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Indicate (X) client(s) to whom this concept project proposal is submitted. Replace any of these with other relevant clients if required.

NB: The instructions in red, throughout the template, should be omitted from the final document.

FINAL REPORT
(2016)

1. PROGRAMME AND PROJECT LEADER INFORMATION

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2. PROJECT INFORMATION

Research Organisation Project number	000279 (WW 08/34)
Project title	Effect of oenological parameters on acetaldehyde kinetics during alcoholic fermentation and its impact on ethanol-producing organisms in South African must/wine.
Short title	Acetaldehyde as affected by oenological parameters, and its impact on ethanol producing organisms

Fruit kind(s)	Grapes (must & wine)		
Start date (mm/yyyy)	04/2012	**End date** (mm/yyyy)	03/2016

Key words	Acetaldehyde, yeasts, oenological parameters, commercial yeast, *Saccharomyces cerevisiae*, non-*Saccharomyces*, Chenin blanc, Pinotage, wine quality

Approved by Research Organisation Programme leader (tick box) ☐

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

3. EXECUTIVE SUMMARY

Acetaldehyde plays a role in the rate of fermentation and the aroma quality of the wine. High concentration levels of acetaldehyde in fermenting must/juice may retard or inhibit yeast ethanol formations, resulting in sluggish or stuck fermentations. It has thus been

established that, depending on its concentration levels, acetaldehyde has an effect on yeast metabolism and can therefore influence fermentation kinetics by the inhibition or stimulation of the lag phase and growth rate, which in turn has an impact on the cell yield (biomass). The overall aim of this project is to study the impact of acetaldehyde, as influenced by different yeast strains and oenological parameters, on fermentation dynamics and wine quality, since specific levels of acetaldehyde could result in stuck fermentations, as well as off-odours in wine.

Ten commercial *Saccharomyces cerevisiae* strains and ten non-commercial non-*Saccharomyces*, with the exception of *Lachancea thermotolerans*, which was isolated from the commercial yeast, Rhythm (Chr. Hansen) were selected from the culture collection of ARC Infruitec-Nietvoorbij. These yeasts were screened in a laboratory trial for their acetaldehyde-producing ability during alcoholic fermentation, and resulted in the selection of a high-, medium- and low-acetaldehyde producing yeast. Three levels of acetaldehyde production (high, medium & low) was selected to have definitive parameters when studying the impact that these different acetaldehyde levels has on the fermentation dynamics. The selected yeasts were the *Saccharomyces cerevisiae* yeasts NT 50 (high), NT 116 (medium) and VIN 13 (low); and the non-*Saccharomyces* yeasts *Torulaspora delbrueckii* (high), *Candida valida* (medium) and *Candida guilliermondii* (low).

Initially, it was decided to use Pinotage and Sauvignon blanc as the two local cultivars on which the study would be performed. However, Chenin blanc replaced Sauvignon blanc as cultivar of choice, since it was felt that the typical methoxypyrazine aromas of Sauvignon blanc could adversely impact the sensorial detection of acetaldehyde in wine.

For the cellar trial Pinotage and Chenin blanc grapes were harvested from Nietvoorbij vineyards and, after crushing, the musts were frozen away at subzero temperatures and later vinified. The above-mentioned selection of *Saccharomyces* yeasts was used individually for vinification of grape must, as well as in varying combinations with the non-*Saccharomyces* yeasts, and resultant wines analysed chemically and evaluated sensorially. The initial sensory evaluation/discussion, amongst judges from Nietvoorbij

and the University of Stellenbosch, showed noticeable differences between treatments, in terms of aroma and residual sugar.

Statistical evaluation of the data from the laboratory and cellar trials clearly showed that yeast strain, time of fermentation and fermentation temperature have an impact on levels of acetaldehyde. It is, therefore, concluded that wines co-inoculated with selective yeasts at low fermentation temperatures will have lower levels of acetaldehyde than wines only inoculated with *Saccharomyces cerevisiae* yeasts.

Since SO_2 has a very high affinity for acetaldehyde, the impact of various concentrations of SO_2 on the levels of acetaldehyde in fermenting must was monitored in a cellar trial, and the resulting effects on fermentation dynamics and final wine quality were monitored. Although it is known that SO_2 impacts wine quality, it was also found in this experimental trial that the varying levels of SO_2 have a direct effect on the acetaldehyde levels during fermentation and in the finished wine.

As expected, there were differences in acetaldehyde levels between yeast strains, although the levels were still within acceptable ranges or limits found in wines. Higher acetaldehyde levels were found in samples inoculated with *Saccharomyces cerevisiae (*because the rate of fermentation of *S. cerevisiae* is much faster than the co-inoculated samples), as well as samples exposed to high SO_2 levels, and at higher fermentation temperatures. Therefore, to ensure lower levels of acetaldehyde in wine, one must preferably co-inoculate with a non-*Saccharomyces* yeast strain at lower fermentation temperatures, while maintaining low levels of SO_2 during fermentation.

4. PROBLEM IDENTIFICATION AND OBJECTIVES

Acetaldehyde (ethanal) is a volatile chemical compound found to play a significant role in wine aroma, colour and microbiological stability. At low levels acetaldehyde can contribute pleasant fruity aromas, and add flavour complexity in full flavoured wines, especially red wines. Increases in acetaldehyde concentration during vinification, generally are in adverse correlation to sensory properties.

Acetaldehyde is formed during the anaerobic fermentation of grape must to wine (Osborne *et al.*, 2000; Jackowetz *et al.*, 2010), and is also the most important carbonyl

compound produced by yeast metabolism during alcoholic fermentation (Nykänen, 1986). Post-fermentation activity, like the oxidation of ethanol in wine, can also lead to the production of acetaldehyde (Wildenradt & Singleton, 1974; Romano *et al.*, 1994; Jackowetz *et al.*, 2010).

High concentration levels of acetaldehyde in fermenting juice is unwanted as it may retard or inhibit yeast ethanol formations, resulting in sluggish or stuck fermentations (Liu & Pilone, 2000), and depending on its concentration levels, acetaldehyde may affect yeast metabolism negatively. Acetaldehyde can, therefore, influence fermentation kinetics by the concentration-dependent inhibition or stimulation of the lag phase and growth rate (Stanley *et al.*, 1993; Liu & Pilone, 2000; Vriesekoop, 2007; Hucker & Vriesekoop, 2008), which in turn has an impact on the cell yield (biomass). The overall aim of this project is to better understand the impact of acetaldehyde, as influenced by different yeast strains and oenological parameters, on fermentation dynamics and wine quality. The purpose of the selection of a high-, medium- and low-acetaldehyde producer was to observe the correlation between the different yeast strains and acetaldehyde production, because the range of acetaldehyde levels in wine is very wide and can have a positive or negative effect on the aroma profile of wine. High intra- or extracellular concentrations or accumulation of acetaldehyde inhibit yeast ethanol fermentations, resulting in sluggish or stuck alcoholic fermentations as sometimes experienced by some winemakers, but overlooking the possible role of acetaldehyde amongst the other possible causes of sluggish or stuck fermentations, the end-result being that the quality of the wine and the economics of wine production is jeopardised.

5. WORKPLAN (MATERIALS AND METHODS)

5.1. *Literature review (continuous)*

The literature review forms part of the MSc study and for a more complete and detailed overview we will refer to the final MSc thesis.

5.2. *Laboratory-scale trials (screening of yeasts)*

Grape musts of two common South African cultivars, Sauvignon blanc (replaced by Chenin blanc – see below) and Pinotage, were procured from the Nietvoorbij farm and stored frozen until the laboratory-scale fermentation trials commenced.

Since it was felt that the aroma profile of Sauvignon blanc could adversely impact the sensorial detection of acetaldehyde in wine, it was decided that Chenin blanc should replace Sauvignon blanc as the white cultivar of choice for future trials. However, due to the unavailability of Chenin blanc during this laboratory trial, it was decided to only use clear Pinotage juice (free run/no skin contact) for the yeast screening, since a single cultivar was sufficient for this purpose. During a pre-trial (pilot) the Pinotage must was divided into different batches and the effects of freezing, racking, fermentation and filtration were investigated for their effects on acetaldehyde production.

The laboratory trials for the screening of the yeasts were done merely to select the highest, medium and lowest (relative) acetaldehyde-producing yeasts. The purpose of this selection was to observe the correlation between the different yeast strains and acetaldehyde production, because the range of acetaldehyde levels in wine is very wide and can have a positive or negative effect on the aroma profile of wine.

Ten *Saccharomyces* and 10 non-*Saccharomyces* yeasts were screened for their acetaldehyde-producing abilities during the first and second laboratory trials, respectively (Table 1).

Yeast strains were sourced from the ARC Microbiology Yeast Culture Collection & Genebank at Nietvoorbij, Stellenbosch. Published literature were used as a guide in the selection process. The Microbiological team at Nietvoorbij assisted with the careful selection and/or procurement of the relevant yeasts. The eventual screening outcome enabled the selection of a high-, medium- and low-acetaldehyde producer, the purpose or reason for the selections being described above.

Standard laboratory protocol was followed for the growing up of the yeasts, as well as for small-scale vinification.

All yeasts were grown up on YPD media and each was inoculated into a 500 mL aliquot of autoclaved grape juice. The inoculations were done in duplicate. Colony forming unit (CFU) measurements were also performed and expressed as CFU/ml.

