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Wine yeasts of New Zealand

An investigation into their distribution, contribution to Sauvignon Blanc aroma and interaction in co-ferments

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A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Sciences

The University of Auckland, 1st July 2010
Sauvignon Blanc is the most important grape variety for New Zealand’s economy with exports of ~$740M in 2008. The two varietal thiols 3-mercaptohexanol (3MH) and 3-mercaptohexyl acetate (3MHA), reminiscent of tropical aromas like passion fruit and grapefruit, have been identified as key aroma compounds in Marlborough Sauvignon Blanc. These compounds are released during fermentation due to the metabolic action of yeast. This thesis investigates the contribution of natural New Zealand yeast isolates to the characteristic aroma of Sauvignon Blanc. Natural *Saccharomyces* and non-*Saccharomyces* isolates were screened for their 3MH and 3MHA production. Four non-*Saccharomyces* yeasts from two species, *Candida zemplinina* and *Pichia kluyveri*, were identified as predominantly producing either 3MH or 3MHA. In subsequent co-fermentation experiments with commercial *S. cerevisiae* strains, the combination of VL3 and *P. kluyveri* I at a ratio of 1:9 showed remarkable increase in 3MHA production. Analysis of nitrogen usage in this co-ferment revealed that *P. kluyveri* I was able to grow on proline as sole nitrogen source in a synthetic medium. Proline is one of the most abundant amino acids in grape juice but cannot be utilized by *S. cerevisiae* as its degradation pathway requires oxygen. Nothing is known about proline utilization in *P. kluyveri* but the data presented here suggests a different utilization pathway than that found in *S. cerevisiae*.

In addition, a *S. cerevisiae* population was isolated for the first time from a New Zealand vineyard, mainly from soil samples. Furthermore, *S. cerevisiae* strains found in a nearby apiary matched genotypes found in the vineyard, providing evidence that bees are transport vectors for *S. cerevisiae*. 
Around here, however, we don’t look backwards for very long. We keep moving forward, opening new doors, and doing new things, because we’re curious and curiosity keeps leading us down new paths.

Walt Disney

Für Opi

(19.03.1916 – 1996)
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# Table of Contents

Abstract....................................................................................................................................................II
Dedication................................................................................................................................................III
Acknowledgements...............................................................................................................................IV
Table of Contents.....................................................................................................................................VI
List of Figures.......................................................................................................................................XI
List of Tables.......................................................................................................................................XVIII
Abbreviations.......................................................................................................................................XX

Chapter 1

General Introduction
1.1 Preface ...............................................................................................................................................2
1.2 Winemaking ......................................................................................................................................3
1.3 Fermentation ...................................................................................................................................4
  1.3.1 Yeasts in winemaking .............................................................................................................6
1.4 Wine aroma ......................................................................................................................................6
1.5 History of wine in New Zealand ......................................................................................................8
1.6 Aims ..................................................................................................................................................10

Chapter 2

Investigating the origin, diversity and dispersal of *Saccharomyces cerevisiae* in New Zealand

2.1 Introduction ........................................................................................................................................12
  2.1.1 Origin of *Saccharomyces cerevisiae* ...................................................................................12
  2.1.2 Transport vectors for *Saccharomyces cerevisiae* ...............................................................17
  2.1.3 Species identification .............................................................................................................18
  2.1.4 Aims ..........................................................................................................................................20
Table of Contents

2.2 Materials and Methods ..............................................................................................................21
  2.2.1 Sample sites ..........................................................................................................................22
  2.2.2 Protocol for the isolation of S. cerevisiae samples (based on Mortimer and Polsinelli 1999) ......................................................................................................................................24
  2.2.3 Species identification .........................................................................................................25
  2.2.4 S. cerevisiae strain differentiation ..................................................................................27
  2.2.5 Glycerol stocks ..................................................................................................................27
  2.3 Results and Discussion ..............................................................................................................29
    2.3.1 Isolation of S. cerevisiae from Matua Valley vineyard ...................................................29
      2.3.1.1 S. cerevisiae Matua Valley vineyard population ......................................................31
      2.3.1.2 Distribution and relationship of the S. cerevisiae vineyard population ........................33
    2.3.2 Isolation of yeasts from beehives ..................................................................................41
    2.3.3 Bees – a transport vector for S. cerevisiae? ....................................................................43
  2.4 Conclusion ..................................................................................................................................46

