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FINAL REPORT
(2016/17)

1. PROGRAMME AND PROJECT LEADER INFORMATION

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2. PROJECT INFORMATION

Research Organisation Project number	WW10-23
Project title	Effect of non-*Saccharomyces* yeast and lactic acid bacteria interactions on malolactic fermentation and wine flavour
Short title	Effect of non-*Saccharomyces* yeast on MLF

Fruit kind(s)	Wine grapes		
Start date (mm/yyyy)	04/2012	**End date** (mm/yyyy)	03/2016

Key words	Chemical analysis, lactic acid bacteria, malolactic fermentation, sensory, yeast, interactions

Approved by Research Organisation Programme leader (tick box) X

3. EXECUTIVE SUMMARY

Objectives & Rationale

The successful induction and completion of malolactic fermentation (MLF) is an on-going problem worldwide, despite the considerable body of knowledge available concerning wine properties, factors affecting lactic acid bacteria (LAB) and developments in strain selection and starter cultures. To date the interaction between various non-*Saccharomyces* yeasts and LAB has received little attention and less is known about the resulting impact on wine flavour. The interaction between commercial *Saccharomyces* yeast, non-*Saccharomyces* reference yeasts and lactic acid bacteria (LAB), and subsequent effects on malolactic fermentation (MLF) and wine flavour were investigated.

Methods

Five *Saccharomyces* and 37 non-*Saccharomyces* yeast strains, which included seven different species, were evaluated in a synthetic grape juice (SJ) medium to determine yeast and LAB compatibility. Selected yeast and lactic acid bacteria strains were also evaluated in small-scale (20 L) Chardonnay, Merlot and Shiraz wine production trials using a standardised protocol. Standard chemical parameters were measured using a WineScan™ FT120 instrument or an OenoFoss™ wine analyser. The major volatile compounds commonly found in wine were determined, using a gas chromatograph coupled to a flame ionization detector (GC-FID). Wines were also subjected to sensory evaluations. All the collected wine data were subjected to statistical analyses (e.g. analysis of variance, principle component analyses, etc.).

Key Results

Most of the non-*Saccharomyces* yeasts evaluated were compatible with LAB and did not inhibit LAB growth, but there were exceptions. LAB inhibition was mainly due to competition for nutrients or production of toxic metabolites by the yeast strains. Wines produced with non-*Saccharomyces* yeasts contained lower alcohol levels and completed MLF in shorter periods than wines produced with *S. cerevisiae* strains only. Wines that underwent simultaneous MLF completed faster than wines that underwent sequential MLF. Non-*Saccharomyces* yeasts in combination different *S. cerevisiae* strains and MLF strategies (no MLF, simultaneous or sequential MLF) produced wines with significantly different chemical composition and sensory properties. Differences in volatile composition did not always translate to perceivable sensory differences. The use of non-*Saccharomyces* yeasts to improve wine flavour and quality is affected by *S. cerevisiae* yeast strain and also grape cultivar. Wines that underwent sequential MLF had more flavour than wines that underwent simultaneous MLF, but this trend varied between cultivars and was affected by yeast strain used. Shiraz wines produced with non-*Saccharomyces* yeasts in combination with *S. cerevisiae* and LAB had more colour than wines produced *S. cerevisiae* only.

Conclusion/Discussion

Commercial and non-commercial non-*Saccharomyces* yeast strains had a beneficial impact on the completion of MLF, which include induced MLF, using commercial cultures, and spontaneous MLF. The use of the suitable non-*Saccharomyces* yeast strains may help to reduce alcohol concentrations, while enhancing wine complexity, which could be used as a unique marketing strategy. The use of non-*Saccharomyces* yeasts and different MLF strategies gives winemakers the tools to produce wines of differing styles and flavour profiles. The benefits of finding a suitable non-*Saccharomyces*, *Saccharomyces* yeast and LAB combinations are faster completion of MLF, enhanced wine complexity and even improved colour. However, if commercial yeast and LAB cultures are used, it may increase wine production costs and winemakers will have to decide whether the benefits of using the aforementioned cultures justify the potential increase in production costs.

4. PROBLEM IDENTIFICATION AND OBJECTIVES

The successful induction and completion of malolactic fermentation (MLF) is an on-going problem worldwide, despite the considerable body of knowledge available concerning wine properties, factors affecting lactic acid bacteria (LAB) and developments in strain selection and starter cultures. The presence of non-*Saccharomyces* yeasts (natural and inoculated) in wine production constitutes another unknown factor for MLF. To date the interaction between various non-*Saccharomyces* yeasts and LAB has received little attention and less is known about the resulting impact on wine flavour. The objective of this project was to investigate the interactions between dominant natural and commercial non-*Saccharomyces* reference yeasts and LAB, and subsequent effects these interactions have on MLF and wine flavour.

