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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 SO_2 and pH (Du

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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-fermentations with *Hanseniaspora vineae*. Domizio, Liu, Bisson, and Barile (2014) reported that mixed fermentations of non-*Saccharomyces* yeasts in complex metabolic 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, astringency, bitterness, body, mouthfeel, fullness, complexity and structure (Rodriguez-Montealegre, Romero-Peces, Chacón-Vozmediano, Martinez-Gascueña, & García-Romero, 2006). Viana and co-workers (2011) found that mixed fermentations of *S. cerevisiae* and *Hanseniaspora uvarum* (anamorph: *Kloeckera 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, Dellacassa, 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 monocarboxylic 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, Merín, Morata, & Farías, 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* (Viniflora® 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 (IWBT, Stellenbosch University, Stellenbosch). The YAN was 133.0 mg/L which was considered insufficient. Therefore, a standard addition of 50 g/hL diammonium hydrogen phosphate (DAP) was added to the juice (Minnaar, Ntushelo, Ngqumba, Van Breda, and 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 pulcherrima and *H. uvarum* were inoculated as wet cultures on day 0 at a concentration of 8.4×10^5 and 6.4×10^5 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 (IWBT, 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 panellists 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 panellist 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 physiochemical 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. pulcherrima* at day 0 were below 1×10^6 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. pulcherrima* 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 > 1×10^6 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. pulcherrima,* 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 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, Ríos-Reina, Ubeda, and Paneque (2019) reported increased alcohol in wines made with *S. cerevisiae* as compared to co-inoculated ferments. 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/

Table 1
Physicochemical characteristics of wines obtained by alcoholic and malolactic of co-inoculated fermentations using mixed cultures of *Saccharomyces* (Sc1), non-*Saccharomyces* and lactic acid bacteria.

Physicochemical characteristics	Syrah must	Reference (*S. cerevisiae*)	*S. cerevisiae*/MLF		*S. cerevisiae*/co-inoculations/MLF			
		Sc1[1]	Sc1 + LAB1[2]	Sc1 + LAB2[3]	Mp[4] + Sc1 + LAB1	Mp + Sc1 + LAB2	Hu[5] + 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
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	3.81 ± 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
L-malic acid (g/L)	3.1	2.02 ± 0.11a	0.21 ± 0.02c	0.22 ± 0.05c	0.26 ± 0.03b	0.24 ± 0.07b	0.28 ± 0.03b	0.27 ± 0.04b
Volatile acidity (g/L)	0.44	0.31 ± 0.03a	0.27 ± 0.02a	0.33 ± 0.01a	0.31 ± 0.03a	0.29 ± 0.03a	0.25 ± 0.02a	0.32 ± 0.01a
°Brix	23.0	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	< 0.2	< 0.2	< 0.2	< 0.2

N A: Not applicable.
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 ($p \leq 0.05$).
** Standard deviation.
[1] Saccharomyces cerevisiae (Sc1 [VIN13], reference).
[2] LAB1: Oenococcus oeni.
[3] LAB2: Lactobacillus plantarum.
[4] Metschnikowia pulcherrima
[5] Hanseniaspora uvarum

Sc2) had an average L-malic acid content of 1.76 g/L. Hranilovic et al. (2017) reported decreased L-malic acid concentrations in mixed co-inoculation fermentations in comparison to *S. cerevisiae* fermentations.

Volatile acidity and pH were not significantly different among any of the fermentations (Sc1/Sc2), including reference fermentations. This agrees with work by Puertas et al. (2018) where Chardonnay must was co-inoculated with *S. cerevisiae/T. delbrucekii.* All wines including reference wines (Sc1/Sc2) fermented to dryness with RS < 0.2 g/L.

3.4. Phenolic compounds

3.4.1. Phenolic acids

Mixed culture co-inoculations (Sc1) 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 2
Physicochemical characteristics of wines obtained by alcoholic and malolactic of co-inoculated fermentations using mixed cultures of *Saccharomyces* (Sc2), non-*Saccharomyces* and lactic acid bacteria.

Physicochemical characteristics	Syrah must	Reference (*S. cerevisiae*)	*S. cerevisiae*/MLF		*S. cerevisiae*/co-inoculations/MLF			
		Sc2[1]	Sc2 + LAB1[2]	Sc2 + LAB2[3]	Mp[4] + Sc2 + LAB1	Mp + Sc2 + LAB2	Hu[5] + 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
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
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
L-malic acid (g/L)	3.1	1.51 ± 0.05a	0.22 ± 0.03c	0.21 ± 0.05c	0.28 ± 0.08b	0.27 ± 0.06b	0.24 ± 0.06b	0.25 ± 0.07b
Volatile acidity (g/L)	0.44	0.39 ± 0.02a	0.41 ± 0.01a	0.39 ± 0.02a	0.38 ± 0.01a	0.40 ± 0.01a	0.43 ± 0.02a	0.39 ± 0.02a
°Brix	23.0	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	< 0.2	< 0.2	< 0.2	< 0.2

N A: Not applicable.
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 ($p \leq 0.05$).
** Standard deviation.
[1] Saccharomyces cerevisiae (Sc2 [NT202], reference).
[2] LAB1: Oenococcus oeni.
[3] LAB2: Lactobacillus plantarum.
[4] Metschnikowia pulcherrima.
[5] Hanseniaspora uvarum.

