9 g/L and 0.5 g/L, respectively and a pH of 3.8. Saccharomyces cerevisiae (Sc1) after MLF, significantly increased the total acidity, compared to reference fermentations (Sc1) and mixed culture co-inoculations after MLF. Mixed culture co-inoculated fermentations had 4.74 g/L total acidity, compared to 5.25 g/L for Sc1 after MLF and 4.90 g/L for reference fermentations.

In mixed culture co-inoculations of H. uvarum after MLF (Sc2), total acidity was 4.69 g/L, compared 4.84 g/L for M. pulcerrima, 5.04 g/L for S. cerevisiae wines after MLF and 4.99 g/L for reference fermentations (Table 2). Hranilovic et al. (2017) reported reduced acetic acid production in mixed co-inoculations of T. delbrueckii/S. cerevisiae, Lb. thermotolerans/S. cerevisiae and S. cerevisiae/Lb. thermotolerans/T. delbrueckii, compared to S. cerevisiae reference wines. Puertas et al. (2018) found increased concentrations of total acidity in Chardonnay wines made with S. cerevisiae, compared to sequentially inoculated must with S. cerevisiae/T. delbrueckii. The production of acetic acid during AF is however dependent on the initial sugar concentration of the must.

Reference fermentations (Sc1/Sc2) and S. cerevisiae wines after MLF had an average of 13.69% alcohol content, compared to mixed culture co-inoculations after MLF of 12.46%. Morales, Fierro-Risco, Rios-Reina, Ubeda, and Paneque (2019) reported increased alcohol in wines made with S. cerevisiae as compared to co-inoculated ferment. Puertas et al. (2018) reported increased concentrations of alcohol in Chardonnay wines made with S. cerevisiae, compared to co-inoculated must with S. cerevisiae/T. delbrueckii. Inoculated strategy was however sequential.

Saccharomyces cerevisiae (Sc1/Sc2) wines after MLF had an average of 0.21 g/L malic acid (L), whereas wines of mixed culture co-inoculations had an average of 0.26 g/L. Reference fermentations (Sc1/
Saccharomyces and lactic acid bacteria.

3.4. Phenolic compounds

3.4.1. Phenolic acids

Mixed culture co-inoculations (Sc2) of H. uvarum after MLF, significantly increased gallic, caffeic, p-coumaric and chlorogenic acids, compared to reference fermentations, S. cerevisiae wines after MLF and mixed culture co-inoculations of M. pulcherrima after MLF (Table 3). Reference fermentations (Sc1) had 17.84 mg/L caffeic and 18.49 mg/L chlorogenic acid as opposed to 27.94 mg/L caffeic and 32.49 mg/L chlorogenic acid in S. cerevisiae wines after MLF and 30.07 mg/L caffeic and 31.35 mg/L chlorogenic acid in mixed culture co-inoculations.

Mixed culture co-inoculations (Sc2) after MLF, significantly increased gallic, p-coumaric and chlorogenic acids in comparison to reference fermentations and S. cerevisiae wines after MLF (Table 4). Caffeic acid was not significantly different among any of the fermentations, including reference fermentations.

Mixed culture co-inoculations (Sc1) of H. uvarum after MLF, significantly increased the total phenolic acids, compared to the rest of the fermentations. For Sc2 wines, mixed culture co-inoculations of M. pulcherrima and H. uvarum after MLF had increased concentrations of total phenolic acids. Total phenolic acids ranged from 83.52 mg/L to 108.27 mg/L for mixed culture co-inoculation after MLF. Saccharomyces cerevisiae (Sc1/Sc2) wines after MLF ranged from 80.59 mg/L to 84.59 mg/L, whereas reference fermentations ranged from 61.65 mg/L to 73.21 mg/L.