5. DETAILED REPORT

a. PERFORMANCE CHART (for the duration of the project)

Milestone	Target Date	Extension Date	Date completed
1. Small-scale wine production trials 2012	March 2012		April 2012
2. Sensory and chemical analyses of 2012 wines	Sep. 2012		Oct. 2012
3. Statistical analyses of 2012 wines	December 2012		Feb. 2013
4. Laboratory scale fermentation trials 2012	July 2012		Feb. 2013
5. Small-scale wine production trials 2013	March 2013		April 2013
6. Chemical and sensory analyses of 2013 wines	Sep. 2013		Feb. 2014
7. Statistical analyses of 2013 data	Oct. 2013		March 2014
8. Laboratory scale fermentation trial 2013	Not part of initial planning*		March 2014
9. Small-scale wine production trials: 2014	March 2014		April 2014
10. Chemical and sensory analyses of 2013 wines (12 months in bottle) and 2012 wines (24 months in bottle)	June 2014		Sep. 2014
11. Chemical and sensory analyses of 2014 wines	Oct. 2014		Oct. 2014
12. Statistical analyses of 2012, 2013 and 2014 chemical and sensory data	Nov. 2014		July 2015
13. Chemical and sensory analyses of 2013 wines (24 months in bottle) and 2014 wines (12 months in bottle)	May 2015	Aug. 2015	Sept. 2015
14. Statistical analyses of data	June 2015	Nov. 2015	Dec. 2015
15. Consumer preference testing of selected wines	Not part of initial planning*	Sept 2015	June 2016
16. Final report	July 2015	May 2017	May 2017
17. Journal publication(s) – final milestone Scientific publications: 3 published;	June 2016		Nov. 2017

3 in progress Popular publications: 1 published; 3 in progress	June 2018 April 2017 June 2018		 Dec. 2017

*Additional milestones added after consultation with Winetech committee.

b) WORKPLAN (MATERIALS AND METHODS)

Yeast and lactic bacteria compatibility

Five *Saccharomyces* and 37 non-*Saccharomyces* yeast strains, which included seven different species (*Candida stellata* (1), *C. zemplinina* (7), *Hanseniaspora uvarum* (11), *Lachancea thermotolerans* (2), *Metschnikowia pulcherrima* (7), *Schizosaccharomyces pombe* (1), *Torulaspora delbrueckii* (8) were evaluated in a synthetic grape juice (SJ) medium described by Costello *et al*. (2003). The detailed materials and methods are discussed in Addendum 1.

Small-scale (20 L) wine production trials

Selected yeast and lactic acid bacteria strains were used in small-scale (20 L) wine production trials using a standardised protocol. Three MLF strategies were applied for wine production: (1) yeast without MLF, (2) yeast with simultaneous MLF (sim MLF) and (3) yeast with sequential MLF (seq MLF). In the treatment where non-*Saccharomyces* yeasts were used, *S. cerevisiae* was only inoculated after 24 hours, whereas the reference/control treatments (*S. cerevisiae* only) were inoculated on day 0. In the case of sim MLF, LAB were inoculated after 24 hours and for seq MLF the LAB were inoculated at the end of alcoholic fermentation. All fermentations were performed in triplicate. Sugar concentration, pH, malic and lactic acid, total acidity (TA), alcohol and volatile acidity (VA) was determined on the wines using a WineScan™ FT120 instrument (Institute for Wine Biotechnology, Stellenbosch University) or an OenoFoss™ wine analyser. Glycerol concentrations were was determined on the wines using a WineScan™. The concentration of 32 major volatile compounds commonly found in wine was determined, using a gas chromatograph coupled to a flame ionization detector (GC-FID). Wines were sensorially evaluated by descriptive evaluations during the annual experimental wine evaluations at Nietvoorbij. The panels consisted of commercial winemakers and Nietvoorbij staff. A ten centimetre unstructured line scale was used and the tasters were asked to score wines for the different aroma and taste descriptors, as well as overall quality. Standard wine tasting glasses were used and the wines were coded and presented in random order. Chemical and sensory data were tested for normality by the Shapiro-Wilk test then subjected to analysis of variance (ANOVA) using the general linear means procedure of SAS 9.2 (SAS Institute Inc. Institute Inc., 2008). Student's *t*-least significant difference (LSD) values were calculated at the 5% probability level (p = 0.05) to facilitate comparison between treatment means.

In 2012, Chardonnay (36) and Shiraz (72) wines were produced and the micro-organisms used are listed in Table 1. Nine yeast strains were used in combination with two fermentation strategies, i.e. (1) yeast fermentation (non-*Saccharomyces* yeast and/or VIN 13) without MLF and (2) non-*Saccharomyces* yeast + VIN 13 + simultaneous MLF, for Chardonnay production. In total, 18 different treatments were applied triplicate. Chardonnay wines were evaluate by a panel of 10 judges, four months after bottling.

Eight yeast strains were used in combination with three fermentation strategies, i.e. (1) yeast fermentation (non-*Saccharomyces* yeast and/or VIN 13) without MLF, (2) non-*Saccharomyces* yeast + VIN 13 + simultaneous MLF and (3) non-*Saccharomyces* yeast + VIN 13 + sequential MLF, for Shiraz production. In total, 24 treatments were applied in triplicate. Detailed materials and methods for Shiraz production are discussed in Addendum 2. Five non-commercial and two commercial non-*Saccharomyces* yeast strains representing five different species were included in this trial based on their performance in previous experiments (Addendum 1). Viniflora oenos was used as a MLF culture because it is a good general MLF culture and is also more sensitive to yeast inhibition than some of the other MLF cultures.

Table 1. Micro-organisms used during 2012 Chardonnay and Shiraz wine production trials.