Chapter 3

Yeasts' influence on the aroma and flavour of New Zealand Sauvignon Blanc

3.1 Introduction ..................................................................................................................................48
  3.1.1 Key aroma compounds of Sauvignon Blanc ....................................................................48
  3.1.2 Precursors of the key aroma compounds in Sauvignon Blanc ............................................50
  3.1.3 Biogenesis of thiols ..............................................................................................................52
  3.1.4 Aims .......................................................................................................................................56
  3.2 Materials and Methods ..............................................................................................................57
    3.2.1 4MMP-cysteine growth assay ..........................................................................................57
      3.2.1.1 Influence of 4MMP-cysteine or cysteine concentration on yeast growth ..................59
    3.2.2 Screening for thiol production .......................................................................................59
      3.2.2.1 Yeast species ..............................................................................................................59
Table of Contents

3.2.2.2  Microfermentation........................................................................................................62
3.2.2.3  Thiol extraction .........................................................................................................65
3.2.2.4  GC-MS analysis .........................................................................................................66
3.2.3  Five litre fermentation..................................................................................................67
  3.2.3.1  Sensory Consumer study............................................................68

3.3  Results and Discussion..................................................................................................70
  3.3.1  4MMP-cysteine growth assay ..................................................................................70
    3.3.1.1  Influence of 4MMP-cysteine concentration on yeast growth..................73
  3.3.2  Yeasts’ influence on thiol production in New Zealand Sauvignon Blanc......75
    3.3.2.1  Screening of *Saccharomyces sensu stricto* group yeasts for 3MH and
             3MHA release..................................................................................................76
    3.3.2.2  Screening of non-*Saccharomyces* yeasts for 3MH and 3MHA release...79
  3.3.3  Co-fermentation of four non-*Saccharomyces* species with commercial *S.
        cerevisiae* strains..........................................................................................82
    3.3.3.1  3MH to 3MHA conversion by non-*Saccharomyces* yeast.................87
    3.3.3.2  Is the 3MH and 3MHA increase in *P. kluyveri* co-ferments due to the
             action of *P. kluyveri*? ..............................................................................93
    3.3.3.3  The generality of the interaction ....................................................................97
    3.3.3.4  Sequential inoculation.....................................................................................99
  3.3.4  Five litre upscale experiment...................................................................................101
    3.3.4.1  Consumer tasting of co-fermented wines ...........................................106

3.4  Conclusion....................................................................................................................110

Chapter 4

Mechanisms underlying interactions in co-ferments

4.1  Introduction ..................................................................................................................112
  4.1.1  Co-fermentations with non-*Saccharomyces* species ..............................112
  4.1.2  Factors influencing yeast survival during fermentation............................113
  4.1.3  Aims.........................................................................................................................116
# Table of Contents

4.2 Materials and Methods ........................................................................................................... 117
  4.2.1 Population dynamics of *P. kluyveri* I and VL3 in single and co-ferments .................. 117
  4.2.2 Assay for inhibitory compounds ................................................................................. 118
  4.2.3 Nutrient assay ............................................................................................................... 120
  4.2.4 Proline assay ............................................................................................................... 121
4.3 Results and Discussion ......................................................................................................... 122
  4.3.1 Population dynamics of *P. kluyveri* I and VL3 in single and co-ferments ............... 122
    4.3.1.1 Is 3MHA increase due to an additive effect? ....................................................... 128
  4.3.2 Assay for inhibitory compounds ................................................................................. 129
  4.3.3 Nutrient assay ............................................................................................................... 134
  4.3.4 Proline assay ............................................................................................................... 138
4.4 Conclusion ................................................................................................................................. 143