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[1]	Sc1 + LAB1[2]	Sc1 + LAB2[3]	Mp[4] + Sc1 + LAB1	Mp + Sc1 + LAB2	Hu[5] + Sc1 + LAB1	Hu + Sc1 + LAB2
Gallic acid (THBA[6])	2.45* ± 1.44c**	2.45 ± 1.05c	2.34 ± 1.23c	2.67 ± 3.23b	2.67 ± 1.64b	2.83 ± 2.15a	2.89 ± 1.26a
Caffeic acid (HCA[7])	17.84 ± 1.12d	27.80 ± 2.25b	27.94 ± 1.89b	28.60 ± 2.88b	28.14 ± 1.46b	31.09 ± 0.15a	32.46 ± 1.06a
p-Coumaric acid (HCA[7])	22.86 ± 2.97c	21.69 ± 2.12c	21.03 ± 0.59c	26.31 ± 4.24b	25.06 ± 2.31b	34.62 ± 2.38a	35.37 ± 1.69a
Chlorogenic acid (ester of HCA[7], two isomers)	18.49 ± 1.52d	32.64 ± 0.15b	32.34 ± 2.95b	25.93 ± 0.72c	25.90 ± 2.84c	36.04 ± 0.38a	37.54 ± 4.55a
Phenolic acids	61.65c	84.59b	83.68b	83.52b	83.79b	104.60a	108.27a
Epigallocatechin 3-*O*-gallate (ester of EGC[8])	1.29 ± 1.67c	1.29 ± 1.05c	1.33 ± 2.42c	1.57 ± 1.58b	1.45 ± 1.98b	1.79 ± 2.17a	1.87 ± 0.11a
(+)-Catechin	6.86 ± 0.38a	6.94 ± 0.55a	6.77 ± 0.19a	5.62 ± 0.47c	5.49 ± 1.53c	6.34 ± 1.37b	6.12 ± 0.62b
Flavan-3-ols	8.15a	8.23a	8.10a	7.19b	6.95b	8.13a	8.00a
Quercetin 3-*O*-rutinoside (rutin)	2.94 ± 1.76c	3.34 ± 0.17b	3.53 ± 0.30b	3.52 ± 0.11b	3.72 ± 0.80b	4.75 ± 0.42a	4.72 ± 0.49a
Quercetin 3-*O*-glucoside (isoquercetin)	1.26 ± 2.68c	1.35 ± 1.01b	1.35 ± 1.97b	1.47 ± 1.29b	1.41 ± 3.71b	1.81 ± 1.21a	1.62 ± 0.93a
Quercetin	2.60 ± 3.93a	2.56 ± 4.38a	2.50 ± 2.92a	1.74 ± 2.39b	1.72 ± 1.95b	1.78 ± 1.19b	1.76 ± 2.17b
Flavonols	6.80c	7.26b	7.39b	6.74c	6.86c	8.34a	8.12a
Malvidin-3-*O*-glucoside (anthocyanin)	63.49 ± 9.71b	65.53 ± 2.97b	65.21 ± 1.46b	82.31 ± 4.04a	81.55 ± 6.37a	67.35 ± 4.62b	67.33 ± 5.94b
Total phenolics	140.11d	165.63c	164.39c	179.77b	178.17b	188.43a	191.74a

* 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 ($p \leq 0.05$).

** 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.

Phenolic acids quantified in the wines represent *ca.* 50% of the total phenolics in the different fermentations, including reference fermentations. Hernández et al. (2007), Chescheir et al. (2015) and Minnaar et al. (2017) reported increased concentrations of phenolic acids after MLF in Tempranillo, Pinot noir and Syrah wines respectively, in sequential inoculations of the grape musts using *Saccharomyces* and non-*Saccharomyces* yeast after MLF. These increased concentrations of phenolic acids indicate that there may have been other sources of phenolic acids in grapes, which most likely originated through the hydrolysis of cinnamoyl-glucoside anthocyanins or from other hydroxycinnamic derivatives by the LAB's enzymatic activity (Hernández et al., 2007). Work by Boido, Lloret, Medina, Carrau, and Dellacassa (2002) reported changes in the glycoside content of Tannat wines during MLF. Medina et al. (2018) describe LAB as a potential source of glycosidic activity.

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 isoquercetin, 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 isoquercetin. *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

Table 4
Average concentrations of phenolic compounds (mg/L) of wines obtained by alcoholic and malolactic co-inoculated fermentations using mixed cultures of *Saccharomyces* (Sc2), non-*Saccharomyces* and lactic acid bacteria.

