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FINAL REPORT
2014

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Project Information

Research Organisation Project number	Ww10/21
Project title	The use of Torulaspora delbrueckii for wine production.

Fruit kind(s)			
Start date (mm/yyyy)	1 April 2008	**End date** (mm/yyyy)	31 March 2013

Project keywords	Non-*Saccharomyces, Torulaspora delbrueckii*, wine production

Approved by Research Organisation Programme leader (tick box) √

THIS REPORT MUST INCLUDE INFORMATION FROM THE **ENTIRE** PROJECT

Executive Summary

It is generally accepted that the wealth of yeast biodiversity with hidden potential, especially for oenology, is largely untapped and it is especially non-*Saccharomyce*s yeasts that fall into this category. The non-*Saccharomyces* yeast, *Torulaspora delbrueckii* (anamorph: *Candida colliculosa*) is a fermentative yeast formerly classified as *Saccharomyces rosei.* It was previously suggested for vinification of musts low in sugar and acid and has been used for the commercial production of red and rosé wines in Italy, albeit some time back (1950's). In more recent times it was found that *T. delbrueckii* in pure culture produced lower levels of volatile acidity than the *S. cerevisiae* strains tested. This yeast has also been used together with *S. cerevisiae* for the production of small-scale Chardonnay, Sauvignon blanc and Chenin blanc wines. The Sauvignon blanc and Chenin blanc wines were both judged to be better than the respective *S. cerevisiae* reference wines. The use of *T. delbrueckii* with and without co-inoculation of *S. cerevisiae* also holds potential for production of wines lower in alcohol. This may possibly be achieved due to a less efficient fermentative ability than the wine yeast, *S. cerevisiae*. Competition for sugars by *T. delbrueckii* may also result in less sugar available for ethanol production.

In this project forty-three South African *Torulaspora delbrueckii* yeast isolates from the ARC Infruitec-Nietvoorbij yeast culture collection, the *T. delbrueckii* type strain, commercial *T. delbrueckii* reference strains and a commercial *Saccharomyces cerevisiae* yeast had their identities confirmed and were characterised using conventional and molecular microbiological techniques. The performances of the yeasts were monitored by means of laboratory-scale fermentations in grape must at 15 ºC and 22 ºC. The fermentation kinetic data showed that the yeasts had varying speeds of fermentations. Biochemical and physiological grouping did not coincide with the fermentation abilities and good fermenters could be found in more than one group. Principle Component Analyses of chemical analytical data (alcohol, volatile acidity, glycerol, total SO_2, residual sugar) showed different groupings. The yeasts that grouped close to the *S. cerevisiae* reference strain showed more acceptable wine chemical profiles, while those further away displayed less acceptable profiles. Three locally isolated strains and one commercial *T. delbrueckii* yeast strain, produced wines with acceptable chemical profiles at both temperatures. These strains also had comparable fermentation kinetics to the *S. cerevisiae* reference.

Subsequently the best nine natural *T. delbrueckii* isolates and reference strains were used in the production of small-scale Chenin blanc and Pinotage wines (2009). The small-scale cellar fermentations were carried out at 15 ºC and 24 ºC. Three of the *T. delbrueckii* yeast isolates and the commercial *T. delbrueckii* strain were used as single inoculants, while the *S. cerevisiae* yeast strain was co-inoculated with the other four *T. delbrueckii* yeast isolates at 0 h, 24 h and 48 h, respectively. The wines were chemically and sensorially evaluated and the data statistically analysed. Yeast lees samples of the must from the single inoculant white wines were collected at the end of the fermentations to determine the survival of the *T. delbrueckii* yeast inoculants. A principle component analysis of the chemical analysis, together with the sensory evaluation data, were used to identify the yeast strains that potentially could produce novel wines, either on their own or as a component of co-inoculated fermentations. The results for the white wine trial showed that two natural *T. delbrueckii* isolates had the potential for producing wines that compared well and even exceeded the quality of that produced by the *S. cerevisiae* reference strain with regards to chemical composition and overall sensory quality. The 48 hour treatment did not give good results and was discontinued. The sensory analyses data of the red wines compared well to the *S. cerevisiae* reference wine, but showed no increase in wine quality as seen as in the white wine trial. Furthermore, no pattern regarding inoculation times was observed.

The two best *T. delbrueckii* yeast strains were subsequently evaluated in Chenin blanc over four vintages (2010-1013) utilising *T. delbrueckii*-only and co-inoculated fermentations with *S. cerevisiae* at 0 and 24 h. Distinct stylistic differences were observed and dependant on the desired wine style, a suitable yeast inoculation treatment can be chosen. *T. delbrueckii*-only treatments lead to semi-sweet wines, and co-inoculation treatments lead to dry wines. The *S. cerevisiae* yeast choice makes a critical difference to the fermentation kinetics and wine style. Excessive volatile acidity production by *S. cerevisiae* can be tempered by the use of *T. delbrueckii*. Overall, two South African *T. delbrueckii* isolates fared very well and in some cases were better than the commercial strains. Generally the red wine evaluations showed that stylistic differences could be obtained, but not necessarily an increase in quality. Preliminary observations on polyphenolic analytic data of red wines lead to a separate project investigating chemical profiles of non-*Saccharomyces* wines. The resultant data is not contained in this report, but in the ww10/25 progress report.

Improved *T. delbrueckii* strains by adaptive selection and the evaluation of a potential hybrid met with limited success and was not pursued further, due to the focus of the project on the best initial isolates. Determination of extra-cellular enzymatic activity showed no activity for the enzymes tested.

In conclusion, two *T. delbrueckii* isolates from an initial 43 isolates can be used singly or in co-inoculation with a *S. cerevisiae* yeast for changing styles of wine, producing novel wines and in some instances improving quality. Lowering of ethanol is only practical where a semi-sweet wine is desired. A 0.3% alcohol reduction can be achieved in dry wine, but is probably of no practical use. The two *T. delbrueckii* isolates compare well to the commercial strains and can be considered for commercialisation. Depending on the fermentation temperature, different *T. delbrueckii* strains will be suitable for specific wine styles and some can even be considered for single inoculations (without *S. cerevisiae*) for industrial fermentations. Other outputs for the project included five posters, two papers, two publications and one MTech graduate.

Problem identification and objectives

Problem being addressed and ultimate aim of the project

Increased local and global competition leading to the ever increasing need for improved wine quality, high alcohols unacceptable to consumers and current resistance by consumers to GMO products are some of the ongoing problems faced by the South African wine industry. In an effort to address this situation, further utilisation of non-*Saccharomyces* yeasts needs to be investigated. The objective of this project is therefore to investigate the potential of *Torulaspora delbrueckii* yeasts to produce novel wine, wine with lower alcohol concentrations (single and co-inoculations with *S. cerevisiae*), while maintaining or increasing wine quality during production of both red and white wines.

Workplan (materials and methods)

1. **Confirmation of identity and uniqueness of strains:**
   - Literature search for information regarding *T. delbrueckii*/*C. colliculosa* yeasts,
   - Selection of *T. delbrueckii/C. colliculosa* isolates from the Nietvoorbij microbiology culture collection.
   - Isolation of *T. delbrueckii* reference yeast from commercial blend.
   - Confirmation of identity by ID32C identification strips and PCR-RFLP analyses.
   - Adaptation of CHEF gel electrophoreses running conditions for *T. delbrueckii*.
   - Comparison of isolates by CHEF gel electrophoreses to exclude identical strains.
2. **Laboratory-scale fermentations:**
   - Investigate selected yeasts in a series of laboratory-scale fermentations to determine fermentation rate in comparison to *S. cerevisiae* reference strains.
   - Determine sugar-alcohol conversion rate in comparison to reference strains.

- Determine chemical analyses profile of laboratory-scale wines.
- Select promising isolates for further fermentation studies with and without co-inoculations of *S. cerevisiae*.
- Review data and plan small-scale winemaking trials in conjunction with statistical advice.

3. **Small-scale wine production:**
   - Small-scale wine production with selected yeasts.
   - Repeat small-scale wine production.
4. **Evaluation of small-scale wines:**
   - Chemical and sensory evaluation of small-scale wines.
5. **Determination of extra-cellular enzymatic activity:**
   - Determination of extra-cellular enzymatic activity (according to methods of Strauss *et al*., 2001) to determine possible oenological potential.

Results and discussion

The results of the project are presented in seven parts (Research results I – VII). Research results I covers the initial laboratory trials and forms the basis for the selection of *T. delbrueckii* yeasts for the first vintage's cellar trials (2009). These trials in Chenin blanc and Pinotage are discussed in Research results II. The third part (Research results III) covers four vintages where Chenin blanc wines were produced with a smaller selection of *T. delbrueckii* yeasts. Research results IV deals with adapted and hybrid *T. delbrueckii* yeasts. Research results V covers red wine production while Research results VI deals with further chemical analyses. Research results VII briefly touches on extra-cellular enzyme activity. Research results I and II (Chenin blanc production) were also submitted in a thesis form for the awarding of an MTech degree for Ms Valmary van Breda.

Research results I

Characterisation of commercial and natural *Torulaspora delbrueckii* wine yeast strains

This section covers the initial screening and characterisation of *T. delbrueckii* isolates under laboratory conditions. This data has been published and only the abstract is shown below. The full article is attached as an addendum to this report (Van Breda *et al.*, 2013).

Abstract

Forty-three South African *Torulaspora delbrueckii* yeast isolates from the ARC Infruitec-Nietvoorbij yeast culture collection, the T. delbrueckii type strain (CBS 1146), one reference *T. delbrueckii* strain (CBS 4663), *T. delbrueckii* strains isolated from commercial yeast blends (Viniflora® Harmony.nsac and Viniflora® Melody.nsac), and a commercial *Saccharomyces cerevisiae* yeast had their identities confirmed and were characterised using conventional and molecular microbiological techniques. These included a selection of growth media as well as CHEF electrophoretic karyotyping and PCR-RFLP analyses. Based on the biochemical and physiological results the strains were divided into 13 groups. The performances of the yeasts were also monitored by means of laboratory-scale fermentations in grape must at 15 ºC and 22 ºC. The fermentation kinetic data showed that at 22 ºC, the yeasts were divided into two distinct groups, a faster and a slower fermenting group. The fermentation curves of the laboratory-scale study at 15 ºC showed that, at this lower temperature, the yeasts also fermented at different speeds, but the fermentation curves showed greater separation. The biochemical and physiological grouping did not coincide with the fermentation abilities and good fermenters could be found in more than one group. Chemical analyses of the resultant wines (alcohol, volatile acidity, glycerol, total SO_2, residual sugar) were used in Principle Component Analyses. The yeasts that grouped close to the *S. cerevisiae* reference strain showed more acceptable wine chemical profiles, while those further away displayed less acceptable profiles.

Three locally isolated strains and one commercial *T. delbrueckii* yeast strain, Viniflora® Harmony.nsac produced wines with acceptable chemical profiles at both temperatures. These strains also had comparable fermentation kinetics to the *S. cerevisiae* reference. Therefore, depending on the fermentation temperature, different *T. delbrueckii* strains will be suitable for specific wine styles and some may even be considered for single inoculations without *S. cerevisiae* in industrial fermentations.

Research results II

The use of *Torulaspora delbrueckii* yeasts strains for small-scale wine production: First vintage trials in Chenin blanc and Pinotage

A draft article with the data emanating from the first vintage (2009) for the production of Chenin blanc and Pinotage is attached as Addendum 2. The article will still be submitted for publication (Van Breda *et al*., 2014). Only the article abstract is shown here.

Abstract

Nine natural *Torulaspora delbrueckii* yeast isolates from the ARC Infruitec-Nietvoorbij yeast culture collection, an isolate from a commercial *T. delbrueckii* yeast blend (Viniflora® Harmony.nsac, CHR Hansen) and a commercial *Saccharomyces cerevisiae* yeast strain were used in the production of small-scale white and red wines. The small-scale cellar fermentations were carried out at 15 ºC and 24 ºC over 32 d and 5 d, respectively. Three of the *T. delbrueckii* yeast isolates and the commercial *T. delbrueckii* strain were used as single inoculants, while the *S. cerevisiae* yeast strain was co-inoculated with the other four *T. delbrueckii* yeast isolates at 0 h, 24 h and 48 h, respectively. The small-scale wines were monitored and fermentation curves construed from the data. The resultant wines were bottled and stored for four months. The wines were chemically and sensorially evaluated and the data statistically analysed. Yeast lees samples of the must from the single inoculant white wines were collected at the end of the fermentations and were analysed for evidence of the survival of the *T. delbrueckii* yeast inoculants. A principle component analysis (PCA) of the chemical analysis results, together with the sensory evaluation results, were used to identify the yeast strains that potentially could produce novel wines, either on their own or as a component of co-inoculated fermentations. The results for the white wine trial showed that two natural *T. delbrueckii* isolates had the potential for producing wines that compared well and even exceeded the quality of that produced by the *S. cerevisiae* reference strain with regards to chemical composition and overall sensory quality. During the red wine production, all fermentations were completed after 5 d with residual sugar values below 4 g/mL. Although other analyses compared well to the *S. cerevisiae* reference wine, the red wine sensory results showed no increased quality as seen in the white wine trial. No pattern regarding inoculation times was observed.

Research results III

Chenin blanc trials: 2010 – 2013

The first vintage cellar trials (Research results II) identified the best *T. delbrueckii* isolates for further investigation and for comparison to commercial reference strains. Some of the commercial *T. delbrueckii* reference strains were not available in the early stages of the project, but were included as they became available. Subsequent trials were done over four vintages (2010 – 2013) in Chenin blanc grape must from the Nietvoorbij Research Farm.

Table 3.1 Yeast strains used in this section.

Strain no.	Obtained/isolated from	Origin	Year isolated	Identity
VIN 13[1]	Anchor Bio-Technologies	SU[2]	unknown	*S. cerevisiae*
VIN 7[1]	Anchor Bio-Technologies	ARC Inf-Nvb[3]	unknown	*S. cerevisiae*
654	ARC Inf-Nvb	Robertson	1995	*T. delbrueckii*
M2/1	ARC Inf-Nvb	Robertson	1998	*T. delbrueckii*
Har[4]	CHR Hansen	unknown	unknown	*T. delbrueckii*
Lall[5]	Lallemand	unknown	n/a	*T. delbrueckii*
Laff[6]	Laffort Oenology	unknown	n/a	*T. delbrueckii*

[1]Commercial *S. cerevisiae* wine yeast. [2]Stellenbosch University. [3]ARC Infruitec-Nietvoorbij Culture Collection. [4]*T. delbrueckii* strain isolated from Viniflora Harmony.nsac commercial active dried yeast blend (CHR Hansen, Denmark). [5]*T. delbrueckii* strain isolated from Enoferm Level 2^{Tm} TD^{Tm} active dried yeast (Lallemand, Canada). [6]*T. delbrueckii* strain isolated from Zymaflore® Alpha TD $^{n.sacch.}$ active dried yeast (Laffort Oenology, France).

Natural and commercial *T. delbrueckii* reference strains were used to produce Chenin blanc wines over four vintages from 2010-2013 (Table 3.1). Small-scale wine production (18L) was carried out in 20L stainless steel canisters fitted with fermentation locks. The two best ARC *T. delbrueckii* isolates (654, M2/1), as identified in the first vintage (Research Results II, 2009 data), were compared to commercial reference yeasts. The reference yeasts were isolates from active dried yeasts Harmony.nsac (Har) from CHR Hansen, Enoferm Level 2^{Tm} TD^{Tm} (Lall) from Lallemand and Zymaflore® Alpha$^{Td.nsacch.}$ (Laff) from Laffort Oenology. The Laffort Oenology yeast was only available from 2011. An active dried *S. cerevisiae* (strain VIN 13, Anchor Bio-Technologies) was also included as a control and for co-inoculation. The inoculation protocol used the *T. delbrueckii* yeasts singularly and in co-inoculations where *S. cerevisiae* was inoculated at time zero and 24 h later (treatments and replicates are shown in Table 3.2). A 48 hour treatment was initially included but as poor wine quality was obtained, this treatment was discontinued (data not shown). From 2011 a spontaneous fermentation treatment was also included to show the influence of the background yeast population. The *T. delbrueckii* yeast inoculum was cultivated in a three step propagation protocol in YPD broth (Merck, South Africa) while the active dried S. cerevisiae was rehydrated and inoculated according to manufacturer's recommendations. The must analyses is given in Table 3.3.