Table 1

| Physicochemical characteristics | Syrah must | Reference (S. cerevisiae) | S. cerevisiae/MLF | S. cerevisiae/co-inoculations/MLF |
|---------------------------------|------------|--------------------------|-------------------|-----------------------------------|
|                                 | Sc1        | Sc1 + LAB1                | Sc1 + LAB2        | Mp1 + Sc1 + LAB1                  |
|                                 |            |                          |                   | Mp + Sc1 + LAB2                   |
|                                 |            |                          |                   | Hu1 + Sc1 + LAB1                  |
|                                 |            |                          |                   | Hu + Sc1 + LAB2                   |
| Total acidity (g/L)             | 7.43       | 4.90 ± 0.32b             | 5.39 ± 0.34a      | 5.11 ± 0.04a                      |
|                                 |            | 4.71 ± 0.63c             | 4.78 ± 0.09c      | 4.76 ± 0.07c                      |
|                                 |            | 4.73 ± 0.05c             | 4.73 ± 0.05c      |                                    |
| pH                              | 3.57       | 3.82 ± 0.09a             | 3.77 ± 0.11a      | 3.88 ± 0.01a                      |
|                                 |            | 3.81 ± 0.07a             | 3.89 ± 0.04a      | 3.74 ± 0.07a                      |
|                                 |            | 3.74 ± 0.07a             | 3.81 ± 0.01a      |                                    |
| Alcohol (% v/v)                 | N D        | 13.54 ± 0.35a            | 13.51 ± 0.44a     | 13.57 ± 0.37a                     |
|                                 |            | 12.49 ± 1.23b            | 12.41 ± 1.16b     | 12.11 ± 0.27b                     |
|                                 |            | 12.76 ± 0.61b            | 12.76 ± 0.61b     |                                    |
| l-Malic acid (g/L)              | 3.40       | 0.21 ± 0.02c             | 0.22 ± 0.05c      | 0.26 ± 0.03b                      |
|                                 |            | 0.24 ± 0.07b             | 0.28 ± 0.03b      | 0.27 ± 0.04b                      |
|                                 |            | 0.27 ± 0.04b             | 0.27 ± 0.04b      |                                    |
| Brix                            | 23.0       | N A                      | N A               | N A                               |
|                                 |            | N A                      | N A               | N A                               |
|                                 |            | N A                      | N A               | N A                               |
| Residual sugar (g/L)            | N A        | < 0.2                    | < 0.2             | < 0.2                             |

** N D: Not detected.

* Different letters in the same row indicate significant differences in the content of the measured parameters among the different treatments according to Fischer's least significant difference test (p ≤ 0.05).

** Standard deviation.

1 Saccharomyces cerevisiae (Sc1 [VIN13], reference).

2 LAB1: Oenococcus oeni.

3 LAB2: Lactobacillus plantarum.

4 Metschnikowia pulcherrima

5 Hanseniaspora uvarum

Table 2

| Physicochemical characteristics | Syrah must | Reference (S. cerevisiae) | S. cerevisiae/MLF | S. cerevisiae/co-inoculations/MLF |
|---------------------------------|------------|--------------------------|-------------------|-----------------------------------|
|                                 | Sc2        | Sc2 + LAB1                | Sc2 + LAB2        | Mp1 + Sc2 + LAB1                  |
|                                 |            |                          |                   | Mp + Sc2 + LAB2                   |
|                                 |            |                          |                   | Hu1 + Sc2 + LAB1                  |
|                                 |            |                          |                   | Hu + Sc2 + LAB2                   |
| Total acidity (g/L)             | 7.43       | 4.99 ± 0.18a             | 5.11 ± 0.20a      | 4.98 ± 0.05a                      |
|                                 |            | 4.86 ± 0.07ba            | 4.83 ± 0.03ba     | 4.73 ± 0.15c                      |
|                                 |            | 4.66 ± 0.12c             | 4.66 ± 0.12c      |                                    |
| pH                              | 3.57       | 3.78 ± 0.05a             | 3.76 ± 0.08a      | 3.82 ± 0.02a                      |
|                                 |            | 3.83 ± 0.02a             | 3.86 ± 0.02a      | 3.79 ± 0.04a                      |
|                                 |            | 3.85 ± 0.04a             | 3.85 ± 0.04a      |                                    |
| Alcohol (% v/v)                 | N D        | 13.84 ± 0.34a            | 14.03 ± 0.27a     | 13.65 ± 0.32a                     |
|                                 |            | 12.26 ± 0.15b            | 12.74 ± 0.48b     | 12.22 ± 0.01b                     |
|                                 |            | 12.75 ± 0.72b            | 12.75 ± 0.72b     |                                    |
| l-Malic acid (g/L)              | 3.1        | 0.22 ± 0.03c             | 0.21 ± 0.05c      | 0.28 ± 0.08b                      |
|                                 |            | 0.27 ± 0.06b             | 0.24 ± 0.06b      | 0.25 ± 0.07b                      |
|                                 |            | 0.25 ± 0.07b             | 0.25 ± 0.07b      |                                    |
| Brix                            | 23.0       | N A                      | N A               | N A                               |
|                                 |            | N A                      | N A               | N A                               |
|                                 |            | N A                      | N A               | N A                               |
| Residual sugar (g/L)            | N A        | < 0.2                    | < 0.2             | < 0.2                             |

** N D: Not detected.

* Different letters in the same row indicate significant differences in the content of the measured parameters among the different treatments according to Fischer's least significant difference test (p ≤ 0.05).

