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FINAL REPORT
FOR 2011/12

PROGRAMME & PROJECT LEADER INFORMATION

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PROJECT INFORMATION

Project number	WW10-22
Project title	Effect of non-*Saccharomyces* yeast on malolactic fermentation
Project Keywords	Lactic acid bacteria, yeast and bacteria interactions, wine quality

Industry programme	**CFPA**	
	Deciduous	
	DFTS	
	Winetech	Microbiology
	Other	

Fruit kind(s)	Wine grapes
Start date (dd/mm/yyyy)	01/04/2009
End date (dd/mm/yyyy)	31/03/2012

FINAL REPORT

1. Executive summary

This project showed that there is a lot of variation among non-*Saccharomyces* yeasts in terms of their fermentation abilities, as well as their impact on MLF. This varied between species, but also between strains within the same species. During the 2010-harvest, three non-*Saccharomyces* strains, i.e. *Kloeckera apiculata*, *Schizosaccharomyces pombe* and *Torulaspora delbrueckii* were used in the production of Chenin blanc and Pinotage wines. Non-*Saccharomyces* fermented wines completed MLF faster than wines fermented with *Saccharomyces cerevisiae* only. Sensory data showed that Chenin blanc wines not having undergone MLF were preferred and scored higher for 'aroma intensity' and 'overall quality'. Pinotage wines not having undergone MLF scored higher for 'aroma intensity' and 'fruity aroma', but Pinotage wines having undergone MLF had more 'body' and scored higher for 'overall quality'. During the 2011-harvest, two commercial *T. delbrueckii* strains were evaluated in Chenin blanc and Pinotage. Malolactic fermentation was completed after three days in all of the Pinotage wines. Due to sluggish alcoholic fermentations of the non-*Saccharomyces* yeasts, the Chenin blanc wines underwent spontaneous MLF. These results showed that non-*Saccharomyces* yeasts have a positive effect on MLF, but it may increase the occurrence of spontaneous MLF, especially in sluggish fermentations. Pinotage wines produced with non-*Saccharomyces* yeasts were considered to be better than wines produced with *Saccharomyces* yeast only. Depending on species and strain selection, non-*Saccharomyces* yeasts have a positive effect MLF and wine quality.

2. Problem identification and objectives

Successful induction and completion of malolactic fermentation (MLF) is an ongoing problem worldwide, despite the considerable body of knowledge concerning wine properties, factors affecting lactic acid bacteria (LAB) and developments in strain selection and starter cultures. The advent of the use of non-*Saccharomyces* yeasts in wine production constitutes another unknown factor for malolactic fermentation. To date the interaction between wild and industrial yeasts and lactic acid bacteria has received little attention. The objective of this project is therefore to investigate the interaction between non-*Saccharomyces* yeasts and LAB, and subsequent effects on MLF and wine quality.

3. Workplan (materials & methods)

<u>Screening of non-*Saccharomyces* yeasts and *O. oeni*</u>

This procedure is based on the screening technique reported by Krieger and Arnink (2003). Frozen Chardonnay juice from 2009 was fermented with different commercial *Saccharomyces cerevisiae*, selective non-*Saccharomyces* yeast and LAB strains listed in Table 1. At the end of AF, a filter disk was impregnated with the wine fermented with the selected yeasts strains and placed on MRS grape juice agar (MRS supplemented with 25% ($^{v}/_{v}$) grape juice), on which specific commercial LAB strains i.e. Viniflora oenos, Viniflora CH16, Enoferm alpha and Lactoenos SB3 was lawned. MRS plates were incubated at 30°C until growth was observed.

Table 1. Yeasts and bacteria strains used in this study.

Species	Strain	Source
Saccharomyces cerevisiae	N 96*	Anchor Yeast
Saccharomyces cerevisiae	VIN 13*	Anchor Yeast
Saccharomyces cerevisiae	NT 202	Anchor Yeast
Saccharomyces cerevisiae	NT 112	Anchor Yeast
Saccharomyces cerevisiae	QA23	Lallemand Inc.
Saccharomyces cerevisiae	BM45	Lallemand Inc.
Saccharomyces cerevisiae	BM4X4	Lallemand Inc.
Saccharomyces cerevisiae	EC1118	Lallemand Inc.
Saccharomyces cerevisiae	FX10	Laffort
Candida pulcherrima	Y0838	ARC Infruitec-Nietvoorbij genebank
Candida pulcherrima	Y0839	ARC Infruitec-Nietvoorbij genebank
Candida pulcherrima	Y0842	ARC Infruitec-Nietvoorbij genebank
Candida pulcherrima	Y0857	ARC Infruitec-Nietvoorbij genebank
Candida pulcherrima	Y0861	ARC Infruitec-Nietvoorbij genebank
Candida zemplinina	Y0841	ARC Infruitec-Nietvoorbij genebank
Candida zemplinina	Y0844	ARC Infruitec-Nietvoorbij genebank
Candida zemplinina	Y0849	ARC Infruitec-Nietvoorbij genebank
Candida zemplinina	Y0872	ARC Infruitec-Nietvoorbij genebank
Candida zemplinina	Y0879	ARC Infruitec-Nietvoorbij genebank
Kloeckera apiculata	Y0840	ARC Infruitec-Nietvoorbij genebank
Kloeckera apiculata	Y0845	ARC Infruitec-Nietvoorbij genebank
Kloeckera apiculata	Y0846	ARC Infruitec-Nietvoorbij genebank
Kloeckera apiculata	Y0858	ARC Infruitec-Nietvoorbij genebank
Kloeckera apiculata	Y0860	ARC Infruitec-Nietvoorbij genebank
Schizosaccharomyces pombe	Y0197	ARC Infruitec-Nietvoorbij genebank
Torulaspora delbrueckii	Y0163	ARC Infruitec-Nietvoorbij genebank
Torulaspora delbrueckii	M2/1	ARC Infruitec-Nietvoorbij culture collection
Torulaspora delbrueckii	M2/15	ARC Infruitec-Nietvoorbij culture collection
Torulaspora delbrueckii	M2/27	ARC Infruitec-Nietvoorbij culture collection
Torulaspora delbrueckii	Harmony	Chr. Hansen A/S
Torulaspora delbrueckii	Level 2^{Td}	Lallemand Inc.
Torulaspora delbrueckii	301	ARC Infruitec-Nietvoorbij culture collection
Torulaspora delbrueckii	654	ARC Infruitec-Nietvoorbij culture collection
*Oenococcus oeni***	Viniflora oenos	Chr. Hansen A/S
Oenococcus oeni	Viniflora CH16	Chr Hansen A/S
Oenococcus oeni	Enoferm alpha	Lallemand Inc
Oenococcus oeni	Lactoenos SB3	Laffort

*Reference yeasts.

**Commercial lactic acid bacteria strains.

Evaluation of commercial MLF strains

A synthetic (chemically defined) medium used by Costello *et al*. (2003) was used to study *Saccharomyces* yeast and LAB compatibility. Five hundred millilitres aliquots of the synthetic juice (SJ) were fermented to dryness by *S. cerevisiae* strains NT 202, NT 112, EC 1118 and FX10. After alcoholic fermentation (AF), 100 mL aliquots were divided into sterile 250 mL bottles and inoculated with commercial MLF starter cultures (Table 1). Both, AF and MLF were conducted at *ca.* 22°C. All commercial cultures were used as prescribed by the suppliers. Progress of MLF in the synthetic wine (SW) was monitored using paper chromatography (Kunkee 1968).

Laboratory-scale wine production trials

The SJ medium of Costello *et al*. (2003) was used to study *Saccharomyces* yeast and LAB compatibility. The following components were excluded from the SJ: D-erythritol, L-rhamnose, D-arabitol, D-mannose, $Zncl_2$, $Co(NO_3)_2.6H_2O$, $Na_2MoO_4.2H_2O$, adenine sulphate, guanine, uracil, xanthine, cytosine, choline chloride and cobalamine. Yeast strains used in this study were grown in Yeast Peptone Dextrose (YPD) broth at 30°C prior to inoculation. Two hundred and fifty millilitres of the SJ were inoculated with pure cultures of the different non-*Saccharomyces* strains and fermented to dryness or for 33 days. Alcoholic fermentation (AF) was carried out in duplicate. After AF, the SW was sterile filtered through a 0.22 µm filter and 100 mL was aliquoted into sterilised 250 mL bottles for the three different treatments. The three treatments were: (1) addition of LAB, (2) addition of nutrients and LAB, and (3) the reduction of inhibitory compounds in SW, prior to the addition of LAB. Treatments 1 and 2 were performed in duplicate. *Oenococcus oeni* strain, V. oenos was used to induce MLF. V. oenos was inoculated as prescribed by supplier. The ethanol concentration of the SW was adjusted to 10% ($^v/_v$) prior to addition of V. oenos. The screening of non-*Saccharomyces* yeasts in SJ was conducted at ±21°C. The Ripper method was used to determine free and total sulphur dioxide (SO_2) and alcohol concentration was determined by Koelenhof Wine Cellar Laboratory.