Table 1. *Saccharomyces* and non-*Saccharomyces* yeasts screened for their acetaldehyde-producing abilities

Commercial *Saccharomyces cerevisiae* yeasts	**Non-Commercial non-*Saccharomyces* yeasts**
VIN 13 (Anchor)	*Hanseniaspora uvarum/ Kloeckera apiculata*
EC1118 (Lallemand)	*Metschnikowia pulcherrima/ Candida pulcherrima*
Zymaflore VL1 (Laffort)	*Torulaspora delbrueckii/ Candida colliculosa*
Zymaflore VL3 (Laffort)	*Pichia fermentans/ Candida lambica*
D47 (Lallemand)	*Hanseniaspora valbyensis*
QA23 (Lallemand)	*Pichia guilliermondii/ Candida guilliermondii*
NT 202 (Anchor)	*Pichia membranifaciens/ Candida valida*
NT 116 (Anchor)	*Pichia kluyveri*
NT 50 (Anchor)	*Lachancea fermentati*
NT 112 (Anchor)	*Lachancea thermotolerans ("Rhythm")/ Candida dattila*

Fermentations were conducted at two temperatures: 15°C (to simulate white wine-making conditions) and 25°C (to simulate red wine-making conditions). The fermentation rates of all the treatments were closely monitored by regularly weighing & recording the mass-loss of fermenting must. Samples were taken at specific intervals during alcoholic fermentation for analyses of acetaldehyde levels (mg/L or ppm) in the fermenting must, as well as analyses of the routine winemaking parameters, i.e. pH, ethanol concentration (vol %), total residual sugar (glucose and fructose) concentration (g/L), titratable acidity (g/L) and malic acid (g/L).

5.3. *Wine-making (with selected yeast strains / combinations) – Cellar trial*

Pinotage and Chenin blanc grapes were harvested from Nietvoorbij vineyards and, after crushing, frozen away at subzero temperatures. The Chenin blanc must was spoilt as a

result of spontaneous fermentation, and therefore only the Pinotage must was vinified according to the blanc-de-noir style.

Once it had been established which yeast strains were the highest-, medium- and lowest-acetaldehyde producers, the final fermentation trial commenced in which the three selected *Saccharomyces cerevisiae* strains were used individually, and in combination with the three selected non-*Saccharomyces* strains in various permutations for the vinification of Pinotage grape must (Table 2). The *Saccharomyces cerevisiae* yeasts were NT 50 (high), NT 116 (medium) and VIN 13 (low); and, the non-*Saccharomyces* yeasts were *Torulaspora delbrueckii* (high), *Candida valida* (medium) and *Candida guilliermondii* (low). For each combination treatment the non-*Saccharomyces* yeasts were inoculated 24 h prior to *Saccharomyces cerevisiae* inoculation. The standard Nietvoorbij winemaking protocol was followed for the vinification process, except for the yeast inoculation. Wine was fermented to dryness, bottled and stored until the sensorial evaluations. Samples for acetaldehyde, routine wine analyses, including alcohol analyses, were taken regularly during the alcoholic fermentation.

Cell counts (CFU) and yeast strain identification during fermentation were also performed in trials where *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts were co-inoculated.

Acetaldehyde was quantified. Routine chemical analysis, i.e. pH, SO_2, TA, VA and alcohol were also performed with OenoFoss. Following an informal wine-tasting (discussion to identify aroma descriptors), whereafter a formal sensory evaluation took place by a panel of expert judges to score the intensity of a set of aroma descriptors on a 10-point line-scale.

Table 2. Yeast strains and combinations for cellar fermentation trial using Pinotage must

Treatment	1st inoculation	2nd inoculation (24 hr later)
1	0	VIN 13*
2	0	NT 50

3	0	NT 116
4	0	VIN 13
5	*Torulaspora delbrueckii*	NT 50
6	*Candida valida*	NT 50
7	*Candida guilliermondii*	NT 50
8	*Torulaspora delbrueckii*	NT 116
9	*Candida valida*	NT 116
10	*Candida guilliermondii*	NT 116
11	*Torulaspora delbrueckii*	VIN 13
12	*Candida valida*	VIN 13
13	*Candida guilliermondii*	VIN 13

*Control
All other yeasts were sourced from the ARC Microbiology Yeast Culture Collection & Genebank at Nietvoorbij, Stellenbosch. Chenin blanc must was not vinified (see above).

5.4. *The effect of SO_2 on the levels of acetaldehyde and the resulting effect on fermentation dynamics and wine quality - Cellar trial*

In an independent cellar trial, different concentrations of SO_2 (0, 50, 100 & 150 ppm) were added to Pinotage and Chenin blanc grape must prior to fermentation (Day 0) to monitor its effect on acetaldehyde levels. In addition to the control (no SO_2 added), initial SO_2 concentrations were manipulated externally by addition of pre-calculated volumes from a stock SO_2 solution to the must samples, to final concentrations of 50, 100 and 150 mg/L. The control had a baseline SO_2 value and was not 0 mg/L. The standard red and white wine-making protocols, with the exception of the SO_2 inoculations, were followed in the Nietvoorbij Cellar. SO_2 additions were done before the *Saccharomyces cerevisiae* (VIN 13) yeast inoculations. Samples for SO_2, acetaldehyde and routine wine analyses were taken daily until the end of fermentation. Wine was fermented to dryness, bottled and stored until such time that the sensorial evaluations would be performed. Acetaldehyde concentrations (mg/L) were quantified using an Arena Enzyme Robot. Routine analyses, which included pH, ethanol (vol %), residual sugar (g/L), malic acid (g/L), volatile- and total acidity (g/L), were performed on an OenoFoss™. Organoleptic evaluations of the finished wines were performed as well.

5.5. *Analyses*

All acetaldehyde analyses were initially done at an external laboratory (GC-FID), until the decision was made to utilise the Arena Enzyme Robot, after collaboration with Dr Wessel du Toit of Department Oenology & Viticulture at Stellenbosch University. The Arena Enzyme Robot, at the university's Central Analytical Facility, is an enzymatic-based method performed on an automated machine. It requires a very small sample volume for analysis (2 mL instead of 20 - 100 mL as needed for the GC-FID method), which is very beneficial for frequent sampling periods, as it will not have a major impact on the sample volume, and has a much faster turnaround time than the GC-FID method. Thus, after a comparative study of similar samples analysed on both the Arena Enzyme Robot and a GC-FID, the results proved to be statistically very similar and the decision was made to do all future acetaldehyde quantification on the Arena Enzyme Robot instead. Therefore, the GC-FID method for acetaldehyde quantification has been replaced with the use of the Arena Enzyme Robot instead.

Routine analyses, which included pH, ethanol (vol %), residual sugar (g/L), malic acid (g/L), volatile- and total acidity (g/L), were performed on an OenoFoss™ at ARC Infruitec-Nietvoorbij.

5.6. *Progress reports*

All progress reports were submitted timeously.

RESULTS AND DISCUSSION

6.1. *Laboratory-scale trials (screening of yeasts)*

Preliminary studies performed showed that treatments such as freezing and settling of must, and filtration before analysis, had no significant effect on acetaldehyde levels (Fig. 1).

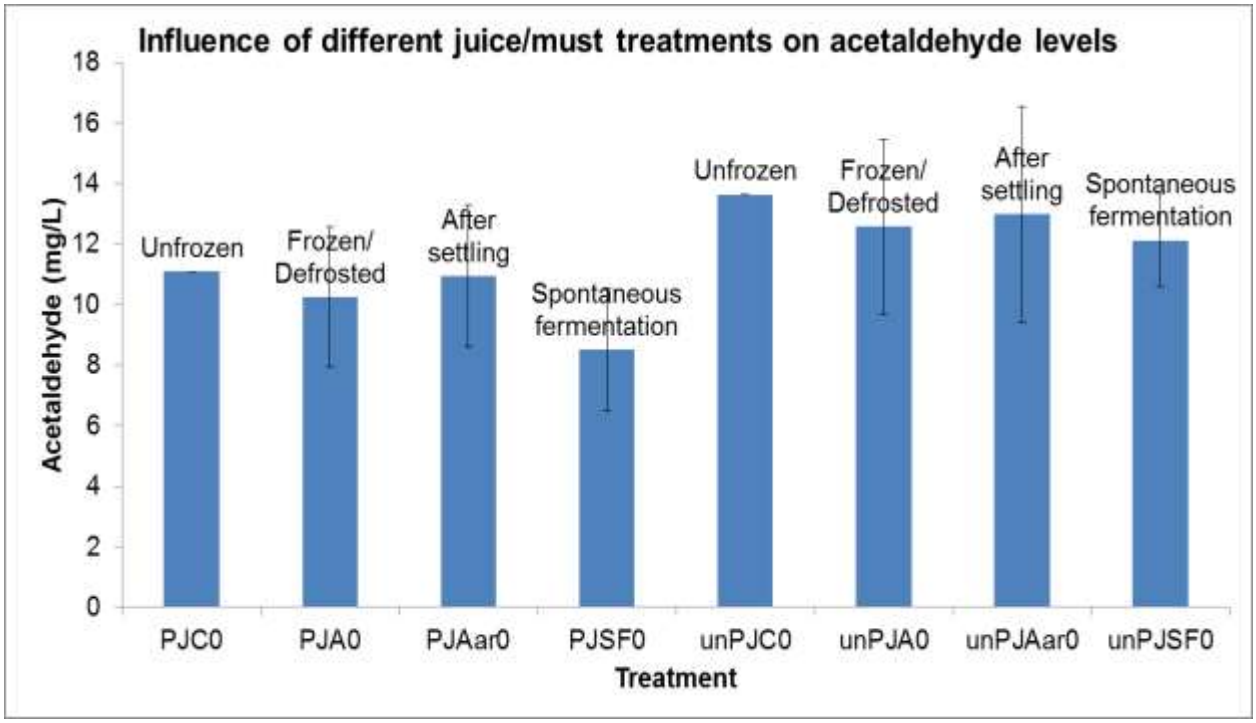


Figure 1. Influence of different Pinotage juice/must treatments on acetaldehyde levels. Abbreviations: PJ, Pinotage juice; C, before freezing; A, after freezing; Aar, after racking; 0, before fermentation (Day 0); SF, spontaneous fermentation; and un, unfiltered. Acetaldehyde analysis (enzyme robot) was performed on unfiltered and filtered (0.22μm) must samples.