** Standard deviation.

1 Saccharomyces cerevisiae (Sc2 [NT202], reference).

2 LAB1: Oenococcus oeni.

3 LAB2: Lactobacillus plantarum.

4 Metschnikowia pulcherrima

5 Hanseniaspora uvarum
During MLF, Medina et al. (2018) describe LAB as a potential source of glycosidic activity. MLF and mixed culture co-inoculations of fermentations (Tables 3 and 4). Concentrations, compared to the rest of the fermentations, including reference and S. cerevisiae levels of 5.89 mg/L, compared to 6.85 mg/L in reference fermentations after MLF were significant compared to the rest of the fermentations, including reference and S. cerevisiae levels of 5.89 mg/L, compared to 6.85 mg/L in reference fermentations after MLF (Table 3). However, for Sc2 wines, increased (+)-catechin concentrations in mixed culture co-inoculations after MLF were found, compared to reference fermentations and S. cerevisiae wines after MLF (Table 4). Total flavan-3-ols were 7.03 mg/L in mixed culture co-inoculations (Sc1) of M. pulcherrima after MLF, compared to 8.06 mg/L in H. uvarum and 8.15 mg/L in S. cerevisiae and reference wines. Contrary to the above, mixed culture co-inoculations (Sc2) of M. pulcherrima and H. uvarum had 7.9 mg/L of total flavan-3-ols as opposed to 6.11 mg/L in S. cerevisiae wines after MLF and 5.82 mg/L in reference fermentations. Syrah wines made with mixed co-inoculations of S. cerevisiae/Lb. thermotolerans/T. delbrueckii had decreased concentrations of total flavan-3-ols, compared to S. cerevisiae wines (Hranilovic et al., 2017). MLF was however not induced. This is in contrast to results reported in this paper. Aglianico red wines co-inoculated with S. cerevisiae and Lb. plantarum (MLF) had increased levels of proanthocyanidins as opposed to S. cerevisiae wines (Suriano, Savino, Basile, Tarricone, & Di Gennario, 2015). Work by Suriano et al. (2015) is in agreement with results of this paper, however, Aglianico grape cultivar was under study. Total flavan-3-ol concentrations ranged from 6.95 mg/L to 8.62 mg/L for mixed culture co-inoculations after MLF. Fermentations of S. cerevisiae after MLF ranged from 5.98 mg/L to 8.23 mg/L, whereas reference fermentations ranged from 5.82 mg/L to 8.23 mg/L.

### 3.4.2. Flavan-3-ols

Mixed culture co-inoculations (Sc1/Sc2) of H. uvarum after MLF, significantly increased epigallocatechin 3-O-gallate (EGCG) concentrations, compared to the rest of the fermentations, including reference fermentations (Tables 3 and 4). Saccharomyces cerevisiae (Sc1/Sc2) after MLF and mixed culture co-inoculations of M. pulcherrima and H. uvarum after MLF were significantly different among each other in EGCG concentrations.

Mixed culture co-inoculations (Sc1) after MLF had (+)-catechin levels of 5.89 mg/L, compared to 6.85 mg/L in reference fermentations and S. cerevisiae (Sc1) wines after MLF (Table 3). However, for Sc2 wines, increased (+)-catechin concentrations in mixed culture co-inoculations after MLF were found, compared to reference fermentations and S. cerevisiae wines after MLF (Table 4). Total flavan-3-ols were 7.03 mg/L in mixed culture co-inoculations (Sc1) of M. pulcherrima after MLF, compared to 8.06 mg/L in H. uvarum and 8.15 mg/L in S. cerevisiae and reference wines. Contrary to the above, mixed culture co-inoculations (Sc2) of M. pulcherrima and H. uvarum had 7.9 mg/L of total flavan-3-ols as opposed to 6.11 mg/L in S. cerevisiae wines after MLF and 5.82 mg/L in reference fermentations. Syrah wines made with mixed co-inoculations of S. cerevisiae/Lb. thermotolerans/T. delbrueckii had decreased concentrations of total flavan-3-ols, compared to S. cerevisiae wines (Hranilovic et al., 2017). MLF was however not induced. This is in contrast to results reported in this paper. Aglianico red wines co-inoculated with S. cerevisiae and Lb. plantarum (MLF) had increased levels of proanthocyanidins as opposed to S. cerevisiae wines (Suriano, Savino, Basile, Tarricone, & Di Gennario, 2015). Work by Suriano et al. (2015) is in agreement with results of this paper, however, Aglianico grape cultivar was under study. Total flavan-3-ol concentrations ranged from 6.95 mg/L to 8.62 mg/L for mixed culture co-inoculations after MLF. Fermentations of S. cerevisiae after MLF ranged from 5.98 mg/L to 8.23 mg/L, whereas reference fermentations ranged from 5.82 mg/L to 8.23 mg/L.