Table 3.2 Yeast treatments used for small-scale Chenin blanc production with single and combined cultures of *T. delbrueckii* and *S. cerevisiae* yeasts.

Treatment and time of the *S. cerevisiae* inoculation	**Treatment abbreviation[1]**	**Replicates per vintage**			
		2010	**2011**	**2012**	**2013**
S. cerevisiae VIN13 reference	S	2	2	3	3
T. delbrueckii 654	6	2	2	3	3
T. delbrueckii 654 & VIN 13 (0h)	6_0	2	2	3	3
T. delbrueckii 654 & VIN 13 (24h)	6_24	2	2	3	3
T. delbrueckii M2/1	M	2	2	3	3
T. delbrueckii M2/1 & VIN 13 (0h)	M_0	2	2	3	3
T. delbrueckii M2/1 & VIN 13 (24h)	M_24	2	2	3	3
T. delbrueckii Har	H	2	2	3	3
T. delbrueckii Har & VIN 13 (0h)	H_0	2	2	3	3
T. delbrueckii Har & VIN 13 (24h)	H_24	2	2	3	3
T. delbrueckii Lall	La	2	2	3	3
T. delbrueckii Lall & VIN 13 (0h)	La_0	2	2	3	3
T. delbrueckii Lall & VIN 13 (24h)	La_24	2	2	3	3
T. delbrueckii Laff	Lf	Nd[2]	2	3	3
T. delbrueckii Laff & VIN 13 (0h	Lf_0	Nd[2]	2	3	3
T. delbrueckii Laff & VIN 13 (24h)	Lf_24	Nd[2]	2	3	3
Spontaneous	Sp	Np[3]	2	3	3

[1]Abbreviations used in multivariate analyses plots. [2]No data as the Laffort yeast was not commercially available in 2010. [3]Np = not produced.

Table 3.3 Chenin blanc base must analyses.

Vintage	**pH**	**Total acidity (g/L)**	**Sugar concentration (°B)**
2010	3.31	7.1	22.4
2011	3.41	7.9	21.6
2012	3.49	7.5	20.4
2013	3.41	8.1	20.5
Average ± standard deviation	*3.41 ± 0.07*	*7.7 ± 0.4*	*21.2 ± 1.0*

Fermentation kinetics and residual sugars

Fermentations were monitored by CO_2 mass loss (Figs 3.1 - 3.4). Towards the end of fermentation the residual sugars were measured and those showing no further reduction in residual sugar were stopped. Over the four vintages a similar fermentation pattern was observed with the single *T. delbrueckii* yeast fermentations being slower than the co-inoculated fermentations (Figs. 3.1 – 3.4). The single *T. delbrueckii* fermentations took on average 31 days to reach a stationary phase, five days longer than the spontaneous fermentations of 26 days (Table 3.4). The co-inoculated fermentations took between 20 and 22 days for the zero and 24 hour inoculations, respectively, which was similar to the *S. cerevisiae* reference fermentations. The residual sugars of the *T. delbrueckii*-only fermentations varied greatly (from < 2g/L to over 10 g/L residual sugar). The spontaneous fermentations varied from dry to incomplete fermentations.

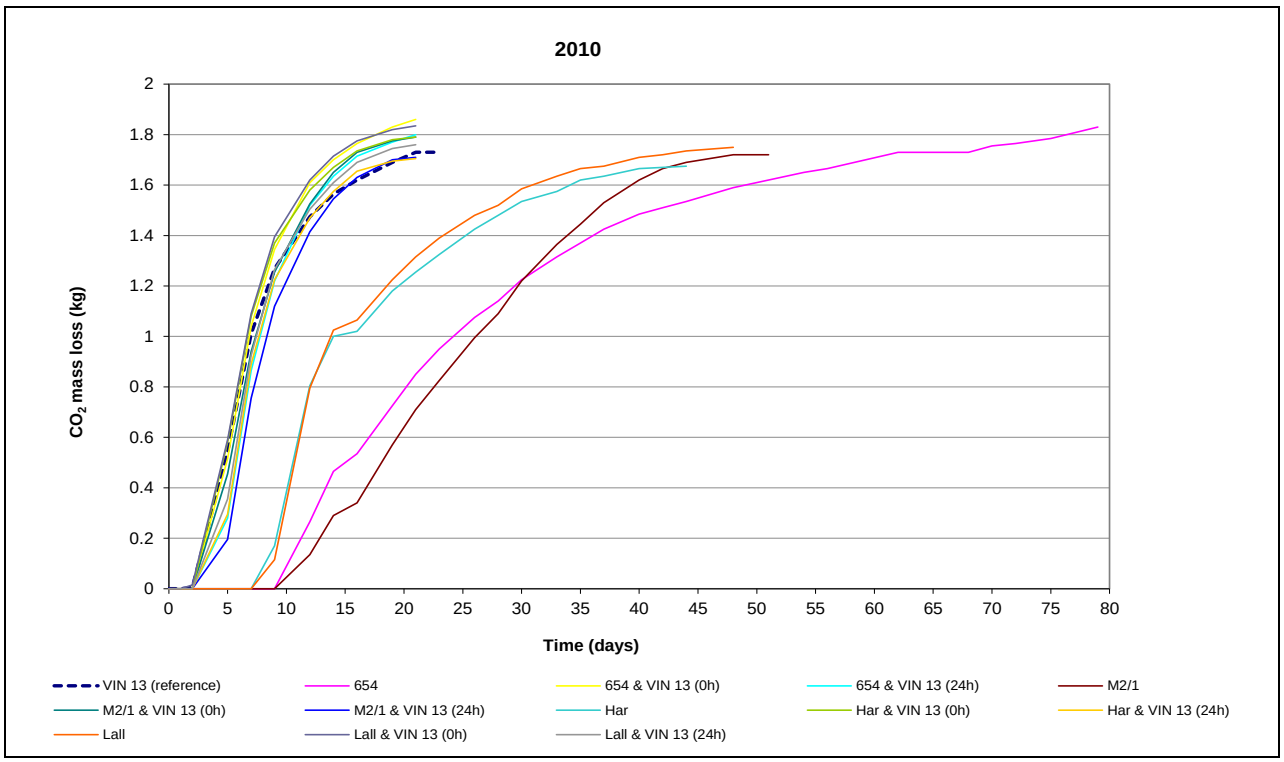


Fig. 3.1 Average fermentation curves (two replicates) based on mass-loss of small-scale Chenin blanc 2010 wines produced by combinations of *T. delbrueckii* and *S. cerevisiae* yeasts (base must: 22.4°B, 7.1 g/L total acidity, pH 3.31). No spontaneous fermentation was conducted in 2010.

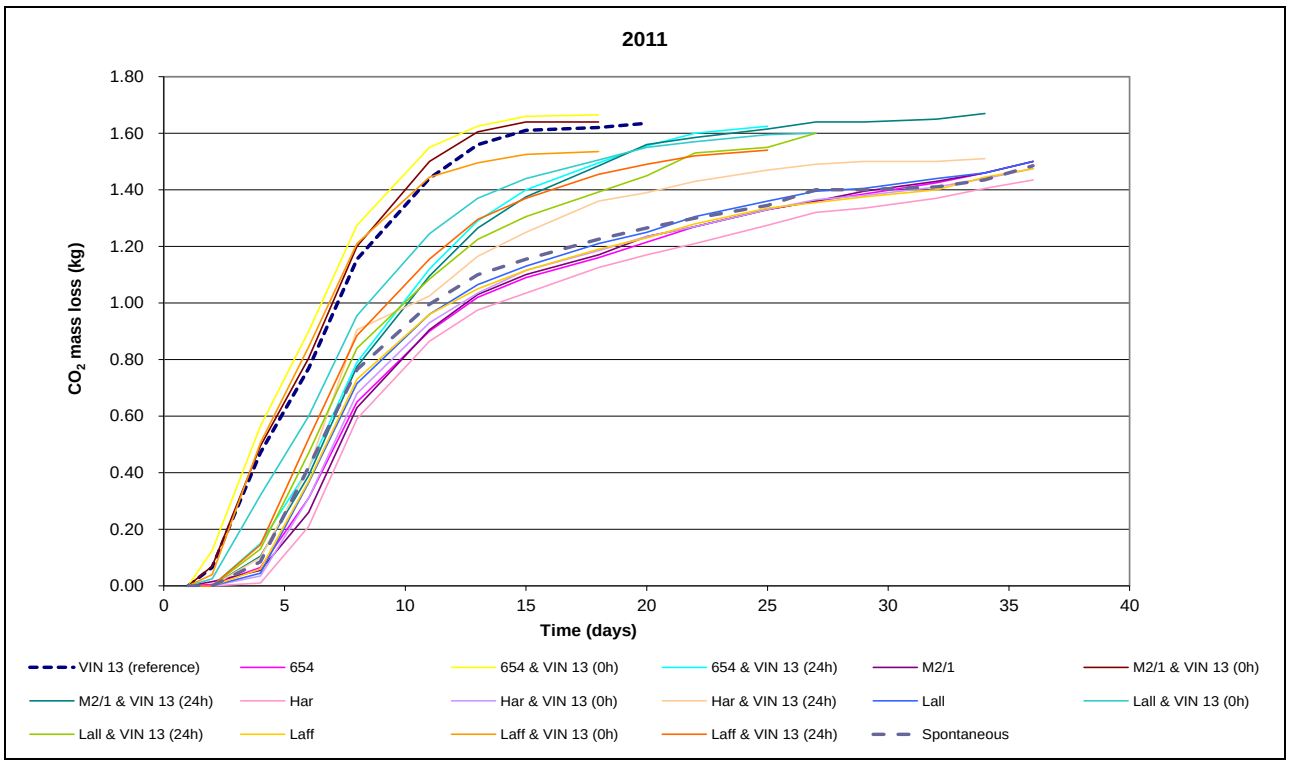


Fig. 3.2 Average fermentation curves (two replicates) based on mass-loss of small-scale Chenin blanc 2011 wines produced by single and combined cultures of *T. delbrueckii* and *S. cerevisiae* yeasts (base must: 21.6°B, 7.9 g/L total acidity, pH 3.41).

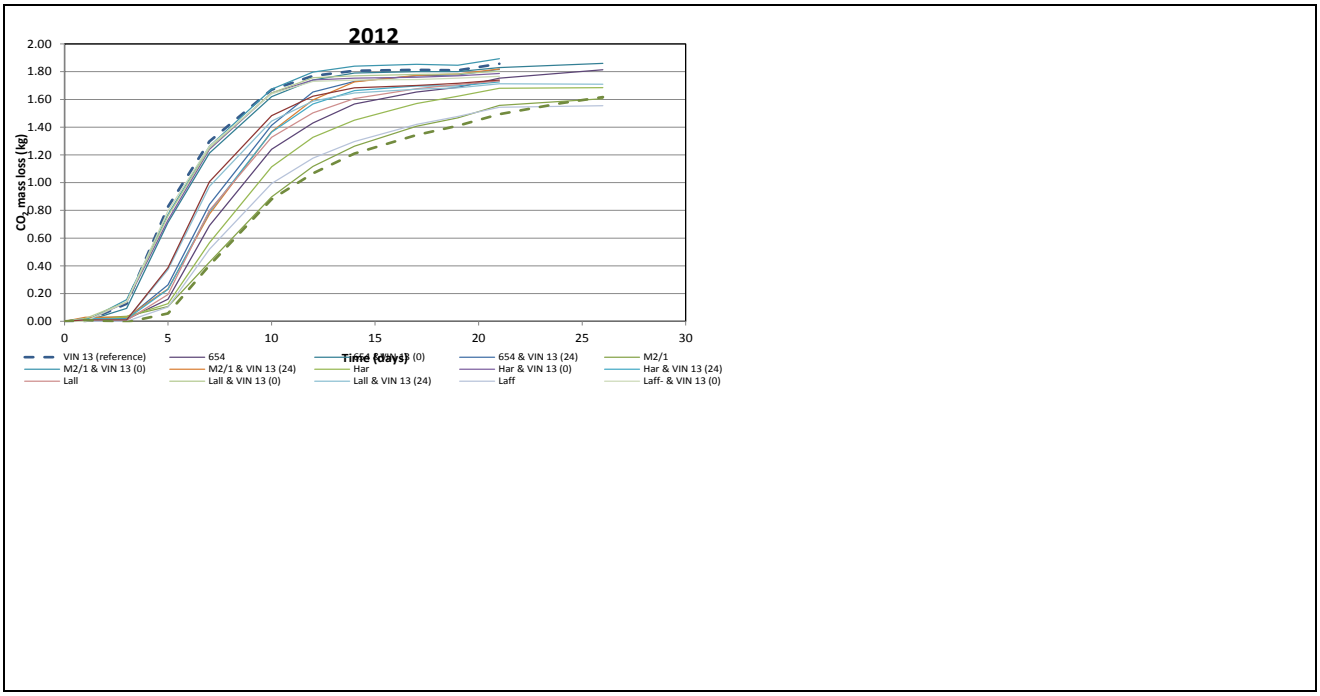


Fig. 3.3 Average fermentation curves (three replicates) based on mass-loss of small-scale Chenin blanc 2012 wines produced by single and combined cultures of *T. delbrueckii* and *S. cerevisiae* yeasts (base must: 20.4°B, 7.5 g/L total acidity, pH 3.49).

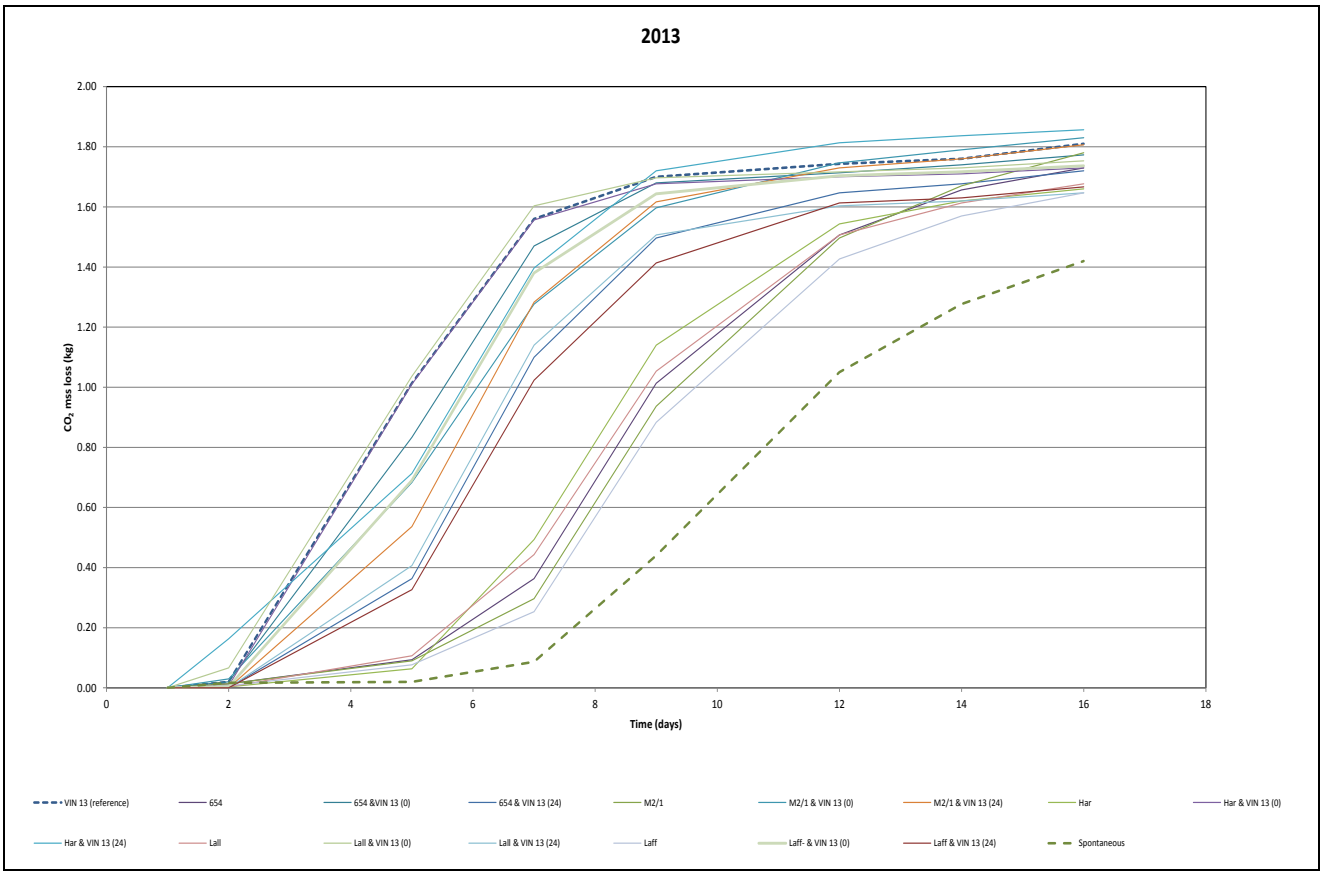


Fig. 3.4. Average fermentation curves (three replicates) based on mass-loss of small-scale Chenin blanc 2013 wines produced by single and combined cultures of *T. delbrueckii* and *S. cerevisiae* yeasts (base must: 20.5°B, 8.1 g/L total acidity, pH 3.41).