Reference code	Species name	Origin
Sc	*Saccharomyces cerevisiae*	VIN 13, commercial strain from Anchor Wine Yeast, South Africa
Hu	*Hanseniaspora uvarum*	Y0858, Natural isolate from Rawsonville area, South Africa
Mp	*Metschnikowia pulcherrima*	Y0839, Natural isolate from Constantia area, South Africa
Cz	*Candida zemplinina*	Y1020, Natural isolate from Constantia area
T3	*Torulaspora delbrueckii*	Level2 ^{TD}, commercial strain from Lallemand Inc., France
T6	*Torulaspora delbrueckii*	Mp/1, Natural isolate from Robertson, South Africa
T7	*Torulaspora delbrueckii*	654, Natural isolate from Stellenbosch, South Africa
L1	*Lanchancea thermotolerans*	Rhythm, commercial strain from Chr. Hansen, Denmark
L2	*Lanchancea thermotolerans*	Y0560, Natural isolate from Robertson
Viniflora oenos	*Oenococcus oeni*	Commercial malolactic fermentation starter from Chr. Hansen

The micro-organisms used in fermentation trials in 2013 and 2014 are listed in Table 2. In 2013, only Shiraz (90) wines were produced, but in 2014, Merlot (45) and Shiraz (90) wines were produced, with the Shiraz trial being a repeat of the 2013 trial. 2013-Shiraz wines were evaluate by a panel of 26 judges, 16 months after bottling. For the Merlot trial, only one *S. cerevisiae* (VIN 13) strain was used and the same non-*Saccharomyces* yeast and MLF strategies were followed as for Shiraz 2013 and 2014 wine production. Detailed materials and methods are listed in Addendum 3. Selected phenolic compounds were quantified in 2014-Shiraz wines using reversed-phase high performance liquid chromatography-photodiode array detection (RP-HPLC-DAD) technique (Addendum 4). Sensory (descriptive evaluation) and physico-chemical / oenological parameters (Winescan® and OenoFoss™) results were compared to phenolic compound concentrations.

Table 2. Micro-organisms used during 2013 and 2014 Shiraz wine production trials.

Reference code	Species name	Origin
Sc1	*Saccharomyces cerevisiae*	VIN 13, commercial strain from Anchor Wine Yeast
Sc2	*Saccharomyces cerevisiae*	NT202, commercial strain from Anchor Wine Yeast
Hu	*Hanseniaspora uvarum*	Natural isolate from Rawsonville area
Mp	*Metschnikowia pulcherrima*	Natural isolate from Constantia area
oeni	*Oenococcus oeni*	Viniflora oenos, commercial malolactic fermentation starter from Chr. Hansen
plan	*Lactobacillus plantarum*	Enoferm V22, commercial malolactic fermentation starter from Lallemand Inc.

c) RESULTS AND DISCUSSION

Yeast and lactic bacteria compatibility

Thirty-seven non-*Saccharomyces* yeast strains, which included seven different species, were evaluated in a chemically defined medium to investigate their fermentation activity and compatibility with a commercial *Oenococcus oeni* strain (Addendum 1).

Candida stellata, *C. zemplinina*, *H. uvarum*, *M. pulcherrima* and *Sc. pombe* strains were shown to be slow to medium fermenters, whereas *L. thermotolerans* and *T. delbrueckii* strains were found to be medium to strong fermenters (Addendum 1). The slow to medium fermenting yeast species should only be used in mixed culture fermentations with *S. cerevisiae* or other strong fermenters, unless the aim is to produce wines that contain residual sugar. Some of *L. thermotolerans* and *T. delbrueckii* strains were comparable to the *S. cerevisiae* reference yeasts and could be used on their own or in combination with *S. cerevisiae*.

None of the non-*Saccharomyces* yeast produced volatile acidity (VA) levels that were above the legal limit of 1.2 g/L. The *Sc. pombe* strain that was used, degraded 77% of the malic acid of the synthetic wine, while the other non-*Saccharomyces* yeast strains varied with regard to their abilities to degrade malic acid (3 to 62% degradation). The duration of MLF in synthetic wine varied among the yeast strains used, but none of the yeasts completely inhibited MLF. Delays with regard to the progression of MLF appear to be strain dependent. In most cases delays in MLF could be alleviated by nutrient supplementation. However, in a few cases delays could not be alleviated, suggesting that the yeast strains produced toxic metabolites that affected LAB growth. SO_2 production was ruled out as a reason for the delays, but other toxic metabolites were not investigated. The metabolites produced by these inhibitory strains need further investigation.

Small-scale Chardonnay wine production trial

In the Chardonnay trial, eight non-*Saccharomyces* (*C. zemplinina, H. uvarum, M. pulcherrima, L. thermotolerans* (2), and *T. delbrueckii* (3)) yeast strains in combination with one *S. cerevisiae* strain (VIN 13) and two MLF strategies were evaluated, *i.e.* (1) yeast (non-*Saccharomyces* yeast and/or *S. cerevisiae*) without MLF and (2) yeast (non-*Saccharomyces* yeast and/or *S. cerevisiae*) with simultaneous MLF (Table 1).

None of the Chardonnay wines contained high residual sugar concentrations at the end of alcoholic fermentation (Table 3). The ethanol concentration of wines produced with non-*Saccharomyces* yeast was lower (0.1 to 0.8% $^{v}/_{v}$) than the *S. cerevisiae* (Sc) reference treatment, even though it was not significantly lower. Wines produced with T3 + Sc that did not undergo MLF contained the lowest alcohol levels (12.6% $^{v}/_{v}$). None of the treatments produced high volatile acidity levels (> 0.8 g/L). Malolactic fermentation was completed after 18 days in most treatments. The only exceptions were *H. uvarum* (Hu) +Sc + sim MLF (23 days) and *L. thermotolerans* L1 + Sc + sim MLF (54 days). This *L. thermotolerans* strain (L2) could have produced toxic metabolites or depleted essential nutrients necessary for LAB growth. Lower LAB counts were observed for the L1 + Sc + sim MLF treatment in comparison with the other treatments.