### 3.4.3. Flavonols

Mixed culture co-inoculations (Sc1) of H. uvarum after MLF had 4.73 mg/L rutin and 1.71 mg/L isorquercetin, compared to 3.62 mg/L and 1.44 mg/L in mixed culture co-inoculations of M. pulcherrima with 3.43 mg/L and 1.35 mg/L in S. cerevisiae wines after MLF (Table 3). Reference fermentations had decreased concentrations of rutin and isorquercetin. Saccharomyces cerevisiae (Sc2) wines after MLF contained on average 4.48 mg/L rutin in comparison to 3.83 mg/L for reference wines and 3.28 mg/L for mixed culture co-inoculation fermentations of M. pulcherrima and 2.47 mg/L for H. uvarum. Isoquercetin reached a concentration of 1.38 mg/L in S. cerevisiae (Sc2) wines after MLF, 1.31 mg/L in mixed culture co-inoculations and 1.20 mg/L in reference fermentations.

Mixed culture co-inoculations of M. pulcherrima (Sc1/Sc2) after MLF

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**Table 3**

Average concentrations of phenolic compounds (mg/L) of wines obtained by alcoholic and malolactic co-inoculated fermentations using mixed cultures of Saccharomyces (Sc1), non-Saccharomyces and lactic acid bacteria.

| Phenolics | Reference (S. cerevisiae) | S. cerevisiae/MLF | S. cerevisiae/co-inoculations/MLF |
|-----------|--------------------------|------------------|----------------------------------|
|           | Sc1                      | Sc1 + LAB1       | Sc1 + LAB2                       |
|           |                          | Mp1 + Sc1 + LAB1 | Mp1 + Sc1 + LAB2 + Hu3 + Sc1 + LAB1 |
|           |                          | Hu + Sc1 + LAB2  |                                  |
| Gallic acid (THBA) | 2.45 ± 1.44a             | 2.45 ± 1.05c     | 2.34 ± 1.23c                     |
| Caffeic acid (HCA)  | 17.84 ± 1.12d            | 27.80 ± 2.25b    | 27.94 ± 1.89b                    |
| p-Coumaric acid (HCA) | 22.86 ± 2.97c            | 21.69 ± 2.12c    | 21.03 ± 0.59c                    |
| Chlorogenic acid (ester of HCA7, two isomers) | 18.49 ± 1.52d | 32.64 ± 0.15b | 32.34 ± 2.95b |
| Phenolic acids    | 61.65c                   | 84.59b           | 83.68b                           |
| Epigallocatechin 3-O-gallate (ester of EGCG) | 1.29 ± 1.67c | 1.29 ± 1.05c | 1.33 ± 2.42c |
| (+)-Catechin      | 6.86 ± 0.38a             | 6.94 ± 0.55a     | 6.77 ± 0.19a                     |
| Flavan-3-ols      | 8.15a                    | 8.23a            | 8.10a                            |
| Quercetin 3-O-rutinoside (rutin) | 2.94 ± 1.76c | 3.34 ± 0.17b | 3.51 ± 0.30b |
| Quercetin 3-O-gallate (isorquercetin) | 1.26 ± 2.68c | 1.35 ± 0.10b | 1.35 ± 1.97b |
| Quercetin         | 2.60 ± 3.93a             | 2.56 ± 4.38a     | 2.50 ± 2.92a                     |
| Flavonols         | 6.80c                    | 7.26b            | 7.39b                            |
| Malvidin-3-O-gallate (anthocyanin) | 63.49 ± 9.71b | 65.53 ± 2.97b | 65.21 ± 1.46b |
| Total phenolics   | 140.11d                  | 165.63c          | 164.39c                          |

* Different letters in the same row indicate significant differences in the content of the measured phenolic compounds among the different treatments according to Fischer’s least significance difference test (p < 0.05).