Table3.4 Residual sugar and number of days of fermentation of Chenin blanc produced between 2010 and 2013.

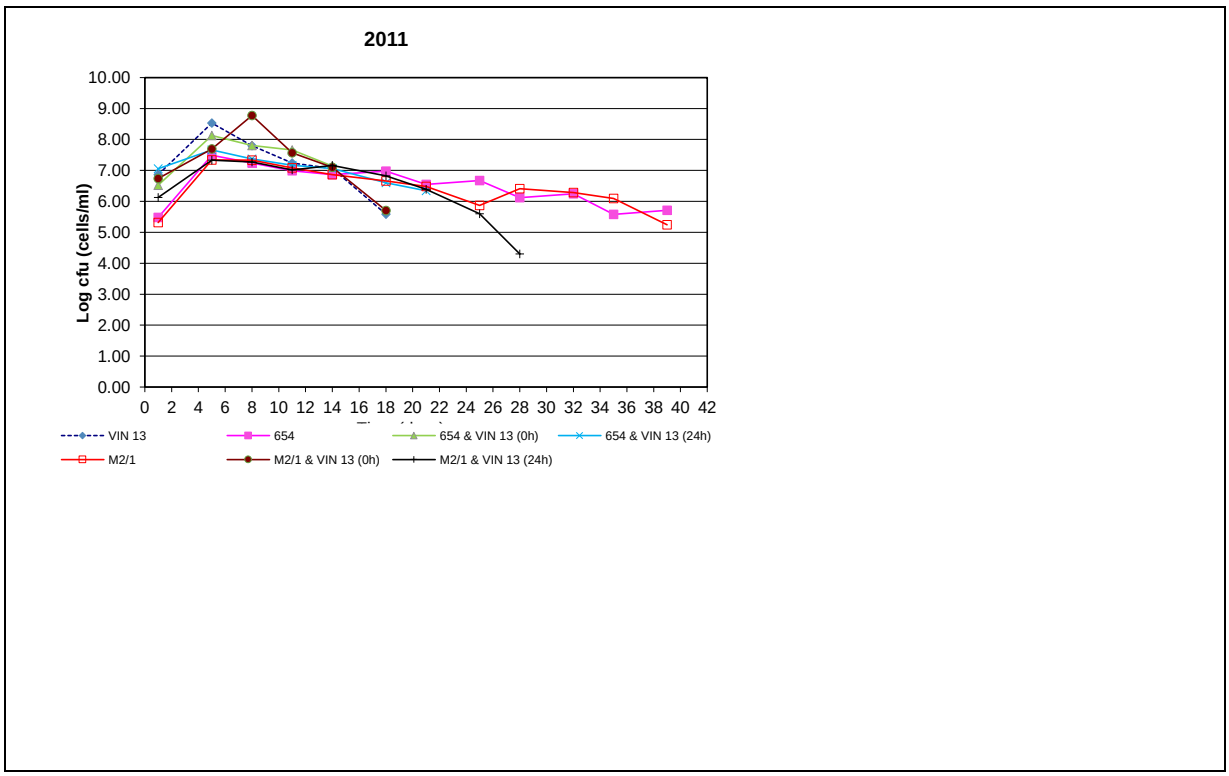
Yeast treatment	Duration of fermentation (Days)			Residual sugar (g/L)			
	Ave.	Range		Ave.	± *SD*[1]	Range	
		Min.	Min.			Min.	Max.
VIN13 reference	20	16	21	2.44	*1.42*	1.35	5.33
654	39	16	79[2]	10.10	*10.84*	1.77	30.78
M2/1	31	16	51	9.89	*9.99*	1.97	30.54
Har	31	16	44	7.04	*7.91*	1.51	22.77
Lall	30	16	48	5.75	*5.94*	1.66	17.14
Laff	26	16	36	8.37	*7.50*	1.73	18.05
Averages of T. delbrueckii-only	*31*			*8.23*			
654 & VIN 13 (0h)	20	16	26	2.56	*1.02*	1.64	4.68
M2/1 & VIN 13 (0h)	19	16	21	1.98	*0.46*	1.38	2.77
Har & VIN 13 (0h)	24	16	36	6.00	*8.31*	1.56	26.11
Lall & VIN 13 (0h)	21	16	27	2.09	*0.67*	1.55	3.32
Laff & VIN 13 (0h)	18	16	21	1.88	*0.33*	1.55	2.53
Average of co-inoculations (0h)	*20*			*2.90*			
654 & VIN 13 (24h)	21	16	25	2.44	*1.12*	1.34	4.48
M2/1 & VIN 13 (24h)	23	16	34	2.25	*0.91*	1.43	4.19
Har & VIN 13 (24h)	23	16	34	2.69	*2.10*	1.31	8.39
Lall & VIN 13 (24h)	23	16	27	2.92	*2.24*	1.05	8.59
Laff & VIN 13 (24h)	21	16	25	2.09	*0.86*	1.20	3.68
Average of co-inoculations (24h)	*22*			*2.48*			
Spontaneous	26	16	36	16.81	*12.35*	2.48	39.07

[1]SD = Standard deviation. [2]Fermentations stopped on day 79.

Survival of *T. delbrueckii* yeasts during fermentation

In order to investigate the extent of *T. delbrueckii* participation and survival during the fermentations, the presence of strains 654 and M2/1 were monitored in their respective single and co-inoculated fermentations during 2011 and 2012. Total cell counts (*T. delbrueckii*, *S. cerevisiae* and any background indigenous yeast populations) initially followed a similar pattern to that of the *S. cerevisiae* reference. An initial increase followed by a slow decrease (Fig. 3.5).

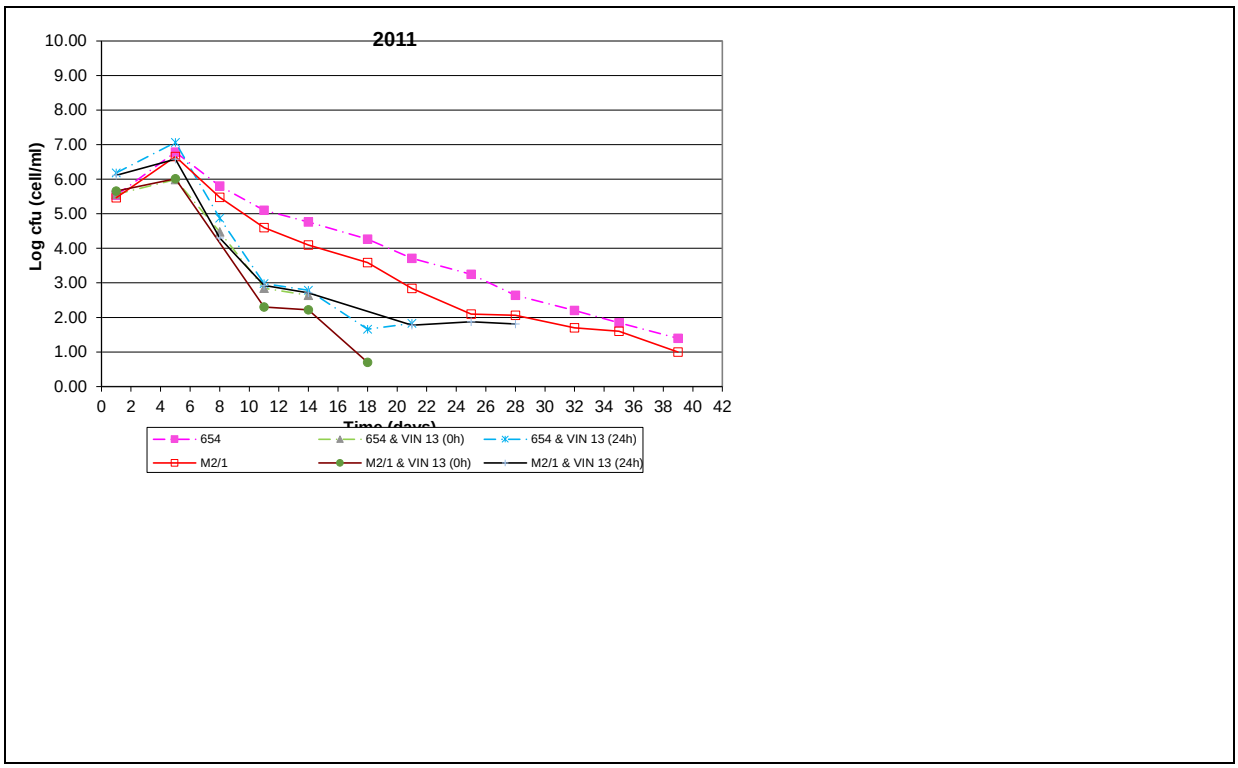
Cell counts done on mannitol agar (representative of the *T. delbrueckii* population only) showed a similar initial pattern of growth (albeit at a lower concentration), but then declined sharply towards the end of fermentation (Fig. 3.6). The initial increase in population was much less in 2012. In contrast an increase in population of *T. delbrueckii* was observed in some cases towards the end of fermentation. In 2011 individual colonies were purified and subjected to identification by ID32C (BioMérieux, South Africa) to confirm identity (Table 3.5). With the exception of the M2/1 24 hour co-inoculated fermentation, the *T. delbrueckii* yeasts were present between 70 to 80% of the duration of the fermentation.



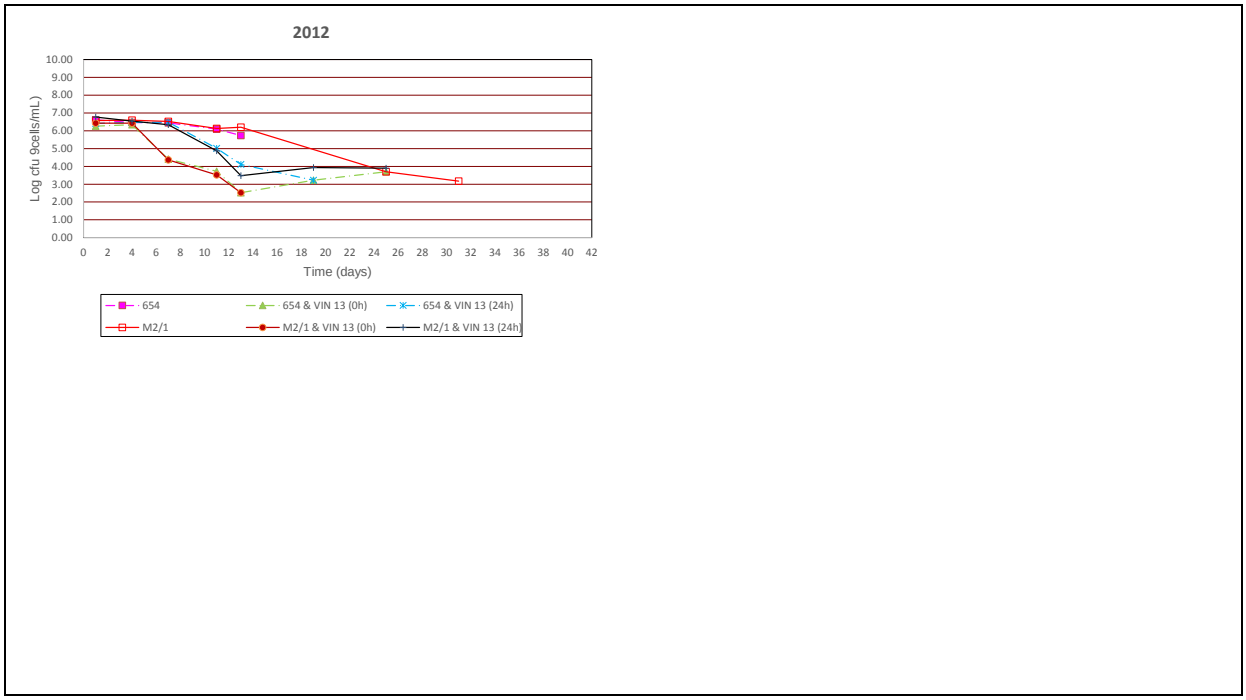
(a)

(b)

Fig. 3.5 Total cell counts (cfu) of *S. cerevisiae* (VIN 13) and *T. delbrueckii* (654 and M2/1) on YPD agar in single and co-inoculated fermentations in (a) 2011 and (b) 2012.



(a)



(b)

Fig. 3.6 *T. delbrueckii* cell counts (cfu) on mannitol agar in single (654, M2/1) and co-inoculated (with VIN 13 after 0 or 24h) fermentations in (a) 2011 and (b) 2012.

Table 3.5 Number of days that *T. delbrueckii* was detected in single and co-inoculated fermentations in 2011.

Fermentation treatment	***Yeast identification by ID32C[1]***	***Last day of T. delbrueckii detection (Days)***	***Total fermentation time (Days)***	***Length of T. delbrueckii presence (%)***
VIN 13	*S. cerevisiae*	Na[2]	20	Na[2]
654	*T. delbrueckii*	32	39	82
654 & VIN 13 (0h)	*T. delbrueckii*	14	20	70
654 & VIN 13 (24h)	*T. delbrueckii*	21	25	84
M2/1	*T. delbrueckii*	32	39	82
M2/1 & VIN 13 (0h)	*Nd[3]*	Nd[3]	18	Nd[3]
M2/1 & VIN 13 (24h)	*T. delbrueckii*	14	35	40

[1] BioMérieux, South Africa. [2]Not applicable. [3]No data available.

Chemical analyses of Chenin blanc wines produced from 2010 to 2011

Standard chemical analyses data showed that volatile acidity analyses, in comparison to the *S. cerevisiae* reference wine, tended to be higher for the *T. delbrueckii*-only wines, but lower than the spontaneous fermentations (Table 3.6). The co-inoculated wine's volatile acidity levels were similar or slightly higher. However, all the volatile acidity levels were still within acceptable limits. Alcohol levels for the *T. delbrueckii* single and co-inoculated fermentations were on average 0.3% lower than the *S. cerevisiae* reference wines and although notable, of limited practical use to the wine industry in pursuit of lower alcohol wines. As previously mentioned, total residual sugar for *T. delbrueckii*-only wines varied across the different strains, but mostly the wines could be classified as semi-sweet (5-10 g/L sugar according to legislation). The co-inoculated fermentations in contrast led to dry wines (< 5 g/L sugar) with the exception of the *T. delbrueckii* Harmony treatment. The spontaneous fermentations also varied, but mostly lead to incomplete fermentations. Glycerol levels varied slightly between treatments, but were generally similar to each other. Total SO_2 levels were only measured for 2010 wines and they were between 1 and 20 g/L higher than the *S. cerevisiae* reference wine (data not shown). The higher total SO_2 was mainly for the single *T. delbrueckii* fermentations with the co-inoculations mostly of a comparable level to the *S. cerevisiae* wines.

Table 3.6 Average chemical analyses of Chenin blanc wines from 2010 to 2013 produced by *S. cerevisiae* and *T. delbrueckii* in single and co-inoculated fermentations.