The sensory data of the Chardonnay wines showed that there were notable differences between the wines produced with the various yeast combinations. The wines varied significantly with regard to 'tropical fruit', 'tree fruit' and 'caramel' aroma. Of the treatments that did not undergo MLF, wines produced with commercial non-*Saccharomyces* yeast L1 + Sc scored the highest for 'tree fruit', 'tropical fruit' and 'caramel' aroma, but wines produced with *T. delbrueckii* T7 + Sc scored the highest for 'overall quality'. All the wines produced in combination with non-*Saccharomyces* yeasts were different from the *S. cerevisiae* reference wines, but most of them scored lower for overall quality.

Chardonnay wines fermented with the different yeasts that underwent simultaneous MLF were different to wines that did not undergo MLF. Wines produced with *M. pulcherrima* (Mp) + Sc + sim MLF scored the highest for 'tropical fruit' aroma, while wines produced with L2 + Sc + sim MLF scored the highest for 'tree fruit' aroma and wines produced with Hu + Sc scored the highest for 'caramel' aroma. Wines produced with T7 + Sc that underwent simultaneous MLF scored

significantly lower for 'overall quality' than the T7 + Sc wines that did not undergo MLF. Some yeast combinations benefitted from simultaneous MLF, whereas others did not. The findings reported in this trial are for non-*Saccharomyces* yeasts in combination with *S. cerevisiae* strain VIN 13 and *O. oeni* strain Viniflora oenos. Other *S. cerevisiae* strains or LAB combinations may give different results.

Table 3. Chemical analyses and duration of simultaneous malolactic fermentation (sim MLF) of 2012-Chardonnay wines produced with different yeast combinations* at the end of alcoholic fermentation.

Treatment	Gluc/Fruc (g/L)	Total acids (g/L)	pH	Ethanol (% v/v)	Volatile acidity (g/L)	Malic acid (g/L)	MLF duration (days)
Sc	0.9a	6.26a	3.47b	**13.4a**	0.34b	1.9a	No MLF
Sc + sim MLF	1.7a	5.51c	3.59a	13.0a	0.40ab	0.0b	**18**
Hu+ Sc	1.4a	6.31a	3.49b	13.2a	0.40ab	2.1a	No MLF
Hu + Sc + sim MLF	3.0a	5.80bc	3.59a	13.1a	0.41ab	0.1b	23
T3 + Sc	1.5a	6.37a	3.49b	**12.6a**	0.39ab	2.2a	No MLF
T3 + Sc + sim MLF	2.1a	5.51c	3.61a	12.9a	0.41ab	0.0b	**18**
Mp + Sc	1.0a	6.30a	3.49b	13.1a	0.39ab	2.1a	No MLF
Mp + Sc + sim MLF	2.3a	5.50c	3.60a	12.9a	0.39ab	0.0b	**18**
L1 + Sc	1.4a	6.20a	3.45b	13.2a	0.39ab	2.1a	No MLF
L1 + Sc + sim MLF	2.6a	5.63bc	3.59a	13.1a	0.35ab	0.1b	18
Cz + Sc	1.3a	6.34a	3.48b	13.1a	0.38ab	2.2a	No MLF
Cz + Sc + sim MLF	2.5a	5.56c	3.62a	12.8a	0.44a	0.0b	**18**
T6 + Sc	0.8a	6.28a	3.50b	13.1a	0.36ab	2.1a	No MLF
T6 + Sc + sim MLF	2.0a	5.53c	3.58a	12.9a	0.38ab	0.0b	**18**
T7 + Sc	2.7a	6.27a	3.48b	13.1a	0.33b	2.1a	No MLF
T7 + Sc + sim MLF	1.4a	5.98ab	3.60a	13.1a	0.33b	0.1b	**18**
L2 + Sc	0.9a	6.32a	3.46b	13.3a	0.39ab	2.1a	No MLF
L2 + Sc + sim MLF	0.8a	5.78bc	3.58a	13.2a	0.39ab	0.5b	**54**

*Sc: *Saccharomyces cerevisiae* strain VIN 13, Hu: *Hanseniaspora uvarum*, T3: *Torulaspora delbrueckii* T3, Mp: *Metschnikowia pulcherrima*, L1: *Lachancea thermotolerans* L1, and, Cz: *Candida zemplinina*, T6: *T. delbrueckii* T6, T7: *T. delbrueckii* T7 and L2: *L. thermotolerans* L2.

Small-scale Shiraz wine production trials

2012 trial

Seven non-*Saccharomyces* strains (*C. zemplinina*, *H. uvarum*, *M. pulcherrima*, *L. thermotolerans* (2), and *T. delbrueckii* (2)), in combination with *S. cerevisiae* (VIN 13) were investigated (Table 1). These yeasts in combination with three MLF strategies (no MLF, simultaneous and sequential MLF) were evaluated. Two of the non-*Saccharomyces* yeast strains, *C. zemplinina* Cz and *L. thermotolerans* L2 had an inhibitory effect on LAB growth, when yeast and LAB were inoculated simultaneously (Addendum 2). However, no inhibition was observed in Shiraz wines undergoing sequential MLF. All wines fermented to dryness and none of the treatments led to high volatile acidity production. The mixed culture fermentations using non-*Saccharomyces* yeasts had lower alcohol (0.1 to 0.5% $^{v}/_{v}$) and lower glycerol levels (0.1 to 0.45 g/L) than wines produced with *S. cerevisiae* (Sc) only. Wines produced with *C. zemplinina* in combination with *S. cerevisiae* that did not undergo MLF produced the lowest alcohol levels (15.5% $^{v}/_{v}$). Malolactic fermentation took less time (6 to 13 days) to complete in wines produced with non-*Saccharomyces* yeasts than in wines produced with only *S. cerevisiae*.