** Standard deviation.

1 Saccharomyces cerevisiae (Sc1 [VIN13], reference wine).
2 LAB1: Oenococcus oeni.
3 LAB2: Lactobacillus plantarum.
4 Metschnikowia pulcherrima.
5 Hanseniaspora uvarum.
6 Trihydroxybenzoic acid.
7 Hydroxycinnamic acid.
8 Epigallocatechin.
were limited to an average of 1.86 mg/L quercetin, whereas S. cerevisiae wines after MLF and reference fermentations, contained an average of 2.46 mg/L. Hernández et al. (2007) reported increased concentrations of myricetin and quercetin in Tempranillo wines that underwent MLF. Romboli, Mangani, Buscioni, Granchi, and Vincenzini (2015) found increased concentrations of quercetin but a reduction of quercetin 3-O-glucoside in Sangiovese wines inoculated with Candida zemplinina, Candida zemplinina (Sc1), and Candida zemplinina (Sc2) after MLF. Contrary to Sc1 fermentations, S. cerevisiae (Sc2) wines after MLF had more total flavonols than mixed culture co-inoculations after MLF, including reference fermentations. Significantly less total flavonols were found in mixed culture co-inoculations of M. pulcherrima (Sc1 and Sc2) and H. uvarum (Sc2) after MLF, compared to S. cerevisiae wines (Sc1 and Sc2) after MLF. Tristeza et al. (2016) found increased concentrations of total flavonols in Negroamaro wines co-inoculated with H. uvarum/S. cerevisiae, compared to S. cerevisiae wines. Vinification was however on a micro-scale. Total flavonol concentrations ranged from 6.40 to 8.34 mg/L for mixed culture co-inoculations after MLF. Fermentations of S. cerevisiae after MLF ranged from 7.26 mg/L to 8.25 mg/L, whereas reference fermentations ranged from 6.80 mg/L to 7.43 mg/L.

### Table 4

| Phenolics | Reference (S. cerevisiae) | S. cerevisiae/MLF | S. cerevisiae/co-inoculations/MLF |
|-----------|--------------------------|------------------|----------------------------------|
|           | Sc2                      | Sc2 + LAB1       | Sc2 + LAB2                       |
|           |                          |                  |                                  |
| Gallic acid (THBA<sup>1</sup>) | 1.99 ± 2.27<sup>1</sup> | 1.95 ± 1.62b | 1.76 ± 0.83b | 2.15 ± 0.91a | 2.15 ± 1.39a | 2.41 ± 2.40a | 2.23 ± 1.93a |
| Caffeic acid (HCA<sup>1</sup>) | 26.87 ± 3.37a | 29.68 ± 1.50a | 28.05 ± 1.53a | 26.07 ± 3.39a | 26.28 ± 0.45a | 27.60 ± 1.47a | 27.41 ± 1.41a |
| p-Coumaric acid (HCA<sup>1</sup>) | 22.66 ± 1.30b | 24.47 ± 1.81b | 23.84 ± 2.03b | 31.60 ± 2.73a | 31.08 ± 3.68a | 33.93 ± 1.08a | 33.52 ± 1.29a |

### Footnotes

1. * Saccharomyces cerevisiae (Sc2 [NT202], reference wine).
2. LAB1: Oenococcus oeni.
3. LAB2: Lactobacillus plantarum.
4. *Metschnikowia pulcherrima*.
5. Hanseniaspora uvarum.
6. Trihydroxybenzoic acid.
7. Hydroxycinnamic acid.
8. Epigallocatechin.

<sup>*</sup> Different letters in the same row indicate significant differences in the content of the measured phenolic compounds among the different treatments according to Fischer's least significant difference test (p < 0.05).
<sup>**</sup> Standard deviation.

