Industry allocated project number	PHI allocated project number

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FINAL REPORT
(2016)

1. PROGRAMME AND PROJECT LEADER INFORMATION

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2. PROJECT INFORMATION

Research Organisation Project number	WW 10-25
Project title	Chemical profiling of non-*Saccharomyces* wines.
Short title	Chemical profiling

Fruit kind(s)	Wine grapes		
Start date (mm/yyyy)	01/04/2013	**End date** (mm/yyyy)	31/03/2016

Key words	Non-*Saccharomyces* yeasts, wine, chemical profiling

Approved by Research Organisation Programme leader (tick box) √

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Version 2015

3. EXECUTIVE SUMMARY

The aim of this project was the chemical analyses of previously produced non-*Saccharomyces* wines (project ww1021) to help the understanding of how these yeasts impact on wine chemical profile. This project was divided into five sub-projects.

The major non-volatile components found in wine are the polyphenols, which are important contributors to wine quality regarding colour, astringency, mouth feel and bitterness. The polyphenols of white grape varieties is found in the flesh of the grape and include phenolic acids, flavanols, flavones, flavanones and flavonols. Red grapes are different in that it is composed of anthocyanins and tannins, which are present in grape skin in addition to the polyphenols found in the flesh. Total flavan-3-ol- and anthocyanin content in Pinotage and Cabernet Franc wines were determined by a spectrophotometric method. An increase in anthocyanin content and colour intensity in both Cabernet Franc and Pinotage wines was observed in wines made with *T. delbrueckii* compared to *S. cerevisiae*. Further analyses quantified the individual anthocyanins and flavanols by high-performance liquid chromatography (HPLC-DAD). Clear differences in the individual phenolic compounds and physicochemical parameters of the Pinotage and Cabernet Franc were found. This more in depth analyses showed that *T. delbrueckii* is the preferred yeast for Pinotage wine production and *S. cerevisiae* the preferred yeast for Cabernet Franc grape must in terms of combined anthocyanins (colour) and flavanol (mouthfeel/tannins) concentrations.

A phenolic study of Chenin blanc wines showed that wines made with one *T. delbrueckii* strain had higher concentrations of (+)-catechin, caffeic acid, ferulic acid and p-coumaric acid compared to the second *T. delbrueckii* strain. Wines made with *S. cerevisiae* had higher concentrations of flavan-3-ols, compared to wines made with *T. delbrueckii*. Sensory evaluation showed the *S. cerevisiae* strain led to more perceived acidity and astringency, while the *T. delbrueckii* strains contributed to body mouthfeel of the wines. This is similar to the conclusions made about the red wines.

Volatile aroma, standard chemical and sensory evaluations showed that vintage influences the effect that *T. delbrueckii* can have on volatile aroma compounds. However, within a vintage, yeast combination and time of inoculation has a definitive role in the chemical fingerprint on the wine. A 24-hour co-inoculation strategy lead to a significantly different wine profiles to that of *S. cerevisiae* and represents a true *T. delbrueckii* imprinted wine. *T. delbrueckii* does not adversely affect SO_2 levels in co-inoculated wines.

In conclusion, *T. delbrueckii* yeasts strains can positively affect non-volatile (mouth-feel and colour) as well as volatile (aroma) compounds. The individual strains also differ in their effect on wine aromatics. The chemical data supports the sensory data.

4. PROBLEM IDENTIFICATION AND OBJECTIVES

Current industry funded projects conducted at the ARC Infruitec-Nietvoorbij, generate a large number of experimental wines, especially those derived from the investigation of the effect of non-*Saccharomyces* yeasts on the quality of wine. These wines are currently only subjected to superficial chemical analysis. The chemical data emanating from the superficial analysis of the wines limits the conclusions that are made regarding the overall quality of the wine. The aim of this project was therefore the chemical analyses of non-*Saccharomyces* produced wines.

5. WORKPLAN (MATERIALS AND METHODS)

Wine were produced as part of project ww10/21 and not during this project. Wines were conserved at 15°C until analysed.

Standard chemical and sensory analyses
The unfermented grape juice was analysed for sugar (°Brix), total titratable acidity and pH (Anonymous, 2002). Standard wine chemical analyses (residual sugar, alcohol, volatile acidity, total acidity and pH) were conducted by FOSS Winescan™ (Central Analytical Facility, Stellenbosch University, Stellenbosch) and SO_2 analyses by the Ripper method. Time of sensory and chemical analyses coincided so that the data-sets could be linked where appropriate.

Spectrophotometric determination of total anthocyanins and flavanols
An ultra violet/visible spectrophotometric technique was optimised and validated for the quantification of total anthocyanins and flavanols. This section was done by a BTech student (Mr Ludwe Nyobo) under the leadership of Mr. P Minnaar.

Polyphenol analyses
Wine samples were subjected to an HPLC-DAD (photo diode array detection) technique that allows for the maximum absorbance of each group of flavonoids, control of peak purity and identification of peaks by means of visible spectra and retention times. This section was done by an MTech student (Miss Zama Ngqumba) under the leadership of Mr. P Minnaar.

Volatile aroma compound analyses
Wines of three vintages were analyzed for 32 volatile aroma compounds by gas chromatography coupled to a flame ionization detector (GC-FID) at the Central Analytical Facility, Stellenbosch University.

Statistical analysis
The analytical data were subjected to ANOVA using General Linear Models Procedure (PROC GLM) of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). Principal Component Analysis (PCA), with the correlation matrix to standardize the data) was performed to examine the relationships among and between the variables and observations. Discriminant analysis (DA) was performed to investigate observation (treatment) separation by the variables. These techniques were executed using XLStat software (XLStat, Version 2011, Addinsoft, New York, USA). The statistical analyses were conducted by Ms N. Ntushelo and Dr M. Booyse.