Ten commercial *Saccharomyces* (Table 1) and 10 non-commercial non-*Saccharomyces* yeasts (Table 1) were screened for their acetaldehyde-producing abilities during fermentation at two temperatures. Evaluation of the data evidently shows the impact of yeast strain, time of fermentation, and fermentation temperature on levels of acetaldehyde (Tables 3 and 4).

The *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts showed different rates of acetaldehyde production at 15 and 25˚C during fermentation. The differences observed between samples at the two temperatures could be due to varying fermentation rates at the different temperatures. Environmental/treatment conditions (i.e. ageing, pH, sugar content, free SO_2, metals, racking, bottling and filtering, turbidity with oxygen exposure), including temperature, reportedly affect acetaldehyde levels. However, findings are controversial, with increases and no increases reported. Increases in production or increased acetaldehyde concentrations in wine at higher temperatures, i.e. 30˚C, have been reported in literature, as also observed in this study

for *C. valida* with increased acetaldehyde production from 15 to 25˚C (Table 3). Production of higher acetaldehyde concentrations achieved during fermentation at the lower temperature, and vice versa, have been reported, and in agreement to findings in this study for *T. delbrueckii* (Table 3). The fluctuation in acetaldehyde levels during fermentation could be as a result of the yeast cells producing acetaldehyde, but also metabolising it at different times, as well as being degraded or absorbed by various substances in the fermentation medium.

The screening trial resulted in a statistically-based selection of the following yeasts: *Saccharomyces cerevisiae* yeasts: NT 50 (high), NT 116 (medium), VIN 13 (low), and non-*Saccharomyces* yeasts: *Torulaspora delbrueckii* (high), *Candida valida* (medium), *Candida guilliermondii* (low).

Table 3. Impact of non-*Saccharomyces* yeasts, fermentation time and temperature on acetaldehyde levels

Non-*Saccharomyces*	**Acetaldehyde concentration (mg/L) at 25°C**						
Yeast	**DAY 2**	**DAY 6**	**DAY 8**	**DAY 11**	**DAY 15**	**DAY 18**	**DAY 22**
K. thermotolerans	25,895±5.89^{ghijklmn}	21,530±0.08^{ijklmnopqr}	22,775±0.12^{ijklmnopq}	24,440±0.57^{hijklmno}	21,770±1.87^{ijklmnopqr}	25,855±0.53^{ghijklmn}	25,250±0.49^{hijklmn}
K. apiculata	33,515±1.52^{efghij}	7,0950.35±st	8,270±0.48^{rst}	8,900±0.99^{qrst}	13,135±0.40^{lmnopqrst}	13,800 ±0.61^{lmnopqrst}	18,950±5.73^{klmnopqrst}
C. pulcherrima	25,325±0.76^{ghijklmn}	9,135±0.16^{qrst}	11,165±1.39^{opqrst}	21,020±4.74^{ijklmnopqrs}	17,145±5.08^{klmnopqrst}	10,330±0.83^{pqrst}	18,250±0.21^{klmnopqrst}
T. delbrueckii	51,675±0.81 ^{cd}	39,915±3.02^{def}	40,405±2.53^{def}	39,950±3.12^{def}	37,525±4.12^{efgh}	37,860±3.46^{defgh}	34,400±3.53^{efghi}
C. lambica	29,950±1.60^{efghijk}	10,460±1.58^{pqrst}	19,260±1.57^{klmnopqrst}	10,865±0.76^{opqrst}	16,160±18.12^{klmnopqrst}	8,985±7.59 ^{qrst}	18,050±19.87^{klmnopqrst}
H. valbyensis	19,505±2.81^{klmnopqrst}	10,405±0.40^{pqrst}	12,410±0.24 ^{nopqrst}	14,110±2.84^{lmnopqrst}	15,180±1.17^{lmnopqrst}	12,695±4.56^{mnopqrst}	16,500 ±1.56^{klmnopqrst}
C. guilliermondii	16,880±0.79^{klmnopqrst}	9,545±3.50^{qrst}	15,555±0.16^{lmnopqrst}	15,885±2.90^{lmnopqrst}	20,430±6.65^{ijklmnopqrst}	10,750 ±2.02^{opqrst}	24,200±2.12^{hijklmnop}
C. valida	8,750±3.82^{rst}	42,165±54.33^{de}	26,975±8.38^{fghijkl}	14,040±2.26^{lmnopqrst}	13,995±5.90^{lmnopqrst}	16,570±6.92^{klmnopqrst}	18,000±5.94^{klmnopqrst}
P. kluyveri	26,645±1.56^{fghijklm}	10,310±0.06^{pqrst}	16,425±4.86^{klmnopqrst}	11,970±2.12^{nopqrst}	39,280±4.24^{defg}	14,710±0.39^{lmnopqrst}	15,350±0.07^{lmnopqrst}
L. fermentati	16,440±3.55^{klmnopqrst}	6,600±0.99^{t}	10,005±2.07^{qrst}	12,600±2.82^{nopqrst}	16,820±3.73^{klmnopqrst}	15,420±1.87^{lmnopqrst}	20,150±1.90^{jklmnopqrst}
NT50 (control)	118,545±3.61^{a}	64,465±0.08^{bc}	65,105±1.00^{bc}	68,040±0.45^{b}	60,650±1.15^{bc}	63,275±0.28^{bc}	59,650±0.35^{bc}
	Acetaldehyde concentration (mg/L) at 15°C						
	DAY 3	**DAY 7**	**DAY 8**	**DAY 11**	**DAY 15**	**DAY 18**	**DAY 22**
K. thermotolerans	30,765±3.06^{i}	18,525±0.53^{mnopqrst}	22,555±1.03^{jklmn}	18,635±0.84^{mnopqrst}	18,460±0.11^{mnopqrst}	20,270±0.57^{klmnopq}	21,000±0.56^{jklmnop}
K. apiculata	30,815±3.44^{i}	17,775±0.54^{nopqrst}	23,260±2.10^{jklmn}	9,875±0.03^{abcdefghz}	16,290±3.79^{pqrstuvw}	9,060±0.59^{abcdefghikj}	8,050±0.07^{cdefg}
C. pulcherrima	30,705±2.75^{i}	12,155±0.60^{ij}	25,935±4.61^{ij}	13,955±1.57^{astuvwxyz}	24,325±6.63^{jkl}	15,340±0.46^{qrstuvwxy}	21,850±4.31^{cdrfg}
T. delbrueckii	52,840±0.94^{fg}	45,825±1.11^{h}	74,240±1.04^{d}	53,345±0.38^{fg}	68,880±1.69^{e}	48,800±1.47^{gh}	54,200±0.14^{f}
C. lambica	10,415±1.25^{abcefgyz}	7,140±0.48^{efghijk}	12,325±0.10^{abcdevwxyz}	7,405±0.28^{defghijk}	13,805±2.42^{astuvwxyz}	10,385±7.09^{abcdefgy}	19,400±13.29^{lmnopqr}
H. valbyensis	23,195±1.11^{jklm}	14,405±0.72^{rstuvwxyz}	22,375±0.21^{jklmn}	13,480±1.21^{abtuwxyz}	18,945±1.36^{mnopqrst}	13,210±0.56^{abcuvwxyz}	16,000±0.56^{pqrstuvw}
C. guilliermondii	20,210±1.15^{lmnopq}	9,850±5.21^{abcdefghiz}	9,610±2.14^{abcdefghiz}	12,535±1.53^{abcdevwxyz}	21,015±1.19^{jklmnop}	4,740±1.01^{hijk}	25,450±8.55^{jk}
C. valida	6,925±0.40^{fghijk}	7,390±0.21^{defghijk}	6,025±0.57^{ghijk}	4,715±0.27^{hijk}	4,380±0.33^{ijk}	3,885±0.44^{k}	4,650±0.21^{ijk}
P. kluyveri	9,395±0.21^{abcdefghiz}	10,845±2.01^{abcdefgxz}	12,790±0.02^{abcuvwxyz}	12,275±1.47^{abcdevwxyz}	16,675±1.08^{astuvwxyz}	6,095±0.41^{ghijk}	13,500±2.12^{abtuvwxyz}
L. fermentati	22,580±4.32^{jklmn}	11,420±1.61^{abcdefwy}	17,855±1.15^{nopqrst}	9,915±0.24^{abcdefgh}	10,670±1.06^{abcdefgyz}	8,435±0.24^{bcdeefghijk}	11,100±0.98^{abcdexyz}
NT50 (control)	96,920±0.69^{c}	104,935±2.38^{b}	112,610±0.96^{a}	107,420±2.34^{a}	99,170±1.71^{c}	75,915±2.59^{d}	65,050±0.35^{e}

Table 4. Impact of *Saccharomyces cerevisiae* yeasts, fermentation time and temperature on acetaldehyde levels