# Chapter 5

## General Discussion

**Introduction** ............................................................................................................................................... 145
  5.1 *S. cerevisiae* population outside the winery ................................................................. 146
  5.2 Influence of natural yeast isolates on Sauvignon Blanc aroma and flavour ............... 149
  5.3 Manipulation of Sauvignon Blanc aroma and flavour using co-fermentation .......... 151
  5.4 Interactions in co-ferments ............................................................................................... 153
  5.5 Are there benefits for yeasts in thiol release? ................................................................. 154
  5.6 Summary ..................................................................................................................................... 157

## Appendices

**Appendix A** ................................................................................................................................................. 158
  Evaluation of two isolation protocols for *Saccharomyces cerevisiae* from natural samples
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.1</td>
<td>Introduction</td>
<td>158</td>
</tr>
<tr>
<td>A.2</td>
<td>Comparison of two isolation protocols</td>
<td>159</td>
</tr>
<tr>
<td>A.2.1</td>
<td>Bark and Soil experiment</td>
<td>163</td>
</tr>
<tr>
<td>A.2.2</td>
<td>Viability of <em>S. cerevisiae</em> and <em>T. delbrueckii</em> in enrichment culture media</td>
<td>165</td>
</tr>
<tr>
<td>A.2.3</td>
<td>PIM2 Test</td>
<td>168</td>
</tr>
<tr>
<td>A.3</td>
<td>Conclusion</td>
<td>173</td>
</tr>
<tr>
<td>Appendix B</td>
<td>Consumer tasting sheets</td>
<td>174</td>
</tr>
<tr>
<td>Appendix C</td>
<td>Fermentation progress of the different microferments</td>
<td>180</td>
</tr>
<tr>
<td>Appendix D</td>
<td>References</td>
<td>184</td>
</tr>
</tbody>
</table>
List of Figures

Figure 1: The winemaking process. The green circles indicate the two main origins for aroma compounds. Modified after Jackson (2000). ................................................................. 4

Figure 2: Different hypothesis on the life cycle of S. cerevisiae during the year. A. based on Hansen (reviewed by Martini 1993); B. based on different researchers reviewed by Brysch-Herzberg (2004) and Martini (1993) and C. based on Martini et al. (1996). ....13

Figure 3: Experimental procedure for the isolation of S. cerevisiae .............................................. 21

Figure 4: Map of the Waimauku region west of Auckland, showing its relative position in New Zealand and the Auckland region (with the Mangere sample site). Indicated are the two different sample sites, Matua Valley vineyard and Beesonline Café respectively. ....23

Figure 5: Map of the Matua Valley winery and their surrounding vineyards. Indicated are the sample sites with more detailed description in the table. ............................................. 24

Figure 6: Life cycle of S. cerevisiae. ........................................................................................................... 32

Figure 7: Microsatellite profiles (genotypes) distinguished by their abundance in space and time. ................................................................................................................................. 34

Figure 8: Distribution of the 41 different genotypes of S. cerevisiae in the Matua Valley Sauvignon Blanc vineyard block over space and time. Red represents genotypes isolated only once, yellow represents genotypes unique to the sample site and date and green correspond to genotypes found at more than one site or date. *1 all these genotypes found in bark, *2 these genotypes were isolated from a buttercup flower in the direct surrounding of the vine on sample site i. All other genotypes were isolated from soil. ...36
Figure 9: Distribution of the six different genotypes isolated more than once from different sample sites and/or dates from the Matua Valley vineyard block. The colours of the boxes represent one sample site each as indicated in the legend below the chart.

Figure 10: Neighbour net of the 41 genotypes found in the Matua Valley Sauvignon Blanc vineyard block. Red dots indicate unique genotypes, yellow are genotypes found at least twice at the same sample site and date, and green dots indicate genotypes found at different sample sites and dates.

Figure 11: Graphic display of where the 6 different genotypes were found. Same colour arrows indicate same genotype. Arrows do not indicate potential travel routes of *S. cerevisiae* but merely try to connect the sites where the same genotypes were found.

Figure 12: Hypothesized pathway of 4MMP release from its cysteine precursor.

Figure 13: Hypothesized pathway of 3MHA production based on Swiegers et al. (2006b).