The yeast, LAB and even the time of MLF induction (simultaneous or sequential) had a significant effect on the volatile composition of the wines. Wines that underwent MLF and wines that did not undergo MLF were significantly different in terms of volatile composition and sensory properties. In this trial, MLF strategy had a greater effect on chemical composition and sensory properties than the yeast combination used. The significant differences observed in the volatile composition

of the wines did not always translate to significant sensory differences. Yeast treatment had a significant effect on 'berry aroma', while MLF strategy had a significant effect on 'berry aroma", acid balance and astringency. Wines produced with *C. zemplinina* in combination with *S. cerevisiae* had significantly more berry aroma than wines produced with *H. uvarum* Hu + Sc, *L. thermotolerans* L2 + Sc, *T. delbrueckii*, T3 + Sc and the *S. cerevisiae* reference treatment. Wines that underwent simultaneous MLF and wines that did not undergo MLF had significantly more 'berry aroma' than wines that underwent sequential MLF. Wines that underwent simultaneous MLF had a better acid balance than wines that underwent sequential MLF, but were also significantly more astringent.

This trial showed that the interactions between non-*Saccharomyces*, *Saccharomyces* yeasts and LAB improve wine flavour and quality, but certain yeast and LAB combinations produced better wines when used as a simultaneous inoculation, while other combinations performed better as a sequential inoculation. In general, wines produced with *M. pulcherrima* or the commercial *L. thermotolerans* strain L1 improved wine quality irrespective of when MLF was induced. It is therefore important to select non-*Saccharomyces, Saccharomyces* yeasts and LAB that are compatible to ensure fast MLF and enhance wine complexity.

2013 and 2014 trials

Based on results from previous trials and literature *H. uvarum* and *M. pulcherrima* were selected for small-scale Shiraz production trials because both these species had *β*-glucosidase activity (Addendum 1), which plays a role in releasing volatile compounds from non-volatile precursors can therefore enhance wine flavour. Both species are also frequently found on grapes and in juice. These non-*Saccharomyces* yeasts were used in combination with two commercial *S. cerevisiae* yeast strains (VIN 13 or NT 202) and two commercial MLF starter cultures (*O. oeni* and *Lactobacillus plantarum*) (Table 2). The aforementioned yeast combinations were used in combination with three MLF strategies (no MLF, simultaneous and sequential MLF). Ninety Shiraz wines (30 treatments in triplicate) were produced per season and the same treatments were investigated during 2013 and 2014-harvest seasons. Similar trends were observed for 2013 and 2014, and these trends were comparable to trends observed in 2012. All wines fermented to dryness and none of the treatments produced high VA levels (< 0.6 g/L), with the wines that did not undergo MLF containing the lowest VA levels (0.19 to 0.43 g/L). Wines produced with non-*Saccharomyces* yeasts in combination with *S. cerevisiae* contained lower alcohol (0.13 to 0.72% $^{v}/_{v}$) and glycerol (0.02 to 0.97 g/L) levels than wines produced with the reference *S. cerevisiae* yeasts only. Wines produced with *M. pulcherrima* in combination with any of the *S. cerevisiae* yeasts contained the lowest alcohol levels and were on average 0.4 to 0.5% $^{v}/_{v}$ lower than the *S. cerevisiae* only wines.

Similar trends with regard to MLF were observed for both vintages. Malolactic fermentation completed in a shorter period in wines produced with non-*Saccharomyces* yeasts in combination with *S. cerevisiae* than wines produced with *S. cerevisiae* only. Wines inoculated with *O. oeni* completed MLF in shorter period than wines inoculated with *Lb. plantarum* and this trend was observed for wines that underwent simultaneous and sequential MLF. Simultaneous MLF completed in a shorter time than sequential MLF. Wines produced with *S. cerevisiae* NT 202 and its combinations completed MLF in less time than wines produced with *S. cerevisiae* VIN 13 and its combinations.

Yeast treatment, MLF strategy and the interaction of yeast treatment and MLF strategy resulted in wines that were significantly different with respect to volatile chemical composition. Even using different LAB strains resulted in significant differences in the volatile composition of the Shiraz wines. Principle component analysis (PCA) of the volatile compounds showed that wines could be differentiated based on the yeast combination, but that vintage effect played a bigger role with regard to separation of the wines (Fig. 1). Within the different yeast clusters, MLF strategy and LAB strain also resulted in further variation.