6. RESULTS AND DISCUSSION

Non-volatile compounds
Polyphenol compounds are the major non-volatile components found in wine and are important contributors to wine quality. They are responsible for important qualities of wine such as colour, astringency, mouth feel and bitterness of the wine. The polyphenol content of white grape varieties differs to that of red grape varieties. White wine is composed of polyphenols found in the flesh of the grape such as phenolic acids, flavan-3-ols, flavones, flavanones and flavonols. Red wine is composed of anthocyanins and tannins, which is present in grape skin in addition to the polyphenols found in the flesh.

Sub-project 1: Spectrophotometric determination of total anthocyanins and flavanols in Pinotage and Cabernet Franc wines
This section formed part of a CPUT BTech degree (Mr Ludwe Nyobo). Total anthocyanins and flavan-3-ols in seventy-two wines, i.e. Pinotage (2009-2013) and Cabernet Franc wines (2010-2013) produced by single and co-inoculations of *T. delbrueckii* and *S. cerevisiae* yeasts were investigated. Chemical analyses were correlated with sensory analyses (mouth-feel and colour). Summarised results are given below, and the full thesis is attached (Addendum 1).

Summary

Wine and grapes contain a large number of phenolic compounds belonging to the classes of non-flavanoids and flavanoids. This study evaluated the effect of *Torulaspora delbrueckii* yeast on the polyphenol compound concentrations of Pinotage and Cabernet Franc wines in terms of colour and mouth feel. A spectrophotometric method was applied for the determination of total flavan-3-ol- and anthocyanin content. Commercial standard were used to establish calibration curves. A panel of 13 experienced wine judges evaluated 72 wine samples, three times a week for three weeks for sensory attributes.

Results for Cabernet Franc wines treated over 2 consecutive vintages made with *S. cerevisiae* and *Torulaspora delbrueckii* yeast strains (VIN13 + 654 and M2/1 only) showed higher anthocyanin content (318.40 mg/L & 316.43 mg/L, respectively) and higher colour intensity (75.33%, M2/1; 74.16%, 654), compared to wines made with *Saccharomyces cerevisiae* yeasts (300.62 mg/L). Similar analyses were done on Pinotage wines made with *T. delbrueckii* only (strain 654) over five consecutive vintages showed higher anthocyanin content (468.63 mg/L) and colour intensity (76.29%), compared to wines made with *S. cerevisiae,* i.e. 426.08 mg/L (anthocyanins) and 65.77%, colour intensity.

A direct proportional relationship between the flavan-3-ol content (mouth-feel) and astringency was observed for Cabernet Franc wines made with 654 *T. delbrueckii* strains. These wines showed lower astringency than the *S. cerevisiae* treatment Cabernet Franc wines. This lead to the judges evaluating the wines as more mature. In conclusion, the use of *T. delbrueckii* on its own leads to more colour in Cabernet Franc and Pinotage wines.

<u>Sub-project 2</u>: Quantification of individual anthocyanins and flavanols in Cabernet Franc and Pinotage wines

This study investigated the effect of *T. delbrueckii* on the anthocyanins, flavan-3-ols and sensorial attributes of Pinotage and Cabernet Franc wines. The results have been published in the South African Journal of Enology and Viticulture (Minnaar *et al*., 2015; Addendum 2). A popular version has been accepted for publication in Winetech Tegnies (Effect of *Torulaspora delbrueckii* Yeast on the Colour and Mouthfeel Properties of Pinotage Wines and Cabernet Franc Wines, Minnaar, P., Jolly, N., Ngqumba, Z. and van Breda, V. The popular version is given below, and the Afrikaans version is attached (Addendum 3).

Summary

Pinotage and Cabernet Franc grape musts were fermented with *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* yeasts. Differences in colour were observed between *S. cerevisiae* Pinotage and *T. delbrueckii* Pinotage wines, whereas differences in berry and herbaceous aroma were observed between *S. cerevisiae* Cabernet Franc and *T. delbrueckii* Cabernet Franc wines. Total anthocyanins (colour compounds) were highest in *S. cerevisiae* Pinotage wines and *T. delbrueckii* Cabernet Franc wines. Tannins/flavanols (mouthfeel compounds) were highest in *T. delbrueckii* Pinotage and *S. cerevisiae* Cabernet Franc wines. The results show that yeast species impact on the anthocyanin and flavanol concentrations within a grape cultivar.

Introduction

Non-*Saccharomyces* yeast species contribute to the aroma and flavour of wine due to metabolic products resulting from their growth. These metabolites include terpenoids, esters, higher alcohols, glycerol, acetaldehyde, acetic acid and succinic acid. The yeasts also play a role in releasing volatile aroma compounds from non-volatile precursors and increasing polysaccharide concentrations in wine. Although far less studied, non-*Saccharomyces* yeasts can affect the phenolic compounds, i.e. anthocyanins and flavanols of wine.

The major non-yeast derived chemical compounds in red wine are the phenolics constituting the sub-groups of flavanols (contribute to mouthfeel) and anthocyanins (contribute to wine colour), which are present in grape skins and seeds. The colour of red wine, which is an important quality parameter, is primarily dependent on anthocyanins. The concentrations of anthocyanins in grape berry skin vary with grape cultivar and are affected by viticultural practices and environmental conditions. The anthocyanin profile of a given grape cultivar is linked to its genetic composition.

The anthocyanin and flavanol profiles of red wine are known to undergo some modification during winemaking because of physicochemical and biological factors. The transformation of grape juice into wine is a complex microbial reaction involving the sequential development of various yeast species responsible for alcoholic fermentation and lactic acid bacteria responsible for malolactic fermentation. These microbial populations can potentially affect the anthocyanin profile of red wine through three distinct processes, i.e. anthocyanin adsorption on cell walls, microbial metabolite-mediated formation of anthocyanin derivatives (acetylated and coumarylated) and anthocyanin hydrolysis. Cell adsorption of anthocyanins is considered a strain-dependent property within a specific yeast species which varies with the polarity of the anthocyanin. During alcoholic fermentation yeasts release secondary metabolic products such as pyruvic acid and acetaldehyde, which react with anthocyanins to produce more or less stable coloured derivatives, such as vitisin A, vitisin B and ethyl-linked anthocyanin-flavanol pigments. It is known that *S. cerevisiae* wine yeasts are among the contributors which decrease the phenolic concentration in wine, but the latest technology using non-*Saccharomyces* yeasts for wine production adds another variable to this topic. Therefore, this study investigated the effect of the non-*Saccharomyces* yeast, *T. delbrueckii* on the anthocyanins, flavanols and sensorial attributes of Pinotage and Cabernet Franc wines.