Figure 14: 4MMP-cysteine growth assay. a) SD medium positive control. b) to g) growth of the six different yeast strains in 3% (w/v) 4MMP-cysteine medium (circle), 1.5% (w/v) cysteine medium (triangle) and negative control medium (bar). Arrows indicate when additional glucose and ammonium sulfate was added to all treatments. n=1.

Figure 15: 4MMP-cysteine growth assay with different concentrations of 4MMP-cysteine and cysteine over time. Values are mean ± standard error of the mean. n=3.

Figure 16: Concentrations of 3MH and 3MHA in wines fermented with different *Saccharomyces sensu stricto* strains. The black lines represent the minimum concentration of 3MH and 3MHA found in Marlborough Sauvignon Blanc (Nicolau et al. 2006). Values are mean ± s.e.m. n=3.
List of Figures

Figure 17: Concentrations of 3MH and 3MHA released by non-Saccharomyces species in Sauvignon Blanc ferments. The black line represents the minimum concentration of 3MH and 3MHA found in Marlborough Sauvignon Blanc (Nicolau et al. 2006). The arrows indicate strains with the highest concentration of 3MH or 3MHA. Values are mean ± s.e.m. n=3. .......................................................... 80

Figure 18: Weight loss for single ferments of C. zemplinina I and II and P. kluyveri I and II. For comparison, the weight loss curve of S. cerevisiae is shown. Values are mean ± s.e.m., n=3. .................................................................................................................................................. 83

Figure 19: 3MH and 3MHA concentrations in co-ferments of VL3 and four non-Saccharomyces (NS) species inoculated at three different ratios. The dashed line represents the mean concentration of 3MH or 3MHA in the VL3 single ferments for comparison. Values are mean ± s.e.m. n=3; * p<0.05, ** p<0.01 calculated using t-tests in comparison to the VL3 single ferment. ........................................................................................................................................ 84

Figure 20: Hypothesis of 3MH to 3MHA conversion a) for S. cerevisiae by Swiegers et al. 2006b, b) for C. zemplinina I and II and c) for P. kluyveri I and II. Including the 3MH and 3MHA concentrations found in single ferments for VL3, C. zemplinina strains and P. kluyveri strains (see also Figure 19) to support the hypothesis. .................................................................................................. 89

Figure 21: 3MH and 3MHA release in the 3MHA conversion experiment. Values are mean ± s.e.m. n=3. * p<0.05, ** p<0.01.......................................................................................................................... 91

Figure 22: Concentrations for 3MH and 3MHA in VL3 single and co-ferments with P. kluyveri I. Here, the inoculum size for VL3 varied from 100% to 0.1% in both single and co-ferments. The inoculum size of P. kluyveri I in the co-ferment was kept constant at 90% (=2.25×10^6 cells mL^{-1}). Values are mean ± s.e.m. n=3. .................................................................................................................. 94

Figure 23: Fermentation progress measured via weight loss of VL3 single and co-ferments with P. kluyveri I. Here, the inoculum size for VL3 varied from 100% (=2.5×10^6 cells mL^{-1}) to 0.1% in both single and co-ferments. The inoculum size of P. kluyveri I in the co-ferment was kept constant at 90% (=2.25×10^6 cells mL^{-1}). Values are mean ± s.e.m. n=3. .................................................................................................................. 96
List of Figures

Figure 24: Concentrations of 3MH and 3MHA in single and co-ferments of five different commercial wine yeasts and *P. kluyveri* I. The co-ferments were inoculated at a 1:9 ratio wine yeast to *P. kluyveri* I with total inoculum size of 2.5×10⁶ cells mL⁻¹. The fermentation was conducted at 14°C. Values are mean ± s.e.m. n=3. **p<0.01 calculated using a t-test. ................................................. 98

Figure 25: Concentration of 3MH and 3MHA in wines inoculated with *P. kluyveri* I, II, *C. zemplinina* I, II and VL3 and VIN7 added after 4 days of fermentation. Values are mean ± s.e.m. n=3................................................................................................................................. 100

Figure 26: Concentrations of 3MH and 3MHA in 5 L wines of single and co-ferments. Co-ferments were initiated with 10% (=2.5×10⁵ cells mL⁻¹) VIN7 and 90% (=2.25×10⁶ cells mL⁻¹) split evenly between the non-*Saccharomyces* yeasts if needed. Values are mean ± s.e.m. n=4, *p<0.05 (calculated with two tailed t-test in comparison to the single VIN7 control). ............................................................................................................................................. 103