Saccharomyces cerevisiae	**Acetaldehyde concentration (mg/L) at 25°C**					**Acetaldehyde concentration (mg/L) at 15°C**			
	DAY 2	**DAY 8**	**DAY 15**	**DAY 22**		**DAY 3**	**DAY 8**	**DAY 15**	**DAY 22**
VIN13	14.755±0.09^{klm}	9.415±0.04^{p}	14.930±3.39^{jklm}	21.575±4.12^{def}		43.890±4.68^{c}	9.685±0.18^{lmnop}	8.825±0.74^{op}	11.140±1.32^{jklmnop}
EC1118	19.910±0.28^{fgh}	12.585±0.61^{mno}	19.715±0.50^{fgh}	25.055±0.70^{bc}		63.430±6.82^{a}	19.145±2.02^{g}	10.945±1.55^{jklmnop}	15.175±2.77^{ghij}
VL1	17.900±1.20^{ghij}	8.780±0.19^{p}	12.665±0.55^{mno}	17.665±0.87^{ghijk}		32.485±0.52^{e}	13.510±0.00^{hijklmn}	10.065±0.77^{lmnop}	13.985±1.22^{hijklm}
VL3	21.690±0.57^{def}	9.040±0.42^{p}	13.470±1.47^{mno}	19.880±4.45^{fgh}		44.500±1.39^{c}	18.820±1.50^{g}	13.000±1.30^{hijklmo}	14.720±0.58^{ghijk}
D47	19.580±1.73^{fgh}	9.305±0.33^{p}	13.350±1.27^{mno}	17.225±0.20^{hijkl}		45.500±4.50^{c}	13.860±0.51^{hijklmn}	8.415±0.37^{p}	10.115±0.35^{lmnop}
QA23	19.330±1.00^{fgh}	11.625±1.97^{npo}	14.535±2.17^{lmn}	16.960±1.65^{hijkl}		37.960±0.82^{d}	16.335±1.61^{ghi}	10.125±0.66^{lmnop}	14.050±1.91^{hijkl}
NT202	25.045±1.83^{bc}	10.530±1.23^{po}	18.035±3.75^{ghi}	20.350±2.51^{efg}		62.030±7.45^{a}	16.530±1.13^{gh}	10.280±0.37^{klmnop}	13.055±0.15^{hijklmno}
NT116	23.300±2.57^{cde}	10.665±0.89^{po}	18.005±0.75^{ghi}	21.110±0^{ef}		54.370±6.51^{b}	15.480±0.45^{ghij}	9.470±0.23^{mnop}	13.000±0.78^{hijklmno}
NT50	63.360±3.81^{a}	14.395±0.02^{lmn}	24.145±0.10^{bcd}	27.070±3.70^{b}		63.33±0.17^{a}	26.755±4.59^{f}	11.140±0.01^{jklmnop}	11.910±0.27^{jklmnop}
NT112	19.770±1.95^{fgh}	9.585±0.60^{p}	15.155±1.33^{ijklm}	19.170±1.38^{fgh}		51.690±1.20^{b}	17.030±0.91^{gh}	7.790±0.21^{pq}	9.335±0.28^{nop}

6.2. *Wine-making (with selected yeast strains / combinations) – Cellar trial*

The cellar trial commenced by inoculation with the highest-, medium- and lowest-acetaldehyde producing yeast strains from both groups in various permutations. For each combination treatment the non-*Saccharomyces* yeasts were inoculated 24 h prior to *Saccharomyces cerevisiae* inoculation.

The levels of acetaldehyde, during alcoholic fermentation, in the different treatments varied from 4.8 – 86.1 mg/L on specific sampling days. This large variation confirmed that certain yeasts can play a role in influencing the levels of acetaldehyde in wines.

During the course of fermentation acetaldehyde levels increase, level off, and decrease (Fig. 2). Yeast strain impacts on the levels of acetaldehyde during fermentation and also in the finished wine (Figs 2 and 3). Variable acetaldehyde yields as affected by fermentation conditions and (dominant) yeast strain(s) involved (large species and strain differences) have been reported in literature, also for certain mutants of *Saccharomyces cerevisiae* accumulating more acetaldehyde in the medium than the parental strain. Acetaldehyde levels in samples inoculated with *Saccharomyces cerevisiae* are generally higher than in samples co-inoculated with non-*Saccharomyces* (Table 5; Fig. 2). All co-inoculations with a non-*Saccharomyces* yeast resulted in lower levels of acetaldehyde than the inoculations with only *Saccharomyces cerevisiae* (Fig. 2; Table 5). Colony forming units (CFU) were monitored for all yeast treatments during fermentation and confirmed that increases in acetaldehyde levels were a reflection of yeast growth during fermentation.

It is also possible for acetaldehyde produced by yeast cells to be re-utilised again, degraded or absorbed by various substances in the fermentation medium.

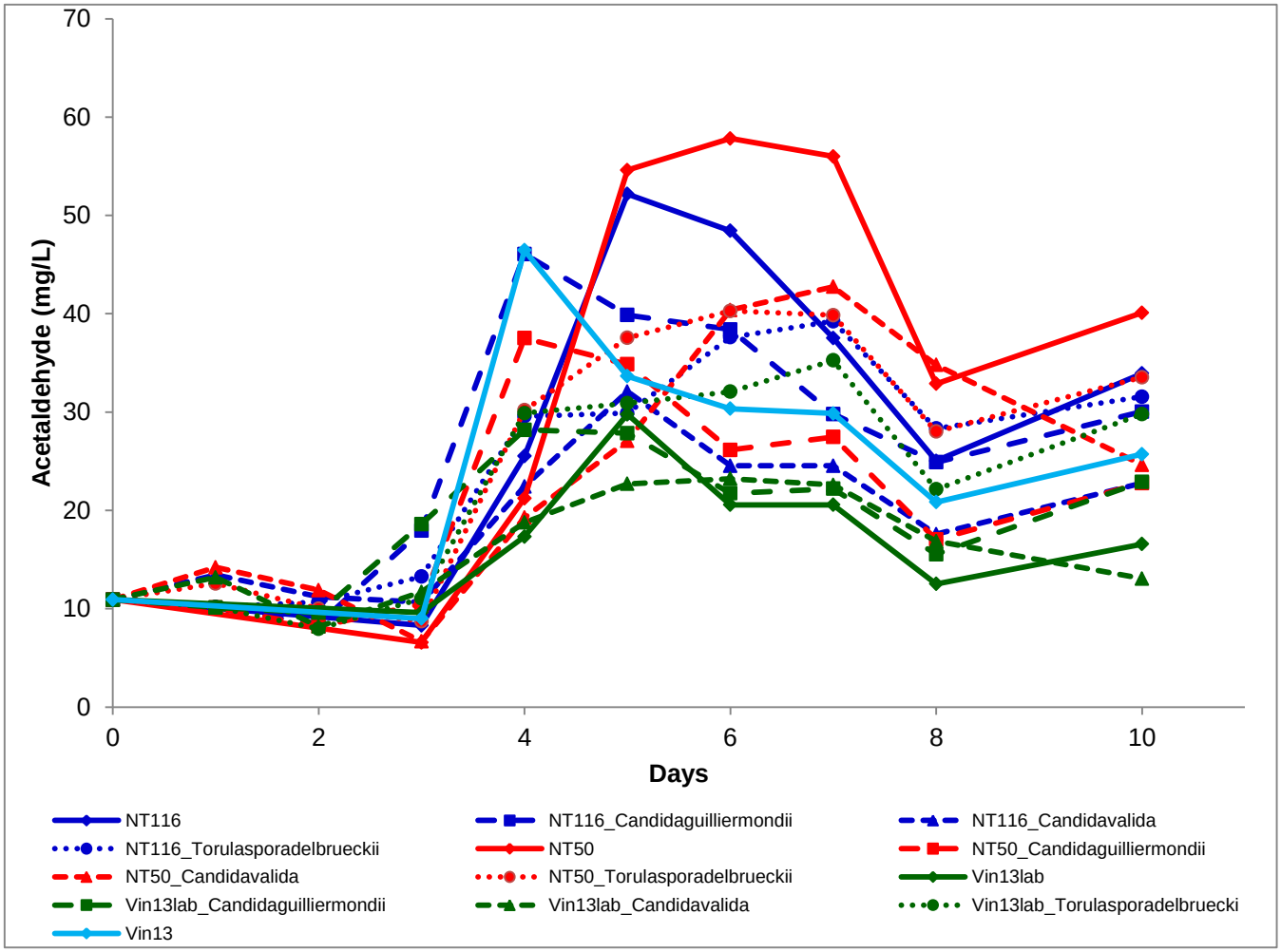


Figure 2. Acetaldehyde production by various *Saccharomyces cerevisiae* strains, and by the same strains co-inoculated with non-*Saccharomyces* yeast species, during the fermentation of Nietvoorbij Pinotage. “Vin13lab” indicates the yeast sourced from the ARC Microbiology Yeast Culture Collection at Nietvoorbij, Stellenbosch. “Vin13” indicates the commercial dry yeast used by Nietvoorbij Cellar.