Small-scale wine production trials of 2010 (1^{st} vintage)

Grapes were obtained from the Nietvoorbij Research Farm and the analysis of the juice after crushing and pressing are listed in Table 2. *Kloeckera apiculata* (Y0858), *Schizosaccharomyces pombe* (Y0197) and *Torulaspora delbrueckii* (M2/15) were used in small-scale (20 L) Chenin blanc (14°C) and Pinotage (23°C) wine production trials during the 2010-harvest. *S. cerevisiae* strain, N 96 was used as the reference yeast. V. oenos inoculated at the end of AF to induce MLF. Two treatments were applied i.e. treatment 1: non-*Saccharomyces* yeast without undergoing MLF and treatment 2: non-*Saccharomyces* yeast with MLF. All fermentations were conducted in duplicate. Juice samples were analysed (pH, total acidity, sugar, free and total SO_2) in the Nietvoorbij cellar laboratory using standard techniques (Iland *et al*., 2000). The progress of MLF was monitored using paper chromatography and Wine Scan analyses (IWBT, Stellenbosch University).

Table 2. Chemical analyses of Chenin blanc and Pinotage juice of the 2010-harvest.

Cultivar	**Sugar (°B)**	**pH**	**Total acid (g/L)**	**Free SO_2 (mg/L)**	**Total SO_2 (mg/L)**
Chenin blanc	18.8	3.12	7.28	2	16
Pinotage	25.0	3.37	5.21	16	25

Sensory evaluation and chemical analyses of 2010 wines

The wines were sensorially evaluated by descriptive analyses during the annual experimental wine evaluations at Nietvoorbij. The panels consisted of commercial winemakers (7) and Nietvoorbij staff (2). A ten centimetre unstructured line scale was used and the judges were asked to score wines for the different aroma and taste descriptors, as well as overall quality. The wines were coded and presented in a random order. The concentrations of the volatile compounds in the wines were measured using Gas chromatography with flame ionization detector (GC-FID) (Analytical Laboratory, Distell, Stellenbosch).

Small-scale wine production trials of 2011 (2^{nd} vintage)

Torulaspora delbrueckii strains isolated from commercial yeast cultures, i.e. Harmony (Chr. Hansen) and Level 2^{Td} (Lallemand), were used in small-scale (20 L) Chenin blanc (14℃) and Pinotage (23℃) wine production trials during the 2011-harvest. VIN 13 was used as the reference yeast. Two treatments were applied, i.e. treatment 1: yeast without undergoing MLF and treatment 2: yeast with MLF. V. oenos was inoculated at the end of the alcoholic fermentation (AF) in the Pinotage wines. All fermentations were conducted in duplicate. Juice samples were analysed (pH, total acidity, sugar, free and total SO_2) in the Nietvoorbij Cellar Laboratory using standard techniques. The total nitrogen content of the juice was determined by Vinlab (Pty) Ltd. The progress of MLF was monitored using paper chromatography and WineScan analyses (IWBT).

Table 3. Chemical analyses of Chenin blanc and Pinotage juice of the 2011-harvest.

Cultivar	**Sugar (ºB)**	**pH**	**Total acidity (g/L)**	**Free SO_2 (mg/L)**	**Total SO_2 (mg/L)**	**YAN (mg/L)**	**FAN (mg/L)**	**NH_4 (mg/L)**
Chenin blanc	22.3	3.34	6.38	1	15	120	90	30
Pinotage	22.1	3.31	5.43	3	14	280	180	100

Sensory evaluation and chemical analyses of 2011 wines

The wines were sensorially evaluated by descriptive analyses during the annual experimental wine evaluations at Nietvoorbij. The panels consisted of commercial winemakers (2) and Nietvoorbij staff (10).). A ten centimetre unstructured line scale was used and the judges were asked to score wines for the different aroma and taste descriptors, as well as overall quality. The wines were coded and presented in a random order. The concentrations of the volatile compounds in the wines were measured using Gas chromatography with flame ionization detector (GC-FID) (Analytical Laboratory, Distell).

4. Results and discussion

Milestone	**Achievement**
1. Literature study	Completed.
2. Screening of non-*Saccharomyces* yeasts and *O. oeni*	Completed. Plate assay needs optimization.
3. Evaluation of commercial LAB strains	Completed. E. alpha and CH16 faster and less sensitive MLF cultures than V. oenos and Lactoenos.
4. Laboratory-scale wine production trials	Completed. *Candida zemplinina* isolates least inhibitory effect on MLF. *Torulaspora delbrueckii* isolates showed good fermentation abilities.
5. Small-scale wine production trials of 2010 (1st vintage)	Completed. 14 Chenin blanc and 14 Pinotage wines produced with three different non-*Saccharomyces* yeasts.
6. Chemical and sensory analyses of 2010 vintage wines	Completed. Non-*Saccharomyces* wines completed MLF faster, and were preferred to reference *Saccharomyces* wines.
7. Small-scale wine production trials of 2011 (2nd vintage)	Completed. 12 Chenin blanc and 12 Pintotage wines produced with two commercial *T. delbrueckii* yeasts.
8. Chemical and sensory analyses of wines 2011 vintage wines	Completed. *T. delbrueckii* wines having undergone MLF preferred to *Saccharomyces* reference wines.

Milestone 1: Literature study

A literature search was performed to find relevant and related information about the effect non-*Saccharomyces* yeasts have on the growth of LAB. Non-*Saccharomyces* yeasts can produce compounds such as acetaldehyde, acetic acid, esters, glycerol, sulphur dioxide, hydrogen sulphide and other by-products (Romano *et al*., 1997, 2003; Ciani *et al*., 2010), which may be inhibiting or stimulating to LAB. Non-*Saccharomyces* yeasts can secrete pectinases, proteases and glycosidases (Fernández *et al*., 2000; Ciani *et al*., 2010). Enzymatic hydrolysis of the proteins into smaller more soluble nitrogen-containing molecules (peptides and amino acids) could stimulate LAB growth. Terpenes and other volatile compounds such as straight-chain and cyclic alcohols, to name just a few, help improve wine aroma. These volatile odorous compounds are present in free form in the grapes or are mainly bind to sugar to form odourless non-volatile glycosidic complexes. β-Glucosidase cleaves off aroma precursors bound to the sugar molecules contributing to wine aroma. These sugar molecules can be utilised by LAB. Wine macro-molecules such as polysaccharides and mannoproteins can also affect LAB growth and Diez *et al*. (2012) found that mannoproteins could stimulate LAB growth.

Some non-*Saccharomyces* yeast such as *Hansenula anomala* (*Pichia anomala*), *Schizosaccharomyces pombe*, *Zygosaccharomyces bailii* and *Issatchenkia orientalis* can degrade malic acid (Saayman & Viljoen-Bloom 2006; Seo *et al*., 2007; Ciani *et al*., 2010), which will affect LAB growth and MLF. During the beginning of fermentation, these yeasts may deplete the nutrients found in wine. These deficiencies, combined with toxic metabolites, can inhibit the growth of LAB (Ribéreau-Gayon *et al*., 2006; Costello *et al*., 2003). It has also been reported that the growth of native yeast species, such as *Saccharomycodes ludwigii*, *Candida pulcherrima* and *Pichia* spp., is antagonistic to the growth of LAB (Fornachon, 1968). However, in the last mentioned study only six non-*Saccharomyces* yeast strains were used and their effect on one *Lactobacillus brevis* strain, one *Lactobacillus hilgardii* strain and two *Leuconostoc mesenteroides* strains were investigated. Mendoza & Farías (2010) evaluated the use of *S. cerevisiae* and *K. apiculata* in mixed fermentations with *O. oeni*. They found that the yeast/LAB interaction did not affect MLF and that sequential inoculation of *O. oeni* allowed better control on the sensory quality of the wine.