Yeast treatment	Volatile acidity (g/L)		Total acidity (g/L)		Glucose (g/L)		Fructose (g/L)		Total sugar (glucose + fructose) (g/L)		Alcohol (% $^{v}/_{v}$)		Glycerol (g/L)	
	Ave	±SD	Ave	±SD	Ave	±SD	Ave	±SD	Ave	±SD	Ave	±SD	Ave	±SD
VIN13 (reference)	0.29	*0.05*	5.77	*0.28*	0.50	*0.21*	1.94	*1.57*	2.44	*1.42*	12.77	*0.77*	6.65	*0.61*
654	0.36	*0.05*	5.69	*0.23*	1.70	*1.35*	8.40	*9.66*	10.10	*10.84*	12.21	*0.80*	6.88	*0.56*
M2/1	0.38	*0.04*	5.69	*0.25*	1.50	*1.49*	8.39	*8.78*	9.89	*9.99*	12.24	*0.88*	6.95	*0.72*
Har	0.42	*0.06*	5.69	*0.25*	0.84	*0.66*	6.20	*7.44*	7.04	*7.91*	12.39	*0.77*	6.44	*0.64*
Lall	0.38	*0.04*	5.64	*0.27*	0.79	*0.63*	4.95	*5.96*	5.75	*5.94*	12.47	*0.73*	6.55	*0.76*
Laff	0.40	*0.04*	5.68	*0.29*	1.38	*0.88*	7.00	*7.47*	8.37	*7.50*	12.03	*0.32*	6.74	*0.69*
654 & VIN 13 (0h)	0.29	*0.05*	5.74	*0.28*	0.85	*0.67*	1.72	*1.19*	2.56	*1.02*	12.71	*0.80*	6.74	*0.70*
M2/1 & VIN 13 (0h)	0.30	*0.04*	5.73	*0.30*	0.56	*0.24*	1.42	*0.54*	1.98	*0.46*	12.72	*0.88*	6.62	*0.63*
Har & VIN 13 (0h)	0.32	*0.04*	5.72	*0.31*	0.98	*1.27*	5.01	*7.27*	6.00	*8.31*	12.51	*0.85*	6.54	*0.71*
Lall & VIN 13 (0h)	0.31	*0.04*	5.70	*0.31*	0.39	*0.26*	1.70	*0.80*	2.09	*0.67*	12.72	*0.82*	6.52	*0.67*
Laff & VIN 13 (0h)	0.29	*0.03*	5.69	*0.30*	0.68	*0.48*	1.20	*0.21*	1.88	*0.33*	12.39	*0.35*	6.46	*0.81*
654 & VIN 13 (24h)	0.33	*0.05*	5.73	*0.25*	0.62	*0.45*	1.82	*1.04*	2.44	*1.12*	12.71	*0.78*	6.94	*0.33*
M2/1 & VIN 13 (24h)	0.34	*0.03*	5.75	*0.28*	0.57	*0.37*	1.68	*0.86*	2.25	*0.91*	12.72	*0.81*	6.87	*0.47*
Har & VIN 13 (24h)	0.36	*0.04*	5.75	*0.23*	0.56	*0.45*	2.13	*1.77*	2.69	*2.10*	12.72	*0.78*	6.84	*0.31*
Lall & VIN 13 (24h)	0.35	*0.07*	5.77	*0.24*	0.93	*1.44*	1.99	*1.22*	2.92	*2.24*	12.68	*0.73*	7.17	*0.51*
Laff & VIN 13 (24h)	0.35	*0.06*	5.84	*0.22*	0.46	*0.26*	1.63	*1.07*	2.09	*0.86*	12.35	*0.32*	6.99	*0.39*
Spontaneous	0.42	*0.08*	5.65	*0.15*	2.97	*2.95*	13.84	*9.81*	16.81	*12.35*	11.65	*0.88*	7.02	*1.15*

Sensory analyses of Chenin blanc wines from 2010 to 2013

A perusal of the sensory data presented in Table 3.7 shows no major differences, with the exception of sweetness intensity, between the *T. delbrueckii* wines in comparison to the *S. cerevisiae* reference wine for the parameters evaluated. These included "fruit and fermentation aroma" intensity, "body/mouth feel" intensity and "overall quality". It would therefore appear superficially that the various yeast treatments had no effect on the sensory attributes of the wines and did not affect the overall quality score, albeit the wines were of different styles. To further investigate the impact on wine style by the various yeast treatments, the chemical and sensory data were combined and subjected to multivariate analyses. The results are discussed in the next section.

Table 3.7 Sensory intensity attributes[1] of Chenin blanc wines from 2010 to 2013 produced by *S. cerevisiae* and *T. delbrueckii* single and co-inoculated fermentations.

Yeast treatment	Fruity & fermentation aroma	Sweetness (%)	Body/Mouth feel (%)	Overall quality (%)
VIN13 reference	49.3ab	16.3b	43.0cde	45.8abc
654	46.3ab	37.5a	47.2abc	43.5abc
M2/1	51.3a	39.0a	48.4ab	47.4ab
Har	47.9ab	30.6ab	46.6abcd	44.5abc
Lall	46.6ab	26.5ab	46.2abcde	45.0abc
Laff	46.1ab	30.6ab	45.2bcde	44.0abc
654 & VIN 13 (0h)	48.4ab	14.2b	44.1bcde	45.3abc
M2/1 & VIN 13 (0h)	45.0b	12.6b	41.4e	42.4bc
Har & VIN 13 (0h)	48.7ab	27.5ab	45.4abcde	47.3ab
Lall & VIN 13 (0h)	49.0ab	14.0b	43.7bcde	46.7ab
Laff & VIN 13 (0h)	46.4ab	15.1b	44.3bcde	41.3c
654 & VIN 13 (24h)	49.6ab	14.9b	43.6bcde	47.3ab
M2/1 & VIN 13 (24h)	46.4ab	15.8b	41.5de	42.8bc
Har & VIN 13 (24h)	47.2ab	16.8b	44.7bcde	44.6abc
Lall & VIN 13 (24h)	46.8ab	16.1b	43.9bcde	45.4abc
Laff & VIN 13 (24h)	46.3ab	16.3b	43.8bcde	44.7abc
Spontaneous	50.5a	42.6a	50.4a	48.4a

[1]Average value of wines. Values within columns, for the same attribute, followed by the same letter do not differ significantly ($p < 0.05$).

Multivariate biometrical analyses of standard chemical and sensory data

The standard chemical and sensory analyses data of the 2010 to 2013 wines were subjected to multivariate analyses by principle component analyses (PCA). The PCA plot showed distinct groupings, with the exception of the 2013 spontaneous fermentation, corresponding to the vintages (Fig. 3.7, circled areas are for illustration only and do not represent statistical significance).The 2010 data does not show due to their being too few data points. The 2011 wines grouped into two groups. The one containing the spontaneous fermentations and most of the single *T. delbrueckii* fermentations and the other group comprising of the co-inoculated and *S. cerevisiae* fermentation. It is thus apparent that vintage differences were the greater driver in wine style differentiation. Separation on the F1 axis was due to differences in pH, sugar analyses (glucose, fructose, total sugar) and all the sensory parameters. Separation on the F2 axis was due to volatile acidity, malic acid and ethanol values.

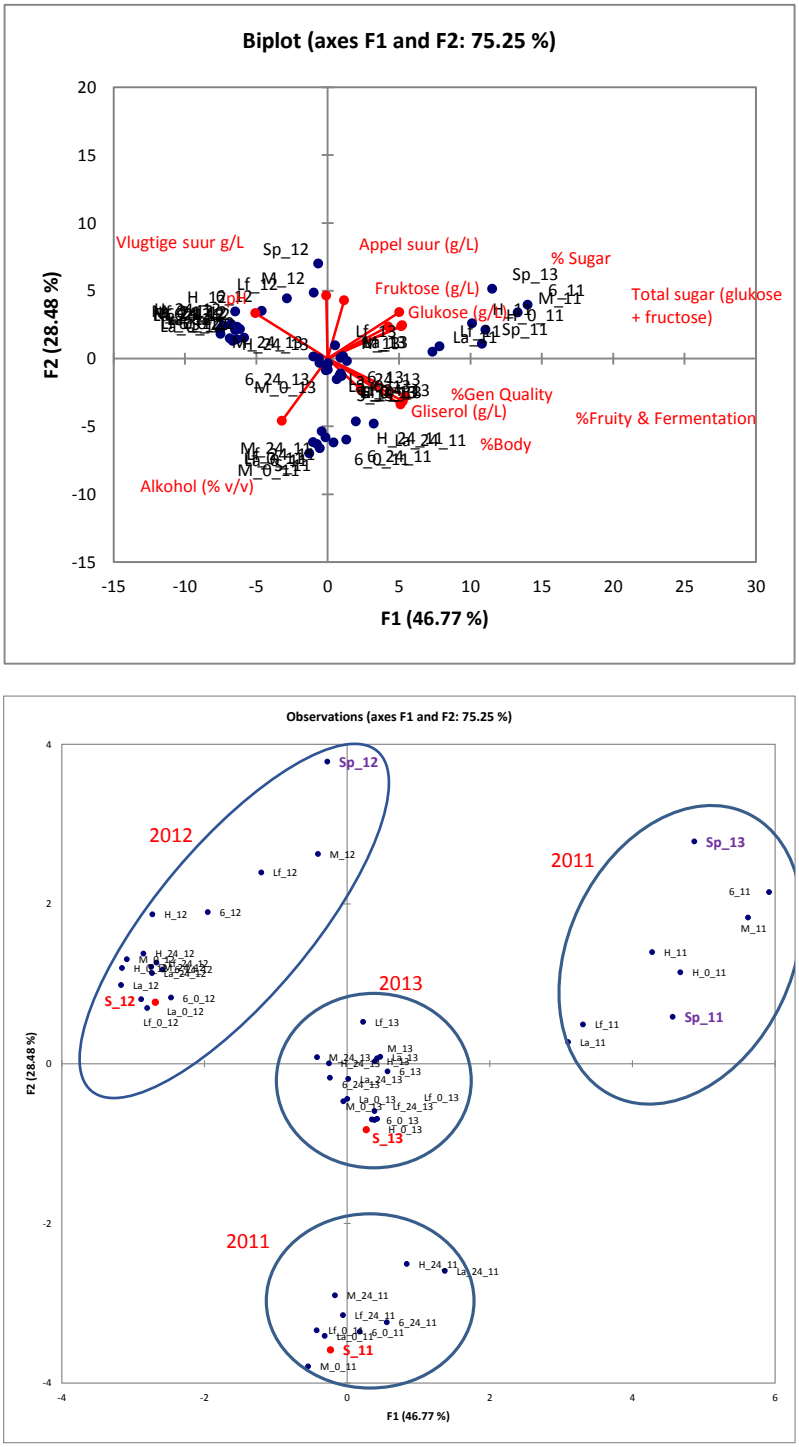


Fig 3.7 Principle Component Analyses of standard chemical and sensory data of Chenin blanc wines 2010-2011. Circled areas are for illustration only and do not represent statistical significance. Treatment abbreviations are shown in Table 2.

Subsequently a discriminant analyses (DA) was done on the data per vintage (Fig. 3.8). With the exception of some outliers, grouping per vintage was 95% significant according to fermentation treatment, i.e. *T. delbrueckii*-only, 0 hours co-inoculation and 24 hours co-inoculation. The *S. cerevisiae* reference fermentation treatment position varied over vintages, sometimes grouping with the *T. delbrueckii*-only (2011-2013) and sometimes with the *T. delbrueckii* 0 hours co-inoculation (2010). In the 2011 to 2013 vintages where the *S. cerevisiae* treatment grouped with *T. delbrueckii*-only treatment can be examples of where the *T. delbrueckii* was out-competed by the background natural *S. cerevisiae* yeast population and the wine profile/style was more akin to a *S. cerevisiae* wine. Likewise, in 2010 where the

S. cerevisiae grouped with *T. delbrueckii* 0 hour treatment can be indicative of the inoculated *S. cerevisiae* outcompeting the *T. delbrueckii* yeast. In the same vintages where the 0 hour co-inoculations grouped separately from the *S. cerevisiae* fermentation shows that the *T. delbrueckii* component was influencing the wine profile and style. This is supported by the spontaneous fermented wines also grouping with the 0 hour co-inoculation (2011-2013). Statistical separation was mostly on the F1 axis and the most important variables are shown in Table 3.8.

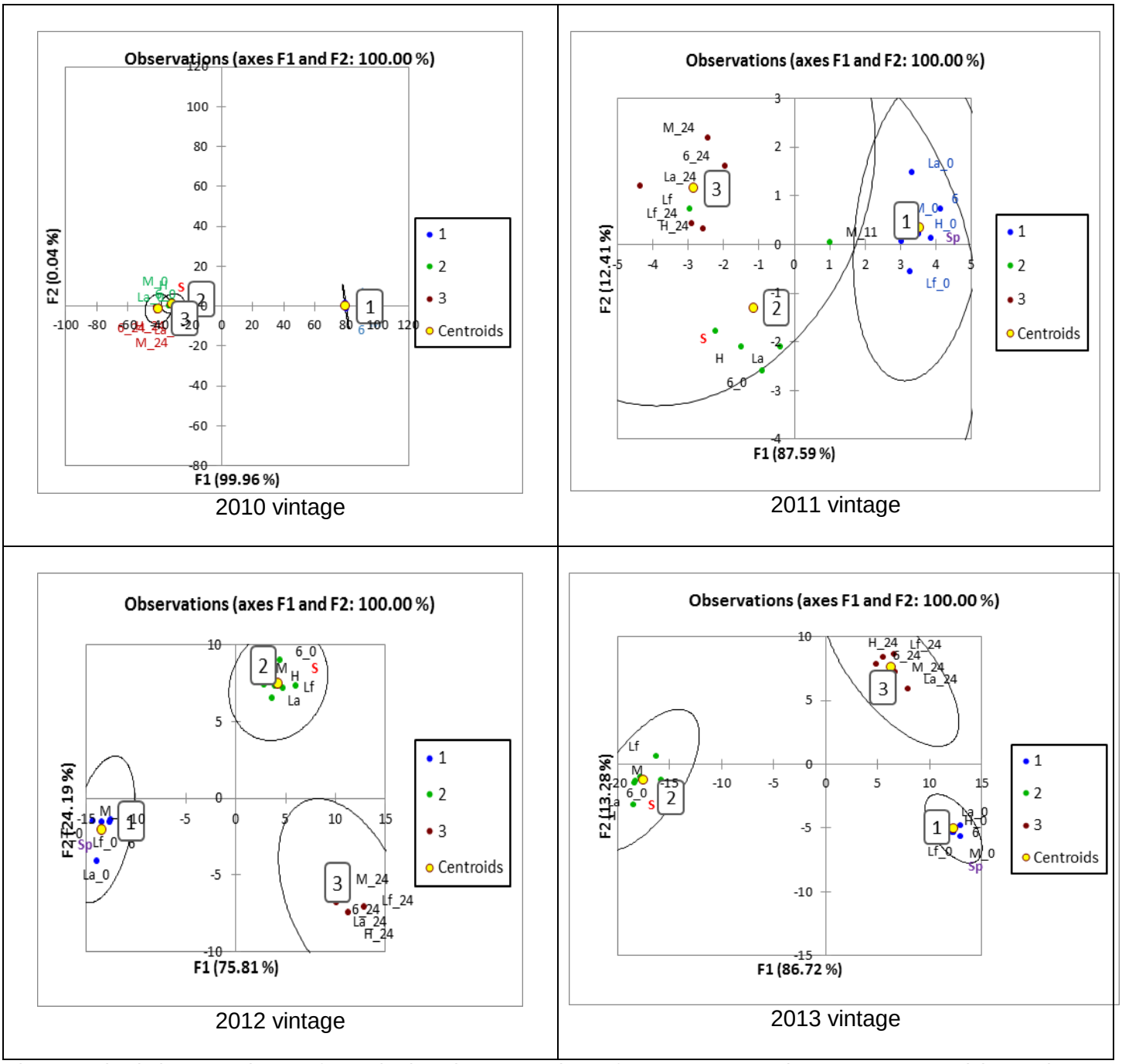


Fig. 3.8 Discriminant analyses of standard and sensory data of 2010 to 2013 wines. S = *S. cerevisiae* and Sp = spontaneous fermentation. Other treatment abbreviations are shown in Table 2.

Table 3.8 Discriminant analyses variables and factor correlations larger than 0.600 driving grouping of wines over vintages.