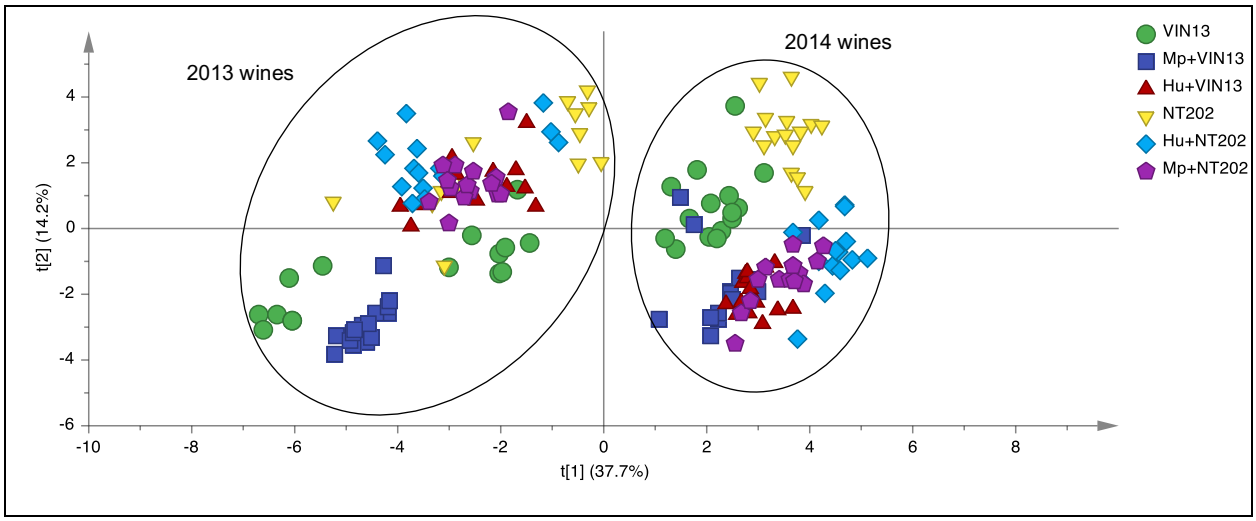

Figure 1. Principle component analysis (PCA) score plot of the volatile compounds of Shiraz wines produced with different yeast combinations and during two different vintages. Abbreviations: VIN13: *Saccharomyces cerevisiae* strain VIN 13, Mp: *Metschnikowia pulcherrima* Hu: *Hanseniaspora uvarum* and NT202: *S. cerevisiae* strain NT 202.

Yeast treatment had a significant effect on 'berry', fruity', 'sweet associated', 'spicy" and 'floral' aroma as well as acid balance, body/mouthfeel and overall quality. LAB strain and MLF strategy had a significant effect on acid balance, body/mouthfeel, bitterness and overall quality. The interaction between yeast, LAB strain and MLF strategy only had a significant effect on body/mouthfeel of the wines. Wines produced with non-*Saccharomyces* yeast scored higher for the various aroma descriptors and overall quality than wines produced with only *S. cerevisiae*. Wines produced with *Lb. plantarum* scored higher for most of the sensory descriptors than wines produced with *O. oeni*. Shiraz wines that underwent sequential MLF scored higher for most of the sensory descriptors than wines that underwent simultaneous MLF, but this trend varies between yeast combinations.

Selected anthocyanins, flavan-3-ols, flavonols and phenolic acids were only quantified for Shiraz wines produced during 2014. Phenolic compound concentrations increased in Shiraz wines made with non-*Saccharomyces* yeasts, in combination with *S. cerevisiae* and LAB. The use of non-*Saccharomyces* yeast and LAB in combination with *S. cerevisiae* had a beneficial effect on Shiraz wine colour.

Small-scale Merlot wine production trials

For the 2014-Merlot trial, the interaction between one commercial *S. cerevisiae* (VIN 13), two non-*Saccharomyces* (*H. uvarum* and *M. pulcherrima*) yeast and two LAB (*Lb. plantarum* and *O. oeni*) strains are described. Similar trends observed for Merlot wines that were observed for Shiraz wines. None of the treatments produced high VA levels, and wines produced with the non-*Saccharomyces* yeasts contained lower alcohol and glycerol levels than wines produced with *S. cerevisiae* only. Wines produced with non-*Saccharomyces* yeasts completed MLF faster than wines inoculated with *S. cerevisiae* only. Wines where *O. oeni* was used to induce MLF completed faster than wines inoculated with *Lb. plantarum*. Wines that underwent simultaneous MLF completed MLF in a shorter period than wines that underwent sequential MLF and this observed for both LAB strains.

As observed with Shiraz wines, Merlot wines produced with the various treatments resulted in wines that were significantly different from each other with respect to volatile chemical composition. Yeast treatment only had a significant effect on 'floral' aroma and overall quality of the Merlot wines, whereas LAB strain and MLF strategy had a significant effect on berry aroma, body/mouthfeel and overall quality. The interaction between yeast treatment, LAB strain and MLF strategy did not have a significant effect on any of the sensory descriptors. Wines produced with

S. cerevisiae only scored higher for most of the descriptors, but wines produced with *M. pulcherrima* in combination with *S. cerevisiae* scored higher for overall quality. Merlot wines that underwent sequential MLF scored higher for most of sensory descriptors, including overall quality than wines that underwent simultaneous MLF. Merlot wines produced with *Lb. plantarum* scored higher for most sensory descriptors and overall quality than wines produced with *O. oeni.* These trends were also observed for Shiraz wines.

d) CONCLUSIONS

This study showed that yeast strain selection is important with regard to rapid completion of MLF and the flavour profiles of wines. Simultaneous MLF is faster than sequential MLF. Most non-*Saccharomyces* yeasts were shown to have a beneficial effect on successful completion of MLF, but there are strains that have the opposite effect. The use of non-*Saccharomyces* yeasts might stimulate the occurrence of spontaneous MLF. The use of the suitable non-*Saccharomyces* yeast strains may help to reduce alcohol concentrations (0.4 – 0.5% $^{v}/_{v}$), while enhancing wine complexity. The use of non-*Saccharomyces* yeasts and different MLF strategies gives the winemakers the tools to produce wines with varying flavour profiles and to improve colour. Non-*Saccharomyces* yeasts (commercial strains or spontaneous fermentations) play an important role due to interactions with MLF cultures and the procedure followed, and will affect quality. Winemakers should make informed decisions about what strategy and cultures to use by reading research work like this and consulting with their local suppliers.