Table 5. Acetaldehyde concentration in Nietvoorbij Pinotage as a function of yeast/combinations

Yeast(s) and combinations	Acetaldehyde concentration (mg/L) ± (Standard deviation)
VIN 13-Candida valida	17.16 (3.19)
VIN 13	18.40 (4.11)
VIN 13-Candida guilliermondii	19.23 (3.04)
NT 116-Candida valida	19.56 (2.83)
NT 50-Candida guilliermondii	21.47 (3.61)
VIN 13-Torulaspora delbrueckii	22.43 (5.56)

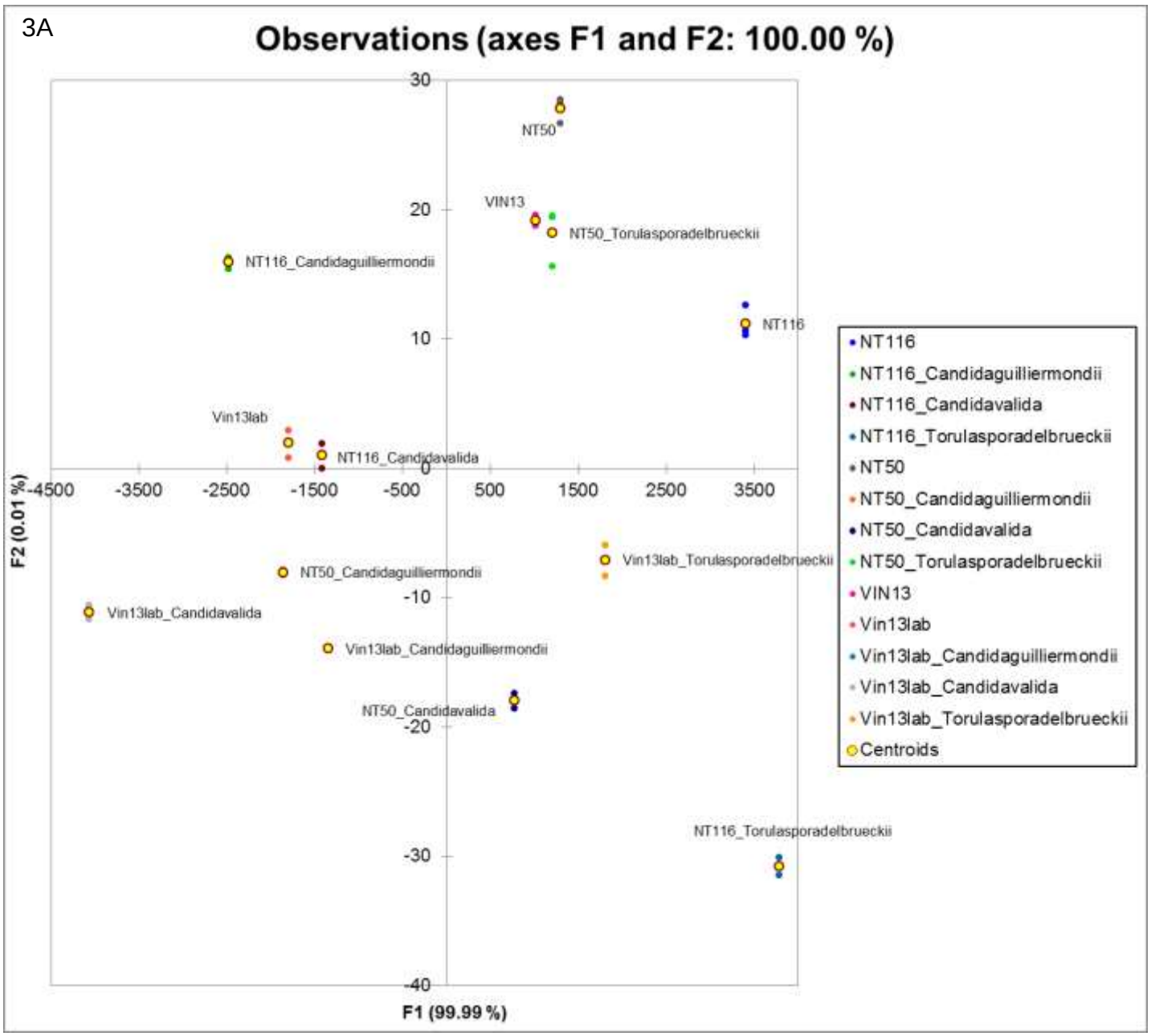
NT 50-Candida valida	24.63 (8.28)
NT 116-Torulaspora delbrueckii	24.84 (7.30)
NT 50-Torulaspora delbrueckii	25.90 (7.80)
NT 116-Candida guilliermondii	26.93 (6.29)
VIN 13*	28.35 (8.51)
NT 116	32.83 (7.38)
NT 50	38.18 (15.67)

*Commercial dry yeast
All other yeasts were sourced from the ARC Microbiology Yeast Culture Collection & Genebank at Nietvoorbij, Stellenbosch.

The sensorial evaluation took the form of an informal tasting and discussion with judges from the university (Stellenbosch) as well as from Nietvoorbij, all in possession of the wine-tasting certificate. During this evaluation a "free profiling" was done where each judge evaluated the wines and gave a list of various descriptors for each wine. This list of free-profiling descriptors also included some of the typical acetaldehyde descriptors. These descriptors were studied, and similar descriptors grouped together under more general headings. These descriptors, as well as those associated with acetaldehyde (obtained from literature), were used in a formal 10-point line scale wine-tasting for both vintages, in which the intensity of each of the aroma descriptors, previously discussed, was evaluated. The aroma descriptors include the “typical” acetaldehyde descriptors nutty, sherry-like, bruised apple, green, grassy and metallic, and other descriptors, i.e. dried fruit, cooked/vegetative, off flavour aroma, fresh vegetative, astringency, caramel-butter, mouthfeel, sweet-associated, floral, alcohol, acidity and berry (Fig. 3A, B).

Many of the treatments were found to have residual sugar levels, which can be attributed to the mixed fermentations (i.e. *Saccharomyces* and non-*Saccharomyces* yeasts) which result in different rates of sugar metabolism (glucose, fructose or sucrose), which could possibly have masked some of the more typical cultivar aromas. Most of the yeasts/combinations presenting higher acetaldehyde levels gave hints of vegetativeness.

3A



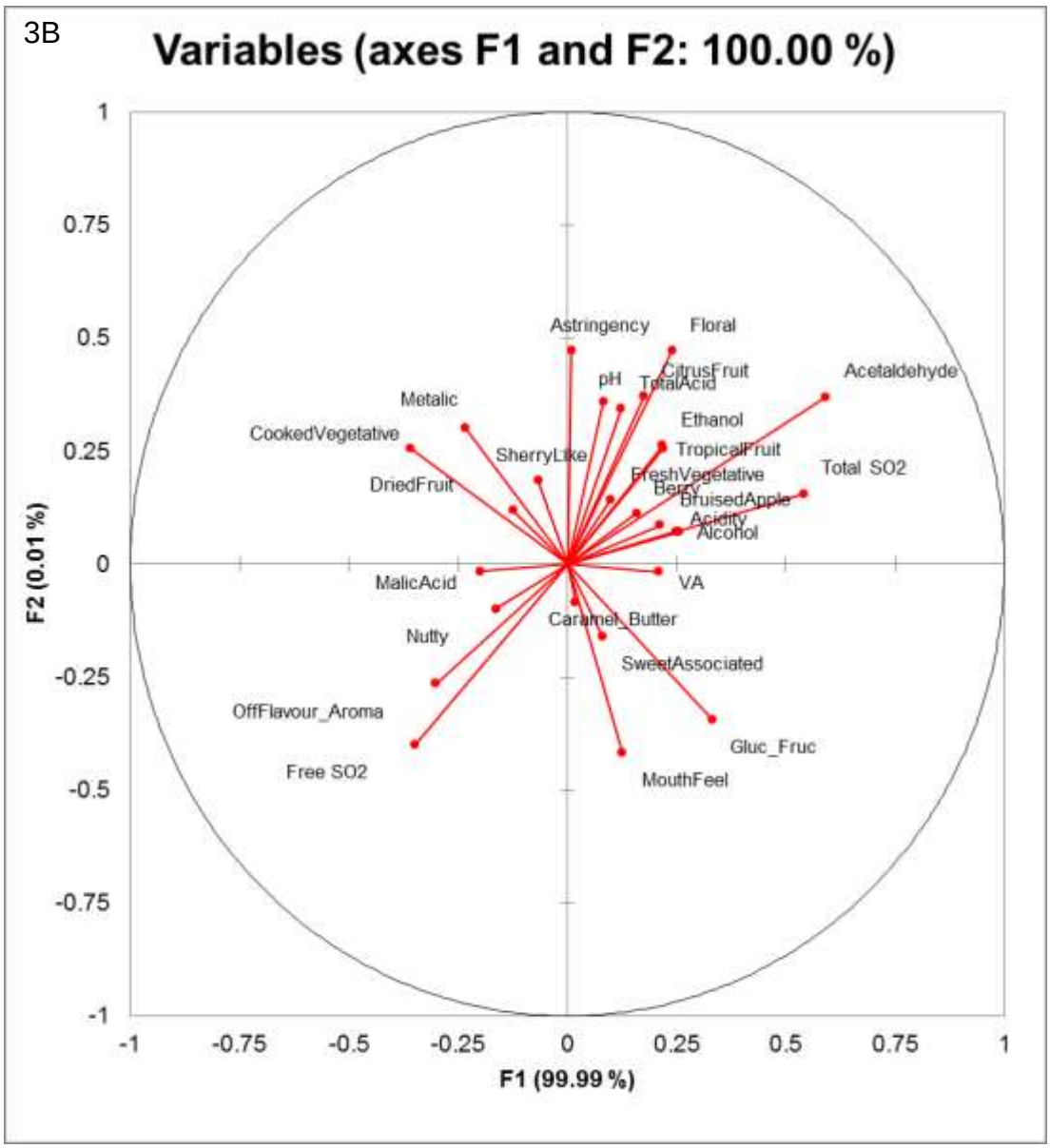


Figure 3 A & B. Discriminant analysis plot of Pinotage finished wine based on sensory and chemical variables for discrimination between classes, i.e. NT 116, NT 116_Candida guilliermondii, NT 116_Candida valida, NT 116_Torulaspora delbrueckii, NT 50, NT 50_Candida guilliermondii, NT 50_Candida valida, NT 50_Torulaspora delbrueckii, VIN 13, VIN 13lab, VIN 13lab_Candida guilliermondii, VIN 13lab_Candida valida, VIN 13lab_Torulaspora delbrueckii. Chemical variables analysed include the routine wine parameters pH, TA, VA, ethanol, malic acid, total sugar (glucose + fructose), free and total SO_2 and acetaldehyde. Sensory descriptors include nutty, sherry like, bruised apple, green, grassy, metallic, nutty, dried fruit, cooked/vegetative, off flavour aroma, fresh vegetative, astringency, caramel-butter, mouthfeel, sweet-associated, floral, alcohol, acidity and berry. “Vin13lab” indicates the yeast sourced from the ARC Microbiology Yeast Culture Collection at Nietvoorbij, Stellenbosch. “Vin13” indicates the commercial dry yeast.