Variable	F1				F2			
	Vintages				Vintages			
	2010	2011	2012	2013	2010	2011	2012	2013
pH	0.945	-0.746	0.587	0.723				
Volatile acidity	0.847	0.693		0.894			-0.708	
Malic acid		0.828		-0.624				
Glukose			-0.759					
Fructose	0.843	0.898	-0.605					
Total sugar	0.849	0.875	-0.619					
Alcohol	-0.887	-0.876	0.670					
Glycerol		-0.657		0.769			-0.830	
% Fruity intensity								
% Sweetness intensity		0.909	-0.708					
% Body intensity		0.878						
% General quality intensity	-0.604					0.663		

Volatile aroma compounds: multivariate biometrical analyses of data of wines from the 2011 vintage

The Chenin blanc 2011 wines were analysed for volatile aroma compounds by GC-FID at the Central Analytical Facility, Stellenbosch University. A discriminant analyses with model selection (propanol, isobutanol, acetoin, hexanol, butanol, isobutyric acid) was done and the results showed three groups of wines (Fig. 3.9) corresponding to the inoculation strategy. There was some overlapping between 0 hour inoculation and *T. delbrueckii*-only. The *S. cerevisiae* reference fermentation (S in Fig. 3.9) grouped with the 0 hour inoculation strategy showing that this strategy had a "*S. cerevisiae*" type of aromatic profile and the *T. delbrueckii* may have had a limited influence on the volatile aromatic profile. The spontaneous fermentation (Sp in Fig. 3.9) grouped with the *T. delbrueckii*-only fermentations showing that these wines were more akin to a controlled spontaneous fermentation. The 24 hour inoculation strategy was significantly different. Separation between the 24 hour inoculation and the other two groups were due differences in butanol and isobutyric acid. The grouping based on the volatile aroma compounds is in agreement with that of the standard chemical and sensory data, with the exception of the *S. cerevisiae* reference (S) and Spontaneous fermentations (Sp) (Fig. 3.8, 2011 vintage).

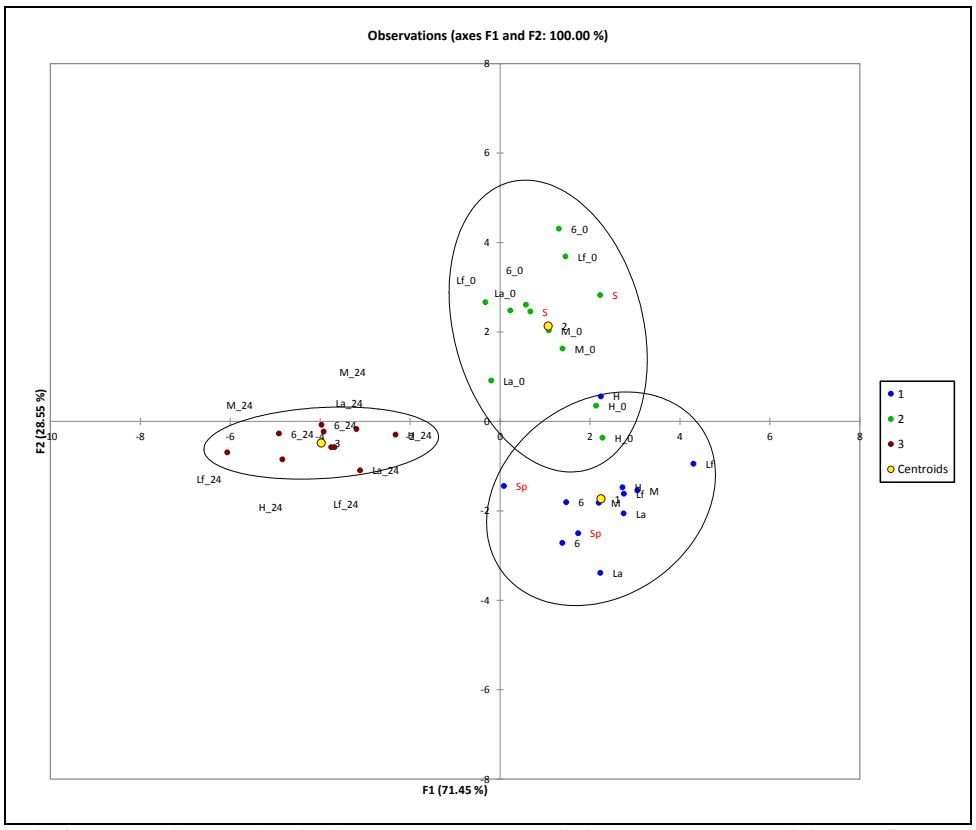


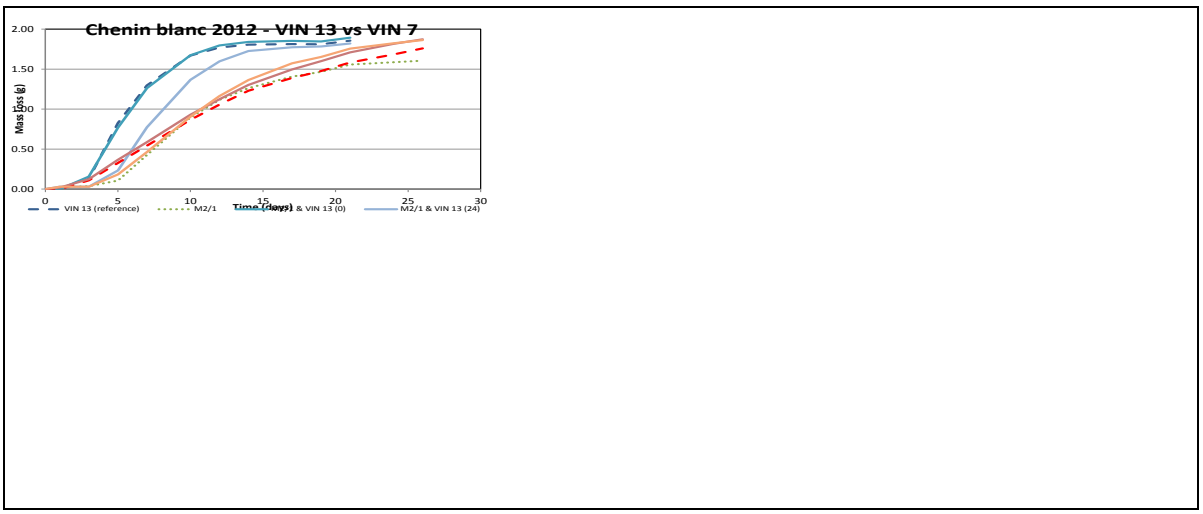
Fig. 3.9. Discriminant analyses of volatile aroma compound data. S = S. cerevisiae and Sp = spontaneous fermentation. Other treatment abbreviations are shown in Table 2.

Co-inoculation with two different *S. cerevisiae* yeasts: strain VIN 13 vs VIN 7

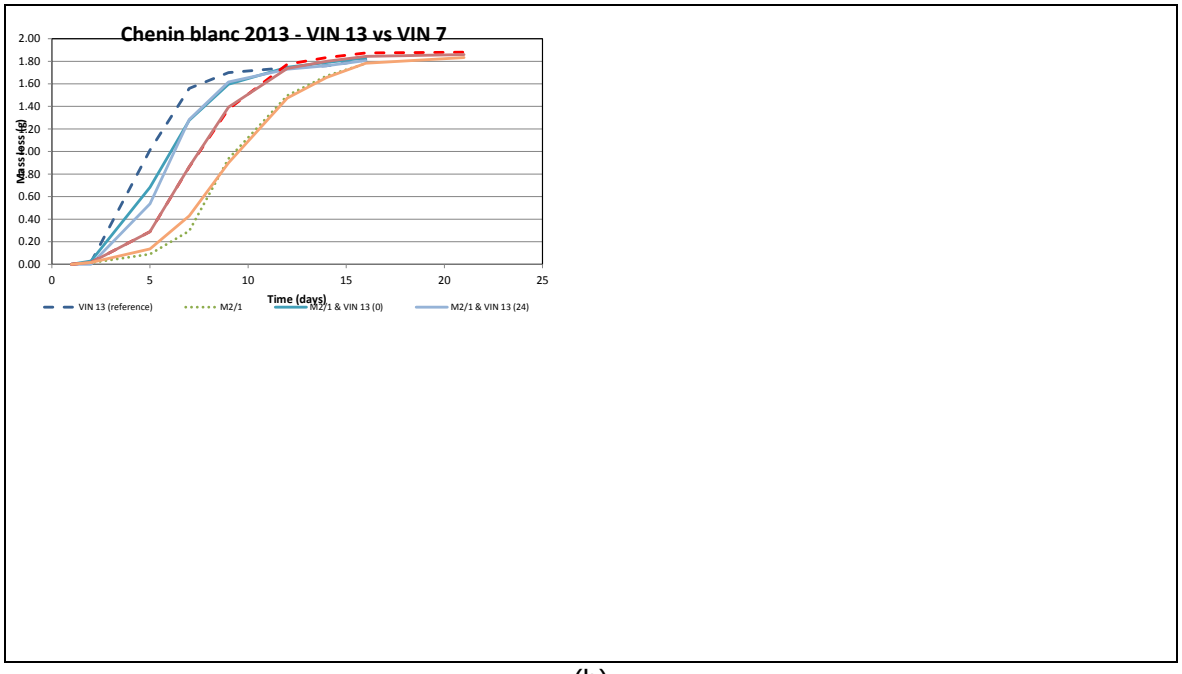
A second commercial *S. cerevisiae* yeast, VIN 7, was also investigated in 2012 and 2013 to ascertain if the results obtained with VIN 13 also apply to another yeast when co-inoculated with *T. delbrueckii* strain M2/1. The *S. cerevisiae* VIN 13 fermentations showed normal fermentation pattern, but in contrast the *S. cerevisiae* VIN 7 fermentations were generally slower for both vintages (Fig. 3.10). The VIN 7 and co-inoculated VIN 7 fermentation curves were very similar to the *T. delbrueckii*-only fermentation.

Population numbers were determined in 2012 only. The population dynamics of VIN 7 appeared to be very different from VIN 13 in the co-inoculations (Fig. 3.11). VIN 7 is a slower fermenter than VIN 13 and the *T. delbrueckii* population remained at higher levels in the VIN 7 co-inoculations than the VIN 13 co-inoculations.

Sensory analytical data differed greatly within replicates, but overall averages between different treatments were similar (Table 3.9). However, the treatments with VIN 13 were always marginally higher than VIN 7 treatments. Chemical analyses (Table 3.10) showed higher volatile acidity for VIN 7 in comparison to VIN 13, however, co-inoculation with *T. delbrueckii* resulted in lower volatile acidity levels. All the wines, with the exception of *T. delbrueckii*-only, were dry. In conclusion the choice of *S. cerevisiae* yeast for co-inoculation does play a role in final wine quality. This is also evident by commercial *T. delbrueckii* yeasts usually being paired with a recommended *S. cerevisiae* yeast.



(a)



(b)

Fig. 3.10 Comparison of *S. cerevisiae* VIN 13 and VIN 7 fermentations co-inoculated with *T. delbrueckii* strains, based on mass loss (a) 2012 and (b) 2013 vintage.

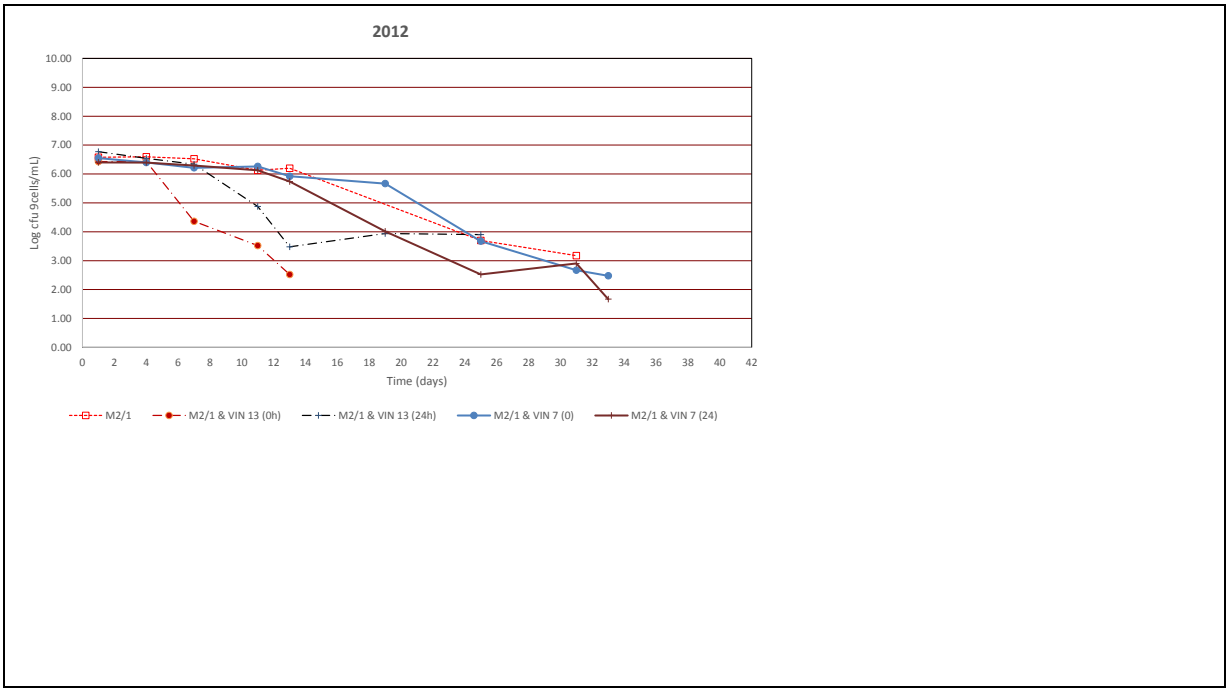


Fig. 3.11 *T. delbrueckii* cell counts (cfu) on mannitol agar in single (M2/1) and co-inoculated (with VIN 13 and VIN 7 after 0 or 24h) fermentations in 2012.

Table 3.9 Sensory data of VIN 13 and VIN 7 wines co-inoculated with *T. delbrueckii* strain M2/1: 2012 and 2013 wines.

Yeast treatment	**Fruity & Fermentation bouquet intensity (%)**		**Sweetness (%)**		**Body (%)**		**General quality (%)**	
	Ave	*± SD*	Ave	*± SD*	Ave	*± SD*	Ave	*± SD*
VIN 13	47.86	*11.32*	17.43	*8.79*	41.99	*11.42*	44.61	*9.78*
VIN 7	49.90	*24.02*	28.90	*7.63*	45.60	*17.45*	42.92	*21.71*
M2/1	49.12	*13.33*	36.32	*16.59*	46.73	*13.77*	45.98	*11.63*
M2/1 & VIN 13 (0h)	43.25	*12.33*	13.89	*9.75*	40.08	*14.06*	40.97	*13.30*
M2/1 & VIN 7 (0h)	42.43	*21.48*	25.85	*5.54*	40.77	*13.81*	35.99	*17.41*
M2/1 & VIN 13 (24h)	44.68	*10.89*	16.64	*6.34*	40.35	*10.71*	41.18	*10.43*
M2/1 & VIN 7 (24)	46.22	*15.96*	25.02	*6.41*	41.95	*15.21*	37.61	*12.56*

Table 3.10 Average chemical data of VIN 13 and VIN 7 co-inoculated with *T. delbrueckii* strain M2/1: 2012 and 2013 wines.

Yeast treatment	**Volatile acidity (g/L)**		**Total acidity (g/L)**		**Glucose (g/L)**		**Fructose (g/L)**		**Total sugar (glucose + fructose) (g/L)**		**Alcohol (% $^{v}/_{v}$)**		**Glycerol (g/L)**	
	Ave	± SD	Ave	*± SD*	Ave	*± SD*	Ave	*± SD*	Ave	*± SD*	Ave	*± SD*	Ave	*± SD*
VIN 13	0.29	0.05	5.77	*0.28*	0.50	*0.21*	1.94	*1.57*	2.44	*1.42*	12.77	*0.77*	6.65	*0.61*
VIN 7	0.95	0.32	6.44	*0.23*	1.36	*0.76*	2.33	*1.70*	3.69	*2.37*	12.15	*0.10*	8.49	*1.35*
M2/1	0.38	0.04	5.69	*0.25*	1.50	*1.49*	8.39	*8.78*	9.89	*9.99*	12.24	*0.88*	6.95	*0.72*
654 & VIN 13 (0h)	0.30	0.04	5.73	*0.30*	0.56	*0.24*	1.42	*0.54*	1.98	*0.46*	12.72	*0.88*	6.62	*0.63*
M2/1 & VIN 7 (0h)	0.89	0.33	6.39	*0.21*	1.07	*0.62*	1.63	*0.84*	2.70	*1.19*	12.13	*0.13*	8.33	*1.32*
M2/1 & VIN 13 (24h)	0.34	0.03	5.75	*0.28*	0.57	*0.37*	1.68	*0.86*	2.25	*0.91*	12.72	*0.81*	6.87	*0.47*
M2/1 & VIN 7 (24h)	0.71	0.18	5.93	*0.26*	0.51	*0.15*	1.29	*0.29*	1.80	*0.40*	12.24	*0.20*	7.51	*0.37*

Research results IV

Other *T. delbrueckii* yeasts

Table 4.1 Yeast strains used in this section.