Figure 27: Fermentation progress of the 5 L single and co-ferments measured via weight loss and Brix measurement. The values for weight loss are mean ± s.e.m. with n=4............................................................................................................................................... 104

Figure 28: Winescan results for the 5 L fermentations. Shown here are the values for residual sugar, total acidity, glycerol, pH and volatile acidity. Values are mean ± s.e.m. n=8 *p<0.05, **p< 0.01 as calculated with t-tests in comparison to the VIN7 single ferment............................................................................................................................................. 105

Figure 29: Results of the consumer wine tasting of three co-ferments with VIN7 in comparison to the single VIN7 ferment. The dashed line in a) indicates the category of neither like nor dislike. Values are mean ± s.e.m, n=64. ................................................................................................................................. 108

Figure 30: VL3 and *P. kluyveri* I together on a YPD plate................................................................. 118

Figure 31: Population dynamics of VL3 (blue) and *P. kluyveri* I (yellow) single ferments and the fermentation progress over time measured in weight loss for VL3 (green) and *P.
List of Figures

kluyveri I (red). Values are mean ± s.e.m. n=6 for population dynamics and n=3 for weight loss data. 122

Figure 32: Frequencies of VL3 and P. kluyveri I in co-ferments inoculated at three different ratios (9:1, 1:1 and 1:9). Values are mean ± s.e.m. n=3. 124

Figure 33: Cell concentrations of VL3 (blue) and P. kluyveri I (yellow) in a 1:9 co-ferment. Grey circles show the total amount of cells mL⁻¹ in the co-ferment. Green triangles show the fermentation progress measured via weight loss. Values are mean ± s.e.m. n=6 for cell concentration and n=3 for weight loss. 125

Figure 34: Inhibitory compound assay with P. kluyveri I. P. kluyveri I cells grown to mid-exponential phase were subjected to different supernatant: fresh grape juice and single ferments of VL3, VIN7 and P. kluyveri I (as control), or co-ferments of VL3/ VIN7 with P. kluyveri I at 1:9 ratio after growth for 34 hours. Values are mean ± s.e.m. n=6. 131

Figure 35: Inhibitory compound assay with VL3. VL3 cells grown to mid-exponential phase were subjected to different supernatants: fresh grape juice and single ferments of P. kluyveri I and VL3 (as control), or co-ferments of VL3 with P. kluyveri I at 1:9 ratio after growth for 34 hours. Values are mean ± s.e.m. n=6. 132

Figure 36: Inhibitory compound assay with VIN7. VIN7 cells grown to mid-exponential phase were subjected to different supernatants: fresh grape juice and single ferments of P. kluyveri I and VIN7 (as control), or co-ferments of VIN7 with P. kluyveri I at 1:9 ratio after growth for 34 hours. Values are mean ± s.e.m. n=6. 133

Figure 37: a) YAN and b) glucose/fructose usage in single and co-ferments with P. kluyveri I. c) weight loss of the respective ferments. Values are mean ± s.e.m. n=3. 135

Figure 38: Population dynamics of the single ferments of VL3, VIN7 and P. kluyveri I in the nutrition experiment. Values are mean ± s.e.m. n=6. 136
List of Figures

Figure 39: Population dynamics of *P. kluyveri* I, VL3 and VIN7 in synthetic media with proline as sole nitrogen source and 200 g L\(^{-1}\) glucose as carbon source. Values are mean ± s.e.m., n=6. .................................................................................................................................................... 139

Figure 40: Comparison of population dynamics in grape juice (previous experiments see Section 4.3.1 population dynamics (blue) and Section 4.3.3 nutrition assay (green)) and in synthetic proline media. Values are mean ± s.e.m. n=6. .................................................................................. 141