Phenolics	Reference (*S. cerevisiae*)	*S. cerevisiae*/MLF		*S. cerevisiae*/co-inoculations/MLF			
	Sc2[1]	Sc2 + LAB1[2]	Sc2 + LAB2[3]	Mp[4] + Sc2 + LAB1	Mp + Sc2 + LAB2	Hu[5] + Sc2 + LAB1	Hu + Sc2 + LAB2
Gallic acid (THBA[6])	1.99* ± 2.27b**	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[7])	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[7])	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
Chlorogenic acid (ester of HCA[7], two isomers)	21.68 ± 1.67c	26.43 ± 0.87b	26.93 ± 2.26b	35.33 ± 0.46a	34.34 ± 2.13a	36.38 ± 1.36a	36.70 ± 0.31a
Phenolic acids	73.21c	82.55b	80.59b	95.16a	93.86a	97.92a	99.86a
Epigallocatechin 3-*O*-gallate (ester of EGC[8])	0.85 ± 4.60d	1.47 ± 2.62c	1.43 ± 0.24c	1.63 ± 0.70b	1.62 ± 0.71b	2.38 ± 0.45a	2.58 ± 0.56a
(+)-Catechin	4.97 ± 0.45d	4.78 ± 0.16d	4.55 ± 0.62d	5.59 ± 0.33b	5.55 ± 0.48b	6.20 ± 0.17a	6.04 ± 0.45a
Flavan-3-ols	5.82d	6.24c	5.98c	7.22b	7.17b	8.59a	8.62a
Quercetin 3-*O*-rutinoside (rutin)	3.83 ± 0.83b	4.47 ± 0.47a	4.50 ± 0.56a	3.32 ± 0.21c	3.24 ± 0.37c	2.50 ± 0.26d	2.44 ± 0.19d
Quercetin 3-*O*-glucoside (isoquercetin)	1.20 ± 1.77b	1.39 ± 1.83a	1.38 ± 2.10a	1.31 ± 0.63a	1.51 ± 1.53a	1.21 ± 1.68b	1.24 ± 2.13b
Quercetin	2.39 ± 1.28b	2.36 ± 4.32b	2.36 ± 2.57b	1.95 ± 1.02c	2.03 ± 2.14c	2.96 ± 2.92a	2.71 ± 2.07a
Flavonols	7.43b	8.23a	8.25a	6.59c	6.79c	6.68c	6.40c
Malvidin-3-*O*-glucoside (anthocyanin)	51.63 ± 2.68c	56.05 ± 2.97b	57.21 ± 1.46b	81.66 ± 4.04a	82.11 ± 6.37a	78.35 ± 4.62a	77.33 ± 5.94a
Total phenolics	138.10c	153.07b	152.04b	190.65a	189.94a	191.55a	192.24a

* 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 ($p \leq 0.05$).

** 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.

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* (*St. bacillaris*)/*S. cerevisiae*, compared to *S. cerevisiae* wines. Grape must was however sequentially inoculated. Izquierdo-Cañas, Garcia-Romero, Mena-Morales, and Gómez-Alonso (2016) found decreased levels of quercetin 3-*O*-glucoside, quercetin 3-*O*-rutinoside and quercetin in Petit Verdot wines made with *S. cerevisiae* after MLF (*O. oeni*), compared to *S. cerevisiae* wines without MLF. This is in contrast to result of this paper. Quercetin concentrations in *S. cerevisiae* wines after MLF increased.

Total flavonols in mixed culture co-inoculations of *M. pulcherrima* (Sc1) after MLF and reference fermentations were both 6.80 mg/L in comparison to 7.33 mg/L in *S. cerevisiae* wines after MLF and 8.23 mg/L in mixed culture co-inoculations of *H. uvarum* 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. Tristezza 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.

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, Martinez, and Ayestaran (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*.

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 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 (2015) and result reported in this paper; Abrahamse and Bartowsky (2012) reported decreased levels of total phenolics in *S. cerevisiae* Syrah 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 Briffox (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[1]	Sc1 + LAB1[2]	Sc1 + LAB2[3]	Mp[4] + Sc1 + LAB1	Mp + Sc1 + LAB2	Hu[5] + Sc1 + LAB1	Hu + Sc1 + LAB2
Acidity	53.9* ± 1.22a**	47.2 ± 2.23c	47.3 ± 2.11c	48.4 ± 0.97b	48.5 ± 0.99b	48.4 ± 1.12b	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 ± 1.11ba	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
Average percentage scores of sensory attributes of wines obtained by alcoholic and malolactic co-inoculated fermentations using mixed cultures of *Saccharomyces* (Sc2), non-*Saccharomyces* and lactic acid bacteria.

Sensory attributes	Reference (*S. cerevisiae*)	*S. cerevisiae*/MLF		*S. cerevisiae*/co-inoculations/MLF			
	Sc2[1]	Sc2 + LAB1[2]	Sc2 + LAB2[3]	Mp[4] + Sc2 + LAB1	Mp + Sc2 + LAB2	Hu[5] + Sc2 + LAB1	Hu + Sc2 + LAB2
Acidity	50.8* ± 2.01a**	47.0 ± 2.11b	48.3 ± 1.98b	46.0 ± 2.22c	46.1 ± 2.88c	47.4 ± 1.99c	47.5 ± 2.44b
Mouthfeel	50.0 ± 1.23c	52.7 ± 0.98b	52.5 ± 2.02b	55.0 ± 2.09a	55.3 ± 2.43a	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] Saccharomyces 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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