Wine yeast may exert a range of effects upon LAB, including inhibition, stimulation, or neutral effect. The interactions between *Saccharomyces cerevisiae* and *O. oeni* have been studied in greater detail, and the same rules should apply to non-*Saccharomyces* yeasts. It is clear, however, that the interaction between *Saccharomyces*/non-*Saccharomyces*/LAB is complex and that there are many factors that play a role.

Milestone 2: Screening of non-*Saccharomyces* yeasts and *O. oeni*

A simple screening technique to determine yeast and LAB compatibility was evaluated. If the yeast and LAB strain was not compatible a clear zone of no growth would be visible around the filter disk. If the yeast and LAB was compatible no zone would be visible around the filter disk. The results of the plate screening technique were inconclusive because all yeast and LAB combination appeared to be compatible, including *Saccharomyces* reference strains previously reported to be incompatible (S. Krieger-Webber, personal communication).

Milestone 3: Evaluation of commercial LAB strains

The aim of this experiment was to determine the effect of *S. cerevisiae* strains, NT 202, NT 112, EC 1118 and FX10, have on sequential MLF in SW. These commercial yeasts were used because it is known what effects they have on LAB and MLF in wine. Malolactic fermentation took longer to complete in the SW fermented with NT 112, than with the other yeasts (Table 4). Malolactic fermentation completed the fastest in SW fermented with NT 202. Enoferm alpha completed MLF the fastest and was compatible with all the yeast strains. This trial showed that LAB strains, E. alpha and CH16 were less affected by yeast selection than V. oenos and Lactoenos and that the wrong yeast and LAB selection could delay MLF.

Table 4. Duration of sequential malolactic fermentation (MLF) in synthetic wine fermented with different commercial yeast strains.

Treatment	MLF (days)
NT 202 + Viniflora CH16	7
NT 202 + Viniflora oenos	20
NT 202 + Enoferm alpha	7
NT 202 + Lactoenos SB3	20
NT 112 + Viniflora CH16	25
NT 112 + Viniflora oenos	32
NT 112 + Enoferm alpha	25
NT 112 + Lactoenos SB3	41
EC 1118 + Viniflora CH16	41
EC 1118 + Viniflora oenos	20
EC 1118 + Enoferm alpha	20
EC 1118 + Lactoenos SB3	20
FX10 + Viniflora CH16	20
FX10 + Viniflora oenos	25
FX10 + Enoferm alpha	20
FX10 + Lactoenos SB3	25

Milestone 4: Laboratory-scale wine production trials
The aim of this trial was to determine the effect non-*Saccharomyces* yeasts have on *O. oeni* strain, V. oenos and MLF in a synthetic grape juice. This medium was chosen because it could help to determine if the different yeast strains stimulated or inhibited LAB growth and MLF. If yeast inhibited LAB growth and MLF, it could be determined whether inhibition was due to nutrient depletion or yeast derived compounds.

Progress of AF (measured in CO_2 weight loss) of the different non-*Saccharomyces* strains in SJ, are shown in Fig. 1. Some non-*Saccharomyces* yeast appeared to be strong fermenters, whereas others appeared to be weak. However, none of the non-*Saccharomyces* yeasts fermented to dryness and a few of the SW samples contained high residual sugar concentrations at the end of AF (Table 5). There were clear differences in the ability of the different non-*Saccharomyces* yeasts to produce SO_2, with *Kloeckera apiculata* Y0840 and *Torulaspora delbrueckii* M2/15 producing the highest total SO_2 levels. There was less variation in the alcohol levels for the *Torulaspora delbrueckii* strains than for the other non-*Saccharomyces* strains, with the *Candida* strains showing the most variation and the highest residual sugar values. Many of the non-*Saccharomyces* yeasts produced relatively high alcohol concentrations.

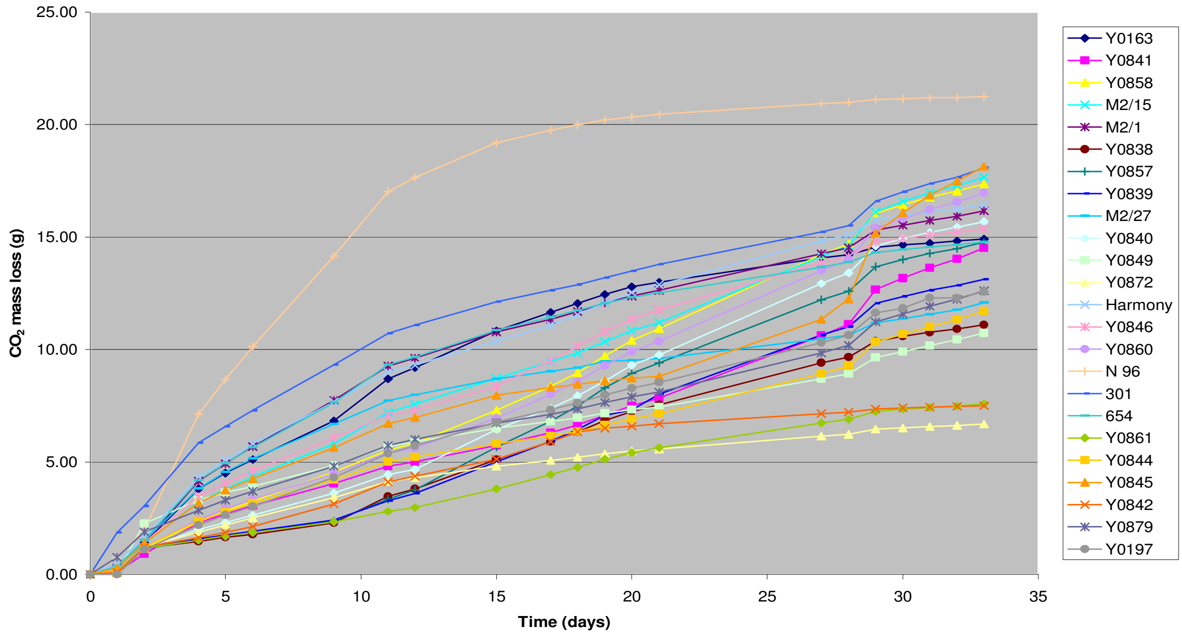

Figure 1. Progress of alcoholic fermentation in a synthetic grape juice fermented with different non-*Saccharomyces* yeasts.

The chemical analyses and duration of MLF of the SW are listed in Tables 5 and 6, respectively. The different non-*Saccharomyces* strains had a definite effect on the progress of MLF (Table 6). In this synthetic medium, most of the non-*Saccharomyces* strains had an inhibitory effect on MLF. Only a few non-*Saccharomyces* strains completed MLF for all three treatments, with *Candida zemplinina* Y0844 performing the best. The only other non-*Saccharomyces* strains where MLF was completed for all three treatments were two *Candida zemplinina* strains, Y0849 and Y0872, and a *Kloeckera apiculata* strain, Y0860. All three treatments of the *S. cerevisiae* reference yeast, N 96 completed MLF. It is clear from treatments 2 and 3 that the inhibition of MLF by some strains was due to nutrient depletion or production of inhibitory components/metabolites.

Table 5. Chemical analyses of the synthetic wine[a] at the end of alcoholic fermentation.

Species	Strain	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Alcohol (% $^v/_v$)	Residual sugar (g/L)
Saccharomyces cerevisiae[b]	N 96	5	17	10.01	3.80
Candida pulcherrima	Y0838	6	18	5.91	65.40
Candida pulcherrima	Y0839	4	13	7.87	58.00
Candida pulcherrima	Y0842	6	12	5.46	73.80
Candida pulcherrima	Y0857	6	18	8.86	38.60
Candida pulcherrima	Y0861	3	11	2.98	104.40
Candida zemplinina	Y0841	5	23	8.69	27.80
Candida zemplinina	Y0844	5	12	5.60	77.60
Candida zemplinina	Y0849	6	13	5.10	93.40
Candida zemplinina	Y0872	8	13	5.45	96.80
Candida zemplinina	Y0879	4	13	8.73	44.80
Kloeckera apiculata	Y0840	5	29	8.04	35.20
Kloeckera apiculata	Y0845	4	8	8.20	18.40
Kloeckera apiculata	Y0846	7	19	5.09	69.60
Kloeckera apiculata	Y0858	6	17	9.67	13.80
Kloeckera apiculata	Y0860	7	23	6.36	67.60
Schizosaccharomyces pombe	Y0197	5	13	9.18	26.00
Torulaspora delbrueckii	Y0163	6	10	7.36	38.25
Torulaspora delbrueckii	M2/1	6	18	9.47	24.55
Torulaspora delbrueckii	M2/15	5	29	9.88	10.20
Torulaspora delbrueckii	M2/27	6	24	8.03	49.60
Torulaspora delbrueckii	Harmony	7	27	8.93	24.00
Torulaspora delbrueckii	301	5	19	9.10	57.80
Torulaspora delbrueckii	654	4	22	8.54	44.60

[a]Average values of the duplicate samples.
[b]Reference yeast.