6.3. *The effect of SO_2 on the levels of acetaldehyde, and the resulting effect on fermentation dynamics and wine quality - Cellar trial*

Acetaldehyde originates as an intermediary or by-product of yeast metabolism during alcoholic fermentation. Acetaldehyde is excreted mainly during the yeast growth period. The highest or peak concentrations of acetaldehyde are formed by yeast metabolism at the very beginning of alcoholic fermentation (first few days), declining gradually towards the end of fermentation due to re-adsorption by yeast.

Acetaldehyde is released by the yeast as the detoxification mechanism for sulphites. Acetaldehyde has a strong affinity for SO_2, therefore, varying concentrations of the one, will influence the free or available levels of the other, with subsequent influence on wine quality. Therefore, the effect of SO_2 addition on the levels of acetaldehyde produced by the *Saccharomyces cerevisiae* yeast VIN 13 during alcoholic fermentation, was investigated. The varying levels of SO_2 had a direct effect on the acetaldehyde levels during fermentation of Chenin blanc grape must. The increased production of acetaldehyde by yeast cells in response to increased levels of SO_2 is evident from figure 4, as well as the decreased production of acetaldehyde as the yeasts die off in the presence of higher ethanol concentration. Additional reasons for this levelling off and decreasing trends, during the latter stages of fermentation, could be due to exposure to oxygen (aldehydes degrade in air via the process of autoxidation), and because of their tendency to oligomerise, polymerise or hydrate. Fermentations under severely repressive conditions, such as juices treated with high SO_2 (metabisulfite) prior to the onset of fermentation, reportedly cause a correspondingly higher production of acetaldehyde by the yeast cells to tie up the SO_2. A possible explanation for this observed trend could be that high SO_2 levels affects the enzyme that converts acetaldehyde into ethanol (i.e. alcohol dehydrogenase), by binding directly with acetaldehyde, thereby preventing its transformation to ethanol.

The fermentation of Pinotage took place on the grape skins and the “punching-down” activity was performed to extract colour and aroma compounds, as well as tannins to which SO_2 and acetaldehyde can bind. Acetaldehyde concentration decreases during the initial stages of fermentation on the grape skins, while the latter part of the fermentation shows an increased and decreased trend up to the end of fermentation (Fig. 4). These decreases could be explained by the binding of acetaldehyde to SO_2,

and to the grape skins and phenolics during fermentation. The “punching-down” activity may also contribute to the loss of SO_2 and acetaldehyde due to their volatility. Increases in observed SO_2 levels (particularly noticeable for low or no added SO_2 concentrations) after a few days of fermentation, could be reflective of SO_2 produced by the yeast. The concentrations of SO_2 used in the experiment were within the legal upper limit of 160 mg/L, imposed by South African regulations.

Groupings based on final SO_2 concentrations can clearly be observed using Principal Component Analysis (PCA) and Discriminant Analysis (DA) (Figs 5 and 6, respectively); SO_2 concentration did, therefore, impact on the groupings observed. Higher acetaldehyde levels are observed with higher levels of SO_2 additions.

The dynamics of acetaldehyde and SO_2 changes throughout fermentation and culminates in the finished wine, with red and white wine making procedures impacting on these dynamics (Figs 4-6). Not only do the patterns of acetaldehyde levels differ during fermentation between red and white musts, but also the levels of acetaldehyde in the finished white wine is more varied than in the case of red wine (Table 6), as influenced by different levels of added SO_2 before fermentation.

Not only do the levels of acetaldehyde in this study fall within reported levels for acetaldehyde in white (11 – 493, average 80 mg/L) and red (4 – 212, average 30 mg/L) wines (Table 4), but increased levels of acetaldehyde as a result of increased levels of SO_2 also changes the wine organoleptically as the flavour threshold values in wine, i.e. 100-125 mg/L is reached (Fig. 4).

Table 6. Acetaldehyde concentration in finished wine as influenced by different SO_2 concentration additions to grape must before fermentation

	Chenin blanc – Finished wine			Pinotage – Finished wine	
Treatment	N	Acetaldehyde (mg/L)		N	Acetaldehyde (mg/L)
0	2	21.13 (0.070)		3	25.78 (3.83)
50	3	44.52 (2.10)		3	24.76 (3.04)
100	3	101.87 (4.72)		3	28.78 (9.45)
150	2	68.20 (1.29)		3	31.09 (0.97)

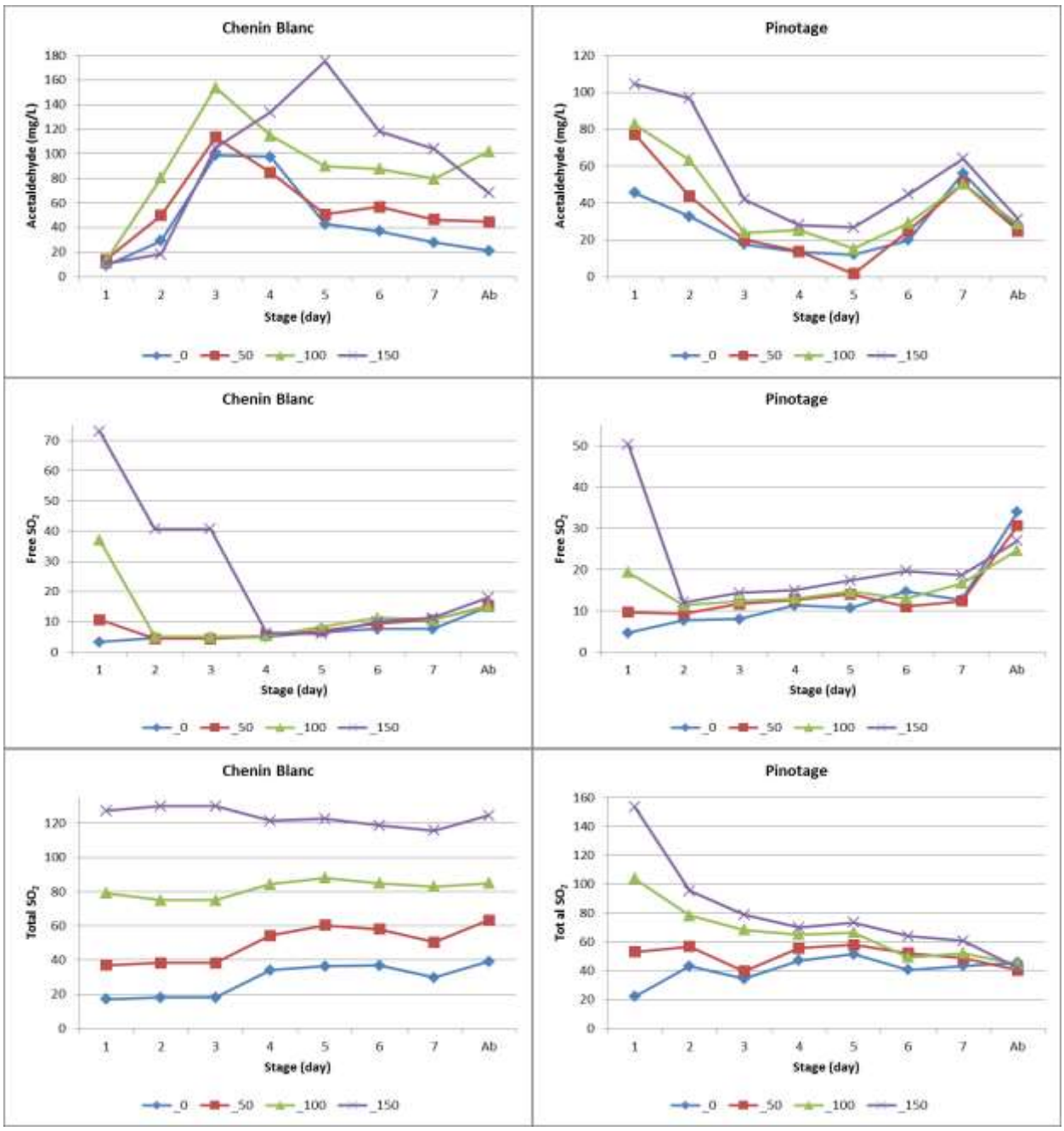


Figure 4. Impact of added SO_2 (0, 50, 100 and 150 ppm) on acetaldehyde production by VIN 13 during fermentation, and changes in the concentration of SO_2 during fermentation. Concentration reflects those of acetaldehyde and free and total SO_2 actually measured during fermentation. SO_2 was added on Day 0, and first measurement made on Day 1. The control (no SO_2 added) had a baseline SO_2 value and was not 0 mg/L. Abbreviations: Ab, after bottling.