Strain no.	Obtained from	Origin	Year isolated	Identity
VIN 13[1]	Anchor Bio-Technologies	SU[2]	unknown	*S. cerevisiae*
VIN 7[1]	Anchor Bio-Technologies	ARC Inf-Nvb[3]	unknown	*S. cerevisiae*
654	ARC Inf-Nvb	Robertson	1995	*T. delbrueckii*
M2/1	ARC Inf-Nvb	Robertson	1998	*T. delbrueckii*
TD Harmony[4]	CHR Hansen	unknown	unknown	*T. delbrueckii*
Td Lallemand[5]	Lallemand	unknown	n/a[6]	*T. delbrueckii*
TdAS/11	ARC Inf-Nvb	New isolates	n/a	*T. delbrueckii*
136/25	ARC Inf-Nvb	New isolates	n/a	*T. delbrueckii*
117/69	ARC Inf-Nvb	H. Hart (ww10/01)	n/a	*T. delbrueckii*/*S. cerevisiae* hybrid[7]
Type strain	CBS	unknown	n/a	*T. delbrueckii* type strain

[1]Commercial *S. cerevisiae* wine yeast. [2]Stellenbosch University. [3]ARC Infruitec-Nietvoorbij Culture Collection. [4]*T. delbrueckii* strain isolated from Harmony.nsac commercial active dried yeast (CHR Hansen, Denmark). [5]*T. delbrueckii* strain isolated from Enoferm Level 2[Tm] TD[Tm] (Lallemand). [6]Not applicable.[7]Hybrid status uncertain.

Adaptive selection trial 1: selected isolates Forty-one *T. delbrueckii* isolates were put through an adaptive selection protocol of six cycles of fermentation and regeneration. This was followed by a comparative fermentation trial of eighteen post-adapted isolates to four reference strains (data not shown). The two best post-adapted isolates fermented on average 6g/L dryer than the best reference strain. Characterisation by electrophorectic karyotyping showed that the two post-adaptive isolates' fingerprints differed from that of the *T. delbrueckii* 2009 yeasts selected for wine production (data not shown). Their fermentation performance was also compared to the 2009 wine yeasts, as well as the newly released strain from Lallemand (designated Td Lallemand) (Fig. 4.1). As the TdAS/11 showed the most promising fermentation curve it was selected for further investigation in wine production trials. Species identity was confirmed by ID32C (BioMérieux).

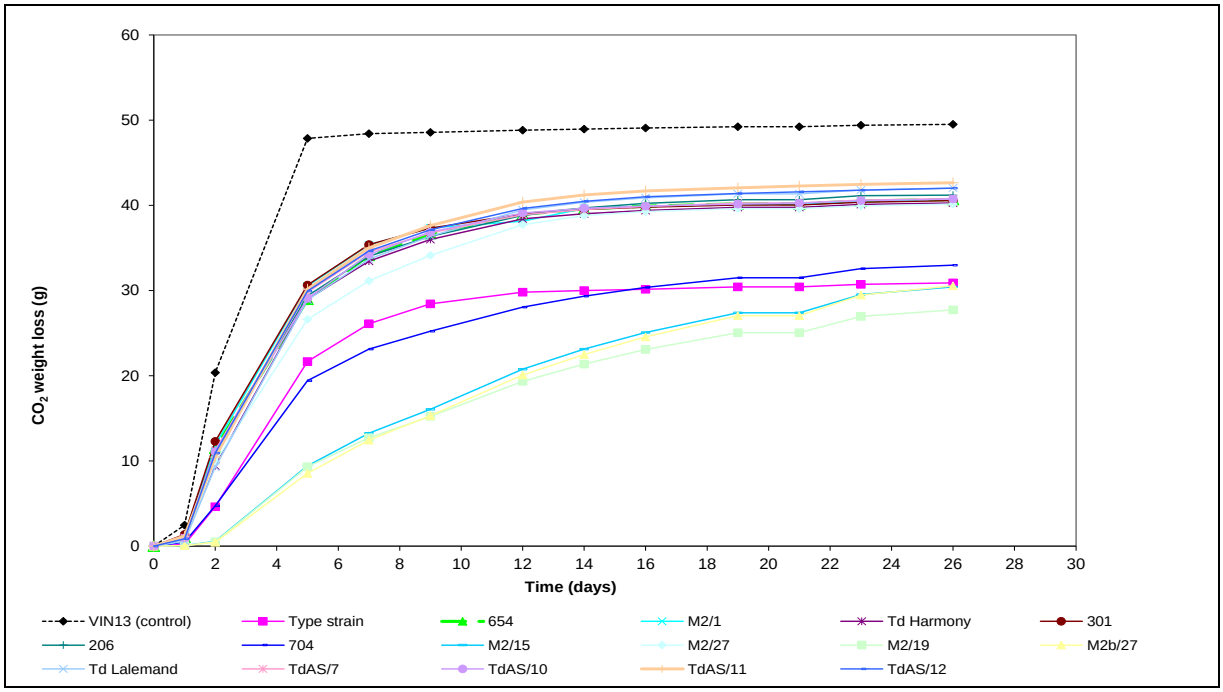


Fig. 4.1 Comparative fermentation curves of a selection of *T. delbrueckii* yeast strains after six cycles of fermentation and regeneration to strains previously used in this project (Fermentation conditions: 22.4°B; *ca*. 22°C; orbital shaker; average of two randomised replicates).

Adaptive selection trial 2: strain 654 In the second trial, strain 654 was put through three cycles of fermentation and regeneration. Eight post-adapted isolates were evaluated in comparison to the 654 parent strain (Fig. 4.2) and their identities confirmed by

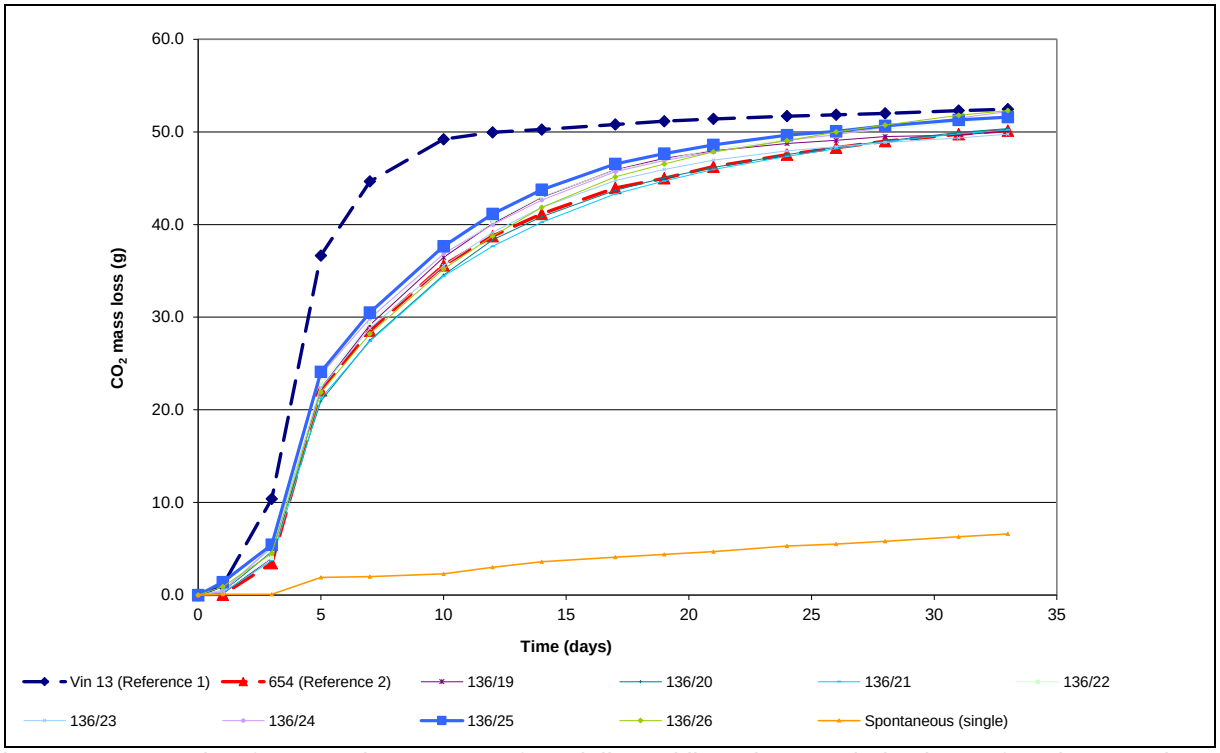


Fig 4.2 Comparative fermentation curves of *T. delbrueckii* strain 654 derivatives after three cycles of fermentation and regeneration (Fermentation conditions: 22.4°B; 15°C; orbital shaker; two replicates randomised).

ID 32C (BioMérieux). The best new isolate, 136/25 (based on the fermentation curves) was also set aside for further investigation in wine production trials.

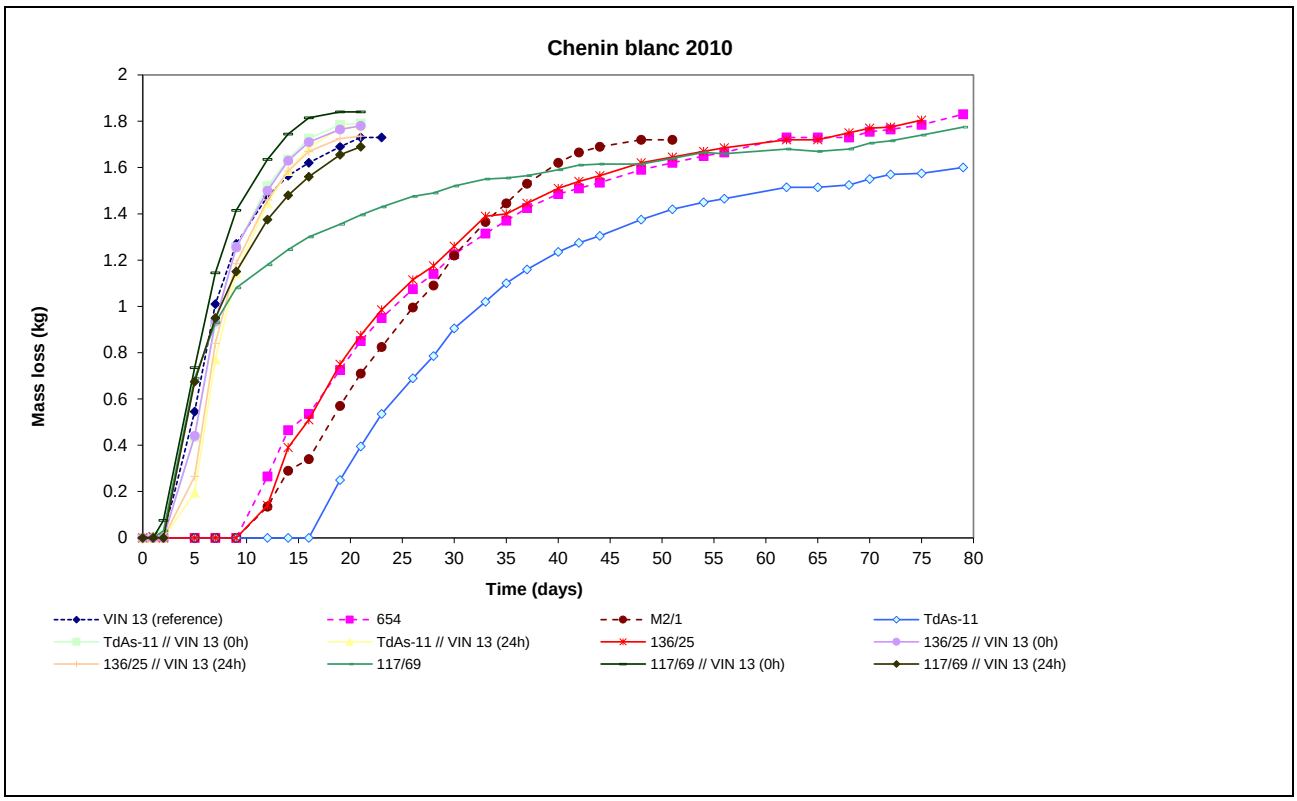
***T. delbrueckii/S. cerevisiae* hybrids** Two potential *S. cerevisiae/T. delbrueckii* hybrid yeasts (117/68, 117/69) were obtained from project ww10/01 (H. Hart, 2010). No identification could be made by ID32C (BioMérieux), although both parent species can be identified by this system. A preliminary comparative fermentation trial indicated that both yeasts had similar fermentation profiles (data not shown). Strain 117/69 was chosen for wine production trials.

Evaluation in small-scale cellar trials

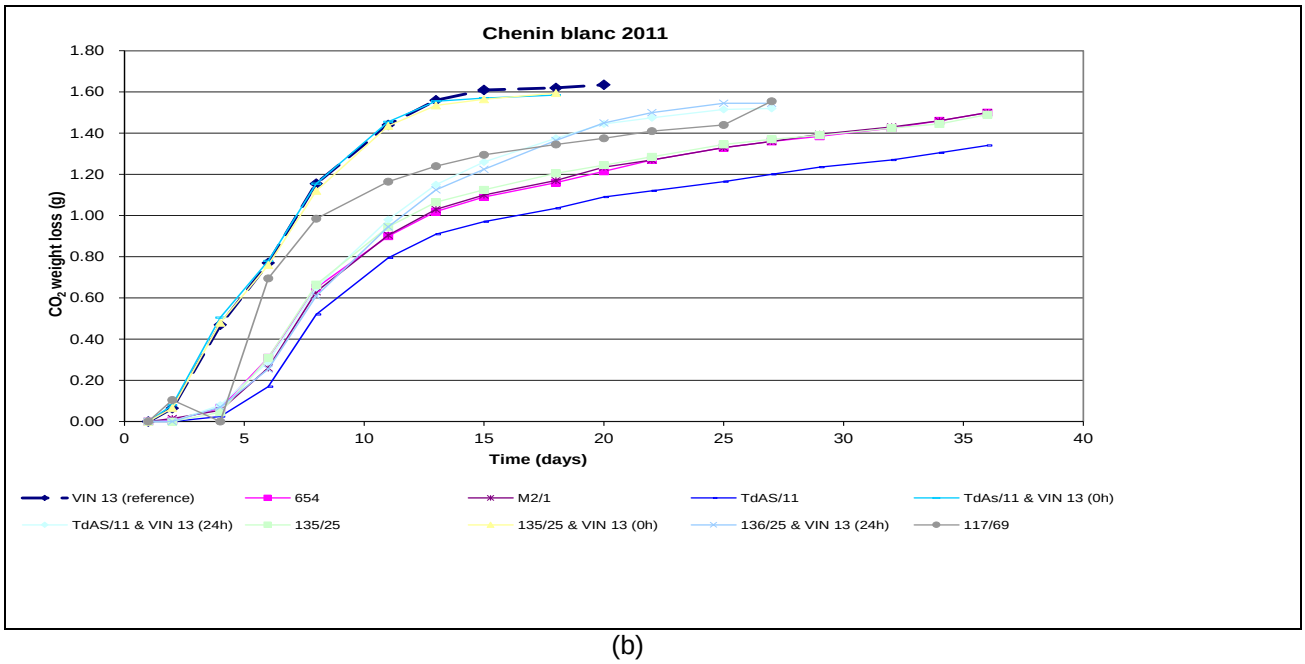
The *T. delbrueckii* yeasts 117/69, TdAs11 and 135/25 were evaluated in small scale cellar trials over two vintages. TdAs/11 turned out to be a slower fermenter than 654 and M2/1 representing the best original *T. delbrueckii* yeasts. The hybrid 117/69 had an intermediate fermentation curve between the *T. delbrueckii* strains and *S. cerevisiae*. Co-inoculation with a *S. cerevisiae* strain improved the fermentation kinetics as previously shown for the *T. delbrueckii* isolates.