### 3.4.4. Malvidin-3-O-glucoside

Mixed culture co-inoculations (Sc1) of *M. pulcherrima* after MLF had 81.93 mg/L malvidin-3-O-glucoside in comparison to an average of 65.78 mg/L for the rest of the fermentations (Table 3). Mixed culture co-inoculations of *M. pulcherrima* and *H. uvarum* (Sc2) after MLF had an average of 79.86 mg/L malvidin-3-O-glucoside, compared to 56.58 mg/L in *S. cerevisiae* wines after MLF and 51.63 mg/L in reference fermentations (Table 4). Malvidin-3-O-glucoside concentrations ranged from 67.33 to 82.31 mg/L for mixed culture co-inoculations after MLF. *Saccharomyces cerevisiae* wines after MLF ranged from 56.0 mg/L to 65.53 mg/L, whereas reference fermentations ranged from 51.63 mg/L to 63.49 mg/L. *Saccharomyces cerevisiae* wines after MLF and mixed culture co-inoculations after MLF had between 20.8% and 59.4% more malvidin-3-O-glucoside respectively, compared to reference fermentations. This is in agreement with work by Burns and Osborne (2015) and Minnaar et al. (2017) who reported increased malvidin-3-O-glucosides in Pinot noir and Syrah wines, respectively of mixed culture inoculations after MLF, compared to fermentations with *Saccharomyces*. Kwaw et al. (2018) inoculated Mulberry juice with *Lb. plantarum*, *Lb. acidophilus* and *Lb. paracasei*. The results showed that lactic acid fermentation impacted on the colour of the juice. Furthermore, the study showed that LAB positively affected the phenolic profile of the juice.

The decreased concentrations of malvidin-3-O-glucosides in reference fermentations and *S. cerevisiae* wines (Sc1/Sc2) after MLF can be due to excessive adsorption of free anthocyanin molecules onto yeast cell walls as proposed by Guadalupe, Martínez, and Ayestarán (2010). Interaction of yeast mannoproteins and arabinogalactans with anthocyanins could be another cause of the decrease in malvidin-3-O-glucosides or the reaction with cell wall proteins. Conversely, the reduced malvidin-3-O-glucosides in reference fermentations indicate that the sugar moiety of malvidin-3-O-glucoside was most likely metabolised by *S. cerevisiae*. 

**Standard deviation.**

1. *Saccharomyces cerevisiae* (Sc2 [NT202], reference wine).
2. LAB1: Oenococcus oeni.
3. LAB2: Lactobacillus plantarum.
4. *Metschnikowia pulcherrima*.
5. Hanseniaspora uvarum.
6. Trihydroxybenzoic acid.
7. Hydroxycinnamic acid.
8. Epigallocatechin.
Mixed culture co-inoculations (Sc1) of *H. uvarum* contained an average of 187.58 mg/L total phenolics, while mixed culture co-inoculations of *M. pulcherrima* were limited to 178.97 mg/L *S. cerevisiae* wines after MLF and reference fermentations had an average of 165.00 mg/L and 140.11 mg/L, respectively. For Sc2 wines, mixed culture co-inoculations of *M. pulcherrima* and *H. uvarum* contained an average of 191.07 mg/L total phenolics, followed by *S. cerevisiae* wines after MLF with 152.55 mg/L and reference fermentations of 138.10 mg/L. Suriano et al. (2015) reported increased concentrations of total phenolics in Aglianico mixed culture co-inoculations, compared to *S. cerevisiae* fermentations. Contrary to work by Suriano and co-workers (2012) reported decreased levels of total phenolics in *S. cerevisiae* wines after MLF (O. oeni) than wines without MLF. Total phenolics in mixed culture co-inoculations reported in this paper were ca. 1.4 times more than reference fermentations, whereas *S. cerevisiae* wines after MLF were ca. 1.2 times more than reference wines.

### 3.5. Sensory analysis

The perception of acidity for *S. cerevisiae* wines (Sc1) after MLF was 47.3%, followed by mixed culture co-inoculations of 48.5%, compared to reference fermentation of 53.9% (Table 5). Contrary to Sc1 fermentations, mixed culture co-inoculations (Sc2) scored 46.7% in acidity perception, *S. cerevisiae* wines after MLF scored 47.71% and references fermentations 50.8% (Table 6). Reference fermentations, both Sc1 and Sc2, were significantly different from the rest of the fermentations.

Fermentations of mixed culture co-inoculations of Sc1 and Sc2, after MLF, scored an average of 51.7% and 55.3% respectively in mouthfeel, followed by *S. cerevisiae* after MLF of 47.1% and 52.6%, respectively.

Mixed culture co-inoculations of Sc1 and Sc2, after MLF scored an average of 40.0% and 37.5% respectively, in astringency, compared to 44.3% and 41.5% of *S. cerevisiae* wines after MLF, respectively. Hranilovic et al. (2017) reported that *S. cerevisiae* and non-*Saccharomyces* combination wines (*M. pulcherrima*) to be more astringent than *S. cerevisiae* reference wines.