Milestone 5: Small-scale wine production trials of 2011 (1st vintage)

Three non-*Saccharomyces* and one *Saccharomyces* reference strain were used to produce Chenin blanc and Pinotage wines. Chenin blanc and Pinotage wine analyses at the end of AF are listed in Tables 7 and 8. All the Chenin blanc wines fermented to dryness (Table 7), but the Pinotage wines still contained some residual sugar (Table 8) at the end of AF. In both cultivars, the indigenous *Saccharomyces* population completed the AF (data not shown). The wines fermented with *Sc. pombe* all underwent partial MLF at the end of AF (Tables 7 and 8).

Table 6. Duration of sequential malolactic fermentation (MLF) in synthetic wine fermented by different yeast strains.

Species	**Strain**	**MLF duration (days)**		
		Treatment 1[a]	**Treatment 2[b]**	**Treatment 3[c]**
Saccharomyces cerevisiae[d]	N 96	19	10	14
Candida pulcherrima	Y0838	>68	39	>68
Candida pulcherrima	Y0839	(42 - >68)	(22 - >68)	>68
Candida pulcherrima	Y0842	(8 - >68)	>68	6
Candida pulcherrima	Y0857	>68+ 42	>68	>68
Candida pulcherrima	Y0861	8	>68 + 10	14
Candida zemplinina	Y0841	(42 - >68)	(17 - >68)	>68
Candida zemplinina	Y0844	8	7	6
Candida zemplinina	Y0849	25	14	5
Candida zemplinina	Y0872	22	27	9
Candida zemplinina	Y0879	35	36	>68
Kloeckera apiculata	Y0840	(42 - >68)	(42 - >68)	50
Kloeckera apiculata	Y0845	MLF completed prior to inoculation		
Kloeckera apiculata	Y0846	(42 - >68)	>68 + 36	16
Kloeckera apiculata	Y0858	>68	>68	Not tested
Kloeckera apiculata	Y0860	15	34	7
Torulaspora delbrueckii	M2/15	>68	>68	>68
Torulaspora delbrueckii	M2/1	42	(8 - >68)	>68
Torulaspora delbrueckii	M2/27	>68	>68	>68
Torulaspora delbrueckii	Y0163	>68	>68	>68
Torulaspora delbrueckii	Harmony	(65 - >68)	(58 - >68)	>68
Torulaspora delbrueckii	301	(35 - >68)	40	>68
Torulaspora delbrueckii	654	34	10	>68
Schizosaccharomyces pombe	Y0197	MLF completed prior to inoculation		

[a]Treatment 1: addition of Viniflora oenos (commercial MLF culture).
[b]Treatment 2: nutrient supplementation and addition of V. oenos.
[c]Treatment 3: detoxification of the fermented synthetic grape juice prior to addition of V. oenos.
[d]Reference yeast.

Table 7. Chemical analyses of the 2010 Chenin blanc wines[a] at the end of alcoholic fermentation.

Treatment	**pH**	**Volatile acidity (g/L)**	**Total acidity (g/L)**	**Malic acid (g/L)**	**Lactic acid (g/L)**	**Alcohol (% v/v)**	**Glycerol (g/L)**	**Residual sugar (g/L)**
N96[b] + MLF	3.14	0.36	7.11	3.70	<0.3	10.87	5.12	0.65
Y0585	3.16	0.37	6.86	3.45	<0.3	10.87	5.38	1.90
Y0197	3.37	0.30	5.79	1.70	<0.3	10.55	4.79	0.90
M2/15	3.25	0.28	6.67	3.41	<0.3	10.38	4.63	0.10
Y0585 + MLF	3.20	0.39	6.88	3.40	<0.3	10.51	5.33	0.80
Y0197 + MLF	3.39	0.27	5.49	0.59	<0.3	10.36	4.82	0.10
M2/15 + MLF	3.24	0.25	6.61	3.34	<0.3	10.38	4.73	0.80

[a]Values in table are averages of the duplicates samples.
[b]Reference yeast.

Table 8. Chemical analyses of 2010 Pinotage wines[a] at the end of alcoholic fermentation.

Treatment	pH	Volatile acidity (g/L)	Total acidy (g/L)	Malic acid (g/L)	Lactic acid (g/L)	Alcohol (% $^v/_v$)	Glycerol (g/L)	Residual sugar (g/L)
N96[b] + MLF	3.78	0.21	5.94	1.57	0.20	14.49	10.21	3.15
Y0585	3.74	0.27	5.80	1.23	0.22	14.36	10.77	2.30
Y0197	3.80	0.27	5.58	0.92	0.24	14.10	10.58	2.70
M2/15	3.77	0.26	5.78	1.14	<0.2	14.44	11.16	3.60
Y0585 + MLF	3.77	0.26	6.07	1.43	0.29	14.52	10.95	3.00
Y0197 + MLF	3.64	0.26	5.94	0.95	<0.2	14.11	10.62	3.10
M2/15 + MLF	3.75	0.29	6.09	1.39	0.28	14.42	11.29	4.25

[a]Values in table are averages of the duplicates samples.
[b]Reference yeast.

All the treatments completed MLF successfully, with the *Sc. pombe* wines finishing MLF the quickest and the reference wines (N 96) taking the longest to complete (Tables 9 and 10). In the Chenin blanc wines, higher LAB numbers resulted in faster MLF. The LAB numbers in the Pinotage wines showed more variation, but there is no clear correlation between LAB numbers and duration of MLF. This trial showed that non-*Saccharomyces* fermented wines completed MLF faster than wines fermented with N 96 (*Saccharomyces* reference yeast).

The non-*Saccharomyces* yeasts did not inhibit MLF as expected from data of the SW trial. Malolactic fermentation actually completed quickly and without any problems, indicating that the compounds that were excluded from the SJ medium were probably essential to LAB growth, which explains the slow MLF progress in the SW.

Table 9. Viable lactic acid bacteria (LAB) count, duration of malolactic fermentation and chemical analyses of 2010 Chenin blanc wines[a] fermented with different yeasts.

Treatment	LAB count (cfu/mL)	MLF (days)	pH	Volatile acidity (g/L)	Total acidity (g/L)	Alcohol (% $^v/_v$)	Glycerol (g/L)
N 96[b] + MLF	1.5E+06	58	3.38	0.51	5.77	11.16	5.49
Y0858 + MLF	1.8E+06	13	3.48	0.54	5.48	10.57	5.35
Y0197 + MLF	1.9E+06	7	3.54	0.35	4.67	10.38	4.97
M2/15 + MLF	1.7E+06	19	3.52	0.37	5.28	10.37	4.79

[a]Values in table are averages of the duplicates samples.
[b]Reference yeast.

Table 10. Viable lactic acid bacteria (LAB) count, duration of malolactic fermentation and chemical analyses of Pinotage wines[a] fermented with different yeasts.

Treatment	LAB count (cfu/mL)	MLF (days)	pH	Volatile acidity (g/L)	Total acidity (g/L)	Alcohol (% $^v/_v$)	Glycerol (g/L)
N 96[b] + MLF	3.7E+06	16	3.81	0.33	4.92	15.27	10.96
Y0858 + MLF	1.8E+06	13	3.80	0.39	5.04	15.33	11.68
Y0197 + MLF	1.8E+06	9	3.83	0.33	4.96	14.83	11.15
M2/15 + MLF	2.5E+06	13	3.78	0.34	5.13	15.15	11.56

[a]Values in table are averages of the duplicates samples.
[b]Reference yeast.

Milestone 6: Chemical and sensory analyses of 2010 wines

The *K. apiculata* treatment without MLF scored the highest for most of the descriptors during the sensory evaluation of the Chenin blanc wines and the *S. pombe* with MLF treatment scored the lowest (Fig. 2). The Chenin blanc wines having undergone MLF scored lower for ‘aroma intensity’ and ‘overall quality’ than the treatments that did not undergo MLF. However, the wines that underwent MLF had more ‘body’ than the wines that did not undergo MLF.