Material and Methods

Pinotage and Cabernet Franc grapes for vinification were obtained from the Nietvoorbij Research Farm of the Agricultural Research Council in Stellenbosch. The two yeast species used as single inoculants for wine production were T. delbrueckii (strain 654) and a commercial *S. cerevisiae* strain (VIN 13, Anchor Bio-Technologies). Three replicates of each yeast treatment were conducted over three vintages (2011-2013). Yeast starter cultures were cultivated in a yeast extract-peptone-dextrose broth and inoculated at a concentration of 1×10^6 cells/mL.

Pinotage and Cabernet Franc grapes were harvested at an average Brix of 25.3°Brix and 23.5°Brix, respectively, over the three consecutive vintages. Wines were made according to a standardised small-scale winemaking procedure in the Nietvoorbij Research Cellar at an ambient temperature of ca. 25°C. Malolactic fermentation was not induced for any of the wines.
Physicochemical parameters, i.e. residual sugar, percentage alcohol, total acidity (TA), pH, volatile acidity (VA), and glycerol were measured using a Foss® Winescan (Chemical Laboratory, IWBT, Stellenbosch University, Stellenbosch).

High-performance liquid chromatography with photodiode array detection (HPLC-DAD) was used for the quantification of anthocyanins and flavanols. The identification of the phenolic compounds was confirmed by their relative retention times and UV-visible absorption characteristics.

Sensory analyses were conducted on the wines five months after bottling. Sensory analyses involved the evaluation of colour intensity, berry aroma, mouthfeel and overall quality for Pinotage and Cabernet Franc wines with the addition of herbaceous aroma for the latter. The judges rated the wine sensory attributes on a 10 cm unstructured line scale. The chemical and sensory data were statistically analysed to help explain differences in the generated data.

Results and Discussion

Pinotage and Cabernet Franc grape musts' sugar averages over three years were slightly different (Table 1) with Cabernet Franc ca. two °Brix higher in total soluble solids than Pinotage.

This was also reflected in alcohol concentration differences of ca. 13% for Pinotage and ca. 15% for Cabernet Franc wines. Total acidity was higher in Pinotage grape must, compared to Cabernet Franc grape must, while the average pH values were similar for both grape cultivars over the three vintages. Considering the expected variability between vintages within a grape cultivar, the analyses of the base musts in terms of physicochemical parameters were of sufficient similarity per cultivar to make comparisons in wines derived from the yeast treatments. Monitoring of the fermentations showed that the *T. delbrueckii* treatments took the same or double the time to ferment to below 0°Brix, compared to *S. cerevisiae* treatments (data not shown). This was expected, as it is known that the *T. delbrueckii* yeasts are slower fermenters than *S. cerevisiae* yeasts. However, the skin contact time, with the exception of the 2013 Cabernet Franc, was the same for a grape cultivar. This ensured that anthocyanin and flavanols extraction were the same for a specific yeast treatment per vintage. The Pinotage wines had a shorter skin contact time (5-7 days), compared to the Cabernet Franc wines (7-14 days) over the three vintages.

Table 1. Physicochemical parameters measured in Pinotage and Cabernet Franc grape musts and wines indicating average values and standard deviations over three consecutive vintages (2011-2013). Wines were produced on small-scale with two different yeast species.

	Pinotage		**Cabernet Franc**	
Parameters	***S. cerevisiae*** **Strain VIN 13**	***T. delbrueckii*** **Strain 654**	***S. cerevisiae*** **Strain VIN 13**	***T. delbrueckii*** **Strain 654**
Base must				
Sugar (°Brix)	23.47 (± 1.97)		25.26 (± 0.73)	
Total acidity (g/L)	6.66 (± 0.36)		5.33 (± 1.01)	
pH	3.36 (± 0.13)		3.52 (± 0.17)	
Wine				
Volatile acidity (g/L)	0.41 (± 0.13)	0.41 (± 0.15)	0.37 (± 0.21)	0.48 (± 0.22)
Residual sugar (g/L)	1.32 (± 0.41)	1.61 (± 0.60)	1.60 (± 0.19)	2.96 (± 0.42)
Alcohol (v/v %)	13.64 (± 1.76)	13.53 (± 1.81)	15.34 (± 0.51)	15.15 (± 0.52)
Glycerol (g/L)	9.76 (± 4.89)	9.67 (± 1.17)	10.05 (± 0.41)	11.03 (± 0.52)

Volatile acidity concentrations of the treated wines were similar (Table 1), except for Cabernet Franc wines fermented with *T. delbrueckii*, which were highest. Glycerol concentrations for both grape cultivars were above 5.2 g/L (Table 1). This is the level where a sweet taste is detected. Cabernet Franc wines were highest in glycerol levels. Glycerol can also contribute to smoothness (mouthfeel) and complexity in wines.

The sensory data showed that Cabernet Franc wines for both yeast treatments showed similar scores for wine colour intensity (Table 2). In contrast, *T. delbrueckii* Pinotage wines were higher in colour intensity, compared to *S. cerevisiae* Pinotage wines. However, the Pinotage wines showed a notably greater standard deviation, compared to Cabernet Franc wines. Cabernet Franc grape must inoculated with *T. delbrueckii*, scored higher in berry and mouthfeel attributes, compared to Cabernet Franc inoculated with *S. cerevisiae*. Overall quality scored highest for Cabernet franc wines made with *T. delbrueckii*. Cabernet Franc wines made with *S. cerevisiae* were higher in colour intensity and mouthfeel than Cabernet Franc wines made with *T. delbrueckii*. Differences in berry attributes for both the grape cultivars were noted between the two treatments. Differences in herbaceous attributes were evident for Cabernet Franc wines between the two treatments.