6. ACCUMULATED OUTPUTS

Students and personnel trained
Maxwell Mewa Ngongang (graduated BTech [2015] and MTech [2016])
Heinrich du Plessis (graduated PhD [March 2018])
Five DST-NRF interns received work experience and two Work Integrated Learning students obtained their diploma.

Publications
Two scientific papers were published in the *South African Journal of Enology and Viticulture* and a third paper was published in *Fermentation*.
Another three scientific papers still to be submitted for publication.
One popular paper published in Winelands and Winetech Technical and another three popular publications are in progress.

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

The data contributes to a better understanding and use of micro-organisms such as *Saccharomyces,* non-*Saccharomyces* yeast and LAB strains, as well as the impact of the interactions on MLF and wine flavour.

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

Results will be presented at local and international conferences, and results will also be published in popular magazines.

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

Student Name and Surname	Student Nationality	Degree (e.g. MSc Agric, MComm)	Level of studies in final year of project	Graduation date	Total cost to industry throughout the project
Honours students					
Maxwell Mewa Ngongang	Cameroonian	BTech		March 2015	R36,000
Masters Students					
Maxwell Mewa Ngongang	Cameroonian	MTech	MTech	Sept 2016	R70,000
PhD students					
Heinrich du Plessis		PhD	PhD	March. 2018	R534,516
Support Personnel (not a requirement for HORTGRO Science)					
Domecia Blaauw (DST-NRF intern)	South African		MSc		0
Jeremy Boonzaier (DST-NRF intern)	South African		MSc		0
Veruscha Paulsen (DST-NRF intern)	South African		MSc		0
Dolly September (DST-NRF intern)	South African		Hons BSc		0
Robyn-Lee Louw (DST-NRF intern)	South African		BSc		0
Sonia Podgorski (Work integrated learning student)	South African	Diploma in Biotechnology			0
Khanyisile Kula (Work integrated learning student)	South African	Diploma in Biotechnology	BTech		0

PERSONS PARTICIPATING IN THE PROJECT (Excluding students)

Initials & Surname	Highest Qualification	Degree/ Diploma registered for	Race (1)	Gender (2)	Institution & Department	Position (3)	Cost to Project R
H. du Plessis	MSc	PhD	B	M	ARC, PHWT	PL	534,516
N. Jolly	PhD		W	M	ARC, PHWT	Co	109,344
P. Minnaar	MSc	PhD	W	M	ARC, PHWT	Co	54,600
M. du Toit	PhD		W	F	SU, DVO & IWBT	Coll	0
H. Nieuwoudt	PhD		W	F	SU, DVO & IWBT	Coll	0
J. Brand	MSc	PhD	W	F	SU, DVO & IWBT	Coll	0
V. Panzeri	MSc		W	F	IGWS & SU	Coll	0
M. Kidd	PhD		W	M	SU, CSC	Coll	0
M. van der Rijst	MSc	PhD	W	F	ARC, Biometry	Co	2,500
N. Ntushelo	MSc		B	F	ARC, Biometry	Co	2,500
R. Hart	MSc	PhD	B	M	ARC, PHWT	Co	34,600
J. Hoff	MSc		B	M	ARC, PHWT	TA	82,516
V. van Breda	MTech		B	F	ARC, PHWT	TA	57,771
S. Ohlson	MTech		B	F	ARC, PHWT	TA	34,000
G. Harold	BSc		B	M	ARC, PHWT	TA	43,509
P. Adonis	Matric		B	F	ARC, PHWT	RA	45,416

(1)Race B = African, Coloured or Indian
W = White

(2)Gender F = Female
M = Male

(3)Position Co = Co-worker (other researcher at your institution)
Coll = Collaborator (participating researcher that does not receive funding for this project from industry)
PF = Post-doctoral fellow
PL = Project leader
RA = Research assistant
TA = Technical assistant/ technician

d) PUBLICATIONS (POPULAR, PRESS RELEASES, SEMI-SCIENTIFIC, SCIENTIFIC)

Du Plessis HW, Jolly NP. (2017) Non-*Saccharomyces* yeasts, malolactic fermentation and Chardonnay flavour. *Winetech Technical Yearbook* 2017, 123-124. (Addendum 5)

Du Plessis HW, du Toit M, Hoff JW, Hart RS, Ndimba BK, Jolly NP. (2017) Characterisation of non-*Saccharomyces* yeasts using different methodologies and evaluation of their compatibility with malolactic fermentation. *South African Journal of Enology and Viticulture* 38, 46-63. (Addendum 1)

Du Plessis H, Du Toit M, Nieuwoudt, H, Van Der Rijst, M, Kidd, M, Jolly, N. (2017). Effect of *Saccharomyces*, non-*Saccharomyces* yeasts and malolactic fermentation strategies on fermentation kinetics and flavor of Shiraz Wines. Fermentation, 3(4), 64. doi:10.3390/fermentation3040064. (Addendum 2)