Bitterness in mixed culture co-inoculations (Sc1) after MLF scored an average of 29.5%. Conversely to the Sc1 fermentations, mixed culture co-inoculations (Sc2) after MLF scored 32.3% in bitterness. Hranilovic and co-workers (2017) reported that mixed cultures co-inoculations of Syrah wines with *S. cerevisiae/T. delbrueckii/Lb. thermotolerans* were more bitter than non-*Saccharomyces* mono-culture fermentations and *S. cerevisiae* ferments. This is in agreement with results reported in this paper for Sc2 mixed culture co-inoculations. *S. cerevisiae* wines (Sc1) after MLF scored 38.0% and reference wines scored 34.7% in bitterness. *S. cerevisiae* wines (Sc2) after MLF, scored 27.7% and reference wines 27.9% in bitterness. Mixed culture co-inoculations after MLF were perceived as better “quality”, compared to reference fermentations and *S. cerevisiae* wines after MLF.

Mixed culture co-inoculations after MLF had on average 187.8 mg/L total phenolics, compared to 152.2 mg/L and 49.1 mg/L for *S. cerevisiae* after MLF and reference fermentations, respectively. Mixed culture co-inoculations after MLF can also lead to an increased aroma and flavour profile, thereby improving wine quality (Jolly, Varela, & Pretorius, 2014). The tasters preferred mixed culture co-inoculated (Sc1) wines, possibly owing to their less astringent and bitterness and improved mouthfeel. Tasters, however, also preferred mixed culture co-inoculation (Sc2) wines, probably because of their decreased astringency and increased mouthfeel. The effect of non-*Saccharomyces* and LAB on sensory attributes may involve the modulation of interacting phenolics, the extent to which may depend upon yeast/bacterial strain and wine chemical composition (López et al., 2011). Differences in phenolics can also be a function of differential or partial adsorption capacities between yeast strains. However, Azzolini et al. (2012) reported no differences in the sensory properties of wines after MLF, as opposed to wines without MLF. Contrary to Azzolini et al. (2012); Gerbaux and Briffoux (2003) reported a loss of colour in Pinot noir wines after MLF.

López et al. (2011) reported that the use of commercial LAB offers less risk compared to spontaneous MLF, and also positively affects the sensory profile of the wine, thereby increasing the complexity of the wine. The use of yeast strains and LAB that adsorb fewer phenolics onto their cell walls, compared to yeasts/bacteria that adsorb more phenolics, may also be beneficial for red wine colour (Morata et al., 2003). Certain yeasts may also improve the wine’s body, mouthfeel, complexity, structure and fullness by releasing polysaccharides and producing glycerol (Domizio et al., 2014; Belda et al., 2016).

### 4. Conclusions

The effect of mixed culture co-inoculations on the physicochemical characteristics, phenolics and sensory attributes of Syrah wines was investigated. Mixed culture co-inoculations strategies of Syrah grape must with non-*Saccharomyces*, *Saccharomyces* and LAB resulted in wines with reduced alcohol and ameliorated phenolics and sensory attributes, when compared to reference wines.

Mixed culture co-inoculations using *S. cerevisiae* cultures together with non-*Saccharomyces* and LAB present a practical way to improve the quality (preference) of Syrah wines. No negative effects of mixed culture co-inoculated MLF on the vinification process of the wines were found. Furthermore, the results indicate a technological advantage in applying this protocol for phenolic acids, flavan-3-ols, flavonols and malvidin-3-O-glucoside with increased concentration in the wines other

### Table 5

Average percentage scores of sensory attributes of wines obtained by alcoholic and malolactic co-inoculated fermentations using mixed cultures of *Saccharomyces* (Sc1), non-*Saccharomyces* and lactic acid bacteria.