Table 2. Average scores (percentage) and standard deviations of sensory attributes in Pinotage (2011-2013) and Cabernet Franc (2011-2013) wines. Wines were produced on small-scale with two different yeast species.

Sensory attributes	Pinotage		Cabernet Franc	
	S. cerevisiae **Strain VIN 13**	***T. delbrueckii*** **Strain 654**	***S. cerevisiae*** **Strain VIN 13**	***T. delbrueckii*** **Strain 654**
Colour intensity	44.24 (± 17.43)	55.33 (± 13.86)	54.33 (± 5.50)	56.33 (± 6.70)
Berry aroma	45.35 (± 9.01)	52.06 (± 11.13)	48.01 (± 5.29)	55.33 (± 11.15)
Herbaceous aroma	NA[1]	NA[1]	39.11 (± 8.00)	36.51 (± 5.51)
Mouthfeel	42.33 (± 7.09)	44.66 (± 8.51)	47.66 (± 4.04)	51.33 (± 8.73)
Overall quality	40.66 (± 7.37)	45.66 (± 7.37)	48.33 (± 5.13)	53.66 (± 10.78)

[1]NA = Not-Applicable

The differences in residual sugar and glycerol levels between the two grape cultivars (Table 1) were evident, but these parameters do not sufficiently explain the differences observed between mouthfeel and overall quality. Table 3 lists the total anthocyanin and flavanol concentrations for Pinotage and Cabernet Franc wines made with two yeast species. Anthocyanins and flavanols measured in Pinotage wines made with *T. delbrueckii* were consistently higher, compared to Pinotage grape must inoculated with *S. cerevisiae*. In contrast, Cabernet Franc wines made with *T. delbrueckii* were lower in anthocyanin and flavanol concentrations than Cabernet Franc wines made with *S. cerevisiae*.

The sensory attribute scores for Pinotage wines correlate with the anthocyanins and flavanol concentrations. Cabernet Franc wines, however, showed a negative correlation between sensory attribute scores and anthocyanin and flavanol concentrations.

Conclusions

Pinotage and Cabernet Franc grape cultivars reacted to both yeast treatments. There are clear differences in sensory scores, phenolic compounds (anthocyanins and flavanols) and physicochemical parameters of Pinotage and Cabernet Franc wines made with *S. cerevisiae* and *T. delbrueckii* yeasts. In this study *T. delbrueckii* is the preferred yeast for Pinotage grape must and *S. cerevisiae* the preferred yeast for Cabernet Franc grape must in terms of anthocyanins (colour) and flavanol (mouthfeel/tannins) concentrations. Only one strain of each yeast species was used in this study. It is expected that a similar trend may be observed for other strains. However, due to the high genetic variability found amongst yeast strains within a species, this observation should be substantiated with further research.

<u>Sub-project 3</u>: Effect of *Torulaspora delbrueckii* yeast strains on phenolics and sensory attributes of Chenin blanc wines

This section formed part of the CPUT MTech degree (Ms Zama Ngqumba) and has been submitted for publication to the South African Journal of Enology and Viticulture (Ngqumba *et al.,* 2016; Addendum 4). The purpose of the investigation was to identify and quantify the phenolic compounds affected by two *Torulaspora delbrueckii* yeast strains (654 and M2/1) during production of Chenin blanc wines in comparison to *Saccharomyces cerevisiae* (strain VIN 13) wines. Forty wines comprising single and co-inoculated wines over three vintages were analysed.

Abstract

The non-*Saccharomyces* yeast, *Torulaspora delbrueckii* contributes positively to the sensory properties of wines by affecting aroma and flavour due to changes in alcohols, esters, fatty acids and lactone levels. One of the lesser-studied aspects of *T. delbrueckii* is their effect on phenolic compounds relating to sensory attributes. An HPLC-DAD technique was used for the quantitation of phenolic compounds in Chenin blanc wines made with *S. cerevisiae* and two *T. delbrueckii* yeasts over three vintages. Chemical and sensory data were subjected to ANOVA and PCA. Differences were observed between the wines made with the two *T. delbrueckii* strains in (+)-catechin, caffeic-, p-coumaric-, and ferulic acid concentrations. Wines made with

the *S. cerevisiae* strain had higher concentrations of flavan-3-ols, compared to wines made with *T. delbrueckii* strains. In this study an association was evident between (+)-catechin and astringency, (-)-epicatechin and body mouthfeel and (-)-epicatechin, (+)-catechin and astringency. The *S. cerevisiae* strain led to more perceived acidity and astringency, while the *T. delbrueckii* strains contributed to body mouthfeel of the wines.