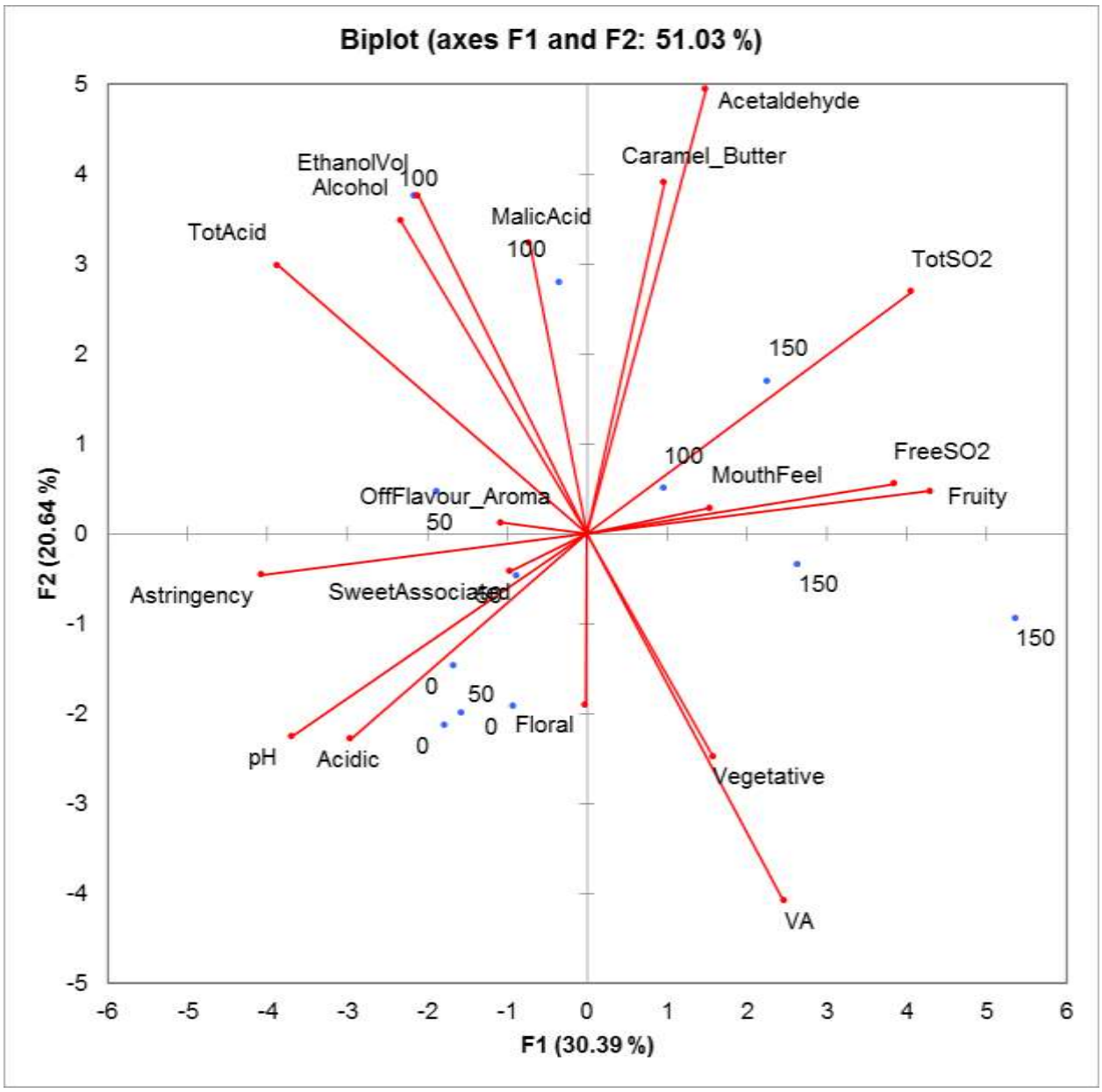


Figure 5. PCA plot of Chenin blanc finished wine based on sensory and chemical variables for discrimination between classes, i.e. 0, 50, 100 and 150 ppm SO_2 added to final concentration before inoculation of grape must with VIN 13. Chemical variables analysed for include the routine wine parameters pH, TA, VA, alcohol, malic acid, free and total SO_2, and acetaldehyde. Sensory descriptors include vegetative, acidic, astringency, caramel-butter, fruity, mouthfeel, off flavours-aromas, sweet associated and floral.

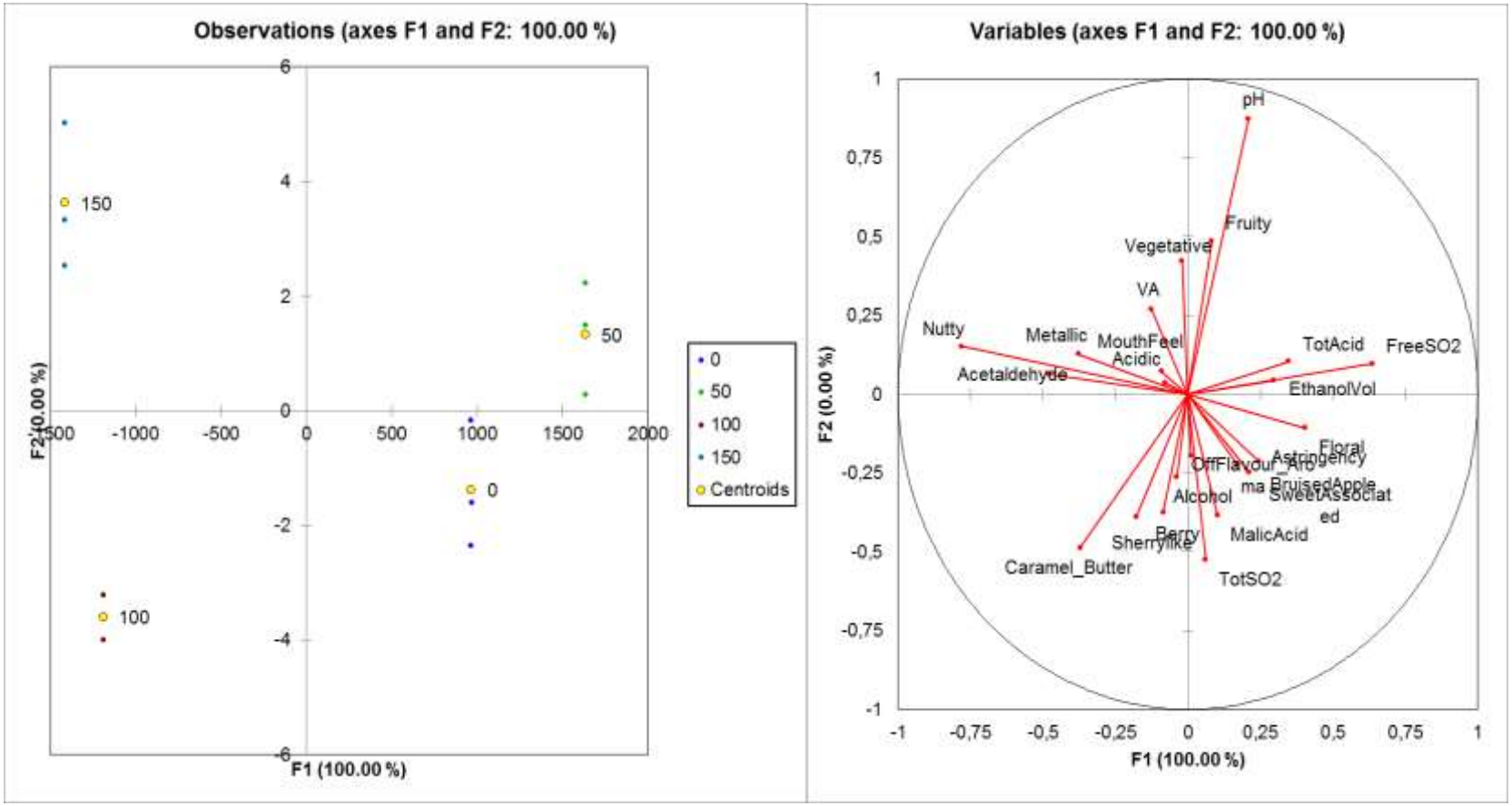


Figure 6. Discriminant analysis plot of Pinotage finished wine based on sensory and chemical variables for discrimination between classes, i.e. 0, 50, 100 and 150 ppm SO_2 added to final concentration before inoculation of grape must with VIN 13. Chemical variables analysed for include the routine wine parameters pH, TA, VA, alcohol, malic acid, free and total SO_2, and acetaldehyde. Sensory descriptors include metallic, bruised apple, nutty, vegetative, acidic, astringency, caramel-butter, fruity, mouthfeel, off flavours-aromas, sweet associated and floral.

6.4. *Analyses* / **6.5.** *Progress reports*

See points 5.5 and 5.6 above.

References

1. Hucker, B. & Vriesekoop, F. (2008) Acetaldehyde stimulates ethanol-stressed Saccharomyces cerevisiae, grown on various carbon sources. Folia Microbiol. 53 (6), 505-508.

2. Jackowetz, J.N., Dierschke, S. & Mira de Orduña, R. (2010) Multifactorial analysis of acetaldehyde kinetics during alcoholic fermentation by Saccharomyces cerevisiae. Food Research Int. 44, 310-316.

3. Liu, S.-Q. & Pilone, G.J. (2000) An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications. Int. J. Food Sci. Technol. 35, 49-61.

4. Nykänen, L. (1986) Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. Am. J. Enol. Vitic. 37 (1), 85-96.

5. Osborne, J.P., Mira de Orduña, R., Pilone, G.J. & Liu, S.-Q. (2000) Acetaldehyde metabolism by wine lactic acid bacteria. FEMS Microbiology Letters 191, 51-55.

6. Romano, P., Suzzi, G., Turbanti, L. and Polsinelli, M. (1994) Acetaldehyde production in Saccharomyces cerevisiae wine yeasts. FEMS Microbiology Letters 118, 213-218.

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8. Wildenradt, H.L. & Singleton, V.L. (1974) The production of aldehydes as a result of oxidation of polyphenolic compounds and its relation to wine aging. Am. J. Enol. Vitic. 25 (2), 119-126.