Minnaar PP, du Plessis HW, Paulsen V, Ntushelo N, Jolly NP, du Toit M. (2017) *Saccharomyces cerevisiae*, non-*Saccharomyces* yeasts and lactic acid bacteria in sequential fermentations: Effect on phenolics and sensory attributes of South African Syrah wines. *South African Journal of Enology and Viticulture* 38 (2), 237-244. (Addendum 4)

e) PRESENTATIONS/PAPERS DELIVERED

Du Plessis HW, Jolly NP, du Toit M. (2012) Effect of non-*Saccharomyces* yeast and lactic bacteria interactions on wine flavour. Presentation at the Institute for Wine Biotechnology laboratory meeting in the JH Neethling building, Stellenbosch University. 22 August 2012.

Du Plessis HW. (2012) Nie-*Saccharomyces* gis en melksuurbakterieë interaksies in wyn. Radio talk in Afrikaans for RSG Agriculture. Radio Elsenburg, Infruitec Campus, Stellenbosch. 19 October 2012.

Du Plessis HW, Jolly NP, du Toit M. (2012) Effect of non-*Saccharomyces* yeast and lactic bacteria interactions on wine flavour. Oral presentation at the 34th South African Society of Enology and Viticulture Congress at Allee Bleue, Franschhoek. 14-16 November 2012.

Du Plessis HW. (2013) Effect of non-*Saccharomyces* yeast and lactic bacteria interactions on malolactic fermentation and wine flavour. Presentation at the IWBT laboratory meeting, Stellenbosch University, 14 August 2013.

Du Plessis HW, Jolly NP, du Toit M. (2013) The impact of yeast and lactic bacteria interactions on malolactic fermentation and wine flavour. Oral presentation at the South African Society for Microbiology Conference at the Forever Resort Warmbaths, Bela Bela, Limpopo, 24-27 November 2013.

Hoff JW, Mewa Ngongang M, du Plessis HW. (2013) The isolation and identification of wine yeasts from spontaneous Shiraz fermentations. Poster presentation at the South African Society for Microbiology Conference at the Forever Resort Warmbaths, Bela Bela, Limpopo, 24-27 November 2013.

Du Plessis HW. (2014) Effect of non-*Saccharomyces* yeast and lactic bacteria interactions on malolactic fermentation and wine flavour. Presentation at the IWBT laboratory meeting, Stellenbosch University, 28 May 2014.

Du Plessis HW, du Toit M, Jolly NP. (2014) Evaluation of Shiraz wines produced with different non-*Saccharomyces* yeast and lactic acid bacteria combinations. Poster presentation at the 2014 Macrowine conference at the Wallenberg Research Centre @ STIAS, Stellenbosch University, 7-10 September, 2014.

Du Plessis HW, du Toit M, Jolly NP. (2014) Impact of non-*Saccharomyces* yeasts on malolactic fermentation and wine flavour. Oral presentation at the 36th South African Society for Enology and Viticulture (SASEV) Conference, Lord Charles Hotel, Somerset-West, 12-14 November 2014.

Hoff JW, Mewa Ngongang M, du Plessis HW. (2014) The isolation and identification of wine yeasts from spontaneous Shiraz fermentations. Poster presentation at the 36th South African Society for Enology and Viticulture (SASEV) Conference, Lord Charles Hotel, Somerset-West, 12-14 November 2014.

Du Plessis HW. (2015) Effect of non-*Saccharomyces* yeast and lactic bacteria interactions on wine flavour. Presentation at the IWBT research hour, Stellenbosch University, 21 October 2015.

Du Plessis HW, du Toit M, Jolly NP. (2016) Impact of non-*Saccharomyces* yeasts on malolactic fermentation and wine flavour. Oral presentation at the South African Society for Microbiology Conference 2016 Biennial congress, Coastlands, Umhlanga, Durban, 17-20 January 2016.

Du Plessis HW. (2016) Effect of non-*Saccharomyces* yeast and lactic bacteria interactions on wine flavour. Presentation at the IWBT research hour, Stellenbosch University, 12 October 2016.

7. BUDGET

TOTAL COST SUMMARY OF THE PROJECT

YEAR	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
2012/13					201,476	65,036	209,700	476,212
2013/14					250,509	57,636	236,028	544,173
2014/15					279,146	64,225	290,540	633,911
2015/16					150,219	34,550	191,339	376,108
2016/17					0			0
Total					**881,350**	**221,447**	**927,607**	**2,030,404**

EVALUATION BY INDUSTRY

This section is for office use only

Project number	

Project name	

Name of Sub-Committee*	

Comments on project

Committee's recommendation (Review panel in the case of PHI)

- Accepted. ☐
- Accepted provisionally if the sub-committee's comments are also addressed. Resubmit this final report by_____ ☐
- Unacceptable. Must resubmit final report. ☐

Chairperson_____ Date_____

*SUB-COMMITTEES

Winetech
Viticulture: Cultivation; Soil Science; Plant Biotechnology; Plant Protection; Plant Improvement;
Oenology: Vinification Technology; Bottling, Packaging and Distribution; Environmental Impact; Brandy and Distilling; Microbiology

Deciduous Fruit
Technical Advisory Committees: Post-Harvest; Crop Production; Crop Protection; Technology Transfer
Peer Work Groups: Post-Harvest; Horticulture; Soil Science; Breeding and Evaluation; Pathology; Entomology