Similar to other *T. delbrueckii*-only wines, chemical analyses (Table 4.2) of the wines showed that the sugar levels ranged from dry to semi-sweet. Volatile acidity levels tended to be higher than the *S. cerevisiae* reference, especially in the 2010 wines. While co-inoculation reduced the volatile acidity levels, it was still higher than the *S. cerevisiae* reference. The volatile acidity levels could therefore be the reason for the low sensory scores (general quality) for the *T. delbrueckii*-only wines (Table 4.2). Co-inoculated wines were similar in sensory properties to

the *S. cerevisiae* reference. The three yeast were not investigated in subsequent vintages, due to the focus of the project being on the two best *T. delbrueckii* isolates, M2/1 and 654.



(a)



(b)

Fig. 4.3. Average fermentation curves (two replicates) based on mass-loss of small-scale Chenin blanc (a) 2010 and (b) 2011 wines produced by combinations of *T. delbrueckii* and *S. cerevisiae* yeasts (base must 2010: 22.4°B, 7.1 g/L total acidity, pH 3.31; and 2011: 21.6°B, 7.9 g/L total acidity, pH3.41)

Table 4.2 Chemical analyses of Chenin blanc 2010 and 2011 produced in small-scale with different combinations of yeasts.

Yeast treatment	Chemical attribute[1]									
	2010					2011				
	Vol. acidity (g/L)	Total sugar (gluc. + fruc) (g/L)	Alco-hol (% v/v)	Gly-cerol (g/L)	Total[2] SO_2 (mg/L)	Vol. acidity (g/L	Total sugar (gluc. + fruc.) (g/L)	Alco-hol (% v/v)	Gly-cerol (g/L)	Total[3] SO_2 (mg/L)
VIN13	0.34	5.11	14.06	7.18	70	0.23	1.7	13.00	7.18	38
654	0.38	7.64	13.50	6.93	67	0.35	27.0	11.40	6.93	46
M2/1	0.42	4.64	13.81	7.78	74	0.36	25.2	11.55	7.78	47
TdAs/11	0.41	6.77	13.71	7.05	64	0.36	31.4	11.14	7.05	47
TdAs/11 & VIN 13 (0h)	0.33	2.73	14.13	7.01	73	0.27	1.8	12.94	7.01	36
TdAs/11 & VIN 13 (24h)	0.34	2.74	14.20	7.16	84	0.31	3.4	12.86	7.16	44
136/25	0.41	11.29	13.47	6.65	77	0.37	16.5	12.14	6.65	46
136/25 & VIN 13 (0h)	0.37	3.18	14.12	7.05	74	0.28	2.1	12.95	7.05	38
136/25 & VIN 13 (24h)	0.35	2.82	14.10	7.09	83	0.31	3.0	12.90	7.09	44
117/69	0.55	27.01	12.29	6.86	106	0.32	19.1	11.82	6.86	48
117/69 & VIN 13 (0h)	0.35	2.21	13.78	7.36	80	Np[4]	Np	Np	Np	Np
117/69 & VIN 13 (24h)	0.50	3.04	13.93	7.45	Np	Np	Np	Np	Np	Np

[1]Average values of two fermentations. Analyses by Winescan (Chemical Laboratory, IWBT, Stellenbosch University, Stellenbosch). Base must analyses was: 22.4°B, 7.1 g/L total acidity and pH of 3.31. [2]Total SO_2 of final wine. [3]Total SO_2 at first racking. [4]Not produced.

Table .4.3 Sensory analyses of Chenin blanc 2010 and 2011 produced in small-scale with different combinations of yeasts.

Yeast treatment	Wine characteristic[1]					
	2010		2011			
	Overall fruit intensity (%)	General quality (%)	Overall fruit intensity (%)	Sweet-ness (%)	Body (%)	General quality (%)
VIN13	55	51	65	13	58	60
654	53	43	58	59	59	55
M2/1	65	51	59	60	62	59
TdAs/11	53	40	64	63	65	59
TdAs/11 & VIN 13 (0h)	54	54	55	5	52	53
TdAs/11 & VIN 13 (24h)	48	46	59	9	54	53
136/25	53	49	55	43	57	54
136/25 & VIN 13 (0h)	57	53	60	5	56	54
136/25 & VIN 13 (24h)	55	54	58	5	55	57
117/69	34	23	63	46	63	62
117/69 & VIN 13 (0h)	56	52	np[3]	np	np	np
117/69 & VIN 13 (24h)	52	51	np	np	np	np

[1]Average value of duplicate wines judged by a panel of thirteen judges. [2]Not produced.

Research results V

Red wine production trials

The initial laboratory trials (Research results I) identified *T. delbrueckii* isolates with potential for red wine production and were first evaluated in 2009 in for Pinotage production (see Research results II). Subsequently the best strains (Table 5.1) were used in subsequent trials (2010 – 2013).

Table 5.1 Yeast strains used in this section.

Strain no.	Obtained from	Origin	Year isolated	Identity
VIN 13[1]	Anchor Bio-Technologies	US[2]	unknown	*S. cerevisiae*
654	ARC Inf-Nvb[3]	Robertson	1995	*T. delbrueckii*
M2/1	ARC Inf-Nvb[3]	Robertson	1998	*T. delbrueckii*

[1]Commercial *S. cerevisiae* wine yeast.[2]Stellenbosch University. [3]ARC Infruitec-Nietvoorbij Culture Collection.

In 2010, Pinotage and Cabernet franc wines were produced using a *S. cerevisiae* reference and the best *T. delbrueckii* yeast strain at that stage of the project, i.e. strain 654. No co-inoculations were done. Chemical (Table 5.2) and sensory (Tables 5.3 and 5.4) analyses for both cultivars did not show any notable differences between the two yeast strains. It was evident from the residual sugar analyses that the background *S. cerevisiae* population must have played a role as the wines were all dry. Preliminary polyphenol analyses on the wines showed interesting trends so the 654 and *S. cerevisiae* treatments were further investigated as part of a new project (ww10/25: Chemical profiling of non-*Saccharomyces* wines). The resultant data of these wines are discussed in that project report.

Table 5.2. Chemical analyses of Pinotage and Cabernet franc 2010 wines produced in small-scale with different yeasts.

Cultivar and yeast treatment[1]	Chemical attribute[2]						
	Vol. acidity (g/L)	Glucose (g/L)	Fructose (g/L)	Total sugar (gluc. + fruc.) (g/L)	Total sugar (g/L)	Alcohol (% v/v)	Glycerol (g/L)
Pinotage							
VIN 13	0.39 ± 0	0.25 ± 0.11	1.25 ± 0.04	1.50 ± 0.10	1.40 ± 0.5	15.14 ± 0.10	11.23 ± 0.05
654	0.33 ± 0.01	0.61 ± 0.04	1.23 ± 0.03	1.84 ± 0.06	1.60 ± 0.6	14.99 ± 0.07	11.23 ± 0.10
Cabernet franc							
VIN 13	0.30 ± 0	0.23 ± 0.08	1.25 ± 0.06	1.48 ± 0.10	0.50 ± 0.1	15.42 ± 0.04	10.15 ± 0.13
654	0.32 ± 001	0.69 ± 0.14	1.80 ± 0.41	2.49 ± 0.30	0.90 ± 1.2	15.23 ± 0.07	10.75 ± 0.03

[1]All wet cultures. [2]Average values of three fermentations. Analyses by Winescan (Chemical Laboratory, IWBT, Stellenbosch University, Stellenbosch). Second total sugar column done by Rebelein method. Base must analyses Pinotage: 25.1°B, 6.7 g/L total acidity and pH of 3.40; Cabernet franc: 24.7°B, 6.5 g/L total acidity and pH of 3.38.

Table 5.3. Sensory analyses of Pinotage 2010 wine produced in small-scale with different yeasts.

Yeast treatment[1]	Wine characteristic[2]			
	Colour intensity	Berry/Cherry/Plum aroma intensity	Body	Overall quality
VIN 13	80.95 ± 0.41	66.19 ± 1.09	56.43 ± 3.57	51.19 ± 3.22
654	80.24 ± 2.06	67.14 ± 3.71	57.86 ± 3.27	52.38 ± 3.67

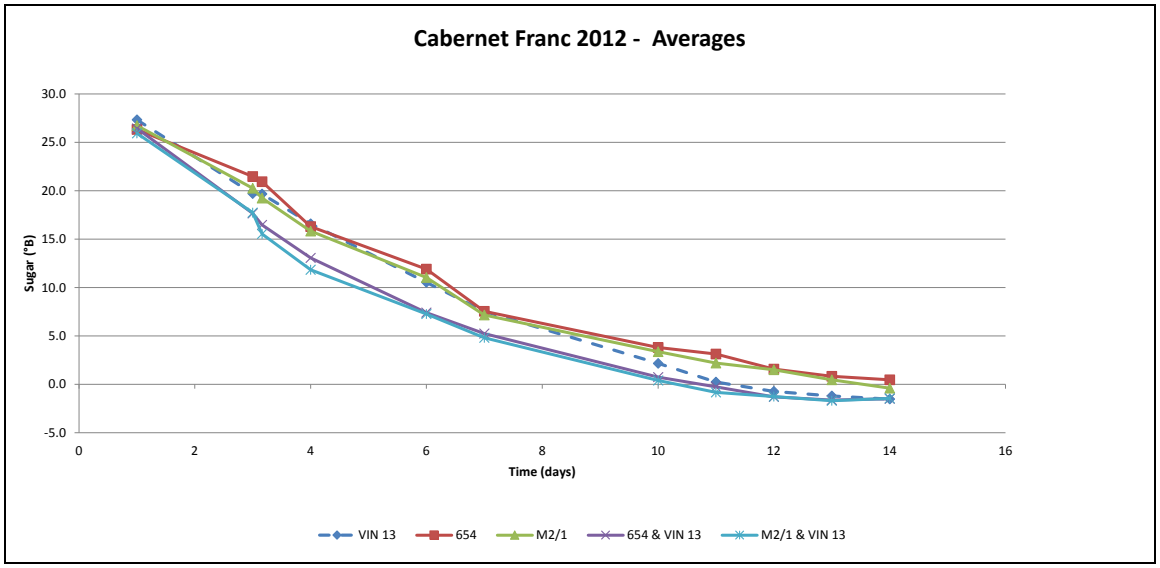
[1] All wet cultures. [2]As evaluated on an 10 cm unstructured line scale from low to high intensity, thin to full bodied and unacceptable to excellent quality. Average value of three replicate wines judged by a panel of seven judges.

Table 5.4. Sensory analyses of Cabernet franc 2010 wine produced in small-scale with different yeasts.

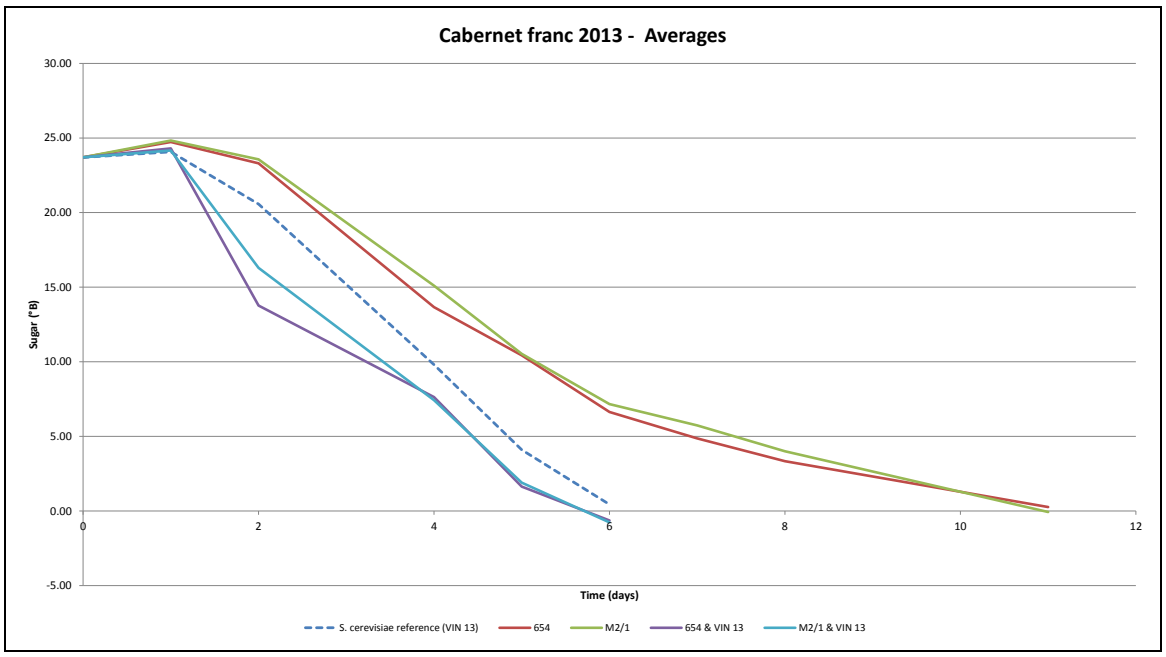
Yeast treatment[1]	Wine characteristic[2]				
	Colour	Berry	Herbaceous	Body	Overall quality
VIN 13	65.00 ± 2.47	50.00 ± 2.58	48.33 ± 4.65	45.71 ± 4.34	43.81 ± 4.06
654	65.24 ± 5.77	49.52 ± 2.70	42.86 ± 2.47	47.62 ± 3.22	43.81 ± 2.18

[1] All wet cultures. [2]As evaluated on an 10 cm unstructured line scale from low to high intensity, thin to full bodied and unacceptable to excellent quality. Average value of three replicate wines judged by a panel of seven judges.

The red wine trials were expanded in 2012 using the two best *T. delbrueckii* strains (654, M2/1) as identified in previous white wine trials. These strains were used on their own and in combination with *S. cerevisiae* for the production of Cabernet franc wine. Fermentation kinetics showed the same pattern in both vintages. The *T. delbrueckii* strains fermented slower than the *S. cerevisiae* reference and *S. cerevisiae* co-inoculated wines. All wines appeared to go to dryness (Figs. 5.6). Although general quality of the wines tended to be similar, stylistic differences could be observed in the sensory data (Fig. 5.7).