Descriptive sensory analyses of the Pinotage wines indicated that there was no or little difference between the treatments (Fig. 3). The *T. delbrueckii* treatment without MLF scored the highest for most of the descriptors and N 96 treatment scored the lowest. Pinotage wines having undergone MLF were less ‘fruity’ than wines not having undergone MLF, but scored higher for ‘vegetative aroma’, ‘body’ and ‘overall quality’. The sensory data showed that MLF negated the aromatic contribution of non-*Saccharomyces* yeasts in Chenin blanc wines, but enhanced the ‘mouth-feel’ and ‘general quality’ of Pinotage wines produced with non-*Saccharomyces* yeasts.

Gas chromatographic analysis of the Chenin blanc wines revealed that the *K. apiculata* treatments, with and without MLF, had noticeable higher ethyl acetate levels than the other treatments (Table 11). The *K. apiculata* treatment with no MLF also contained slightly higher levels of the aromatic esters, iso-amyl acetate and hexyl acetate. The concentrations of the other esters did not differ or differed only slightly and were therefore not included. None of the treatments contained high methanol levels (Table 12). The *Sc. pombe* treatments contained slightly lower levels of most of the higher alcohols than the other treatments, except for iso-butanol, which was slightly higher than the other treatments. Wines fermented with N 96 contained higher volatile acidity levels than wines produced with the non-*Saccharomyces* yeasts. The concentration of hexanoic and octanoic acid was also higher in the N 96 wines (Table 13).

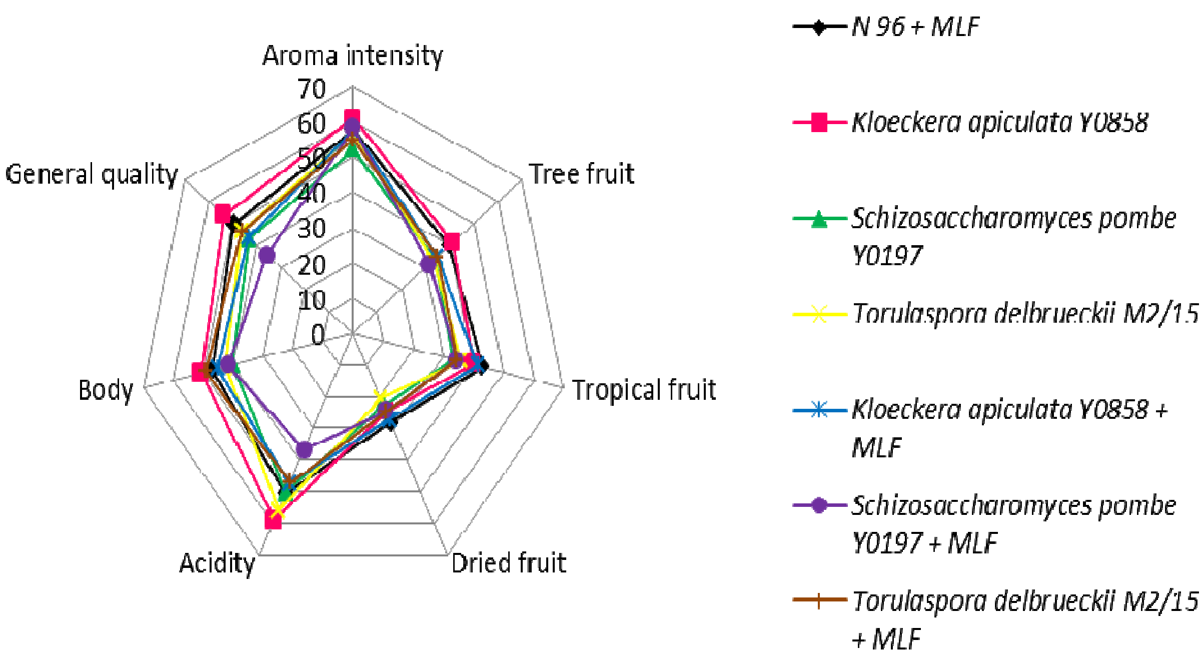

Figure 2. Descriptive sensory analyses of Chenin blanc wines of 2010.

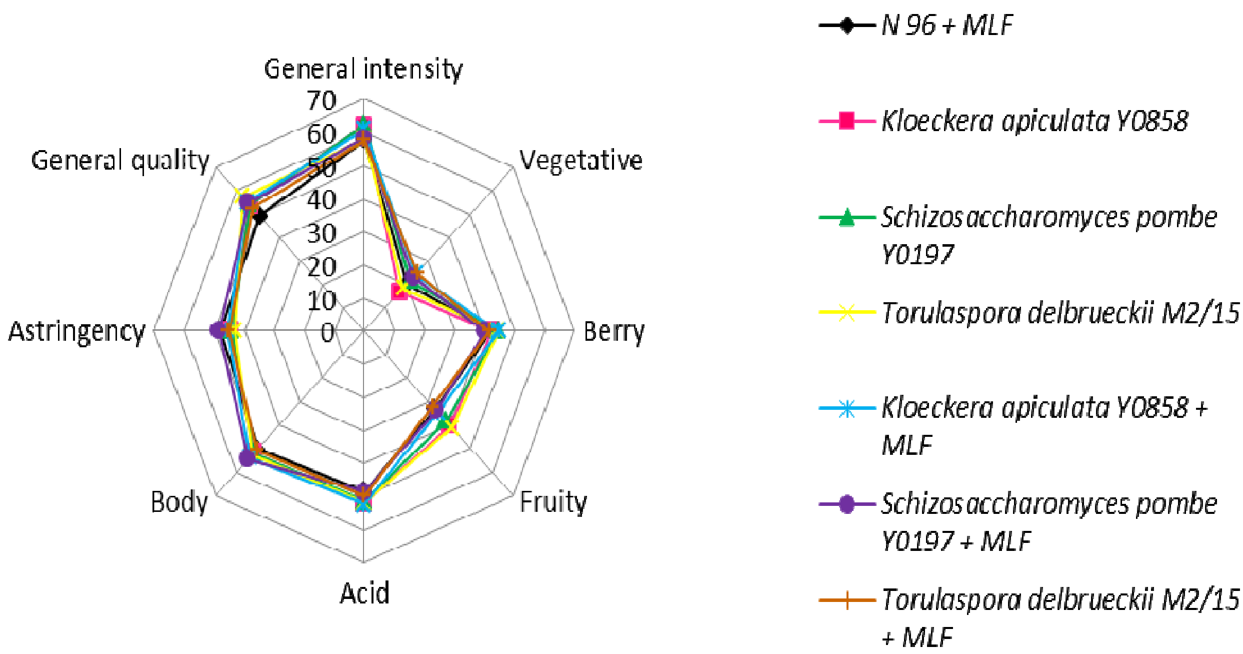

Figure 3. Descriptive sensory analyses of Pinotage wines of 2010.

Table 11. Ester concentrations (mg/L) in Chenin blanc wines[a] of 2010.

Treatment	Ethyl acetate	iso-Amyl acetate	Hexyl acetate	Ethyl lactate	Ethyl caprylate	Di-ethyl succinate
N 96[b] + MLF	72.08	5.85	0.33	82.53	0.79	0.71
Ka[c] Y0858	121.75	8.48	0.47	5.21	0.77	0.49
Sp[d] Y0197	76.48	7.75	0.34	3.04	1.03	0.55
Td[e] M2/15	88.42	7.93	0.39	3.85	0.77	0.68
Ka Y0858 + MLF	172.62	8.03	0.41	71.40	0.94	0.58
Sp Y0197 + MLF	78.45	6.49	0.24	27.50	0.94	0.59
Td M2/15 + MLF	95.93	7.56	0.37	58.20	1.18	0.71

[a]Values in table are averages of two replicates.
[b]Reference yeast, [c]*Kloeckera apiculata*, [d]*Schizosaccharomyces pombe* and [e]*Torulaspora delbrueckii.*

Table 12. Higher alcohol concentrations (mg/L) in Chenin blanc wines[a] of 2010.