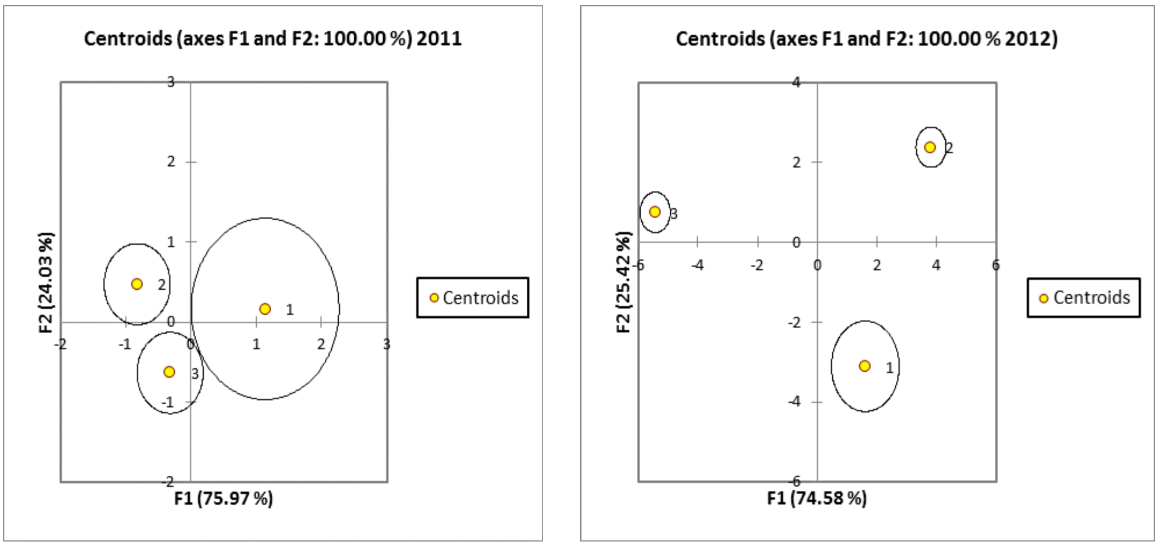
Volatile compounds

<u>Sub-project 4</u>: Volatile aroma compounds

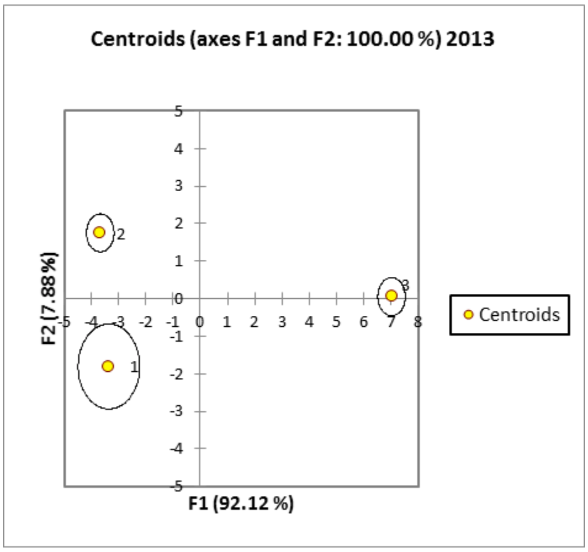
Chenin blanc wines from three vintages (2011 to 2013) were analysed for 32 volatile aroma compounds by gas chromatography coupled to a flame ionization detector (GC-FID) at the Central Analytical Facility, Stellenbosch University. These wines represented three treatments. Treatment 1 was a reference inoculated with *S. cerevisiae* only. Treatment 2 was *T. delbrueckii* co-inoculated at time zero simultaneously with *S. cerevisiae*, and Treatment 3 was a *T. delbrueckii* inoculation followed 24 hours later with a *S. cerevisiae* inoculation. The *T. delbrueckii* yeasts consisted of two natural isolates and three *T. delbrueckii* commercial yeast strains.

Principle Component Analyses (PCA) and Discriminant Analyses (DA) were carried out to mine the data-set. Initial indications were that vintage was a greater driver of differentiation than yeast treatment (data not shown), and is consistent with the variability in grape must composition expected between vintages. Based on the above finding, the data was subsequently analysed per vintage.

Discriminant analyses of esters, alcohols and fatty acid totals showed that each group of compounds were playing a role in differentiation of the wines from the three treatments (data not shown). Further analyses considering each of 28 volatile aroma compounds individually showed separation between the three treatments over the three vintages studied (Fig. 1). Differentiation on the F1 axis was between 74 and 92% and was driven by 17, 16 and eight of the volatile compounds for the 2011, 2012 and 2013 wines, respectively. However, over all three vintages six compounds consistently appeared to play the biggest role in the differentiation. They were in no specific order: 2-phenyl ethanol (floral, rose), diethyl succinate (fruity), iso-valeric acid (rancid, cheese, sweaty), acetic acid (vinegar pungent), ethyl acetate (varnish, nail polish, fruity) and isobutyric acid (pungent). However, the contribution by the other compounds cannot be disregarded, as these can all interact with, or be masked by each other.