| Sensory attributes | Reference (S. cerevisiae) | S. cerevisiae/MLF | S. cerevisiae/co-inoculations/MLF |
|--------------------|--------------------------|-------------------|----------------------------------|
|                    | Sc1                      | Sc1 + LAB1        | Sc1 + LAB2                       | Sc2 + LAB1 | Sc2 + LAB2 |
|                    |                          |                   |                                 | Mp1 + Sc1 + LAB1 | Mp1 + Sc1 + LAB2 |
|                    |                          |                   |                                 | Hu3 + Sc1 + LAB1 | Hu3 + Sc1 + LAB2 |
| Acidity            | 53.9 ± 1.22**            | 47.2 ± 2.23c      | 47.3 ± 2.11c                     | 48.4 ± 0.97b    | 48.5 ± 0.99b    | 48.4 ± 1.12c    | 48.6 ± 2.01b    |
| Mouthfeel          | 45.2 ± 0.89c             | 47.4 ± 1.44b      | 46.8 ± 0.98b                     | 52.1 ± 1.09a    | 51.5 ± 0.78a    | 51.7 ± 1.01a    | 51.3 ± 1.45a    |
| Astringency        | 42.1 ± 1.11a             | 44.9 ± 1.89a      | 43.7 ± 0.99a                     | 40.3 ± 1.19c    | 40.4 ± 1.45c    | 39.6 ± 2.02c    | 39.8 ± 2.02c    |
| Bitterness         | 34.7 ± 2.34ba            | 38.2 ± 2.11a      | 37.9 ± 1.12a                     | 30.1 ± 2.01c    | 29.1 ± 1.89c    | 29.7 ± 2.01c    | 29.0 ± 1.44c    |
| Preference (quality) | 51.0 ± 1.21c            | 53.9 ± 2.32b      | 53.0 ± 1.09b                     | 55.9 ± 1.11a    | 55.6 ± 0.98a    | 56.9 ± 11.0a    | 55.8 ± 2.34a    |

* Different letters in the same row indicate significant differences in the content of the measured compounds among the different treatments according to Fischer’s least significant difference test (p < 0.05).

** Standard deviation.

1 *Saccharomyces cerevisiae* (Sc1, [VIN13], reference wine).

2 LAB1: Oenococcus oeni.

3 LAB2: Lactobacillus plantarum.

4 Metschnikowia pulcherrima.

5 Hanseniaspora uvarum.
Table 6

| Sensory attributes | Reference (S. cerevisiae) | S. cerevisiae/MLF | S. cerevisiae/co-inoculations/MLF |
|--------------------|-------------------------|-------------------|----------------------------------|
|                    | Sc1                     | Sc2 + LAB1        | Sc2 + LAB2                       |
| Acidity            | 50.8 ± 2.01**           | 47.0 ± 3.11b      | 48.3 ± 1.98b                     |
|                    |                         | 46.0 ± 2.22c      | 46.1 ± 2.88c                     |
|                    |                         | 47.4 ± 1.99c      | 47.5 ± 2.48b                     |
| Mouthfeel          | 50.0 ± 1.22c            | 52.7 ± 0.98b      | 52.5 ± 2.02b                     |
|                    |                         | 55.0 ± 2.09b      | 55.3 ± 2.48b                     |
|                    |                         | 55.7 ± 2.22a      | 55.3 ± 1.89a                     |
| Astringency        | 42.0 ± 1.89a            | 41.7 ± 1.44a      | 41.2 ± 2.44a                     |
|                    |                         | 38.0 ± 1.89b      | 37.3 ± 1.22b                     |
|                    |                         | 37.4 ± 2.08b      | 37.1 ± 1.79b                     |
| Bitterness         | 27.9 ± 2.03b            | 27.4 ± 1.09b      | 27.9 ± 1.88b                     |
|                    |                         | 31.6 ± 1.22a      | 31.8 ± 2.01a                     |
|                    |                         | 32.9 ± 2.23a      | 32.8 ± 2.77a                     |
| Preference (quality) | 52.0 ± 2.45d          | 54.1 ± 2.33c      | 56.2 ± 1.99c                     |
|                    |                         | 61.5 ± 2.11b      | 60.5 ± 2.11b                     |
|                    |                         | 64.0 ± 1.34a      | 63.6 ± 1.99a                     |

* Different letters in the same row indicate significant differences in the content of the measured compounds among the different treatments according to Fischer’s least significant difference test (p < 0.05).

** Standard deviation.

1 Sc2 cerevisiae (Sc2, [NT202], reference wine).
2 LAB1: Oenococcus oeni.
3 LAB2: Lactobacillus plantarum.
4 Metschnikowia pulcherrima.
5 Hanseniaspora uvarum.

than reference wines, but is dependent on the yeast and LAB strains used. The results also suggest that mixed culture co-inoculations are feasible strategies to consider for Syrah wines in comparison to AF, but success is subject to the selection of the yeast/LAB combination. The use of other red grape cultivars may have a different outcome using the identified yeast/LAB combination strategy. The interactions between different yeasts and LAB during fermentation and the modalities of inoculation are complex and therefore need further investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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