Treatment	Methanol	n-Propanol	iso-Butanol	n-Butanol	iso-Amyl alcohol	2-Phenyl ethanol
N 96[b] + MLF	17.05	39.93	12.29	1.21	140.80	11.68
Ka[c] Y0858	16.57	38.05	13.26	1.57	137.41	8.39
Sp[d] Y0197	17.18	37.24	16.28	1.38	129.99	7.77
Td[e] M2/15	14.93	40.96	14.15	1.52	126.47	8.08
Ka Y0858 + MLF	15.18	36.35	13.80	1.56	125.72	8.03
Sp Y0197 + MLF	15.90	35.71	16.84	1.34	121.17	7.67
Td M2/15 + MLF	15.07	41.15	14.74	1.56	127.11	8.08

[a]Values in table are averages of two replicates.
[b]Reference yeast, [c]*Kloeckera apiculata*, [d]*Schizosaccharomyces pombe* and [e]*Torulaspora delbrueckii.*

Table 13. Volatile acid concentrations (mg/L) in Chenin blanc wines[a] of 2010.

Treatment	Acetic acid	Propionic acid	Hexanoic acid	Octanoic acid	Decanoic acid	n-Butyric acid
N 96[b] + MLF	296.43	18.28	8.76	11.98	2.18	2.35
Ka[c] Y0858	151.76	16.52	5.91	8.63	1.83	1.37
Sp[d] Y0197	120.44	16.44	5.94	8.43	1.73	1.52
Td[e] M2/15	83.38	11.63	6.07	8.76	1.99	1.70
Ka Y0858 + MLF	271.34	14.04	5.68	7.90	1.55	1.98
Sp Y0197 + MLF	183.60	15.58	6.19	8.84	2.01	1.97
Td M2/15 + MLF	136.68	12.59	6.62	9.28	2.24	2.15

[a]Values in table are averages of two replicates.
[b]Reference yeast, [c]*Kloeckera apiculata*, [d]*Schizosaccharomyces pombe* and [e]*Torulaspora delbrueckii.*

The GC data of the Pinotage wines confirmed the sensory evaluation results, with only a few compounds differing for the various treatments. These compounds are listed in Tables 14 and 15. Compounds not included in Tables 14 and 15 did not differ much. As observed in the Chenin blanc wines, the *K. apiculata* treatments contained higher ethyl acetate levels. As expected, ethyl lactate and acetic acid levels were noticeably higher in the wines having undergone MLF (Table 14). N 96 wines contained higher iso-valeric acid levels than the other wines. There were clear differences in the levels of higher alcohols for the various treatments. The N 96 wines contained the lowest methanol and the highest propanol levels. The *K. apiculata* treatments also contained higher iso-butanol levels than the other treatments.

Table 14. Concentrations of volatile compounds (mg/L) in Pinotage wines[a] of 2010.

Treatment	Ethyl acetate	iso-Amyl acetate	Ethyl lactate	Acetoin	Acetic acid	iso-Valeric acid
N 96[b] + MLF	62.73	2.07	80.38	13.71	263.51	**7.45**
Ka[c] Y0858	102.52	3.01	23.51	4.69	249.94	4.96
Sp[d] Y0197	68.64	2.33	20.44	4.31	253.71	4.62
Td[e] M2/15	74.84	2.75	23.44	6.60	242.09	5.37
Ka Y0858 + MLF	112.23	2.79	85.31	11.65	316.15	5.20
Sp Y0197 + MLF	76.35	2.28	70.30	14.75	362.04	4.37
Td M2/15 + MLF	77.81	2.37	82.09	12.90	320.92	5.97

[a]Values in table are averages of two replicates.
[b]Reference yeast, [c]*Kloeckera apiculata*, [d]*Schizosaccharomyces pombe* and [e]*Torulaspora delbrueckii.*

Table 15. Higher alcohol concentrations (mg/L) in Pinotage wines[a] of 2010.

Treatment	Methanol	n-Propanol	iso-Butanol	iso-Amyl alcohol	2-Phenyl ethanol
N 96[b] + MLF	67.07	152.13	30.65	194.08	17.68
Ka[c] Y0858	78.17	134.27	52.18	189.15	16.65
Sp[d] Y0197	81.24	109.03	38.27	208.67	19.84
Td[e] M2/15	79.27	133.08	42.49	201.46	20.63
Ka Y0858 + MLF	85.20	137.78	52.22	189.23	16.77
Sp Y0197 + MLF	79.65	104.86	38.78	206.34	19.58
Td M2/15 + MLF	81.43	118.39	43.19	198.43	20.62

[a]Values in table are averages of two replicates.
[b]Reference yeast, [c]*Kloeckera apiculata*, [d]*Schizosaccharomyces pombe* and [e]*Torulaspora delbrueckii.*

Milestone 7: Small-scale wine production trials of 2011 (2nd vintage)
Purified *T. delbrueckii* isolates from commercial cultures, Harmony and Level 2[Td], were evaluated in Chenin blanc and Pinotage during the 2011-harvest. *S. cerevisiae*, VIN 13 was used as reference yeast. The nitrogen level of the Chenin blanc was lower than that of the Pinotage juice (Table 3). The initial SO_2 concentration was very low for both cultivars and no SO_2 was added.

All the treatments of the Pinotage wines fermented to dryness and the analyses of the wines after AF are listed in Table 16. The analyses at the end of AF were very similar, with one or two treatments showing a slight variation. Malolactic fermentation completed quickly and all the treatments were finished after three days (Table 17). The chemical analyses of the wines after MLF were also very similar.

Table 16. Chemical analyses of 2011 Pinotage wines[a] at the end of alcoholic fermentation.

Treatment	pH	Volatile acidity (g/L)	Total acidity (g/L)	Malic acid (g/L)	Glucose (g/L)	Fructose (g/L)	Alcohol (% v/v)	Glycerol (g/L)
VIN 13[b]	3.45	0.47	6.34	2.20	0.55	0.85	12.85	9.54
Harmony[c]	3.46	0.45	5.96	1.41	0.33	0.90	12.79	9.48
Level 2[Td]*	3.43	0.45	6.16	1.55	0.28	1.20	12.83	9.31

[a]Values in table are averages of two replicates.
[b]Reference yeast
[c]Isolate from commercial *Torulaspora delbrueckii* culture of Chr. Hansen A/S.
*Isolate from commercial *Torulaspora delbrueckii* culture of Lallemand Inc.

Table 17. Duration of malolactic fermentation (MLF) and chemical analyses of 2011 Pinotage wines[a] fermented with different yeasts.

Treatment	Duration of MLF (days)	pH	Volatile acidity (g/L)	Total acidity (g/L)	Alcohol (% v/v)	Glycerol (g/L)
VIN 13[b]	3	3.63	0.50	5.12	12.86	10.45
Harmony[c]	3	3.57	0.58	5.23	12.91	10.05
Level 2[Td]*	3	3.50	0.54	5.18	12.80	10.28

[a]Values in table are averages of two replicates.
[b]Reference yeast
*Isolate from commercial *Torulaspora delbrueckii* culture of Lallemand Inc.

Chenin blanc juice inoculated with the different *T. delbrueckii* strains struggled with AF at 14ºC and only the VIN 13 treatment fermented to dryness. As a result the temperature was increased to 20ºC. The fermentations remained sluggish and on completion still contained some residual sugar, most of which was fructose (Table 18). The AF of the *T. delbrueckii* treatments took between 50-60 days to complete (glucose levels) and during this time spontaneous MLF occurred in most of the samples, including the treatments that were not supposed to undergo MLF. Level 2[Td] wines completed MLF faster than the other treatments, showing that there are some strain differences. The only treatments that did not undergo MLF were the VIN 13 treatments (reference strain). After 80 days, MLF did not even start in the VIN 13 treatment that was inoculated for MLF (Table 19). Two different commercial MLF starters, V. oenos and Enoferm alpha, were used to induce MLF, without success. This trial confirmed that non-*Saccharomyces* yeasts have a positive effect on the occurrence of MLF.

Table 18. Chemical analyses of 2011 Chenin blanc wines[a] at the end of alcoholic fermentation.

Treatment	pH	Volatile acidity (g/L)	Malic acid (g/L)	Glucose (g/L)	Fructose (g/L)	Alcohol (% $^{v}/_{v}$)
VIN 13[b]	3.31	0.30	2.94	<0.3	1.98	12.73
Harmony[c]	3.43	0.45	0.73	1.00	14.46	11.90
Level 2[Td*]	3.44	0.39	0.46	<0.3	6.95	12.25
VIN 13 + MLF[d]	3.37	0.30	2.86	<0.3	1.36	12.72
Harmony + MLF[d]	3.44	0.52	0.58	1.34	15.02	11.80
Level 2[Td] + MLF[d]	3.48	0.38	0.43	<0.3	2.99	12.55

[a]Values in table are averages of two replicates.
[b]Reference yeast
[c]Isolate from commercial *Torulaspora delbrueckii* culture of Chr. Hansen A/S.
*Isolate from commercial *Torulaspora delbrueckii* culture of Lallemand Inc.
[d]Spontaneous MLF

Table 19. Duration of spontaneous malolactic fermentation (MLF) in Chenin blanc wines[a] of 2011 fermented with different yeasts.