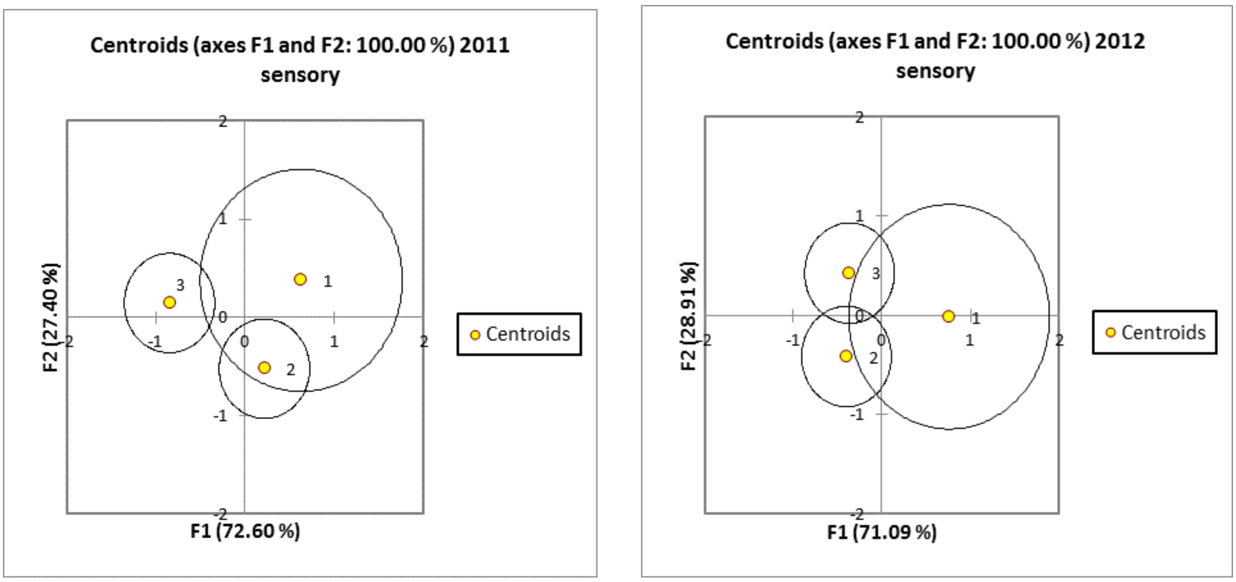
2011 **2012**



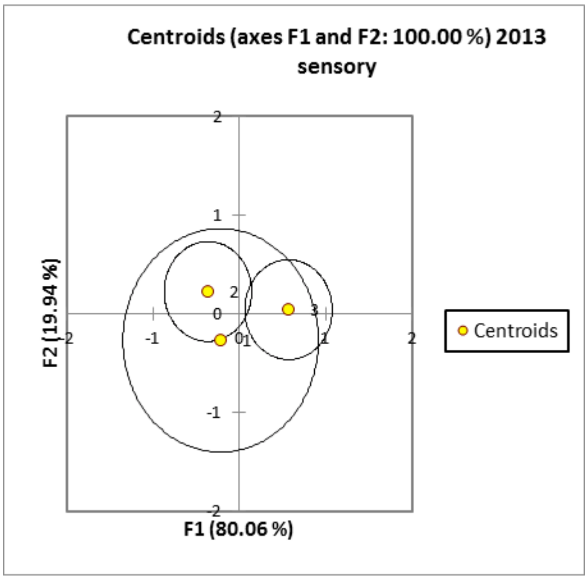
2013

Fig. 1. Discrminant analyses of 28 volatile compounds of Chenin blanc wines produced over three vintages (2011-2013). Treatment 1 = *S. cerevisiae* only; treatment 2 = *T. delbrueckii* and *S. cerevisiae* co-inoculated at time zero; Treatment 3 = S. *cerevisiae* inculated 24 hours after *T. delbrueckii*.

Discriminant analyses of the sensory data showed that there is an overlapping of the three different yeast treatments (Fig. 2). This is in contrast to the analytical data as shown in Fig. 1. It is thus apparent that the sensory panel, as a whole, was not able to distinguish between the three different treatments. Considering the discriminant analyses results, it can however, be concluded that individual tasters who may be more sensitive to certain aromas, were able to distinguish between the treatments.



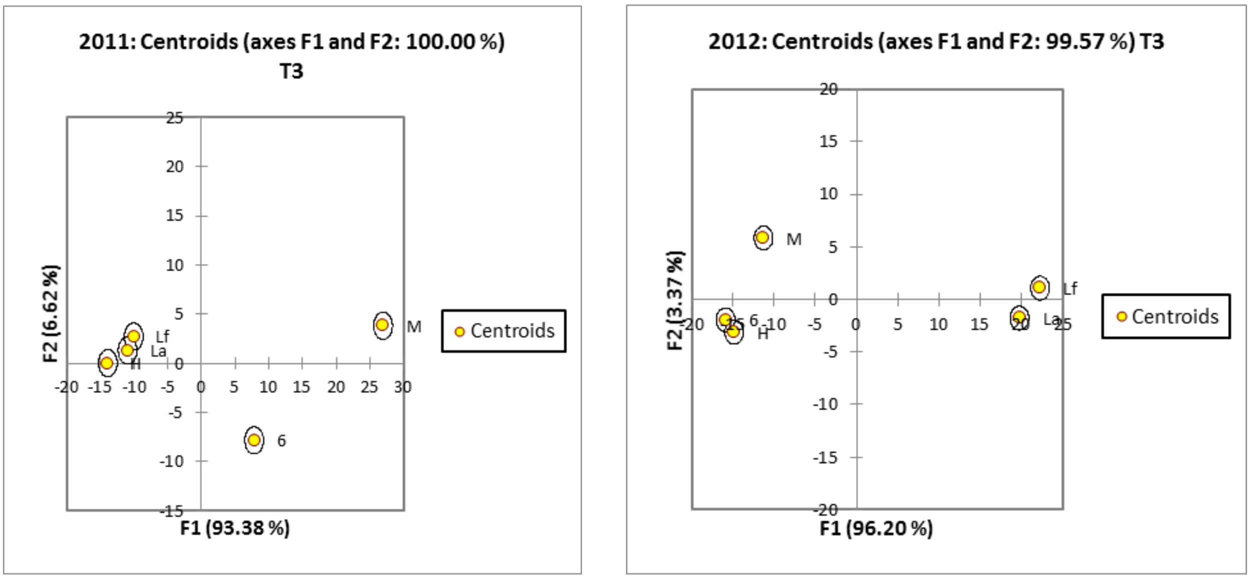
2011 **2012**



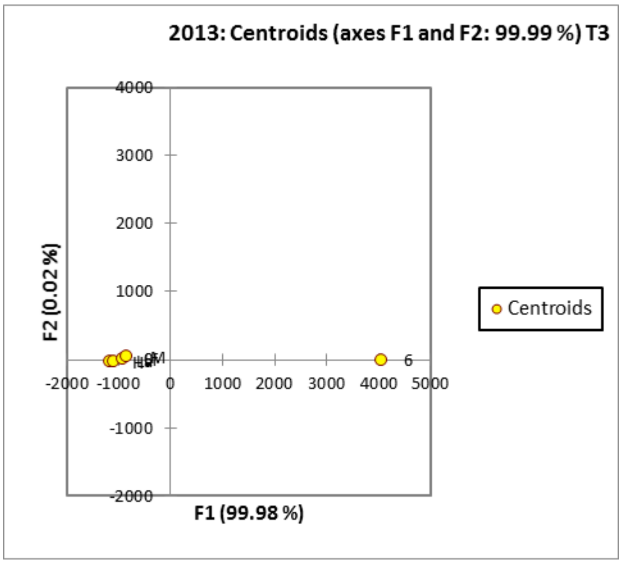
2013

Fig. 2. Discrminant analyses of sensory data of Chenin blanc wines produced over three vintages (2011-2013). Treatment 1 = *S. cerevisiae* only; treatment 2 = *T. delbrueckii* and *S. cerevisiae* co-inoculated at time zero; Treatment 3 = S. *cerevisiae* inoculated 24 hours after *T. delbrueckii*.