Figure 41: Total cell numbers for *T. delbrueckii* and *S. cerevisiae* after incubation in SelMed and PIM1. 10, 10\(^3\) and 10\(^5\) represent the starting concentration (cells mL\(^{-1}\)) of *S. cerevisiae* in the media. mean ± s.e.m.; n=3 for samples with starting concentration of 10 *S. cerevisiae* cells mL\(^{-1}\) and n=2 for all others. .................................................................................. 162

Figure 42: a) sterile bark pieces submerged in lab yeast mix; b) sterile soil submerged in lab yeast mix.................................................................................................................................................. 163

Figure 43: Total cell concentration for *T. delbrueckii* and *S. cerevisiae* after recovering from bark and soil samples using different pre-isolation techniques and two different media, SelMed and PIM1 respectively. Initial concentration of 10\(^3\) cells mL\(^{-1}\) *S. cerevisiae* in 10\(^8\) cells mL\(^{-1}\) of *T. delbrueckii* cells. Mean ± s.e.m.; n=3. .............................................................................................. 164

Figure 44: Total cell count for *S. cerevisiae* and *T. delbrueckii* after five days (red) compared to day zero (yellow). Mean ± s.e.m. (on day five); n=9 for counts on day five. .............................................................................................................................................. 166

Figure 45: Cell counts of living (white) and dead (blue) *S. cerevisiae* and *T. delbrueckii* cells after five days of incubation in SelMed and PIM1 respectively. Mean ± s.e.m.; n=9. .............................................................................................................................................. 167

Figure 46: Changes of living (open triangle and square) to dead (blue triangle and square) *T. delbrueckii* cells in SelMed (square) and PIM1 (triangle) over 48 hours. Mean ± s.e.m.; n=3. .............................................................................................................................................. 168
Figure 47: Consumer tasting sheet to rate the aroma and flavor of each wine.................174

Figure 48: Consumer tasting: difference test example sheet...........................................175

Figure 49: Fermentation progress measured via weight loss for the Saccharomyces
senso stricto group yeasts screened for 3MH and 3MHA release (see Figure 16). Values
are mean ± s.e.m, n=3. .................................................................................................................. 180

Figure 50: Fermentation progress measured via weight loss for the two Candida species
releasing high concentrations of 3MH and the two Pichia species releasing high
concentrations of 3MHA (see Figure 17). Values are mean ± s.e.m., n=3. ......................181

Figure 51: Fermentation progress measured via weight loss for the non-Saccharomyces
species screened for 3MH and 3MHA production (see Figure 17). Values are mean ±
s.e.m., n=3........................................................................................................................................... 182

Figure 52: Fermentation progress measured via weight loss in single and co-ferments of
VL3 and C. zemplinina I and II and P. kluyveri I and II. Only the single ferments are
shown in the legend as all the other co-ferments regardless of the starting ratio cluster
right below the VL3 single ferment indicating a finished fermentation. These ferments
were conducted at 25°C. Also see Figure 19. Values are mean ± s.e.m., n=3 .................183
List of Tables

Table 1: Components of SelMed and YPD media (all %=w/v, except ethanol which is v/v). .................................................................25

Table 2: Microsatellite primer set used for *S. cerevisiae* strain differentiation..........................28

Table 3: Restriction patterns of isolates from Matua Valley vineyard samples obtained by digesting ITS sequences with *Hinfl* and *HaeIII*, respectively. The numbers reflect band sizes in base pairs, judged visually comparing them to a size marker. The numbers refer to the frequencies of the particular restriction pattern. The 26S DNA of a member of each pattern was sequenced and the closest match found in the Genbank database (NCBI) is listed with its accession number and similarity. ..........................................................30

Table 4: Yeasts isolated from beehives in Mangere Bridge and Beesonlive in winter (June to September 2005) and spring (November 2005). Total bp refers to the size of the ITS PCR product as visually compared to a size marker. The numbers of *Hinfl* and *HaeIII* reflect band sizes in base pairs (bp) as compared to a size marker on a 1.5% (w/v) agarose gel. *26S DNA comparison instead of ITS sequence.* ...........................................42

Table 5: Organoleptic characteristics of volatile thiols identified in Sauvignon Blanc. ¹Tominaga *et al.* (1998a), ²Darriet *et al.* (1995), ³Tominaga *et al.* (1996);* in aqueous alcohol solution 12% v/v. .................................................................................49