Treatment	Duration of MLF (days)
VIN 13[b]	>80
Harmony[c]	55[d]
Level 2[Td*]	53[d]

[a]Values in table are averages of four replicates.
[b]Reference yeast
[c]Isolate from commercial *Torulaspora delbrueckii* culture of Chr. Hansen A/S.
*Isolate from commercial *Torulaspora delbrueckii* culture of Lallemand Inc.
[d]Spontaneous MLF

Milestone 8: Sensory evaluation and chemical analyses of 2011 wines

Results of sensory evaluation of the Pinotage wines are shown in Figure 4. Pinotage wines having undergone MLF scored higher than the wines not having undergone MLF. The wines produced with *T. delbrueckii* yeasts scored higher than the wines fermented with the *Saccharomyces* yeast, VIN 13.

Due to the sluggish fermentation of the Chenin blanc wines and the difference in residual sugars, it was decided to only evaluate the aroma and not the taste of the wine. The reference wine, VIN 13 scored the highest for 'general aroma intensity', 'tree fruit' and tropical 'fruit', whereas the *T. delbrueckii* wines were considered to have a more 'dried fruit' character (Fig. 5). The *T. delbrueckii* wines also scored higher for 'other' aroma, which referred to sweet associated aromas or in some cases off-flavours. The volatile chemical composition of the Chenin blanc wines was not determined.

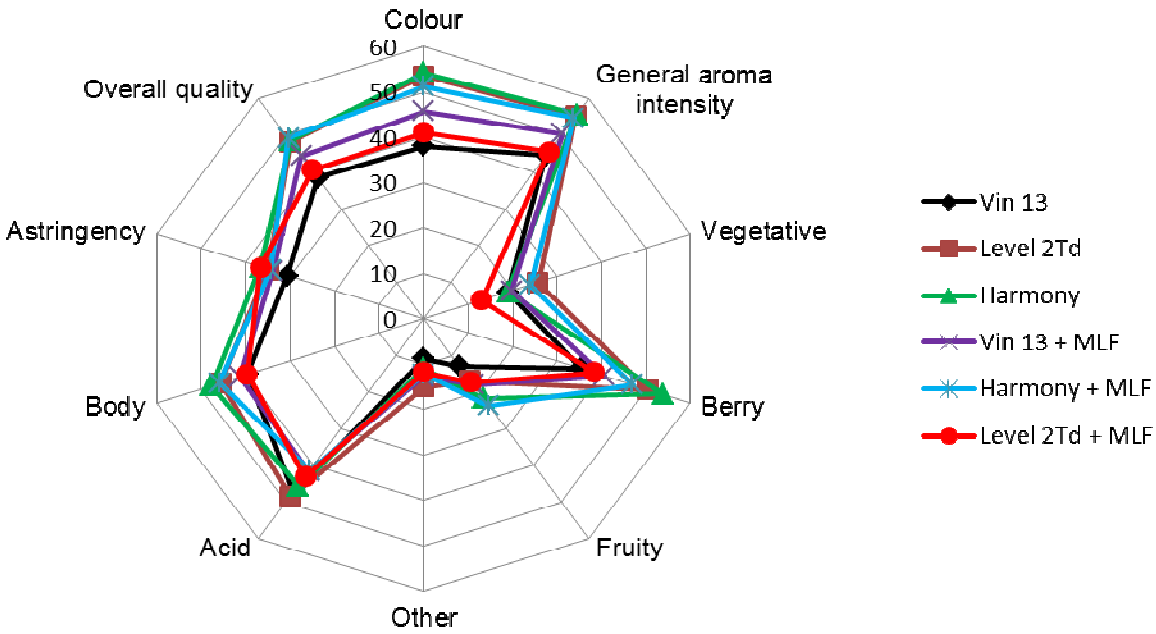

Figure 4. Descriptive sensory analyses of Pinotage wines of 2011.

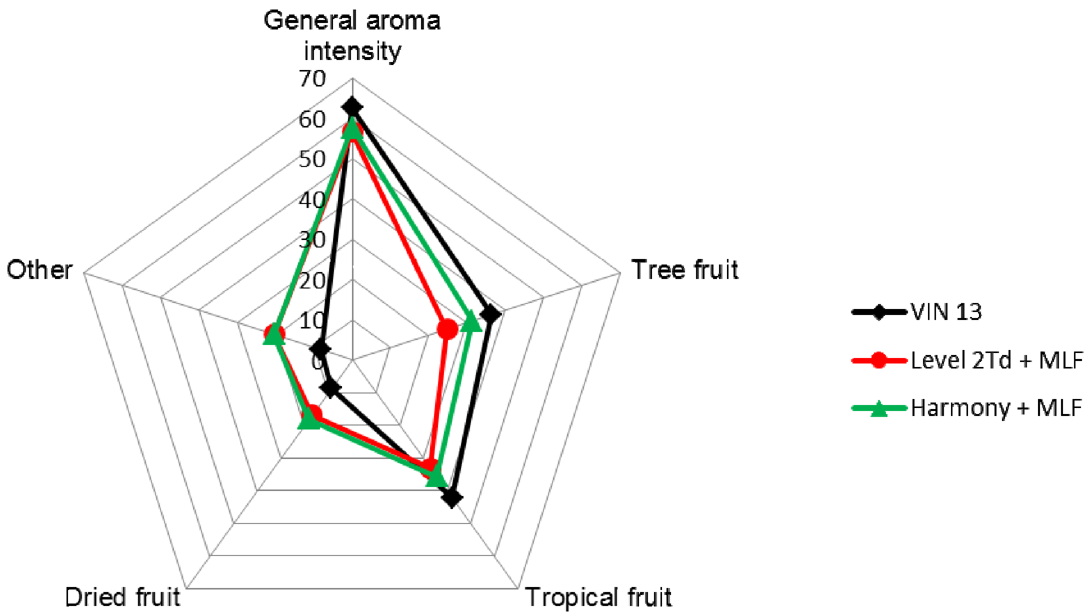

Figure 5. Descriptive aroma analyses of Chenin blanc wines of 2011.

Results of the GC analyses of the Pinotage wines are listed in Tables 20 to 22. Except for hexyl acetate, Pinotage wines having undergone MLF contained higher esters levels than the wines not having undergone MLF (Table 20).

Table 20. Concentrations of esters (mg/L) in Pinotage wines[a] of 2011.

Treatment	Ethyl acetate	iso-Amyl acetate	Ethyl caproate	Hexyl acetate	2-Phenylethyl acetate
VIN 13[b]	52.44	0.94	0.31	0.00	0.04
Harmony[c]	53.60	1.33	0.23	0.12	0.08
Level 2[Td*]	55.22	1.28	0.12	0.16	0.09
VIN 13 + MLF	64.08	0.94	0.32	0.00	0.06
Harmony + MLF	79.73	1.62	0.35	0.03	0.09
Level 2[Td] + MLF	67.32	1.58	0.29	0.03	0.10

[a]Values in table are averages of two replicates.
[b]Reference yeast
[c]Isolate from commercial *Torulaspora delbrueckii* culture of Chr. Hansen A/S.
*Isolate from commercial *Torulaspora delbrueckii* culture of Lallemand Inc.

Methanol, propanol and iso-butanol levels are higher in Pinotage wines having undergone MLF (Table 21). The different yeast strains also produced varying levels of higher alcohols. The levels of propanol and iso-amyl alcohol were higher in VIN 13 wines than the *T. delbrueckii* wines.

Table 21. Higher alcohol concentrations (mg/L) in Pinotage wines[a] of 2011.

Treatment	Methanol	n-Propanol	iso-Butanol	iso-Amyl alcohol	2-Phenyl ethanol
VIN 13[b]	47.18	113.60	34.29	171.07	19.73
Harmony[c]	43.66	71.96	42.06	158.82	18.68
Level 2[Td*]	32.39	75.78	47.91	164.66	21.44
VIN 13 + MLF	55.47	118.69	35.00	165.36	18.68
Harmony + MLF	48.33	73.52	43.36	162.52	19.50
Level 2[Td] + MLF	55.54	76.42	48.93	165.35	21.65

[a]Values in table are averages of two replicates.
[b]Reference yeast
[c]Isolate from commercial *Torulaspora delbrueckii* culture of Chr. Hansen A/S.
*Isolate from commercial *Torulaspora delbrueckii* culture of Lallemand Inc.