Further statistical analyses of volatile aroma compound data of treatment 3 only (*T. delbrueckii* co-inoculated with *S. cerevisiae* 24 hours later) showed strain variation regarding the effect on volatile aroma compound concentrations (Fig. 3). This effect differed over the three vintages. Generally, the non-local strains (Lf, La, H) clustered separately from the local isolated strains (M and S) and separation was mainly along the F1 axis (92 to 99%). Nineteen, 22 and 14 compounds were responsible for the separation between the treatments for the 2011, 2012 and 2013 wines, respectively. No specific compound could be consistently linked to the differentiation between the treatments, which can be explained by the genetic variation between the strains, and differences in the must composition (complex nutrients, nitrogen and pre-cursors) over the three vintages.



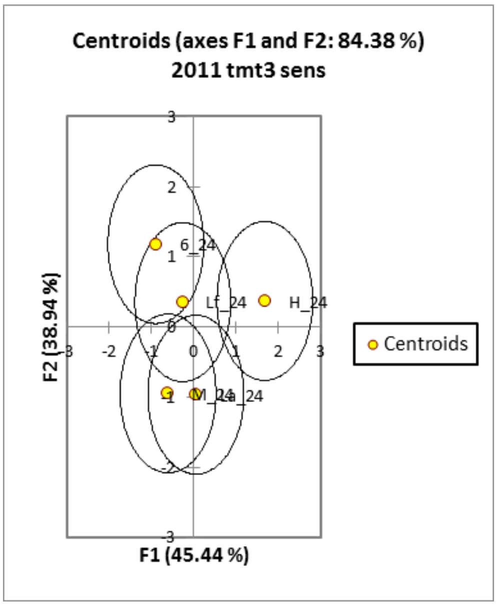
2011 **2012**

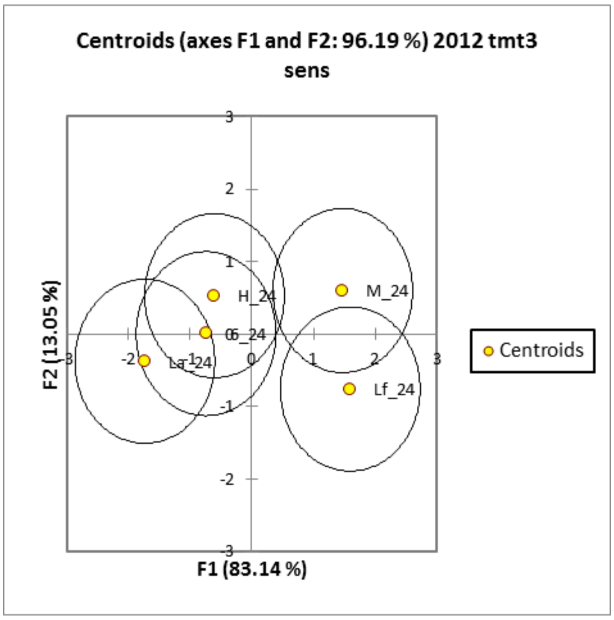


2013

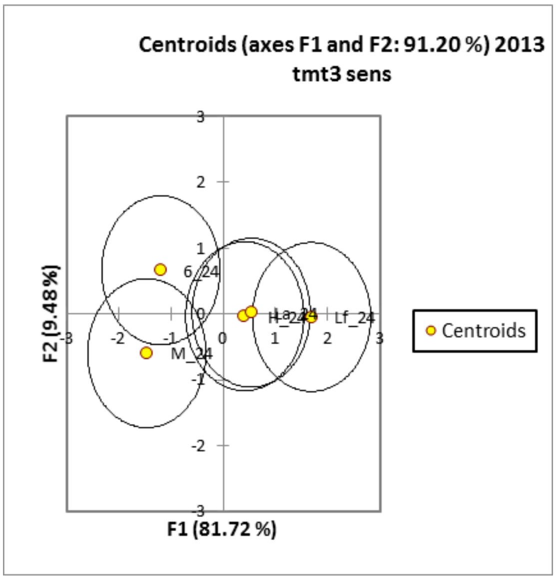
Fig. 3. Discriminant analyses of 32 volatile compounds produced by five *T. delbrueckii* strains co-inoculated with *S. cerevisiae* 24 hours after the *T. delbrueckii* (Treatment 3). Lf, La, H are non-local strains, and M and S are locally isolated *T. delbrueckii* strains.

Analyses of the sensory data of treatment 3 only showed overlapping between the wines made by the five *T. delbrueckii* strains, but separation between some individual strains (Fig. 4). The differences between the wines were for “sugar” perception in 2011 and 2012, although the wines were all dry (2.13 g/L ± 0.97). For the 2013 wines differentiation was based on “Fruity”, “body” and “general quality”. This confirms the results obtained with the polyphenol data-set.





2011 **2012**



2013

Fig. 4. Discrminant analyses of sensory data of Chenin blanc wines produced over three vintages (2011-2013) for Treatment 3 (S. *cerevisiae* inoculated 24 hours after *T. delbrueckii*).

Other analyses

Sub-project 5: SO_2 levels

As higher SO_2 levels have previously been noted in wines where *T. delbrueckii* was the only yeast inoculated, the total SO_2 levels were measured. The co-inoculated wines mostly had comparable total SO_2 levels to the *S. cerevisiae* wines, and the use of the strains in this study will not adversely affect SO_2 levels in wine.

7. COMPLETE THE FOLLOWING TABLE

Milestone	Target Date	Extension Date	Date completed	Achievement
1. Collection of wines from ARC Research Cellar	Wines withdrawn as required.		December 2015	Wines collected and analyses.
2.Semi quantitative analyses of red wine samples for polyphenols	May 2013		May 2013	Wines analysed.
3.Quantitative analyses of red wine samples for polyphenols	June 2014		June 2014	Data published.
4. Quantitative analyses of white wine samples for polyphenols	June 2015		June 2015	Data submitted for publication.
5. GC-FID analyses	September 2014		June 2015	Wines from three vintages analysed.
6. Sensory analyses	December 2014		December 2014	Sensory analyses done.
7. Compilation of all chemical and sensory data	June 2015	June 2016	June 2016	Compilation completed.