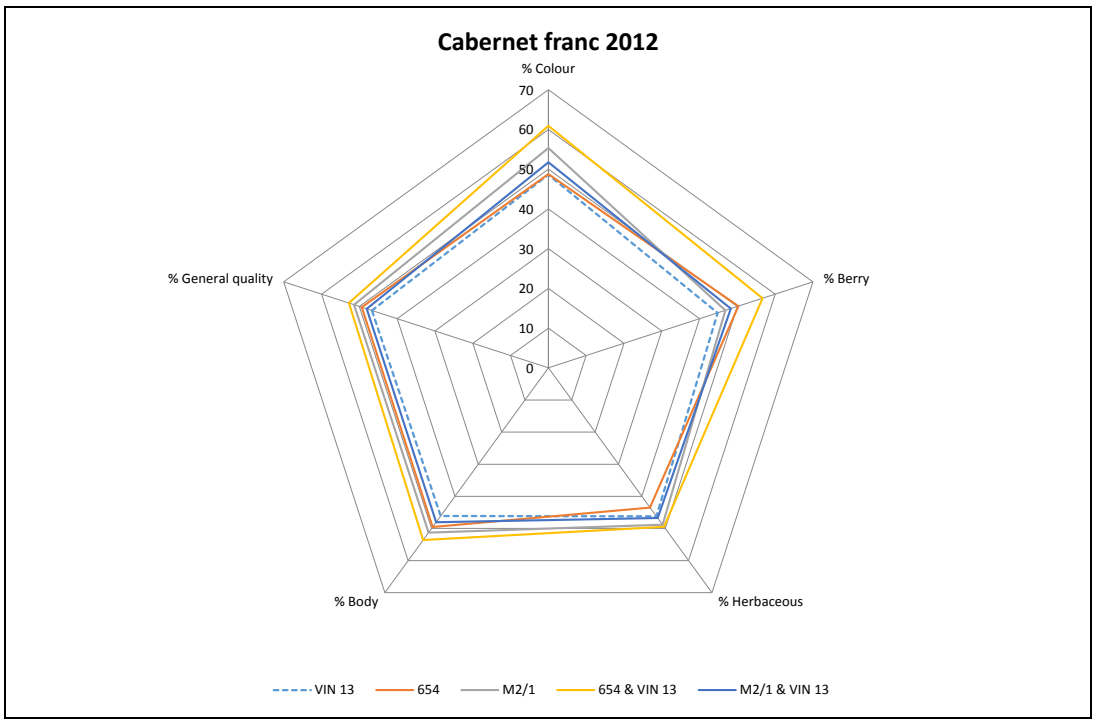


(a)

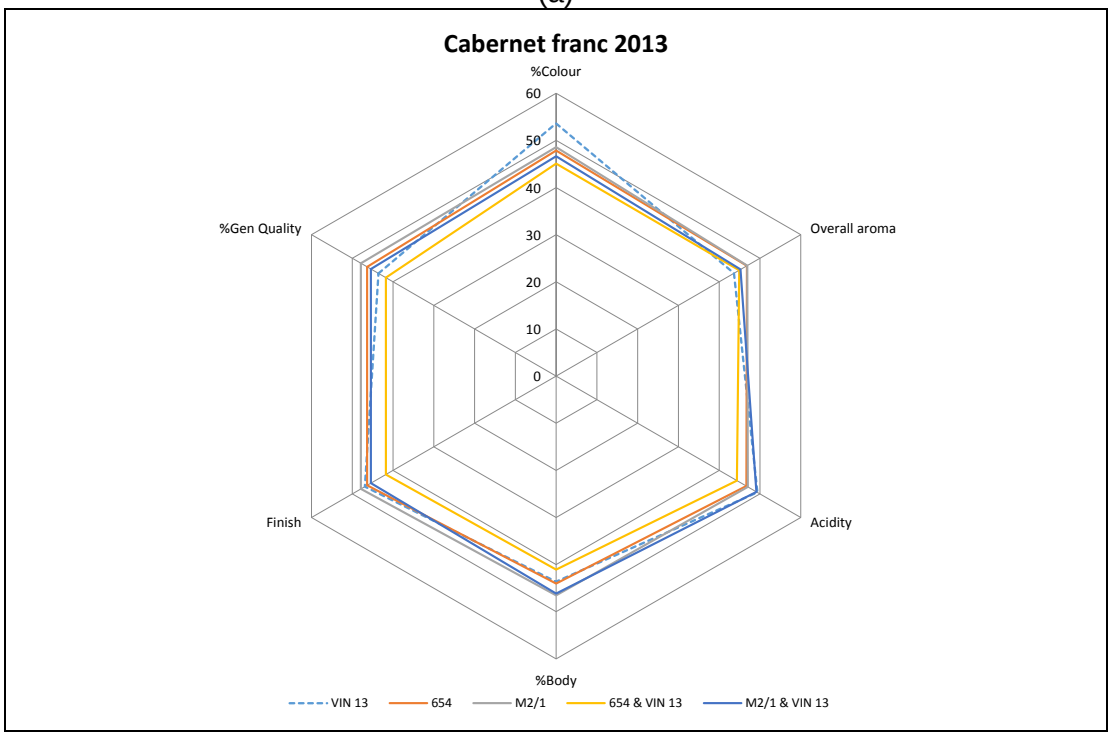


(b)

Fig. 5.6. Cabernet franc fermentation curves of (a) 2012 and (b) 2013 wines singly and co-inoculated with *T. delbrueckii* strains.



(a)



(b)

Fig. 5.7. Sensory profiles of Cabernet franc wines in (a) 2012 and (b) 2013 produced by *T. delbrueckii* in single and co-inoculations.

Research results VI

Further chemical investigations

The improved mouthfeel of some of the 2009 wines were investigated further. Preliminary work indicated that there may be some interaction between *T. delbrueckii* and polyphenols measured by means of a liquid chromatographic technique (UV detection). The polyphenols measured in the *T. delbrueckii* wines were consistently lower than those in *S. cerevisiae* wines for both Chenin blanc and Pinotage (Figs 6.1 and 6.2), although the body of the wines were unchanged or rated higher than *S. cerevisiae* wines. Although differences in residual sugar are evident, these parameters did not sufficiently explain the apparent inconsistency and necessitated further investigation. Pinotage and Cabernet franc wines were produced in subsequent vintages. A new project was initiated (ww10/25: Chemical profiling of non-*Saccharomyces* wines) where the wines could be investigated in more depth on a chemical level.

A selection of ten 2009 wines were sent for volatile thiol analyses in Europe as a result of a goodwill offer by a yeast manufacturer. However, the data showed no differences between yeast treatments (data not shown).

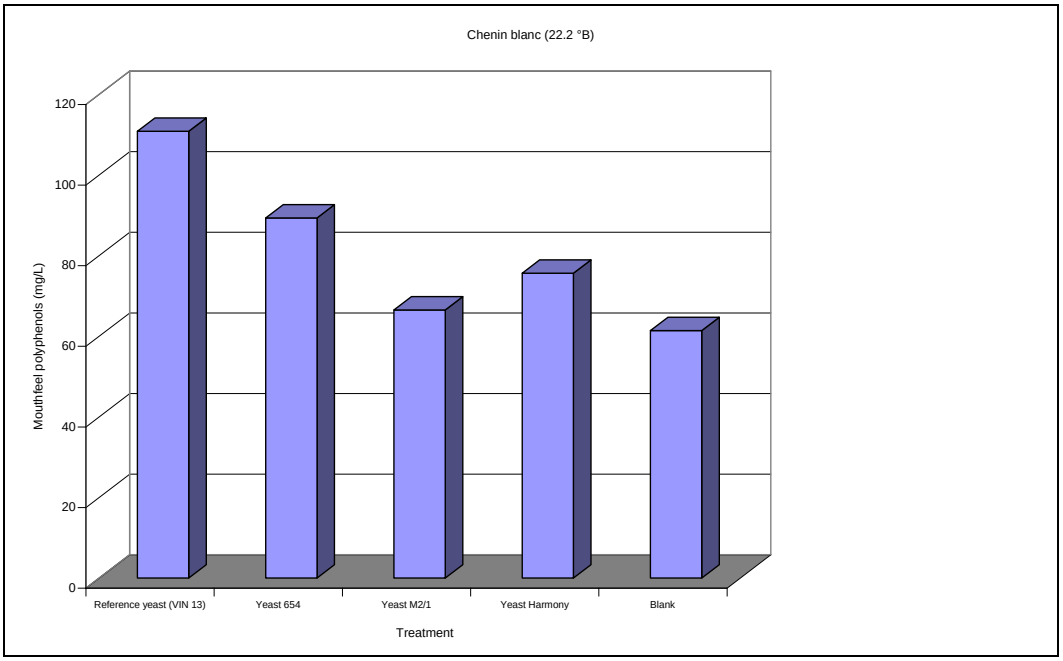


Fig. 6.1 Impact of *T. delbrueckii* yeasts on the polyphenol compounds in Chenin blanc wine (VIN 13 = *S. cerevisiae*; 654, M2/1 and Harmony = different *T. delbrueckii* strains).

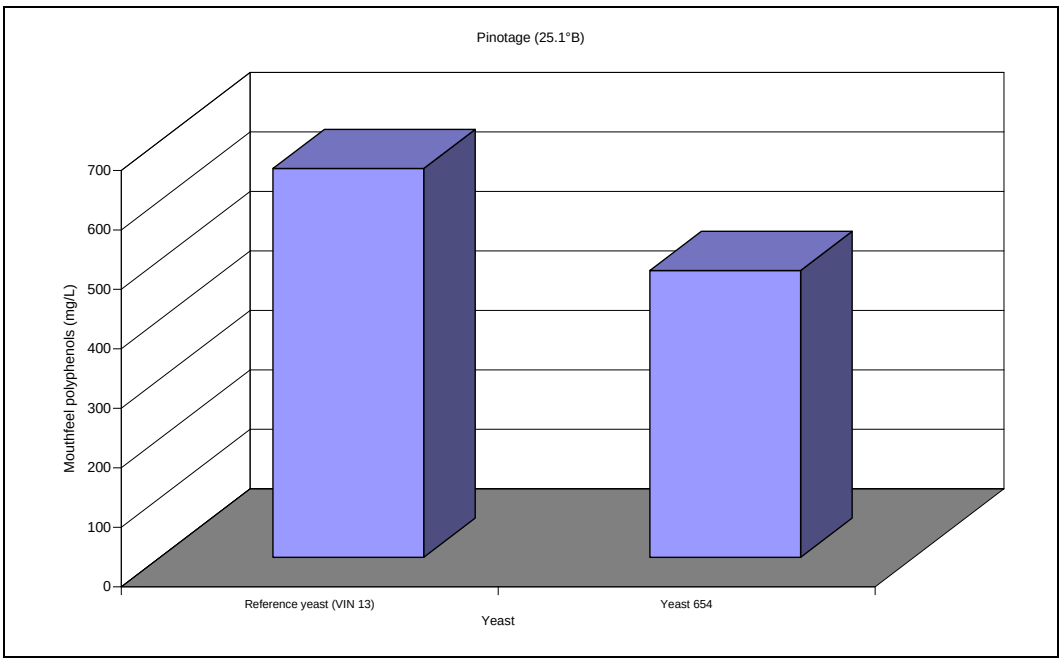


Fig. 6.2 Impact of *T. delbrueckii* yeasts on the polyphenol compounds in Pinotage wine (VIN 13 = *S. cerevisiae*; 654 = *T. delbrueckii*).

Research results VII

Determination of extra-cellular enzymatic activity

Protease (important role during autolysis) and β-Glucosidase (releasing flavour compounds from glycosidically-bound non-volatile precursors) activity of the *T. delbrueckii* strains were tested according to the plate methods employed by Strauss *et al*. (2001). No activity was noted (data not shown) which follows the same trends noted by Strauss *et al.* (2001) who also investigated a range of South African non-*Saccharomyces* yeasts.

Strauss, M.L.A., Jolly, N., Lambrechts, M.G. & Van Rensburg, P., 2001. Screening for the production of extracellular hydrolytic enzymes by non-Saccharomyces wine yeasts. J. Appl. Microbiol. 91, 182-190

Complete the following table

Milestone	Target Date	Extension Date	Date Completed	Achievement
1. Confirmation of identity and uniqueness of selected strains.	2009	2009	2009	Objective completed: An initial selection of 48 *T. delbrueckii* strains was made.
2. Laboratory-scale fermentations.	2009	2009	2009	Objective completed: Laboratory-scale fermentation trials were carried out at 15 and 22°C. Seven yeasts showing potential chosen for white and red wine production, respectively in 2009.
3. Small-scale wine production: 1st vintage (2009).	2009	2009	2009	Objective completed: Selected yeasts were used for production of Chenin blanc and Pinotage wine using single inoculations, and staggered co-inoculations with *S. cerevisiae.*

4. Evaluation of small-scale wines: 1st vintage (2009).	2010	2010	2010	Objective completed: Improvement in white wine quality achieved. Not in red wine.
5. Determination of extra-cellular enzymatic activity.	2010	2010	2010	Objective completed: Protease and β Glucosidase activity absent.
6. Recommendations for project extension.	2010	2010	2010	It was recommended that extension be granted to accommodate at least another two vintages. Funding was approved for 2011/12 and a second motivation was submitted for 2012/13.
7. Further selections, improvements and comparisons.	2010	2010	2010	Two further isolates, a potential hybrid and a newly released commercial strain were investigated.
8. Small-scale wine production: 2nd vintage (2010).	2010	2010	2010	Wine production trials were completed in 2010.
9. Evaluation of small-scale wines: 2nd vintage (2010).	2011	2011	2011	Potential for *T. delbrueckii* as single inoculants was shown.
10. Further chemical investigations.	2012	2012	2012	Both single and co-inoculation strategies with a South African isolate show potential. South African isolate possibly better than commercial strains.
11. Small-scale wine production and evaluation: 3rd vintage (2011).	2011	2011	2011	Both single and co-inoculation strategies with a South African isolate show potential. South African isolate possibly better than commercial strains.
12. Preliminary discussions with yeast manufacturers.	2012	2013	2013	Company A not interested. Company B may be interested at a later stage.
13. Small-scale wine production: red wines.	2013	2013	2013	Wine produced in 2012 and 2013. Chemical and sensory analyses completed.
14. Small-scale wine production: white wines.	2013	2013	2013	White wine produced in 2012 and 2013. Chemical and sensory analyses completed.
15. Journal publication/s – final milestone.	2014	2015	2015	First publication completed in 2013, second due in 2014 and third due in 2015.

Accumulated outputs

2009: One poster
2010: One poster
2011: Two posters
2012: One publication in a proceeding
2013: One scientific publication
2014: One poster to be presented, one publication to be submitted, one paper to be presented.

Conclusions

Two *T. delbrueckii* isolates from an initial 43 isolates that were screened can be used singly or in co-inoculation with a *S. cerevisiae* yeast for changing wine styles, producing novel wines and in some instances improving quality. Lowering of ethanol is only practical where a semi-sweet wine is desired, while a 0.3% alcohol reduction achieved in dry wine production. The two best *T. delbrueckii* isolates compare well to the commercial strains.

Technology development, products and patents

The two best *T. delbrueckii* isolates can be considered for commercialisation.

Suggestions for technology transfer

List any suggestions you may have for technology transfer

Human resources development/training

Student level (BSc, MSc, PhD, Post doc)	Cost to Project
1. MTech (Ms V van Breda)	R120,540
2.	
3.	
4.	
5.	

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

Jolly, N.P., van Breda, V. & Booyse, M., 2012. Comparison between South African *Torulaspora delbrueckii* isolates and commercial strains for use as single inoculants in wine production. Oeno 2011, Actes de colloques du 9e symposium international d' oenologie de Bordeaux, Dunod, Paris, pp 570 - 574.

Van Breda, V., Jolly, N., & van Wyk, J., 2013. Characterisation of commercial and natural *Torulaspora delbrueckii* wine yeast strains. Int. J. Food Microbiol. 163, 80-88.

Van Breda, V., Jolly, N., Booyse, M., and van Wyk, J. The use of *Torulaspora delbrueckii* yeasts strains for small-scale wine production (to be submitted for publication in 2014).

Presentations/papers delivered

Posters:

Van Breda V., Jolly N.P. & Van Wyk, J., 2009. *Torulaspora delbrueckii*: A preliminary investigation for wine production. Poster 4th International SASEV Conference on Enology & Viticulture – Beyond 2010, 28-30 July 2009, Cape Town International Convention Centre, Cape Town, South Africa.

Van Breda, V., Jolly, N.P. & Van Wyk, J., 2010. A preliminary investigation into the use of *Torulaspora delbrueckii* for wine production at lower and higher temperatures. Poster 15th World Congress of Food Science and Technology, Cape Town International Convention Centre, Cape Town, South Africa, 22-26 August 2010.

Jolly, N.P., van Breda, V. & Booyse, M., 2011. Comparison between South African Torulaspora delbrueckii isolates and commercial strains for use as single inoculants in wine production. Poster 9th International Symposium of Oenology, 15-17 June 2011, Palais de la Bourse, Bordeaux, France.

Jolly, N., Van Breda, V. & Booyse., M., 2011. Wine production with *Torulaspora delbrueckii* yeast strains. Poster SASM 2011 (South African Society for Microbiology Conference), 6-8 November 2011, Cape Town.

Van Breda V., Jolly N.P. van Wyk J., 2014. The use of *Torulaspora delbrueckii* yeast strains for the production of small-scale wines. Poster 36th SASEV conference, 12-14 November 2014 (accepted for presentation).

Papers delivered

Jolly, N., Van Breda, V. & Van Wyk, J., 2010. Paper: *Torulaspora delbrueckii*: the new wine yeast. Paper 32nd Conference of the Society for Enology and Viticulture, 18-19 November, Lord Charles Hotel, Somerset West.

Jolly, N., Van Breda, V. & Booyse, 2014. Chenin blanc wine production with Torulaspora delbrueckii and Saccharomyces cerevisiae yeasts. Proposed paper 31st International Specialised Symposium on Yeast (ISSY 31), 9-12 October 2014, Vipava and Nova Gorica, Slovenia (abstract accepted for presentation).

Total cost summary of the project

TOTAL COST IN REAL TERMS	Year	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
YEAR 1	2008/09					200,046		208,211	408,257
YEAR 2	2009/10					299,724		464,343	764,067
YEAR 3	2010/11					242,613		252,516	495,129
YEAR 4	2011/12					250,080	100,386	260,288	510,368
YEAR 5	2012/13					248,213	79,773	258,334	586,334
TOTAL						**1,240,676**	**180,159**	**1,443,692**	**2,764,155**