Table 6: Yeast species employed for the 4MMP growth assay..................................................57

Table 7: Media used in the 4MMP growth assay (all %=w/v).......................................................58

Table 8: *Saccharomyces sensu stricto* yeasts screened for 3MH and 3MHA release.............60

Table 9: Non-*Saccharomyces* yeasts screened for 3MH and 3MHA release.........................61
Table 10: Commercial yeast strains used in co-fermentation experiments together with their respective suppliers. ........................................................................................................................... 62

Table 11: Inoculum concentrations for VL3 and *P. kluyveri* I to test the effect of varying inoculum concentrations on 3MH and 3MHA release in VL3 single fermentations and co-ferments with *P. kluyveri* I. .......................................................................................................................... 64

Table 12: Proportions of yeasts employed in the five litre fermentations. ............................................. 67

Table 13: Preferences of the different groups concerning aroma, taste and the most fruity and green. ............................................................................................................................................ 109

Table 14: Set up of inhibitory compound assay for *P. kluyveri* I; co= co-ferment. ........ 119

Table 15: Set up of inhibitory compound assay for VL3 and VIN7; co= co-ferment ...... 120

Table 16: Comparison of the media used by Mortimer and Polsinelli (1999) and Sniegowski *et al.* (2002) for isolation of *Saccharomyces* species (all concentrations w/v unless otherwise indicated). .................................................................................................................... 160

Table 17: Yeast mixtures utilized to explore the limitations of the isolation protocols of Mortimer and Polsinelli (1999) and Sniegowski *et al.* (2002). ................................................................. 161

Table 18: Composition of PIM2 medium and four other media to test the single components of the PIM2 medium (all% w/v, unless otherwise indicated). ....................... 169

Table 19: Yeast species utilized in the PIM2 medium test ................................................................. 170

Table 20: PIM 2 medium test with different *Saccharomyces* and non-*Saccharomyces* isolates. ................................................................................................................................. 171
Abbreviations

# number

(NH₄)₂SO₄ ammonium sulfate

°C degrees Celsius

3MH 3-mercaptohexan-1-ol

3MHA 3-mercaptohexyl acetate

4M2M2MB 4-methoxy-2-methyl-2-mercaptobutane

4MMP 4-mercapto-4-methylpentan-2-one

AA amino acid(s)

AWRI Australian Wine Research Institute

BC before christ

BHA butylated hydroxyanisole

bp base pair

CBS Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands)

cfu(s) colony forming unit(s)

CO₂ carbon dioxide

DMDC dimethyl dicarbonate

DNA deoxyribonucleic acid

dNTP(s) deoxynucleotide triphosphate(s)

ESR Environmental Science and Research Institute

FRST Foundation for Research, Science and Technology
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>gt</td>
<td>genotype</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>ITS</td>
<td>internal transcribed spacer</td>
</tr>
<tr>
<td>Kg, g, µg, ng</td>
<td>kilogram, gram, microgram, nanogram</td>
</tr>
<tr>
<td>L, ml, µl</td>
<td>litre, millilitre, microlitre</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>magnesium chloride</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>n</td>
<td>number</td>
</tr>
<tr>
<td>Na-acetate</td>
<td>sodium acetate</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
</tr>
<tr>
<td>p</td>
<td>probability value</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>pHMB</td>
<td>sodium-4-(hydroxymercuri)benzoate</td>
</tr>
<tr>
<td>rDNA</td>
<td>ribosomal DNA</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>s.e.m.</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SD medium</td>
<td>synthetic defined medium</td>
</tr>
<tr>
<td>SO₄</td>
<td>sulfate</td>
</tr>
<tr>
<td>TA</td>
<td>titratable acidity</td>
</tr>
<tr>
<td>YAN</td>
<td>yeast available nitrogen</td>
</tr>
<tr>
<td>YNB –aa-c-n</td>
<td>yeast nitrogen base, without amino acids, carbon and nitrogen source</td>
</tr>
<tr>
<td>YPD</td>
<td>yeast extract, peptone, dextrose rich medium</td>
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</tbody>
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