8. Statistical analyses of all data	July 2015	June 2016	June 2016	Analyses completed.
9. Interpretation of data and technology transfer to stakeholders	July 2015	June 2016	June 2016	Completed.
5. Journal publication(s) – final milestone			June 2016	One published article, one submitted for publication, one popular article submitted for publication.

8. CONCLUSIONS

- The use of *T. delbrueckii* on its own leads to more colour in Cabernet Franc and Pinotage wines. This can be determined by a spectrophotometric method measuring total polyphenols, making it more accessible for cellar staff to use. *T. delbrueckii* wines were also judged to have lower astringency, and therefore more mature.

- In terms of combined results of analyses of red wine pigments (malvidin) and flavanol (mouthfeel/tannins) concentrations, *T. delbrueckii* is, however, the preferred yeast for Pinotage, and *S. cerevisiae* the preferred yeast for Cabernet Franc wines. This method requires more specialised equipment and expertise.

- Quantification of phenolic compounds and sensory data of Chenin blanc wines showed that *S. cerevisiae* led to more perceived acidity and astringency, while the *T. delbrueckii* strains contributed to body mouthfeel of the wines. This is similar to the conclusions made about the red wines.

- Vintage influences the effect that *T. delbrueckii* can have on volatile aroma compounds. However, within a vintage, yeast combination and time of inoculation has a definitive role in the chemical fingerprint on the wine.

- *T. delbrueckii* yeasts strains can positively affect non-volatile (mouth-feel and colour) and volatile (aroma) compounds. The individual strains also differ in their effect on wine aromatics. The sensory data is supported by chemical data.

9. ACCUMULATED OUTPUTS

<u>Students and interns trained</u>:

- BTech student graduated (CPUT), October 2014 (Mr Ludwe Nyobo).
- One MTech graduated (CPUT), September 2016 (Ms Zama Ngqumba).
- One DST-NRF intern trained, 2015 (Ms Veruscha Paulsen).
- One DST-NRF intern trained, 2016 (Ms Melanie Vermeulen).

<u>Presentations at conferences</u>:

- One poster presentation at ARC Post Graduates Conference and Awards Ceremony, June 2014 (Ms Z. Ngqumba et al 2014).
- One oral presentation at 36th Conference of the South African Society for Enology and Viticulture, November 2014 (P.P. Minnaar et al., 2014).
- One poster presentation at the 7th International Conference on Polyphenols and Health, Tours, France, October 2015 (P.P. Minnaar et al., 2015).

Publications:

- One article published in the South African Journal of Enology and Viticulture, 2015 (Minnaar *et al*. 2015).
- One article submitted for publication in the South African Journal of Enology and Viticulture (Minnaar *et al*., December 2016).
- One popular publication accepted for publication in Winelands (Winetech Tegnies).

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

-

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

-

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

Student Name and Surname	**Student Nationality**	**Degree** (e.g. MSc Agric, MComm)	**Level of studies in final year of project**	**Graduation date**	**Total cost to industry throughout the project**
BTech/Honours students					
Ludwe Nyobo	South African	BTech	B Tech	October 2014	0
Masters Students					
Zama Ngqumba	South African	MTech	3rd year	September 2016	0
PhD students					
Phillip Minnaar	South African	PhD	PhD	December 2017	0
Postdocs					
-					
Support Personnel					
Veruscha Paulsen	South African	N/A	N/A	N/A	0
Melanie Vermeulen	South African	N/A	N/A	N/A	0

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

Minnaar PP, Ntushelo N, Ngqumba Z, Van Breda V, Jolly NP (2015). Effect of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* yeasts on the anthocyanins and flavanols of Cabernet Franc and Pinotage wines. South African Journal of Enology and Viticulture 36, 50-58.

Ngqumba Z, Ntushelo N, Ximba BJ, Jolly NP, Minnaar PP (2016). Effect of *Torulaspora delbrueckii* yeast strains on phenolics and sensory attributes of Chenin blanc wines. South African Journal of Enology and Viticulture (submitted for publication, December 2016).

e) PRESENTATIONS/PAPERS DELIVERED

Ngqumba Z, Minnaar PP, Jolly NP, Ximba BJ (2014). The application of an HPLC technique for the quantification of flavanols and flavonols in Chenin blanc wines produced by *T. delbrueckii* yeast. ARC Post Graduates Conference and Awards Ceremony, June 2014 (Poster).

Minnaar PP, Ntushelo N, Ngqumba Z, Van Breda V, Jolly NP (2014) Effect of *Torulaspora delbrueckii* Yeast on the Anthocyanin and Flavanol Concentrations of Cabernet Franc and Pinotage Wines. 36th South African Society for Enology and Viticulture (SASEV) Conference, Lord Charles Hotel, Somerset-West, South Africa, 12 - 14 November 2014 (Oral).

Minnaar P, Ntushelo N, Ngqumba Z, Jolly N, (2015) Effect of *Torulaspora delbrueckii* yeast on the anthocyanin and flavanol concentrations of Cabernet Franc and Pinotage wines. 7th International Conference on Polyphenols and Health, Tours, France, 27-30 October 2015 (Poster).

10. BUDGET

a) TOTAL COST SUMMARY OF THE PROJECT

YEAR	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
2013/14					303 544		315 934	619 478
2014/15					320 219		333 289	653 508
TOTAL					623 753		649 223	**1 272 976**

EVALUATION BY INDUSTRY

This section is for office use only

Project number	

Project name	

Name of Sub-Committee*	

Comments on project

Committee's recommendation

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

Chairperson____ Date___________________

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*SUB-COMMITTEES

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

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