7. COMPLETE THE FOLLOWING TABLE

Milestone	Target Date	Extension Date	Date completed	Achievement
1. Literature review (continuous)	Continue up and until submission of thesis in		2016 (MSc thesis to be handed in end of	A preliminary literature review was already handed in with the project proposal. A

	2016		2016)	popular publication was published in Wineland Magazine. M.Sc. proposal/ oral presentation was presented on 24 May 2012 at the Post-graduate Forum of the Biochemistry department at University Stellenbosch.
2. Procurement of grapes and yeast	2012-2015 harvests		2016	Completed
3. Laboratory-scale fermentation trials (Screening of yeasts): Screening of Saccharomyces and non-Saccharomyces yeasts, and selecting a high, medium & low acetaldehyde-producing yeast strain from each genera.	2012		2013	Completed
4. Wine-making (with selected yeast strains / combinations)	2013		2014	Completed
5. Wine-making (with SO_2 adjustments)	2014		2015	Completed
6. Monitoring acetaldehyde metabolism by wine LAB in synthetic medium	2013		2013	Additional experiment performed to what was proposed in project proposal.
7. Quantification of acetaldehyde	2012-2015 harvests		2016	Completed
8. Routine or standard wine analyses	2012-2015		2016	Completed
9. Sensorial evaluations	2012-2015		2016	Completed
10. Progress/Final	2012-2015		2016	Completed

Reports				
11. Journal publication(s)	2016/17		2016/17	2016/17
12. Popular publication(s)	2016/17		2016/17	One completed. One more to follow scientific publication
13. MSc thesis/dissertation – final milestone	2016/17		2016/17	Graduation December 2016/March 2017

8. CONCLUSIONS

Successful screening of various *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts resulted in a statistically-based selection of high-, medium- & low acetaldehyde-producing yeast strains from each genera. The high-, medium- & low acetaldehyde-producing yeasts were combined in various permutations in a cellar trial. The impact of yeast strain, time of fermentation, and fermentation temperature on levels of acetaldehyde, is evident.

In the cellar trial that monitored the effect of varying SO_2 levels on acetaldehyde levels in fermenting must, the varying levels of SO_2 had a direct effect on the acetaldehyde levels during fermentation and in the finished wine.

Although there were differences in acetaldehyde levels between yeast strains used in this study, the levels were still within acceptable ranges or limits found in wines. Acetaldehyde levels in samples inoculated with *Saccharomyces cerevisiae* are generally higher than in samples co-inoculated with non-*Saccharomyces*. Higher levels of SO_2 can trigger increased production of acetaldehyde by yeasts in fermenting musts. Yeast metabolism, specifically the production of acetaldehyde by different yeast strains, is also influenced by the fermentation temperature. Therefore, co-inoculation with selective non-*Saccharomyces* yeasts at low fermentation temperatures, as well as maintaining low levels of SO_2 during fermentation, may result in wines with lower levels of acetaldehyde.

In the additional experiment that was not in the project proposal, acetaldehyde metabolism by wine LAB in synthetic medium was monitored. None of the Nietvoorbij LAB strains that were screened showed any degradation of acetaldehyde.

Mr FM October successfully registered (first year of registration 2012) for a M.Sc. study on this project and aims to submit his dissertation in 2017 for graduation in March 2018.

9. ACCUMULATED OUTPUTS

Dr Van Jaarsveld managed to secure additional collaboration on this project with Dr Wessel du Toit from the Department Oenology & Viticulture at Stellenbosch University.

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

Co-inoculation with selective non-*Saccharomyces* yeasts at low fermentation temperatures, as well as maintaining low levels of SO_2 during fermentation, may result in wines with lower levels of acetaldehyde, subject to local conditions. Such knowledge proves valuable in optimising the alcoholic fermentation process, indirectly enhancing the safety and quality of wine.

Patents: N.A.

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

OCTOBER F.M., ROHWER J. & VAN JAARSVELD F.P., 2013. The effect of yeast strains and oenological parameters on acetaldehyde production and wine quality during alcoholic fermentation. (English). (ROHWER J., US, Department of Biochemistry). July 2012 progress report to Winetech.

OCTOBER F.M., ROHWER J., DU TOIT W. & VAN JAARSVELD F.P., 2013. The effect of yeast strains and oenological parameters on acetaldehyde production and wine quality during alcoholic fermentation. (English). (ROHWER J., US, Department of Biochemistry, DU TOIT W., US, Department of Oenology and Viticulture). July 2013 progress report to Winetech.

OCTOBER F.M., ROHWER J., DU TOIT W. & VAN JAARSVELD F.P., 2013. The effect of yeast strains and oenological parameters on acetaldehyde production and wine quality during alcoholic fermentation. (English). (ROHWER J., US, Department of Biochemistry, DU TOIT W., US, Department of Oenology and Viticulture). July 2014 progress report to Winetech.

OCTOBER F.M., ROHWER J., DU TOIT W. & VAN JAARSVELD F.P., 2013. The effect of yeast strains and oenological parameters on acetaldehyde production and wine quality during alcoholic fermentation. (English). (ROHWER J., US, Department of Biochemistry, DU TOIT W., US, Department of Oenology and Viticulture). March 2016 final report to Winetech.

OCTOBER F.M., ROHWER J., DU TOIT W. & VAN JAARSVELD F.P., 2014. The effect of yeast strains and oenological parameters on acetaldehyde production and wine quality during alcoholic fermentation. (English). (ROHWER J., US, Department of Biochemistry, DU TOIT W., US, Department of Oenology and Viticulture). March 2014 progress report to The Department of Biochemistry at Stellenbosch University who has introduced progress report forms for M and D students starting from the beginning of 2014. The Progress Report on project WW 08/34 reported on progress so far and aims and milestones for the next period.

OCTOBER F.M., ROHWER J., DU TOIT W. & VAN JAARSVELD F.P., 2014. The effect of yeast strains and oenological parameters on acetaldehyde production and wine quality during alcoholic fermentation. (English). (ROHWER J., US, Department of Biochemistry, DU TOIT W., US, Department of Oenology and Viticulture). July 2015 progress report to The Department of Biochemistry at Stellenbosch University. The Progress Report on project WW 08/34 reported on progress and aims and milestones for the next period.

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

Student Name and Surname	Student Nationality	Degree (e.g. MSc Agric, MComm)	Level of studies in final year of project	Graduation date	Total cost to industry throughout the project
Honours students					
ND Biotech 3rd year (2013) - Ms N. Qokela (Student/WIL)	South African	ND Biotechnology 3rd year		2013	16 660
ND Biotech 3rd year and B Tech 4th year (2012/3) - Ms V. Nqkenqka (Student/WIL)*	South African	ND Biotech 3rd year / B Tech 4th year		2013	14 700

ND Biotech 3rd year (2014) - Ms N. Phaladi (Student/WIL)	South African	ND Biotechnology 3rd year		2014	14 700
Masters Students					
MSc (2012-2016) - Mr FM October	South African	MSc		2016/17	
PhD students					
PhD - Mr HW du Plessis	South African	PhD		2017	
PhD - Mr Boredi Silas Chidi (ARC-PDP)	South African	PhD		2016	
Postdocs					
PhD - Mr Boredi Silas Chidi (DST-NRF-PDP)	South African	Post-doc		-	
Support Personnel					
C. Paulsen and cellar team	South African	Winemaker / B. Agric. Oenology and Viticulture		-	
Ms Marieta van der Rijst	South African	Statistician / MSc Biometry		-	

* The student we hosted did very very well with a final mark of 72, being 2nd with a class pass. Message received from Dr. Karabo S. Ntwampe [EngD, HDHET*, MSAIChe, MWISA), Senior Lecturer/3rd year and WIL co-ordinator, Biotechnology, Cape Peninsula University of Technology, Department of Agricultural and Food Sciences.

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

Van Jaarsveld F, October F. (2015) Asetaldehied in wyn. *Winetech Tegnies* nr 310 – June 2015, 73-76.

e) PRESENTATIONS/PAPERS DELIVERED

- VAN JAARSVELD F.P., OCTOBER F.M., ROHWER J. & DU TOIT W., 2013. Effect of oenological parameters on acetaldehyde kinetics. 35th SASEV/ WINETECH CONFERENCE. 14 November, 2013. (ROHWER J., DU TOIT W., US).
- A presentation in Italy on 23 October 2012 titled: "Acetaldehyde in Wine".
- Mr October completed an oral presentation (2013) of the MSc project proposal as a university requirement.
- Mr October completed an oral presentation (2015) of the MSc project progress as a university requirement.

10. BUDGET

a) TOTAL COST SUMMARY OF THE PROJECT

YEAR	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
2012/13					127 032		132 218	**259 250**
2013/14*					144 817*		150 729	**295 546**
2014/15					234 569		244 141	**478 710**

*Represents actual cost (received), although the estimated budget for project was R179 663 (Winetech), R186 997 (other) and R366 660 (total).

b) FINAL BUDGET/FINANCIALS OF PROJECT

Please ensure that the budget is sufficiently detailed and add notes to explain all significant variations from the budget – you may submit this in an EXCEL document. Please report on the budget for the entire duration of the project. Add additional rows if required.

Project duration	Proposed budget	Actual cost incurred	Variance	Notes
TOTAL INCOME				
Industry Funding	506 420	506420	0	Winetech

Project duration	Proposed budget	Actual cost incurred	Variance	Notes
PHI Funding	-	-	-	-
Other Funding	300 000	300 000	0	THRIP
TOTAL EXPENDITURE				
Running Expenses	174039	174039	0	-
General operating costs (printing, communication, etc.)				
Local Travel				
Publication costs				
Lab Analysis				
Lab Consumables				
Other				
Running expenses SUB-TOTAL				
HR Administration and Project Management	230 135	230 135	0	**-**
HR Technical				
HR Research				
Student Bursaries				
HR SUB-TOTAL				
OTHER EXPENSES	108 253	108 253	0	Overheads
SURPLUS / DEFICIT				

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_____

*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