Pinotage wines having undergone MLF contained higher acetic acid and iso-valeric acid levels than wines not having undergone MLF (Table 22). None of the yeasts produced high acetic acid levels. The different yeast strains produced varying levels of volatile acids. VIN 13 produced the higher levels of propionic acid than the *T. delbrueckii* yeasts.

Table 22. Volatile acid concentrations (mg/L) in Pinotage wines[a] of 2011.

Treatment	Acetic acid	Propionic acid	iso-Valeric acid	Hexanoic acid	Octanoic acid	Decanoic acid
VIN 13[b]	242.08	50.03	5.61	2.08	1.76	0.20
Harmony[c]	226.47	31.44	4.39	2.49	2.00	0.18
Level 2[Td*]	240.38	32.66	4.72	2.23	1.83	0.22
VIN 13 + MLF	399.66	52.15	17.73	2.62	2.62	1.79
Harmony + MLF	414.83	34.29	15.60	2.59	2.16	0.41
Level 2[Td] + MLF	381.21	32.32	13.76	2.37	1.86	0.18

[a]Values in table are averages of two replicates.
[b]Reference yeast
[c]Isolate from commercial *Torulaspora delbrueckii* culture of Chr. Hansen A/S.
*Isolate from commercial *Torulaspora delbrueckii* culture of Lallemand Inc.

Discussion

Results showed that there is a lot of variation among non-*Saccharomyces* yeasts in terms of their fermentation abilities, as well as their impact on MLF. This varied between species, but also between strains within the same species. Some of the non-*Saccharomyces* yeast strain showed a lot of potential and will be investigated further, to improve on available commercial yeasts. Non-*Saccharomyces* yeasts have a positive effect on MLF, but it may increase the occurrence of spontaneous MLF, especially in sluggish fermentations. Non-*Saccharomyces* wines were considered to be better and more complex than wines fermented with *Saccharomyces* yeasts only, and this was shown with the non-*Saccharomyces* isolates and commercial strains. Wines produced with non-*Saccharomyces* yeasts, which underwent MLF scored higher for 'overall quality' and were generally better than wines not having undergone MLF. Depending on strain selection, non-*Saccharomyces* yeasts have a positive effect on MLF and wine quality. This knowledge should help the industry to alleviate some of the MLF problems and improve the quality of South African wines.

Literature cited

Ciani, M., Comitini, F., Mannazzu, I. & Domizio, P., 2010. Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking. FEMS Yeast Res 10, 123-133.

Costello, P.J., Henschke, P.A. & Markides, A.J., 2003. Standardised methodology for testing malolactic bacteria and wine yeast compatibility. *Australian Journal of Grape and Wine Research* 9, 127-137.

Diez, L., Guadalupe, Z., Ayestarán, B., & Ruiz-Larrea, F., 2010. Effect of yeast mannoproteins and grape polysaccharides on the growth of wine lactic acid and acetic acid bacteria. *Journal of Agricultural and Food Chemistry*, 58, 7731-7739.

Fernández, M., Úbeda, J.F. & Briones, A.I., 2000. Typing of non-*Saccharomyces* yeasts with enzymatic activities of interest in wine-making. *International Journal of Food Microbiology* 59, 29-36.

Fornachon, J.C.M., 1968. Influence of different yeasts on the growth of lactic acid bacteria in wine. *Journal of the Science of Food and Agriculture* 19, 374-8.

Iland, P.G., Ewart, A., Sitters, J., Markides, A. & Bruer, N., 2000. *Techniques for chemical analysis and quality monitoring during winemaking*. Patrick Iland Promotions, Campbelltown, Australia.

Krieger, S. & Arnink, K., 2003. Malolactic fermentation- A review of recent research on timing of inoculation and possible yeast-bacteria combinations. *Proceedings of the 32nd Annual New York Wine Industry Workshop*, Geneva, NY, 2-4 April 2003. pp. 60-73.

Kunkee, R.E., 1968. A simplified chromatographic procedure for detection of malolactic fermentation. *Wines and Vines* 49, 23-24.
Mendoza, L. & Farías, M.E., 2010. Improvement of wine organoleptic characteristics by non-*Saccharomyces* yeasts. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology* 2, 908-919.
Ribéreau-Gayon, P., Dubourdieu, D., Donéche, B. and Lonvaud, A., 2006. The Microbiology of Wine and Vinifications. *Handbook of Enology*. John Wiley & Sons Ltd. Chichester, England.
Romano, P., Fiore, C., Paraggio, M., Caruso, M. & Capece, A., 2003. Function of yeast species and strains in wine flavour. *International Journal of Food Microbiology* 86, 169-180.
Romano, P., Suzzi, G., Domizio, P. & Fatichenti, F., 1997. Secondary products formation as a tool for discriminating non-Saccharomyces wine strains. *Antonie van Leeuwenhoek* 71, 239-242.
Saayman, M. & Viljoen-Bloom, M., 2006. The biochemistry of malic acid metabolism by wine yeasts - a review. *South African Journal of Enology and Viticulture* 27, 113-122.
Seo, S-H., Rhee, C-H. & Park, H-D., 2007. Degradation of malic acid by *Issatchenkia orientalis* KMBL 5774, an acidophilic yeast strain isolated from Korean grape wine pomace. *The Journal of Microbiology* 45, 521-527.

5. Accumulated outputs

List ALL the outputs from the start of the project.
The year of each output must also be indicated.

Technology development, products and patents

Indicate the commercial potential of this project (intellectual property rights or a commercial product(s)).

Several non-*Saccharomyces* yeasts were evaluated and some have the potential to be commercialised.

Human resources development/training

Indicate the number and level (e.g. MSc, PhD, post doc) of students/support personnel that were trained as well as their cost to industry through this project. Add in more lines if necessary.

	Student level (BSc, MSc, PhD, Post doc)	Cost to project (R)
1.	Mr H.W. du Plessis (PhD)	R103,446
2.	Mr J.W. Hoff (MSc)	R44,520
3.	Ms V.M. Breda (MTech)	R29,680
4.	Ms S. Podgorski (ND Biotechnology)	R6,000

Publications (popular, press releases, semi-scientific, scientific)

Heinrich du Plessis & Neil Jolly. Impact of non-*Saccharomyces* yeasts on malolactic fermentation and flavour in Chenin blanc wines (Popular – submitted to Winelands).
Heinrich du Plessis & Neil Jolly. Impact of non-*Saccharomyces* yeasts on malolactic fermentation and flavour in Pinotage wines (Popular – submitted to Winelands).
Heinrich du Plessis & Neil Jolly. Wines produced using *Torulaspora delbrueckii* yeasts (Popular – to be submitted to Winelands).
H.W. du Plessis, N.S. Ntushelo and N.P. Jolly. Impact of non-*Saccharomyces* yeasts on malolactic fermentation and flavour in Chenin blanc and Pinotage wines. (Scientific – to be submitted to *South African Journal of Enology and Viticulture*).

Presentations/papers delivered

Du Plessis, H.W. & Jolly, N.P. 2010. Effect of non-*Saccharomyces* yeast on malolactic fermentation. 32nd Conference of the Society of Enology and Viticulture, Somerset West, South Africa. Poster

Du Plessis, H.W., van Breda, V.M., Hoff, J.W., Ohlson, S. & Jolly, N.P., 2011. Effect of non-*Saccharomyces* yeasts on malolactic fermentation and wine flavour. South African Society for Microbiology Conference 2011. 6-9 November 2011. Poster

Du Plessis, H.W. 2012. Other. Nie-*Saccharomyces* gis en melksuurbakterieë interaksies in wyn. Radio Elsenburg. 19 October 2012. Radio talk

4. Total cost summary of project

	Year	CFPA	Deciduous	DFTS	Winetech	THRIP	Other	TOTAL
Total cost in real terms for year 1	2009/10				146,762		152,752	**299,514**
Total cost in real terms for year 2	2010/11				161,438		164,135	**325,573**
Total cost in real terms for year 3	2011/12				211,168	105,569	334,296	**651,033**
Total cost in real terms for year 4								
Total cost in real terms for year 5								
TOTAL					**519,368**	**105,569**	**651,183**	**1,276,120**